



JID Open

A Phase 2 Randomized Trial of Apremilast in Patients with Atopic Dermatitis

Eric L. Simpson¹, Shinichi Imafuku², Yves Poulin³, Benjamin Ungar⁴, Lisa Zhou⁴, Kunal Malik⁴, Huei-Chi Wen⁴, Hui Xu⁴, Yeriel D. Estrada⁴, Xiangyu Peng⁴, Mindy Chen⁵, Nilam Shah⁵, Mayte Suarez-Farinas⁴, Ana B. Pavel⁴, Kristine Nograles⁵ and Emma Guttman-Yassky⁴

A phase 2, double-blind, placebo-controlled trial evaluated apremilast efficacy, safety, and pharmacodynamics in adults with moderate to severe atopic dermatitis. Patients were randomly assigned to receive placebo, apremilast 30 mg twice daily (APR30), or apremilast 40 mg twice daily (APR40) for 12 weeks. During weeks 12–24, all patients received APR30 or APR40. A biopsy substudy evaluated atopic dermatitis-related biomarkers. Among 185 randomly assigned intent-to-treat patients at week 12, a dose-response relationship was observed; APR40 ($n = 63$), but not APR30 ($n = 58$), led to statistically significant improvements (vs. placebo, $n = 64$) in Eczema Area and Severity Index (mean [standard deviation] percent change from baseline = -31.6% [44.6] vs. -11.0% [71.2], $P < 0.04$; primary endpoint). mRNA expression of T helper type 17/T helper type 22-related markers (IL-17A, IL-22, and S100A7/A8; $P < 0.05$) showed the highest reductions with APR40, with minimal changes in other immune axes. Safety with APR30 was largely consistent with apremilast's known profile (common adverse events: nausea, diarrhea, headache, and nasopharyngitis). With APR40, adverse events were more frequent, and cellulitis occurred ($n = 6$). An independent safety monitoring committee discontinued the APR40 dosage. APR40 showed modest efficacy and decreased atopic dermatitis-related biomarkers in moderate to severe atopic dermatitis patients. Adverse events, including cellulitis, were more frequent with APR40, which was discontinued during the trial. Clinical Trial Registration Number: NCT02087943 (clinicaltrials.gov).

Journal of Investigative Dermatology (2019) **139**, 1063–1072; doi:10.1016/j.jid.2018.10.043

INTRODUCTION

Atopic dermatitis (AD) is a heterogeneous, chronic, inflammatory skin disorder with clinical features that include highly pruritic, eczematous lesions (Bieber, 2008; Eichenfield et al., 2014a, 2014b). Genetic and environmental factors are believed to underlie dysfunctional T-helper (Th) cell type 1, Th2, and Th17 innate and adaptive immune responses in patients with AD (Bieber, 2008; Brunner et al., 2017; Czarnecki et al., 2014; Gittler et al., 2012; Guttman-Yassky and Krueger, 2017; Guttman-Yassky et al., 2011; Park et al., 2013). Some patients with AD exhibit elevated serum levels of total and allergen-specific IgE (extrinsic AD), whereas others have normal IgE levels and negative results for serum allergen-specific IgE (intrinsic AD) (Eichenfield et al., 2014b; Park et al., 2013; Park et al., 2008). Although total IgE levels may be more elevated in patients with greater AD disease severity, there is substantial variability in the general

US population, making the clinical significance of this distinction unclear (Eichenfield et al., 2014b).

AD is treated primarily with topical therapies, mainly with intermittent, preventive application of topical corticosteroids and/or topical calcineurin inhibitors, paired with daily emollient use (McGregor et al., 2017). Intermittent corticosteroid use poses minimal risk of corticosteroid-associated adverse effects like skin atrophy, striae formation in sensitive or thin-skinned areas, and systemic effects (Hanifin et al., 2002). The rate of long-term adherence to topical therapy, however, is low (Snyder et al., 2015). Off-label use of other conventional systemic oral immunomodulatory agents such as cyclosporine, azathioprine, and methotrexate in adults with AD can be effective, but patients have reported adverse events (AEs) or ineffectiveness as reasons for discontinuation of treatment; use of these drugs poses well-known safety limitations and requires regular laboratory monitoring (Roekevisch et al., 2018; Sidbury et al., 2014). Thus, better systemic treatment options are needed, particularly for patients with AD who do not adequately respond to topical therapy. Dupilumab, a subcutaneous injectable biologic therapy that targets the IL-4 and IL-13 receptor (Beck et al., 2014), was approved by the US Food and Drug Administration for the treatment of adult patients with moderate to severe AD (Jensterle et al., 2015). The need for oral treatment options remains a priority in this disease state.

Apremilast, an orally available phosphodiesterase 4 (PDE4) inhibitor that is approved for the treatment of adults with moderate to severe psoriasis and active psoriatic arthritis (Celgene, 2017), regulates a number of the proinflammatory signals involved in AD, including IL-17, IL-22, IL-13, IL-31,

¹Oregon Health and Science University, Portland, Oregon, USA; ²Fukuoka University, Fukuoka, Japan; ³Laval University, Quebec City, Quebec, Canada; ⁴Icahn School of Medicine at the Mount Sinai Medical Center, New York, New York, USA; and ⁵Celgene Corporation, Summit, New Jersey, USA

Correspondence: Eric L. Simpson, Department of Dermatology, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97239, USA. E-mail: simpson@ohsu.edu

Abbreviations: AD, atopic dermatitis; AE, adverse event; CI, confidence interval; EASI, Eczema Area and Severity Index; EoS, eosinophil; ET, epidermal thickness; PDE4, phosphodiesterase 4; RT-PCR, real-time PCR; sPGA-A, static physician's global assessment—acute signs; Th, T helper cell

Received 25 May 2018; revised 11 September 2018; accepted 4 October 2018; accepted manuscript published online 5 December 2018; corrected proof published online 15 February 2019

IL-33, IL-5, and the alarmins S100A7, S100A8, and S100A12 (Adams et al., 2015; Gottlieb et al., 2013; Schafer et al., 2014). In patients with moderate to severe psoriasis receiving apremilast 30 mg twice daily for 16 weeks, 29%–41% achieved a 75% or greater reduction from baseline in Psoriasis Area and Severity Index score; continued treatment up to 52 weeks is marked by sustained Psoriasis Area and Severity Index response and an acceptable safety profile (Papp et al., 2012, 2015; Paul et al., 2015; Reich et al., 2017; Crowley et al., 2017). In small, single-center, open-label pilot studies, findings with apremilast have been mixed in patients with moderate to severe AD (Samrao et al., 2012; Volf et al., 2012). A new topical PDE4 inhibitor, crisaborole, is available for the treatment of mild to moderate AD in children and adults (Pfizer, 2017; Paller et al., 2016), suggesting that systemic PDE4 inhibition may benefit patients with moderate to severe AD.

The objective of this phase 2 study was to evaluate the efficacy, safety, and pharmacodynamics profile of apremilast for the treatment of adult patients with moderate to severe AD.

RESULTS

Patients

A total of 191 patients were enrolled and randomly assigned to treatment groups. However, because of the unanticipated death of an investigator at one of the study sites, the six patients enrolled at that site were excluded from the intent-to-treat population, because their data could not be confirmed by the investigator and were deemed invalid according to International Council for Harmonisation Good Clinical Practice guidelines. Consequently, 185 patients constituted the intent-to-treat population (placebo, $n = 64$; apremilast 40 mg twice daily, $n = 63$; apremilast 30 mg twice daily, $n = 58$). Of these, 146 (78.9%) completed week 12, with comparable completion rates among the placebo and apremilast treatment groups (78%–79%) (see [Supplementary Figure S1 online](#)). The most common reasons for discontinuation were lack of efficacy in the placebo and apremilast 30 mg twice daily arms and AEs in the apremilast 40 mg twice daily arm. A benefit-risk assessment by the sponsor's independent data and safety monitoring board and an external data monitoring committee recommended that the apremilast 40 mg twice daily dose be discontinued and that patients in this arm be reassigned to receive apremilast 30 mg twice daily. Two patients taking apremilast 40 mg twice daily were reassigned to receive apremilast 30 mg twice daily on or after the week 20 visit.

