

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 ^aRegeneron Pharmaceuticals, Tarrytown; ^bthe Laboratory for Investigative Dermatology, Rockefeller University, New York; ^cSanofi, Bridgewater; and ^dthe 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, Inovaderm 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

© 2014 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

<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-4R α :	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-4R α) is a requisite receptor for signaling from both cytokines, we tested whether blocking IL-4R α could modify molecular mechanisms of AD pathogenesis in the skin. Dupilumab, a fully human mAb to IL-4R α 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 AD¹⁶ 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) score¹⁹ at baseline and week 4 (Table I).

RNA analyses

Expression profiling was performed to evaluate the effects of IL-4R α 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

TABLE I. Baseline and treatment response in biopsy substudy participants and the parent trials

Characteristic	Biopsy substudy			Combined parent trials		
	Placebo (n = 4)	Dupilumab, 150 mg (n = 7)	Dupilumab, 300 mg (n = 7)	Placebo (n = 16)	Dupilumab, 150 mg (n = 22)	Dupilumab, 300 mg (n = 21)
Age (y), mean (SD)	40.8 (26.1)	38.0 (10.4)	60.6 (15.9)	37.4 (17.2)	42.5 (11.4)	45.4 (15.9)
Male sex, no. (%)	3 (75)	5 (71)	6 (86)	11 (69)	12 (55)	10 (48)
Baseline EASI score, mean (SD)	23.6 (7.7)	30.5 (16.3)	28.2 (8.1)	22.8 (12.0)	30.0 (17.0)	27.4 (11.2)
Percentage change in EASI score, week 4, mean (SD)	-23.6 (8.1)	-43.8 (41.1)	-68.9 (22.5)	-25.4 (35.0)	-55.0 (30.4)	-64.3 (25.8)
EASI-50 responders at week 4 (%)	0*	42.9	85.7	18.8	54.5	71.4
Baseline serum total IgE (kU/L), geometric mean (95% CI)	2,833 (269.9-29,736.1)	1,090.3 (226.3-5,252.8)	193.2 (34.2-1,090.7)	2,227.8 (605.8-8,188.0)	1,965.0 (885.9-4,358.3)	521.1 (186.3-1,457.7)
Percentage change in IgE level at week 4, mean (SD)	13.5*	-7 (24)	-13 (13)	10 (30)	-2 (26)	-4 (22)
Phadiatop result positive at baseline, no. (%)	4 (100)	6 (86)†	5 (71)	14 (88)	20 (91)	17 (81)

*Week 4 data were only available for 2 of 4 patients in the placebo group.

†Baseline Phadiatop data were only available from 6 of 7 patients.

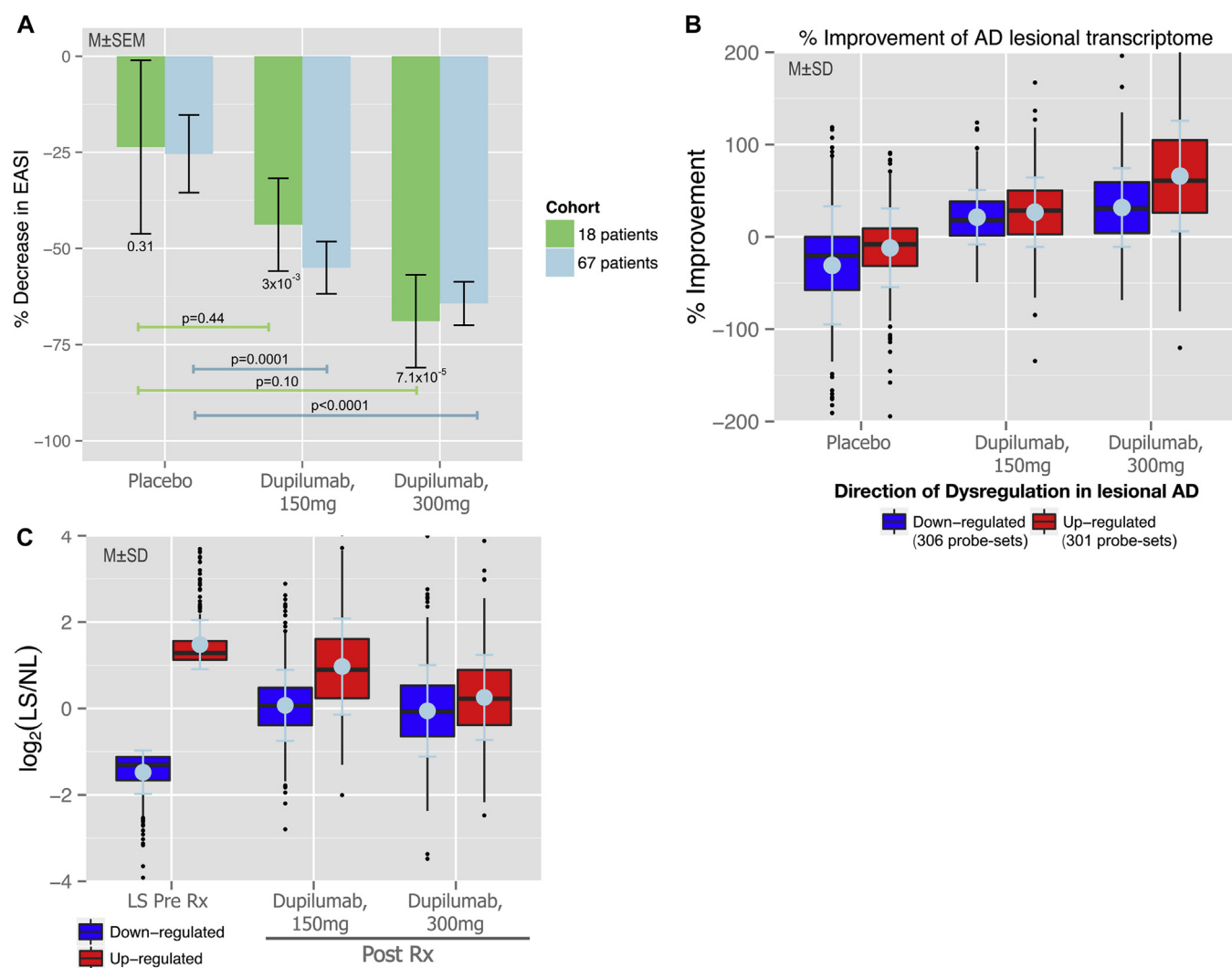


FIG 1. Clinical and molecular changes with dupilumab. **A**, Clinical responses (percentage change in EASI score) in the substudy were similar to parent trials. Bar plots represent means $\pm$ SEMs. **B**, Dose-dependent changes in the AD transcriptome with dupilumab (150/300 mg) versus placebo. Bar plots represent means $\pm$ SEMs. **C**, Transcriptomic differences between lesional and nonlesional skin showed dose-dependent decreases with dupilumab. Red indicates upregulated and blue indicates downregulated genes in the AD transcriptome (lesional vs nonlesional). Week 4 NLs were not available for the placebo group. Bar plots represent means $\pm$ SDs. *P* values of changes from baseline or between groups are listed.

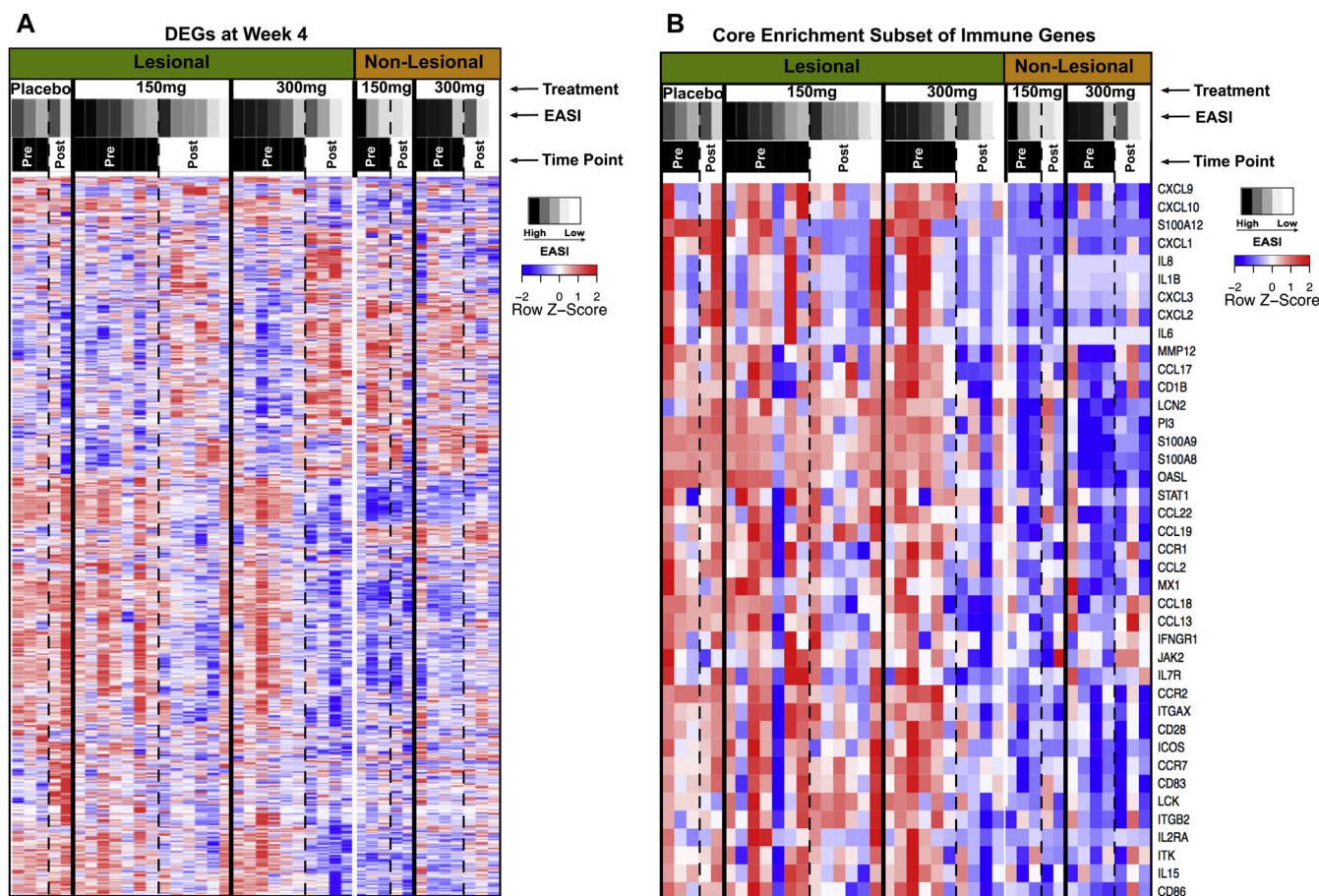


FIG 2. Pretreatment versus posttreatment gene expression profiles. **A**, Differentially expressed genes (DEG) with all treatments. **B**, Heat map of an immune gene subset. Upregulation of inflammatory genes characterizes lesional skin at baseline. Whereas inflammatory genes were strongly downregulated with 300 mg of dupilumab (red to blue transition in pretreatment vs posttreatment lesional samples), no changes were seen with placebo. Red indicates gene upregulation and blue indicates gene downregulation with treatment.

changes were of greater magnitude than concurrent decreases in EASI scores (Fig 1, A, and Table I). For example, there was a 74% difference between the 300-mg dupilumab group compared with the placebo arm (+52% improvement in the 300-mg group vs an overall exacerbation of -21.5% in the placebo group). In contrast, only a 45.3% difference between the 300-mg dupilumab and placebo arms was observed for the percentage change in EASI scores (Fig 1, A and B). The molecular differences between lesional and nonlesional skin before treatment were minimized in a dose-dependent manner, which was most evident in the 300-mg dupilumab group (Fig 1, C).

Differential gene expression with treatment at week 4

Additional differences in gene expression not limited to the previously defined AD transcriptome between week 4 and baseline were determined in LSs of patients treated with dupilumab and placebo (Fig 2, A, and see Table E3 in this article's Online Repository at www.jacionline.org). As shown in the heat map in Fig 2, A, dose-dependent changes with treatment were most evident in the 300-mg dupilumab group. Strikingly, the LS transcriptome after treatment with 300 mg of dupilumab resembles that of nonlesional

skin (Fig 2, A). At week 4, 821 probes (473 upregulated and 348 downregulated) were significantly modulated with 300 mg of dupilumab versus only 275 probes with placebo (105 and 160 upregulated and downregulated, respectively; >2-fold change, $P < .05$; see Table E3). Markers of epidermal proliferation (*MKI67*), keratin 16 (*K16*), keratin 6B (*K6B*), and multiple inflammatory mediators were among the large set of genes downregulated by dupilumab (Fig 2, A, and see Table E3). Among the top genes upregulated with dupilumab were those with structural (eg, *MATN4*), lipid metabolism (eg, *PLIN4*, *ADIPOQ*, and *PLIN1*), and barrier-related (eg, *CLDN8*, *ELN*, and *CLDN11*) functions (see Table E3). Expression of some of these genes decreased in the placebo group (eg, *PLIN4*, *CLDN11*, and *ADIPOQ*). Among the most significantly downregulated immune genes were inflammatory markers (*MMP12* and *S100A12*), T-cell markers (eg, *ITK* and *ICOS*), T_H2 and eosinophil-attracting chemokines (eg, *CCL13* and *CCL26*), DC antigens (*ITGAX/CD11c*, *CD1b*, and *CD83*), and keratinocyte-associated mediators (*IL6*, *IL8*, and *IL7R*; Figs 2 and 3 and see Tables E3 and E4 in this article's Online Repository at www.jacionline.org). By using Gene Set Enrichment Analysis to evaluate changes in an immune gene-subset, a prominent molecular signal of inflammation was seen in lesional skin at baseline (Fig 2, B, and see Table E4). Dose-dependent decreases were observed

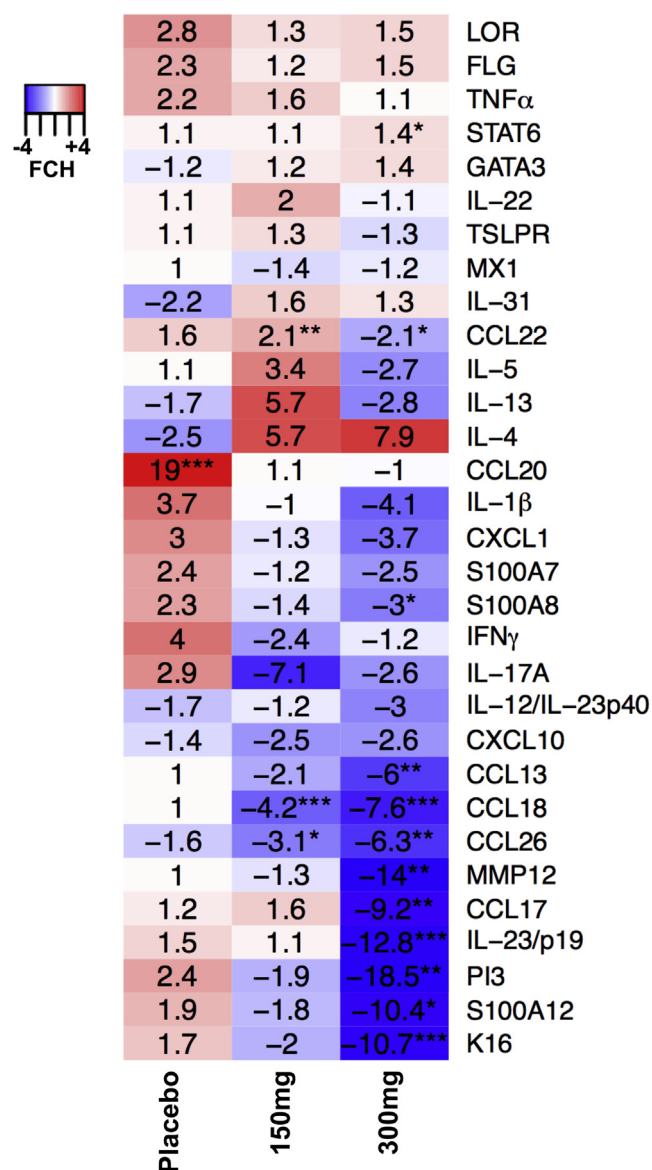


FIG 3. A summary heat map of all quantitative RT-PCR mRNA expression changes with treatments. Dose-dependent inhibition of epidermal proliferation and inflammatory markers were observed in biopsy specimens from patients treated with 150 or 300 mg of dupilumab for 4 weeks compared with exacerbations with placebo. Values represent the mean fold change (FCH) $\pm$ SEM. * $P < .1$, ** $P < .05$, and *** $P < .01$.

with dupilumab, with lesional skin showing a more nonlesional immune phenotype with 300 mg of dupilumab. No significant changes in this immune subset were seen with placebo (Fig 2, B).

Dose-dependent mRNA suppressions of immune and epidermal responses measured by using qRT-PCR

To measure modulation of various immune axes activated in lesional skin, we performed qRT-PCR for a wide array of mRNAs shown in Figs 3 and 4 (red indicates upregulation and blue indicates downregulation). With 300 mg of dupilumab, there was strong and significant modulation of T_H2 -associated chemokines (*CCL13*, *CCL17*, *CCL18*, and *CCL26*) and some epidermal products, particularly the proliferation marker *K16* and elafin (*PI3*),

whereas increased immune activation was observed with placebo (Figs 3 and 4 and see Fig E2 in this article's Online Repository at www.jacionline.org). No significant changes with treatment were observed in mRNAs of major T_H2 cytokines (*IL4*, *IL13*, *IL5*, and *IL31*; see Fig E3 in this article's Online Repository at www.jacionline.org).

Although one might hypothesize that inhibiting the T_H2 pathway would upregulate T_H1 activity, increases in T_H1 /IFN- γ -related gene expression were not observed with dupilumab treatment. In fact, small decreases in expression of these genes (*IFNG*, *OASL*, *MX1*, and *CXCL10*) were detected by using arrays and qRT-PCR for the treatment arms, whereas expression of some of these genes increased with placebo (Figs 3 and 4 and see Tables E2 and E3).

IL17A and *IL22* mRNAs were not significantly reduced at week 4 with dupilumab. However, significant suppression of IL-17/IL-22-modulated genes (ie, *CXCL1*, *CXCL2*, *PI3*, *IL-23p19/IL-23A*, and *S100* genes) was observed with dupilumab treatment compared with placebo. *CCL20* expression significantly increased with placebo. Of the IL-17/IL-22-modulated genes, *PI3* and *S100A12* showed the greatest suppression with 300 mg of dupilumab (Figs 3 and 4 and see Fig E3).

The barrier defect in patients with AD is characterized by hyperplasia (as measured by the proliferation marker *K16*), induction of *S100As*, and suppression of terminal differentiation (ie, *FLG* and *LOR*).^{9,11} Four weeks of 300 mg of dupilumab resulted in significant suppression of *K16* (-10.7 -fold change, $P < .001$, Fig 3). We also observed significant decreases in expression of *S100A* genes (ie, *S100A12* and *S100A8*) and a modest trend of increases in terminal differentiation proteins (Figs 3 and 4). Dose-dependent increases in terminal differentiation genes were observed with dupilumab after adjusting for changes in hyperplasia (as measured by *K16* mRNA changes, see Fig E4 in this article's Online Repository at www.jacionline.org).

When assessing dupilumab's effects on gene signatures for epidermal differentiation, cytokine-treated keratinocytes, and various immune axes,²²⁻²⁴ dose-dependent responses from baseline were observed, with concurrent exacerbations with placebo (Fig 5 and see Table E5 in this article's Online Repository at www.jacionline.org). Substantial suppression of the T_H17 signature was observed in the 300-mg dupilumab group (with a large exacerbation in the placebo group).

Correlation with improvements in disease activity

We evaluated the association of the markers measured by using qRT-PCR with clinical improvement by determining the Pearson correlations of the change with treatment of each variable measured in lesional skin with EASI score improvements (data not shown). Reductions in *CCL26* and *CCL13* expression had the highest correlation with improvement in percentage change in the EASI score (*CCL26*: $r = 0.8$, $P = .005$; *CCL13*: $r = 0.55$, $P = .1$). In patients who achieved 50% or greater improvement in EASI scores, *K16* showed the highest correlation with clinical improvement ($r = 0.98$, $P = .01$; data not shown).

DISCUSSION

This is the first evaluation of a targeted immune antagonist in patients with AD that correlates mechanistic changes with clinical measures of disease. Previous studies with broad

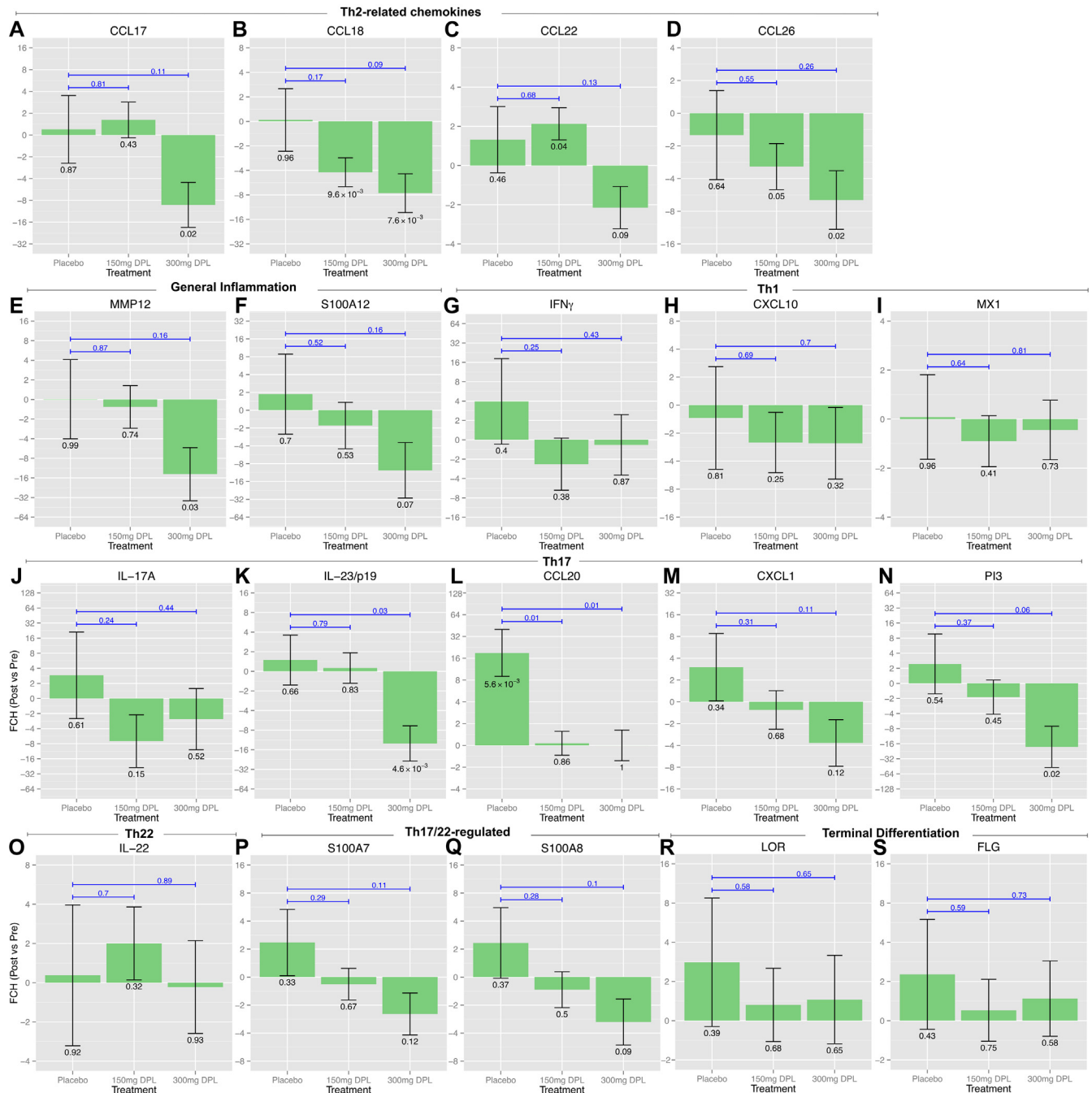


FIG 4. Dupilumab (DPL)- and placebo-induced lesional skin changes in inflammatory and epidermal markers, as quantified by using qRT-PCR. Panels A through S show posttreatment versus pretreatment mRNA expression differences (fold change) of selected genes, as grouped by inflammatory pathway, in lesional skin at week 4 of treatment. Values represent means $\pm$ SEMs with posttreatment versus pretreatment *P* values compared with baseline. FCH, Fold change.

immune suppressants (ie, CsA, efalizumab, and alefacept) demonstrated clinical improvements (using EASI or SCORAD scores),²⁵⁻²⁷ but most lacked evaluations of molecular markers. We recently established a relationship between activation of cytokine pathways and epidermal alterations in skin and clinical disease activity in a CsA study.²⁴ However, studies with targeted immune antagonists are needed to understand the contribution

of specific cytokine pathways to the epidermal pathology in patients with AD.

In this study statistically significant, dose-dependent improvements of the AD transcriptome were observed in patients treated with 4 weeks of dupilumab compared with placebo.

Dupilumab suppressed mRNA expressions of genes related to activation of T cells, DCs, eosinophils, inflammatory pathways,

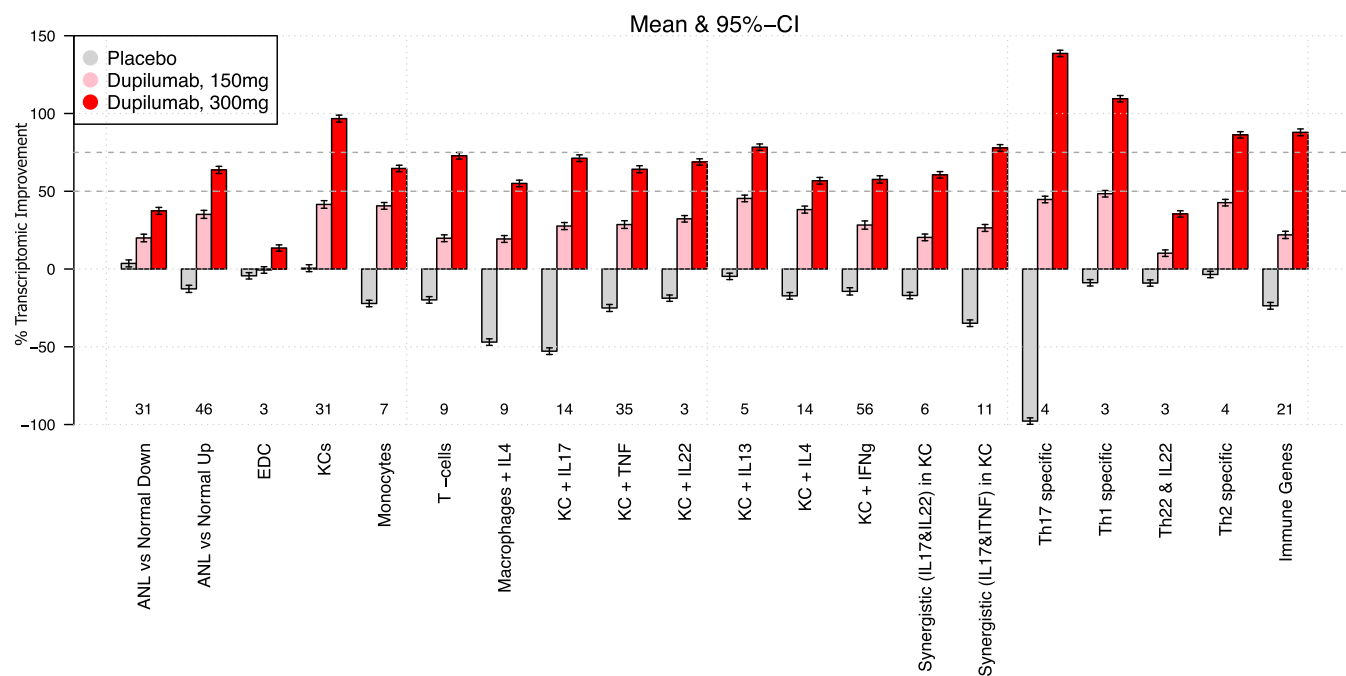


FIG 5. Dose-dependent genomic changes were observed by using microarrays in previously defined immune pathways and epidermal gene subsets with dupilumab. *Transcriptomic improvement* refers to transition toward a noninflammatory molecular phenotype. Values represent means $\pm$ 95% CIs. Numbers in the bottom row represent the number of probes representing AD genes in each pathway. Detailed gene lists are provided in Table E5.

and T_H2 -inducing chemokines in skin lesions, with increases or insignificant decreases observed with placebo. Although we did not detect significant reductions in IL-17A or IL-22 levels, large reductions in expression of IL-17-related genes, such as *elafin* (*PI3*), *IL23p19/IL23A*, and *S100A8* and trends for suppression (*CXCL1* and *S100A7*) were found with 300 mg of dupilumab. Furthermore, with placebo, there was an overall exacerbation of the inflammatory AD lesional phenotype, with large increases in expression of T_H17 -associated genes, particularly *CCL20*. These results are consistent with recent findings from flaky tail mice suggesting IL-4 signaling might be regulated, at least in part, by the IL-17 pathway (IL-17A deficiency attenuated T_H2 inflammation in this model).²⁸

The treatment was associated with a dose-response reversal of the epidermal lesional phenotype. In particular, major suppressions of hyperplasia-related genes (eg, *K16*) and reductions in expression of *S100A* genes were evident with 300 mg of dupilumab by using microarrays and qRT-PCR. The reduction in *K16* expression was of greater magnitude than that observed in patients with similar disease severity treated for 12 weeks with 5 mg/kg CsA.²⁴ We also measured changes in other critical epidermal alterations in AD skin, including significant increases in claudin and lipid product levels with 300 mg of dupilumab, whereas decreases were observed in the placebo group. A trend of dose-dependent increases in expression of differentiation genes (*LOR* and *FLG*) was observed after adjusting for suppression of epidermal hyperplasia, as demonstrated by *K16* reductions with dupilumab. Increases in *LOR* and *FLG* expression were also seen with placebo, but these can be attributed to the increased epidermal hyperplasia in the placebo arm and were not evident after adjusting for *K16* expression. These data suggest that IL-4/IL-13 blockade might restore abnormal

lipid and differentiation alterations in the skin of patients with AD.²⁹

No significant differences were found in either clinical or tissue (molecular) responses to treatment between patients with normal or increased serum total IgE levels.¹⁶ These results suggest the hypothesized model of barrier inhibition through activation of T_H2 cytokines could be operative in most patients with AD, regardless of IgE levels, and that targeting T_H2 inflammation might be an efficacious treatment for patients with either intrinsic or extrinsic AD.^{18,30}

These data are consistent with the suggested roles of IL-4 and IL-13 in patients with AD and the proposal that dual antagonism of these cytokines with a single agent has the potential to potently inhibit T_H2 effector responses. The IL-4 and IL-13 cytokines have been suggested to exert several effects in AD, including activation and survival of T_H2 T cells, induction of differentiation and activation of myeloid and atopic DCs, activation of B cells, stimulation of IgE class-switching, and eosinophil recruitment.³¹⁻³⁴ IL-4 and IL-13 also suppress lipid production and keratinocyte differentiation, ultimately disrupting the epidermal barrier, with possible feedback pathways that could induce epidermal hyperplasia.^{32,35-37}

These data support AD as an immune-driven disease and establish IL-4 and IL-13 as pathogenic cytokines in patients with AD, driving complex T_H2 -centered inflammation that regulates epidermal responses. The data also provide further evidence that IL-4 is a key regulator of T_H17 activity in AD skin. Because IL-4R α is expressed on many inflammatory cells involved in AD (eg, keratinocytes, T cells, DCs, and eosinophils),^{13,37} blocking signaling through this receptor might exert a range of anti-inflammatory effects beyond the known T_H2 axis. Modulating IL-4/IL-13 signaling through IL-4R α inhibition might be an effective method for treating existing skin lesions and for

limiting disease exacerbations. These results suggest that inhibition of a single target has the potential to reverse AD pathomechanisms, opening the door to a new era of targeted treatment for this common and debilitating inflammatory skin disease. Dupilumab trials to evaluate longer-term disease suppression, including cellular and molecular analyses of changes in epidermal pathology and immune abnormalities, are underway. These larger studies of longer treatment duration will not only clarify the effect of dupilumab on lesional skin but might also provide greater insight as to whether nonlesional skin is normalized.²² These studies might also be used to compare the long-term dupilumab data with studies using conventional treatments (ie, CsA and NB-UVB),^{18,24} as well as the respective molecular remnants across conventional and specific therapeutic agents.^{38,39} Molecular effects of dupilumab on the AD transcriptome in larger studies should also consider recognized AD subtypes based on IgE level, ethnicity, and other variables.⁴⁰

We thank all patients who participated in the study; as well as Linda Williams, Usman Chaudhry, Scott J. Mellis, Steven P. Weinstein, Melissa Hager, E. Jay Bienen, Chaim "Josh" Cantor, Haobo Ren, Robert Phillips, Warren Brooks.

Clinical implications: Targeted therapeutics might open the door to a new treatment paradigm for patients with AD.

REFERENCES

- Hanifin JM, Reed ML. A population-based survey of eczema prevalence in the United States. *Dermatitis* 2007;18:82-91.
- Fowler JF, Duh MS, Rovba L, Buteau S, Pinheiro L, Lobo F, et al. The direct and indirect cost burden of atopic dermatitis: an employer-payer perspective. *Manag Care Interface* 2007;20:26-32.
- Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: a systematic review. *Pediatr Dermatol* 2008;25:1-6.
- McKenna SP, Doward LC. Quality of life of children with atopic dermatitis and their families. *Curr Opin Allergy Clin Immunol* 2008;8:228-31.
- Chamlin SL, Chren MM. Quality-of-life outcomes and measurement in childhood atopic dermatitis. *Immunol Allergy Clin North Am* 2010;30:281-8.
- Zachariae R, Zachariae C, Ibsen HH, Mortensen JT, Wulf HC. Psychological symptoms and quality of life of dermatology outpatients and hospitalized dermatology patients. *Acta Derm Venereol* 2004;84:205-12.
- Kimata H. Prevalence of suicidal ideation in patients with atopic dermatitis. *Suicide Life Threat Behav* 2006;36:120-4.
- Dieris-Hirce J, Gieler U, Kupfer JP, Milch WE. [Suicidal ideation, anxiety and depression in adult patients with atopic dermatitis]. *Hautarzt* 2009;60:641-6.
- Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—part II: immune cell subsets and therapeutic concepts. *J Allergy Clin Immunol* 2011;127:1420-32.
- Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483-94.
- Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—part I: clinical and pathologic concepts. *J Allergy Clin Immunol* 2011;127:1110-8.
- Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, De Benedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007;120:150-5.
- Sehra S, Yao Y, Howell MD, Nguyen ET, Kansas GS, Leung DY, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol* 2010;184:3186-90.
- Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunol Rev* 2011;242:233-46.
- Suarez-Farinas M, Dhingra N, Gittler J, Shemer A, Cardinale I, de Guzman Strong C, et al. Intrinsic atopic dermatitis shows similar T_H2 and higher T_H17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 2013;132:361-70.
- Beck LA, Thaçi D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al. Dupilumab for the treatment of adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014;371:130-9.
- Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013;368:2455-66.
- Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583-93.
- Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *EASI Evaluator Group. Exp Dermatol* 2001;10:11-8.
- Suarez-Farinas M, Pellegrino M, Wittkowski KM, Magnasco MO. Harshlight: a "corrective make-up" program for microarray chips. *BMC Bioinformatics* 2005;6:294.
- Wu Z, Izratty R, Gentleman R, Martinez-Murillo F, Spencer F. A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 2004;99:909-17.
- Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011;127:954-64.
- Dhingra N, Gulati N, Guttman-Yassky E. Mechanisms of contact sensitization offer insights into the role of barrier defects vs. intrinsic immune abnormalities as drivers of atopic dermatitis. *J Invest Dermatol* 2013;133:2311-4.
- Khattari S, Shemer A, Rozenblit M, Dhingra N, Czarnowicki T, Finney R, et al. Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology. *J Allergy Clin Immunol* 2014;133:1626-34.
- Sowden JM, Berth-Jones J, Ross JS, Motley RJ, Marks R, Finlay AY, et al. Double-blind, controlled, crossover study of cyclosporin in adults with severe refractory atopic dermatitis. *Lancet* 1991;338:137-40.
- Takiguchi R, Tofte S, Simpson B, Harper E, Blauvelt A, Hanifin J, et al. Efalizumab for severe atopic dermatitis: a pilot study in adults. *J Am Acad Dermatol* 2007;56:222-7.
- Moul DK, Routhouska SB, Robinson MR, Korman NJ. Alefacept for moderate to severe atopic dermatitis: a pilot study in adults. *J Am Acad Dermatol* 2008;58:984-9.
- Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol* 2014;134:2122-30.
- Cole C, Kroboth K, Schurch NJ, Sandilands A, Sherstnev A, O'Regan GM, et al. Filaggrin-stratified transcriptomic analysis of pediatric skin identifies mechanistic pathways in patients with atopic dermatitis. *J Allergy Clin Immunol* 2014;134:82-91.
- Gittler JK, Shemer A, Suarez-Farinas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344-54.
- Brandt EB, Sivaprasad U. Th2 cytokines and atopic dermatitis. *J Clin Cell Immunol* 2011;2:110.
- Howell MD, Fairchild HR, Kim BE, Bin L, Boguniewicz M, Redzic JS, et al. Th2 cytokines act on S100/A11 to downregulate keratinocyte differentiation. *J Invest Dermatol* 2008;128:2248-58.
- Bao L, Shi VY, Chan LS. IL-4 up-regulates epidermal chemotactic, angiogenic, and pro-inflammatory genes and down-regulates antimicrobial genes in vivo and in vitro: relevant in the pathogenesis of atopic dermatitis. *Cytokine* 2013;61:419-25.
- Izuhara K, Arima K, Yasunaga S. IL-4 and IL-13: their pathological roles in allergic diseases and their potential in developing new therapies. *Curr Drug Targets Inflamm Allergy* 2002;1:263-9.
- Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. *Clin Immunol* 2008;126:332-7.
- Danso MO, van Drongelen V, Mulder A, van Esch J, Scott H, van Smeden J, et al. TNF-alpha and Th2 cytokines induce atopic dermatitis-like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents. *J Invest Dermatol* 2014;134:1941-50.
- Goenka S, Kaplan MH. Transcriptional regulation by STAT6. *Immunol Res* 2011;50:87-96.
- Suarez-Farinas M, Gittler JK, Shemer A, Cardinale I, Krueger JG, Guttman-Yassky E. Residual genomic signature of atopic dermatitis despite clinical resolution with narrow-band UVB. *J Allergy Clin Immunol* 2013;131:577-9.
- Rozenblit M, Suarez-Farinas M, Shemer A, Khattari S, Gilleaudeau P, Sullivan-Whalen M, et al. Residual genomic profile after cyclosporine treatment may offer insights into atopic dermatitis reoccurrence. *J Allergy Clin Immunol* 2014;134:955-7.
- Leung DY, Guttman-Yassky E. Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches. *J Allergy Clin Immunol* 2014;134:769-79.