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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Targeting IL-13 with tralokinumab normalizes type 2 inflammation in atopic dermatitis both early and at 2 years

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Abstract

Background: Tralokinumab is a monoclonal antibody that specifically neutralizes interleukin (IL)-13, a key driver of skin inflammation and barrier abnormalities in atopic dermatitis (AD). This study evaluated early and 2-year impacts of IL-13 neutralization on skin and serum biomarkers following tralokinumab treatment in adults with moderate-to-severe AD.

Methods: Skin biopsies and blood samples were evaluated from a subset of patients enrolled in the Phase 3 ECZTRA 1 (NCT03131648) and the long-term extension ECZTEND (NCT03587805) trials. Gene expression was assessed by RNA sequencing; protein expression was assessed by immunohistochemistry and immunoassay.

Results: Tralokinumab improved the transcriptomic profile of lesional skin by Week 4. Mean improvements in the expression of genes dysregulated in AD were 39% at Week 16 and 85% at 2 years with tralokinumab, with 15% worsening at Week 16 with placebo. At Week 16, tralokinumab significantly decreased type 2 serum biomarkers (CCL17/TARC, periostin, and IgE), reduced epidermal thickness versus placebo, and increased loricrin coverage versus baseline. Two years of tralokinumab treatment significantly reduced expression of genes in the Th2 (*IL4R*, *IL31*, *CCL17*, and *CCL26*),

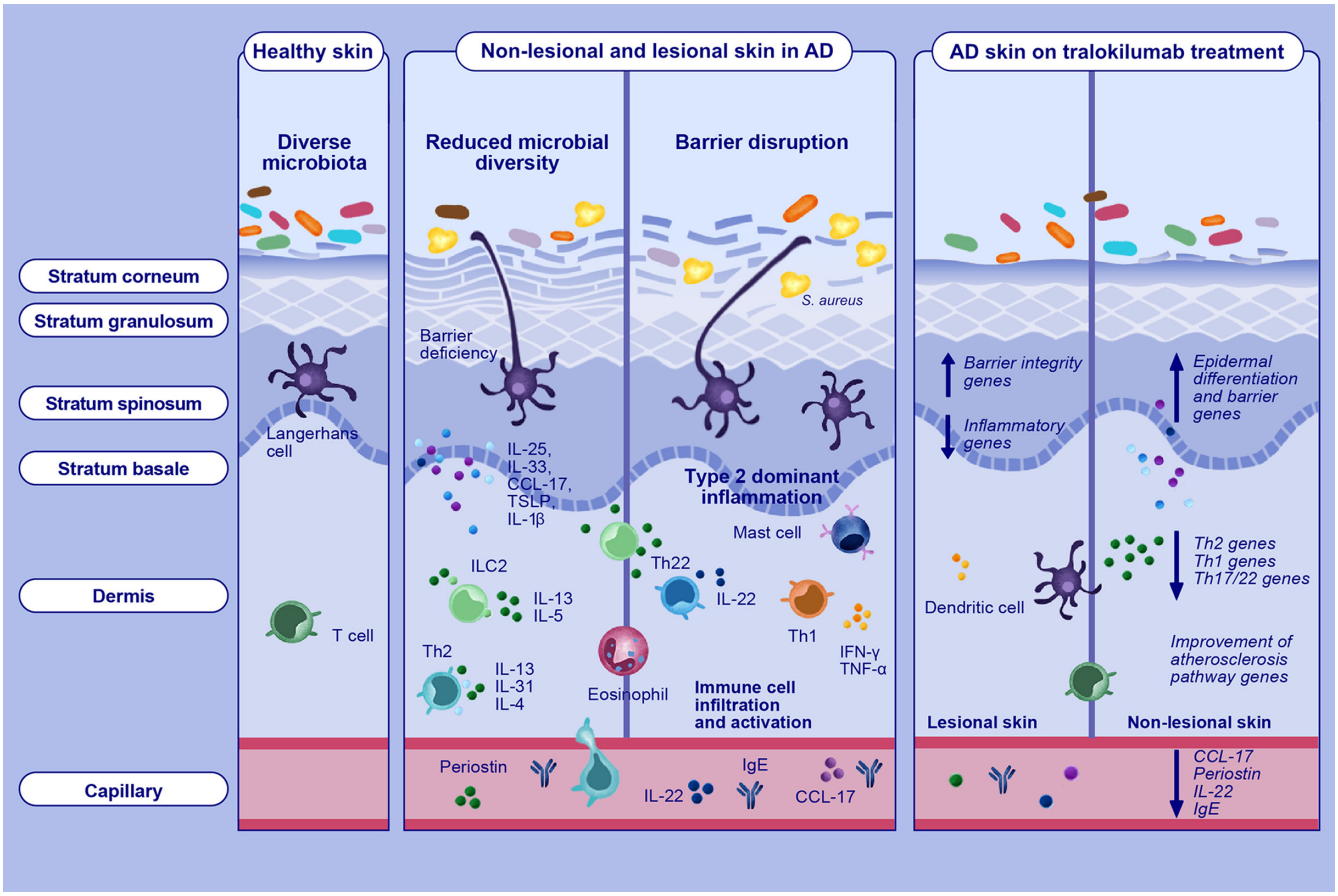
Th1 (*IFNG*), and Th17/Th22 (*IL22*, *S100A7*, *S100A8*, and *S100A9*) pathways as well as increased expression of epidermal differentiation and barrier genes (*CLDN1* and *LOR*). Tralokinumab also shifted atherosclerosis signaling pathway genes (*SELE*, *IL-37*, and *S100A8*) toward non-lesional expression.

Conclusion: Tralokinumab treatment improved epidermal pathology, reduced systemic markers of type 2 inflammation, and shifted expression of key AD biomarkers in skin towards non-lesional levels, further highlighting the key role of IL-13 in the pathogenesis of AD.

Clinical Trial Registration: NCT03131648, NCT03587805.

KEYWORDS

atopic dermatitis, biomarkers, gene expression, tralokinumab, type 2 inflammation



GRAPHICAL ABSTRACT

This study evaluated early and 2-year impacts of IL-13 neutralization on skin and serum biomarkers following tralokinumab treatment in adults with moderate-to- severe AD. Specific targeting of IL-13 with tralokinumab improved epidermal pathology and normalized expression of key biomarkers in serum and lesional skin in patients with moderate-to-severe AD. These data further support the central role of IL-13 in driving AD pathology and highlight that inhibition of IL-13 alone is sufficient for normalizing the molecular phenotype of AD. Abbreviations: AD, atopic dermatitis; CCL-17, CC-motif chemokine ligand 17; IFN-γ, interferon γ; IgE, immunoglobulin E; IL, interleukin; ILC2, group 2 innate lymphoid cells; Th, T helper cell; TNF-α; tumor necrosis factor α; TSLP, thymic stromal lymphopoietin.

1 | INTRODUCTION

Atopic dermatitis (AD), a chronic inflammatory skin disease characterized by recurrent eczematous lesions and intense pruritus, can be associated with pain, sleep loss, and reduced quality of life.[1,2] The pathogenesis of AD involves both genetic and environmental factors, contributing to defects in the epidermal barrier, abnormalities in the skin microbiome, and immune dysregulation

in both lesional and non-lesional skin areas.[2] There is growing recognition of AD as a systemic disease; studies have shown associations between AD, or AD severity, and atopic diseases, particularly asthma, allergic rhinitis, and food allergy, psychosocial outcomes, and cardiovascular risk.[2–4] A meta-analysis derived AD transcriptome was enriched for inflammatory and barrier pathways, as well as atherosclerosis and lipid metabolism pathways.[5] The AD immune signature is predominantly type 2-skewed, involving infiltration and activation of type 2 innate lymphoid cells and T helper (Th) 2 cells, as well as increased expression of type 2 cytokines, including interleukin (IL)-4 and IL-13.[6] In particular, IL-13 has been identified as a key driver of skin inflammation and barrier abnormalities in AD.[7–10]

Tralokinumab, a monoclonal antibody approved for the treatment of adults with moderate-to-severe AD, binds to IL-13 with high affinity, preventing receptor interaction and IL-13 signaling.[11,12] Tralokinumab has demonstrated efficacy and safety for treatment of moderate-to-severe AD in pivotal Phase 3 trials over 16 weeks[13,14] and improved microbial dysbiosis in AD skin by Week 8 of treatment.[15] High levels of at least 75% improvement in Eczema Area and Severity Index (EASI-75) and Investigator's Global Assessment (IGA) 0/1 responses have also been sustained through 2 years of continued treatment.[16]

In the context of AD, biomarkers may provide important information for several uses, including diagnosis, guiding treatment decisions, evaluating disease severity, and monitoring response to treatment.[17] To date, the biomarker with the most supporting evidence is serum CC chemokine ligand (CCL)17/thymus- and activation-regulated chemokine (TARC).[17] Additional emerging biomarkers that have been reported to correlate with AD severity or response to therapy include the following: CCL22/MDC, CCL26/Eotaxin-3, CCL27/CTACK, and eosinophil counts in serum; CCL18/PARC, IL-13, and IL-22 in skin; and matrix metalloproteinase (MMP)12 in both serum and skin.[17] CCL17, CCL22, CCL18, and CCL27 likely promote AD by attracting circulating T cells, particularly Th2 cells and cutaneous lymphocyte-associated antigen (CLA) + memory T cells, to inflamed skin (via CCR4, CCR10, and CCR8, respectively).[18,19] IL-13, produced by Th2 and innate immune cells, promotes barrier dysfunction and skin infection by inhibiting keratinocyte production of skin barrier proteins, lipids, and antimicrobial peptides, promotes skin thickening by increasing production of collagen, induces additional immune cell recruitment, and contributes to itch by stimulating sensory neurons.[7] Eosinophils, drawn to sites of inflammation by CCL26,[20] exacerbate inflammation and contribute to barrier disruption.[21] MMP12 is a marker of general inflammation and likely contributes to tissue remodeling in AD.[22] Additional proposed biomarkers include total IgE and periostin, as serum levels are elevated in AD patients; however, levels of these proteins are inconsistently correlated with disease severity.[17]

Inhibition of IL-4 and IL-13 signaling using dupilumab was previously shown to reduce expression of type 2 biomarkers and improve epidermal pathology in lesional skin at Week 16.[23] Cellular and molecular changes in AD skin and blood over time following selective therapeutic targeting of IL-13 have not been previously assessed. Here, we investigated the short- and long-term impacts of IL-13 neutralization on skin and serum biomarkers following tralokinumab treatment in adult patients with moderate-to-severe AD.

2 | METHODS

2.1 | Study design

A subpopulation of patients from the phase 3 study, ECZTRA 1 (NCT03131648), and the open-label extension trial, ECZTEND (NCT03587805), with relevant samples available, were included in these analyses. These trials have been described in detail elsewhere.[14,16] Briefly, ECZTRA 1 was a 52-week, multinational, randomized, double-blinded, placebo-controlled trial in adults with moderate-to-severe AD. Patients were randomized 3:1 to subcutaneous tralokinumab 300 mg, after a 600-mg loading dose on Day 0, or placebo every other week for 16 weeks (Figure S1A). At Week 16, tralokinumab-treated patients who achieved prespecified clinical response criteria were re-randomized 2:2:1 to tralokinumab every 2 weeks or every 4 weeks or placebo for a 36-week maintenance period. Patients who did not meet the prespecified clinical response criteria at Week 16 were transferred to open-label treatment for 36 weeks.

ECZTEND is an ongoing, 5-year, open-label, single-arm, multicenter, long-term extension trial in patients who complete tralokinumab parent trials, including ECZTRA 1. Patients in ECZTEND received subcutaneous tralokinumab 300 mg every 2 weeks after a 600-mg loading dose. Endpoints evaluating AD extent and severity, including the proportion of patients achieving EASI-75, percent improvement in EASI from baseline, and improvement in worst weekly pruritus Numeric Rating Scale (NRS) from baseline, were evaluated in both trials.

Both trials were sponsored by LEO Pharma and conducted in accordance with the ethical principles derived from the Declaration of Helsinki and Good Clinical Practice guidelines and were approved by the local institutional review board or ethics committee of each institution. All patients provided written informed consent.

2.2 | Assessments

2.2.1 | Biopsy and blood collection

Blood samples were collected at baseline and at Weeks 4, 8, and 16 (Figure S1A). Skin biopsy samples (3 mm) were collected from lesional and non-lesional skin at baseline and 2 years of tralokinumab treatment (52 weeks in ECZTRA 1 and 52 weeks in ECZTEND) and from lesional skin at Week 4 and Week 16 of treatment (Figure S1A).

2.2.2 | Serum biomarkers

Serum protein biomarkers were analyzed as follows: CCL17/TARC and periostin by ELISA (R&D Systems, cat. # DDN00 and Thermofisher, cat. # EHPOSTN, respectively); IL-13 and IL-22 by the Singulex Erenna Assay (EMD Millipore cat. # 03–0109 and # 03–0059); and IgE by ImmunoCap (ImmunoCap-250, Phadia, using Total IgE kit reference 52-5292-EN/04).

2.2.3 | Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed and paraffin-embedded tissues using purified mouse and rabbit anti-human antibodies (Table S1). Epidermal thickness and amount of staining were quantified per biopsy length using computer-assisted analysis software (Visiopharm Integrator Software; Version 2018.9.2). See the Methods section in the supplemental materials for details.

2.2.4 | RNA extraction, sequencing, and qPCR

RNA was isolated from skin biopsies using the miRNeasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. On-column DNase digestion was performed during the RNA extraction. RNA concentration was determined using the Qubit 2.0 fluorometer (Thermo Fisher Scientific). On average, an RNA concentration of 136.2 ng/μL was obtained, ranging from 0 to 467.2 ng/μL. Primers used for RT-qPCR are listed in Table S2.

Next-generation sequencing was performed on Illumina NovaSeq 6000 (Illumina, San Diego, CA) with single-ended 100 read cycles. Sequencing data were generated from 174 samples (from 48 subjects). Each sample was split into seven runs for sequencing, with each run conducted in four lanes, resulting in 28 FASTQ files of sequence reads per sample. After pre-processing, the counts of mapped reads for each of 33,121 genes were extracted from the respective 28 FASTQ files and were then aggregated as a summarized count for each gene in each sample. Minimal pre-filtering was performed to keep only 25,269 genes with at least 10 reads total. RNA-seq sample quality was assessed using FastQC (Cambridge, United Kingdom) and MultiQC.[24] Samples were aligned to the human reference genome on the basis of the STAR RNA-seq aligner,[25] and mapped reads were assigned to genomic features by featureCounts.[26] The human reference genome GRCh37 was used. The Star software version 2.4.2a (Subread/FeatureCounts 1.5.0.post3) was used for alignment. Data were subsequently $\log_2$ transformed with voom transform[27] and modeled using a mixed-effects model with time, treatment, and tissue as a fixed effect and random effect (using the R limma package) for each patient. The Benjamini-Hochberg procedure was used to adjust *p*-values for

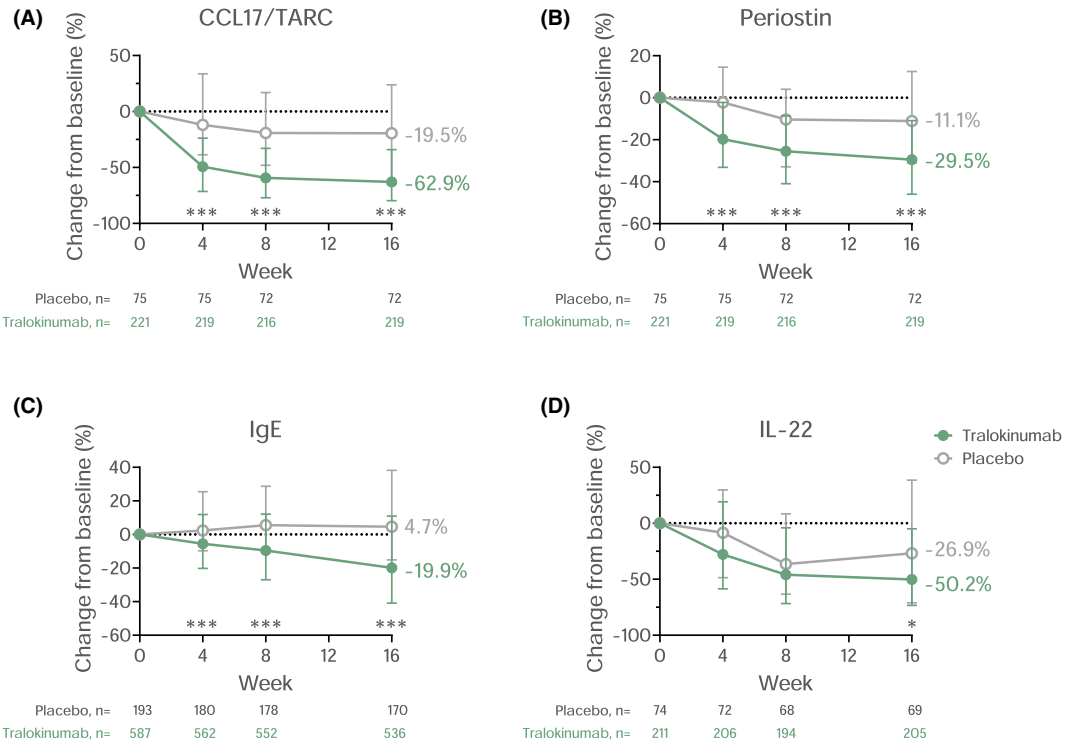


FIGURE 1 Tralokinumab treatment reduces atopic dermatitis (AD)-associated serum proteins over 16 weeks. (A–D) Median percentage change from baseline over 16 weeks in (A). CCL17/TARC (B). Periostin (C). IgE (D). IL-22 in serum of patients who received tralokinumab or placebo. Error bars show interquartile range. Number of patients per group is listed below each graph. *p*-values shown for comparison between tralokinumab and placebo groups at Week 4, 8, and 16; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

multiple hypotheses by controlling the false discovery rate (FDR). qPCR data analyses were done using Biogazelle's qbase+ (www.qbaseplus.com).

2.3 | Statistical analysis

For the estimation of median change from baseline (Figure 1) an empirical approach was used and only patients with pairwise complete measurements were included, no imputation was performed. *p*-values for the test of differing medians between treatment arms are based on Wilcoxon rank test. Spearman rank correlation of change in biomarkers with change in EASI was conducted (Table S3); only measurements where both EASI and biomarker measurements were complete are included. Treatment, time, and tissue effects on skin biomarkers were estimated by linear mixed-effect models with treatment, time, and tissue, and their interaction, as fixed effects and random effects for each patient. Contrasts of interest (e.g., fold-changes vs. baseline, fold-changes in lesional vs. non-lesional tissue) (Figure 4; Table S4) were estimated with empirical Bayes and tested for their statistical significance with moderated *t*-tests. Percentage improvement in skin biomarkers (Figure 3; Figure S4) was estimated by the ratio between post-treatment lesional changes and baseline lesional versus non-lesional changes. Comparison in improvement across treatments and timepoints was performed with the Wilcoxon rank test. Inference on histological biomarkers change from baseline (Figure 2B) was carried out with two-tailed paired *t*-tests on log-transformed expression/levels. At a specific timepoint (Week 4, 8, 12, or 16), only pairwise complete observations were used in the calculations. At a specific timepoint, the hypothesis of the difference between treatments (tralokinumab vs. placebo) was tested with two-tailed unpaired *t*-tests. The shape of biomarkers distribution was assessed with histograms and summary statistics, including a set of representative quantiles.

Gene set variation analysis (GSVA) was performed as previously described[28] using unsupervised sample-wise enrichment in the R software GSVA package with the *z*-score method, and Spearman correlations were performed with the R stats package.[29,30]

3 | RESULTS

3.1 | Patient disposition and baseline characteristics

All 802 patients randomized in ECZTRA 1 had blood samples collected; 299 ($n=223$, tralokinumab; $n=76$, placebo) patients were randomly selected to be included in these analyses (Figure S1B). Among patients with analyzed serum samples, a subgroup of 50 patients ($n=35$, tralokinumab; $n=15$, placebo) consented to donate skin biopsies. Of the skin biopsy subgroup, a further subgroup of 13 patients, who were treated with tralokinumab in ECZTRA 1 and continued into ECZTEND, provided additional skin samples at 2 years of treatment (Figure S1B).

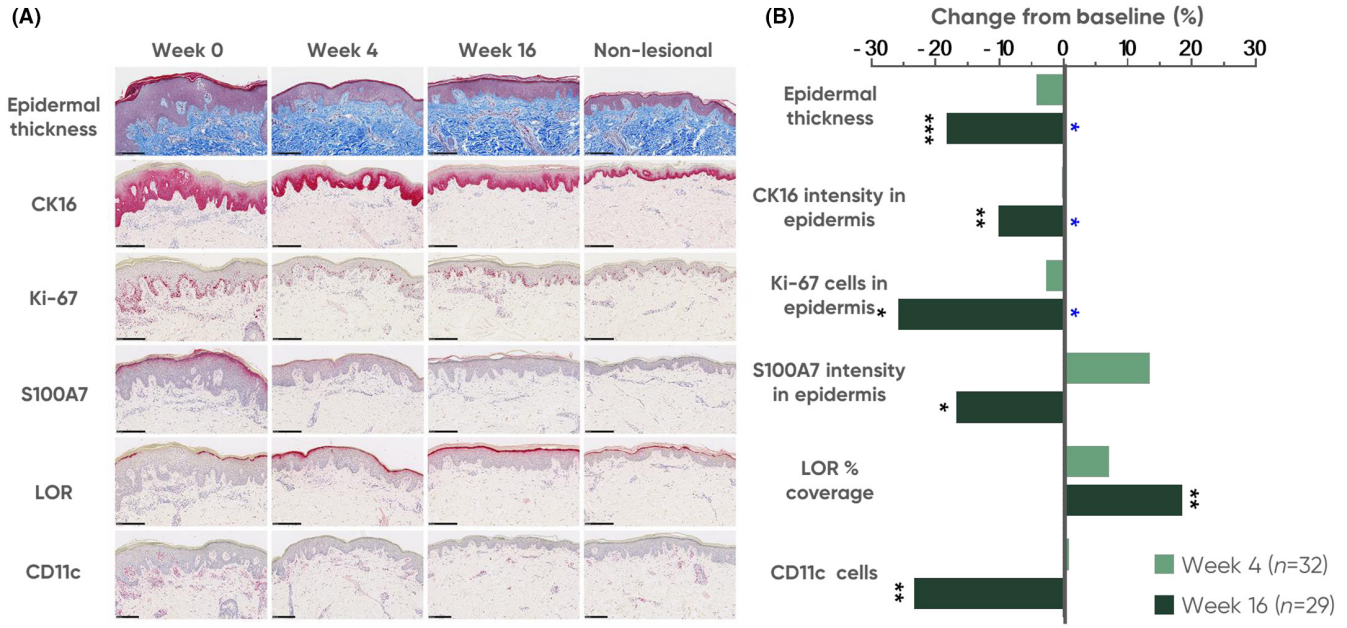


FIGURE 2 Tralokinumab treatment normalizes atopic dermatitis (AD)-associated epidermal barrier pathology over 16 weeks. (A) Representative histology panels from non-lesional or lesional sites at the indicated time points following initiation of tralokinumab treatment in one patient who achieved primary endpoints. Panels for epidermal thickness show Masson's Trichrome staining; remaining panels show immunostaining (red) for the indicated markers. (B) Percentage change from baseline to Week 4 or Week 16 of tralokinumab treatment in the indicated measures of epidermal barrier pathology. All statistics presented are for Week 16. $^{*}p<.05$, $^{**}p<.01$, $^{***}p<.001$. Black stars, significance of comparison between Week 16 and baseline; blue stars, significance of comparison between tralokinumab and placebo groups (data for placebo [$n=14$ at Week 4/16] not shown).

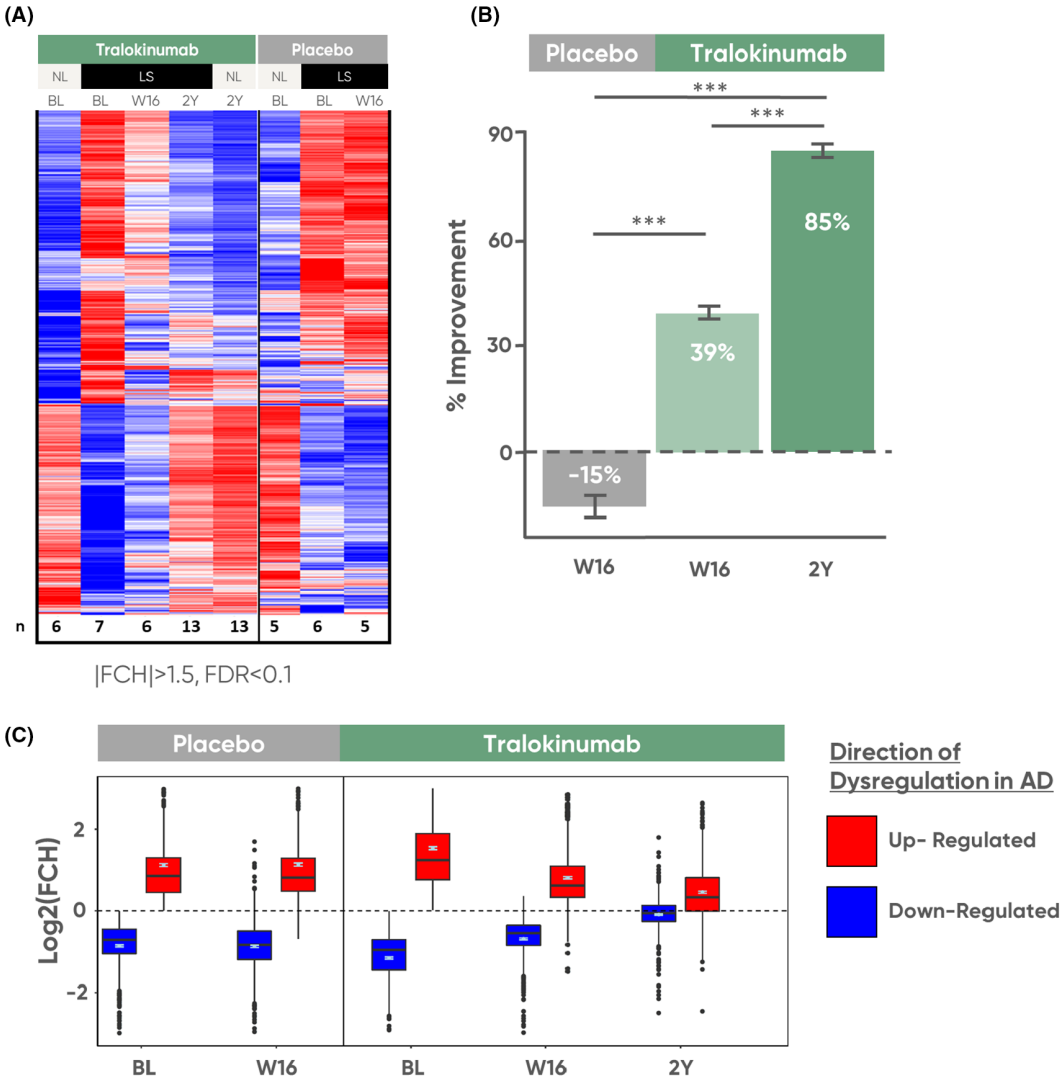


FIGURE 3 Tralokinumab treatment further improves the transcriptomic profile in lesional skin from Week 16 to 2 years. (A) Summary heat map of mean expression levels of differentially expressed genes (defined by criteria of FCH ≥1.5 and FDR <0.1) in lesional and non-lesional skin of patients receiving tralokinumab or placebo at the indicated timepoints. (B) Overall percentage improvement in differentially expressed genes (as in A) at Week 16 and 2 years relative to baseline in lesional skin. (C). Effect of tralokinumab or placebo on expression of genes upregulated (*red*) or downregulated (*blue*) in atopic dermatitis (AD). Dotted line represents expression level in non-lesional skin. ***$p < .001$. Error bars superimposed on plots represent means ± SEM. ***$p < 0.001$ 2Y, 2 years; AD, atopic dermatitis; BL, baseline; FCH, fold change; FDR, false discovery rate; LS, lesional; NL, non-lesional; W16, Week 16.

Baseline characteristics were largely consistent between the subgroups analyzed and the overall population randomized in ECZTRA 1 (Table 1). However, the smaller 2-year biopsy subgroup included a higher proportion of moderate patients (IGA 3), but higher mean EASI compared to all randomized patients.

3.2 | IL-13 neutralization with tralokinumab reduces AD-associated serum protein expression and epidermal pathology by Week 16

At baseline, levels of serum biomarkers including IL-13 and IL-22 were elevated relative to published reference values in healthy controls,[31,32] with 86% of patients having baseline serum IgE above 150 IU/mL. Serum levels of IL-13 were positively correlated with levels of CCL17/TARC, periostin, and IL-22 (Figure S2A). Serum levels of all four molecules correlated with baseline EASI (Figure S2B). Tralokinumab treatment led to decreased serum levels from baseline of the type 2 biomarkers CCL17/TARC, periostin, and IgE by Week 4, with further reductions at Week 16 (Figure 1A–C). Serum levels of IL-22 were similarly reduced following tralokinumab treatment (Figure 1D). The change from baseline to Week 16 was significantly greater with tralokinumab treatment than placebo for all four serum biomarkers (Figure 1A–D).

At Week 16, improvements in EASI and pruritus NRS were greater for patients receiving tralokinumab versus placebo (Table 2). Changes in these clinical measures were largely consistent across

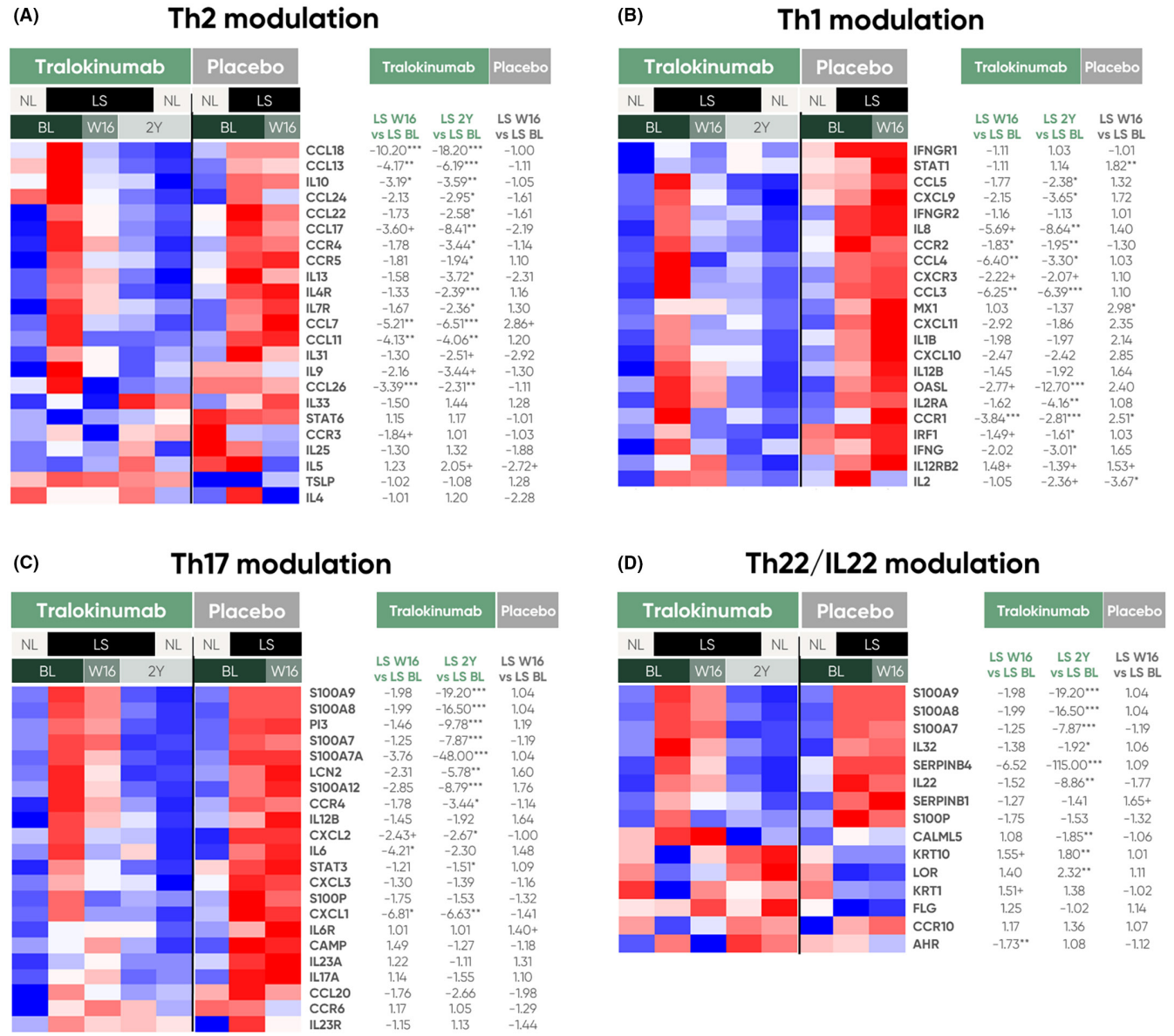


FIGURE 4 Treatment with tralokinumab improves expression of Th cell pathway genes over 2 years. (A–D) Heat maps of mean expression levels of differentially expressed genes in the (A). Th2 (B). Th1 (C). Th17 and (D). Th22/IL-22 gene set pathways. Tables show FCHs between the indicated groups. $^{+}p<.1$, $^{*}p<.05$, $^{**}p<0.01$ $^{***}P<0.001$ 2Y, 2 years; BL, baseline; FCH, fold change; LS, lesional; NL, non-lesional; W16, Week 16.

the different biomarker subgroups. Change in EASI from baseline to Week 16 correlated with changes in serum CCL17/TARC, periostin, and IL-22 (Table S3).

At baseline, biopsies from lesional skin showed increased epidermal hyperplasia compared to non-lesional biopsies (Figure 2). At Week 16, tralokinumab treatment demonstrated significantly reduced epidermal thickness and reduced expression of the cell proliferation markers CK16 and Ki-67 compared to baseline; these improvements in epidermal hyperplasia were significantly greater in tralokinumab-treated patients versus placebo (Figure 2). Expression of the antimicrobial peptide S100A7 and the immune cell marker CD11c were significantly reduced relative to baseline after 16 weeks of tralokinumab treatment (Figure 2). Compared to baseline, there was significantly increased coverage of the skin barrier protein loricrin in lesions of tralokinumab-treated patients at Week 16 (Figure 2).

3.3 | Gene expression in lesional skin further shifts toward a non-lesional transcriptional profile over 2 years of IL-13 neutralization with tralokinumab

RNA-seq was used to identify 1908 genes that were differentially expressed between lesional and non-lesional skin at baseline using criteria of fold change >2 and false discovery rate <0.05 (Figure S3). Increasing improvement towards a non-lesional profile was observed

TABLE 1 Baseline demographics and clinical characteristics for randomized subjects in parent study (ECZTRA 1) and in the serum biomarker and skin biopsy subgroups.

		Serum biomarker subgroup ($N = 299$)		Skin biopsy subgroups		
				Week 16 biopsy ($n = 50$)		2-year biopsy ($n = 13$)
	All randomized ($N = 802$)	Tralokinumab ($n = 223$)	Placebo ($n = 76$)	Tralokinumab ($n = 35$)	Placebo ($n = 15$)	Tralokinumab ($n = 13$)
Age						
Mean (SD)	38.8 (14.1)	39.9 (15.4)	39.7 (16.0)	39.2 (13.9)	39.5 (14.3)	45.4 (11.1)
Sex						
Male, *n* (%)	474 (59.1)	115 (51.6%)	47 (61.8%)	20 (57.1)	9 (60.0)	8 (61.5%)
Female, *n* (%)	328 (40.9)	108 (48.4%)	29 (38.2%)	15 (42.9)	6 (40.0)	5 (38.5%)
Race						
White, *n* (%)	564 (70.3)	145 (65.0%)	50 (65.8%)	25 (71.4)	12 (80.0)	11 (84.6%)
Black, *n* (%)	59 (7.4)	19 (8.5%)	10 (13.2%)	2 (5.7)	1 (6.7)	1 (7.7%)
Asian, *n* (%)	160 (20.0)	52 (23.3%)	15 (19.7%)	8 (22.9)	2 (13.3)	1 (7.7%)
IGA severity						
Moderate disease, *n* (%)	391 (48.8)	108 (48.4%)	44 (57.9%)	16 (45.7)	10 (66.7)	10 (76.9%)
Severe disease, *n* (%)	407 (50.7)	115 (51.6%)	32 (42.1%)	19 (54.3)	5 (33.3)	3 (23.1%)
EASI						
Mean (SD), *n*	32.4 (13.8), 798	33.2 (14.9), 223	32.0 (13.1), 76	34.0 (14.0), 35	33.5 (15.3), 15	35.3 (15.5), 13
SCORAD						
Mean (SD), *n*	70.6 (12.9), 798	71.6 (13.1), 223	70.5 (12.3), 76	73.4 (13.4), 35	72.4 (11.8), 15	73.2 (15), 13
DLQI						
Mean (SD), *n*	16.9 (7.0), 785	17.0 (7.4), 221	15.9 (6.6), 75	17.6 (5.0), 34	17.0 (6.5), 15	17.4 (7), 12
Worst daily pruritus NRS (weekly average)						
Mean (SD), *n*	7.7 (1.4), 793	7.9 (1.4), 222	7.6 (1.5), 75	8.0 (1.2), 35	7.9 (1.4), 15	7.9 (1.4), 13

Abbreviations: DLQI, Dermatology Life Quality Index; EASI, Eczema Area and Severity Index; IGA, Investigator's Global Assessment; *n*, number of subjects in analysis set; NRS, numeric rating scale; SCORAD, SCORing Atopic Dermatitis; SD, standard deviation.

TABLE 2 Clinical response to tralokinumab at Week 16 and 2 years in the serum biomarker and skin biopsy subgroups.

	All patients (*N* = 802)		Serum biomarker subgroup (N = 299)		Skin biopsy subgroups			
					Week 16 biopsy (*n* = 50)		2-year biopsy (*n* = 13)	
	Tralokinumab (*n* = 601)	Placebo (*n* = 197)	Tralokinumab (*n* = 223)	Placebo (*n* = 76)	Tralokinumab (*n* = 35)	Placebo (*n* = 15)	Tralokinumab (*n* = 13)	
Week 16							2 years	
EASI-75 (%)	33.4	17.3	38.6	19.7	25.7	20.0	61.5	
Mean EASI change (%)	−55.6	−36.7	−51.5	−33.0	−53.5	−28.3	−78.1	
Mean EASI change	−17.6 (561)	−12.4 (182)	−18.0	−11.0	−18.4 (31)	−10.1	−27.2	
Mean pruritus NRS change	−2.9 (514)	−2.2 (167)	−2.9 (202)	−1.9 (72)	−2.8 (29)	−2.1	−5.2	

Note: Data presented as observed ignoring rescue medication use. For EASI-75, patients with missing data at Week 16 were imputed as non-responders.
Abbreviations: EASI, Eczema Area and Severity Index; *n*, number of subjects in analysis set; NRS, numeric rating scale.

in samples from patients receiving tralokinumab ($n = 35$) but not placebo ($n = 15$) over 16 weeks of treatment (Figure S3A). Altered gene expression relative to baseline was apparent by Week 4 of tralokinumab treatment (Figure S3B). At Week 16, the expression of genes dysregulated in AD improved 46.6% in tralokinumab-treated patients compared to 16.4% improvement in patients receiving placebo (Figure S3B). Gene expression approached non-lesional levels in tralokinumab-treated patients at Week 16, while minimal changes were observed in patients receiving placebo (Figure S3C).

Transcriptomic analysis of a subgroup ($n = 13$) of tralokinumab-treated patients with biopsy data available at both Week 16 and 2 years identified 1274 differentially expressed genes between lesional and non-lesional skin at baseline using criteria of fold change >1.5 and false discovery rate <0.1 (Figure 3). A noticeable shift in genomic profile was seen from baseline to Week 16 in patients receiving tralokinumab, but not placebo, with further shifts toward a non-lesional profile by 2 years of tralokinumab treatment (Figure 3A). In this subgroup, tralokinumab improved expression of genes dysregulated in AD by 39% at Week 16 and 85% at 2 years, compared to a worsening to 15% more lesional phenotype in patients receiving placebo at Week 16 (Figure 3B). Tralokinumab-induced reversal of the transcriptional dysregulation (in both up- and downregulated genes) in lesional relative to non-lesional skin at Week 16 and 2 years, compared to limited changes with placebo (Figure 3C). Interestingly, at 2 years, tralokinumab treatment also modified the transcriptomic profile of non-lesional skin (Figure 3A). These changes in transcriptomic profile at 2 years of tralokinumab treatment were accompanied by further improvements in EASI and pruritus NRS (Table 2).

3.4 | IL-13 neutralization with tralokinumab modulates key immune pathways in skin

Gene set enrichment analysis was used to evaluate the impact of tralokinumab relative to placebo on previously reported gene signatures related to key immune pathways. At Week 16, patients receiving tralokinumab had significant decreases in mean expression of Th2, Th22/IL-22, and Th17 gene signatures in lesional skin relative to patients receiving placebo (Figure S4A). Tralokinumab treatment shifted the expression of several key type 2 markers in lesional skin, including *CCL18*, *CCL22*, and *IL4R*, towards non-lesional skin levels (Figure S4B).

Examination of the 2-year subgroup showed tralokinumab treatment continued to modulate gene expression related to Th cell function over 2 years, with further shifts towards non-lesional profiles from Week 16 to 2 years (Figure 4). Key genes downregulated with tralokinumab treatment included the Th2 pathway genes *IL4R*, *CCL17*, and *CCL26*; the Th1 pathway gene *IFNG*; the Th17 and Th22 pathway genes *S100A7*, *S100A8*, and *S100A9*; and the Th22 pathway gene *IL22* (Figure 4). Tralokinumab also significantly reduced expression of the itch-associated cytokine gene *IL31* at 2 years (Figure 4A).

Long-term tralokinumab treatment led to upregulation of epidermal differentiation complex (EDC) and cornified envelope (CE) genes

to levels comparable to non-lesional skin, including *CLDN1* and *LOR* (Figure S5A). Notably, many of these genes, including *LOR*, also showed increased expression in non-lesional skin from baseline to 2 years (Figure S5A). Interestingly, a strong shift toward non-lesional expression patterns was also observed in atherosclerosis signaling pathway genes, including vascular inflammation-associated genes (e.g., *SELE*, *IL37*, and *S100A8*) after 2 years of tralokinumab treatment (Figure S5B).

Expression changes of key immune and barrier genes from baseline to 2-years were validated by qPCR (Table S4). In lesional skin, significant reductions were observed in genes including *MMP12*, *PI3*, *IL4R*, *CCL17*, *CCL18*, *IL13*, *S100A7*, and *S100A8*; in non-lesional skin *FLG* expression increased, and *IFNG* decreased, significantly.

4 | DISCUSSION

Previous data have shown that targeting both the IL-4 and IL-13 pathways, via targeting of the shared IL-4Ra subunit, improves the skin abnormalities characterizing AD. This study further increases our understanding of the pathophysiology of AD, as it shows for the first time that specific targeting of IL-13, but not IL-4 signaling, with tralokinumab, normalizes expression of key AD biomarkers in serum and lesional skin of patients with moderate-to-severe AD. Treatment with tralokinumab improved epidermal pathology and reduced levels of inflammatory mediators and abnormal skin barrier markers towards those of uninvolved skin, while also modulating subclinical inflammation seen in non-lesional skin. Improved gene expression was apparent by Week 4, and further improvements in the transcriptomic profile of lesional skin were observed through 2 years of tralokinumab treatment. These molecular data align with previous studies showing clinical improvement with tralokinumab as early as Week 4 for some patients, progressive improvement in clinical response over 1 year, and high levels of disease control at 2 years of treatment in ECZTEND.[14,16]

The increased shift toward a non-lesional transcriptional profile over 2 years of IL-13 inhibition, including improvement in markers of AD skin pathology, Th2 inflammation, and atherosclerosis, together with the sustained clinical response observed in ECZTEND,[16] may represent an example of disease modification. To be considered disease-modifying, a treatment should improve clinical signs and symptoms of a condition and delay or prevent underlying pathology; such a bar can only be met when clinical data are accompanied by strong supportive evidence from a biomarker program.[33] This study is the first long-term investigation of key biomarkers in combination with known clinical outcomes[16] over 2 years of biologic treatment for patients with AD.

In addition to Th2 inflammation, IL-13 neutralization modulated Th17 gene signatures, which have been described to be elevated in chronic AD lesions, particularly in children and people of Asian descent, who comprised approximately 20% of the study population at baseline. However, the importance of this pathway is not clear, as anti-IL-17A targeting has not been effective for AD treatment.[2] Although the mechanism by which tralokinumab reduces Th17- and Th-22-regulated genes is unknown, similar suppression of these pathways was also observed following targeting of both IL-4 and IL-13 signaling with dupilumab.[23] As the reduction in Th17 and Th22 gene signatures occurred more slowly than changes in Th2 signature, these changes could be secondary effects of improvements in the skin barrier and inflammatory environment upon targeting the IL-13 cytokine.

In alignment with previous reports that type 2 cytokines suppress production of lipids and structural proteins in the skin barrier,[21] we found that specific targeting of IL-13 with tralokinumab increased loricrin coverage by Week 16 and increased expression of EDC and CE genes to near non-lesional levels by 2 years. Tralokinumab significantly reduced the epidermal hyperplasia characteristic of AD lesions over 16 weeks, as shown by decreased epidermal thickness and reduced Ki67 and CK16 staining. Interestingly, we observed CK16 staining in the basal layer of non-lesional skin, which differs from previously reported staining patterns. We confirmed this expression pattern using in situ hybridization of CK16 mRNA in the basal layer of both non-lesional AD and healthy skin biopsies (data not shown), indicating that the CK16 antibody used has higher sensitivity than those used in previous publications. In patients with AD, non-lesional skin is also characterized by barrier defects and immune abnormalities.[34] The transcriptome of non-lesional AD skin, which is characterized by reduced expression of EDC and terminal differentiation genes, represents an intermediate between healthy and lesional skin, with the degree of dysregulation correlating with disease severity.[34] In this study, 2 years of tralokinumab treatment led to an improvement in several genes identified as dysregulated in non-lesional skin,[34] including markers of barrier integrity (*LOR*, *FLG*) and skin inflammation (*CCL18*, *IFNG*), suggesting that IL-13 targeting moves the non-lesional skin phenotype toward a healthy transcriptomic profile. Several key immune and barrier genes were not differentially expressed in skin between baseline and 2 years including, for example, periostin, which was significantly reduced in serum at Week 16; this could potentially reflect the smaller sample size at 2 years or variance between skin and serum.

In addition to skin inflammation and barrier disruption, an altered epidermal microbiome, dominated by increased *Staphylococcus aureus* colonization, is an important hallmark of AD skin.[21] The reduced type 2 inflammation and improved barrier integrity observed in this study mirror the recent results demonstrating increased microbial diversity and decreased *S. aureus* colonization in patients treated with tralokinumab in the ECZTRA 1 trial.[15] Collectively, these data show that specific targeting of IL-13 improves multiple molecular hallmarks of AD, providing a likely underlying mechanism for the improved clinical signs observed in patients treated with tralokinumab.

The atherosclerosis pathway, which includes genes associated with vascular inflammation, was first associated with AD through a meta-analysis derived AD transcriptome.[5] There is also evidence suggesting that modulation of the vagus nerve may regulate inflammation associated with both atherosclerosis and AD.[35–37] The observed shift of atherosclerosis pathway genes towards a non-lesional profile following 2 years of tralokinumab treatment could

therefore be secondary to the overall AD improvement rather than a direct effect of IL-13 inhibition.

Specific inhibition of IL-13 reversed the molecular phenotype of AD, including the significantly reducing expression of markers associated with AD severity (*CCL17*, *CCL22*, *CCL26*, and *IL22*) and itch (*IL4R*, *IL13*, and *IL31*).[17,38] Together with improvements in EASI and pruritus NRS with tralokinumab treatment, our results highlight the role of IL-13 as a key driver of AD pathophysiology. These data align with previous results showing that 16 weeks of treatment with dupilumab, which targets both IL-4 and IL-13 signaling, suppressed molecular markers of inflammation in serum and lesional skin and improved epidermal barrier markers in AD patients.[23] These results support the role of targeted biologic therapy for long-term AD management, and the idea that disease modification may occur with IL-13 targeting treatment.

Limitations of the current study include the lack of a healthy skin comparison group at any timepoint and the lack of a placebo comparison group and serum sample analysis at 2 years. In addition to its limited sample size, the cohort of patients with 2 years of tralokinumab treatment may be enriched for patients with clinical improvement, as patients had to opt in to continue into ECZTEND.[16] The 2-year biopsy cohort also had some variance in baseline characteristics compared to the larger subgroups–notably more moderate disease and a lower proportion of Asian patients–which could have contributed to observed changes between Week 16 and 2 years.

A further limitation is the fact that bulk RNA sequencing does not provide information on cell type-specific changes and therefore may miss changes in low frequency cell types.[39] Another possible confounding factor is the use of TCS, which was allowed as rescue medication if considered medically necessary by the investigator through Week 16 in ECZTRA 1 with optional use permitted in ECZTEND. There were no restrictions on TCS use prior to sample collection and there is evidence that TCS can modulate expression of immune and barrier genes in skin.[21]

In summary, tralokinumab treatment significantly improved aberrant immune activation in skin and blood, epidermal barrier pathology, and markers of atherosclerosis in adult patients with moderate-to-severe AD. These data further support the central role of IL-13 in driving AD pathology and highlight that inhibition of IL-13 alone is sufficient for normalizing the molecular phenotype of AD.

AUTHOR CONTRIBUTIONS

All authors contributed to the acquisition, analysis, or interpretation of data; critically reviewed and revised the manuscript for important intellectual content; approved the final draft for publication; and are accountable for their contributions and the integrity of the work.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request. All requests are reviewed independently of LEO Pharma by an external Patient and Scientific Review Board. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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