Demographic and baseline clinical characteristics were generally comparable across treatment groups, with the exceptions that the placebo group had a smaller proportion of male patients, the apremilast 40 mg twice daily group had a smaller percentage of patients with a static physician's global assessment—acute signs (sPGA-A) rating of severe, and the apremilast 30 mg twice daily group had greater prior use of systemic immunosuppressive therapy ([Table 1](#)). During the placebo-controlled period, use of permitted concomitant therapies for AD, which included low-potency topical corticosteroids and oral antihistamines, oral leukotriene antagonists, inhaled corticosteroids, skin emollients,

Table 1. Demographic and baseline clinical characteristics

Characteristics	Placebo $n = 64$	Apremilast 30 mg Twice Daily $n = 58$	Apremilast 40 mg Twice Daily $n = 63$
Age in years, mean	37.7	39.2	38.3
Male, n (%)	25 (39.1)	31 (53.4)	31 (49.2)
BMI in kg/m^2 , mean	26.90	27.16	27.03
Weight in kg, mean	72.90	76.49	77.07
Duration in years of AD, mean	24.2	24.1	25.0
EASI score (0–72), mean	24.0	24.2	23.6
EASI score >20 , n (%)	31 (48.4)	30 (51.7)	30 (47.6)
Affected % BSA, mean	39.59	42.03	43.16
sPGA-A = 3 (moderate), n (%)	46 (71.9)	40 (69.0)	51 (81.0)
sPGA-A = 4 (severe), n (%)	18 (28.1)	18 (31.0)	12 (19.0)
Prior use of systemic immunosuppressive therapy, n (%)	7 (10.9)	11 (19.0)	8 (12.7)
Japanese, n (%)	18 (28.1)	13 (22.4)	16 (25.4)

Abbreviations: AD, atopic dermatitis; BMI, body mass index; BSA, body surface area; EASI, Eczema Area and Severity Index; sPGA-A, static physician global assessment—acute signs.

moisturizers, sunscreen, and cosmetics, was slightly higher in the placebo group (24/64, 37.5%) and the apremilast 30 mg twice daily group (20/58, 34.5%) compared with the apremilast 40 mg twice daily group (16/63, 25.4%). In the placebo group, 6 of 64 (9.4%) and 5 of 64 (7.8%) patients were treated with betamethasone butyrate propionate and zinc oxide, respectively, whereas no patients in the apremilast 40 mg twice daily and apremilast 30 mg twice daily groups were treated with either dermatologic agent.

Clinical efficacy

At week 12, patients who received apremilast 40 mg twice daily showed significantly greater percent improvement from baseline in Eczema Area and Severity Index (EASI) score versus placebo (mean [standard deviation] = -31.6% [44.6] vs. -11.0% [71.2], $P < 0.04$; effect size [95% confidence interval {CI}] = -20.6 [-39.7 to -1.5]). Patients who received apremilast 30 mg twice daily showed a trend toward greater EASI improvement at week 12 (mean [standard deviation] = -26.0% [40.1]; effect size [95% CI] = -15.0 [-34.5 to 4.5]) versus placebo but did not reach statistical significance ([Figure 1a](#)). At week 24, with the observed data, mean (standard deviation) percent change in EASI score improved from baseline among patients initially randomly assigned to receive apremilast (apremilast 40 mg twice daily: -51.1% [42.2]; apremilast 30 mg twice daily: -49.6% [33.1]); placebo patients who switched to apremilast at week 12 showed week 24 EASI improvements consistent with apremilast-treated patients (placebo/apremilast 40 mg twice daily: -58.2% [30.8]; placebo/apremilast 30 mg twice daily: -49.4% [53.5]). A $\geq 50\%$ reduction in EASI score at week 12 was achieved by 32.8% (21/64, placebo), 42.9% (27/63, apremilast 40 mg twice daily), and 31.0% (18/58, apremilast 30 mg twice daily) of patients (P not statistically significant for both apremilast dosages vs. placebo;

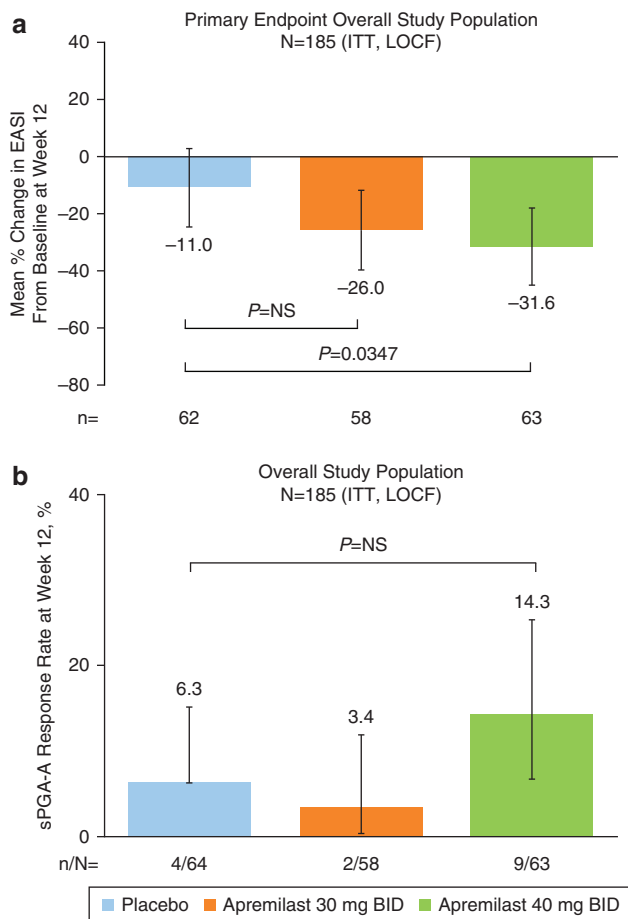


Figure 1. Week 12 mean percent change in EASI score and week 12 achievement of sPGA-A response. Error bars represent 95% confidence interval. sPGA-A response indicates a score of 0 (cleared) or 1 (almost cleared) and 2-point or greater reduction from baseline. EASI, Eczema Area and Severity Index; ITT, intent-to-treat; LOCF, last observation carried forward; n/N, number of patients with sPGA-A response/number of patients in treatment group from ITT population; NS, not significant; sPGA-A, static physician's global assessment—acute signs.

apremilast 40 mg twice daily effect size [95% CI] = 9.9 [−6.7 to 26.6]; apremilast 30 mg twice daily effect size [95% CI] = −1.5 [−18.0 to 14.9]). During the active treatment phase, the proportion of responders with $\geq 50\%$ reduction in EASI score increased through week 24 among patients initially randomly assigned to receive apremilast 40 mg twice daily and 30 mg twice daily (as-observed proportions, 62.2% [28/45] and 57.1% [24/42], respectively).

sPGA-A response at week 12 was achieved by 6.3% (4/64, placebo), 14.3% (9/63, apremilast 40 mg twice daily), and 3.4% (2/58, apremilast 30 mg twice daily) of patients (*P* not statistically significant for both apremilast dosages vs. placebo; apremilast 40 mg twice daily effect size [95% CI] = 8.0 [−2.3 to 18.2]; apremilast 30 mg twice daily effect size [95% CI] = −2.7 [−10.2 to 4.8]) (Figure 1b). During the active treatment phase, the proportion of sPGA-A responders was generally maintained among patients initially randomly assigned to receive apremilast 40 mg twice daily and increased through week 24 among patients initially randomly assigned to receive apremilast 30 mg twice daily (as-observed proportions, 15.6% [7/45] and 14.3% [6/42], respectively).

At week 12, mean percent change from baseline in pruritus visual analog scale score was −3.9%, −16.2%, and −9.8% for the placebo, apremilast 40 mg twice daily, and apremilast 30 mg twice daily groups, respectively (*P* not significant for both apremilast dosages vs. placebo). At week 12, apremilast 40 mg twice daily was associated with a significantly greater percent improvement (decrease) from baseline Dermatology Life Quality Index versus placebo (least-squares mean [standard error] = −27.3% [8.4] vs. 2.7% [8.4], *P* < 0.05); least-squares mean (standard error) improvement with apremilast 30 mg twice daily was −13.3% (8.7) (*P* not significant).

Subgroup analyses

At week 12, numerically greater improvements from baseline in EASI scores were observed with apremilast treatment versus placebo among patients with baseline IgE levels less than 360 $\mu\text{g/L}$ (i.e., intrinsic AD) compared with baseline IgE levels of 360 $\mu\text{g/L}$ or greater (i.e., extrinsic AD) and among patients with lower baseline eosinophil (EoS) level ($<0.39 \times 10^9/\text{L}$) versus higher baseline EoS level ($\geq 0.39 \times 10^9/\text{L}$); however, these changes did not achieve statistical significance (*P* not significant for both apremilast dosages vs. placebo) (Figure 2a). A similar pattern was observed with sPGA-A response achievement among the subgroups (Figure 2b). Additional subgroup analyses of change from baseline in EASI score at week 12 showed a significant difference favoring apremilast 40 mg twice daily over placebo among patients in Japan versus those in North America; this difference did not reach statistical significance with the apremilast 30 mg twice daily dosage.

Pharmacodynamics results

Demographic and baseline clinical characteristics of the biopsy substudy patients are summarized in [Supplementary Table S1 online](#); clinical efficacy findings based on EASI assessment at week 12 are summarized in [Supplementary Table S2 online](#). In all groups at baseline, epidermal thickness (ET) and K16 protein expression were significantly greater in lesional skin than nonlesional skin (*P* < 0.01 and *P* < 0.001, respectively). A change from baseline in ET of −11.4% at week 12 was observed in lesions from patients treated with apremilast 40 mg twice daily that trended toward significance (*P* < 0.1 vs. baseline). In comparison, no significant changes in ET were observed with apremilast 30 mg twice daily or placebo (both *P*-values not significant vs. baseline). K16 protein expression in the apremilast 40 mg twice daily group was significantly decreased (*P* < 0.001) at week 12, and the placebo and apremilast 30 mg twice daily groups showed nonsignificant changes. Only the apremilast 40 mg twice daily group had significantly more Ki67⁺ cells in baseline lesional versus nonlesional tissues. Ki67⁺ cell counts showed a statistically significant decline of −40.2% with apremilast 40 mg twice daily treatment at week 12 versus baseline (*P* < 0.001); no significant changes in Ki67 counts were noted with apremilast 30 mg twice daily or placebo. Changes from baseline at week 12 in ET, K16, and Ki67⁺ cells, based on immunohistochemistry studies and real-time polymerase chain reaction (RT-PCR) for K16 mRNA expression from a representative patient in the apremilast 40 mg twice daily group, are shown in Figure 3.

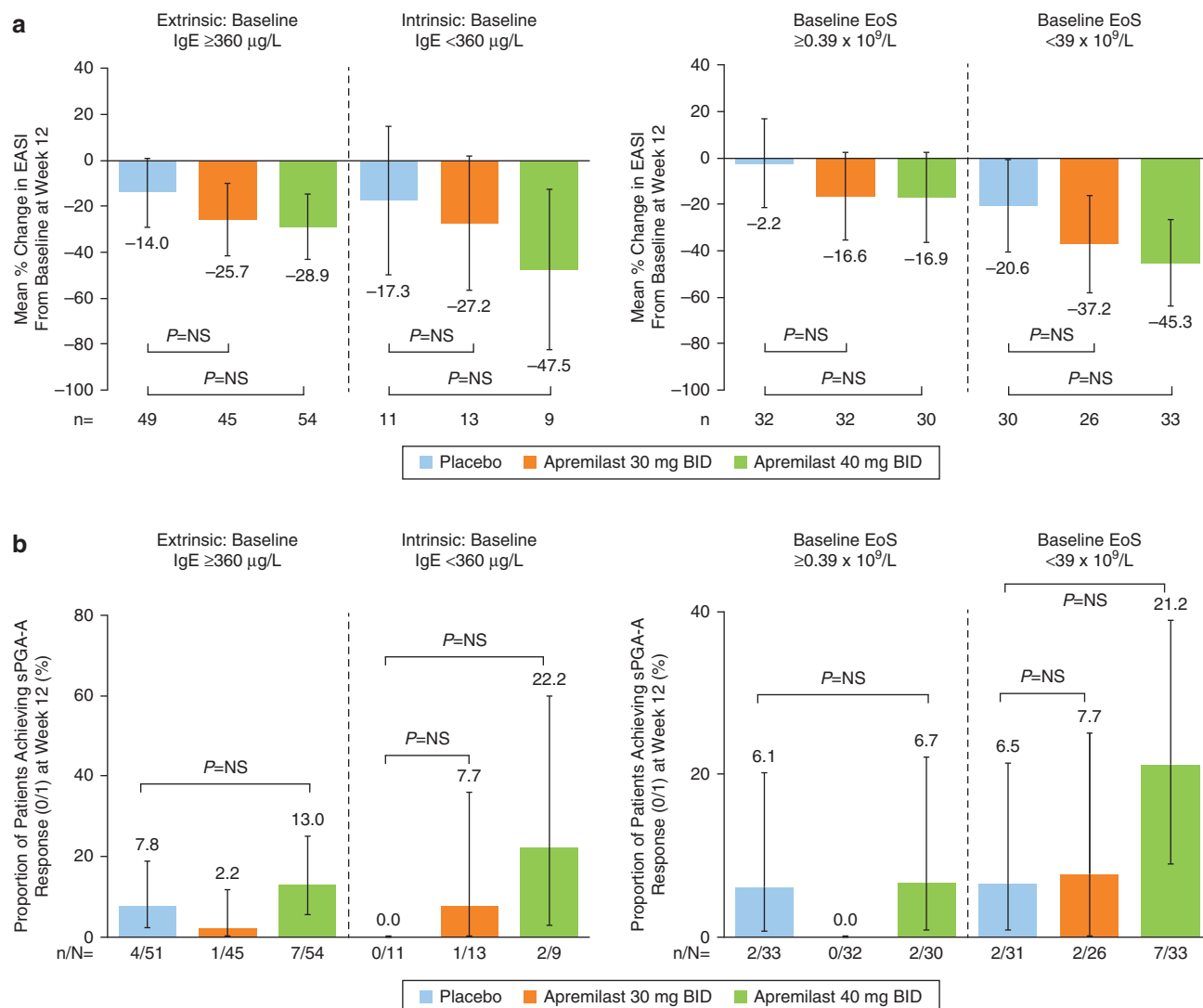


Figure 2. Week 12 mean percent change in EASI score and week 12 achievement of sPGA-A response, subgroups. ITT, LOCF. Error bars represent 95% confidence interval. sPGA-A response indicates a score of 0 (cleared) or 1 (almost cleared) and 2-point or greater reduction from baseline. EASI, Eczema Area and Severity Index; EoS, eosinophil; ITT, intent-to-treat; LOCF, last observation carried forward; n/N, number of patients with sPGA-A response/number of patients in treatment group from ITT population; NS, not significant; sPGA-A, static physician's global assessment—acute signs.

Heatmaps from gene array and RT-PCR assays of representative immune genes in pretreatment and posttreatment AD lesions are shown in [Supplementary Figure S2a online](#) and [Figure 4](#). Gene array analysis showed that the apremilast 40 mg twice daily group had the highest baseline dysregulation in lesional skin; at week 12, the apremilast 40 mg twice daily group showed reductions in T helper (Th) 17/Th22 genes and related markers, including IL-22 and IL-19, that trended toward significance ($P < 0.1$ for both). Other general inflammatory (MMP12) and Th17/Th22 (IL-17, S100A12) markers were decreased by both apremilast 40 mg twice daily and placebo (S100A9, CXCL1-2, STAT3). When comparing treatment effects of apremilast versus placebo, apremilast 40 mg twice daily showed more substantial declines in expression of Th17/Th22-associated markers (S100A12, IL-19, IL-22), with no notable changes in other immune axes ([Figure S2b](#)). The apremilast 30 mg twice daily

group did not show substantial changes from baseline at week 12.

RT-PCR mRNA changes mirrored gene array findings. At baseline, the apremilast 40 mg twice daily group had the highest dysregulation. At week 12, Th17 (IL-17A, IL-12B/IL-12/23p40) and Th17/Th22-associated markers (IL-22, S100A7/S100A8, S100A12) were significantly reduced only with apremilast 40 mg twice daily ($P < 0.05$ for all). Significant or major reductions from baseline were observed in Th2-associated cytokines IL-13, IL-31, and CCL17 in both the placebo and apremilast 40 mg twice daily groups. No significant changes from baseline were observed in the apremilast 30 mg twice daily group. Additional findings from the immunohistochemistry, gene array, and RT-PCR analyses are summarized in the [Supplementary Materials](#), [Supplementary Tables S1 and S3](#), and [Supplementary Figure S3 online](#).

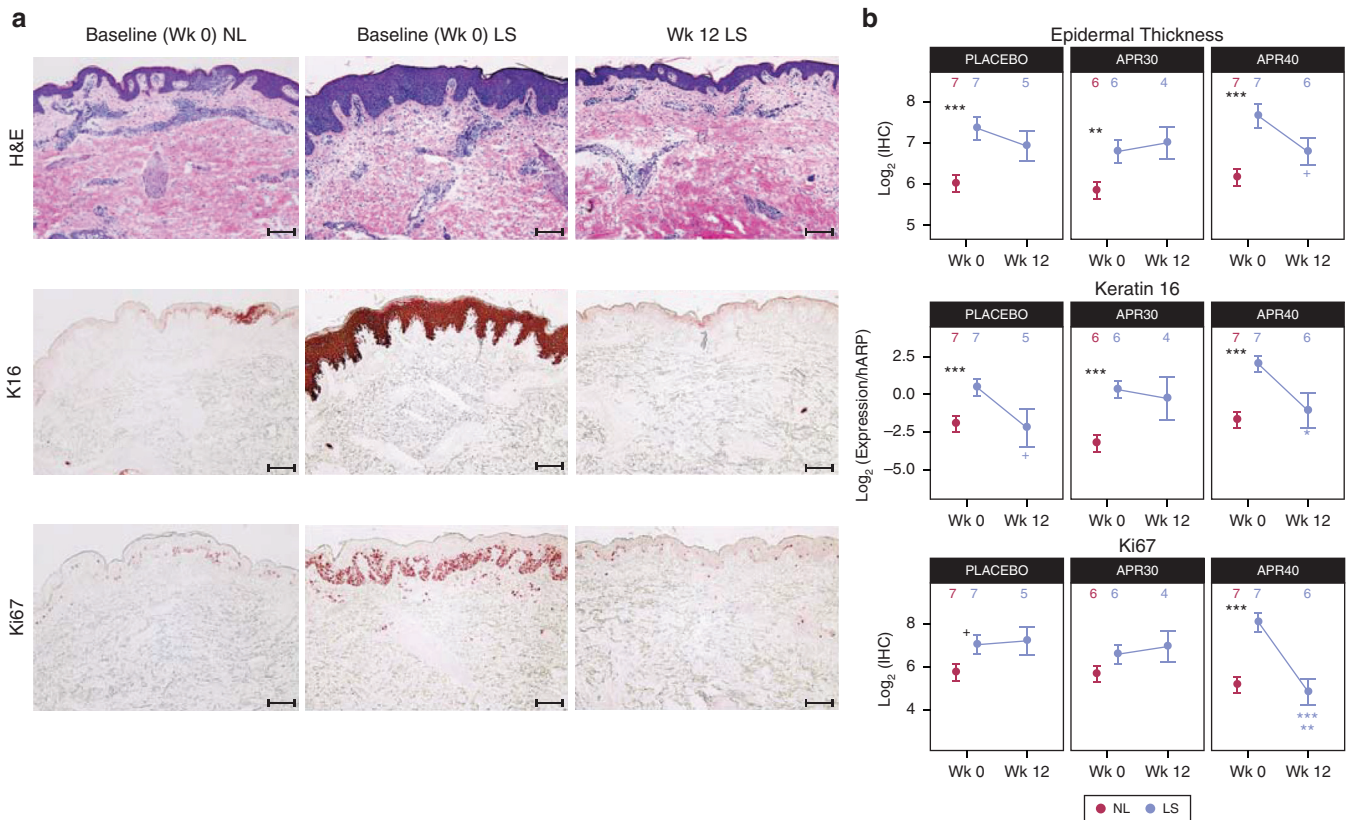


Figure 3. Representative immunohistochemical images and quantification of cellular markers. (a) Hematoxylin and eosin staining of a representative patient receiving apremilast 40 mg twice daily. Scale bar = 100 μ m. (b) Epidermal thickness, K16 mRNA levels, and Ki67 cell counts. Black asterisks indicate a significant difference in change, NL versus LS; blue asterisks, top indicates significant versus baseline; bottom indicates significant versus placebo. Red or blue numbers indicate NL or LS sample number. $^+P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. APR30, apremilast 30 mg twice daily; APR40, apremilast 40 mg twice daily; LS, lesional; NL, nonlesional.

Safety

During weeks 0–12 and throughout the full 24-week apremilast exposure period, most AEs were mild to moderate in severity, and few AEs were serious or led to treatment withdrawal (Table 2). The incidence of AEs, AEs leading to treatment withdrawal, and serious AEs was higher in patients receiving apremilast 40 mg twice daily than in patients receiving apremilast 30 mg twice daily. Because of an unexpected elevated incidence of cellulitis (detailed in this section) among patients receiving apremilast 40 mg twice daily, the independent safety monitoring committees discontinued the apremilast 40 mg twice daily treatment arm on November 20, 2015. No patients were in the placebo-controlled phase at the time of this determination; nearly all had completed the 24-week visit, and two patients initially assigned to receive apremilast 40 mg twice daily were switched to apremilast 30 mg twice daily.

The most common AEs ($\geq 5\%$ of patients in any treatment group) during the placebo-controlled period included diarrhea, nausea, headache, nasopharyngitis, upper respiratory tract infection, abdominal discomfort, cellulitis, and dyspepsia. The incidence of drug-related AEs, serious AEs, or AEs leading to withdrawal did not increase with apremilast exposure through week 24 (Table 2).

A total of six serious AEs were reported for five patients during the study; four serious AEs were reported for three

patients during the placebo-controlled period, and two occurred after week 12. During the placebo-controlled period, these included squamous cell carcinoma ($n = 1$, apremilast 30 mg twice daily), suicidal ideation ($n = 1$, apremilast 40 mg twice daily), and cellulitis and glomerulonephritis ($n = 1$, apremilast 40 mg twice daily). The two new serious AEs reported after week 12 included pneumonia ($n = 1$, apremilast 30 mg twice daily) and cellulitis ($n = 1$, apremilast 40 mg twice daily). Six patients receiving apremilast 40 mg twice daily experienced cellulitis (four cases during weeks 0–12, and two cases during weeks 12–24); all involved the lower extremities. Among four patients with nonserious cases of cellulitis, one patient had a history of peripheral neuropathy of the left foot corresponding to the same leg involved with cellulitis, and the other three patients with nonserious cases had no relevant medical history. Two cases of cellulitis were considered serious; one case occurred in a patient with a history of diabetes mellitus and was suspected to be potentially related to apremilast treatment. The second case, which occurred in a patient with a history of diabetes and streptococcal cellulitis, was not considered related to apremilast treatment; this patient discontinued because of complications from glomerulonephritis related to cellulitis. No other patients who developed cellulitis withdrew from the study; one case of nonserious cellulitis was considered by the investigator as suspected to be related to apremilast. No deaths occurred in the study.

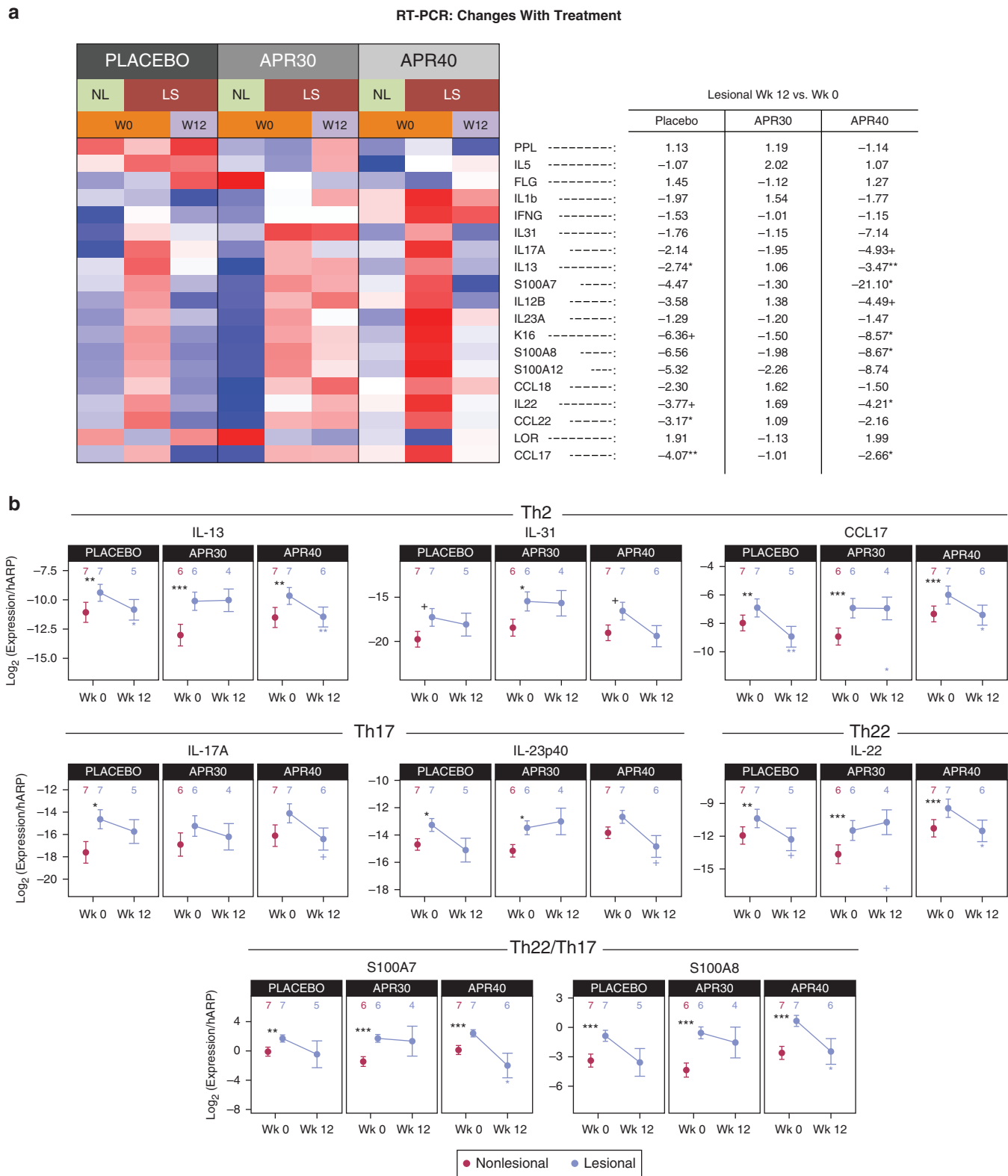


Figure 4. mRNA levels of immune and epidermal markers in skin treated with apremilast or placebo. (a) Heatmap of mean mRNA expression levels of representative immune and barrier markers in pretreatment and posttreatment atopic dermatitis lesions. (b) Line graphs of representative immune marker mRNA changes with treatment. Immune genes are grouped by major inflammatory/regulatory pathways. Black asterisks denote significance in change between nonlesional and lesional skin, top blue asterisks denote significance in change from baseline, and bottom blue asterisks denote significance of treatment effect between apremilast 40 mg twice daily and placebo. Red or blue numbers denote sample number for nonlesional or lesional skin, respectively. $^+P < 0.1$. $^*P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. APR30, apremilast 30 mg twice daily; APR40, apremilast 40 mg twice daily; LS, lesional NL, nonlesional.

Table 2. Overview of adverse events

	Placebo Controlled Period, Weeks 0–12 ¹			Apremilast Exposure Period, Weeks 0 to ≤24 ²	
Adverse Events	Placebo (n = 64)	Apremilast		Apremilast	
Patients, n (%)		30 mg Twice Daily (n = 58)	40 mg Twice Daily (n = 63)	30 mg Twice Daily (n = 82)	40 mg Twice Daily (n = 86)
Any AE	30 (46.9)	36 (62.1)	44 (69.8)	49 (59.8)	61 (70.9)
Any drug-related AE	8 (12.5)	26 (44.8)	27 (42.9)	29 (35.4)	38 (44.2)
Any severe AE	0 (0.0)	0 (0.0)	1 (1.6)	1 (1.2)	1 (1.2)
Any serious AE	0 (0.0)	1 (1.7)	2 (3.2)	2 (2.4)	3 (3.5)
Any AE leading to study drug interruption	3 (4.7)	0 (0.0)	4 (6.3)	0 (0.0)	8 (9.3)
Any AE leading to study drug withdrawal	1 (1.6)	2 (3.4)	6 (9.5)	3 (3.7)	9 (10.5)
Any AE leading to death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AEs reported by ≥5% of patients in any treatment group, n (%)					
Diarrhea	1 (1.6)	10 (17.2)	15 (23.8)	11 (13.4)	21 (24.4)
Nausea	1 (1.6)	9 (15.5)	7 (11.1)	10 (12.2)	14 (16.3)
Headache	5 (7.8)	4 (6.9)	8 (12.7)	7 (8.5)	8 (9.3)
Nasopharyngitis	1 (1.6)	6 (10.3)	6 (9.5)	8 (9.8)	14 (16.3)
Upper respiratory tract infection	8 (12.5)	2 (3.4)	3 (4.8)	8 (9.8)	6 (7.0)
Abdominal discomfort	0 (0.0)	3 (5.2)	2 (3.2)	3 (3.7)	2 (2.3)
Cellulitis	0 (0.0)	0 (0.0)	4 (6.3)	0 (0.0)	6 (7.0)
Vomiting	0 (0.0)	0 (0.0)	3 (4.8)	1 (1.2)	5 (5.8)
Dyspepsia	1 (1.6)	3 (5.2)	0 (0.0)	3 (3.7)	1 (1.2)
AEs leading to drug withdrawal in patients during the apremilast exposure period, n (%)					
Abdominal discomfort	—	—	—	0 (0.0)	2 (2.3)
Diarrhea	—	—	—	0 (0.0)	2 (2.3)
Headache	—	—	—	0 (0.0)	2 (2.3)
Pneumonia	—	—	—	1 (1.2)	0 (0.0)
Squamous cell carcinoma	—	—	—	1 (1.2)	0 (0.0)
Suicidal ideation	—	—	—	0 (0.0)	1 (1.2)
Anxiety	—	—	—	1 (1.2)	0 (0.0)
Migraine	—	—	—	0 (0.0)	1 (1.2)
Cardiac failure	—	—	—	0 (0.0)	1 (1.2)
Myalgia	—	—	—	0 (0.0)	1 (1.2)
Glomerulonephritis	—	—	—	0 (0.0)	1 (1.2)

Abbreviations: AE, adverse event.

¹Based on safety population.

²Includes all apremilast exposure data; patients could have started receiving apremilast at week 0 or week 12.

DISCUSSION

In adult patients with moderate to severe AD, apremilast 40 mg twice daily showed modest but statistically significant improvement in EASI score (from baseline) at week 12; sPGA-A response was numerically better than placebo, but this was not statistically significant. Patients receiving apremilast 40 mg twice daily also reported significantly greater improvement in quality of life at week 12 versus placebo. Use of permitted concomitant dermatologic medications was slightly higher in the placebo and apremilast 30 mg twice daily groups than in the apremilast 40 mg twice daily group, and this may have attenuated differences between the placebo and apremilast 40 mg twice daily groups in efficacy or biomarker outcomes. However, the AEs observed with apremilast 40 mg twice daily conferred a benefit-to-risk profile that was not deemed appropriate for further investigation in AD.

The tissue biomarker data were consistent with clinical results, with the apremilast 40 mg twice daily group showing the greatest molecular and cellular improvements. These

included reductions in epidermal hyperplasia (i.e., K16, Ki67, and ET) and inflammatory markers associated with Th17/Th22 pathways. In contrast, the apremilast 30 mg twice daily group did not show substantial biomarker changes compared with placebo. This likely reflects the expected lower dose effect on the measured biomarkers, which may have affected the ability to detect changes from baseline in this treatment group.

Clinical subgroup analyses suggest better response to apremilast treatment in patients with intrinsic AD compared with patients with extrinsic AD. Prior research has shown that intrinsic AD is associated with greater Th17 expression in lesional skin compared with extrinsic AD (Suarez-Farinas et al., 2013). Apremilast is known to modulate Th17 cytokines in psoriasis (Gottlieb et al., 2013) and appears to modulate the same pathway in AD patients based on the current findings. However, these results should be interpreted with caution because of the small sample size.

Pruritus is a central clinical feature of AD. In this study, improvements in pruritus with either dosage of apremilast

were not significantly different from placebo at week 12. By contrast, apremilast 30 mg twice daily significantly improved pruritus in phase 3 trials in moderate to severe psoriasis (Papp et al., 2015; Paul et al., 2015). It is unclear whether this divergence is due to a difference in the mechanism underlying pruritus in AD versus psoriasis or is because apremilast primarily modulates the Th17 pathway (Schafer et al., 2014), which may be a key disease driver of psoriasis but currently is not recognized as pathogenic in AD, although this axis is increased in AD, and particularly in certain disease subsets such as in Asian patients and those with early-onset pediatric AD (Bieber, 2008; Brunner et al., 2017; Esaki et al., 2016; Guttman-Yassky and Krueger, 2017; Guttman-Yassky et al., 2017; Noda et al., 2015; Tabarkiewicz et al., 2015). Our findings are somewhat surprising given the clinical efficacy of the topical PDE4 inhibitor crisaborole ointment (2%) (Pfizer, 2017; Paller et al., 2016). Differences in results from the trials with crisaborole versus apremilast might be attributable to route of administration, selected patient populations (i.e., children and adults vs. adults only), and baseline disease severity (i.e., mild to moderate vs. moderate to severe AD). More broadly, interpretation of the current findings may be limited to patients in North America. The small number of patients from Japan and lack of patients from other regions may limit meaningful conclusions about Japanese patients and patients from other racial groups.

The safety and tolerability of apremilast 30 mg twice daily observed in this study are largely consistent with the known safety profile of apremilast from phase 3 clinical trials in patients with psoriasis and psoriatic arthritis (Cutolo et al., 2016; Edwards et al., 2016; Kavanaugh et al., 2015; Papp et al., 2015; Paul et al., 2015). Most AEs were mild or moderate in severity, and few led to discontinuation of treatment; the most common AEs included diarrhea, nausea, headache, and nasopharyngitis. Overall, more AEs were observed with apremilast 40 mg twice daily than with apremilast 30 mg twice daily. There were six cases of cellulitis among patients receiving apremilast 40 mg twice daily; no cases of cellulitis were observed among patients receiving apremilast 30 mg twice daily or placebo. After a benefit-risk assessment by the independent safety monitoring committees, the apremilast 40 mg twice daily dosage was discontinued in November 2015, after most patients had completed the 24-week clinic visit; the remaining patients in this arm ($n = 2$) were switched to the apremilast 30 mg twice daily group. Cellulitis may be a phenomenon unique to patients with AD, because bacterial skin infections are often seen in patients with AD (Petry et al., 2012). In patients with psoriasis or psoriatic arthritis, apremilast 30 mg twice daily treatment was not associated with an increased incidence of infection or cellulitis (Crowley et al., 2017; Mease et al., 2017).

Treatment with apremilast 40 mg twice daily over 12 weeks showed modest efficacy in patients with moderate to severe AD. No significant differences were observed between apremilast 30 mg twice daily and placebo by week 12, although there were efficacy endpoints that were numerically improved at week 12 and continued to improve with continuous treatment through week 24. Biomarker analyses in tissues showed decreases in Th17/Th22-related markers

with apremilast 40 mg twice daily versus placebo, with no substantial changes from baseline observed with apremilast 30 mg twice daily. The safety of apremilast 30 mg twice daily was generally similar to the known safety profile of apremilast in psoriasis and psoriatic arthritis patients, although more AEs, including cellulitis, were reported in patients treated with the 40 mg twice daily dosage.

MATERIALS AND METHODS

Patients

Adult patients (≥ 18 years of age) were eligible to participate if they had moderate to severe AD (i.e., AD-affected body surface area $\geq 10\%$, EASI score ≥ 12 , and sPGA-A score ≥ 3 [moderate to severe]) not adequately controlled by a stable regimen (≥ 4 weeks) of topical corticosteroids or topical calcineurin inhibitors within 6 months of screening or if they were considered inappropriate candidates for topical therapy. Additional details of the inclusion/exclusion criteria are provided in the [Supplementary Materials](#). All patients provided written informed consent before entering the study, and the protocol was approved by each site's institutional review board or independent ethics committee.

Study design

This was a phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group study (NCT02087943) conducted from June 2014 to February 2016 at 28 study centers in North America and Japan. Eligible patients were randomly assigned (1:1:1) to receive placebo, apremilast 30 mg twice daily, or apremilast 40 mg twice daily for 12 weeks (placebo-controlled period) with a permuted block method and were stratified by geographic region (North America and Japan) and, within region, by baseline EASI score (≤ 20 or > 20) with an interactive web-response or interactive voice-response system. Apremilast was titrated in 10-mg increments over the first 8 days of treatment. At week 12, patients initially randomly assigned to receive placebo were reassigned randomly (1:1) to double-blind treatment with apremilast 30 mg twice daily or 40 mg twice daily through week 24 (with dose titration over the first 8 days); patients initially randomly assigned to receive apremilast continued to receive their assigned dosage. To maintain double-blind conditions for patients and clinical study personnel throughout the 24-week trial, assigned treatments were dispensed only by authorized personnel at each study site in coded, identically appearing blister cards containing the assigned treatment and dose. Patients who completed or discontinued early entered a 6-week posttreatment observational period. Concomitant medications that were permitted and not permitted are listed in the [Supplementary Materials](#).

Assessments

Clinical efficacy was assessed at weeks 2, 4, 8, and 12 during the placebo-controlled phase; at weeks 16, 20, and 24 during the active-treatment phase; and at the end of the 6-week posttreatment observation period. The primary efficacy endpoint was the percent change from baseline in EASI score at week 12. The proportion of patients who achieved an sPGA-A response (score of 0 [cleared] or 1 [almost cleared] and ≥ 2 -point reduction from baseline) at week 12 was a secondary endpoint, as was achievement of a 50% or greater improvement from baseline EASI score. Predefined exploratory endpoints included the percent change from baseline in average pruritus visual analog scale (cm) score (range: 0 [no itching] to 10 [worst itching imaginable]) (Kunz et al., 1997); percent change from

baseline in Dermatology Life Quality Index score (range: 0 [no effect on life] to 30 [extremely large effect on life]) at week 12; percent change from baseline in EASI score at week 24; and the proportion of patients who achieved an sPGA-A response at week 24. Post hoc analyses examined percent change from baseline in EASI score and sPGA-A response at week 12 among subgroups of patients defined by baseline IgE level less than 360 µg/L (intrinsic AD) versus 360 µg/L or greater (extrinsic AD), as well as among patients with low baseline EoS levels ($<0.39 \times 10^9/L$) versus high baseline EoS levels ($\geq 0.39 \times 10^9/L$), based on the median EoS level of the study population at baseline, $0.39 \times 10^9/L$. Safety was evaluated based on AEs, vital signs, physical examinations, laboratory evaluations, and electrocardiograms.

Pharmacodynamics assessments

Blood samples were obtained at baseline; weeks 4, 12, 16, and 24; and the final study visit. IgE levels and EoS counts were determined; samples were also used to examine standard clinical chemistry and hematology parameters. For the biopsy substudy, pretreatment (week 0) lesional and nonlesional (>1 cm from active lesions) skin was obtained from 21 adult patients (see the [Supplementary Materials](#) and [Supplementary Table S1](#) for further details). For immunohistochemistry, frozen optimum cutting temperature-embedded cryostat sections were labeled using purified mouse antihuman monoclonal antibodies (see the [Supplementary Materials](#) and [Supplementary Table S3](#) for further details), as described previously (Suarez-Farinas et al., 2011; Tintle et al., 2011). Changes from baseline at week 12 in ET and two markers of epidermal proliferation, K16 and Ki67, were examined. ET and cell counts were quantified with ImageJ, version 1.42 (National Institutes of Health, Bethesda, MD). For gene expression analysis of key AD-related genes, RNA was extracted, followed by quantitative RT-PCR and Affymetrix Human U133Plus 2.0 gene arrays (Affymetrix, Santa Clara, CA) analyses, as previously described (Tintle et al., 2011) (see the [Supplementary Materials](#) and [Supplementary Tables S4](#) and [S5](#) online for further details).

Statistical analysis

Efficacy data were assessed in the intent-to-treat population, which included all randomized patients who received at least one dose of study medication according to the group to which they were randomly assigned; safety analyses included all patients who received at least one dose of study medication. A planned sample size of 189 patients would provide 80% power with a two-sided significance level of 0.05, allowing for 20% discontinuation by week 12. Comparisons for the primary endpoint (percent change from baseline in EASI score at week 12) were performed using an analysis of covariance model, with treatment and baseline stratification as factors; missing values were imputed using the last-observation-carried-forward methodology. The same analysis of covariance model was used for analyses of percent change from baseline in pruritus visual analog scale and change from baseline in Dermatology Life Quality Index score at week 12. For the sPGA-A and $\geq 50\%$ improvement in EASI score analyses, treatment comparisons were performed using a Cochran-Mantel-Haenszel test, with missing values imputed using last-observation-carried-forward methodology. Percent change from baseline in EASI score at week 24 was analyzed with descriptive statistics and data as observed. Details of statistical analyses for the biopsy substudy are presented in the [Supplementary Materials](#).

CONFLICT OF INTEREST

ELS reports grants, grants paid to institution, personal fees, and/or nonfinancial support from AbbVie, Anacor Pharma, Celgene Corporation, Dermira, Eli Lilly, Galderma, GlaxoSmithKline, LEO Pharma, MedImmune, Menlo Therapeutics, Novartis, Pfizer, Regeneron Pharmaceuticals, Sanofi Genzyme, Valeant Pharmaceuticals, Roivant Sciences, Tioga Pharmaceuticals, and Vanda Pharmaceuticals. SI receives honoraria as an advisor and as a speaker from Celgene Corporation and Maruho. YP has received grants, board membership fees, and/or speaker fees from AbbVie, Amgen, Baxter, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene Corporation, Janssen, Eli Lilly, EMD Serono, Galderma, GlaxoSmithKline, LEO Pharma, Merck, Novartis, Pfizer, Regeneron, Sanofi, and UCB Pharma. MC and NS are employed by Celgene Corporation. KN was employed by Celgene Corporation during manuscript development. MS-F has received grants/grants pending (paid to Mount Sinai Health System) from Celgene Corporation, Genisphere, and Pfizer; consultancy fees from New Enterprise Associates; and travel fees from DBV Technologies. EG-Y has received grants (paid to Mount Sinai Health System), consulting fees, and/or honoraria from AbbVie, Allergan, Anacor, Asana Bioscience, Bristol-Myers Squibb, Celgene Corporation, Celus Therapeutics, Curadim Pharma, Dermira, Draize, Eli Lilly, Escalier, Galderma, Genentech, Glenmark, Janssen Biotech, Kymab Limited, Kyowa Kirin, Lead Pharma, LEO Pharma, Merck Pharmaceuticals, Novartis, Pfizer, Regeneron, Sanofi, Sienna Biopharmaceuticals, Stiefel/GlaxoSmithKline, Theravance, and Vitae. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

The authors wish to thank the study sponsor, Celgene Corporation. The authors received editorial support in the preparation of this report from Karen Dougherty and Amy Shaberman of Peloton Advantage, LLC, Parsippany, NJ, funded by Celgene Corporation, Summit, NJ. The authors, however, directed and are fully responsible for all content and editorial decisions for this manuscript. All authors had full access to all the data, and data are available upon request.

Celgene is committed to responsible and transparent sharing of clinical trial data with patients, health care practitioners, and independent researchers for the purpose of improving scientific and medical knowledge, as well as fostering innovative treatment approaches. For more information, please visit <https://www.celgene.com/research-development/clinical-trials/clinical-trials-data-sharing>.

Gene expression data are available at Gene Expression Omnibus: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120899>.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.10.043>.

REFERENCES

- Adams M, Horan G, Schafer PH. Apremilast regulates human keratinocyte gene expression in vitro and reduces antigen-driven dermatitis in vivo. Poster presented at: Congress of the European Academy of Dermatology and Venereology. October 7–11, 2015; Copenhagen, Denmark.
- Beck LA, Thaci D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014;371:130–9.
- Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483–94.
- Brunner PM, Guttman-Yassky E, Leung DY. The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies. *J Allergy Clin Immunol* 2017;139(4 Suppl):S65–76.
- Celgene. Otezla[package insert]. Summit, NJ: Celgene Corporation; June 2017.
- Crowley J, Thaci D, Joly P, Peris K, Papp KA, Goncalves J, et al. Long-term safety and tolerability of apremilast in patients with psoriasis: pooled safety analysis for ≥ 156 weeks from 2 phase 3, randomized, controlled trials (ESTEEM 1 and 2). *J Am Acad Dermatol* 2017;77:310–7.
- Cutolo M, Myerson GE, Fleischmann R, Liote F, Diaz-Gonzalez F, Van den Bosch F, et al. A phase III, randomized, controlled trial of apremilast in patients with psoriatic arthritis: results of the PALACE 2 trial. *J Rheumatol* 2016;43:1724–34.
- Czarnowicki T, Krueger JG, Guttman-Yassky E. Skin barrier and immune dysregulation in atopic dermatitis: an evolving story with important clinical implications. *J Allergy Clin Immunol Pract* 2014;2:371–9.

- Edwards CJ, Blanco FJ, Crowley J, Birbara CA, Jaworski J, Aelion J, et al. Apremilast, an oral phosphodiesterase 4 inhibitor, in patients with psoriatic arthritis and current skin involvement: a phase III, randomised, controlled trial (PALACE 3). *Ann Rheum Dis* 2016;75:1065–73.
- Eichenfield LF, Tom WL, Berger TG, Krol A, Paller AS, Schwarzenberger K, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *J Am Acad Dermatol* 2014a;71:116–32.
- Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol* 2014b;70:338–51.
- Esaki H, Brunner PM, Renert-Yuval Y, Czarnowicki T, Huynh T, Tran G, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. *J Allergy Clin Immunol* 2016;138:1639–51.
- Gittler JK, Shemer A, Suarez-Farinas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T_H2/T_H22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344–54.
- Gottlieb AB, Matheson RT, Menter A, Leonardi CL, Day RM, Hu CC, et al. Efficacy, tolerability, and pharmacodynamics of apremilast in recalcitrant plaque psoriasis: a phase II open-label study. *J Drugs Dermatol* 2013;12:888–97.
- Guttman-Yassky E, Krueger JG. Atopic dermatitis and psoriasis: two different immune diseases or one spectrum? *Curr Opin Immunol* 2017;48:68–73.
- Guttman-Yassky E, Krueger JG, Lebowitz MG. Systemic immune mechanisms in atopic dermatitis and psoriasis with implications for treatment. *Exp Dermatol* 2017;27:409–17.
- Guttman-Yassky E, Nogales KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—part II: immune cell subsets and therapeutic concepts. *J Allergy Clin Immunol* 2011;127:1420–32.
- Hanifin J, Gupta AK, Rajagopalan R. Intermittent dosing of fluticasone propionate cream for reducing the risk of relapse in atopic dermatitis patients. *Br J Dermatol* 2002;147:528–37.
- Jensterle M, Salamun V, Kocjan T, Vrtacnik Bokal E, Janez A. Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. *J Ovarian Res* 2015;8:32.
- Kavanaugh A, Mease PJ, Gomez-Reino JJ, Adebajo AO, Wollenhaupt J, Gladman DD, et al. Longterm (52-week) results of a phase III randomized, controlled trial of apremilast in patients with psoriatic arthritis. *J Rheumatol* 2015;42:479–88.
- Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195:10–9.
- McGregor SP, Farhangian ME, Huang KE, Feldman SR. Treatment of atopic dermatitis in the United States: analysis of data from the National Ambulatory Medical Care Survey. *J Drugs Dermatol* 2017;16:250–5.
- Mease PJ, Gladman DD, Gomez-Reino JJ, Hall S, Kavanaugh A, Lespessailles E, et al. Consistent safety profile with up to 4 years of apremilast treatment: analysis of data from 1,493 subjects with psoriatic arthritis in 3 large, phase III, long-term studies [abstract]. *Arthritis Rheumatol* 2017;69(Suppl. 10):604.
- Noda S, Suarez-Farinas M, Ungar B, Kim SJ, de Guzman Strong C, Xu H, et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J Allergy Clin Immunol* 2015;136:1254–64.
- Paller AS, Tom WL, Lebowitz MG, Blumenthal RL, Boguniewicz M, Call RS, et al. Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. *J Am Acad Dermatol* 2016;75:494–503.
- Papp K, Cather JC, Rosoph L, Sofen H, Langley RG, Matheson RT, et al. Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial. *Lancet* 2012;380(9843):738–46.
- Papp K, Reich K, Leonardi CL, Kircik L, Chimenti S, Langley RG, et al. Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, in patients with moderate to severe plaque psoriasis: results of a phase III, randomized, controlled trial (Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis [ESTEEM 1]). *J Am Acad Dermatol* 2015;73:37–49.
- Park CO, Lee HJ, Lee JH, Wu WH, Chang NS, Hua L, et al. Increased expression of CC chemokine ligand 18 in extrinsic atopic dermatitis patients. *Exp Dermatol* 2008;17:24–9.
- Park CO, Noh S, Jin S, Lee NR, Lee YS, Lee H, et al. Insight into newly discovered innate immune modulation in atopic dermatitis. *Exp Dermatol* 2013;22:6–9.
- Paul C, Cather J, Gooderham M, Poulin Y, Mrowietz U, Ferrandiz C, et al. Efficacy and safety of apremilast, an oral phosphodiesterase 4 inhibitor, in patients with moderate to severe plaque psoriasis over 52 weeks: a phase III, randomized, controlled trial (ESTEEM 2). *Br J Dermatol* 2015;173:1387–99.
- Petry V, Bessa GR, Poziomczyk CS, Oliveira CF, Weber MB, Bonamigo RR, et al. Bacterial skin colonization and infections in patients with atopic dermatitis. *An Bras Dermatol* 2012;87:729–34.
- Pfizer. Eucrisa [package insert]. New York, NY: Pfizer; October 2017.
- Reich K, Gooderham M, Green L, Bewley A, Zhang Z, Khanskaya I, et al. The efficacy and safety of apremilast, etanercept, and placebo, in patients with moderate to severe plaque psoriasis: 52-week results from a phase 3b, randomized, placebo-controlled trial (LIBERATE). *J Eur Acad Dermatol Venereol* 2017;31:507–17.
- Roekevich E, Schram ME, Leeflang MMG, Brouwer MWD, Gerbens LAA, Bos JD, et al. Methotrexate versus azathioprine in patients with atopic dermatitis: 2-year follow-up data. *J Allergy Clin Immunol* 2018;141:825–7.
- Samrao A, Berry TM, Goreski R, Simpson EL. A pilot study of an oral phosphodiesterase inhibitor (apremilast) for atopic dermatitis in adults. *Arch Dermatol* 2012;148:890–7.
- Schafer PH, Parton A, Capone L, Cedzik D, Brady H, Evans JF, et al. Apremilast is a selective PDE4 inhibitor with regulatory effects on innate immunity. *Cell Signal* 2014;26:2016–29.
- Sidbury R, Davis DM, Cohen DE, Cordoro KM, Berger TG, Bergman JN, et al. Guidelines of care for the management of atopic dermatitis: section 3. Management and treatment with phototherapy and systemic agents. *J Am Acad Dermatol* 2014;71:327–49.
- Snyder A, Farhangian M, Feldman SR. A review of patient adherence to topical therapies for treatment of atopic dermatitis. *Cutis* 2015;96:397–401.
- Suarez-Farinas M, Dhingra N, Gittler J, Shemer A, Cardinale I, de Guzman Strong C, et al. Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 2013;132:361–70.
- Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nogales K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011;127:954–64.
- Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. *Arch Immunol Ther Exp (Warsz)* 2015;63:435–49.
- Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583–93.
- Volf EM, Au S-C, Dumont N, Scheinman P. A phase 2, open-label, investigator-initiated study to evaluate the safety and efficacy of apremilast in subjects with recalcitrant allergic contact or atopic dermatitis. *J Drugs Dermatol* 2012;11:341–6.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

Supplementary Material

A Phase 2 Randomized Trial of Apremilast in Patients With Atopic Dermatitis

Eric Simpson¹, Shinichi Imafuku², Yves Poulin³, Benjamin Ungar⁴, Lisa Zhou⁴, Kunal Malik⁴, Huei-Chi Wen,⁴ Hui Xu⁴, Yeriel D. Estrada⁴, Xiangyu Peng⁴, Mindy Chen⁵, Nilam Shah⁵, Mayte Suarez-Farinas⁴, Ana B. Pavel⁴, Kristine Nograles⁵, Emma Guttman-Yassky⁴

¹Oregon Health and Science University, Portland, OR, USA; ²Fukuoka University, Fukuoka, Japan; ³Laval University, Quebec City, Quebec, Canada; ⁴Icahn School of Medicine at the Mount Sinai Medical Center, New York, NY, USA; ⁵Celgene Corporation, Summit NJ, USA

Patient Inclusion and Exclusion Criteria

1. Eligible patients were aged ≥ 18 years, had a documented diagnosis of AD for ≥ 12 months, and met Hanifin and Rajka criteria for AD at screening (Hanifin and Rajka, 1980).
2. Patients with moderate to severe AD at screening (i.e., AD-affected body surface area [BSA] $\geq 10\%$, Eczema Area and Severity Index [EASI] score ≥ 12 , and static Physician's Global Assessment–Acute Signs [sPGA-A] score ≥ 3 [moderate to severe]) who were candidates for systemic therapy were considered for inclusion.
3. Eligible patients had AD that was not adequately controlled by a stable regimen (≥ 4 weeks) of topical corticosteroids or topical calcineurin inhibitors within 6 months of screening, or was considered inappropriate for topical therapy because of side effects or safety risks.
4. Key exclusion criteria were clinically significant or major uncontrolled disease, active or history of incompletely treated tuberculosis, history of malignancy within the past 5 years (except treated [cured] squamous cell or basal cell in situ carcinomas or cervical intraepithelial neoplasia or carcinoma in situ of the cervix with no evidence of recurrence within 5 years prior to screening), unstable asthma, significant infection within 2 weeks of screening, active skin infection, use of phototherapy or systemic immunosuppressive drugs within 4 weeks, use of interferon- γ within 12 weeks, and use of biologics within 12 to 24 weeks of baseline.
5. All patients provided written informed consent before entering the study, and the protocol was approved by each site's institutional review board/independent ethics committee.

Concomitant Medications

1. Concomitant use of low-potency topical corticosteroids and oral antihistamines was permitted when used on a stable regimen from ≥ 2 weeks prior to baseline through study Week 12, except within 24 hours before each visit; patients were also permitted to use a stable regimen (from ≥ 2 weeks prior to baseline through the Week 24) of oral leukotriene antagonists, inhaled corticosteroids, or other anti-allergic/anti-inflammatory medications for the treatment of allergic rhinitis or asthma; skin emollients, moisturizers, sunscreen, and cosmetics; and a stable regimen (≥ 2 weeks prior to the baseline visit through the Week 24, except within 24 hours of each study visit) of bathing with emollients, oils, bleach, or salts.
2. Not permitted was use of low-potency topical corticosteroids with occlusive dressing, mid- to high-potency topical corticosteroids, or topical calcineurin inhibitors. Also prohibited was use of oral or topical herbal preparations with immunomodulatory properties; topical antipruritic, analgesic, or antihistamine medications; and use of CYP450 inducers, such as rifampin, phenobarbital, carbamazepine, or St. John's wort.

Cellulitis Cases in the Apremilast 40 mg BID Treatment Arm

Case 1: A 39-year-old black man reported a nonserious AE of moderate cellulitis on Study Day 27. The dose of apremilast was not changed due to the event. The investigator considered the cellulitis as suspected to be related to apremilast. The event was resolved on Study Day 40. A relevant medical history of peripheral neuropathy (left foot) was reported.

Case 2: A 24-year-old white woman reported a nonserious AE of moderate cellulitis on Study Day 32 (4 days after the patient's last dose of apremilast). The investigator considered the

cellulitis as not suspected to be related to apremilast. The event was resolved on Study Day 43. No relevant medical history was reported.

Case 3: A 65-year-old white man reported a nonserious AE of mild cellulitis on Study Day 40. The apremilast dose was not changed due to the event. The investigator considered the cellulitis as not suspected to be related to apremilast. The event was resolved on Study Day 58. No relevant medical history was reported.

Case 4: A 37-year-old white man reported a nonserious AE of moderate cellulitis on Study Day 84. