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Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab

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Background: IL-22 is potentially a pathogenic cytokine in patients with atopic dermatitis (AD), but the molecular effects of IL-22 antagonism have not been defined in human subjects.
Objective: We sought to evaluate the cellular and molecular effects of IL-22 blockade in tissues from patients with moderate-to-severe AD.
Methods: We assessed lesional and nonlesional skin from 59 patients with moderate-to-severe AD treated with anti–IL-22 (fezakinumab) versus placebo (2:1) using transcriptomic and immunohistochemistry analyses.
Results: Greater reversal of the AD genomic profile was seen with fezakinumab versus placebo, namely 25.3% versus 10.5% at 4 weeks ($P = 1.7 \times 10^{-5}$) and 65.5% versus 13.9% at 12 weeks ($P = 9.5 \times 10^{-19}$), respectively. Because IL-22 blockade showed clinical efficacy only in patients with severe AD, we used baseline median IL-22 mRNA expression to stratify for high (n = 30) and low (n = 29) IL-22 expression groups. Much stronger mean transcriptomic improvements were seen with fezakinumab in the IL-22–high drug-treated group (82.8% and 139.4% at 4 and 12 weeks, respectively) than in the respective IL-22–high placebo-treated group (39.6% and 56.3% at 4 and 12 weeks) or the IL-22–low groups. Significant downregulations of multiple immune pathways, including T_H1/*CXCL9*, T_H2/*CCL18*/*CCL22*, T_H17/*CCL20*/*DEFB4A*, and T_H22/*IL22*/*S100A's*, were restricted to the IL-22–high drug group ($P < .05$). Consistently, tissue predictors of clinical response were mostly genes involved in T-cell and dendritic cell activation and differentiation.
Conclusions: This is the first report showing a profound effect of IL-22 blockade on multiple inflammatory pathways in AD. These data, supported by robust effects in patients with high IL-22 baseline expression, suggest a central role for IL-22 in AD, indicating the need for a precision medicine approach for improving therapeutic outcomes in patients with AD.

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, with increasing prevalence.[1-3] Both immune activation and barrier impairment are characteristic of the disease,[4] but their exact interplay is unknown.[5] IL-4 and IL-13, the 2 lead T_H2 cytokines, are upregulated in patients with AD, and their inhibition through dupilumab, an IL-4 receptor α blocker, showed significant clinical efficacy in patients with moderate-to-severe AD.[6-8] However, unlike psoriasis, another common inflammatory disease, in which approximately 75% of patients achieve 90% or greater skin

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Abbreviations used
AD: Atopic dermatitis
AUC: Area under the curve
DC: Dendritic cell
DEG: Differentially expressed gene
FCH: Fold change
FDR: False discovery rate
IL-22R: IL-22 receptor
LCE: Late cornified envelope
MADAD: Meta-analysis–derived atopic dermatitis transcriptome

clearance (Psoriasis Area and Severity Index [PASI90] responses) with single IL-23 cytokine antagonism,[9] only approximately 30% of patients with AD receiving dupilumab achieve a 90% reduction in their Eczema Area and Severity Index (EASI90).[8] Thus other pathways beyond the T_H2 axis may play an important role in AD.

IL-22, an α-helical cytokine of the IL-20 subfamily,[10] is strongly upregulated in patients with AD.[11,12] Initially, data from murine models attributed IL-22 production to T_H17 cells.[13,14] More recently, human studies identified a distinct T_H22 cell subset that uniquely produces IL-22, but not IL-17, and that is responsible for the majority of IL-22 production.[12,15,16] Expression of the IL-22 receptor (IL-22R), consisting of the IL-22R1 and IL-10 receptor 2 subunits, is limited to epithelial cells in the skin (keratinocytes), lung, and gut.[17,18] IL-22 initiates immune responses in these organs, mediating skin and mucosal defense mechanisms.[15,19,20] Under physiologic conditions, IL-22 is a homeostatic cytokine that preserves the integrity of these epithelia against pathogenic invadors.[15] When present at increased levels, IL-22 acts as a proinflammatory cytokine that, in synergy with IL-17, triggers upregulation of antimicrobial peptides, including β-defensins and S100A proteins, in the epidermis.[21,22] IL-22 has also been postulated to be a main driver of epidermal hyperplasia and barrier defects[12,23] by promoting keratinocyte proliferation and inhibiting terminal differentiation, respectively.[23-25] Furthermore, induction of IL-22 in murine skin causes an AD-like pruritic phenotype with strong T_H2 skewing, downregulation of epidermal terminal differentiation, and enhanced dermatitis on epicutaneous allergen exposure.[26]

Taken together with the association of IL-22 and AD disease severity,[11,12] these observations suggest that IL-22 might be central to AD pathogenesis. This assumption was recently confirmed in a phase 2a clinical trial investigating the IL-22–blocking mAb fezakinumab (ILV-094) in patients with moderate-to-severe AD, showing significant clinical improvements versus placebo in patients with severe disease.[27] Thus we investigated lesional and nonlesional skin biopsy specimens from these patients treated with fezakinumab (n = 39) compared with placebo (n = 20). We found that IL-22 blockade leads to reversal of multiple pathologic features in AD skin, reducing the overall inflammatory burden and epidermal pathology characteristic of the disease. The treatment effect observed in tissues was particularly pronounced in patients with high IL-22 baseline expression, indicating the possibility of future development of a precision medicine approach in patients with AD.

METHODS

Study patients and skin samples

Skin biopsy specimens were obtained from adults with moderate-to-severe chronic AD in a randomized, placebo-controlled, multicenter, phase 2a clinical trial (NCT01941537) of IL-22 blockade with the mAb fezakinumab (ILV-094).[27] Patients were randomized 2:1 to either intravenous drug (n = 40) or placebo (n = 20), with a loading drug dose of 600 mg at baseline (day 0), followed by 300 mg at weeks 2, 4, 6, 8, and 10 (last dose).

Lesional and nonlesional biopsy specimens were obtained before (baseline/week 0), during (week 4), and after (week 12) treatment. Biopsy specimens were not available from 1 drug-treated patient. Overall, 59, 52, and 50 lesional and 53, 44, and 44 nonlesional biopsy specimens were available for microarray analyses of 0, 4, and 12 weeks, respectively. Reasons for decreasing numbers of biopsy specimens were dropouts because lack of efficacy (n = 3), time restraints (n = 1), serious adverse events (n = 2), and loss to follow-up (n = 1), as previously described,[27] and some patients withdrew consent for biopsy during the study. Only emollients were allowed, without additional topical or systemic treatments during study participation.[27]

Immunohistochemistry

Immunohistochemistry staining was performed on frozen cryostat tissue sections by using purified mouse anti-human mAbs, as previously reported,[28,29] and stainings were quantified by using computer-assisted image analysis software (ImageJ 1.42; National Institutes of Health, Bethesda, Md).

Quantitative RT-PCR and microarray analysis

RNA from skin biopsy specimens was extracted for RT-PCR with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), as previously described.[30] Expression levels were normalized to human acidic ribosomal protein. HGU133Plus2.0 microarrays (Affymetrix, Santa Clara, Calif) were used, as previously described.[30-32] Microarray data are available through the Gene Expression Omnibus (GSE99802).

Statistical analyses

Gene expression profiling with Affymetrix Human U133Plus 2.0 arrays was processed by using standard R packages, as described in the Methods section in this article's Online Repository at www.jacionline.org, and modeled by using a mixed-effects model, with treatment, visit, tissue, and baseline IL-22 status as a fixed interaction term and a random effect for each patient using the R *limma package*. *P* values were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure, which controls the false discovery rate (FDR). Probes with FDRs of less than 0.05 and fold changes (FCH) of greater than 2 in any comparison were considered differentially expressed. Baseline median IL-22 expression by means of microarray or RT-PCR was used to stratify for high (n = 30) and low (n = 29) IL-22 expression groups for respective analyses. RT-PCR expression and immunohistochemistry data were first $\log_2$ transformed and then modeled by using mixed-effect models framework in R software.

To classify responders versus nonresponders using baseline gene expression data, we considered the receiver operating characteristic area under the curve (AUC), a widely used measure of performance for classification and diagnostic. For expanded statistical methods, see the Methods section in this article's Online Repository.

