Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis

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Background: Dupilumab is an IL-4 receptor α mAb inhibiting signaling of IL-4 and IL-13, key drivers of type 2–driven inflammation, as demonstrated by its efficacy in patients with atopic/allergic diseases.

Objective: This placebo-controlled, double-blind trial (NCT01979016) evaluated the efficacy, safety, and effects of dupilumab on molecular/cellular lesional and nonlesional skin phenotypes and systemic type 2 biomarkers of patients with moderate-to-severe atopic dermatitis (AD).

Methods: Skin biopsy specimens and blood were evaluated from 54 patients randomized 1:1 to weekly subcutaneous doses of 200 mg of dupilumab or placebo for 16 weeks.

Results: Dupilumab (vs placebo) significantly improved clinical signs and symptoms of AD, was well tolerated, and progressively shifted the lesional transcriptome toward a nonlesional phenotype (weeks 4–16). Mean improvements in a meta-analysis–derived AD transcriptome (genes differentially expressed between lesional and nonlesional skin) were 68.8% and 110.8% with dupilumab and −10.5% and 55.0% with placebo (weeks 4 and 16, respectively; $P < .001$). Dupilumab significantly reduced expression of genes involved in type 2 inflammation (*IL13*, *IL31*, *CCL17*, *CCL18*, and *CCL26*), epidermal hyperplasia (keratin 16 *[K16]* and *MKi67*), T cells, dendritic cells (*ICOS*, *CD11c*, and *CTLA4*), and T_H17/T_H22 activity (*IL17A*, *IL-22*, and *S100As*) and concurrently increased expression of epidermal differentiation, barrier, and lipid metabolism genes (filaggrin *[FLG]*, loricrin *[LOR]*, claudins, and *ELOVL3*). Dupilumab reduced lesional epidermal thickness versus placebo (week 4, $P = .001$; week 16, $P = .0002$). Improvements in clinical and histologic measures correlated significantly with modulation of gene expression. Dupilumab also significantly suppressed type 2 serum biomarkers, including CCL17, CCL18, periostin, and total and allergen-specific IgEs.

Conclusion: Dupilumab-mediated inhibition of IL-4/IL-13 signaling through IL-4 receptor α blockade significantly and

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progressively improved disease activity, suppressed cellular/molecular cutaneous markers of inflammation and systemic measures of type 2 inflammation, and reversed AD-associated epidermal abnormalities. (J Allergy Clin Immunol 2019;143:155-72.)

Key words: *Atopic dermatitis, IL-4 receptor α inhibition, dupilumab, transcriptome, gene expression, skin, type 2 inflammation, epidermal pathology*

Abbreviations used

AD: Atopic dermatitis
CsA: Cyclosporin A
DC: Dendritic cell
EASI: Eczema Area and Severity Index
ECP: Eosinophilic cationic protein
EDC: Epidermal differentiation complex
ET: Epidermal thickness
FCH: Fold change
FDR: False discovery rate
FLG: Filaggrin
IL-4Rα: IL-4 receptor α
K16: Keratin 16
LOR: Loricrin
MADAD: Meta-analysis–derived atopic dermatitis
NB-UVB: Narrow-band UVB
PARC: Pulmonary and activation-regulated chemokine
qRT-PCR: Quantitative real-time PCR
SCORAD: SCORing Atopic Dermatitis
TEAE: Treatment-emergent adverse event

Atopic dermatitis (AD) is a common inflammatory skin disease of increasing prevalence that affects 3% to 10% of adults and 10% to 20% of children globally.[1-5] The disease is moderate to severe in up to one third of patients, many of whom require systemic treatment.[6,7] The profound symptom burden, systemic inflammation, and comorbidities in patients with moderate-to-severe AD[8-15] have a substantial effect on the quality of life of patients and their families and present a large burden to health care systems.[16-19]

Therapeutic options are limited for patients with moderate-to-severe AD who had an inadequate response to topical treatments. They include phototherapy (narrow-band UVB [NB-UVB] or UVA1) and immunosuppressants (oral corticosteroids, cyclosporin A [CsA], and others),[1-5,7,20] which have variable efficacy and are associated with multiple adverse effects on long-term use. Therefore there has been a large unmet need for safe and effective treatments for this patient population.

Dupilumab is the first targeted biologic agent approved in the European Union, United States, Japan, and other countries for the treatment of adults with inadequately controlled moderate-to-severe AD. It is a fully human VelocImmune-derived mAb[21,22] targeting IL-4 receptor α (IL-4Rα), the shared subunit of the type 2 cytokines IL-4 and IL-13, inhibiting signaling of both cytokines. In clinical trials dupilumab consistently showed robust efficacy and acceptable safety in adults with moderate-to-severe AD, asthma, chronic rhinosinusitis with nasal polyposis, or eosinophilic esophagitis,[23-34] supporting the notion that these atopic disorders share a type 2–centered pathogenesis.

Although preliminary data suggest that other type 2 antagonists targeting IL-13 or IL-31 receptor A might be beneficial in patients with AD,[35-37] these effects seem less robust than those of dupilumab, suggesting dual inhibition of IL-4 and IL-13 might be necessary for optimal control of moderate-to-severe AD. The limited efficacy of other type 2 immune inhibitors, such as mepolizumab (anti–IL-5) and omalizumab (anti-IgE), also supports IL-4 and IL-13 as critical mediators of AD pathogenesis.[38-43] Targeting other pathways, such as the T_H17/IL-23 or T_H22 pathways, with ustekinumab (anti–IL-12/23 p40) or fezakinumab/ILV-094 (anti–IL-22) has been less efficacious relative to what has been observed in dupilumab AD trials to date.[44,45]

Scientific evidence supports the view of AD as a systemic immune-driven disease similar to psoriasis, another common inflammatory skin disease.[7-9,11,23,46-49] Both diseases are characterized by induction of polar cytokine pathways, with psoriasis viewed primarily as T_H17/IL-23 driven, whereas type 2 pathways predominate in patients with AD. Signals of the T_H22, T_H17, and T_H1 axes are also observed in patients with AD, but their role in pathogenesis is not proved.[50-53]

AD and psoriasis also have significant epidermal alterations, including increased epidermal hyperplasia and upregulated epidermal expression of antimicrobial proteins (S100A7/S100A8/S100A9). AD is uniquely characterized by suppression of terminal differentiation markers in lesional and nonlesional skin.[7,54,55] In patients with psoriasis, increased understanding of pathogenic pathways led to rapid development of multiple biotherapeutics,[7,56-66] which in turn demonstrated the relation between immune suppression and reversal of epidermal pathology.[58,60,61,67]

The cytokines IL-4 and IL-13 were suggested to have direct effects on the epidermis in patients with AD, including (1) inhibition of terminal differentiation with potential for feedback hyperplasia (keratinocyte hyperproliferation),[1,3,57,68-72] (2) induction of spongiosis,[73] (3) inhibition of lipid synthesis,[73] (4) inhibition of synthesis of antimicrobial peptides,[74-77] and (5) promotion of binding and colonization by *Staphylococcus aureus*.[78] IL-4 and IL-13 have also been suggested to induce type 2 activation and differentiation of dendritic cells (DCs), promote B-cell activation and IgE class-switching, and recruit eosinophils.[79-81]

Systemic upregulation of type 2 inflammation in patients with AD has also been demonstrated by increased serum concentrations of CCL17 (thymus and activation-regulated chemokine [TARC]), CCL18 (pulmonary and activation-regulated chemokine [PARC]), eosinophilic cationic protein (ECP), and periostin, which correlated with AD severity.[82-89]

Most patients with AD (or other atopic diseases) have increased IgE levels and antigen-specific IgE sensitization, as demonstrated by increased serum concentrations of various IgEs specific to common allergens (eg, house dust mite, staphylococcal enterotoxins, cat and dog dander, *Cladosporium* species, and grass).[90-95] Nevertheless, although higher concentrations of total and specific IgEs have been associated with higher clinical AD severity at baseline,[11,90] treatment with broad-target agents, such as CsA or phototherapy, does not consistently reduce IgE levels, an observation suggesting that these treatments do not adequately suppress IL-4 and IL-13 activity[47,96,97] and might even lead to increased IgE levels.[98]

Previously, we reported that in a short (4-week) phase 1b trial, inhibition of type 2 inflammation with dupilumab modifies

molecular mechanisms in skin of patients with AD, thus establishing a central pathogenic role for IL-4 and IL-13.[46,99] However, that trial was conducted in only a small number of patients across several dupilumab dose and placebo groups, and it lacked cellular correlates and long-term evaluations of genomic and cellular markers.[46]

We now present results from a larger 16-week study evaluating the efficacy and safety of dupilumab in patients with moderate-to-severe AD, with a focus on the effect of treatment on molecular and cellular phenotypes of lesional and nonlesional skin biopsy specimens and on systemic type 2 biomarkers. Treatment with dupilumab (compared with placebo) progressively improved clinical AD severity and resulted in molecular suppression of AD genomic and cellular measures of inflammation and systemic type 2 biomarkers as early as week 4, and continued improvement beyond 16 weeks of treatment included reversal of AD-associated epidermal pathology.

METHODS

Study design

This was a randomized, placebo-controlled, double-blind, phase 2 trial (ClinicalTrials.gov: NCT01979016) conducted at 5 medical centers in the United States and Canada to assess the efficacy and safety of dupilumab compared with placebo in patients with moderate-to-severe AD. Molecular and cellular phenotypes of lesional and nonlesional skin and pharmacodynamic effects on serum type 2 biomarkers were evaluated. Patients (54/66 screened) were randomized 1:1 to weekly subcutaneous injections of 200 mg of dupilumab or placebo after a 400 mg loading dose or placebo on day 1, for a total of 16 weeks. The 16-week treatment period was followed by a 16-week safety follow-up period (until week 32).

The study was conducted in accordance with current US Food and Drug Administration regulations and guidelines and International Conference on Harmonization guidelines on Good Clinical Practice; all participants provided written informed consent under institutional review board approved protocols before participation (additional information is available in the Methods section in this article's Online Repository at www.jacionline.org).

Patients

Eligible patients were 18 years old or older, had moderate-to-severe AD (Eczema Area and Severity Index [EASI] score ≥16 at baseline) chronically for 3 or more years, and had an inadequate response to topical medications within 6 months before screening. Use of topical corticosteroids or calcineurin antagonists was prohibited 1 week before and during the study. Patients were allowed to use only bland topical emollients during the study. Oral immunosuppressant agents and phototherapy were prohibited for 4 weeks before and during the trial (see the Methods section in this article's Online Repository for additional details).

Study end points

Efficacy end points included mean percentage change from baseline to week 16 in EASI scores (primary end point) and peak pruritus numeric rating scale scores and proportions of patients achieving reductions of 50% or greater, 75% or greater, and 90% or greater from baseline in EASI and SCOring Atopic Dermatitis (SCORAD) scores at week 16. Additional efficacy end points included mean (percentage) changes from baseline to week 16 in total SCORAD score, Patient-Oriented Eczema Measure score, and total Global Individual Signs Score and Global Individual Signs Score components. All efficacy end points are reported in detail in Table E1 in this article's Online Repository at www.jacionline.org.

Safety was assessed from baseline through week 32 based on the incidence of treatment-emergent adverse events (TEAEs; see Table E2 in this article's Online Repository at www.jacionline.org). Exploratory assessments included genomic changes in active AD lesions and changes in the AD transcriptome defined by gene expression differences between lesional and nonlesional skin and assessment of effects of dupilumab treatment on epidermal hyperplasia, as defined by changes in epidermal thickness (ET), keratin 16 (K16) expression or $Ki67^+$ cells, and inflammatory cells in tissues.[46-48,100] An exploratory assessment was also conducted of treatment-related changes in the serum biomarkers CCL17, CCL18, periostin, ECP, total IgE, and allergen-specific IgEs (gray alder, *Alternaria tenuis*, Bermuda grass, silver birch, cat dander, *Cladosporium* species, German cockroach, *Dermatophagoides farinae*, dog dander, elm, Johnson grass, white oak, ragweed, sage mugwort, Timothy grass, white ash, staphylococcal enterotoxin A, and staphylococcal enterotoxin B).

Assessments

Biopsy and blood collection. Biopsy specimens (6 mm) were collected from lesional and nonlesional skin at baseline and at weeks 4 (lesional skin only) and 16. Blood samples were collected at similar time points. Posttreatment biopsy specimens were taken from the same location as pretreatment biopsy specimens approximately 1 cm from prior biopsy scars. All skin and blood analyses were performed while investigators were blinded to treatment allocation.

Immunohistochemistry. Immunohistochemistry was performed on frozen OCT-embedded cryostat tissue sections by using purified mouse anti-human mAbs, as previously described (see Table E3 in this article's Online Repository at www.jacionline.org).[48,54] ET was measured by using computer-assisted image analysis software of hematoxylin and eosin–stained sections (ImageJ 1.42 software; National Institutes of Health, Bethesda, Md), as previously described.[47]

Quantitative real-time PCR and gene-array analysis. RNA was extracted, followed by hybridization to Affymetrix Human U133Plus 2.0 gene arrays (Affymetrix, Santa Clara, Calif) or by using quantitative real-time PCR (qRT-PCR), as previously described (see Table E4 in this article's Online Repository at www.jacionline.org).[48,100] Expression of target genes was determined by using qRT-PCR and normalized to the housekeeping gene human acidic ribosomal protein for analyses.

Circulating biomarkers. Levels of serum total and allergen-specific IgE and ECP were measured by using the ImmunoCAP method.[101,102] Only patients with positive results (≥0.1 kU/L) for allergen-specific IgEs at baseline were included in IgE analyses. Serum CCL17 and CCL18 levels were measured by using Quantikine ELISA kits (R&D Systems, Minneapolis, Minn), and periostin levels were measured by using the R&D Systems DuoSet ELISA kit.

Statistical analyses

Sample size was determined to achieve 79% power to detect a 40% difference between dupilumab and placebo with respect to percentage change in EASI score from baseline to week 16, assuming the common SD is 50% with a 2-sided test at the .05 significance level.

All clinical and circulating biomarker analyses were performed by using SAS (SAS Institute, Cary, NC). Analysis of secondary efficacy end points and exploratory variables were not adjusted for multiplicity, and thus nominal *P* values are provided. Additional details of the analyses performed are reported in the Methods section in this article's Online Repository.

RNA, serum, and correlation analyses were performed in Bioconductor R packages (open source software available from www.bioconductor.org). Linear mixed-effect models were used to assess treatment changes in $\log_2$-transformed qRT-PCR measurements (normalized to human acidic ribosomal protein), with tissue (lesional/nonlesional) and its interaction with time as a fixed factor. qRT-PCR values of less than the limit of detection were imputed as 20% of the minimal value over the limit of detection. Missing values for all mechanistic data were not imputed. The histologic score is a combined score of ET and mRNA expression of K16, as obtained by averaging the z score of both measures.

Quality control of microarrays used standard metrics and R package microarray quality control. Images were scrutinized for spatial artifacts by using Harshlight (Bioconductor).[103] Expression measures were obtained by using the GC Robust Multi-array Average algorithm.[104] Probe sets with expression of greater than 3 in at least 15 samples were kept for analyses.

Expression values were modeled by using mixed-effect models, with treatment and time as fixed factors and a random effect for each patient. Fold changes (FCHs) for comparisons of interest were estimated, and hypothesis testing was conducted with contrasts under the general framework for linear models in R *limma* package. *P* values from moderated (paired) *t* tests were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure, which controls the false discovery rate (FDR).[105] Genes with FDR of less than 0.05 and FCH of 2 or greater were declared differentially expressed. Hierarchical clustering of mean expression profiles was performed with Euclidean distance and a McQuitty agglomeration scheme.[54,104]

AD-associated immune or barrier gene subsets were quantified by using Gene Set Variation Analysis, an unsupervised sample-wise enrichment method that produces a score of activity for a gene subset or pathways for each sample.[106] Modeling was performed by using the same approach described for genes, including adjustment for multiplicity. The association of biomarkers and gene sets with clinical responses was evaluated by using Spearman correlation coefficients. An unsupervised clustering analysis using Spearman correlation and a McQuitty agglomeration scheme is presented.

RESULTS

Patients

Of 66 patients screened, 54 (81.8%) were randomized to receive 200 mg of dupilumab every week (n = 27) or placebo every week (n = 27). Most patients in the placebo (25/27 [92.6%]) and dupilumab (26/27 [96.3%]) groups completed the study to week 16 (see Fig E1 in this article's Online Repository at www.jacionline.org). Baseline demographics and disease characteristics were not statistically different between treatment groups, except for ET of nonlesional skin ($P = .010$, see Table E5 in this article's Online Repository at www.jacionline.org).

Skin biopsy specimens for exploratory analyses were available for analysis from 27 patients in the dupilumab group and from 25 patients in the placebo group. Numbers of lesional and nonlesional samples collected at baseline, week 4, and week 16 from dupilumab- and placebo-treated patients available for analyses are reported in Table E6 in this article's Online Repository at www.jacionline.org. The number of serum samples collected at baseline, week 4, and week 16 from dupilumab- and placebo-treated patients available for analyses are provided in Table E7 in this article's Online Repository at www.jacionline.org.

Efficacy

Dupilumab, compared with placebo, significantly improved measures of clinical efficacy at week 16, including EASI scores ($P < .0001$; see Table E8 and Fig E2, *A*, in this article's Online Repository at www.jacionline.org) and peak pruritus numeric rating scale scores ($P = .003$; see Table E8 and Fig E2, *D*). Proportions of patients with reductions of 50% or greater, 75% or greater, and 90% or greater from baseline in EASI scores at week 16 were also significantly greater with dupilumab than placebo (see Fig E2, *B*). An illustration of clinical improvement with dupilumab in representative patients is shown in Fig E2, *C*. Dupilumab also significantly improved other clinical outcomes relative to placebo, except for the proportions of patients with reductions of 75% or greater and 90% or greater from baseline in SCORAD scores (see Table E8).

Safety

The overall incidence of TEAEs was generally similar in the 2 study groups: 24 (88.9%) patients in the dupilumab group versus 23 (85.2%) patients in the placebo group (see Table E2). No deaths were reported in the study. The incidence of serious TEAEs was numerically greater in the placebo group than in the dupilumab group, whereas the incidence of TEAEs resulting in permanent treatment discontinuation was similar in the 2 treatment groups (see Table E2). The most common TEAEs reported in this study are shown in Table E2.

Suppression of inflammatory cell infiltrates in dupilumab-treated skin

Immunostaining was performed to measure cellular infiltrates of $CD3^+$, $CD8^+$, and $CD1b^+$ T cells; various DC markers, including $CD83^+$, $CD11c^+$, $CD206^+$, $Fc\varepsilon RI^+$, $CD1c^+$, and $CD1a^+$ Langerhans cells; and major basic protein–positive eosinophils in lesional and nonlesional skin after 4 and 16 weeks of treatment (see Fig E3 in this article's Online Repository at www.jacionline.org). Marked and significant reductions in infiltrates at week 16 were only seen with dupilumab, with the exception of major basic protein–positive eosinophils, numbers of which were also reduced with placebo (see Table E9 in this article's Online Repository at www.jacionline.org). As early as week 4, DC markers ($CD11c^+$ and $CD1c^+$ cells) showed significant reductions with dupilumab versus placebo (see Table E9). $Fc\varepsilon RI^+$ cells were significantly reduced from baseline at week 16 with dupilumab ($P < .05$ for mean percentage change from baseline and vs placebo). There were no significant differences between treatment groups in $CD8^+$ or $CD1b^+$ T cells or in $CD83^+$ DCs (see Table E9). Nonlesional skin, which had significantly less increased infiltrates relative to control tissues at baseline, also showed no significant differences (data not shown).

Progressive shift of AD molecular phenotype from lesional to nonlesional

Affymetrix U133Plus 2.0 gene arrays (Affymetrix) were performed to define the AD skin phenotype or transcriptome at baseline (differentially expressed genes between lesional and nonlesional skin). Criteria of FCH of 2 or greater and FDR of less than 0.05 were used. These differentially expressed genes were used to assess the overall effects of dupilumab versus placebo on the AD transcriptome. In samples from patients with AD collected in this study, the baseline transcriptome of lesional versus nonlesional skin was defined by 527 upregulated and 508 downregulated probes in the placebo group and 411 upregulated and 336 downregulated probes in the dupilumab group (Fig 1, *A*).

At week 16, no gene probes in the dupilumab group met the statistical criteria for being differentially regulated, whereas in the placebo group the number of differentially regulated gene probes increased relative to baseline (Fig 1, *A* and *B*). Overall, dupilumab significantly altered gene expression in lesional skin at both weeks 4 and 16 versus baseline; there was no comparable modulation in placebo-treated patients.

Similar genomic changes with dupilumab (vs placebo) were observed after evaluating a recently published, robust, meta-analysis–derived atopic dermatitis (MADAD) transcriptome derived from a meta-analysis of multiple study transcriptomes.[107] Based on the MADAD transcriptome, significantly altered gene expression (vs baseline) was already evident in lesions of dupilumab-treated patients at week 4, with a further shift to a

A

Study transcriptome

Total Number of DEGs
Criteria: FCH>2, FDR<0.05

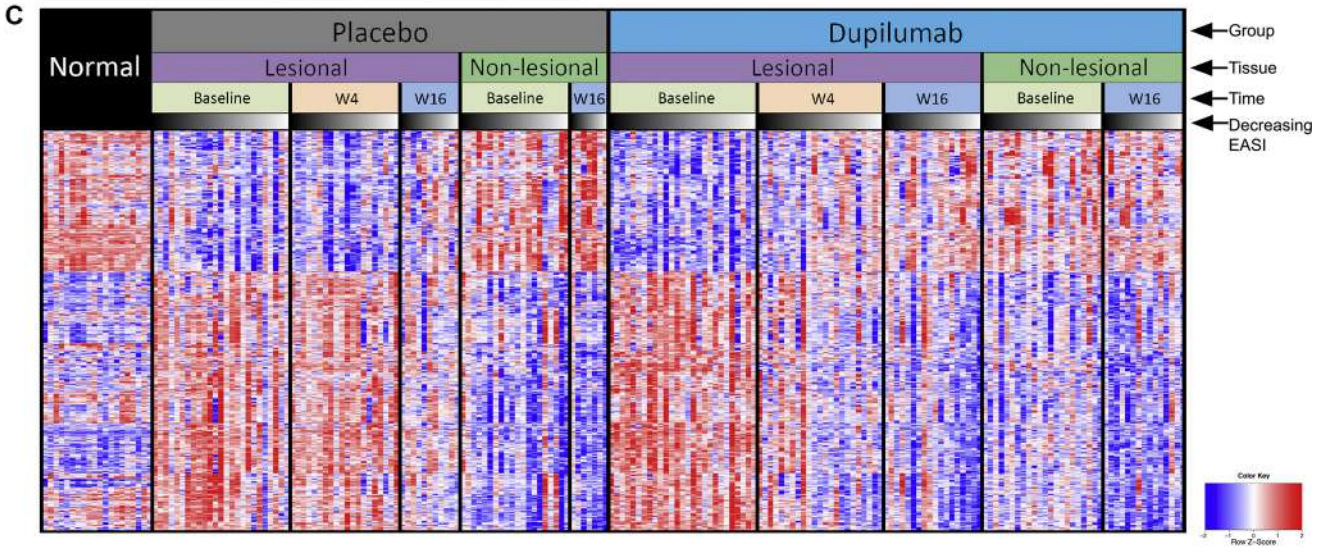
Comparison	Placebo		Dupilumab	
	Up	Down	Up	Down
LS vs. NL at W0	527	508	411	336
LS vs. NL at W16	612	516	0	0
LS Changes at W4	0	0	49	145
LS Changes at W16	1	1	376	554

B

MADAD

Total Number of DEGs
Criteria: FCH>2, FDR<0.05

Comparison	Placebo		Dupilumab	
	Up	Down	Up	Down
LS vs. NL at W0	173	105	164	111
LS vs. NL at W16	74	51	0	0
LS Changes at W4	0	0	16	65
LS Changes at W16	1	1	89	210



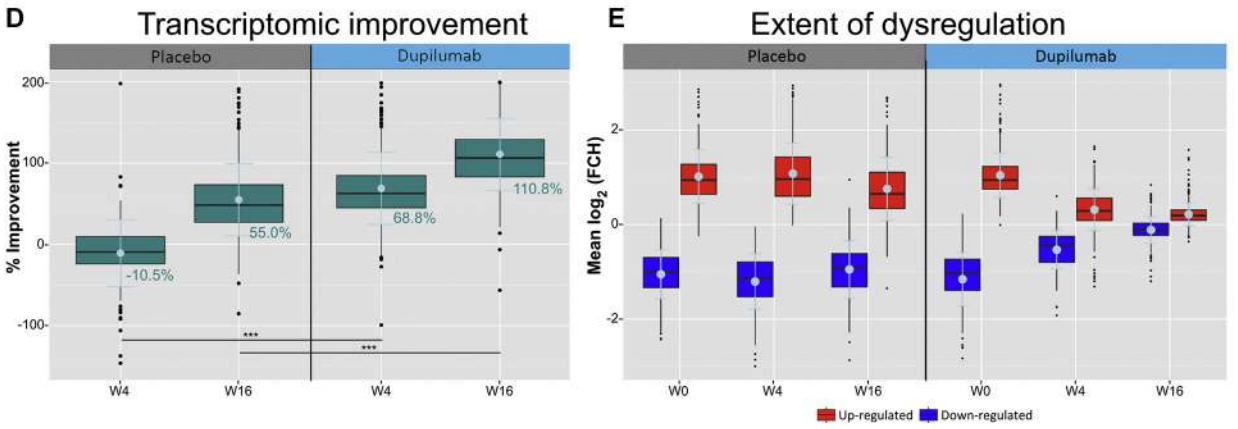
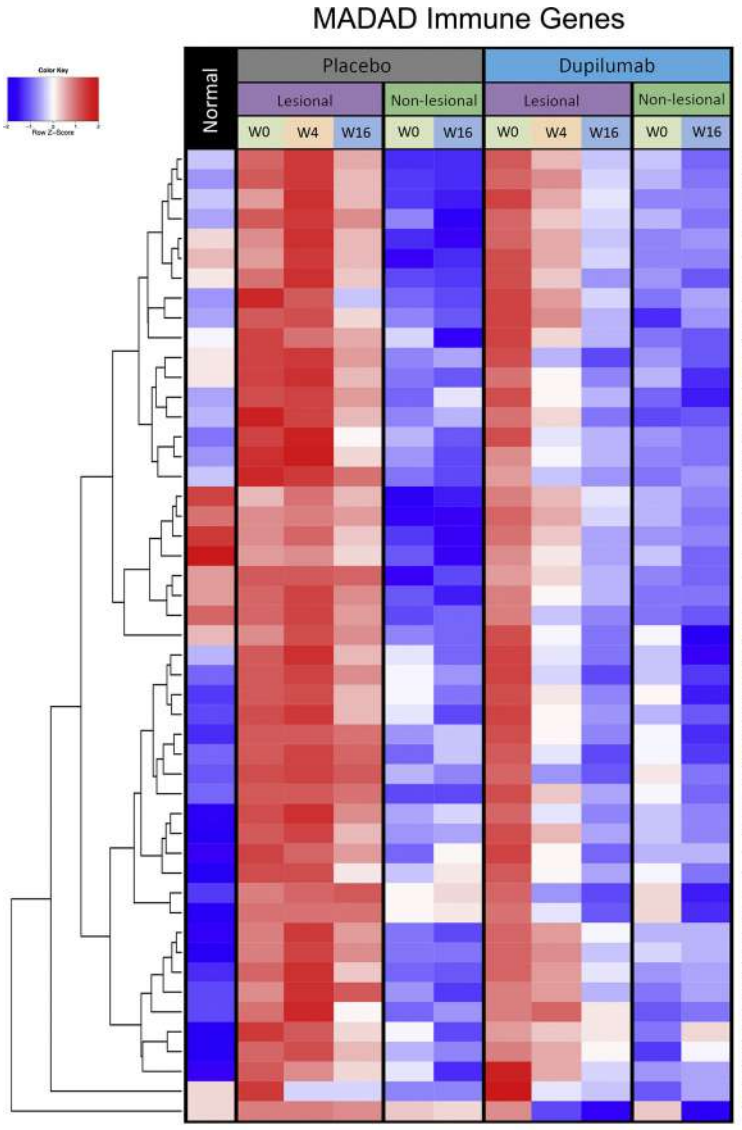


FIG 1. Transcriptomic changes. **A** and **B,** Numbers of differentially expressed genes *(DEGs)* with criteria of an FCH of greater than 2 and FDR of less than 0.05 upregulated *(Up)* and downregulated *(Down)* at baseline *(W0)* and week 16 *(W16)* in lesional *(LS)* versus nonlesional *(NL)* skin in the placebo and dupilumab groups and number of genes differentially modified in LS skin at week 4 *(W4)* and week 16 versus baseline in the study transcriptome (Fig 1, *A*) and the MADAD transcriptome (Fig 1, *B*). **C,** Heat map of the MADAD transcriptome with comparisons to healthy subjects *(Normal)*. Sample data are ordered at each time point and tissue type by decreasing EASI score (indicated by the *gray scale bar*; *black* indicates highest EASI score, and *white* indicates lowest EASI score). **D,** Overall percentage improvement in the MADAD transcriptome at W4 and W16 is shown. ***$P < .001$ at both time points for dupilumab versus placebo comparison. **E,** Effect of dupilumab on expression of genes upregulated *(red)* or downregulated *(blue)* in lesional versus nonlesional skin in the MADAD transcriptome. *Blue bars* superimposed on the plots represent means ± SEMs.

more nonlesional phenotype at week 16 (red-to-blue or blue-to-red transitions; Fig 1, *C*). At week 16, lesions from dupilumab-treated patients had expression levels similar to those of nonlesional skin. No major changes were seen in lesions from placebo-treated patients at week 4 versus baseline, whereas some changes were noted in placebo-treated AD lesions at



	Week 4 vs. Week 0			Week 16 vs. Week 0		
	Placebo	Dupilumab	TE	Placebo	Dupilumab	TE
CD3E:	1.25	−1.37	−1.72	−1.23	−2.08**	−1.69
LCK:	1.12	−1.15	−1.29	−1.46	−1.86*	−1.28
IL7R:	1.41	−1.34	−1.89	−1.10	−1.80**	−1.64
JAK3:	1.11	−1.35	−1.51	−1.22	−1.77**	−1.46
CD2:	1.46	−1.28	−1.87	−1.16	−2.06*	−1.77
CD3G:	1.48	−1.41	−2.09	−1.10	−2.07*	−1.88
CD5:	1.32	−1.56	−2.06	−1.38	−2.59**	−1.88
IL2RA:	−1.15	−1.25	−1.09	−1.80	−1.61+	1.12
CD80:	1.07	−1.24	−1.33	−1.33	−1.92*	−1.44
STAT1:	−1.20	−1.61	−1.34	−1.45	−2.16**	−1.48
CD86:	1.07	−1.95*	−2.08	−1.32	−2.56**	−1.95
CXCR4:	1.08	−1.46	−1.58	−1.45	−2.01**	−1.39
CCR5:	1.04	−1.58+	−1.64	−1.28	−1.87**	−1.47
CCL4:	−1.13	−1.26	−1.12	−1.52	−1.80*	−1.19
S100A12:	1.51	−5.97**	−9.01+	−5.25	−9.04**	−1.72
IL22:	1.26	−1.93	−2.43	−2.91	−2.55+	1.14
IL19:	−1.10	−1.70	−1.54	−1.45	−2.01+	−1.39
IL23A:	1.26	−1.18	−1.49	1.03	−1.54*	−1.59
IL32:	1.07	−1.19	−1.28	−1.04	−1.67*	−1.61
CD3D:	1.15	−1.32	−1.53	−1.21	−1.93**	−1.60
ITGB2:	1.04	−1.38	−1.43	−1.28	−1.99**	−1.55
CCL19:	1.00	−1.18	−1.18	−1.01	−1.61+	−1.58
CCL5:	1.13	−1.36	−1.53	−1.16	−1.71+	−1.48
CCR2:	1.13	−1.60+	−1.80	−1.16	−1.87**	−1.61
LCN2:	1.26	−1.88	−2.38	−1.02	−2.78**	−2.74
PI3:	1.29	−2.72**	−3.51+	−1.65	−4.65**	−2.81
CCR1:	1.08	−2.16**	−2.34	−1.22	−3.31**	−2.71
S100A9:	1.10	−3.27**	−3.59	−2.21	−9.95**	−4.50
OASL:	1.18	−4.67**	−5.50+	−2.42	−10.20**	−4.21
ITGAX:	1.01	−1.93**	−1.95	−1.07	−2.91**	−2.71
CD1B:	1.13	−2.93*	−3.31	−1.12	−6.97**	−6.23
CCL17:	1.14	−5.41**	−6.17+	−1.15	−7.85**	−6.80
CCL22:	1.01	−1.61	−1.62	−1.10	−2.63**	−2.39
CXCL1:	1.35	−4.23**	−5.72+	−1.85	−7.93**	−4.28
CCR7:	1.07	−1.43	−1.54	−1.39	−2.23**	−1.61
CXCL3:	−1.10	−1.63	−1.48	−1.29	−2.37**	−1.84
CXCL2:	−1.01	−2.35*	−2.32	−2.28	−3.53**	−1.55
CCL18:	1.20	−5.62**	−6.77**	1.28	−9.32**	−11.90**
MMP12:	1.10	−9.96**	−11.00+	1.01	−49.60**	−50.30**
ITK:	1.36	−1.21	−1.65	−1.07	−1.66*	−1.54
CTLA4:	1.57	−1.33	−2.09	−1.08	−3.46**	−3.22
ICOS:	1.46	−1.29	−1.89	−1.60	−2.23*	−1.39
CD28:	1.58	−1.10	−1.75	1.23	−2.00*	−2.47
CCL20:	1.60	1.11	−1.44	−1.76	−1.52	1.16
CXCL10:	−1.30	−1.23	1.06	−3.07	−1.58	1.94
CXCL9:	1.11	−1.18	−1.30	−1.44	−1.61	−1.12
IL12RB2:	−1.16	−1.58+	−1.36	−1.42	−2.23**	−1.57
CCL1:	−1.73	−2.23**	−1.29	−1.71	−2.34**	−1.36
CCL26:	1.04	−20.30**	−21.00**	−1.12	−46.00**	−41.10**

FIG 2. MADAD immune genes. Heat map of mean expression levels of a curated list of immune genes in the MADAD transcriptome. The table shows gene symbols with signed FCHs at week 4 *(W4)* and week 16 *(W16)* versus week 0 *(W0)*. Treatment effect *(TE)* is the comparison of change in dupilumab versus change in placebo at week 4 or week 16. +$P < .1$, *$P < .05$, and **$P < .01$ (P values are adjusted).

week 16 (Fig 1, *C*, and see Table E10 in this article's Online Repository at www.jacionline.org).

With dupilumab treatment, there were mean genomic improvements in the MADAD transcriptome of 68.8% (73.8% decreases in upregulated genes and 59.7% increases in downregulated genes) at week 4 and 110.8% (120.1% decreases in upregulated genes and 93.7% increases in downregulated genes) at week 16 ($P < .001$ vs placebo; Fig 1, *D*). In placebo-treated patients we observed a worsening of lesional skin by 10.5% to a more lesional molecular phenotype (6.7% increase in upregulated genes and 17.3% decrease in downregulated genes) in the MADAD transcriptome at week 4, with an overall improvement of 55.0% (59.2% decreases in upregulated genes and 47.1% increases in downregulated genes) at week 16 (Fig 1, *D*). Fig 1, *E*, shows the dupilumab-induced reversal of the baseline genomic dysregulation between lesional and nonlesional skin of patients with AD (in both upregulated and downregulated genes) at weeks 4 and 16 versus minimal changes with placebo.

Dupilumab suppression of type 2, T_H17, and T_H22 inflammatory pathways in lesional skin

As measured by using microarray expression profiling, dupilumab showed significant modulation at week 4 and progressive suppression through week 16 of key immune genes in the MADAD transcriptome.[46-48,107] These included genes related to inflammatory proteases (*MMP12*, *SERPINB4 [SCCA1/2]*, *MMP3*, and *MMP1*), DCs (*CD1b*, *ITGAX/CD11c*, and *CD1c*), T-cell activation (granzyme B, *ICOS*, and *CCR7*), the type 2 pathway (*CCL26*, *IL13RA2*, *CCL17*, *CCL18*, *CCL13*, *CCL22*, and *DPP4*), the T_H17/T_H22 pathway (*CXCL1/CXCL2*, *LCN2*, and *S100A9/S100A12*), and negative regulators (*CTLA4*; Fig 2 and see Table E10).

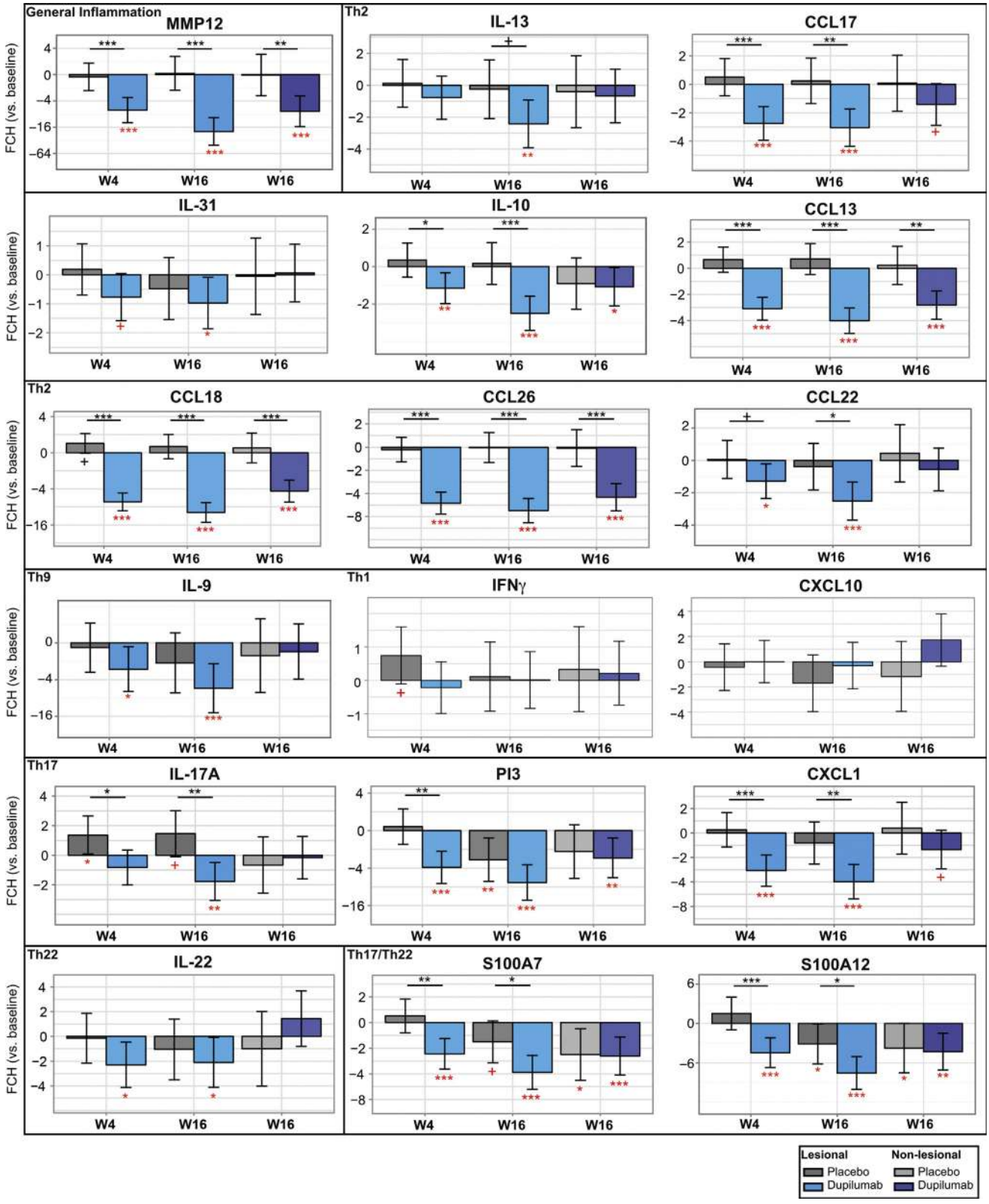
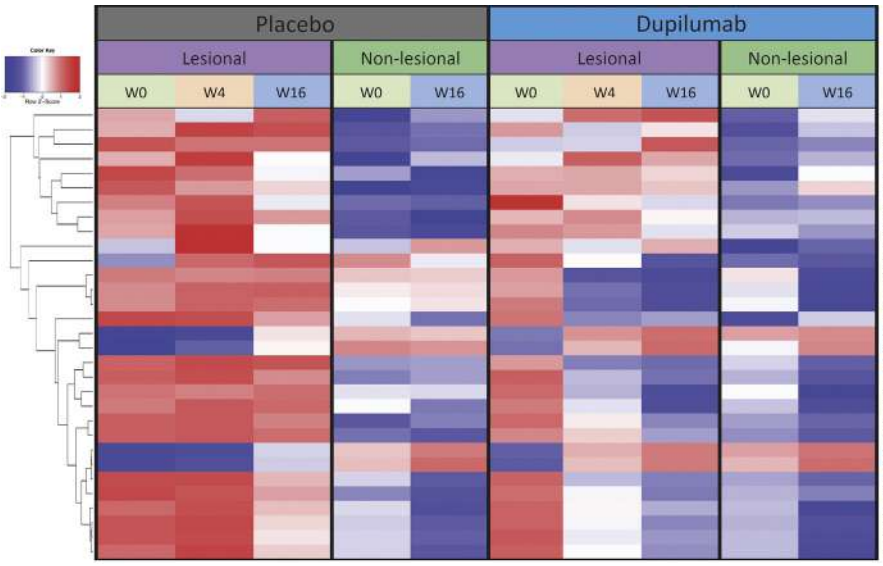


FIG 3. FCHs in inflammatory markers measured by using qRT-PCR. FCHs in inflammatory markers in lesional skin at week 4 *(W4)* and week 16 *(W16)* and at week 16 in nonlesional skin versus baseline. *Black asterisks*, Significance of comparison between placebo and dupilumab; *red asterisks*, significance of comparison with baseline. $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$.



	Week 4 vs. Week 0			Week 16 vs. Week 0		
	Placebo	Dupilumab	TE	Placebo	Dupilumab	TE
IL4	−1.15	1.23	1.42	1.11	1.29	1.16
IL23p19	1.12	−1.12+	−1.26+	1.10	−1.06	−1.17
IL5	−1.03	1.00	1.03	−1.03	1.14	1.17
CCL20	1.37	1.48	1.08	−1.17	1.23	1.44
CXCL10	−1.16	1.01	1.17	−1.80	−1.11	1.62
IL1b	−1.17	1.00	1.17	−1.30	−1.06	1.23
IL31	1.07	−1.30+	−1.39	−1.18	−1.40*	−1.19
FOXP3	1.22	1.08	−1.13	1.01	−1.14	−1.15
IL23p40	1.83+	−1.07	−1.96	−1.33	−1.62	−1.22
IFNG	1.30+	−1.08	−1.40+	1.04	1.00	−1.04
IL17A	1.61*	−1.33	−2.14*	1.66+	−1.85**	−3.07**
CCL26	−1.08	−5.34**	−4.96**	−1.01	−6.70**	−6.62**
CCL13	1.26	−2.92**	−3.67**	1.28	−4.02**	−5.12**
CCL18	1.43+	−6.59**	−9.40**	1.26	−9.90**	−12.50**
IL22	−1.05	−2.22*	−2.11	−1.44	−2.07*	−1.44
LOR	1.07	1.84**	1.71+	2.07**	2.09**	1.01
FLG	1.24	1.45*	1.17	1.69*	1.71**	1.01
CCL17	1.19	−2.59**	−3.09**	1.09	−2.88**	−3.13**
CXCL1	1.10	−2.90**	−3.18**	−1.32	−3.97**	−3.00**
MMP12	−1.13	−6.50**	−5.77**	1.07	−20.10**	−21.40**
IL10	1.13	−1.49**	−1.68*	1.06	−2.37**	−2.51**
CCL22	1.02	−1.56*	−1.59+	−1.15	−2.39**	−2.09*
IL13	1.04	−1.31	−1.37	−1.09	−2.31**	−2.12+
LOR/K16	1.12	7.64**	6.81**	4.95**	12.70**	2.57
FLG/K16	1.28	6.09**	4.75**	4.01**	10.50**	2.61
K16	−1.03	−4.19**	−4.06**	−2.36**	−6.11**	−2.59*
IL9	−1.19	−2.69*	−2.26	−2.12	−5.52**	−2.60
S100A7	1.20	−2.33**	−2.78**	−1.68+	−3.83**	−2.28*
S100A8	1.22	−3.56**	−4.35**	−2.80**	−7.66**	−2.73*
PI3	1.15	−3.90**	−4.50**	−2.92**	−6.79**	−2.33
S100A12	1.55	−3.62**	−5.62**	−2.45*	−8.86**	−3.61*

FIG 4. Heat map of genes measured by using qRT-PCR and cellular infiltrates. Heat map of mean gene expressions measured by using qRT-PCR in lesional skin at week 0 *(W0)*, week 4 *(W4)*, and week 16 *(W16)* and nonlesional skin at weeks 0 and 16 in the placebo and dupilumab groups. The table shows FCHs at week 4 and week 16 versus week 0. Treatment effect *(TE)* is the comparison of change in dupilumab versus change in placebo at week 4 and week 16. Expression ratios of FLG/K16 and LOR/K16 are included. $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$.

To more accurately quantify low-expressing genes not always captured on microarrays, we also performed qRT-PCR; representative markers of the various immune axes associated with AD were included. Highly significant reductions in mRNA expression of the general inflammatory marker MMP12 were seen in dupilumab- versus placebo-treated lesional and nonlesional skin at both weeks 4 and 16 (Figs 3 and 4). As expected, dupilumab significantly suppressed mRNA expression of genes of type 2 inflammation regulated by IL-4Rα–mediated signaling (Figs 3 and 4 and see Fig E4 in this article's Online Repository at www.jacionline.org). Robust and significant reductions in expression of the type 2–polarizing chemokines CCL13/monocyte chemoattractant protein 4, CCL18/PARC, CCL26/Eotaxin-3, CCL17/TARC, and CCL22/macrophage-derived chemokine were observed in lesional skin at weeks 4 and 16. A reduction of *IL13* gene expression at week 16 ($P < .01$ vs baseline and $P < .10$ vs placebo) was also observed. Dupilumab, but not placebo, reduced expression of the itch-associated cytokine IL-31 ($P < .05$ vs baseline at week 16) and the regulatory cytokine IL-10 ($P < .001$ vs baseline and vs placebo at week 16) in lesional skin (Figs 3 and 4). Progressive suppression of the T_H9 cytokine IL-9 was observed in lesions treated with dupilumab but not placebo ($P < .001$ vs baseline for dupilumab at week 16). No significant changes were noted in expression of the T_H1/IFN-γ markers IFN-γ and CXCL10 in skin from dupilumab-treated patients, as measured by using qRT-PCR. Consistent with array results, dupilumab also significantly suppressed mRNA expression of T_H17- and T_H22-regulated genes in lesional skin, including *IL17A* ($P < .05$ vs placebo at both weeks 4 and 16), *CXCL1* ($P < .001$ vs baseline and $P < .01$ vs placebo), *PI3*, *IL22*, and the IL-17/IL-22–regulated *S100A9* and *S100A12* ($P < .001$ vs baseline and $P < .05$ vs placebo; Figs 2-4 and see Fig E4). Expression of several genes decreased from baseline in nonlesional skin by week 16, including *LCN2*, *PI3*, *CCR1*, *S100A9*, *ITGAX*, *CD1b*, *CCL17*, *CCL22*, *CCL18*, *MMP12*, and *CCL26*; Fig 2). No significant changes from baseline with dupilumab were observed in mRNA expression of *IL4*, *IL5*, *IL1B*, *FOXP3*, *IL23p40*, or *CCL20*. *IL23p19* expression was significantly reduced with dupilumab (vs placebo) at week 4 ($P < .05$), but this result was not replicated at week 16 (Fig 4 and see Fig E4).

Reversal of epidermal responses with dupilumab

Compared with placebo, dupilumab significantly modulated genes related to epidermal pathology in patients with AD, including reduced epidermal proliferation measures (*MKi67*, *K16*, *IL24*, and *IL26*) and increased lipid metabolism and barrier junction genes (*ELOVL3*, *FAR2*, claudin 8 *[CLDN8]*, *CLDN23*, and aquaporin 9 *[AQPN9]*), which often are downregulated in AD lesions.[46,50,54,107] These changes were particularly evident at week 16 (see Table E10).

Changes in epidermal hyperplasia were evaluated in dupilumab- and placebo-treated AD tissues by assessing changes from baseline at weeks 4 and 16 in ET and keratinocyte proliferation markers (K16 protein immunostaining and mRNA expression by using RT-PCR and $Ki67^+$ cellular counts) (Fig 5, *A–F*). Consistent with the transcriptome-profiling results, median (quartile 1-quartile 3) ET of lesional skin at baseline was similar in the dupilumab (128 μm; quartile 1-quartile 3, 99.3-156.0 μm) and placebo (125 μm; quartile 1-quartile 3, 106.0-172.4 μm) groups. Robust decreases from baseline in median lesional ET of −23% (week 4) and −44% (week 16) were observed with dupilumab treatment versus median changes from baseline of −5% (week 4) and +4% (week 16) in the placebo group. The difference in ET reduction between the dupilumab and placebo groups was statistically significant at both weeks 4 and 16 ($P < .01$ at both time points; Fig 5, *A* and *D*). No significant changes were noted in the ET of nonlesional skin (median,

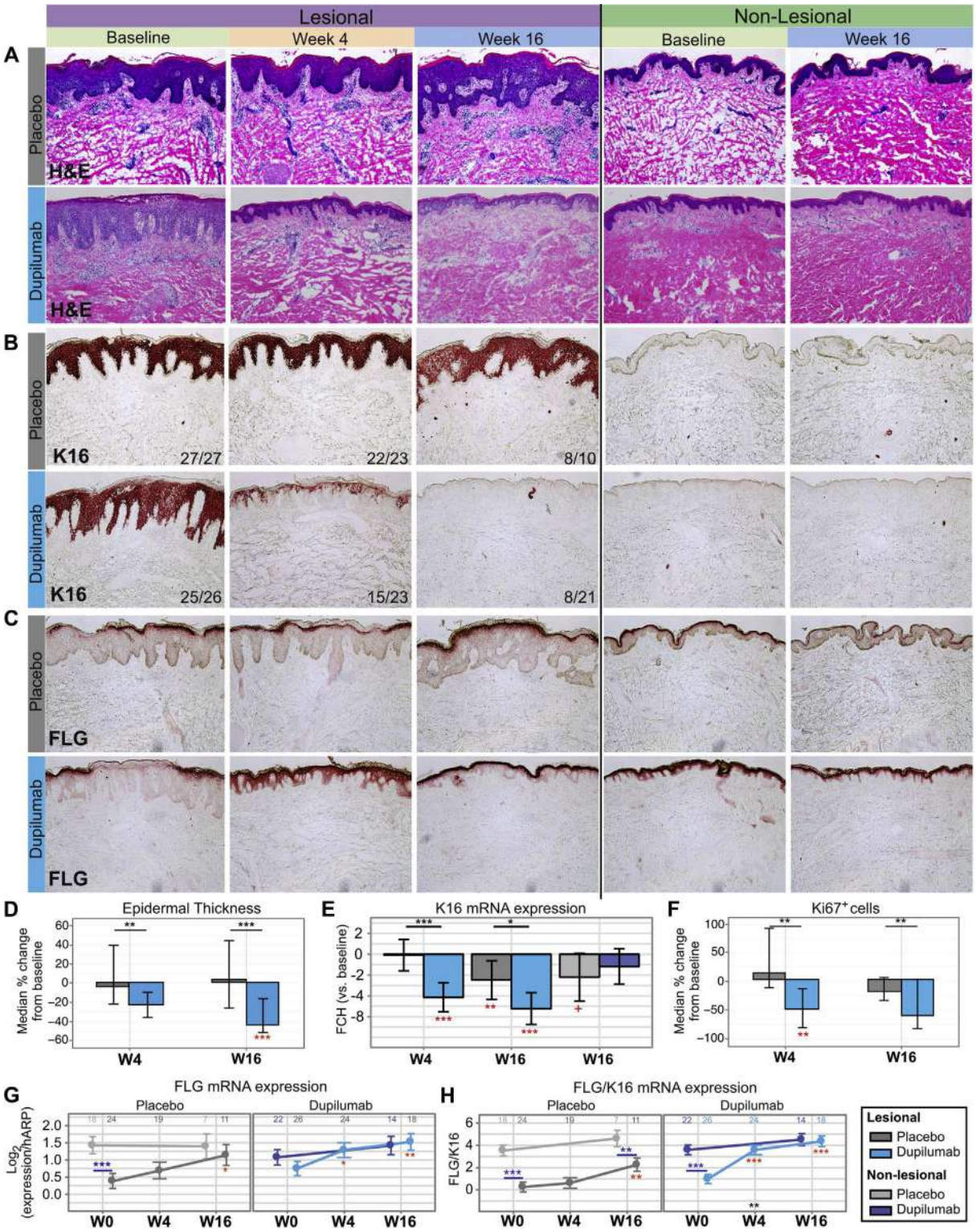


FIG 5. Histologic and barrier changes. **A-C,** Representative histologic images in the placebo and dupilumab groups of hematoxylin and eosin (*H&E*; Fig 5, *A*), K16 (Fig 5, *B*), and FLG (Fig 5, *C*). **D,** Median percentage change in ET at week 4 *(W4)* and week 16 *(W16)* versus baseline in lesional skin (± first and third quartiles). **E,** FCH of K16 mRNA expression in lesional and nonlesional skin at weeks 4 and 16 versus baseline. **F,** Median percentage change in $Ki67^+$ cell counts at weeks 4 and 16 versus baseline in lesional skin (± first and third quartiles). **G,** Log_2 expression/human acidic ribosomal protein *(hARP)* of FLG in lesional skin (at weeks 0, 4, and 16) and nonlesional skin (weeks 0 and 16). Numbers for tissue and time point are shown at top of graph. **H,** Ratio of FLG to K16 expression in lesional skin (at weeks 0, 4, and 16) and nonlesional skin (weeks 0 and 16). Numbers for tissue and time point are shown at top of graph. $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$. *Black asterisks*, Significance of comparison between placebo and dupilumab groups; *red asterisks*, significance of comparison versus baseline; *blue asterisks*, significance of comparison between lesional and nonlesional skin.

A

Spearman Correlation vs. % decrease in EASI at Week 16		
Marker	ρ	p-value
% decrease in SCORAD	0.936	0.000
Ki67	0.659	0.005
Histological Score	0.624	0.010
S100A12	0.590	0.013
S100A8	0.581	0.014
FCERI$^+$ cells	0.575	0.020
S100A7	0.536	0.027
Serum CCL18	0.522	0.031
IL-5	0.522	0.032
CD206$^+$ cells	0.513	0.042
IL-31	0.506	0.038
IL-9	0.486	0.048
PI3	0.483	0.049
CCL22	0.470	0.057
IL13	0.458	0.065
IL-10	0.403	0.109
Epidermal Thickness	0.402	0.123
CD1a$^+$ cells	0.381	0.145
IL-12/23p40	0.366	0.149
MMP12	0.347	0.172
CD11c$^+$ cells	0.330	0.212
CCL20	0.300	0.241
Serum CCL17	0.297	0.247
CCL17	0.288	0.262
CXCL1	0.282	0.273
CD3$^+$ cells	0.280	0.294
CCL18	0.278	0.279
K16	0.264	0.307
IFNγ	0.236	0.361
CD1b$^+$ cells	0.236	0.380
FOXP3	0.233	0.368
Serum Periostin	0.227	0.399
CXCL10	0.158	0.544
IL-1β	0.146	0.576
CD1c$^+$ cells	0.141	0.602
CCL26	0.109	0.677
IL-23p19	0.105	0.687
CCL13	0.102	0.698
IL-17A	0.085	0.746
IL-4	-0.030	0.910
Eosin Cationic Protein	-0.079	0.770
Serum IgE	-0.100	0.724
MBP$^+$ cells	-0.150	0.580
FLG	-0.170	0.513
IL-22	-0.208	0.422
CD83$^+$ cells	-0.237	0.377
CD8$^+$ cells	-0.330	0.212
LOR	-0.407	0.105

B

Spearman Correlation vs. Histological Score Improvement at Week 16		
Marker	ρ	p-value
Epidermal Thickness	0.806	0.000
Ki67	0.729	0.002
PI3	0.712	0.003
% decrease in EASI	0.624	0.010
% decrease in SCORAD	0.591	0.018
S100A12	0.578	0.019
IL-31	0.575	0.020
S100A8	0.547	0.031
K16	0.541	0.033
CD11c$^+$ cells	0.532	0.036
IL-12/23p40	0.515	0.041
CCL22	0.479	0.062
CD3$^+$ cells	0.468	0.070
FCERI$^+$ cells	0.456	0.078
CD206$^+$ cells	0.444	0.085
CXCL1	0.438	0.091
CCL17	0.412	0.114
IL-9	0.401	0.124
CCL20	0.382	0.145
CD1b$^+$ cells	0.359	0.172
Serum CCL18	0.359	0.173
IL-23p19	0.329	0.213
Serum CCL17	0.326	0.217
IL-1β	0.303	0.254
CCL26	0.297	0.263
CD1a$^+$ cells	0.294	0.268
S100A7	0.294	0.268
Serum Periostin	0.289	0.295
Serum IgE	0.289	0.295
MMP12	0.218	0.417
FOXP3	0.185	0.491
MBP$^+$ cells	0.183	0.498
IL-13	0.182	0.499
CXCL10	0.124	0.648
CCL18	0.085	0.755
CD1c$^+$ cells	0.074	0.788
IL-10	0.051	0.850
IL-17A	-0.041	0.879
CD8$^+$ cells	-0.044	0.874
CCL13	-0.079	0.771
IL-5	-0.082	0.763
IL-22	-0.160	0.554
IFNγ	-0.162	0.549
CD83$^+$ cells	-0.279	0.294
IL-4	-0.306	0.249
FLG	-0.535	0.035
Eosin Cationic Protein	-0.585	0.019
LOR	-0.688	0.004

C

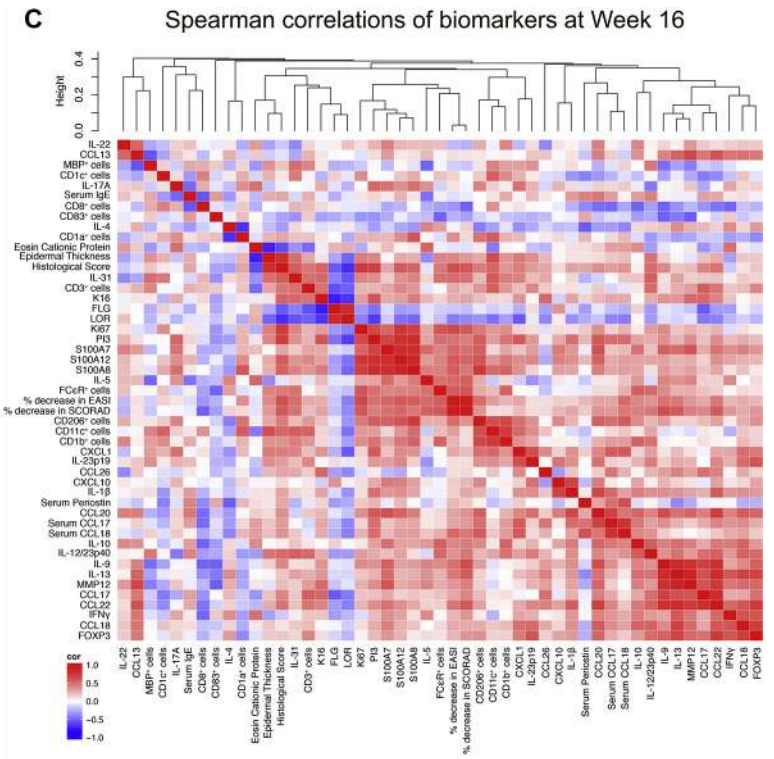


FIG 6. Clinical and histologic correlations. **A** and **B,** Tables of Spearman correlations of change at week 16 versus baseline in clinical measures, gene expression, and cell counts with percentage decrease in EASI score (Fig 6, *A*) and histologic score (composite *z* score of ET and K16) improvement (Fig 6, *B*). **C,** Heat map of Spearman correlation matrix of measured values at week 16 ordered by unsupervised clustering (and shown by dendrogram).

−8% [−18.3%, 4.8%] in the dupilumab group vs −1% [−14.9%, 43.7%] in the placebo group, $P = .55$; Fig 5, *A*).

Significant reduction in *K16* mRNA expression was observed in lesional skin of dupilumab- versus placebo-treated patients at week 4 ($P < .001$) and week 16 ($P < .05$; Fig 5, *E*). Qualitative assessment of K16 protein immunostaining confirmed the *K16* mRNA results, with no significant change in K16 staining observed in the majority of patients in the placebo group, whereas most dupilumab-treated patients progressively lost the K16 staining through week 16 (the number of K16-positive patients out of the total number of patients treated with dupilumab or placebo at each week is provided; Fig 5, *B*). Significant reductions from baseline in Ki67$^+$ cell counts were also observed in lesional skin of dupilumab-treated patients at week 4 (median, −51% [quartile 1-quartile 3, −83.28% to −16.84%) versus baseline ($P = .0074$), but not at week 16 (−62% [−84.77% to 0.36%]) versus baseline ($P = .2979$; Fig 5, *F* and Table E9). The reductions were not statistically significant in placebo-treated patients at week 4 (+10.7%; −15.13, 87.80) versus baseline ($P = .1434$) and at week 16 (−20.7%; −36.44, 3.17) versus baseline ($P = .1016$). However, the differences were statistically significant with dupilumab versus placebo at week 4 ($P = .0013$) and at week 16 ($P = .0062$; Fig 5, *F* and Table E9).

Increased expression of epidermal terminal differentiation markers with dupilumab treatment

Areas with a lesional phenotype before treatment were characterized by discontinuous and relatively faint expression of filaggrin (FLG; Fig 5, *C*). After 16 weeks of dupilumab treatment, FLG showed stronger and more continuous granular layer expression in samples from areas that had been lesional at baseline (Fig 5, *C*). Baseline mRNA expressions of both *FLG* and loricrin *(LOR)* are reduced in AD lesional versus nonlesional skin,[47,54,72] with significant increases (vs baseline) with dupilumab treatment ($P < .01$) in lesional mRNA expression of both genes to levels similar to that in nonlesional skin at week 16 (Fig 5, *G*, and see Fig E5, *A*, in this article's Online Repository at www.jacionline.org). These increases were even more pronounced when adjusted for the reduction in epidermal hyperplasia (as measured by changes in *K16* mRNA expression; Fig 5, *H*, and see Fig E5, *B*).

Correlation of biomarkers with clinical improvement and reductions in epidermal hyperplasia with dupilumab

We identified markers associated with improvements in clinical, molecular, and histologic measures of AD by

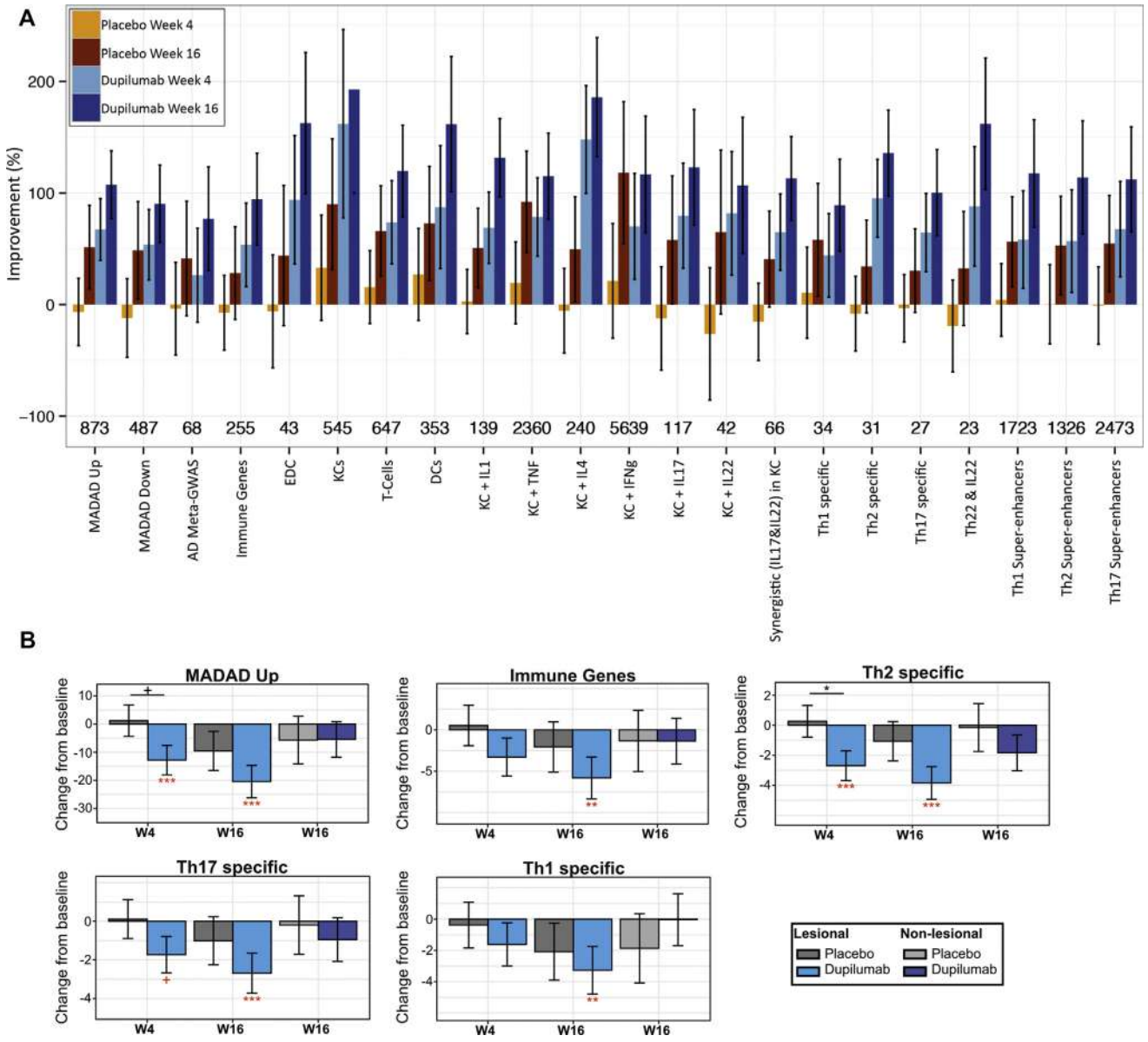


FIG 7. Gene Set Variation Analysis (GSVA). **A,** Improvements in GSVA scores of different gene sets in lesional skin at week 4 *(W4)* and week 16 *(W16)* in placebo and dupilumab groups, with numbers at the bottom of the graph indicating number of genes in each gene set. **B,** Change from baseline *(W0)* of GSVA scores of select gene sets. $+P < .1$, $*P < .05$, $**P < .01$, $***P < .001$. *Black asterisks,* Significance of comparison between placebo and dupilumab; *red asterisks,* significance of comparison versus baseline.

determining correlations of each marker measured in lesional skin using qRT-PCR and immunohistochemistry, and serum biomarkers, with improvements in EASI and SCORAD (clinical disease reversal) scores and reductions in epidermal hyperplasia (histologic disease reversal, as measured based on a combined histologic score of ET and mRNA expression of *K16*; Fig 6). Spearman correlation coefficients of skin and serum biomarkers with percentage change in clinical scores (EASI and SCORAD) and histologic score improvements at week 16 are shown in Fig 6, *A* and *B*, respectively. A heat map showing the relations between biomarkers and with disease scores at week 16 is shown in Fig 6, *C*. SCORAD and EASI disease activity measures were highly correlated with each other and with the histologic score improvement at week 16 (Fig 6, *A* and *B*). Strong, statistically significant, and direct correlations of the hyperplasia-related measures of epidermal proliferation ($Ki67^+$ cells) and histologic score ($P < .01$) were observed with percentage change from baseline in EASI scores at week 16 (Fig 6, *A*). The $Ki67^+$ cell count was also highly correlated with histologic score improvement at week 16 (Fig 6, *B*). Numbers of $Fc\varepsilon RI^+$ and $CD206^+$ DCs and mRNA expression levels of *IL5*, *IL31*, *IL9*, and the IL-22/IL-17–regulated genes (*S100A8*, *S100A12*, *S100A7*, and *PI3*) in the skin were also highly correlated (correlation defined as $\rho \geq 0.2$ and $P < .05$) with the percentage change from baseline in EASI score at week 16 (Fig 6, *A*). Expression of *PI3*, *S100A12*, *S100A8*, and *IL31* was also strongly correlated with histologic

improvement (Fig 6, *B*). The reduction in serum CCL18 expression was highly correlated with the percentage change from baseline in EASI score at week 16 but not with histologic score improvement. $CD11c^+$ cells and *IL-12/23p40* expression were correlated with histologic scores but not the percentage change from baseline in EASI score at week 16 (Fig 6, *A* and *B*). Of note, increases in differentiation markers (*FLG* and *LOR*, $P < .05$) were negatively correlated with histologic scores but not with the percentage change from baseline in EASI scores at week 16 (Fig 6).

Dupilumab induced progressive improvements in inflammatory and barrier responses

We evaluated the effect of dupilumab compared with placebo treatment on expression of previously reported gene signatures[46-48,108] for upregulated and downregulated genes in the AD transcriptome of genes identified in genome-wide association studies of AD.[109] These genes include keratinocyte, T-cell, and DC gene subsets; primary cytokine-treated keratinocytes; epidermal differentiation complex (EDC) genes; and other genes from specific immune pathway subsets or their superenhancers (Fig 7, *A*).[46,107,108,110-119] Only modest improvements were observed in most gene signatures in placebo-treated lesional skin at week 16, with either no improvement or more inflammatory molecular phenotypes at week 4. Dramatic improvements (sometimes exceeding 100%) were observed in dupilumab-treated patients at week 16, with changes already evident at week 4. For example, the gene subsets from untreated keratinocytes and keratinocytes treated with IL-4[46] showed greater than 100% improvement after 4 weeks of treatment with dupilumab compared with placebo. In addition, almost complete improvement toward a more nonlesional molecular phenotype was seen at week 4 in the EDC gene signature.[46,50,55,74] Dupilumab induced significant progressive changes through week 16 that extended beyond the expected type 2 pathway, including suppression of key AD responses in lesional skin (eg, T_H22 and T_H17 pathways) and in cytokine-treated keratinocytes, including innate cytokines.[46,74,107,108,111,120]

Overall, expression signatures of keratinocytes treated with IL-1, TNF-α, or IFN-γ at week 16 were markedly reduced with dupilumab relative to baseline,[46,118] although similar reductions in IFN-γ at week 16 were seen with both dupilumab and placebo. Mean FCH reductions in selected gene signatures in AD lesional and nonlesional skin, including upregulated genes in the MADAD transcriptome; immune genes; and T_H2-, T_H17-, and T_H1-specific genes (Fig 7, *B*) were significantly greater with dupilumab but not placebo.

Dupilumab suppressed circulating markers of type 2 inflammation

Dupilumab (vs placebo) significantly reduced circulating serum concentrations of CCL17, CCL18, and periostin from baseline through week 16 (see Fig E6 and Table E11 in this article's Online Repository at www.jacionline.org). Dupilumab also progressively and significantly reduced total IgE concentrations (see Fig E6 and Table E11), as well as concentrations of multiple allergen-specific IgEs. These included allergen-specific IgEs previously associated with AD and other atopic or allergic type 2 manifestations (eg, staphylococcal enterotoxin A, −61% vs 0%; *Dermatophagoides farinae*, −39% vs 0%; see Table E12 in this article's Online Repository at www.jacionline.org).[90-95] Although concentrations of most specific IgEs were significantly reduced only with dupilumab (except that of sage mugwort *[Artemisia vulgaris]*, $P = .10$), particularly large (>50%) and significant reductions were seen in those of cockroach, staphylococcal enterotoxin A, *Cladosporium* species, Bermuda grass, silver birch, white oak, elm, white ash, Johnson grass, and cat dander (see Table E12). Baseline serum total IgE concentrations were greater than the normal range (119 kU/L) in both groups (see Table E5). The proportion of patients with relatively normal baseline IgE concentrations (<150 kU/L, n = 10) who had positive results for at least 1 of the allergen-specific IgEs tested in the study was 20% (n = 2).

DISCUSSION

The clinical efficacy of dupilumab in patients with moderate-to-severe AD has proved the central role of IL-4 and IL-13 signaling in the pathogenesis of AD. In this study dupilumab targeting helps us better understand the specific pathways that drive AD and mediate its clinical benefit.

This study evaluated both short-term (4 weeks) and longer-term (16 weeks) effects of dupilumab-mediated IL-4Rα blockade on barrier and immune pathomechanisms in the skin of patients with moderate-to-severe AD. Clinical efficacy, safety, and suppression of type 2–related systemic biomarkers were also evaluated. This study demonstrated strong correlations between molecular, histologic, and clinical changes. Most previous studies of broad[121] immune suppressants (including CsA, methotrexate, and NB-UVB) assessed long-term clinical improvements in patients with moderate-to-severe AD but did not perform molecular and cellular correlation analyses in skin biopsy specimens.[4,47,48] Although CsA and NB-UVB have been shown to broadly modulate both immune (including type 2 pathway) and epidermal responses, these treatments have direct effects on keratinocytes and therefore cannot prove the pathogenic role of the type 2 axis in patients with AD.[47,48]

The clinical efficacy and safety of dupilumab monotherapy in this study was consistent with those observed in larger phase 3 AD trials.[24-28] Dupilumab significantly improved AD signs and symptoms, and injection-site reactions and conjunctivitis in this trial were more frequent with dupilumab than with placebo. Aggregated data from dupilumab studies in patients with AD[122] indicate an increased incidence (relative to placebo) of conjunctivitis; however, most cases of conjunctivitis were mild or moderate and resolved or were resolving during study treatment. Of note, a dupilumab-associated increase in conjunctivitis has not been seen in studies of dupilumab in patients with other indications, such as asthma[29,30,33,34] and chronic rhinosinusitis with nasal polyposis.[31]

The parallel, longer-term tissue reversibility of key AD epidermal and immune features with dupilumab treatment was not evaluated. We previously reported significant dose-dependent shifts of the AD transcriptome in patients with AD treated with 4 weeks of dupilumab compared with placebo.[46] Although that small study was the first to associate clinical improvement with suppression of type 2 and other inflammatory markers, it lacked histologic correlates of resolution of the AD-related epidermal pathology. Furthermore, it evaluated only a small number of

patients after 4 weeks of treatment and did not address how specific inhibition of the type 2 pathway affects the AD molecular and cellular profiles with extended treatment.[23,46]

This is the most comprehensive study to establish the ability of dual inhibition of IL-4 and IL-13 signaling to modulate the immune response and epidermal barrier pathomechanisms that typify AD in addition to reversing clinical disease activity. It is also the first study to evaluate tissue responses around the time at which most patients have a maximal clinical response to dupilumab, thus allowing correlation of the extent of disease reversal in skin lesions and circulating markers with clinical responses.[25,26]

Compared with placebo, dupilumab progressively and significantly reduced cellular infiltrates, including $CD3^+$ T cells and various DC subsets, such as $Fc\varepsilon RI^+$ cells, in AD lesions at weeks 4 and/or 16. Dupilumab also induced a strong, rapid, and significant improvement (68.8%) in a robust genomic AD signature, the MADAD transcriptome,[107] after only 4 weeks of treatment, whereas a 10.5% worsening of the AD transcriptome was observed in the placebo group. Progressive modulation of the lesional AD expression profile was achieved at week 16, with an improvement of 110.8% in the dupilumab group but only 55% in placebo-treated patients. This improvement in the placebo group could be explained in part by the much higher dropout rate caused by lack of efficacy in this group. Placebo-treated patients who did not experience relief or even experienced disease exacerbation might have been more likely to drop out of the trial after week 4 but before reaching the 16-week time point. Therefore the placebo group patients remaining at week 16 perhaps represent those with particularly favorable responses to placebo. At week 16, we observed a reversal of the lesional AD phenotype to a level beyond even that of nonlesional skin, and we observed a large shift toward the nonlesional phenotype as early as week 4.

The ability of targeted IL-4Rα blockade with dupilumab to revert the AD skin transcriptome toward that of nonlesional skin is at least comparable with that achievable with a maximal dose of CsA, a broad T- and B-cell immune suppressant[123-126] that is not approved for AD in the United States and use of which is limited because of toxicity.

Blocking IL-4Rα signaling with dupilumab progressively reversed the characteristic epidermal hyperplasia of AD lesions[1,7,55] after 4 and 16 weeks of treatment, as indicated by significant reductions in ET, $Ki67^+$ cells, and K16 protein and gene expression. In parallel, we observed significant increases in mRNA expression of terminal differentiation markers known to be downregulated in patients with AD, including *FLG* and *LOR*, and this was particularly evident after accounting for the concurrent decrease in epidermal proliferation (relative to K16 expression). These increases were coupled with protein localization of FLG that was more homogeneous than the faint and discontinuous staining observed at baseline. These results provide clinical evidence to support the hypothesis from *in vitro* and mouse experiments that IL-4 and IL-13 directly impair the skin barrier and suppress expression of *FLG*, *LOR*, and other EDC and lipid genes.[70-73] Dupilumab treatment significantly increased expression of genes related to lipid metabolism (*ELOVL3* and *FAR2*) and tight junction genes (claudins), genes previously found to be downregulated in AD lesions and also suppressed *in vitro* by type 2 cytokines.[46,50,54,107,127,128] These results confirm previous analyses that showed a negative correlation between type 2 cytokine levels and lipid measures in patients with AD[107] and demonstrate the role of IL-4 and IL-13 in skin barrier dysfunction in patients with AD.

Dupilumab reversed key immune and barrier mechanisms dysregulated in AD skin, with effects extending beyond those expected from dual inhibition of IL-4 and IL-13 signaling, suggesting that chronic type 2 inflammation has secondary effects on other immune axes. Dupilumab potently inhibited type 2 pathway genes as early as week 4, with progressive reductions at week 16 (eg, *IL13*, *CCL17*, *CCL18*, and *CCL26*) and no comparable reductions in the placebo group. *DPP4*, an IL-13–regulated gene being explored as a potential predictive marker for IL-13–specific inhibition in patients with AD, was also downregulated at week 16. Aside from suppression of type 2 genes regulated by IL-4 and/or IL-13 signaling, as expected, dupilumab also significantly downregulated mRNA expression of inflammatory proteases (*MMP12* and *SERPINB4*), T-cell activation (*ICOS* and *CCR7*), and T_H22 and T_H17 pathway genes (eg, *IL17A*, *IL22*, and *S100As*) after 4 and 16 weeks of treatment. Suppression of the T_H17/T_H22 axes correlated with clinical improvements and resolution of epidermal hyperplasia.

Although dupilumab's effects have proved that the type 2 pathway is central to AD pathogenesis, previous studies have shown that the T_H22 and T_H17 pathways can also play contributory roles in the AD phenotype and its epidermal pathology.[1,3-5,7,20,51,53,129,130] However, consistent robust efficacy in patients with AD has been achieved only with dupilumab; inconclusive or less impressive results were obtained with anti–IL-17/IL-23 and anti–IL-22/ILV-094 approaches, suggesting that these axes are not central pathogenic drivers relative to the type 2 axis.[44,45] Future studies with specific IL-22, IL-17, and IL-23 antagonists should determine whether cytokines other than the type 2 IL-4 and IL-13 cytokines have a direct role in AD or are secondary inflammatory effects of chronic type 2 activation.

IL-22 has been linked to epidermal hyperplasia in patients with AD and to inhibition of keratinocyte terminal differentiation[120,131-136] and induction of the S100As,[120,136] processes that further induce hyperplasia.[135,136] IL-22 has also been implicated in expression of antimicrobial peptides and in reduction of *S aureus* colonization.[137]

IL-17 strongly induces antimicrobial peptides, such as β-defensins, cathelicidin, and S100A8.[120,134] In addition to IL-4, IL-17 can further inhibit some barrier measures that are downregulated in patients with AD, including FLG, LOR, and tight junctions[138,139]; it was recently implicated in epidermal proliferation.[140] IL-17 is also thought to play a role in host defense against *S aureus*, which in turn upregulates IL-17.[141,142] Reversal of histologic hyperplasia and/or improvement in AD disease activity with dupilumab treatment was significantly associated not only with reductions in expression of the *S100A7*, *S100A8*, and *S100A12* genes, which have been shown to be induced by IL-17/IL-22,[120] but also with suppression of type $2/T_H2$ measures in the skin (IL-31) and serum (CCL18/PARC). The significant negative correlation between the reduction in epidermal hyperplasia and increases in expression of the terminal differentiation genes *LOR* and *FLG* demonstrates an improvement in skin barrier integrity. Although the reduction in expression of T_H17/T_H22-related genes with IL-4/IL-13 inhibition is not entirely understood, they might result in part from suppression of IL-4/IL-13–mediated induction of DC differentiation[143,144] and might reflect wider networks of inhibition resulting from expression of IL-4Rα on a variety of cells in skin (eg, keratinocytes, DCs, T cells, eosinophils, and mast cells). Data from a study in flaky-tail mice support the existence of IL-4/IL-17 coregulatory networks.[145]

Although the type 2/T_H2 axis is common to all patient populations, various AD phenotypes, such as intrinsic, Asian, and especially pediatric AD, exhibit greater expression of T_H22 and T_H17 cytokines.[1,3,7,130,146-148] Dupilumab appears to have similar efficacy in pediatric and Japanese patients, thus supporting the notion that these other inflammatory pathways are secondary to type 2 inflammation.[149-152]

This study defined robust, progressive, tissue-transcriptional signatures of therapeutic response to dupilumab that could serve as a point of reference for future therapeutic studies across a variety of AD patient populations. It defines a set of skin and circulating biomarkers that correlate with dupilumab-mediated clinical improvements in AD across the atopic or "allergic" spectrum and/or epidermal hyperplasia. Many of these measures are key pathogenic elements of AD and have been linked to successful treatment responses with broad immune-targeting agents, such as CsA and even NB-UVB, in patients with AD.[11,47,48] These measures include significant associations between clinical improvement and type 2/T_H2 markers (eg, IL-13, CCL18, CCL17, CCL26) in the skin and circulation (CCL18, CCL17, and IgE), as well as T_H17/T_H22 pathway–related genes (eg, *S100As*, *CXCL1*, and *PI3*). Changes in markers of epidermal hyperplasia (ET, K16, Ki67, and histological score) and general inflammation (eg, MMP12) were also significantly associated with clinical improvement.

An additional exploratory analysis, which was not previously reported in the longer-term dupilumab studies, was to evaluate the effects of dupilumab on atopic or allergic and type 2 serum biomarkers. Circulating type 2 chemokines have been implicated as biomarkers of AD severity at baseline or as markers of treatment response, and they include CCL17, CCL18, ECP, and periostin.[11,89,153-158] CCL17 and CCL18 are chemokines that attract and activate inflammatory cells expressing CCR4 and CCR8 receptors, whereas the extracellular matrix protein periostin amplifies keratinocyte responses. In our study dupilumab significantly suppressed serum levels of all of these biomarkers, as well as total and allergen-specific IgE concentrations. Although serum total and allergen-specific IgE concentrations were previously associated with greater severity of AD and other type 2 atopic/allergic diseases,[90-95] broad-based treatments have largely failed to cause downregulation of IgE.[47,96-98] Further studies are needed to evaluate the clinical effect on other atopic comorbidities of the dupilumab-induced modulation of circulatory type 2 biomarkers in our study.

Limitations of the current study include the fact that the dose and regimen investigated are different from the approved dose (300 mg every 2 weeks) and the regimens used in the larger phase 3 AD trials (300 mg weekly and 300 mg every 2 weeks),[25-28] as well as the fact that analyses were conducted only to week 16.

Overall, these data further confirm AD as a type 2–driven disease in which IL-4 and IL-13 have a central role. In addition, our results suggest that IL-4/IL-13 signaling contributes to regulation of the IL-17 and IL-22 cytokine networks in patients with AD, given the significant modulation of these pathways that results from IL-4Rα inhibition. These data demonstrate that inhibition of IL-4Rα, which results in dual inhibition of IL-4/IL-13 signaling, can effectively suppress key pathogenic processes in patients with AD, thus supporting the notion that cytokines induce and perpetuate the epidermal alterations in patients with AD.[2,3,68-70,73,120,129,132,133,136] In parallel with increased cytokine activation in skin lesions before treatment,[8-11,49] patients with severe AD have robust systemic cytokine induction, as reflected by the broad abnormalities already observed at the nonlesional skin level.[11,54] Dupilumab significantly improves immune activation in both the skin and blood compartments in parallel with reversal of the pathologic epidermal responses and clinical disease activity. Future studies should also address interactions between the skin microbiome and resolution of clinical disease and tissue inflammation in patients with moderate-to-severe AD.

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Key messages

- **Targeted dual inhibition of IL-4 and IL-13 signaling through anti–IL-4Rα blockade with dupilumab rapidly and progressively suppressed cellular and molecular cutaneous markers of inflammation from week 4 through week 16 and reversed associated epidermal abnormalities while improving disease severity scores and symptoms in patients with moderate-to-severe AD.**
- **Dupilumab significantly suppressed systemic type 2 inflammatory biomarkers in serum, including the type 2–driven chemokines CCL17 and CCL18, periostin, and total and allergen-specific IgEs.**
- **These data further confirm AD as a type 2–driven disease in which IL-4 and IL-13 play a central role in sustaining systemic and cutaneous type 2–driven inflammation and epidermal alterations.**

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