

Effect of short-term liver X receptor activation on epidermal barrier features in mild to moderate atopic dermatitis

A randomized controlled trial

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ABSTRACT

Background: Liver X receptors (LXRs) are involved in maintaining epidermal barrier and suppressing inflammatory responses in model systems. The LXR agonist VTP-38543 showed promising results in improving barrier function and inflammatory responses in model systems.

Objective: To assess the safety, tolerability, cellular and molecular changes, and clinical efficacy of the topical VTP-38543 in adults with mild to moderate atopic dermatitis (AD).

Methods: A total of 104 ambulatory patients with mild to moderate AD were enrolled in this randomized, double-blind, vehicle-controlled trial between December 2015 and September 2016. VTP-38543 cream in 3 concentrations (0.05%, 0.15%, and 1.0%) or placebo was applied twice daily for 28 days. Pretreatment and post-treatment skin biopsy specimens were obtained from a subset of 33 patients. Changes in SCORing of Atopic Dermatitis, Eczema Area and Severity Index, Investigator's Global Assessment, and tissue biomarkers (by real-time polymerase chain reaction and immunostaining) were evaluated.

Results: Topical VTP-38543 was safe and well tolerated. VTP-38543 significantly increased messenger RNA (mRNA) expression of epidermal barrier differentiation (loricrin and filaggrin, $P = .02$) and lipid (adenosine triphosphate-binding cassette subfamily G member 1 and sterol regulatory element binding protein 1c, $P < .01$) measures and reduced epidermal hyperplasia markers (thickness, keratin 16 mRNA). VTP-38543 nonsignificantly suppressed cellular infiltrates and down-regulated mRNA expression of several T_H17/T_H22 -related (phosphatidylinositol 3, S100 calcium-binding protein A12) and innate immunity (interleukin 6) markers.

Conclusion: Topical VTP-38543 is safe and well tolerated. Its application led to improvement in barrier differentiation and lipids. Longer-term studies are needed to clarify whether a barrier-based approach can induce meaningful suppression of immune abnormalities.

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Introduction

The prevalence of atopic dermatitis (AD) has increased during the last decade to affect 17% to 24% of children and 4% to 10% of adults.^{1,2} AD disease pathogenesis involves T_H2/T_H22 immune activation and barrier dysregulation.^{3–5} The epidermal barrier abnormalities in AD include defects in terminal differentiation (eg, loricrin [LOR], filaggrin [FLG]), tight junctions (TJs), lipids, S100 calcium-binding protein As (S100As), antimicrobial peptides (AMPs),

and epidermal hyperplasia.^{6,7} Compromised epidermal barrier also plays a major role in allergic sensitization, possibly leading to other atopic associations, including asthma, allergic rhinitis, and food allergies.⁸ Topical treatments for AD are limited to emollients, including ceramide-based products,^{9,10} immunosuppressants (corticosteroids, calcineurin inhibitors),^{11,12} and most recently the phosphodiesterase inhibitor crisaborole.¹³ These treatments have limited efficacy or adverse effects that prohibit long-term use. Thus, a large unmet need exists for safer, better topical treatments in both adults and children.

The liver X receptors (LXRs) (isoforms α [NR1H3] and β [NR1H2]) are members of the nuclear receptor superfamily, heterodimerizing with RXR for gene regulation.¹⁴ These transcription factors bind to hormone response elements on target gene regulatory regions.¹⁵ Although LXR β is ubiquitous, LXR α is commonly expressed in liver, adipose tissue, macrophages, intestine, and kidneys.¹⁶ Both isoforms are expressed in skin, specifically in keratinocytes and fibroblasts, and are key regulators of epidermal homeostasis and inflammation. Their activation has been implicated in epidermal differentiation, lipid synthesis, suppression of epidermal proliferation, and attenuation of inflammatory responses, ultimately leading to improved barrier homeostasis and reduced skin inflammation.^{17–19}

The broad effects of LXRs on epidermal barrier and inflammation make them attractive potential drug targets for AD.^{20,21} In vitro and human epidermal keratinocyte studies showed that the LXR ligand T1317 induces LOR and FLG expression.^{22,23} Sterol regulatory element binding protein 1c (SREBP1c) and adenosine triphosphate-binding cassette subfamily G member 1 (ABCG1) genes, critical modulators of lipid homeostasis, are both modulated by LXRs.^{24,25} LXR activation increased ABCG1 messenger RNA (mRNA) expression in model systems,²⁴ and the synthetic LXR agonist T0901317 up-regulated SREBP1c expression,²⁵ induced lipid synthesis,²² and normalized epidermal structure and function in a murine model of AD.²⁶ In addition, LXR activation led to significant reduction in innate immune markers (interleukin 6) in human keratinocytes and in mouse models.^{17,26}

Our objective was to evaluate the safety and tolerability of VTP-38543 cream, a potent LXR β -selective ligand (see pharmacology summary in the eAppendix), and its effects on the epidermal barrier, immune profile, and disease severity of patients with mild to moderate AD. Results showed that topical VTP-38543 is safe and well

tolerated. In addition, significant improvement in barrier measures (terminal differentiation and lipids) and marginal cytokine modulation were observed.

Methods

Study Design, Population, Participant Flow, and Interventions

This randomized, double-blind, vehicle-controlled study was approved by the IRB Services (Aurora, Ontario, Canada) to preliminarily evaluate the safety, clinical efficacy, and molecular skin changes after administration of VTP-38543 in adult patients with mild to moderate AD. Eighty-seven patients with mild to moderate AD (40 male and 47 female; mean age, 34.7 years; mean SCORing of Atopic Dermatitis [SCORAD] score, 38.7; mean Eczema Area and Severity Index [EASI] score, 6.9; mean Investigator Global's Assessment [IGA] score, 2.5), evaluated in 15 outpatient dermatology clinics, completed the study (eFig 1). Study participants gave written and informed consent.

As depicted in Figure 1, 3 ascending VTP-38543 doses (0.05%, 0.15%, and 1.0%) were evaluated. Dose panel 1 included 17 patients with AD in the 0.05% VTP-38543 group and 8 in the matching placebo group (vehicle). Dose panel 2 included 16 patients in the VTP-38543, 0.15%, group and 10 in the matching placebo group (vehicle), whereas dose panel 3 contained 19 in the VTP-38543, 1%, group and 17 in the matching placebo group (vehicle). Dose administration began with dose panel 1, escalating to the next dose to be administered in dose panel 2 after review of safety and tolerability data obtained from the first 9 patients through day 28 in dose panel 1. Likewise, escalation to the highest dose to be administered in dose panel 3 was initiated after review of safety and tolerability data obtained from the first 9 patients through day 28 in dose panel 2.

All compounds were applied to AD lesions (excluding face, scalp, and genitals) twice daily for 28 days. Patients were assessed at days 0 (baseline), 7, 14, 28 (primary end point), and 35 (follow-up) (Fig 1). Several indexes of clinical efficacy were determined: SCORAD, EASI, and IGA. Overall, 52 patients completed treatment using VTP-38543 (0.05%: n = 17; 0.15%: n = 16; 1.0%: n = 19). Thirty-five patients completed treatment with vehicle (placebo) application (dose panel 1: n = 8; panel 2: n = 10; panel 3: n = 17).

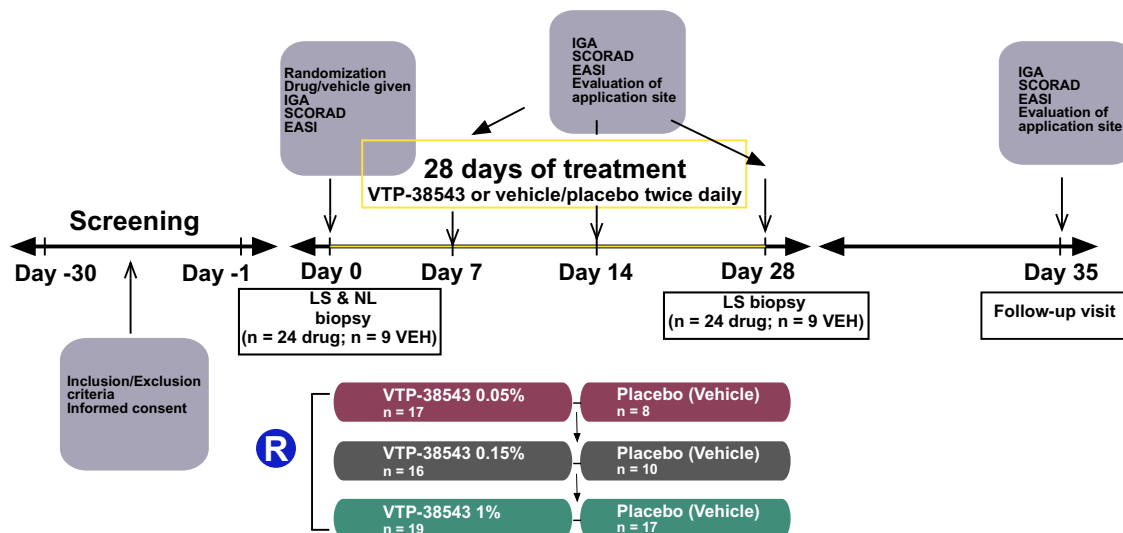


Figure 1. Study design. After a screening period, patients were randomized in a dose escalation study design to 3 ascending VTP-38543 cohorts or matching vehicle (VEH) control (placebo) applied twice daily to atopic dermatitis lesions. EASI indicates Eczema Area and Severity Index; IGA, Investigator's Global Assessment; LS, lesional; NL, nonlesional; and SCORAD: SCORing of Atopic Dermatitis.

Patients treated with systemic immunosuppressants in the last 4 weeks, topical steroids or immunomodulators in the last 2 weeks, and participating in other interventional trials 30 days before enrollment were excluded (eTable 1). Patients were not allowed to use other treatments during the study. Bland nonmedicated emollients were applied to AD lesions for at least 7 days before baseline and entire body (excluding treated lesions) throughout the study.

For immunohistochemical analysis and gene expression assessments, a subset of patients from each active treatment and vehicle arms had undergone 4-mm punch biopsies of lesional and nonlesional tissues (≥ 10 cm from active lesions) at baseline and after 28 days of treatment (lesional only: $n = 33$; VTP-38543: $n = 24$; vehicle: $n = 9$). Clinical efficacy evaluations were performed on the entire cohort ($n = 87$) and on the biopsy subpopulation ($n = 33$). Histologic and gene expression analyses were only performed for the biopsy cohort.

Randomization

After eligibility screening, a computer-generated scheme randomized patients. This was an escalating dose trial, and at each dose level study participants were randomized between active drug and corresponding vehicle. No randomization restrictions were applied. Study investigators, research coordinators, and the patients with AD were masked to treatment allocation until after database lock.

Study Outcomes

The primary objective of this trial was to assess safety and tolerability of VTP-38543 after 28 days of twice-weekly dosing in patients with mild to moderate AD. SCORAD, EASI, and IGA scores were captured before the first dosing and then on days 7, 14, and 28 and during the follow-up visits. The study was not powered for clinical significance, and statistical comparison of efficacy measures was not planned in the protocol. Secondary outcomes included preliminary assessment of biomarker efficacy by obtaining skin biopsy specimens from adult patients before dosing and after 28 days of topical dosing with VTP-38543 to evaluate histologic, immunohistologic, and gene expression changes. Immunostaining, immunofluorescence, and real-time polymerase chain reaction (RT-PCR) were used for biomarker measurements, as detailed below.

Immunostaining and Immunofluorescence

Immunohistochemical staining was performed on frozen optimal cutting temperature (OCT)-embedded cryostat sections using purified mouse anti-human monoclonal antibodies (eTable 3), as previously described.^{27,28} Immunofluorescence staining for neutral lipids with Nile red was performed as previously reported.^{29,30} Epidermal thickness and cell counts were quantified with ImageJ version 1.42 (National Institutes of Health, Bethesda, MD), and immunofluorescence was imaged with MetaView Software (Visitron Systems, Puchheim, Germany).³¹ For further details, see the eAppendix.

Quantitative RT-PCR

RNA was extracted for RT-PCR with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY) as previously described.^{31,32} Expression values were normalized to human acidic ribosomal protein. Primers and probes are listed in eTable 4.

Sample Size

Sample size was estimated based on a minimum number necessary to obtain a preliminary clinical assessment regarding the

drug's safety profile at a given dose, before continuing with dose escalation. It was not based on statistical considerations.

Statistical Analysis

Statistical analyses were performed using Python (<https://www.python.org>) and R (<https://www.r-project.org/>) and associated packages. All 87 participants were included in analyses of clinical efficacy, assessed using percentage change from baseline in SCORAD, EASI, and IGA at 28 days to avoid statistical bias toward patients with high initial severity. Statistical analysis of efficacy data was performed post hoc because only descriptive statistics were planned for the primary analysis. Statistical testing was performed using a 2-sided Wilcoxon rank-sum test. Means and medians of the all the data are given in eTable 5 and eTable 6. Histologic and transcriptional analyses were only available for the biopsy cohort ($n = 33$). Changes in epidermal thickness, cellular infiltrates, and mRNA expressions were quantified as fold changes from baseline and $\log_2$ transformed. A 2-sided Wilcoxon rank-sum test was used to compare vehicle to each VTP-38543 dosage group. Unsupervised hierarchical clustering of variables or samples was performed by using the correlation coefficient as a distance metric with a mean agglomeration algorithm and represented as a heatmap.

Spearman rank correlations were used to correlate clinical responses (percent change in SCORAD or EASI) with histologic and transcriptional markers. Before fold change and $\log_2$ transformation, the half-minimum values of each histologic and transcriptional marker were added to the respective columns to remove zeros from the data.

Responders were defined with a threshold of 25% or greater reduction in SCORAD or EASI. With these criteria, a total of 21 drug-treated responders were identified, 9 of these in the biopsy substudy. Patients receiving placebo were not considered responders, even if they met the criteria.

No formal interim analysis was performed. For dose escalation the sponsor, medical monitor, and medical expert jointly evaluated the safety and tolerability of VTP-38543 after 28 days of dosing of the first 9 patients in each dose panel.

Intolerability was determined by investigator opinion, taking into account all information from adverse events, vital signs, physical examination findings, laboratory abnormalities, electrocardiographic findings, and pharmacokinetic data (if available). Dose-limiting adverse effects were defined as active-drug-related AEs that were considered severe in nature, intolerable, or that, in the opinion of the investigator, the sponsor, and/or the medical monitor, would place individuals at medical risk if a higher dose of VTP-38543 were to be administered. If dose-limiting adverse effects occurred, the remaining doses of that dose panel were not administered and the next higher-dose panel was not initiated.

For additional information, see the eAppendix.

Results

Study Population

A total of 104 patients with mild to moderate AD met eligibility criteria and were randomized in this double-blind, vehicle-controlled trial under institutional review board approved protocols between December 2015 and September 2016. A total of 87 patients completed the study; 52 treated with VTP-38543 (0.05%: $n = 17$; 0.15%: $n = 16$; 1.0%: $n = 19$) and 35 with parallel vehicles. A total of 33 patients participated in the biopsy cohort. Baseline demographics were comparable between groups ($P > .10$ for all) (Table 1), as well as between the biopsy and entire patient cohorts ($P > .10$) (Table 1). Figure 1 and eFigure 1 show an overview of the

Table 1
Baseline Patient Characteristics

Characteristic	VEH (n = 35)	VTP-38543				Full cohort (n = 87)	P value	Biopsy cohort (n = 33)	P value
		0.05% (n = 17)	0.15% (n = 16)	1.00% (n = 19)	Drug (n = 52)				
Sex									
Male	20	6	7	7	20	40	.37	16	.38
Female	15	11	9	12	32	47	.31	17	.35
Race									
White, non-Hispanic	15	10	10	11	31	46	.23	22	.67
Black, non-Hispanic	18	4	2	5	11	29	.90	6	1
Hispanic	0	0	1	0	1	1	.78	0	NA
Asian	2	3	3	3	9	11	.48	5	.48
SCORAD score, mean (SD)	38.6 (11.9)	37.2 (11.5)	43.9 (11.0)	35.8 (10.7)	38.7 (11.6)	38.7 (11.7)	.25	37.0 (10.6)	.25
EASI score, mean (SD)	6.5 (3.8)	6.9 (4.0)	7.8 (4.3)	6.9 (5.3)	7.2 (4.6)	6.9 (4.3)	.34	6.7 (3.3)	.49
IGA score, mean (SD)	2.5 (0.5)	2.6 (0.5)	2.8 (0.4)	2.3 (0.5)	2.5 (0.5)	2.5 (0.5)	.08	2.5 (0.5)	.08
Age, mean (SD), y	31.4 (11.6)	39.1 (15.5)	36.1 (13.3)	35.7 (9.8)	36.9 (13.0)	34.7 (12.8)	.18	35.2 (13.5)	.13
Height, mean (SD), cm	172.4 (7.9)	165.5 (9.3)	169.9 (9.6)	170.6 (9.5)	168.7 (9.7)	170.2 (9.2)	.97	169.9 (11.5)	.83
Weight, mean (SD), kg	84.2 (17.1)	75.0 (16.3)	79.8 (16.3)	83.9 (19.0)	79.8 (17.7)	81.6 (17.6)	.23	78.6 (17.3)	.19
BMI, mean (SD)	28.2 (4.9)	27.4 (5.0)	27.8 (5.1)	28.7 (5.9)	28.0 (5.4)	28.1 (5.2)	.79	27.3 (5.2)	.72

Abbreviations: BMI, body mass index; EASI, Eczema Area and Severity Index; NA, not applicable; SCORAD: Scoring of Atopic Dermatitis; VEH, vehicle.

study design. Table 1 gives the baseline demographics. For additional information see the eAppendix.

Treatment-Emergent Adverse Events

VTP-38543 was generally well tolerated. A total of 68 treatment-emergent adverse events (TEAEs) were reported by 48 patients (46.6%) across all treatments during the study. The number and types of TEAEs were generally similar across treatment regimens, except for application site events, which were nonsignificantly higher in the highest VTP-38543 concentration group of 1% (21% vs 5% in the 2 lower-dose groups and 10% in the vehicle group). All the application site events were considered mild, and no study patient discontinued participation because of this. The most frequently reported TEAEs overall were application site events (n = 14 total) followed by nasopharyngitis (n = 9) and headache (n = 5). TEAEs were mild; there were no reports of severe TEAEs. Administration site pruritus, application site pain, application site reaction, body tinea, postprocedural infection, mild leukopenia, and AD in remote locations were observed in a subset of patients (eTable 2). Adverse events in the drug-treated groups were similar to the vehicle-treated group.

Clinical Measures

Although statistical comparison of efficacy data was not planned, we assessed clinical efficacy, as measured by the change in SCORAD and EASI from baseline to 28 days in the entire cohort (eFig 2A and C), and in the biopsy subgroup (eFig 2B and D). A more negative change in SCORAD and EASI indicates greater improvement. For the entire cohort, VTP-38543 (in any concentration) did not show significant decreases in SCORAD and EASI compared with vehicle ($P > .30$ for all) (eFig 2A and C). In the biopsy arm, the entire drug group had significant decreases in SCORAD compared with vehicle (mean, -17.1% ; median, -21.00% ; $P = .08$) (eFig 2B). Dose-response patterns were not evident, and in the biopsy cohort, 0.05% VTP-38543 notably had greater and more significant decreases in SCORAD and EASI compared with other concentrations ($P \leq .05$) (eFig 2B and D and Table 1).

In both the entire group ($r = 0.83$, $P < .001$) (eFig 2E) and the substudy cohort ($r = 0.85$, $P < .001$) (eFig 2F), SCORAD and EASI improvements were highly correlated using Spearman correlations. For both cohorts, patients with higher baseline disease severity (eFig 2E and 2F) had greater responses. Patients were classified as

responders and nonresponders using a 25% or greater reduction in SCORAD and EASI as the criteria for responders.

Epidermal Pathologic Normalization

To evaluate the barrier effects of VTP-38543 application, we performed RT-PCR on pretreatment and posttreatment lesional and nonlesional biopsy specimens from patients treated with vehicle and 3 ascending doses of VTP-38543. Our panel included epidermal barrier genes reported as LXR-modulated in model systems, including the terminal differentiation genes *LOR* and *FLG* as well as the lipid homeostasis genes *ABCG1* and *SREBP1c*, which are vital for barrier integrity.^{24,25,33,34} VTP-38543 significantly increased *LOR*, *FLG*, *ABCG1*, and *SREBP1c* mRNA expressions vs vehicle ($P \leq .02$ for all) (Fig 2A). Changes in these markers demonstrated clear dose-response effects, with VTP-38543, 0.15% and 1%, showing highest up-regulations (Fig 2B).

To corroborate our RT-PCR results, we performed immunohistochemical analysis. Although baseline AD lesions showed faint, skipped, and irregular FLG expression, increased staining intensity and restoration of continuous FLG expression to baseline nonlesional levels was observed after treatment only in drug-treated but not in vehicle-treated lesions (Fig 2C–H). Because LXR receptor activation plays a major role in lipid metabolism and transport,^{17,35} we used Nile red immunofluorescence staining to enhance visualization of stratum corneum extracellular neutral lipids in baseline lesional and nonlesional AD (Fig 2I and K) vs day 28 AD lesions (Fig 2J) treated with VTP-38543. Contrary to nonlesional skin, which showed extracellular lipids organized in lamellar sheets interspersed between compacted corneocytes and widening of the spaces between lamellar bodies, pretreatment lesions showed decreased stratum corneum corneocyte compaction and reduced lipid layering. Posttreatment lesions demonstrated noticeably augmented layers of extracellular lipids, similar to baseline nonlesional skin.

VTP-38543 Effects on Epidermal Pathologic and Cellular Infiltrates

Immunostaining for hematoxylin-eosin and select epidermal hyperplasia and T-cell and dendritic cell markers was performed in VTP-38543 pretreatment and post-treatment biopsy specimens (representative pictures from a responder are presented in Fig 3). Both baseline lesional and nonlesional skin demonstrated epidermal

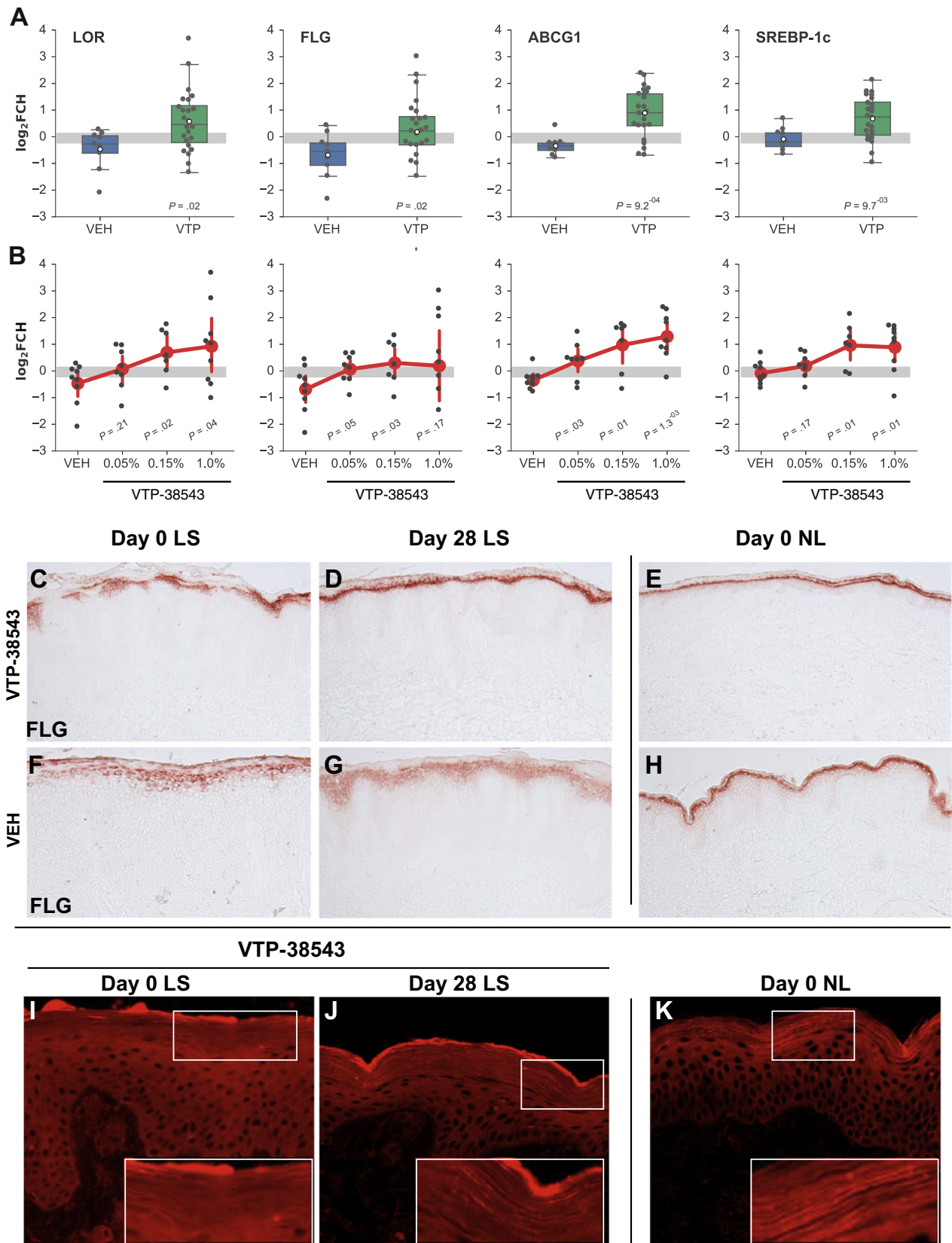


Figure 2. A, Real-time polymerase chain reaction messenger RNA expressions of terminal differentiation and lipid markers. Mean expression estimates normalized to human acidic ribosomal protein are represented in a $\log_2$ fold change (FCH) human acidic ribosomal protein scale. Black horizontal lines in boxes indicate medians, points indicate means, whiskers represent values within 1.5 times the interquartile range, and top and bottom of the boxes represent 75th and 25th percentiles, respectively. B, Dose responses to VTP-38543 for loricrin (LOR), filaggrin (FLG), ABCG1, and SREBP1c. P values indicate comparisons to vehicle (VEH). Red circles represent means, and the range of the red lines represent a 95% confidence interval. The black data points represent individual patients. Representative FLG immunostaining of pretreatment (C, E, F, H) and posttreatment VTP-38543–treated (D) or VEH-treated (G) skin. Representative Nile red immunofluorescence staining of pretreatment (I, K) and posttreatment (J) tissues of a VTP-38543–treated patient (original magnification, $\times 10$). LS indicates lesional; NS, nonlesional.

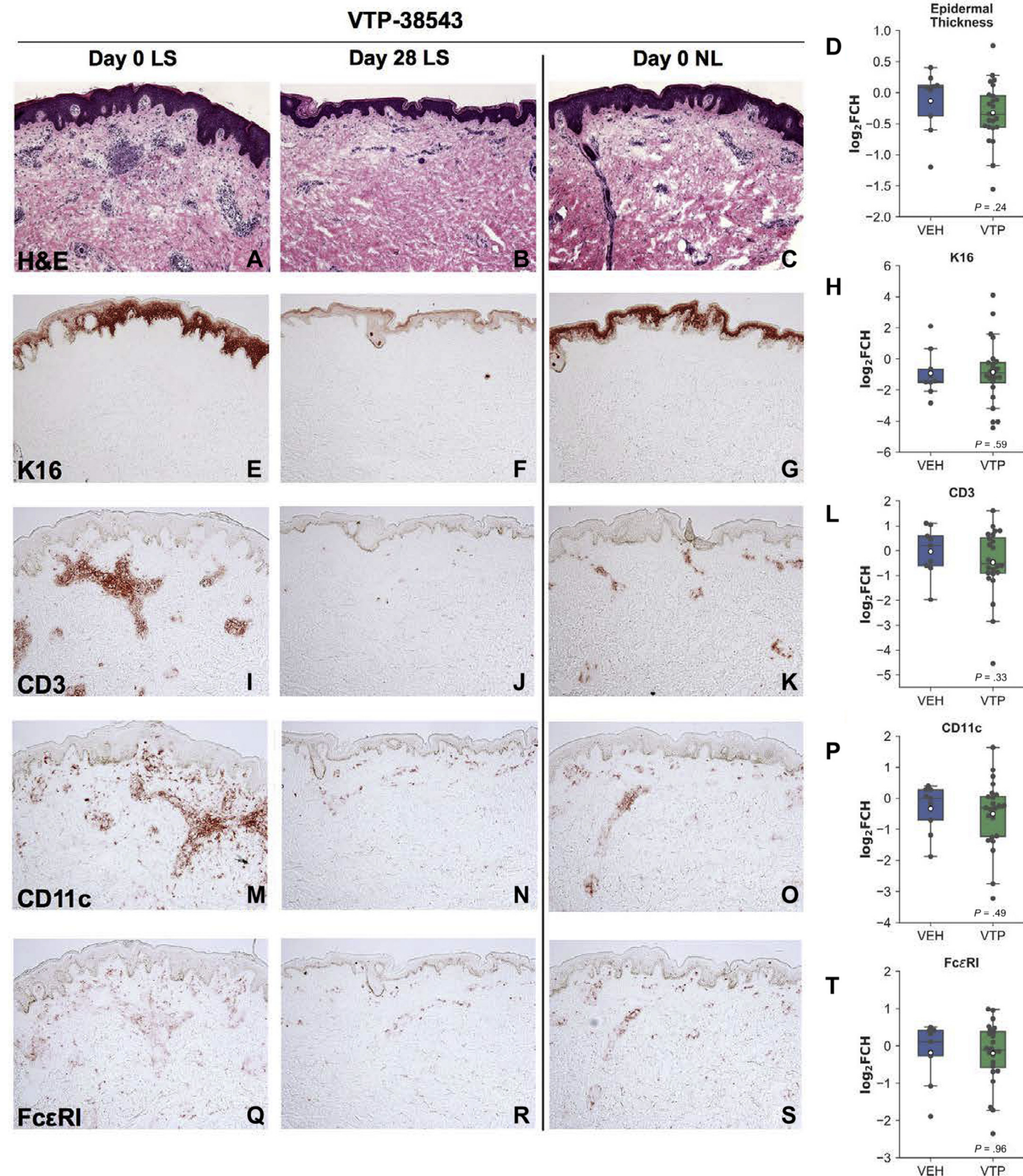


Figure 3. Representative hematoxylin-eosin (H&E; A–C) and immunohistochemical immunostainings (K16, E–G; CD3, I–K; CD11c, M–O; FcεRI, Q–S) of pretreatment nonlesional (NL) skin and pre- and post-VTP-38543-responsive lesions. Box plots are quantified as log₂ fold change (FCH) from day 0 values. Black horizontal lines in boxes indicate medians, white points indicate means, whiskers represent values within 1.5 times the interquartile range, and top and bottom of the boxes represent 75th and 25th percentiles, respectively. *P* values indicate comparisons to vehicle (VEH). LS indicates lesional; mDCs, myeloid dendritic cells.

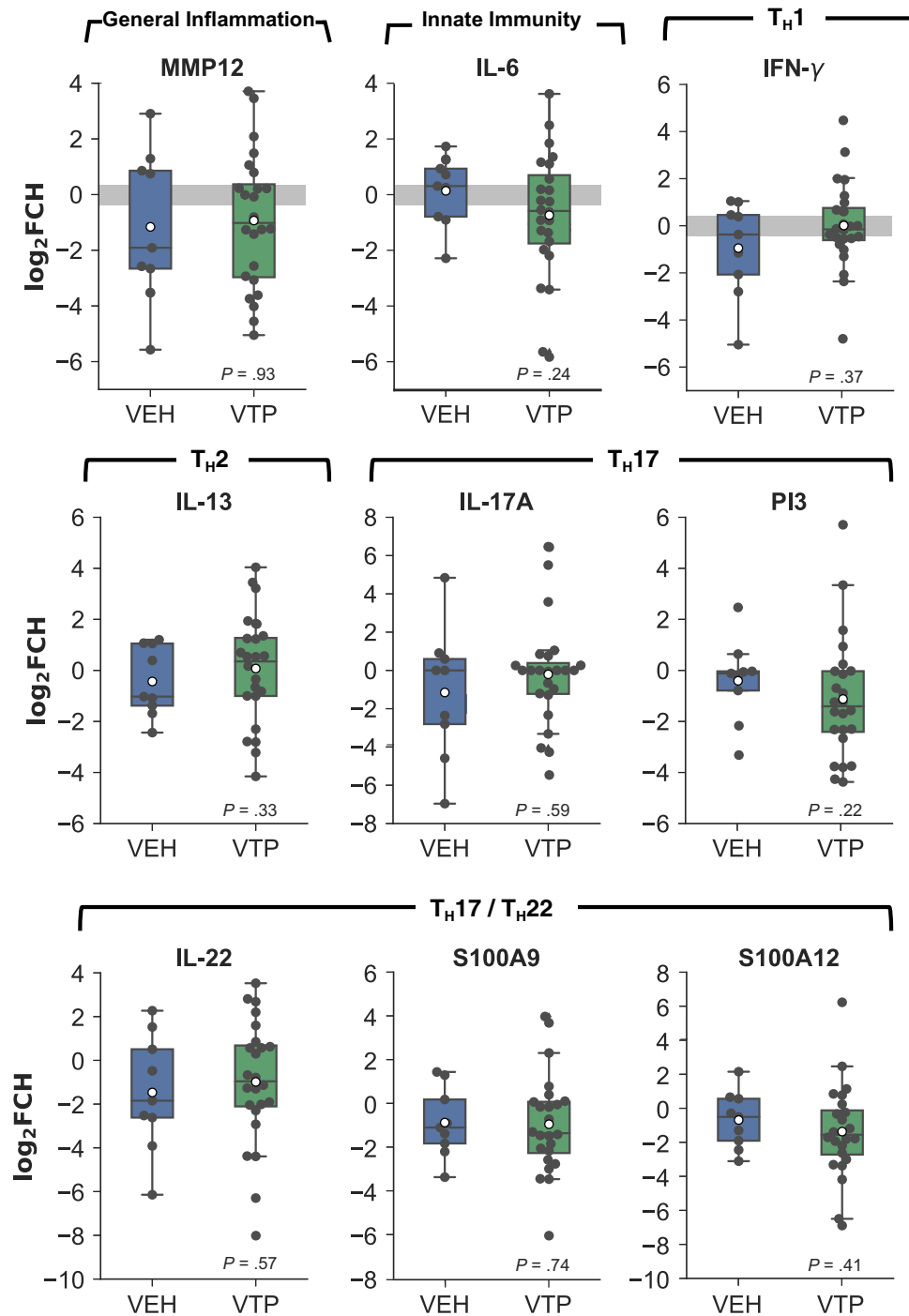


Figure 4. Real-time polymerase chain reaction messenger RNA expressions of major cytokine axes and inflammatory markers. Data were grouped according to the major inflammatory and regulatory pathways represented. Black horizontal lines in boxes indicate medians, white points indicate means, whiskers represent values within 1.5 times the interquartile range, and top and bottom of the boxes represent 75th and 25th percentiles, respectively. Black points indicate individual patients. *P* values indicate comparisons to vehicle (VEH). FCH indicates fold change; PCR, VEH, vehicle.

hyperplasia as seen by the increased epidermal thickness on hematoxylin-eosin and widespread epidermal expression of keratin 16 (K16) (indicating epidermal proliferation) in both lesional and nonlesional baseline tissues (Fig 3A, C, E, and G). At day 28, VTP-38543-treated lesions demonstrated nonsignificant reductions in epidermal thickness from baseline (Fig 3B and D and eFig 3; $P = .24$). Although K16 protein expression by immunostaining largely resolved in drug-responsive lesions (Fig 3E–G) compared with baseline tissues in 12 of 24 VTP-38543-treated patients (50%), mRNA

expression changes were not significant ($P = .59$) (Fig 3H). $CD3^+$ T cells, $CD11c^+$ myeloid dendritic cells, and $FCeRI^+$ dendritic cells were decreased in drug-treated lesional skin (Fig 3L, P, and T) without an associated dose response (eFig 3).

Effect of Short-Term VTP-38543 Application on T_H2 and T_H17 Markers

To evaluate for inflammatory cytokines, we evaluated lesional changes with treatment in mRNA expressions of a panel of T_H1 , T_H2 ,

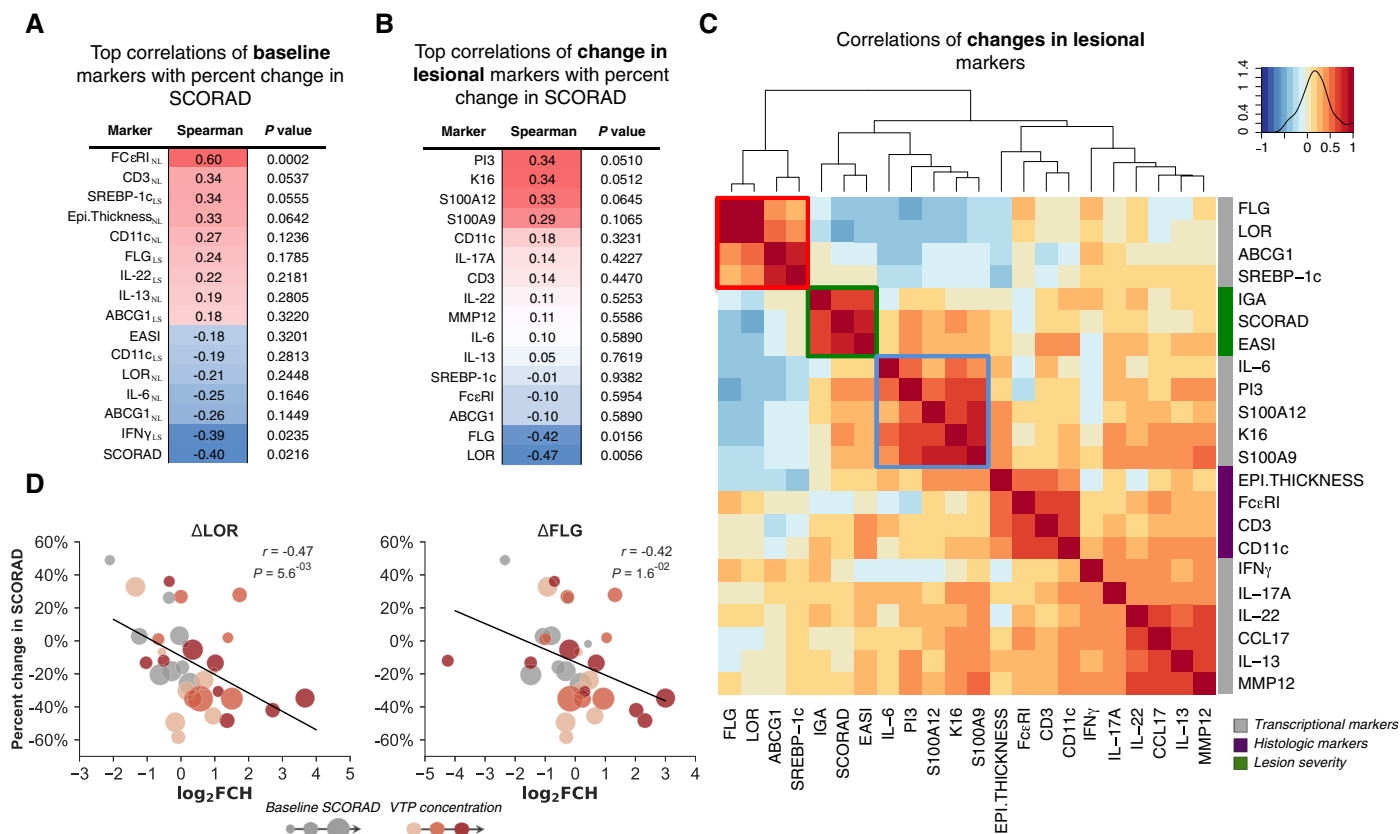


Figure 5. Biomarkers and clinical scores Spearman correlations. A and B, Color intensity represents correlation (red: positive correlation; blue: negative correlation). Color intensity represents the strength of the correlation (–1 to 1). Negative correlations indicate the marker increases with clinical improvement. C, Correlation scatterplots of change in loricrin (LOR) and flaggrin (FLG) with percent change in SCORAD of Atopic Dermatitis (SCORAD) score. D, Spearman correlation heatmap and dendrogram summarizing correlations between changes in clinical (green), transcriptional (grey), and histologic (purple) markers in lesional skin. Red box includes epidermal barrier genes, green box clusters clinical measures, and blue box contains immune markers. Epi indicates epidermal.

and T_H17/T_H22 cytokines by RT-PCR (Fig 4 and eFig 3). Nonsignificant (vs vehicle) reductions in selected T_H17 (phosphatidylinositol 3 [PI3]), innate (interleukin [IL] 6), and T_H17/T_H22-related (S100A12) markers were noted. VTP-38543, 0.05%, demonstrated the greatest reductions vs vehicle for many markers, including in general inflammation (matrix metalloproteinase 12), T_H17 (IL-17A, PI3), and T_H17/T_H22-related markers (IL-22, S100A9, S100A12; $P = 0.05$ for VTP-38543, 0.05%–treated lesions) (eFig 3). IL-6, previously shown to be modulated by LXR synthetic agonists,³⁶ was down-regulated by VTP-38543 application and was the only cytokine that showed dose-response effect (Fig 4 and eFig 3).

Skin Biomarkers Correlate with Drug-Induced Clinical Improvement

To identify biomarkers of response to treatment, we next evaluated associations of different AD severity measures (SCORAD, EASI, IGA) with all evaluated cellular and molecular measures, using unsupervised hierarchical clustering with Spearman correlation coefficients. Figure 5A and B presents positive (red) and negative (blue) correlations between percent change in SCORAD and baseline lesional or nonlesional values or with the change (baseline to day 28) in lesional biomarkers, respectively; the color intensity reflects strength of correlations (full correlation lists appear in eTable 7 and eTable 8). More significant negative correlations with SCORAD improvement in Figure 5A suggest that highest baseline levels are associated with better treatment responses (eg, lesional interferon γ : $r = 0.39$, $P = .02$). Significant positive correlations indicate that higher SCORAD improvements are associated with lower baseline

values. Lower baseline nonlesional cell counts (FCeRI⁺ inflammatory dendritic epidermal cells: $r = 0.60$, $P < .001$; CD3⁺ T cells: $r = 0.34$, $P = .05$), nonlesional epidermal thickness ($r = 0.33$, $P = .06$), and pretreatment lesional SREBPc1 mRNA levels ($r = 0.34$, $P = 0.05$) were most strongly positively correlated with treatment response (percent change in SCORAD). In Figure 5B (changes in lesional skin only), greater positive correlations suggest positive correlations between decreases in biomarkers and SCORAD improvement, and negative correlations indicate an inverse relationship, with greater decreases or increases in biomarkers correlated with higher clinical improvement. The strongest positive correlations between treatment response and change in lesional biomarkers were with the IL-17-related marker PI3 ($r = 0.34$, $P = 0.05$) and the proliferation marker K16 ($r = 0.34$, $P = 0.05$), signifying greatest reductions in these markers with treatment response. The most significant negative correlations (and highest overall correlations) with percent change in SCORAD were with the epidermal differentiation markers LOR ($r = -0.47$, $P = 0.005$) and FLG ($r = -0.42$, $P = .01$) (Fig 5B and C), implying greatest increases in these markers with treatment response. Parallel data with percent change in EASI scores appear in eFigure 4.

Correlations between percent change in clinical measures (SCORAD, EASI, IGA) with lesional log₂-fold changes in cellular and molecular markers are summarized as a heatmap in Figure 5D. Changes in the barrier markers FLG, LOR, ABCG1, and SREBP1c all clustered together and were adjacent to changes in clinical measures. Another nearby cluster consisted of changes in immune measures (IL-6, PI3, K16, S100As). All correlations are detailed in eFigure 7. Individual representative of the SCORAD and EASI correlation plots appear in eFigures 5 and 6.

Discussion

AD pathogenesis involves T_H2/T_H22 cytokine dysregulation and epidermal barrier abnormalities.^{4,37} The barrier abnormalities in AD include increased epidermal proliferation and inhibition of terminal differentiation and lipid secretion.^{28,38,39} Prior studies in model systems showed that LXR agonists have a dual function. They improve epidermal barrier integrity increasing lipid secretion, lamellar body formation, and keratinocyte differentiation as well as reduce epidermal proliferation, coupled with anti-inflammatory properties, including modulation of innate (inducible nitric oxide synthase, IL-1, tumor necrosis factor) and innate immune (IL-6) measures.^{19,36,40} The combined effects on barrier and immune regulation make LXR agonists appealing therapeutic options for AD.^{37,41,42} However, no data on LXR agonists are available in human skin and specifically in AD tissues.

Expression levels of barrier measures (LOR, FLG, lipids) have been previously reported to strongly negatively correlate with both AD clinical severity and immune measures,^{28,43,44} highlighting the importance of the barrier-immune interplay in creating the AD phenotype. This is further delineated by correlations between early defects in functional barrier impairment (by transepidermal water loss) in young children with AD and clinical and immune measures.⁴⁵ Furthermore, multiple genes involved in diverse aspects of the barrier integrity are dysregulated in both involved and uninvolved AD skin, including FLG.^{28,43,46} FLG and its degradation products have vital roles in skin hydration, pH preservation, barrier permeability, and microbial defence.^{47,48} Patients with FLG mutations have earlier AD onset, more severe disease,^{49–51} and increased allergen sensitization.⁵¹ FLG expression is amenable to up-regulation using different compounds in mice models^{52,53} and in humans using topical emollients, glucocorticosteroids, or calcineurin antagonists.^{10,54} A recent study in mice and keratinocyte cultures suggested that a barrier-enhancing product may also alleviate inflammation, using a topical compound JTC801, which promoted FLG expression, and attenuated AD-like skin inflammation.⁵² A topical LXR agonist in mice improves epidermal barrier measures, reducing epidermal proliferation and increasing terminal differentiation markers (LOR, FLG).²¹ Such an approach could be beneficial in human AD, where safe and effective topical treatments that improve barrier integrity and reduce skin inflammation are lacking.^{38,55}

Abnormal lipid composition was demonstrated in lesional and nonlesional AD skin.⁶ SREBP1c and ABCG1 are critical players in lipid homeostasis and epidermal barrier integrity maintenance. SREBP1c, linked to regulation of fatty acid and cholesterol metabolism,⁵⁶ was suggested to participate in lipid down-regulation of AD, and a prior study displayed down-regulation of SREBP1 in human AD skin.⁵⁷ LXR activation was shown to increase ABCG1 and SREBP1c expression in model systems.^{24,25} In addition, topical LXR activators increased fatty acids, epidermal cholesterol and sphingolipid synthesis, and LB secretion in model systems.^{24,34}

This is the first randomized, double-blind, vehicle-controlled study that evaluates the safety, tolerability, and molecular changes of 3 ascending doses (0.05%, 0.15%, and 1%) of a topical LXR agonist, VTP-38543, in adults with mild to moderate AD. This is the first proof in humans that an LXR agonist can significantly modulate terminal differentiation (LOR, FLG) and lipid (ABCG1, SREBP1c) measures, increasing them in a concentration-dependent fashion, further highlighted by the highest correlations between VTP-38543 treatment responses and lesional mRNA changes in LOR and FLG. We also demonstrated using Nile red staining that AD lesions regained nearly normal lipid architecture and distribution after treatment, further supporting the barrier restorative effects of VTP-38543 treatment. This is also the first study that tests whether improvement of the barrier profile can lead to AD resolution and restore the immune imbalance.

AD possibly develops in conjunction with allergen penetration through a compromised barrier, leading to other atopic disorders in 50% of patients with AD.^{8,38} Ongoing efforts are made to identify the optimal approach to prevent AD and potentially the entire atopic march. Several preliminary studies found that early barrier repair through intense emolliation reduces AD development.^{58,59} We found that the inert moisturizer petrolatum improved barrier function with increased FLG and AMP expression in adults with and without AD.¹⁰ Because VTP-38543 has beneficial effects of epidermal barrier, in addition to its possible uses for treatment of AD, it would be interesting to study if it could prevent AD and other atopic disorders.

Although in model systems LXR agonists also ameliorate inflammation, we observed only modest, non-dose-dependent anti-inflammatory effects with VTP-38543, including trends of reduction in several T_H17/T_H22 -related (PI3, S100A12) and innate (IL-6) markers, with greatest correlations with treatment responses seen with changes in PI3. These results are in line with the modest changes observed in clinical efficacy parameters. Interestingly, changes in IL-6, PI3, and S100A12 clustered together and adjacent to changes in clinical measures, as well as to changes in barrier parameters FLG, LOR, ABCG1, and SREBP1c, inferring that clinical alleviation with VTP-38543 relates to improvement of both epidermal barrier and cytokine dysregulation disease aspects. The lack of significant changes in inflammatory measures despite the significant barrier improvement could potentially be attributed to the short-term (4 weeks) application of the study product. Although this period is sufficient to modulate epidermal measures, which are direct drug targets, downstream inhibition of inflammatory networks and clinical improvement may require longer treatment periods. Intriguingly, significant clinical improvement was exclusively observed in the biopsy cohort, which may possibly reflect the increased adherence in this patient subgroup. However, the lack of clinical improvement in the entire patient cohort highlights the importance of immune dysregulation as a primary driver of the AD phenotype.

In summary, short-term application of the LXR agonist VTP-38543 cream was safe and well tolerated. In addition, it had a dose-response epidermal barrier restoration, with marginal down-regulation of $T_H17/T_H22/T_H2$ -related markers in lesions of patients with mild to moderate AD. Larger studies that account for baseline AD severity will need to determine whether prolonged treatment is also able to modulate the characteristic immune dysregulation of AD and the prophylactic potential of this compound in atopic disorders.

Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.anai.2018.03.013>.

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Supplementary Data

Nonclinical Pharmacology Summary

VTP-38543 is a potent liver X receptor (LXR) β -selective ligand that is also selective against other nuclear hormone receptors. Its binding affinity for LXR α and LXR β is 295 and 18 nM, respectively, and in cell-based transfection assays, its concentration that gives half-maximal response for transcriptional activation is 283 nM for LXR α and 12 nM for LXR β .

Supplementary Methods

A total of 169 patients were assessed for eligibility, and 104 patients who met all criteria required (eTable 1) were randomized in this double-blind, vehicle-controlled trial (NCT02655679) under institutional review board–approved protocols. Three ascending VTP-38543 doses (0.05%, 0.15%, and 1%) were evaluated in 3 patient cohorts. Dose panel 1 enrolled 30 patients, randomizing 21 to VTP-38543, 0.05%, and 9 to matching placebo (vehicle). Dose panel 2 enrolled 30 patients and randomized 19 to VTP-38543, 0.15%, and 11 to matching placebo (vehicle). Dose panel 3 enrolled 44 patients and randomized 24 to VTP-38543, 1%, and 20 to a matching vehicle. Dose administration began in dose panel 1 with each patient receiving VTP-38543, 0.05%, or matching vehicle. Dosing was twice daily for 28 consecutive days. Escalation to the next highest dose to be administered occurred after review of the safety and tolerability data obtained from the first 9 patients through day 28. Review of the safety and tolerability data from the first 9 patients in dose panel 3 was also done to ensure continued safety.

A total of 87 patients with mild to moderate atopic dermatitis (AD) (40 men and 47 women; mean age, 34.7 years; mean SCORing of Atopic Dermatitis [SCORAD] score, 38.7; mean Eczema Area and Severity Index [EASI] score, 6.9; mean Investigator's Global Assessment [IGA] score, 2.5) completed the trial. Of all patients allocated to VTP-38543, 1 was withdrawn because of inability to comply with study protocol, 1 was withdrawn because of a positive pregnancy test result, 3 patients were lost to follow-up, 6 patients withdrew consent, and 1 patient discontinued the intervention because of adverse effects. Thus, 52 patients completed VTP-38543 treatment and were analyzed (dose panel 1: $n = 17$; dose panel 2: $n = 16$; dose panel 3: $n = 19$). Of all patients allocated to placebo (vehicle), day 0 SCORAD was unavailable for 1, 2 were lost to follow-up, and 2 patients withdrew consent; thus, 35 patients completed treatment with vehicle (dose panel 1: $n = 8$; dose panel 2: $n = 10$; dose panel 3: $n = 17$). Patients were required to apply a bland nonmedicated emollient on skin, including AD lesions, for at least 7 days before day 0 and continue at the same frequency on their entire body, excluding the AD lesions being treated, throughout the study (the application of the bland emollient continued throughout the study for AD lesions not treated with study drug). Several indexes of clinical efficacy were determined: SCORAD, EASI, and IGA.

Patients treated with systemic immunosuppressants in the last 4 weeks, patients treated with topical steroids or immunomodulators in the last 2 weeks, and patients participating in other interventional trials 30 days before treatment were excluded (eTable 1). A computer-generated randomization scheme allocated patients to each drug panel or vehicle, and patients and investigators were masked to treatments. Study drug was administered by designated site staff in the morning of scheduled visit days, whereas evening doses were applied by the patients at home. Most adverse events were mild, and no serious adverse events were reported in all groups (eTable 2).

For biomarker assessments, 4-mm punch biopsy specimens of paired nonlesional (>10 cm from active lesions) and lesional tissues

($n = 33$; 24 from drug, 9 from vehicle) were taken on day 0. On day 28, only lesional biopsies ($n = 33$; 24 from drug, 9 from vehicle) were obtained. Analysis of clinical efficacy was performed on the full cohort (87 patients) and the biopsy substudy cohort (33 patients). Histologic and transcriptional analyses were performed for the biopsy substudy cohort only. Baseline demographics among vehicle and treatment groups did not significantly differ from either the full study cohort ($P > .10$ for all (Table 1) or the biopsy substudy cohort ($P > .10$) (Table 1). See Figure 1A and eFigure 1 for overview of study design.

Immunohistochemistry

Immunohistochemical (IHC) staining was performed on frozen ornithine carbamoyltransferase-embedded cryostat sections using purified mouse anti-human monoclonal antibodies (eTable 3), as previously described.^{1,2} Immunofluorescence staining for neutral lipids with Nile red was performed as previously reported.^{3–5} For IHC analysis, biotin-labeled horse anti-mouse secondary antibodies (Vector Laboratories, Burlingame, CA) were used to detect the primary antibodies, and the staining signal was developed with chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich, St Louis, MO). Epidermal thickness and positive cells per millimeter were manually quantified by using computer-assisted image analysis software (ImageJ, version 1.42; National Institutes of Health, Bethesda, MD).

Real-time polymerase chain reaction

RNA was extracted for real-time polymerase chain reaction with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY) as previously described.^{6,7} Expression values were normalized to the housekeeping gene *hARP*. Primers and probes are listed in eTable 4.

Supplementary Statistical Methods

Normalization and correlation analysis

Statistical analysis was performed using Python's *scipy* and *sklearn* statistics packages.^{8,9} Figures were generated using *heatmap3* in R Studio and Python's *seaborn* and *matplotlib*.^{10–12} All 87 patients were included in analyses of clinical efficacy of drug during the study course, assessed using the 28-day percent change in SCORAD and EASI (percent change = day 28/day 0 – 1). Percent change was chosen to avoid statistical bias toward patients with high initial severity. Correlations of disease severity metrics (EASI, SCORAD, IGA) were obtained using the Spearman rank correlation coefficient. This test was chosen because of the low sample size and nonnormal distribution of these parameters.

Histologic and transcriptional analyses was performed for only the biopsy substudy cohort. First, zeros in both the transcriptional and histologic data were removed by imputing the lower threshold of detection. For each marker, the minimum value across all conditions and time points was computed. After identifying the overall minimum value for each feature, half this value was added to every measurement of that feature vector to remove zeros from the data set. After this transformation, fold changes (from day 0) for epidermal thickness, cellular infiltrates, and messenger RNA expression change values were calculated, then $\log_2$ transformed to give the 28-day log fold change for each marker. A 2-sided Wilcoxon rank-sum test was used to compare controls and treatment subgroups and the treatment group as a whole. This test was chosen because of the low sample size and nonnormal data distribution of these biomarkers. The Spearman rank correlations were used to correlate clinical responses (percent change) with histologic and transcriptional markers ($\log_2$ fold change).

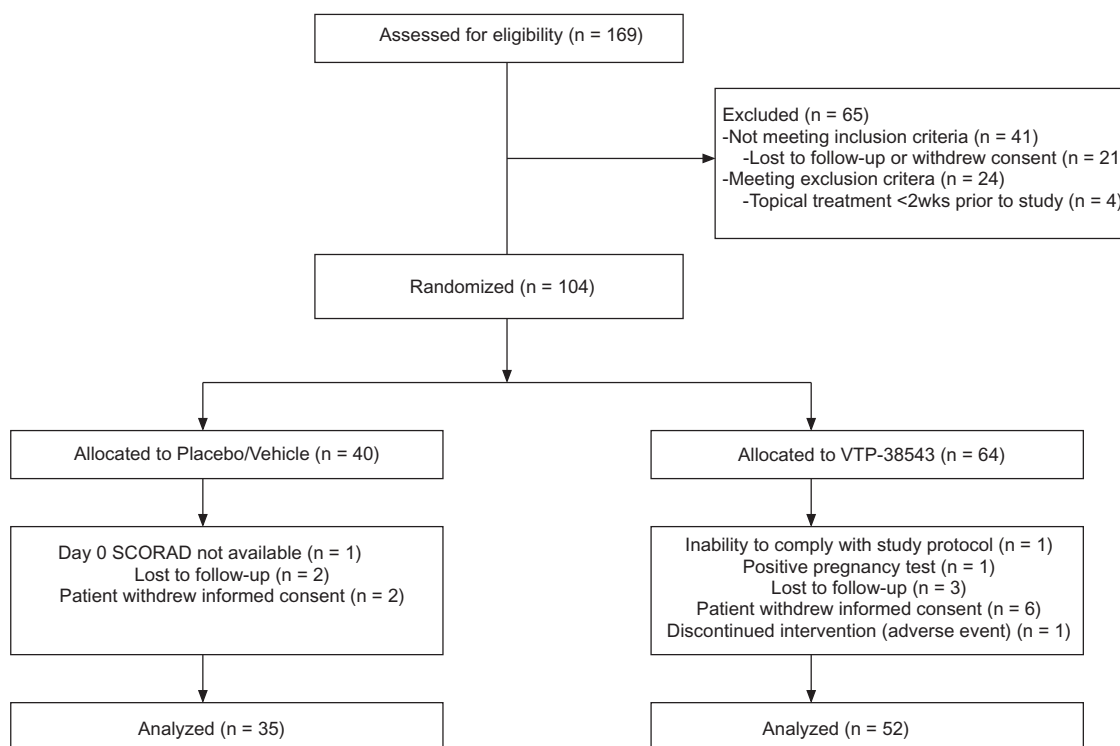
Responder analysis

To identify molecular predictors of drug response, we sought to identify a subset of drug-treated patients who saw the greatest

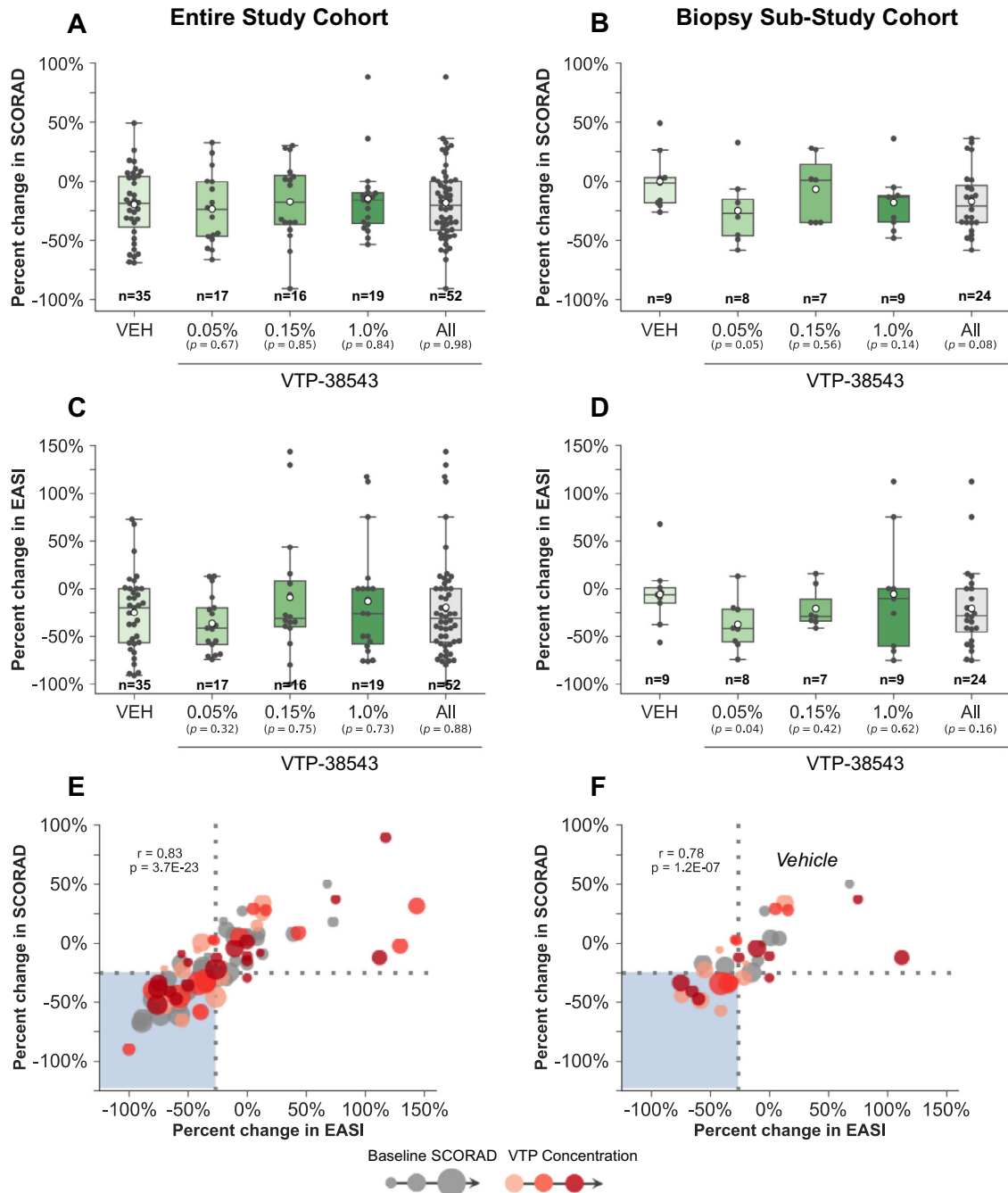
clinical improvement in lesion severity. On the basis of the EASI and SCORAD SD, patients were defined as clinical responders if they had a greater than 25% reduction in severity by both SCORAD and EASI metrics. With these criteria, a total of 21 drug-treated responders were identified from the full study cohort. Of these, 9 belonged to the biopsy substudy cohort. Patients receiving placebo were not considered responders, even if severity reduction criteria were met.

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eFigure 1. CONSORT diagram of patient disposition. AD indicates atopic dermatitis; SCORAD: SCORing of Atopic Dermatitis.



eFigure 2. Clinical improvement with VTP-38545 and vehicle (VEH). A-D, Box plots depicting clinical responses to VEH and VTP-38545, 0.05%, 0.15%, and 1%, groups and the entire VTP-38543 study cohort, measured as percent change in SCORAD and Eczema Area and Severity (EASI) score. A more negative percent change in SCORAD or EASI scores indicates a greater reduction in clinical severity. All *P* values indicate comparisons to VEH. Black horizontal lines in boxes indicate medians, white points indicate means, whiskers represent values within 1.5 times the interquartile range, and the top and bottom of boxes represent 75th and 25th percentiles, respectively. Black points represent individual patients. Scatter plot showing correlations between percent changes in SCORAD and EASI scores for the entire study cohort (E) and the biopsy sub-study cohort (F). The shaded blue region, defined by 25% reduction in both SCORAD and EASI scores, categorizes clinical responders. Bubble size indicates baseline SCORAD. Bubble color represents drug strength.

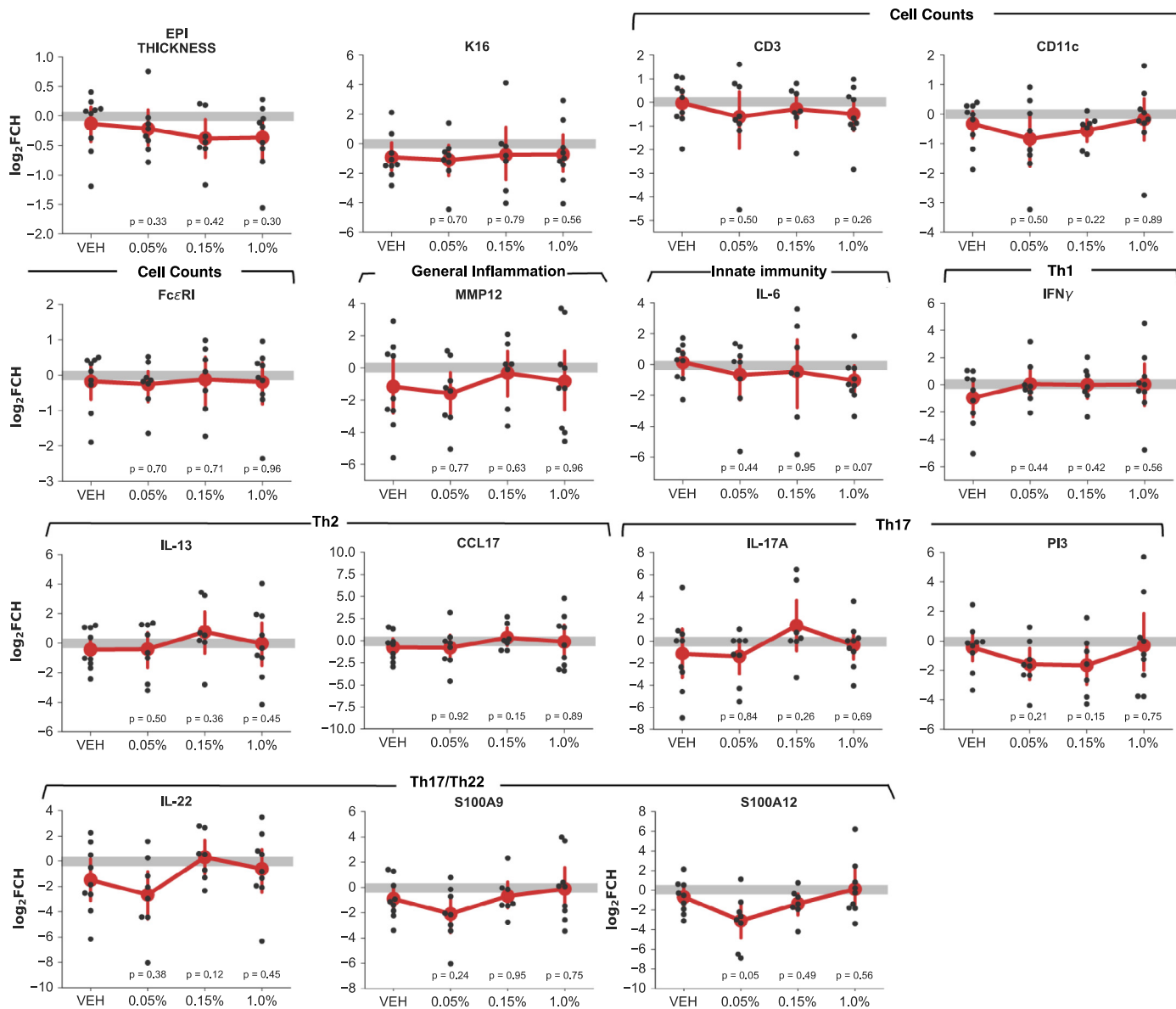
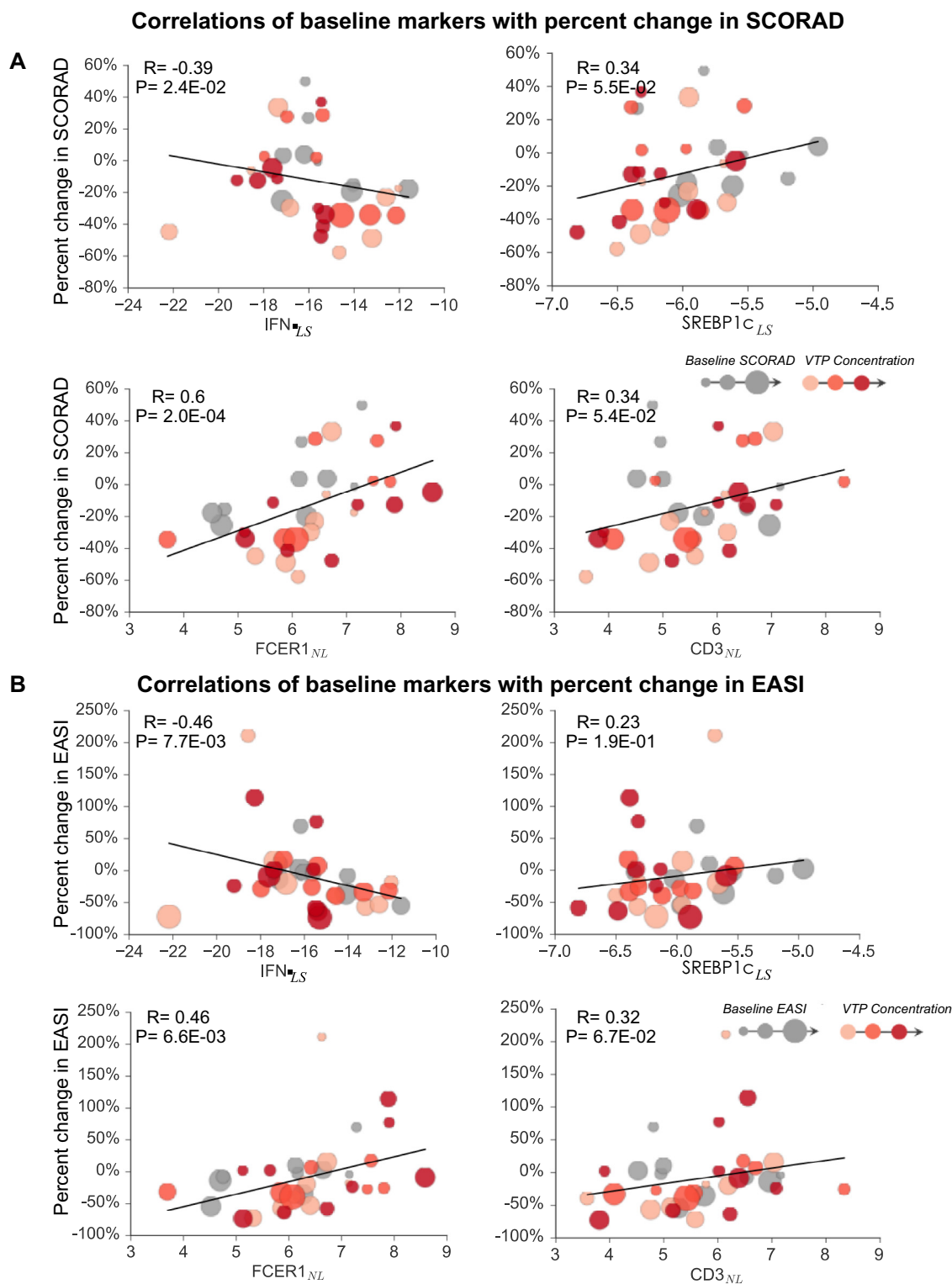


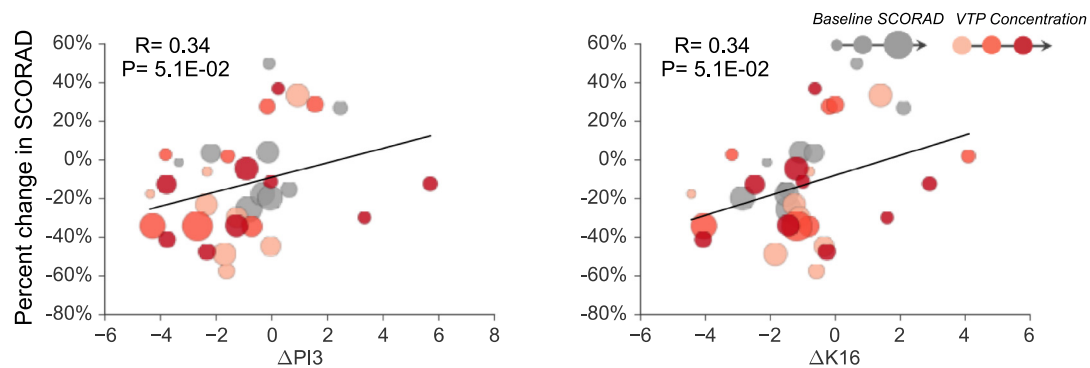
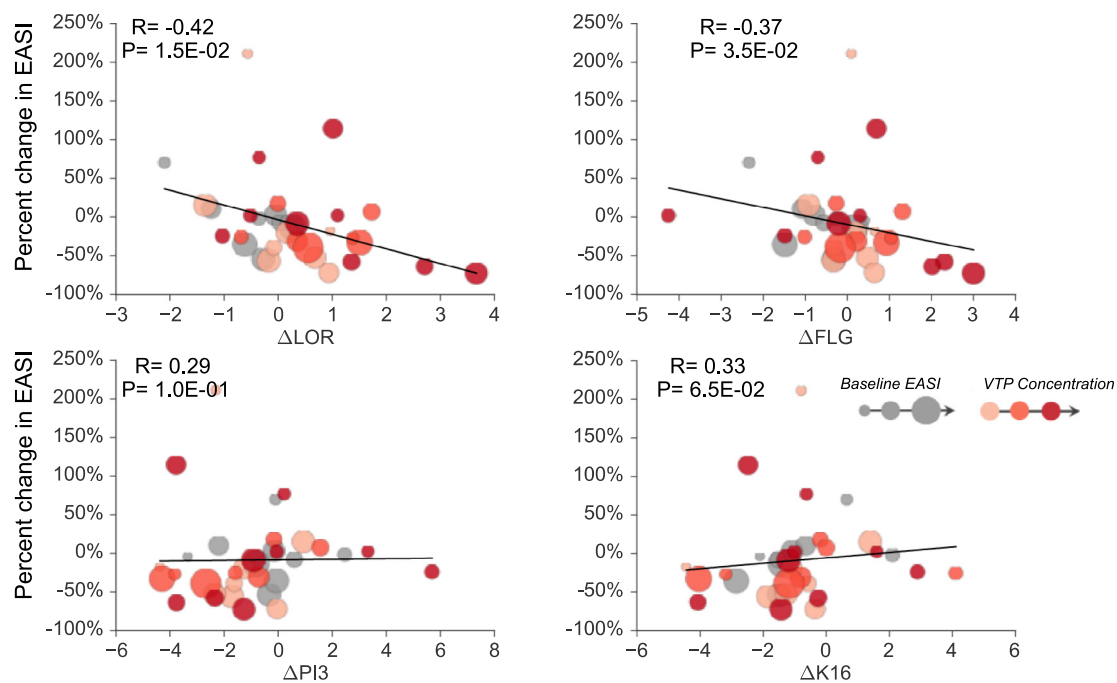
Figure 3. Dose responses of epidermal measures, cellular infiltrates, and real-time polymerase chain reaction–derived messenger RNA expression of major cytokine axes and inflammatory markers represented as line graphs. Data were grouped according to the major inflammatory or regulatory pathway represented. Red points represent means, and vertical red lines represent a 95% confidence interval. Black points indicate individual patients. *P* values indicate comparisons with vehicle (VEH). FCH indicates fold change; IFN, interferon; IL, interleukin; K16, keratin 16; MMP, matrix metalloproteinase; PI3, phosphatidylinositol 3.

Correlations of baseline markers with percent change in EASI			Correlations of change in markers with percent change in EASI		
marker	spearman	p-value	marker	spearman	p-value
FCεRI _{NL}	0.45	0.0083	K16	0.33	0.0647
CD3 _{NL}	0.30	0.0897	PI3	0.29	0.1016
ABCG1 _{LS}	0.30	0.0914	IL-17A	0.26	0.1498
CD11c _{NL}	0.27	0.1278	CD11c	0.22	0.2113
Epi.Thickness _{NL}	0.23	0.1962	CD3	0.22	0.2293
IGA	-0.23	0.2041	S100A9	0.21	0.2398
MMP12 _{LS}	-0.23	0.2026	S100A12	0.20	0.2729
CCL17 _{LS}	-0.24	0.1880	MMP12	0.16	0.3764
EASI	-0.24	0.1847	IL-13	0.13	0.4800
ABCG1 _{NL}	-0.25	0.1551	IL-22	0.10	0.5793
SREBP-1c _{NL}	-0.26	0.1459	CCL17	0.06	0.7283
IL-13 _{LS}	-0.26	0.1362	SREBP-1c	-0.07	0.6801
SCORAD	-0.32	0.0678	FcεRI	-0.08	0.6398
CD11c _{LS}	-0.34	0.0500	ABCG1	-0.09	0.6148
IFN _{LS}	-0.35	0.0479	FLG	-0.37	0.0350
CD3 _{LS}	-0.40	0.0206	LOR	-0.42	0.0154

eFigure 4. Spearman rank correlations between percent change in Eczema Area and Severity Index (EASI) and baseline lesional and nonlesional as well as change in lesional (LS) markers. Color intensity represents correlation (red: positive correlation; blue: negative correlation). Negative correlations indicate the marker increases with clinical improvement. All values are log₂. EPI indicates epidermal; IFN, interferon; IL, interleukin; K16, keratin 16; MMP, matrix metalloproteinase; PI3, phosphatidylinositol 3. SCORAD, SCORing of Atopic Dermatitis.



eFigure 5. Spearman correlations between messenger RNA expression of markers at baseline in lesional (LS) or nonlesional skin with percent change in SCORing of Atopic Dermatitis (SCORAD) (A) or Eczema and Area Severity Index EASI (B). A more negative percent SCORAD or EASI change indicates greater reduction in clinical severity. Bubble size indicates baseline SCORAD and EASI scores, whereas bubble color represents drug concentration.

A Correlations of change in lesional markers with percent change in SCORAD**B Correlations of change in lesional markers with percent change in EASI**

eFigure 6. Spearman correlation scatterplots between changes in messenger RNA expression of markers in lesional (LS) skin with treatment and percent change in SCORing of Atopic Dermatitis (SCORAD) (A) or Eczema and Area Severity Index (EASI) (B) score. A more negative percent SCORAD or EASI change indicates greater reduction in clinical severity. Bubble size indicates baseline SCORAD and EASI scores, whereas bubble color represents drug concentration.

	IGA	SCORAD	EASI	K16	IL6	IL13	FLG	ABCG1	SERBP1c	IL17A	IFNg	IL22	CCL17	LOR	PI3	MMP12	S100A9	S100A12	EPI.THICK	CD3	CD11c	FCER1
IGA	1	8.57E-05	0.0001	0.882	0.381	0.568	0.390	0.716	0.742	0.333	0.263	0.154	0.723	0.239	0.373	0.356	0.894	0.517	0.723	0.499	0.181	0.442
SCORAD	0.630	1	1.18E-07	0.051	0.589	0.762	0.016	0.601	0.941	0.423	0.931	0.525	0.928	0.006	0.047	0.559	0.106	0.065	0.598	0.447	0.323	0.595
EASI	0.614	0.775	1	0.081	0.503	0.168	0.024	0.356	0.728	0.160	0.640	0.168	0.413	0.049	0.036	0.189	0.096	0.107	0.321	0.037	0.036	0.872
K16	0.027	0.342	0.308	1	0.003	0.050	0.029	0.427	0.640	0.008	0.963	0.208	0.047	0.010	4.39E-05	0.033	3.36E-07	3.88E-05	0.016	0.277	0.083	0.802
IL6	-0.157	0.098	0.121	0.494	1	0.376	0.013	0.008	0.191	0.191	0.322	0.833	0.378	0.029	0.002	0.559	0.011	0.073	0.065	0.067	0.825	0.438
IL13	0.103	0.055	0.246	0.344	0.159	1	0.486	0.943	0.690	0.008	0.125	0.002	5.10E-06	0.795	0.039	2.77E-06	0.048	0.399	0.182	0.089	0.016	0.068
FLG	-0.155	-0.417	-0.393	-0.381	-0.430	-0.13	1	0.006	0.075	0.735	0.095	0.656	0.500	1.49E-14	0.002	0.708	0.093	0.023	0.163	0.780	0.848	0.256
ABCG1	-0.066	-0.095	-0.166	-0.143	-0.453	0.01	0.465	1	4.30E-10	0.507	0.185	0.779	0.971	0.004	0.041	0.918	0.564	0.390	0.109	0.137	0.672	0.864
SERBP1c	-0.060	-0.013	-0.063	-0.085	-0.234	0.07	0.315	0.849	1	0.272	0.328	0.421	0.386	0.024	0.209	0.676	0.708	0.392	0.030	0.506	0.888	0.962
IL17A	0.174	0.144	0.250	0.454	0.234	0.46	0.061	0.120	0.197	1	0.013	0.015	0.017	0.902	0.230	0.014	0.041	0.479	0.588	0.079	0.012	0.435
IFNg	0.200	-0.016	-0.084	0.008	-0.178	0.27	0.296	0.237	0.176	0.429	1	0.024	0.008	0.381	0.677	0.029	0.698	0.710	0.475	0.564	0.203	0.459
IL22	0.254	0.115	0.246	0.225	0.038	0.52	0.081	0.051	0.145	0.420	0.392	1	2.07E-05	0.549	0.218	6.54E-06	0.019	0.089	0.675	0.340	0.147	0.142
CCL17	0.064	0.016	0.147	0.349	0.159	0.70	-0.122	0.007	0.156	0.414	0.453	0.669	1	0.530	0.273	1.07E-05	0.035	0.074	0.327	0.137	0.023	0.053
LOR	-0.211	-0.472	-0.346	-0.443	-0.380	-0.05	0.925	0.487	0.391	0.022	0.158	0.108	-0.113	1	0.015	0.835	0.114	0.028	0.101	0.997	0.721	0.268
PI3	0.160	0.348	0.366	0.649	0.530	0.36	-0.509	-0.358	-0.225	0.215	-0.075	0.220	0.197	-0.421	1	0.046	2.57E-05	0.001	0.496	0.429	0.345	0.218
MMP12	0.166	0.106	0.235	0.373	0.106	0.72	0.068	-0.019	0.076	0.426	0.381	0.697	0.685	0.038	0.350	1	0.003	0.096	0.346	0.074	0.016	0.115
S100A9	0.024	0.287	0.294	0.757	0.438	0.35	-0.297	-0.104	-0.068	0.358	0.070	0.406	0.369	-0.280	0.663	0.500	1	1.05E-07	0.010	0.352	0.214	0.614
S100A12	0.117	0.326	0.286	0.652	0.316	0.15	-0.394	-0.155	-0.154	0.128	-0.067	0.300	0.315	-0.383	0.561	0.295	0.777	1	0.015	0.513	0.429	0.686
EPI.THICKNESS	-0.064	0.095	0.178	0.417	0.325	0.24	-0.248	-0.284	-0.379	0.098	-0.129	-0.076	0.176	-0.291	0.123	0.169	0.442	0.421	1	0.001	0.009	0.000
CD3	0.122	0.137	0.365	0.195	0.323	0.30	-0.050	-0.265	-0.120	0.310	0.104	0.171	0.265	-0.001	0.142	0.315	0.167	0.118	0.551	1	6.08E-06	0.000
CD11c	0.239	0.177	0.367	0.306	0.040	0.42	0.035	-0.077	0.025	0.433	0.228	0.258	0.395	0.065	0.170	0.414	0.222	0.142	0.448	0.699	1	0.000
FCER1	-0.139	-0.096	0.029	0.045	-0.140	0.32	0.204	0.031	-0.009	0.141	0.133	0.261	0.340	0.199	-0.220	0.279	0.091	0.073	0.585	0.616	0.602	1

Figure 7. Spearman Correlation Matrix of Changes in Clinical, Transcriptional, and Histologic Markers After Treatment With VTP-38543^a. EASI, Eczema Area and Severity Index; EPI, epidermal; IGA, Investigator's Global Assessment; SCORAD, SCORing of Atopic Dermatitis, ^aValues below the diagonal values of 1 indicate Spearman correlation coefficients for any 2 respective markers, and values above the diagonal values of 1 indicate the respective *P* values for those same 2 markers.

eTable 1
Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Confirmed diagnosis of mild to moderate AD with a minimum of 3% to a maximum of 15% BSA, excluding scalp, palms, soles, face, and genitals, and IGA score of 2 or 3	Unwilling or unable to provide written informed consent
Clinically insignificant screening laboratory test results as determined by investigator	Current treatment or history of treatment for AD with systemic medications within 4 weeks of day 0 and/or treatment with topical agents within 2 weeks of day 0. For parenteral biological or monoclonal antibody agents, no treatment for at least 5 half-lives of the parenteral agent. Treatment with systemic medications and topical agents was further prohibited for the duration of the trial with the exception of bland nonmedicated emollients that do not contain urea and ceramide.
Men or women, 18 to 65 years of age	As determined by the investigator history or presence of significant cardiovascular, pulmonary, hepatic, renal, hematologic, coagulation, gastrointestinal, endocrine, immunologic, dermatologic (other than AD), neurologic, or psychiatric condition that, in the opinion of the investigator would interfere with study evaluations or put the patient at undue risk
Body mass index of 18 to 35	Evidence of organ dysfunction or any clinically significant deviation from normal in vital signs, physical examination, clinical laboratory and ECG findings that as determined by the investigator would put patient at undue risk or interfere with study evaluations
WOCBP and men with partners who are WOCBP must agree that they and/or their partner will use effective contraception method from screening through the 30 days after the final dose of the study drug	5. ALT, AST, or total bilirubin exceeding 1.5 times the upper limit of normal at screening (may be repeated once to confirm)
Men with partners who are WOCBP must agree that they and/or their partner will use an effective contraception method from screening through the 30 days following the final dose of study drug	6. Any major surgery within 3 months of screening
Women must have a negative serum β -hCG pregnancy test result at the screening visit and a negative urine pregnancy test result at the baseline visit	7.
Voluntarily consent to participate in the trial	8. History or presence of alcoholism or drug abuse (guidance reference: <i>DSM-V</i> , diagnostic criteria for drug and alcohol abuse or equivalent)
	9.
	History of hypersensitivity or idiosyncratic reaction to drugs from multiple drug classes
	Use of any prescription medication within 14 days (or, if known, for at least 5 half-lives, if longer) before day 0, which in the opinion of the investigator would place the patient at undue risk or interfere with the study evaluation
	Use of sedative antihistamine within 14 days before day 0
	Use of topical products containing urea and/or ceramides within 14 days before day 0
	Use of a medical device for the treatment of AD within 14 days before day 0
	Blood donation or significant blood loss within 8 weeks before day 0
	Plasma donation within 7 days before day 0
	Blood transfusion within 4 weeks of screening
	Inability to undergo a venipuncture and/or tolerate venous access
	Participation in another clinical trial within 30 days before day 0 or, if known, within 6 half-lives of the experimental drug, whichever is longer
	Positive serum pregnancy test result (women) at screening or positive urine pregnancy test result with confirmatory serum pregnancy test on day 0
	Hemoglobin >5% below the testing laboratory's lower limits of normal by sex
	Prisoners or individuals who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical disease (eg, infectious disease) must not be enrolled into this study
	Excessive sun exposures, use of tanning booths or other UV light sources 4 weeks before day 0
	No planned trips to a sunny climate or use of tanning booths or other UV light sources during the study
	Any other sound medical, psychiatric, and /or social reason as determined by the investigator

Abbreviations: AD, atopic dermatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; *DSM-V*, *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*; IGA, Investigator's Global Assessment; SCORAD: SCORing of Atopic Dermatitis; WOCBP: women of child-bearing potential.

eTable 2
Number (Percentage) of Adverse Events in the Safety Population^a

Adverse event	Vehicle (placebo) (n = 40)	VTP-38543, 0.05% (n = 20)	VTP-38543, 0.15% (n = 19)	VTP-38543, 1% (n = 24)	P value ^b
Any related adverse event	4 (10)	1 (5)	3 (15.7)	6 (25)	.55
Administration site pruritus	0	1 (5)	0	0	>.99
Application site pain	2 (5)	1 (5)	1 (5.3)	3 (12.5)	.70
Application site pruritus	1 (2.5)	0	1 (5.3)	1 (4.2)	>.99
Application site reaction	1 (2.5)	0	0	2 (8.3)	>.99
Body tinea	0	0	0	1 (4.2)	>.99
Postprocedural infection	0	0	1 (5.3)	0	>.99
Leukopenia	0	0	0	1 (4.2)	>.99
Dermatitis ectopic	0	0	1 (5.3)	0	>.99

^aSafety population is defined as randomized patients who received at least one dose of VTP-38543.

^bP values were determined by the Fisher exact test comparisons of the pooled VTP-38543 population with vehicle.

eTable 3
Antibodies Used for Immunohistochemical Analyses

Antigen	Manufacturer	Clone ^a	Isotype	Dilution
Keratin 16	Sigma-Aldrich, St Louis, MO	K8.12	IgG1	1:100
CD3	BD Biosciences, San Jose, CA	SK7	IgG1	1:100
CD11c	BD Biosciences	B-ly6	IgG1	1:100
FCeRI	Thermo Scientific, Waltham, MA	CRA1 (AER37)	IgG2bk	1:100

^aAntibodies used in this experiment were mouse monoclonal antibodies unless stated otherwise.

eTable 4
Primers and Probes Used for Real-Time Polymerase Chain Reaction

Marker	Probe
K16	Hs00955082_g1
S100A12	Hs00942835_g1
IL-13	Hs00174379_m1
IFN- γ	Hs00989291_m1
IL-17A	Hs00174383_m1
Elafin/PI3	Hs00160066_m1
IL-22	Hs01574154_m1
S100A9	Hs00610058_m1
CCL17	Hs00171074_m1
FLG	Hs00856927_g1
LOR	Hs01894962_s1
MMP12	Hs00899662_m1
ABCG1	Hs00245154_m1
SREBP1c	Hs01088691_m1
IL-6	Hs00985639_m1

Abbreviations: FLG, filaggrin; IFN, interferon; IL, interleukin; K16, keratin 16; LOR, loricrin; MMP, matrix metalloproteinase; PI3, phosphatidylinositol 3.

eTable 5
Mean Percent Change in Transcriptional and Histologic Assessments in VEH- and VTP-38543–Treated AD Lesions^a

	VEH			0.05%			0.15%			1.00%			All ^b		
	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value
LOR	−0.4673	0.7233	NA	0.0761	1.0541	.17	0.6965	1.6205	.01	0.9242	1.8977	.02	0.5751	1.4898	.01
FLG	−0.6872	0.6210	NA	0.0678	1.0481	.05	0.2991	1.2304	.03	0.1938	1.1437	.28	0.1825	1.1348	.09
ABCG1	−0.3437	0.7880	NA	0.3739	1.2959	.01	0.9758	1.9667	2.20 ^{−03}	1.2892	2.4439	8.80 ^{−05}	0.8927	1.8566	4.10 ^{−04}
SREBP-1c	−0.0875	0.9411	NA	0.1912	1.1417	.21	0.9585	1.9432	5.10 ^{−03}	0.8869	1.8492	.01	0.6759	1.5976	.01
EPI thickness	−0.1334	0.9117	NA	−0.2192	0.8591	.72	−0.3817	0.7675	.33	−0.3664	0.7757	.36	−0.3218	0.8001	.33
K16	−0.9223	0.5277	NA	−1.1150	0.4617	.80	−0.7543	0.5929	.87	−0.7241	0.6054	.81	−0.8632	0.5497	.93
CD3	−0.0311	0.9787	NA	−0.6166	0.6522	.42	−0.2806	0.8232	.62	−0.4992	0.7075	.36	−0.4746	0.7197	.37
CD11c	−0.3269	0.7973	NA	−0.8343	0.5609	.34	−0.5529	0.6817	.52	−0.1701	0.8888	.74	−0.5031	0.7056	.65
CCL17	−0.7569	0.5918	NA	−0.7953	0.5762	.96	0.3321	1.2589	.17	−0.1373	0.9092	.58	−0.2197	0.8587	.52
FCeRI	−0.1799	0.8828	NA	−0.2582	0.8361	.83	−0.1232	0.9182	.90	−0.1963	0.8728	.96	−0.1956	0.8732	.96
MMP12	−1.1602	0.4475	NA	−1.5822	0.3340	.72	−0.3197	0.8012	.51	−0.8297	0.5626	.81	−0.9318	0.5242	.81
IFN- γ	−0.9484	0.5182	NA	0.0574	1.0406	.27	0.0031	1.0021	.31	0.0323	1.0226	.37	0.0321	1.0225	.19
IL-13	−0.4335	0.7405	NA	−0.4086	0.7534	.97	0.7598	1.6932	.19	−0.0303	0.9792	.67	0.0740	1.0527	.51
IL-17A	−1.1526	0.4498	NA	−1.4033	0.3781	.86	1.3737	2.5914	.16	−0.3695	0.7741	.56	−0.2056	0.8671	.41
PI3	−0.4310	0.7418	NA	−1.5896	0.3323	.16	−1.6602	0.3164	.20	−0.3093	0.8070	.91	−1.1301	0.4569	.42
IL-22	−1.4841	0.3575	NA	−2.6553	0.1587	.41	0.3135	1.2427	.15	−0.6135	0.6536	.51	−1.0237	0.4918	.67
IL-6	0.1368	1.0994	NA	−0.6643	0.6310	.38	−0.4573	0.7284	.62	−1.0236	0.4919	.08	−0.7387	0.5993	.28
S100A9	−0.8973	0.5369	NA	−2.0886	0.2351	.20	−0.6807	0.6239	.79	−0.1158	0.9229	.45	−0.9382	0.5219	.96
S100A12	−0.7021	0.6147	NA	−3.0858	0.1178	.03	−1.3638	0.3886	.43	0.1162	1.0839	.46	−1.3828	0.3835	.49

Abbreviations: AD, atopic dermatitis; EPI, epidermal; FCH, fold change; FLG, filaggrin; IFN, interferon; IL, interleukin; K16, keratin 16; LOR, loricrin; MMP, matrix metalloproteinase; NA, not applicable; PI3, phosphatidylinositol 3; VEH, vehicle.

^aP values indicate comparisons to VEH.

^bAll indicates the pooled VTP-38543 population.

eTable 6Median Percent Change in Transcriptional and Histologic Assessments in Vehicle- and VTP-38543-Treated AD Lesions^a

	VEH			0.05%			0.15%			1.00%			All ^b		
	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value	logFCH	FCH	P value
LOR	−0.2728	0.8277	NA	0.0425	1.0299	.21	0.5651	1.4795	.02	1.0168	2.0234	.04	0.4596	1.3752	.02
FLG	−0.5568	0.6798	NA	0.1527	1.1117	.05	0.2275	1.1708	.03	0.3137	1.2429	.17	0.2170	1.1623	.02
ABCG1	−0.3522	0.7834	NA	0.4223	1.3401	.03	1.5646	2.9580	.01	1.1191	2.1721	1.30 ^{−03}	0.8999	1.8659	9.20 ^{−04}
SREBP-1c	−0.1938	0.8743	NA	0.1942	1.1441	.17	1.0120	2.0166	.01	1.1890	2.2800	.01	0.7388	1.6687	9.70 ^{−03}
EPI thickness	0.0785	1.0559	NA	−0.2826	0.8221	.33	−0.4469	0.7336	.42	−0.1852	0.8795	.30	−0.3472	0.7861	.24
K16	−1.4168	0.3745	NA	−0.9422	0.5204	.70	−0.8090	0.5708	.79	−0.9938	0.5022	.56	−0.9014	0.5354	.59
CD3	0.2074	1.1546	NA	−0.6713	0.6280	.50	−0.3742	0.7716	.63	−0.6767	0.6256	.26	−0.5173	0.6987	.33
CD11c	−0.0067	0.9954	NA	−0.8907	0.5394	.50	−0.3522	0.7834	.22	−0.1791	0.8833	.89	−0.3111	0.8060	.49
CCL17	−0.4380	0.7382	NA	−0.5022	0.7061	.92	0.0000	1.0000	.15	−0.5162	0.6992	.89	−0.2610	0.8345	.51
FCeR1	0.1106	1.0796	NA	−0.1624	0.8936	.70	0.1000	1.0718	.71	−0.0684	0.9537	.96	−0.1052	0.9297	.96
MMP12	−1.9102	0.2661	NA	−1.3251	0.3991	.77	0.2197	1.1645	.63	−1.2555	0.4189	.96	−1.0189	0.4935	.93
IFN-γ	−0.3726	0.7724	NA	−0.2277	0.8540	.44	−0.1553	0.8979	.42	0.0195	1.0136	.56	−0.1328	0.9121	.37
IL-13	−1.0267	0.4908	NA	−0.0439	0.9701	.50	0.5354	1.4493	.36	−0.3432	0.7883	.45	0.3526	1.2769	.33
IL-17A	0.0000	1.0000	NA	−0.6050	0.6575	.84	0.2567	1.1947	.26	0.0000	1.0000	.69	0.0000	1.0000	.59
PI3	−0.1423	0.9061	NA	−1.6650	0.3153	.21	−1.5781	0.3349	.15	−0.9003	0.5358	.75	−1.4168	0.3746	.22
IL-22	−1.8599	0.2755	NA	−2.5066	0.1760	.38	0.5327	1.4467	.12	−0.8225	0.5655	.45	−0.9955	0.5016	.57
IL-6	0.3065	1.2367	NA	0.1759	1.1297	.44	−0.5497	0.6832	.95	−1.2916	0.4085	.07	−0.5864	0.6660	.24
S100A9	−1.1249	0.4585	NA	−2.0972	0.2337	.24	−1.2919	0.4084	.95	0.0782	1.0557	.75	−1.3491	0.3925	.74
S100A12	−0.5197	0.6975	NA	−1.4058	0.3774	.05	−0.2583	0.8361	.49	−1.5666	0.3376	.56	−1.5666	0.3376	.41

Abbreviations: AD, atopic dermatitis; EPI, epidermal; FCH, fold change; FLG, filaggrin; IFN, interferon; IL, interleukin; K16, keratin 16; LOR, loricrin; MMP, matrix metalloproteinase; NA, not applicable; PI3, phosphatidylinositol 3; VEH, vehicle.

^aP values indicate comparisons to VEH.

^bAll indicates the pooled VTP-38543 population.

eTable 7

Correlations of Baseline Markers and Percent Change in SCORAD Scores

Marker	Spearman r	P value
FCER1_NL	0.60	<.001
CD3_NL	0.34	.054
SERBP1c_LS	0.34	.056
EPI thickness_NL	0.33	.06
CD11c_NL	0.27	0.1236
FLG_LS	0.24	0.1785
IL-22_LS	0.22	0.2181
IL-13_NL	0.19	0.2805
ABCG1_LS	0.18	0.3220
FCeR1_LS	0.16	0.3735
MMP12_NL	0.16	0.3868
LOR_LS	0.14	0.4526
IL22_NL	0.08	0.6402
S100A9_LS	0.08	0.6559
CCL17_NL	0.08	0.6619
EPI thickness_LS	0.07	0.6883
S100A12_LS	0.06	0.7395
IL-13_LS	0.02	0.9323
IL-17A_LS	0.00	0.9985
K16_LS	0.00	0.9823
PI3_LS	−0.02	0.9177
S100A12_NL	−0.03	0.8899
CCL17_LS	−0.03	0.8789
IL-17A_NL	−0.04	0.8138
MMP12_LS	−0.06	0.7549
S100A9_NL	−0.06	0.7276
SERBP1c_NL	−0.08	0.6552
IL-6_LS	−0.08	0.6545
FLG_NL	−0.11	0.5368
PI3_NL	−0.11	0.5363
CD3_LS	−0.11	0.5350
K16_NL	−0.12	0.5210
IFN-γ_NL	−0.14	0.4347
IGA	−0.16	0.3744
EASI	−0.18	0.3201
CD11c_LS	−0.19	0.2813
LOR_NL	−0.21	0.2448
IL-6_NL	−0.25	0.1646
ABCG1_NL	−0.26	0.1449
IFN-γ_LS	−0.39	0.0235

Abbreviations: AD, atopic dermatitis; EPI, epidermal; FCH, fold change; FLG, filaggrin; IFN, interferon; IL, interleukin; K16, keratin 16; LOR, loricrin; LS, lesional; MMP, matrix metalloproteinase; NA, not applicable; NL, nonlesional; PI3, phosphatidylinositol 3; SCORAD, SCORing of Atopic Dermatitis; VEH, vehicle.

eTable 8

Correlations of Change in Markers and Percent Change in SCORAD Scores

Marker	Spearman r	P value
EASI	0.78	1.1783 ^{−07}
IGA	0.63	<.001
PI3	0.35	.047
K16	0.34	.051
S100A12	0.33	.06
S100A9	0.29	.11
CD11c	0.18	.32
IL-17A	0.14	.42
CD3	0.14	.45
IL-22	0.11	.52
MMP12	0.11	.56
IL-6	0.10	.5890
EPI thickness	0.10	.60
IL-13	0.05	.76
CCL17	0.02	.93
SERBP1c	−0.01	.94
IFN-γ	−0.02	.93
ABCG1	−0.09	.60
FCeR1	−0.10	.60
FLG	−0.42	.02
LOR	−0.47	.006

Abbreviations: AD, atopic dermatitis; EPI, epidermal; FCH, fold change; FLG, filaggrin; IFN, interferon; IL, interleukin; K16, keratin 16; LOR, loricrin; LS, lesional; MMP, matrix metalloproteinase; NA, not applicable; NL, nonlesional; PI3, phosphatidylinositol 3; SCORAD, SCORing of Atopic Dermatitis; VEH, vehicle.