RESULTS

Study population

As recently reported, clinical scores (SCORAD, Investigator Global Assessment, and body surface area scores) significantly improved in patients with severe AD (baseline SCORAD score ≥ 50) starting at 6 to 8 weeks of IL-22 antagonism (administered intravenously every other week between 0 and

TABLE I. Demographics and clinical characteristics of study participants at baseline

	Entire cohort			IL-22–high patients			IL-22–low patients			DRUG: IL-22-high vs IL-22-low	PLACEBO: IL-22-high vs IL-22-low
	Placebo (n = 20)	Drug (n = 39)	*P* value*	Placebo (n = 9)	Drug (n = 21)	*P* value*	Placebo (n = 11)	Drug (n = 18)	*P* value*	*P* value*	*P* value*
Age (y), mean (SD)	41.3 (16.32)	40.44 (15.1)	.85	43 (18.21)	39.95 (16.12)	.67	39.9 (15.37)	41 (14.18)	.85	.83	.69
Sex, no, (%)											
Female	11 (55.0)	17 (43.6)	.43	4 (44.4)	10 (47.6)	1	7 (63.6)	7 (38.88)	.26	.75	.65
Male	9 (45.0)	22 (56.4)		5 (55.6)	11 (52.4)		4 (36.4)	11 (61.11)			
Race, no. (%)											
Asian	4 (20.00)	11 (28.21)	.51	1 (11.11)	5 (23.80)	.59	3 (27.27)	6 (33.33)	1	.20	.60
African American	10 (50.00)	13 (33.33)		4 (44.44)	5 (23.80)		6 (54.54)	8 (44.44)			
White	6 (30.00)	15 (38.46)		4 (44.44)	11 (52.38)		2 (18.18)	4 (22.22)			
Total serum IgE (kU/L), mean (SD)	6,592 (9,720)	3,661 (4,619)	.21	5,782 (8,848)	4,521 (5,635)	.70	7,254 (10,761)	2,657 (2,893)	.19	.20	.74
SCORAD score, mean (SD)†	55.53 (13.36)	53.82 (13.03)	.64	56.8 (12.43)	56.6 (15.64)	.97	54.5 (14.58)	50.57 (8.40)	.43	.14	.71
SCORAD score (range)	34.5-89	36-84.5		45.8-77.6	36-84.5		34.5-89	36.9-70.8		—	—
IGA, no. (%)‡											
Moderate (3)	15 (75.00)	31 (79.49)	.82	7 (77.77)	15 (71.43)	1	8 (72.72)	16 (88.88)	.34	.42	1
Severe (4)	5 (25.00)	7 (17.95)		2 (22.22)	5 (23.81)		3 (27.27)	2 (11.11)			
Very severe (5)	0 (0.00)	1 (2.56)		0 (0.00)	1 (4.76)		0 (0.00)	0 (0.00)			
BSA, mean (SD)§	38.15 (24.26)	43.28 (27.80)	.47	42.11 (30.1)	46.43 (29.72)	.72	34.91 (19.20)	39.61 (25.72)	.58	.45	.55

Data are presented as means ± SDs or percentages.

BSA, Body surface area; *IGA*, Investigator Global Assessment.

*For numeric variables (age, SCORAD score, BSA score, and total serum IgE level), differences between means by treatment were tested by using a 2-tailed Student *t* test for independent samples. Proportions by treatment for categorical variables (sex, race, and IGA score) were compared by using a Fisher exact test.

†SCORAD scores range from 0 to 103, with higher scores indicating greater severity.

‡The IGA of the severity of AD was scored on a scale of 0 (clear) to 5 (very severe).

§Body surface area was graded from 0% (no skin involvement) to 100% (total skin involvement).

10 weeks), with progressive improvements for another 10 weeks after the last dose until end of study (week 20) compared with placebo.[27] Baseline patients' characteristics are shown in Table I.

Improvement of the AD transcriptome

We performed Affymetrix U133Plus 2.0 gene arrays to define the AD molecular skin phenotype or transcriptome (defined as the differentially expressed genes [DEGs] between lesional and nonlesional skin) by using criteria of FCHs of 2 or greater and FDRs of less than 0.05 (see Table E1 in this article's Online Repository at www.jacionline.org) and depicted DEGs in a heat map (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). DEG expression at baseline (week 0) was comparable between the drug- and placebo-treated patients, as shown in a principal component analysis (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). Progressive changes in gene-expression profiles were observed during fezakinumab treatment, as seen by using a blue-to-red and red-to-blue (or lighter red) shift until week 12, whereas almost no changes were observed with placebo (see Fig E1, *A*).

Baseline dysregulation of the upregulated and downregulated AD-related gene expression profile (lesional vs nonlesional, mean $\log_2$ FCH) approached the profile of nonlesional skin at week 12 in drug-treated patients (see Fig E1, *B*) but not in placebo-treated patients (see Fig E1, *C*). Among upregulated genes that reverted to nonlesional expression levels at week 12 in the drug group were markers associated with general inflammation *(MMP12)* and T-cell activation (*CD28*, *ICOS*, and *IL7R*), as well as T_H2-associated (*CCL17* and *CCL22*) and T_H17/T_H22-associated (*S100A8*, *S100A9*, *S100A12*, *PI3*/elafin, *DEFB4A*, *LCN2*, *IL36G*, and *CCL20*; see Table E1) mediators. Among downregulated genes reverting by week 12 were betacellulin (an epidermal growth factor receptor ligand), the lipid-associated mediator *HSD11B1*, the hypoxia-induced factor *HIF3A*, and the negative regulator of inflammation *IL37* (see Table E1). Similar genomic changes in patients receiving drug versus placebo treatment were observed when we evaluated an established robust meta-analysis–derived atopic dermatitis transcriptome (MADAD) based on several previous AD studies (Fig 1, *A-C*, and see Table E2 in this article's Online Repository at www.jacionline.org).[6,29,32-34] Again, baseline DEG expression was comparable between the drug and placebo groups (see Fig E2, *B*).

Treatment effects were also reflected by stronger mean improvements of upregulated and downregulated genes in the fezakinumab (51.5% in upregulated and 117.1% in downregulated genes) versus placebo (14.5% in upregulated and 11.7% in downregulated genes) groups at week 12 versus week 0 within the MADAD (Fig 1, *D*), which is comparable with the overall transcriptome (see Fig E1, *D*). Overall mean transcriptomic improvements (combining both upregulated and downregulated genes) were 65.5% in drug-treated patients, as opposed to only 13.9%

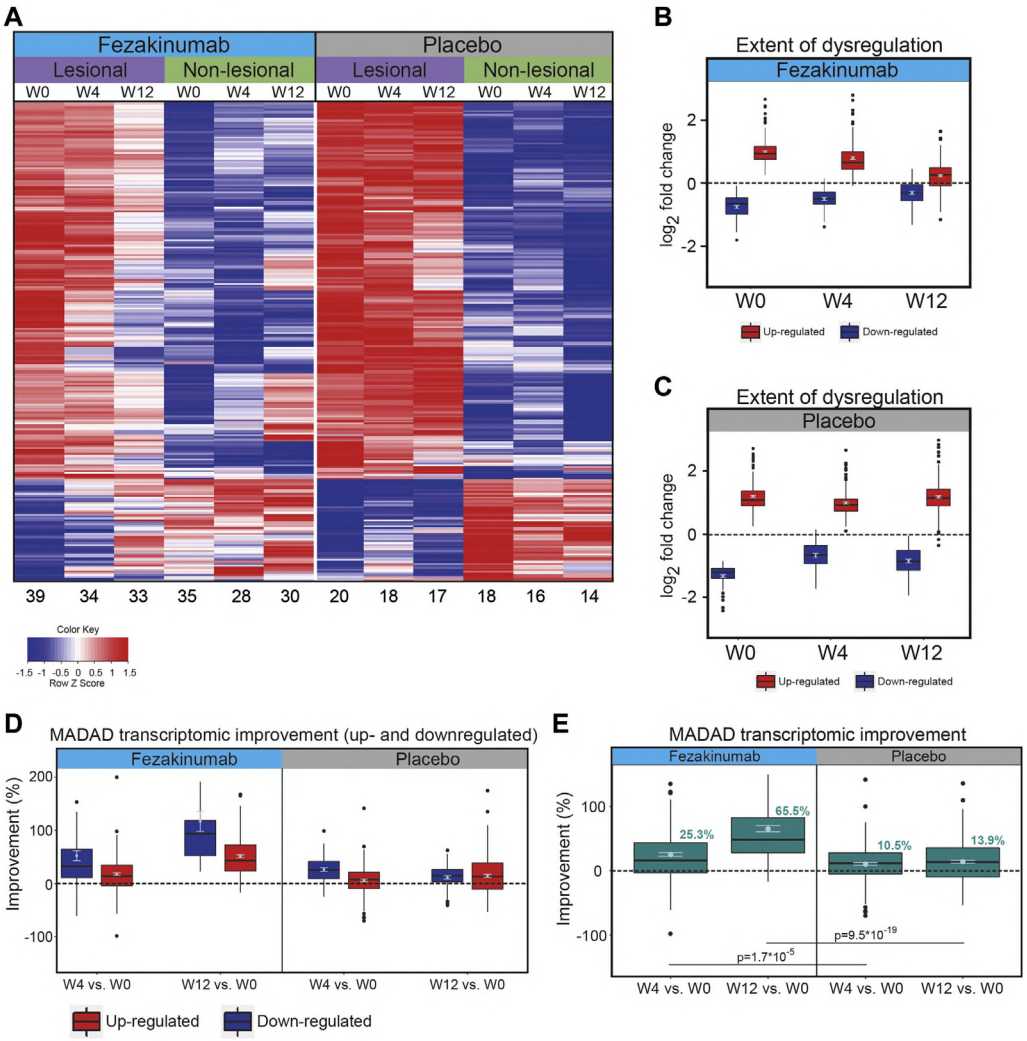


FIG 1. Molecular skin changes with fezakinumab. **A,** Heat map with mean expression levels of the MADAD gene profile, depicting DEGs (defined as FCH > 2 and FDR < 0.05 between lesional and nonlesional skin) at weeks 0, 4, and 12 of treatment with fezakinumab or placebo ordered by unsupervised hierarchical clustering. **B** and **C,** Box plots show overall dysregulation of the AD-related MADAD transcriptome in drug- and placebo-treated patients, respectively, at each time point, depicted as $\log_2$ FCH of lesional genes that were upregulated *(red boxes)* or downregulated *(blue boxes)* compared with nonlesional skin. **D** and **E,** Percentage improvement of baseline dysregulation in the MADAD transcriptome at weeks 4 and 12 versus week 0 in the fezakinumab versus placebo arm depicted separately for upregulated and downregulated genes (Fig 1, *D*) and for overall improvements (Fig 1, *E*). Medians and quartiles are depicted as box plots, with means ± SEMs. Numbers of biopsy specimens analyzed are given below the heat map. *W*, Week.

in placebo-treated patients within the MADAD (Fig 1, *E*), similar to the overall transcriptome (see Fig E1, *E*), with significantly stronger improvements in the drug versus placebo groups ($P = 9.5 \times 10^{-19}$ [Fig 1, *E*] and $P = 9.1 \times 10^{-11}$ [see Fig E1, *E*]).

Fezakinumab effects depend on high baseline IL-22 expression

Recently, we showed that IL-22 blockade elicits significant clinical effects only in patients with severe AD.[27] To assess whether baseline IL-22 skin expression influences fezakinumab responses, we used median IL-22 expression at week 0 (baseline) to stratify for IL-22–high (n = 30) and IL-22–low (n = 29) groups (Table I and see Table E3 in this article's Online Repository at www.jacionline.org) and depicted mean (Fig 2, *A*) or individual (see Fig E3 in this article's Online Repository at www.jacionline.org) expression levels in heat maps. Baseline measures did not differ between groups (Table I), and a principal component analysis plot showed comparable baseline gene expression between the drug and placebo groups among both IL-22–high and IL-22–low patients (see Fig E2, *C*). We found that molecular drug effects were only present in the high baseline IL-22 drug group but not in low IL-22 drug or placebo groups (Fig 2, *A*). In fact, while the drug IL-22–high group gradually changed from red to blue and blue to red (whereas respective placebo showed minimal changes), the IL-22–low drug group (and placebo) showed exacerbations, with increased red intensity over time (Fig 2, *A*). Much stronger mean transcriptomic improvements

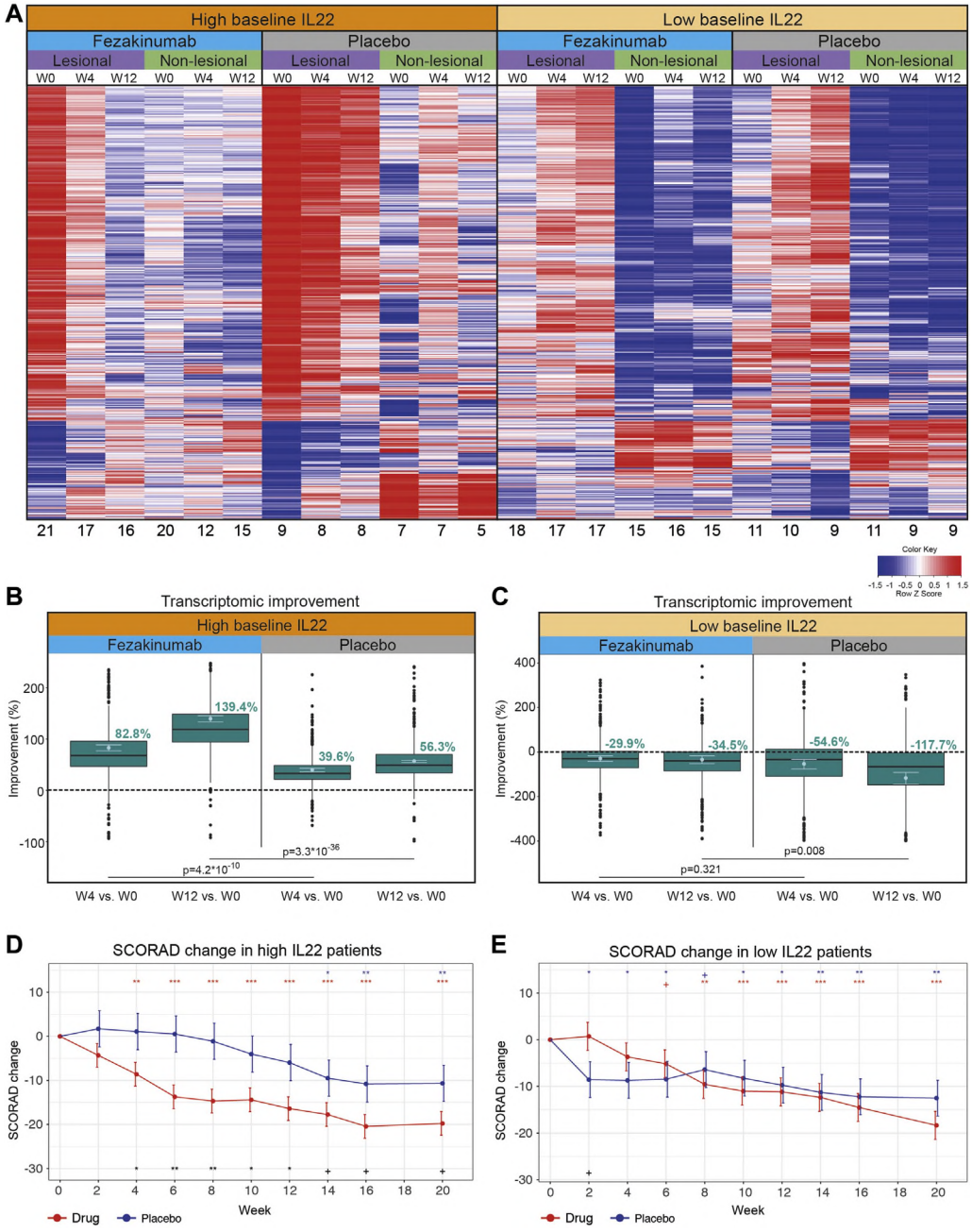
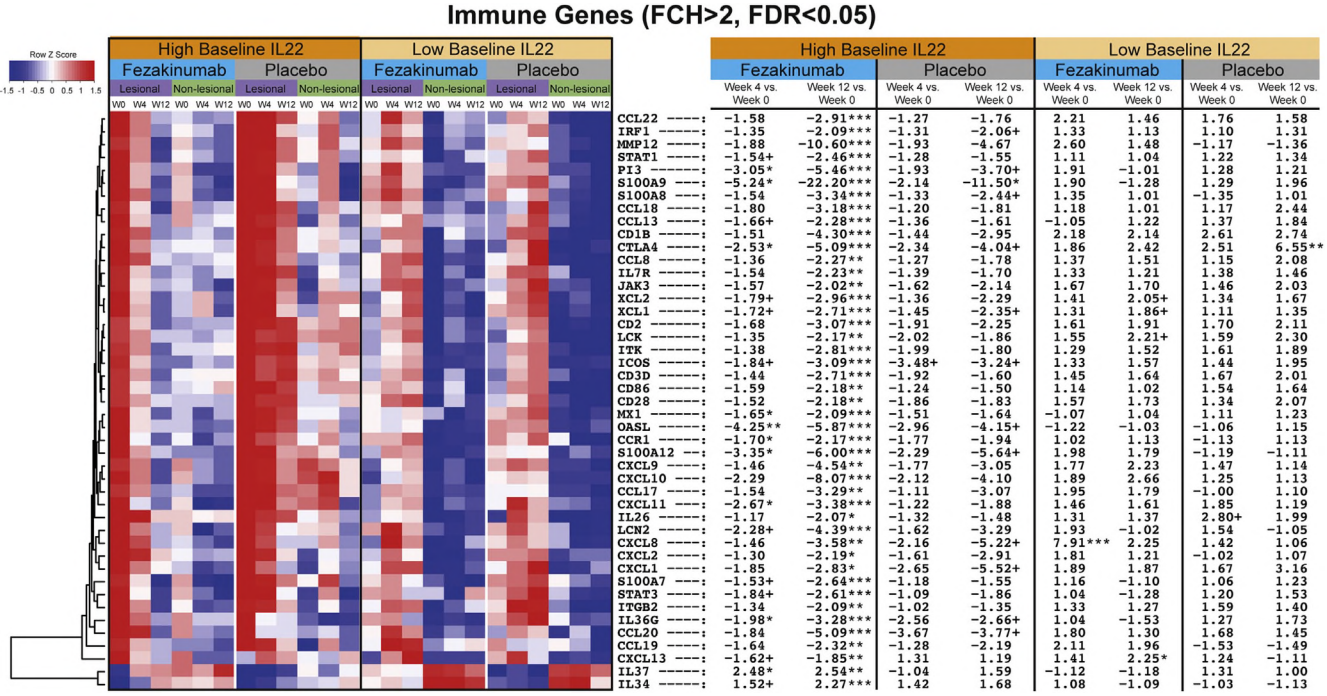


FIG 2. Molecular and clinical changes with fezakinumab stratified for baseline IL-22 mRNA expression. **A,** Heat map with mean expression levels of DEGs (defined as FCH > 2 and FDR < 0.05 between lesional and nonlesional skin) at weeks 0, 4, and 12 of treatment with fezakinumab or placebo ordered by unsupervised hierarchical clustering. High and low IL-22 baseline expression was defined as populations with greater than and less than median IL-22 expression at week 0, respectively. **B** and **C,** Box plots showing percentage overall transcriptomic improvements at weeks 4 and 12 compared with baseline for both IL-22–high (Fig 2, *B*) and IL-22–low (Fig 2, *C*) populations. Medians and quartiles are depicted as box plots with means ± SEMs. **D** and **E,** SCORAD scores are depicted as the change in mean from baseline ± SEM. Data were analyzed by using mixed-effects model repeat measurement. *Red* and *blue asterisks* indicate significant change from baseline for each arm. *Black asterisks* at the bottom indicate significant differences between drug and placebo arm. $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$. Numbers of biopsy specimens analyzed are presented below the heat map. *W*, Week.

were seen with fezakinumab administration in the IL-22–high group (82.8% and 139.4% at weeks 4 and 12, respectively) than in the respective placebo group (39.6% at week 4 and 56.3% at week 12; Fig 2, *B*). We also observed significantly greater responses in the IL-22–high group treated with fezakinumab compared with the fezakinumab-treated IL-22–low group, which even showed exacerbation of their genomic fingerprinting (−29.9% and −34.5% at weeks 4 and 12; Fig 2, *C*, and see



	High Baseline IL22				Low Baseline IL22			
	Fezakinumab		Placebo		Fezakinumab		Placebo	
	Week 4 vs. Week 0	Week 12 vs. Week 0	Week 4 vs. Week 0	Week 12 vs. Week 0	Week 4 vs. Week 0	Week 12 vs. Week 0	Week 4 vs. Week 0	Week 12 vs. Week 0
CCL22	−1.58	−2.91***	−1.27	−1.76	2.21	1.46	1.76	1.58
IRF1	−1.35	−2.09***	−1.31	−2.06+	1.33	1.13	1.10	1.31
MMP12	−1.88	−10.60***	−1.93	−4.67	2.60	1.48	−1.17	−1.36
STAT1	−1.54+	−2.46***	−1.28	−1.55	1.11	1.04	1.22	1.34
PI3	−3.05*	−5.46***	−1.93	−3.70+	1.91	−1.01	1.28	1.21
S100A9	−5.24*	−22.20***	−2.14	−11.50*	1.90	−1.28	1.29	1.96
S100A8	−1.54	−3.34***	−1.33	−2.44+	1.35	1.01	−1.35	1.01
CCL18	−1.80	−3.18***	−1.20	−1.81	1.18	1.01	1.17	2.44
CCL13	−1.66+	−2.28***	−1.36	−1.61	−1.05	1.22	1.37	1.84
CD1B	−1.51	−4.30***	−1.44	−2.95	2.18	2.14	2.61	2.74
CTLA4	−2.53*	−5.09***	−2.34	−4.04+	1.86	2.42	2.51	6.55**
CCL8	−1.36	−2.27**	−1.27	−1.78	1.37	1.51	1.15	2.08
IL7R	−1.54	−2.23**	−1.39	−1.70	1.33	1.21	1.38	1.46
JAK3	−1.57	−2.02**	−1.62	−2.14	1.67	1.70	1.46	2.03
XCL2	−1.79+	−2.96***	−1.36	−2.29	1.41	2.05+	1.34	1.67
XCL1	−1.72+	−2.71***	−1.45	−2.35+	1.31	1.86+	1.11	1.35
CD2	−1.68	−3.07***	−1.91	−2.25	1.61	1.91	1.70	2.11
LCK	−1.35	−2.17**	−2.02	−1.86	1.55	2.21+	1.59	2.30
ITK	−1.38	−2.81***	−1.99	−1.80	1.29	1.52	1.61	1.89
ICOS	−1.84+	−3.09***	−3.48+	−3.24+	1.33	1.57	1.44	1.95
CD3D	−1.44	−2.71***	−1.92	−1.60	1.45	1.64	1.67	2.01
CD86	−1.59	−2.18**	−1.24	−1.50	1.14	1.02	1.54	1.64
CD28	−1.52	−2.18**	−1.86	−1.83	1.57	1.73	1.34	2.07
MX1	−1.65*	−2.09***	−1.51	−1.64	−1.07	1.04	1.11	1.23
OASL	−4.25**	−5.87***	−2.96	−4.15+	−1.22	−1.03	−1.06	1.15
CCR1	−1.70*	−2.17***	−1.77	−1.94	1.02	1.13	−1.13	1.13
S100A12	−3.35*	−6.00***	−2.29	−5.64+	1.98	1.79	−1.19	−1.11
CXCL9	−1.46	−4.54**	−1.77	−3.05	1.77	2.23	1.47	1.14
CXCL10	−2.29	−8.07***	−2.12	−4.10	1.89	2.66	1.25	1.13
CCL17	−1.54	−3.29**	−1.11	−3.07	1.95	1.79	−1.00	1.10
CXCL11	−2.67*	−3.38***	−1.22	−1.88	1.46	1.61	1.85	1.19
IL26	−1.17	−2.07*	−1.32	−1.48	1.31	1.37	2.80+	1.99
LCN2	−2.28+	−4.39***	−1.62	−3.29	1.93	−1.02	1.54	−1.05
CXCL8	−1.46	−3.58**	−2.16	−5.22+	7.91***	2.25	1.42	1.06
CXCL2	−1.30	−2.19*	−1.61	−2.91	1.81	1.21	−1.02	1.07
CXCL1	−1.85	−2.83*	−2.65	−5.52+	1.89	1.87	1.67	3.16
S100A7	−1.53+	−2.64***	−1.18	−1.55	1.16	−1.10	1.06	1.23
STAT3	−1.84+	−2.61***	−1.09	−1.86	1.04	−1.28	1.20	1.53
ITGB2	−1.34	−2.09**	−1.02	−1.35	1.33	1.27	1.59	1.40
IL36G	−1.98*	−3.28***	−2.56	−2.66+	1.04	−1.53	1.27	1.73
CCL20	−1.84	−5.09***	−3.67	−3.77+	1.80	1.30	1.68	1.45
CCL19	−1.64	−2.32**	−1.28	−2.19	2.11	1.96	−1.53	−1.49
CXCL13	−1.62+	−1.85**	1.31	1.19	1.41	2.25*	1.24	−1.11
IL37	2.48*	2.54**	−1.04	1.59	−1.12	−1.18	1.31	1.00
IL34	1.52+	2.27***	1.42	1.68	1.08	−1.09	−1.03	−1.13

FIG 3. Immune gene regulation by fezakinumab treatment. Heat map of immune-associated DEG subsets ordered by unsupervised hierarchical clustering using microarray data. DEGs were defined by FCHs of greater than 2 and FDRs of less than 0.05 between weeks 4 or 12 versus baseline (week 0) in any group comparison. FDR-adjusted *P* values: +*P* < .1, **P* < .05, ***P* < .01, and ****P* < .001. *W*, Week.

Fig E4 in this article's Online Repository at www.jacionline.org). The respective IL-22–low placebo-treated patients had even greater molecular exacerbations (−54.6% and −117.7% at weeks 4 and 12; Fig 2, *C*).

Improvements by upregulated and downregulated genes are displayed in Fig E5, *A* and *B*, in this article's Online Repository at www.jacionline.org. In addition to a resolution in genomic dysregulation between lesional and nonlesional skin with drug treatment (see Fig E5, *C*), the IL-22–high drug-treated group also showed significant improvements in SCORAD scores compared with baseline and placebo-treated patients (Fig 2, *D*), including week 12 (primary end point) and week 20 (end of follow-up), whereas smaller clinical improvements were seen in the IL-22–low group, which did not show significant differences compared with the placebo group (Fig 2, *E*). Comparing only drug-treated patients in the IL-22–high versus IL-22–low groups, mean SCORAD score decreases from baseline were consistently stronger at all time points in IL-22–high patients (eg, −8.6 at week 4 and −16.4 at week 12; Fig 2, *D*) than in IL-22–low patients (eg, −3.7 at week 4 and −11.2 at week 12; Fig 2, *E*), with the smallest differences at the end of the study (week 20 [ie, 8 weeks after the last drug dose]; Fig 2, *D* and *E*).

Fezakinumab broadly decreases immune activation in skin

We next investigated the effects of IL-22 blockade on suppressing inflammatory AD pathways using a previously defined immune gene subset,[7,30,34] as displayed in a heat map showing DEGs at week 4 or week 12 when compared with baseline values (Fig 3). We found significant decreases in levels of multiple inflammatory mediators in the IL-22–high group treated with fezakinumab but not in the placebo or IL-22–low groups, which again showed a trend toward molecular worsening (Fig 3). Significant suppressions included genes representing mediators of general inflammation *(MMP12)*, T-cell activation (*ICOS* and *CD86/CD28*), innate immune responses (*MX1* and *CXCL8*), and molecules associated with T_H1 (*IRF1*, *CXCL9*, *CXCL10*, and *CXCL11*), T_H2 (*CCL13*, *CCL17*, *CCL18*, and *CCL22*), T_H17 (*CCL20*, *PI3*/elafin, *CXCL1*, and *IL36G*), and T_H17/T_H22 activation (*S100A7*, *S100A8*, and *S100A12*). Fezakinumab treatment also increased expression of IL-34 and IL-37, 2 epidermal negative regulators of inflammation[35,36] that often show reduced expression in AD lesions compared with nonlesional and normal tissues (Fig 3).[33,36]

Tight junction proteins, such as claudins, which are frequently downregulated in patients with AD, potentially contributing to barrier defects,[37] were significantly upregulated by fezakinumab treatment only in the IL-22–high group (see Fig E6 in this article's Online Repository at www.jacionline.org). However, other mediators of skin barrier function (ie, terminal differentiation) that are also defective in patients with AD[38] showed only modest or no upregulation, including loricrin, filaggrin, envoplakin, late cornified envelope (LCE) 1B, LCE1E, LCE2B, and periplakin (see Table E2).

Modulation of immune and barrier responses with fezakinumab measured by using RT-PCR and immunohistochemistry

We confirmed regulation of selected immune and barrier markers using RT-PCR (Fig 4 and see Fig E7 in this article's

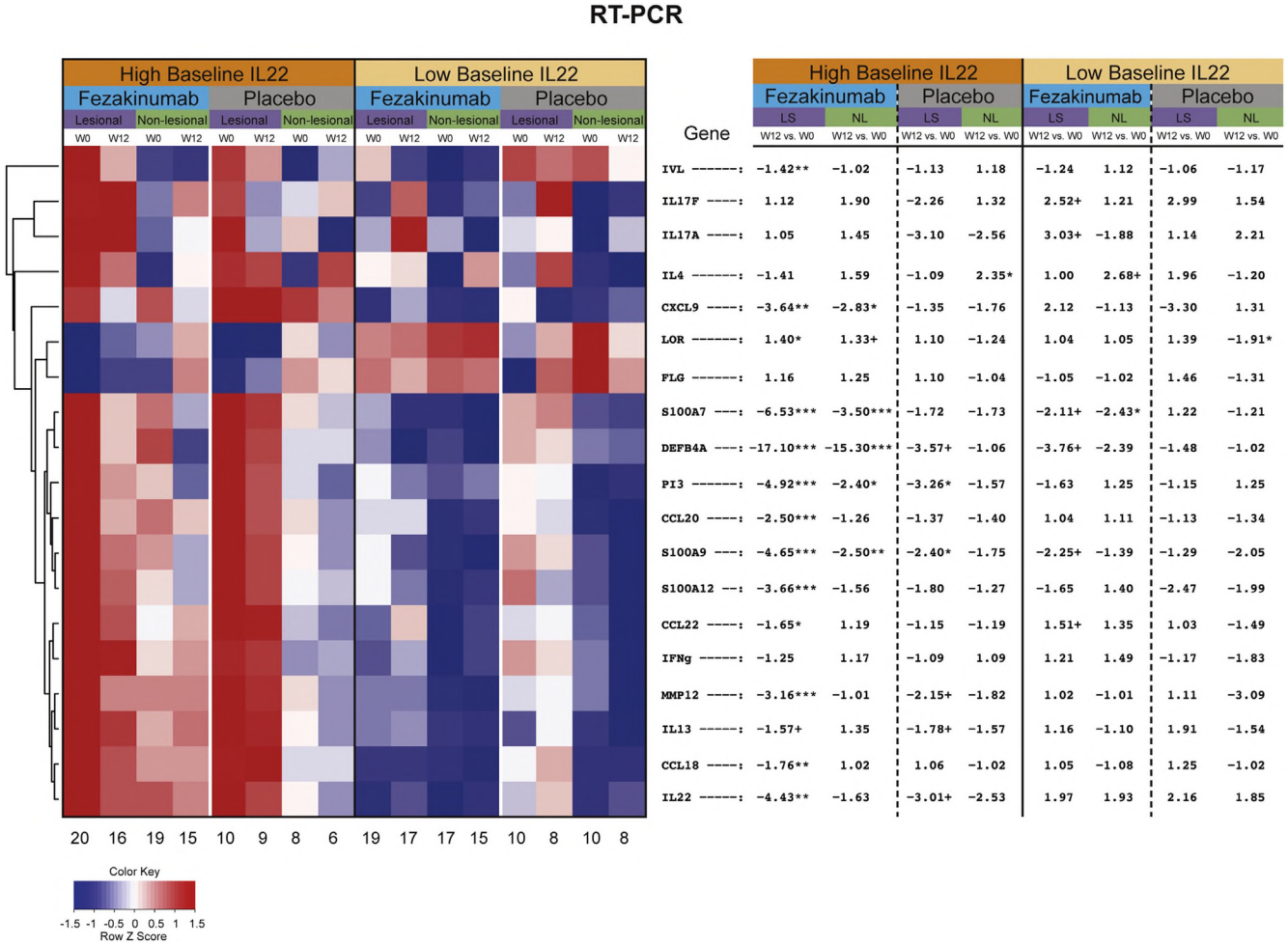


FIG 4. Quantitative RT-PCR analysis (mRNA expression/human acidic ribosomal protein) of lesional and nonlesional skin biopsy specimens depicted as mean expression levels (heat map) or FCHs of week 12 versus baseline (week 0): $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$. Genes are ordered by unsupervised hierarchical clustering. Numbers of biopsy specimens analyzed are given below the heat map. *LS*, Lesional; *NL*, nonlesional; *W*, week.

Online Repository at www.jacionline.org). Consistent with microarray data, fezakinumab treatment downregulated the general inflammation marker *MMP12* and also decreased mRNA expression of mediators associated with T_H1 *(CXCL9)*, T_H2 (*CCL18* and *CCL22*), T_H17 (*DEFB4A*, *CCL20*, and *PI3*/elafin), and T_H17/T_H22 (*S100A7*, *S100A9*, and *S100A12*) activation (Fig 4), which are all produced by activated keratinocytes.[39-43]

Among cytokines primarily produced by T cells, only *IL22* (T_H22), but not *IFNG* (T_H1), *IL4*/*IL13* (T_H2), or *IL17A*/*IL17F* (T_H17), showed significant downregulation with fezakinumab. Epidermal differentiation genes were minimally restored (loricrin and filaggrin) upon IL-22 blockade. Again, consistent fezakinumab effects were only seen in samples with high baseline IL-22 levels but not in the placebo or IL-22–low groups (Fig 4). Importantly, detection of IL-22 by using RT-PCR was highly correlated with microarray data, which led to comparable patient stratification (see Fig E8 and Table E4 in this article's Online Repository at www.jacionline.org).

Strong fezakinumab effects on markers of epidermal activation were confirmed by using immunohistochemistry, showing strong decreases in *S100A7* staining by using fezakinumab but not placebo treatment (Fig 5, *A*). Epidermal thickness (Fig 5, *B*) and $CD3^+$ T-cell and $CD11c^+$ dendritic cell (DC) counts (Fig 5, *C-E*) showed an overall tendency to decrease only in fezakinumab-treated patients, with a trend for exacerbations in placebo-treated patients (Fig 5, *B-E*). However, these differences were statistically significant only for $Fc\varepsilon RI^+$ cells at week 12 fezakinumab versus placebo (Fig 5, *E*). Thus IL-22 antagonism primarily modulates epidermal but less so cellular responses in the IL-22–high group.

Predictor of fezakinumab treatment response

Because clinical responses to fezakinumab had primarily been observed in patients with severe AD,[27] we wanted to determine whether baseline genomic dysregulation correlated with clinical responses. Because fezakinumab continued to improve clinical scores until week 20/end of study (ie, 10 weeks after the last drug dose),[27] we correlated decreases in SCORAD scores from week 0 to week 20 with mean upregulated and downregulated genes of the AD transcriptome (lesional vs nonlesional skin) at baseline (week 0), as represented by respective *z* scores. Both

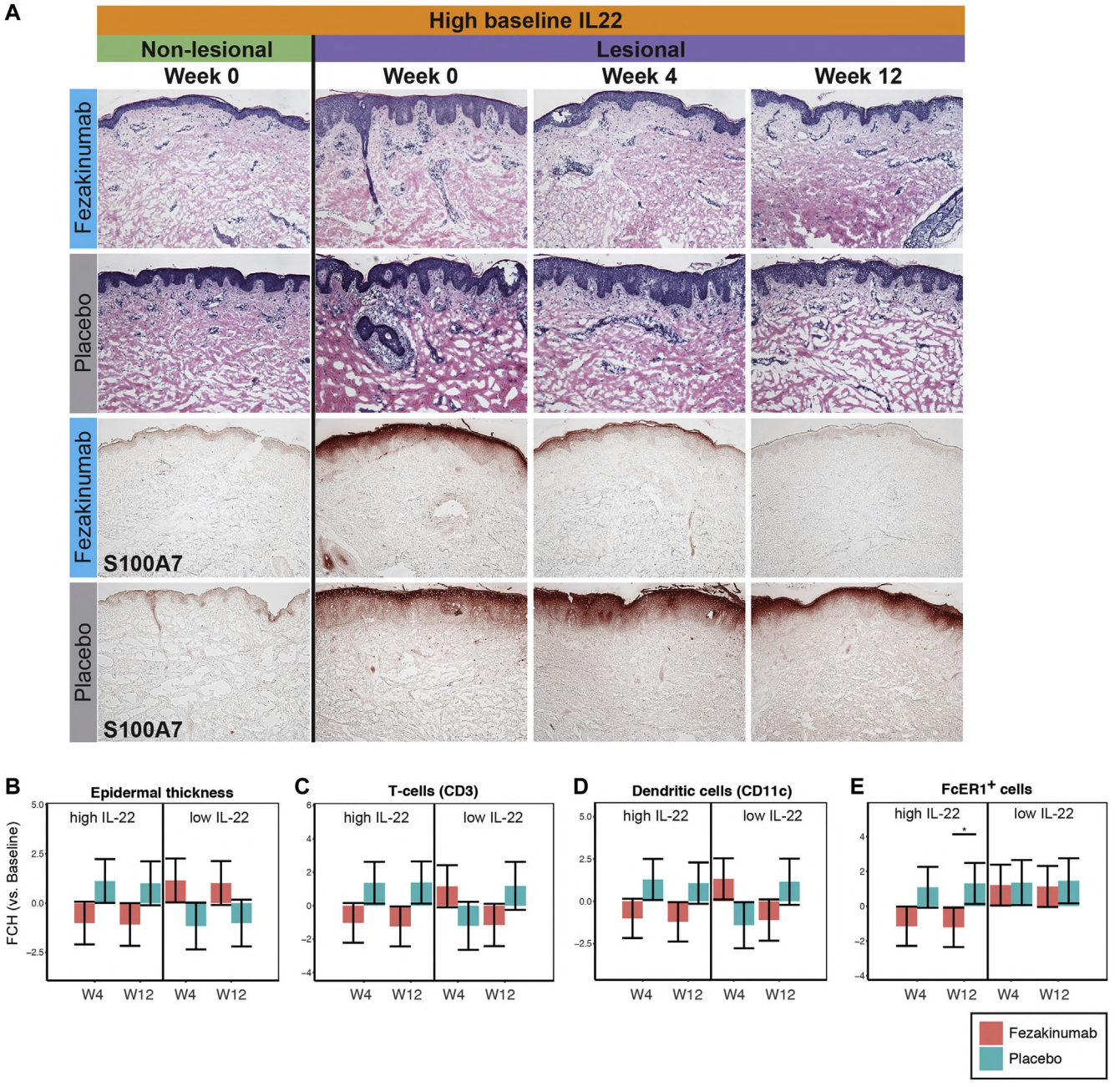
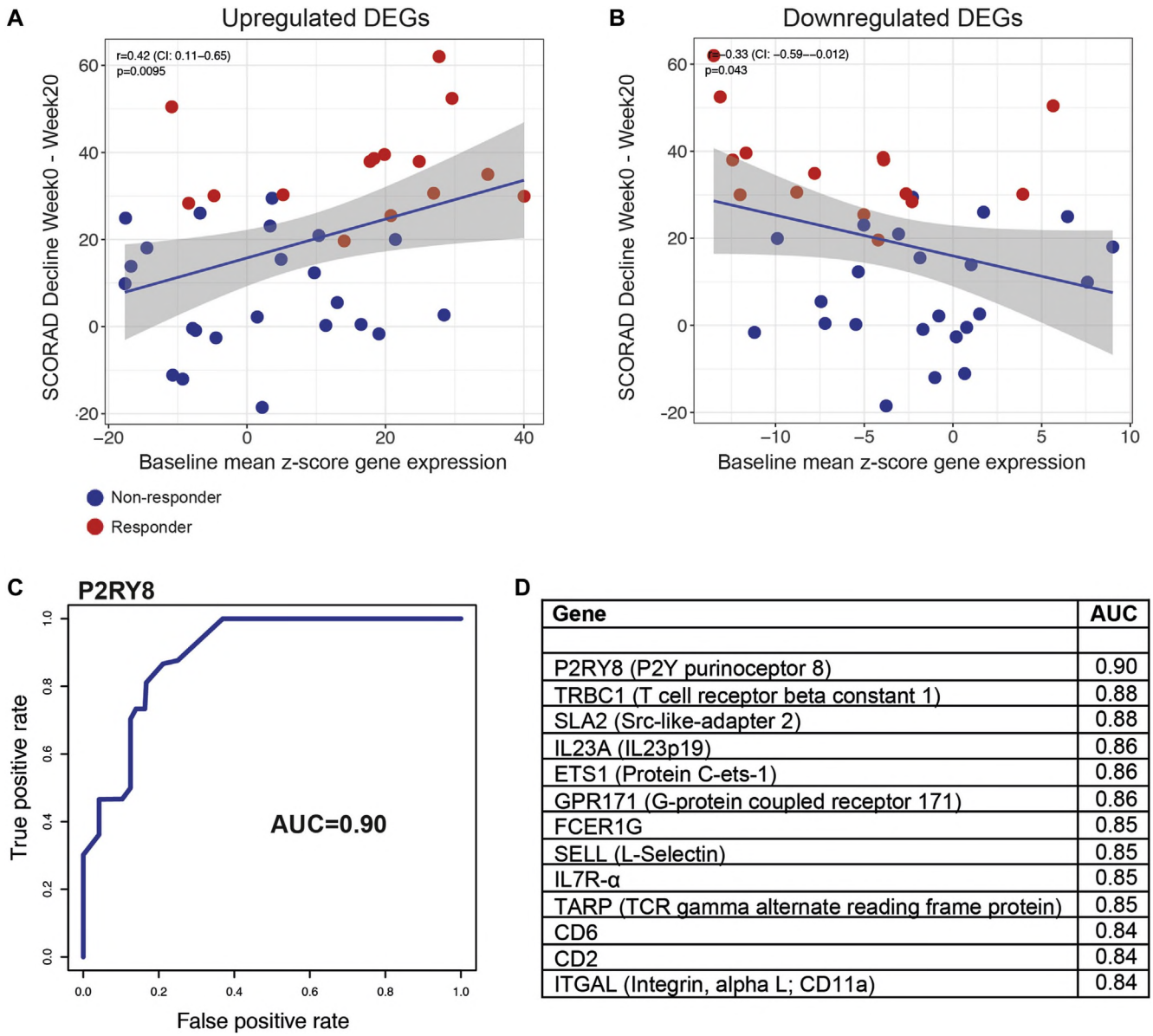


FIG 5. A, Representative pictures of skin samples using hematoxylin and eosin and S100A7 immunostaining of fezakinumab- and placebo-treated patients with high IL-22 levels at baseline. **B-E,** FCH of week 4 and 12 levels versus baseline of epidermal thickness (Fig 5, *B*) and immunohistochemistry cell counts (Fig 5, *C-E*). **P* < .05. *W,* Week.

upregulated and downregulated DEGs at week 0 of drug-treated patients showed significant positive (Fig 6, *A*) and negative (Fig 6, *B*) correlations with SCORAD score decrease until week 20, respectively. When differentiating responders and nonresponders (as defined by SCORAD50 response [ie, a ≥50% decrease from baseline SCORAD score until week 20]), there was a trend for stronger baseline dysregulation in the clinical responder group (red dots) than in the nonresponder group (blue dots, Fig 6, *A* and *B*), corroborating that the magnitude of transcriptomic dysregulation at baseline correlates with clinical treatment responses to fezakinumab.[27] Although there were also 5 placebo responders (see Fig E9 in this article's Online Repository at www.jacionline.org), a heat map shows that these few respective placebo-treated patients had less baseline disease activity and thus might be subjected to more biological fluctuation (see Fig E9). By contrast, the rest of the placebo-treated patients (n = 15) were clinical nonresponders, and these subjects were more comparable in baseline disease activity with drug responders (see Fig E9).

We next aimed to assess whether baseline expression of individual DEGs (FCH > 2 and FDR < 0.05) in fezakinumab-treated patients can predict SCORAD responses at week 20 by



Gene	AUC
P2RY8 (P2Y purinoceptor 8)	0.90
TRBC1 (T cell receptor beta constant 1)	0.88
SLA2 (Src-like-adapter 2)	0.88
IL23A (IL23p19)	0.86
ETS1 (Protein C-ets-1)	0.86
GPR171 (G-protein coupled receptor 171)	0.86
FCER1G	0.85
SELL (L-Selectin)	0.85
IL7R-α	0.85
TARP (TCR gamma alternate reading frame protein)	0.85
CD6	0.84
CD2	0.84
ITGAL (Integrin, alpha L; CD11a)	0.84

FIG 6. A and **B,** Spearman correlation of SCORAD decline (weeks 0-20), with baseline genomic dysregulation depicted as mean *z* score gene expression of week 0 lesional skin, separately shown for upregulated (Fig 6, *A*) and downregulated (Fig 6, *B*) DEGs. Treatment response (*red* and *blue dots*) was defined as SCORAD50 response (responders had a ≥50% decrease in baseline SCORAD until week 20) of fezakinumab-treated patients. **C,** Receiver operating characteristic curve of *P2RY8* baseline expression as a predictor of week 20 SCORAD responses. **D,** Genes with top AUC of baseline expression of DEGs (lesional vs nonlesional skin).

calculating their AUCs in a receiver operating characteristic curve. Indeed, we found several genes to show high AUC levels, which identifies better predictive values (ie, greater true-positive and lower false-positive rates).

P2RY8 gene expression showed maximum predictive values with AUCs of 0.90 (Fig 6, *C*). *P2RY8* is a G protein–coupled receptor activated by adenosine and uridine nucleotides that can rearrange with the thymic stromal lymphopoietin (TSLP) receptor subunit *CRLF2* in T- and B-cell malignancies.[44]

Other top genes showing the highest AUCs (Fig 6, *D*) were largely genes regulating adaptive immune and DC responses. These included lymphocyte activation genes, such as T-cell receptor beta constant 1 *(TRBC1)*, Src like adapter 2 *(SLA2)*, IL-23p19 involved in T-cell priming *(IL23A)*, receptor subunit for IL-7 that regulates T- and B-cell development *(IL7RA)*, T-cell receptor γ alternate reading frame protein *(TARP)*, *CD6* (T-cell activation), *CD2* (T- and natural killer cell marker), the T-cell homing molecules CD11a *(ITGAL)* and SELL (L-selectin/*CD62L*), and the γ chain of the high-affinity IgE receptor (FcεRI; *FCER1G*), which is found, among others, on allergic DCs.[45] IL-7R is also a subunit of the thymic stromal lymphopoietin receptor on DCs,[46,47] which can also express IL-23A, all mediators showing high AUCs (Fig 6, *D*).

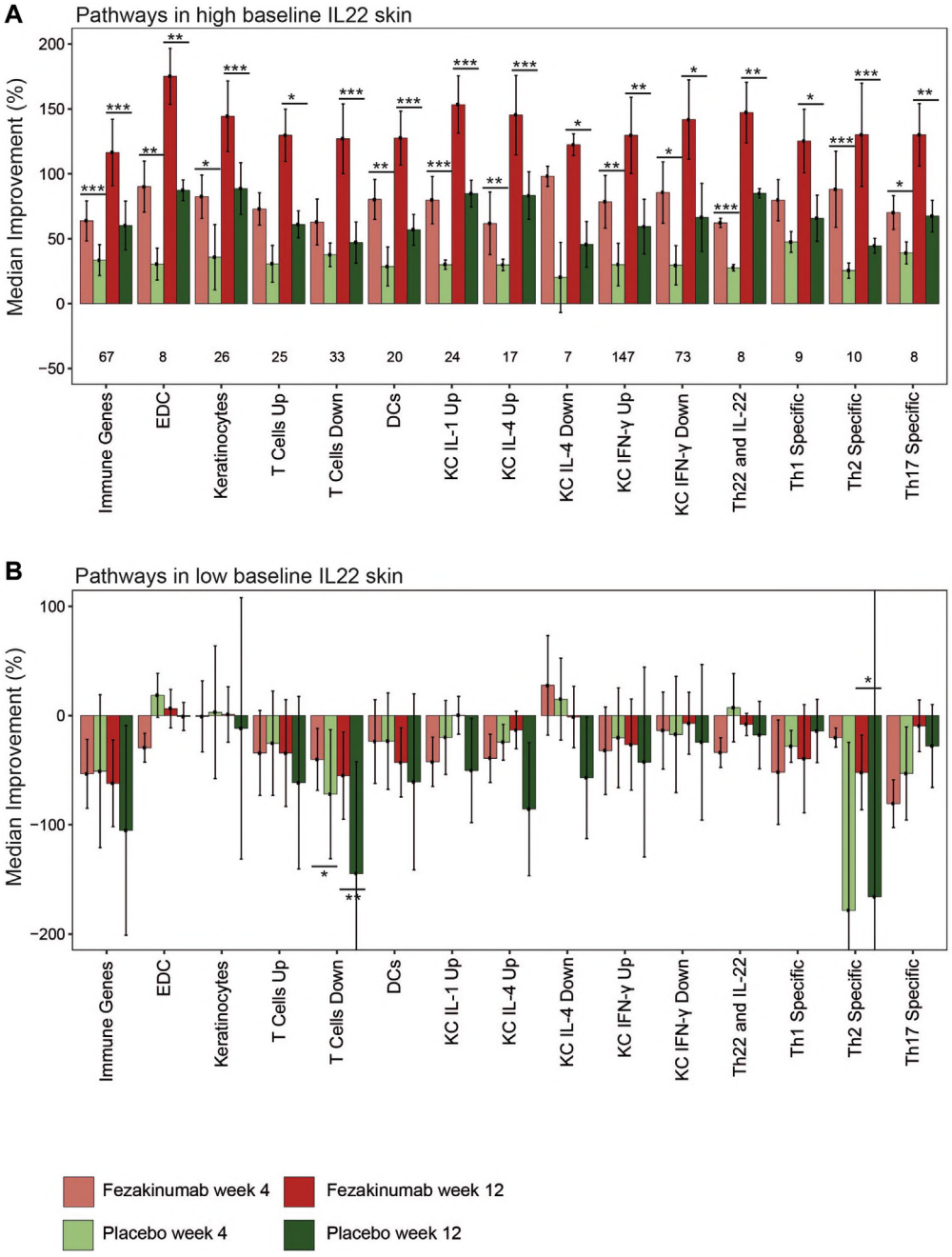


FIG 7. Percentage improvement of baseline dysregulation of selected AD-related gene signatures at weeks 4 and 12 in the fezakinumab versus placebo arms stratified for high **(A)** and low **(B)** baseline IL-22 expression (with median IL-22 expression being the cutoff), with numbers at the bottom of the graph indicating numbers of genes in each gene set. *$P < .05$, **$P < .01$, and ***$P < .001$. Medians ± interquartile ranges are shown. *EDC,* Epidermal differentiation complex; *KC,* keratinocytes.

ETS1, another gene that we found to have a high predictive value is a transcription factor that controls cytokine/chemokine activation in many cells. *ETS1* is also a negative regulator of T_H17 differentiation, and is increased in allergic disease, such as allergic rhinitis.[48-50] Overall, markers of T-cell and DC activation were able to best predict clinical responses to fezakinumab.

Effects on gene signatures

We evaluated fezakinumab's effects on selected gene signatures for upregulated or downregulated genes within the AD transcriptome.[7,28,33,34,51-55] These included keratinocyte, T-cell, and DC gene subsets; immune genes; and keratinocyte or T-cell cytokine responses (Fig 7). In the IL-22–high subset, fezakinumab showed improvement of a broad array of response pathways

that were higher and significantly stronger than placebo (Fig 7, *A*). By contrast, IL-22–low groups (in both the fezakinumab or placebo groups) did not show significant improvements and even had a tendency toward exacerbation (Fig 7, *B*).

We also performed pathway enrichment analyses[56] of genes that were significantly modulated by fezakinumab as opposed to placebo in the IL-22–high patient group by using the functional-based pathway database Kyoto Encyclopedia of Genes and Genomes.[57] We found several significantly regulated pathways with fezakinumab treatment (FDR < 0.05), including chemokine signaling, cytokine–cytokine receptor interaction, cell adhesion molecules, T-cell receptor signaling, and the nuclear factor κB signaling pathways (see Table E5 in this article's Online Repository at www.jacionline.org).

DISCUSSION

IL-22–producing cells have been suggested to be pathogenically linked to AD, with increased IL-22 expression in both skin and blood compartments.[11,12] Increased IL-22 levels have also been shown in patients with other inflammatory diseases, including psoriasis, inflammatory bowel diseases, and rheumatoid arthritis.[15] In patients with Crohn disease, greater baseline serum IL-22 levels were associated with greater likelihood of an anti–IL-23 therapeutic response.[58]

Our study is the first to characterize the effects of IL-22 blockade on a molecular level and to link tissue responses to clinical disease improvement in a human disease. Although AD is a disease of considerable heterogeneity[59-64] in baseline biomarker expression or "endotypes,"[65] its relevance to treatment stratification has not been investigated so far. Thus this study provides a first glimpse into a potential precision medicine approach in AD, where patients with increased IL-22 expression had greater disease improvement, and baseline gene expression of selected genes can potentially predict clinical outcomes to a targeted therapeutic agent.

Fezakinumab treatment resulted in suppression of mRNA expression of multiple genes related to polar cytokine axes, including T_H1, T_H2, and T_H17, in addition to the T_H22 pathway, the obvious drug target. The fact that treatment effects were restricted to patients with high IL-22 baseline expression underscores disease heterogeneity,[5,66,67] which perhaps necessitates different therapeutic approaches in different patient subsets[59-61,63,68] or use of broader inhibitors, such as Janus kinase antagonists,[69] to achieve complete disease resolution in a majority of patients, as achieved in those with psoriasis.[59] These data suggest that precision medicine approaches that identify genes or gene subsets in responders to particular drugs might help determine the patients who could best benefit from certain targeted therapies, avoiding or reducing unnecessary costs or inconveniences associated with suboptimal drug effects for a certain patient, as attempted in other inflammatory conditions, such as asthma.[70,71] Such an approach would not only be among the first precision medicine attempts in patients with inflammatory skin diseases but also the first in patients with AD.

Previously, we showed that high/low SCORAD baseline stratification elicited different therapeutic responses in these patients.[27] Although baseline IL-22 levels and disease severity measures (SCORAD scores) are not well correlated, their independent behavior provides alternative ways to successfully stratify the therapeutic responses to fezakinumab, both yielding to highly significant changes in disease scores (SCORAD) in drug- versus placebo-treated patients. In patients with low IL-22 baseline levels, we even observed the phenomenon of improving clinical scores but worsening molecular signatures in biopsy specimens, which has previously also been observed with dupilumab.[7] As mentioned, SCORAD scores and molecular inflammation are not always correlated because key components of SCORAD assessments are subjective (itch and sleep), which can contribute particularly to the high placebo effects typically seen in patients with AD.[72] Importantly, there was no significant difference in SCORAD responses between drug- and placebo-treated patients in the IL-22–low group, as opposed to the IL-22–high group. Thus molecular analyses from biopsy specimens might offer more objective and reproducible assessments of overall disease activity, but this hypothesis requires confirmation in future studies.

The strong inhibitory effects of fezakinumab on a multitude of inflammatory mediators that are mostly produced by keratinocytes, together with the fact that these cells express the IL-22R,[18] suggest a primary effect of IL-22 blockade on ameliorating epidermal responses in patients with AD.[32,33,36] This establishes a basis to propose that the epidermis can regulate skin-related inflammatory responses through cytokines and other mediators that are dysregulated in response to a single pathogenic "driver," such as IL-22. Potential epidermal contributors could include proinflammatory mediators, such as CCL20 or S100A proteins, or inhibitory cytokines, such as IL-34 and IL-37.[33,35,36] The concept of "feed-forward" inflammation through keratinocytes has previously been suggested for psoriasis,[73] but there has not been a direct proof of this concept using cytokine antagonists because IL-17 and TNF-α have broad expression on keratinocytes and other cutaneous cell types.[73-75] However, in this AD study a keratinocyte-specific cytokine has been antagonized,[18] and thus changes in inflammatory products in the skin must be mediated through the diseased epidermis.[76] Furthermore, epidermal responses might even affect systemic inflammation in patients with AD. Data from mouse models suggest the idea that in some circumstances the epidermis can produce mediators driving not only complex skin but also systemic inflammation.[77] One example is IL-17C overproduction in mouse epidermis, which leads not only to psoriasiform skin manifestations but also to vascular inflammation.[78] However, whether this is relevant to human disease requires further clarification, but some systemic inflammatory components have been shown to correlate to skin disease severity.[11,79-81]

Although fezakinumab showed clear epidermal effects, resolution of immune cell infiltrates might occur only after normalization of the epidermal pathology. This is also possibly suggested by the progressive clinical effects of fezakinumab treatment well beyond the last treatment dose (at week 10) until the end of the study (week 20),[27] together with a tendency toward decreased immune cell counts at the week 12 biopsy. A central role of immune infiltrates is particularly suggested by our finding that the set of genes that best predicted fezakinumab responses were genes of adaptive immune activation, including T-cell priming, as well as markers of DC biology. However, the hypothesis that IL-22 first targets keratinocyte-based products, with later translation to other immune components, needs proof in longer studies with biopsies at later time points.

Some barrier components, such as claudins and lipid-associated mediators, which are often highly suppressed in

patients with AD,[33,36,82,83] were upregulated by fezakinumab treatment. Nevertheless, full restoration of the epidermal abnormalities might take longer, and perhaps better improvements in tissue expression of these markers would have been obtained at the week 20 time point, as would need to be determined by future longer studies. These extended studies should also evaluate whether tissue responses continue to improve long after the end of treatment, as suggested by clinical data.[27] These studies should not only identify drug responders but also investigate whether IL-22 blockade could have long-lasting or perhaps even disease-modifying effects in particular subsets of patients with AD. Results will also need to be assessed in defined AD cohorts, such as intrinsic versus extrinsic[60] or pediatric versus adult patients,[63] and in patients of different ethnicities.[61] In addition to the lack of long-term follow-up and inability to evaluate different AD categories because of the relatively small sample size, another limitation of our study is the fact that predictors of therapeutic response still need validation in an independent patient cohort.

In sum, molecular profiling of AD skin lesions not only provides an objective approach to evaluating treatment response of new agents[7] but also advances our understanding of AD pathomechanisms. Given selective expression of IL-22R on epithelial cells, inhibition of multiple immune axes by fezakinumab provides one of the clearest examples of how epidermal responses to T cell–derived cytokines can "feed forward" inflammation to amplify immune responses that are likely geared to protective immunity.

Clinical implications: Stratification of cytokine expression at baseline might help future precision medicine approaches to effectively treat subsets of patients with AD who might benefit from IL-22 antagonism or other specific blockers.

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