

Clinical Communications

Tape-strips provide a minimally invasive approach to track therapeutic response to topical corticosteroids in atopic dermatitis patients

Caroline Meyer Olesen, MD^{a,*}, Ana B. Pavel, PhD^{b,c,*},
 Gianni Wu, BS^{b,d,*}, Daniela Mikhaylov, BA^b,
 Ester Del Duca, MD^{b,e}, Yeriel Estrada, BS^b,
 James G. Krueger, MD, PhD^f, Ning Zhang, MD^b,
 Maja-Lisa Clausen, MD, PhD^a, Tove Agner, MD, DMSc^a,
 and Emma Guttman-Yassky, MD, PhD^b

Clinical Implications

- Tape-strips captured suppression of T_H2 and T_H17/T_H22-related markers and increased expression of barrier-related genes in response to mometasone treatment for atopic dermatitis, in line with previous biopsy studies. Tape-strips are a reliable, minimally invasive alternative to skin biopsies for tracking cutaneous treatment responses.

Atopic dermatitis (AD) is a common inflammatory skin disease, largely characterized by T_H2/T_H22 polarization with variable T_H1/T_H17 skewing and epidermal barrier impairment.¹ Skin biopsies represent the criterion standard, and have been instrumental in elucidating key AD mechanisms, facilitating rapid therapeutic development.¹ These investigations have also highlighted the heterogeneous nature of AD across multiple endotypes, emphasizing the potential of a precision medicine approach in AD disease management.¹ However, biopsies are invasive, painful, and cause scarring, limiting their use in longitudinal or large-scale studies, particularly in pediatric populations. Thus, identification of a less-invasive alternative is warranted.

Tape-stripping is a minimally invasive approach that has been studied in AD.^{2,3} Although previous biopsy studies have assessed AD therapeutic response biomarkers with topical treatments,⁴ this has not yet been explored using tape-strips, with only 1 previous tape-strip study evaluating protein biomarker changes in patients with AD following emollient application.⁵ Because topical corticosteroids (TCSs) are the mainstay of AD treatment, in this real-life clinical setting study, we aimed to identify gene-expression changes in a panel of 95 inflammatory and barrier-related markers, previously associated with AD,² in tape-stripped skin from adults with AD after topical treatment with mometasone furoate cream 0.1%.

Thirteen European-Caucasian patients with mild to severe AD (age range, 20-55 years; median age, 31 years; median Eczema Area and Severity Index score, 9.7; median SCORing Atopic Dermatitis [SCORAD] score, 43.7) were enrolled after informed consent was obtained (Table I). Inclusion criteria were AD diagnosis, 18 years or older, no phototherapy or systemic treatment for 4 weeks, or topical anti-inflammatory treatment,

within 7 days, before first visit. Patients were evaluated before and after TCS treatment (mean duration, 33 days). From each patient, 20 D-squame tape-strips (CuDerm Corp, Dallas, Texas) were collected from lesional (baseline and posttreatment) and nonlesional skin (baseline only) from the upper extremities, totaling to 60 tape-strips collected per patient. Disease severity was evaluated with Eczema Area and Severity Index and SCORAD, and barrier function was assessed by transepidermal water loss on lesional sites before sampling. RNA was extracted, followed by quantitative RT-PCR, as described.² Spearman correlations were used to assess associations between biomarker expression and clinical improvement scores. Additional details on Methods and Statistical analysis can be found in this article's Online Repository at www.jaci-inpractice.org. With informed consent, a graphical depiction of the tape-stripping process is shown in Figure E1 in this article's Online Repository at www.jaci-inpractice.org. This study was approved by Danish Data Protection Agency and local ethics committee.

Overall, we were able to evaluate 39 of 39 samples (13 pre-treatment lesional, 13 posttreatment lesional, and 13 nonlesional samples). RNA extracted from 20 tape-strips collected from each site represent 1 sample. We detected all 95 gene-targets, and Eczema Area and Severity Index score and SCORAD score significantly improved with treatment (Table I). At baseline, the expression of inflammatory and barrier measures in lesional versus nonlesional AD tape-stripped skin was largely in line with previous AD tape-strip and biopsy reports (Figure 1, A).^{1,2,4} Innate immunity (IL-17C), T_H2- (CCL7, IL-1RL1/ST2/IL-33R), T_H1- (IFN- γ), and T_H17/T_H22- (IL-36A/G, PI3, CAMP/LL37, S100As)-related markers ($P < .05$), and measures of epidermal hyperplasia (Ki67, KRT16, SERPINB3) significantly increased ($P < .05$ for SERPINB3), whereas terminal differentiation markers (FLG, FLG2, LOR, PSORS1C2) decreased ($P < .1$ for PSORS1C2 and LOR; Figure 1, A).

Next, we analyzed lesional expression changes following mometasone treatment. We observed significant, or trending toward significant, decreases in cellular infiltrate measures, including dendritic cells (CD209) and T cells/natural killer cells/T-cell activation (CD3, IL-2, ICOS, GZMA) ($P < .05$ for CD209, IL-2, GZMA; Figure 1, A). Markers of innate immunity (IL-17C), T_H2- (IL-13, CCL13, CCL7), T_H22- (IL-22), T_H1- (IFN- γ), and T_H17- (IL-17F, IL-19, IL-21) pathways, and regulatory T cells (IL-10) also significantly decreased ($P < .05$; Figure 1, A-C). IL-31, a T_H2-related itch cytokine,¹ trended toward decreased expression ($P < .1$; Figure 1, A). Terminal differentiation measures (FLG, FLG2, LOR) were significantly upregulated after mometasone application ($P < .05$; Figure 1, A and D), whereas other barrier-related genes (PSORS1C2, CDSN, LCE2B, ELOVL3) displayed similar, albeit nonsignificant, trends (Figure 1, A). Furthermore, key AD markers showed significant improvements in posttreatment lesional skin beyond both lesional and nonlesional skin at baseline (Figure 1, B-D).

Changes in mRNA expression of immune and barrier-related genes after treatment were correlated with improvements in clinical (SCORAD) and functional barrier (transepidermal water loss) measures (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Significant positive correlations were

TABLE 1. Patient characteristics and clinical measurements at baseline and follow-up

Characteristic	Baseline	Follow-up
No. of patients	13	13
Age (y), median (IQR)	31 (27-39)	—
Sex		
Female, n (%)	6 (46.2)	—
Atopy history, n (%)		
History of asthma	4 (30.8)	—
History hay fever	7 (53.8)	—
First-degree relative with AD	9 (69.2)	—
<i>FLG</i> -mutation carrier, n (%)	4 (40.0)	—
EASI score, median (IQR)	9.7 (6.7-15.5)	6.8 (4.2-14.2)*
Change in EASI score from baseline, mean $\pm$ SD	—	5.1 $\pm$ 7.9
Percentage change in EASI score from baseline, % (SD)	—	22.7 (46.9)
EASI-50, n (%)	—	4 (30.8)
SCORAD score, median (IQR)	43.7 (33.4-57.7)	32.0 (24.9-46.0)†
Change in SCORAD score from baseline, mean $\pm$ SD	—	12.0 $\pm$ 15.2
Percentage change in SCORAD score from baseline, % (SD)	—	23.9 (28.9)
SCORAD-50, n (%)	—	2 (15.4)
Lesional TEWL, median (IQR)	31.4 (16.9-50.2)	21.8 (13.9-26.7)

FLG, Filaggrin gene; *EASI*, Eczema Area and Severity Index; *EASI/SCORAD-50*, proportion of patients experiencing at least a 50% reduction from baseline *EASI/SCORAD* score; *IQR*, interquartile range; *TEWL*, transepidermal water loss.

* $P < .05$, EASI significant decrease from baseline.

† $P < .01$, SCORAD significant decrease from baseline.

obtained between improvements in SCORAD score and reduction in T_H17 measures (IL-17A/F) and IL-3, a cytokine produced by activated T cells and other cells including basophils, mast cells, and eosinophils, which are associated with AD pathogenesis ($r > 0.4$; $P < .05$; Table E1). Studies have linked IL-3 with increased type 2 cytokines (IL-4, IL-5, IL-13) and T_H2 cell differentiation,⁶ and have shown an association of IL-3 and asthma disease severity.⁷ Our data correlating IL-3 with improvement in these clinical measures may therefore suggest a role of IL-3 in other atopic, type 2—high conditions, such as AD. Significant inverse correlations were obtained between SCORAD score improvement and expression changes of barrier-related genes (FA2H, LOR, FLG2) and the negative immune regulator, IL-37 ($r < -0.4$; $P < .05$; Table E1). Improvement in transepidermal water loss also significantly positively correlated with reduction in IL-17F ($r > 0.4$; $P < .05$) and negatively correlated with terminal differentiation measures (LOR, LCE2B) and IL-37 ($r < -0.4$; $P < .05$) (Table E1). Of note, IL-37, an epithelial-derived cytokine, has known anti-inflammatory and immune-regulatory actions in both the innate and adaptive immune systems.⁸ IL-37 upregulation is also associated with disease improvement in response to treatments,⁹ suggesting that it could serve as a reliable biomarker to monitor cutaneous therapeutic response and possibly explain the correlation between IL-37 and AD severity scores.

Finally, we compared our results with the results of a previously published AD study⁴ that used skin biopsies to evaluate transcriptomic responses before and after treatment with triamcinolone acetonide cream 0.025%, a less potent TCS compared with mometasone furoate cream 0.1%. Because this was a real-life clinical setting study, we did not obtain skin biopsies, which would have allowed for direct inpatient comparisons. Nevertheless, we observed largely concordant biomarker modulation. T-cell/natural killer-cell activation (GZMA), T_H2 (IL-13), and T_H17/T_H22 (IL-21, IL-22)

markers were similarly significantly modulated with 4 weeks of mometasone, assessed by tape-strips, as well as with 16 weeks of triamcinolone, assessed by biopsies.⁴ In contrast, epidermal hyperplasia marker (KRT16) and T_H17/T_H22 -regulated S100As were significantly downregulated with TCSs in skin biopsies but not tape-strips, whereas significant increases in terminal differentiation measures (FLG, LOR) were observed with TCSs in tape-strips, but not skin biopsies at week 4.⁴

Although methodological differences limit direct comparisons, differences in sampling techniques may also help explain differential gene-expression modulation. Consecutive tape-stripping samples the stratum corneum and the upper granular layer,³ and skin biopsies sample the entire epidermis and dermis. Thus, tape-strips may be better positioned to detect treatment changes in stratum corneum measures (FLG, LOR), whereas biopsies may be more suitable for detecting gene-expression changes associated with deeper epidermal layers, such as S100As and hyperplasia markers. Yet, in our study, tape-strips detected significant baseline expression differences of S100As, SERPINB3, and PI3 in lesional versus nonlesional skin, with a loss of significance when evaluating lesional changes with treatment. Decreased lesional stratum corneum thickness after treatment may increase the advancement of tape-stripping into the epidermis, resulting in the sampling of deeper epidermal compartments, thereby masking potential treatment-related decreases in these markers.

Tape-stripping has a few limitations, including the relatively laborious process and the potential variability in depth of tape-stripping. However, previous studies have shown that more than 15 tapes reach the granular layer of the skin.^{2,3}

In conclusion, using tape-strips, we show that mometasone significantly suppressed T_H2 and T_H17/T_H22 signaling and increased expression of barrier-related genes, in line with previous skin biopsy studies.⁴ As such, tape-strips are a promising minimally invasive alternative to skin biopsies, and may provide

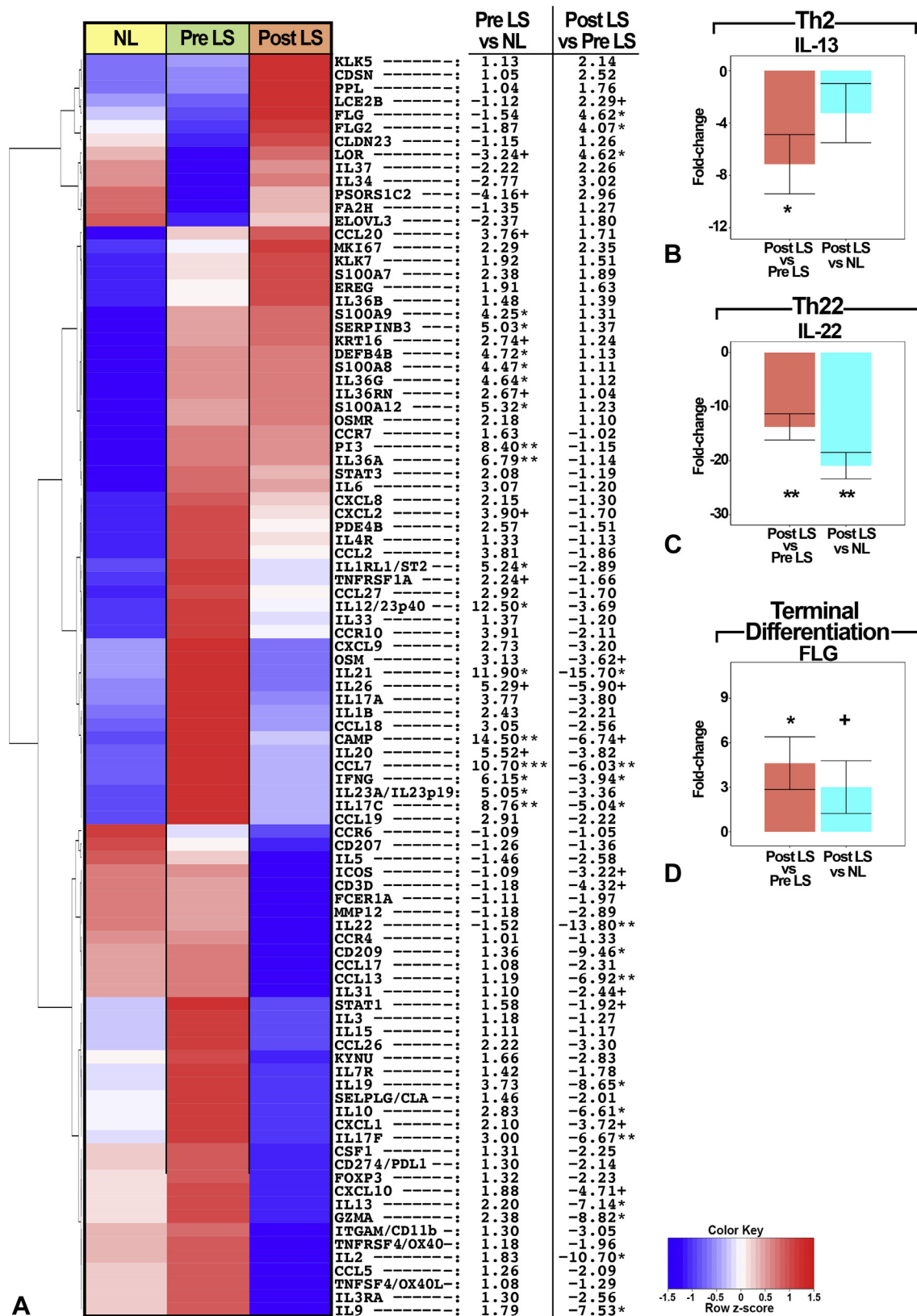


FIGURE 1. (A) Summary heatmap of gene expression of AD biomarkers measured by quantitative RT-PCR. Red denotes higher and blue denotes lower mean expression levels. Table displays fold-changes in pretreatment lesional vs nonlesional skin and posttreatment vs pretreatment lesional skin. **(B-D)** Barplots show fold-changes in posttreatment vs pretreatment lesional expression and posttreatment lesional versus nonlesional expression of key AD mediators. *FLG*, Filaggrin; *LS*, lesional; *NL*, nonlesional. *** $P < .001$, ** $P < .01$, * $P < .05$, + $P < .1$.

insight into the mechanistic basis for treatment responders/nonresponders, help identify novel therapeutic targets, and facilitate personalized medicine approaches.

^aDepartment of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

^bDepartment of Dermatology, and Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York, NY

^cDepartment of Biomedical Engineering, University of Mississippi, Oxford, MS

^dDownstate Medical Center, College of Medicine, State University of New York, Brooklyn, NY

^eDepartment of Dermatology, University of Rome Tor Vergata, Rome, Italy

^fThe Laboratory for Investigative Dermatology, The Rockefeller University Hospital, New York, NY

^gThese authors contributed equally to this work.

Conflicts of interest: J. G. Krueger is an employee of the Rockefeller University and has received research support (grants paid to his institution) and/or personal fees from Pfizer, Amgen, Janssen, Lilly, Merck, Novartis, Kadmon, Dermira, Boehringer, Innovaderm, Kyowa, BMS, Serono, BiogenIdec, Delenex, AbbVie, Sanofi, Baxter, Paraxel, Xenoport, and Kineta. T. Agner is an advisor/investigator or speaker for Pfizer Inc, AbbVie, Eli Lilly, LEO Pharma, Regeneron, and Sanofi-Genzyme. E. Guttman-Yassky is an employee of Mount Sinai and has received research funds (grants paid to the institution) from AbbVie, Celgene, Eli Lilly, Janssen, MedImmune/AstraZeneca, Novartis, Pfizer, Regeneron, Vitae, Glenmark, Galderma, Asana, Innovaderm, Dermira, and UCB and is also a consultant for Sanofi Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, AnaptysBio, Dermira, Galderma, Glenmark, Novartis, Pfizer, Vitae, Leo Pharma, AbbVie, Eli Lilly, Kyowa, Mitsubishi Tanabe, Asana Biosciences, and Promius. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 10, 2020; revised August 13, 2020; accepted for publication August 15, 2020.

Available online ■ ■ ■

Corresponding author: Emma Guttman-Yassky, MD, PhD, Department of Dermatology and Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, 5 E. 98th St, New York, NY 10029. Or: Tove Agner,

MD, DMSc, Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Bispebjerg Bakke 23, 2400 København, Denmark. E-mail: tove.agner@regionh.dk. Or: Emma.Guttman@mountsinai.org.

2213-2198

© 2020 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2020.08.037>

REFERENCES

1. Nomura T, Wu J, Kabashima K, Guttman-Yassky E. Endophenotypic variations of atopic dermatitis by age, race, and ethnicity. *J Allergy Clin Immunol Pract* 2020;8:1840-52.
2. Guttman-Yassky E, Diaz A, Pavel AB, Fernandes M, Lefferdink R, Erickson T, et al. Use of tape strips to detect immune and barrier abnormalities in the skin of children with early-onset atopic dermatitis. *JAMA Dermatol* 2019;155:1358-70.
3. Dyjack N, Goleva E, Rios C, Kim BE, Bin L, Taylor P, et al. Minimally invasive skin tape strip RNA sequencing identifies novel characteristics of the type 2-high atopic dermatitis disease endotype. *J Allergy Clin Immunol* 2018;141:1298-309.
4. Brunner PM, Khattri S, Garcet S, Finney R, Oliva M, Dutt R, et al. A mild topical steroid leads to progressive anti-inflammatory effects in the skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol* 2016;138:169-78.
5. Koppes SA, Brans R, Ljubojevic Hadzavdic S, Frings-Dresen MH, Rustemeyer T, Kezic S. Stratum corneum tape stripping: monitoring of inflammatory mediators in atopic dermatitis patients using topical therapy. *Int Arch Allergy Immunol* 2016;170:187-93.
6. Kumar A, Rani L, Mhaske ST, Pote ST, Behera S, Mishra GC, et al. IL-3 receptor expression on activated human Th cells is regulated by IL-4, and IL-3 synergizes with IL-4 to enhance Th2 cell differentiation. *J Immunol* 2020;204:819.
7. Robinson DS, Ying S, Bentley AM, Meng Q, North J, Durham SR, et al. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993;92:397-403.
8. Pan Y, Wen X, Hao D, Wang Y, Wang L, He G, et al. The role of IL-37 in skin and connective tissue diseases. *Biomed Pharmacother* 2020;122:109705.
9. Bissonnette R, Pavel AB, Diaz A, Werth JL, Zang C, Vranic I, et al. Crisaborole and atopic dermatitis skin biomarkers: an inpatient randomized trial. *J Allergy Clin Immunol* 2019;144:1274-89.

ONLINE REPOSITORY

METHODS

Patient population

Thirteen European-Caucasian patients with mild to severe AD (median age, 31 years [20-55]; females, 46%; median Eczema Area and Severity Index score, 9.7; median SCORAD score, 43.7) were recruited from the Department of Dermatology, Bispebjerg Hospital, from April to September 2019 (Table I). Inclusion criteria were diagnosis of AD according to the UK criteria, 18 years or older, no treatment with phototherapy or systemic immunosuppressive drugs for 4 weeks before baseline, and no use of topical anti-inflammatory treatments within 7 days of first visit. Patients were evaluated before and after treatment with mometasone furoate 0.1% cream (mean, 33 days; most patients were treated between 2 and 4 weeks), instructed not to use emollients within 24 hours of each visit, and to use mometasone once daily between the baseline and follow-up visits. Mometasone furoate 0.1% cream was used according to Danish guidelines. The study was approved by the Danish Data Protection Agency and the local ethics committee. Written informed consent was obtained from all patients.

Skin sampling and severity assessment

Twenty tape-strips (D-squame 3.8 cm²; CuDerm) were collected from lesional (baseline and posttreatment) and nonlesional skin (baseline only) from upper extremities. Tapes were pressed against the skin for 10 seconds to apply standardized pressure (225 g/cm²) with the D-squame pressurizer device. Disease severity was evaluated with Eczema Area and Severity Index and SCORAD. Skin barrier function was assessed by transepidermal water loss on lesional sites before sampling.

Quantitative real-time PCR

RNA was extracted from pooled tape-strips using the miR-Neasy Mini Kit (Qiagen, Hilden, Germany). Total RNA (500 pg) was used for each sample followed by conversion to cDNA and preamplification. For the quantitative RT-PCR, Taqman Low Density Array Cards were used. PCR primers: CCL13-Hs00234646_m1; CCL17-Hs00171074_m1; CCL18-Hs00268113_m1; CCL19-Hs00171149_m1; CCL2-Hs00234140_m1; CCL20-Hs00355476_m1; CCL26-Hs00171146_m1; CCL27-Hs00171157_m1; CCL5-Hs00982282_m1; CCL7-Hs00171147_m1; CCR10-Hs00706455_s1; CCR4-Hs99999919_m1; CCR6-Hs01890706_s1; CCR7-Hs01013469_m1; ITGAM-Hs00167304_m1; CD209-Hs01588349_m1; CD3D-Hs00174158_m1; CDSN-Hs00169911_m1; SELPLG-Hs05033974_s1; CLDN23-Hs01013638_s1; CSF1-Hs00174164_m1; GZMA-Hs00989184_m1; CXCL1-Hs00236937_m1; CXCL10-Hs00171042_m1; CXCL2-Hs00601975_m1; CXCL9-Hs00171065_m1; PI3-Hs00160066_m1; ELOVL3-Hs00537016_m1; EREG-Hs00914313_m1; FA2H-Hs00757813_m1; FCER1A-

Hs00758600_m1; FLG-Hs00856927_g1; FLG2-Hs00418578_m1; FOXP3-Hs01085834_m1; DEFB4B-Hs00823638_m1; ICOS-Hs00359999_m1; IFNG-Hs00989291_m1; IL10-Hs00961622_m1; IL12B-Hs01011518_m1; IL13-Hs00174379_m1; IL15-Hs01003716_m1; IL17C-Hs00171163_m1; IL19-Hs00604657_m1; IL1B-Hs01555410_m1; IL2-Hs00174114_m1; IL20-Hs00218888_m1; IL21-Hs00222327_m1; IL22-Hs01574154_m1; IL23A-Hs00372324_m1; IL26-Hs00218189_m1; IL3-Hs00174117_m1; IL33-Hs04931857_m1; IL34-Hs01050926_m1; IL36A-Hs00205367_m1; IL36B-Hs00758166_m1; IL36G-Hs00219742_m1; IL37-Hs00367201_m1; IL3RA-Hs00608141_m1; IL4R-Hs00965056_m1; IL5-Hs00174200_m1; IL6-Hs00985639_m1; CXCL8-Hs00174103_m1; IL36RN-Hs01104220_g1; IL7R-Hs00902334_m1; KRT16-Hs00955082_g1; KLK5-Hs01548153_m1; KLK7-Hs00192503_m1; KYNU-Hs0114105_m1; CD207-Hs00210453_m1; LCE2B-Hs04194422_s1; CAMP-Hs00189038_m1; LOR-Hs01894962_s1; MKI67-Hs04260396_g1; MMP12-Hs00159178_m1; OSM-Hs00171165_m1; OSMR-Hs00384276_m1; TNFRSF4-Hs00937195_g1; TNFSF4-Hs04400731_m1; PDE4B-Hs00277080_m1; CD274-Hs00204257_m1; PPL-Hs00160312_m1; PSORS1C2-Hs00204152_m1; S100A12-Hs00942835_g1; S100A7-Hs01923188_u1; S100A8-Hs00374264_g1; S100A9-Hs00610058_m1; SERPINB3-Hs00199468_m1; IL1RL1-Hs00249384_m1; STAT1-Hs01013996_m1; STAT3-Hs00374280_m1; TNFRSF1A-Hs01042313_m1; IL-9-Hs00914237_m1; IL-17A-Hs00174383_m1; IL-17F-Hs01028648_m1; IL-31-Hs01098710_m1.

Statistical analyses

Statistical analysis was performed using the statistical language R (www.R-project.org). Linear mixed-effect models were used to analyze treatment changes in log₂ quantitative RT-PCR data with tissue type (lesional/nonlesional) and its interaction with time as a fixed factor. Expression values were modeled by using mixed-effect models, with treatment and time as fixed factors and a random effect for each patient. Mean estimate expression of all markers was summarized in a heat map in which hierarchical clustering was performed with Euclidean distance and a McQuitty agglomeration scheme. Fold-changes in lesional skin after TCS treatment were calculated, and hypothesis testing conducted with contrasts under the general framework for linear models in the R limma package. *P* values from moderated (paired) *t* tests were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure. Correlations between biomarker expression and clinical improvement scores were evaluated by Spearman correlation coefficients.

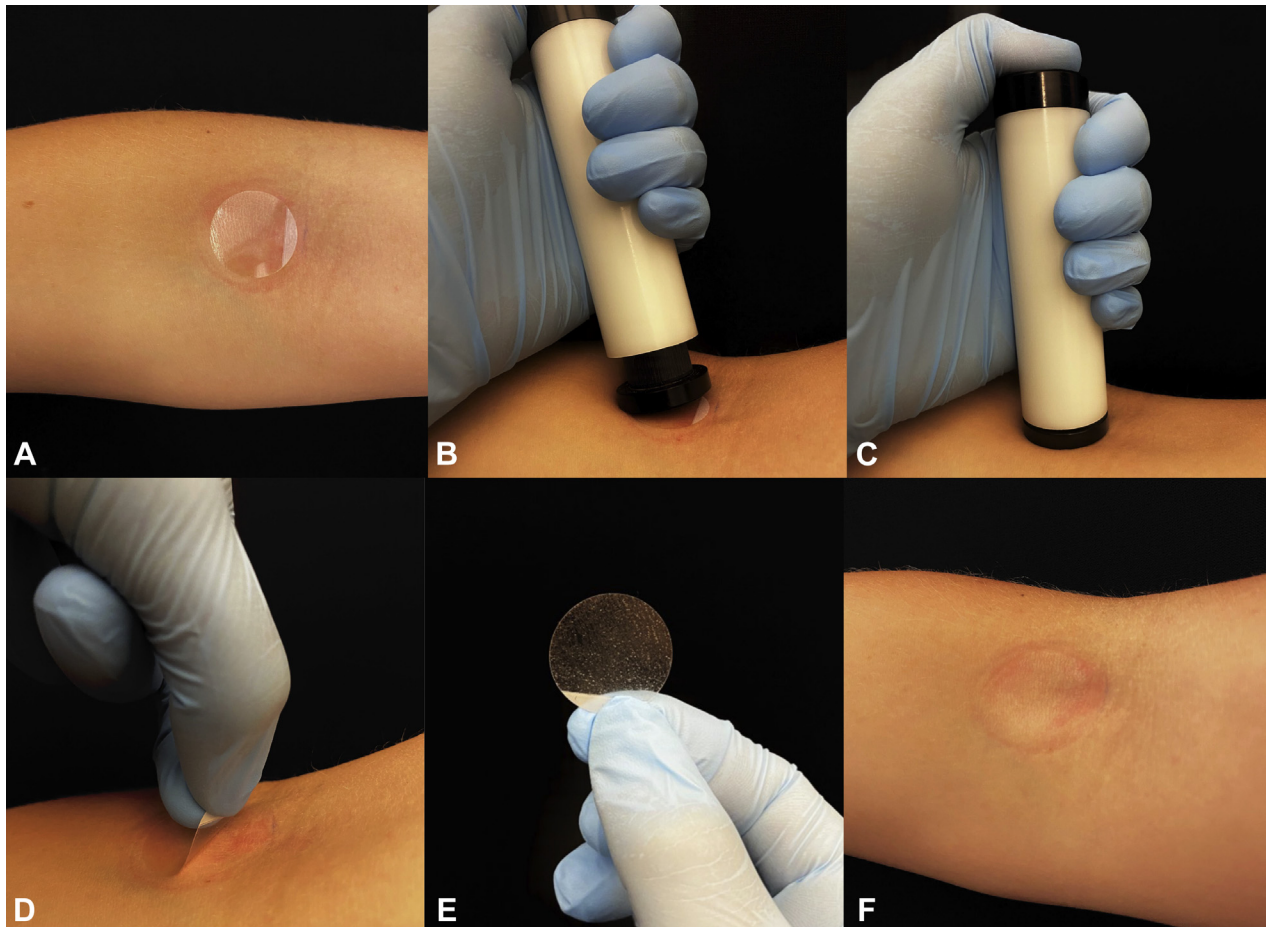


FIGURE E1. (A) The tape-strip is applied to the skin of interest on the upper extremity. (B and C) The tape-strip is pressed against the skin for 10 seconds with a standardized pressure using the D-squame pressurizer device. (D) The tape-strip is gently stripped off the skin. (E) Tape-strip with epidermal material can be used for subsequent molecular profiling. (F) After 20 consecutive tape-strips, the sampled area appears red and shiny without bleeding.

TABLE E1. Spearman correlations of changes in AD biomarker gene expression with improvement in clinical measures

Correlations between lesional markers and change in SCORAD		
Marker	<i>r</i>	<i>P</i> value
TEWL	0.74	.009
IL-3	0.62	.03
IL-17F	0.61	.03
IL-17A	0.57	.05
IL-34	−0.54	.07
LCE2B	−0.55	.06
FLG	−0.56	.06
EREG	−0.58	.05
FLG2	−0.60	.04
LOR	−0.65	.02
FA2H	−0.66	.02
IL-37	−0.71	.009
Correlations between lesional markers and change in TEWL		
Markers	<i>r</i>	<i>P</i> value
IL-17F	0.61	.04
IL-3	0.57	.06
CCL7	0.53	.08
FLG2	−0.50	.1
LCE2B	−0.62	.04
LOR	−0.68	.02
IL-37	−0.68	.02

TEWL, Transepidermal water loss.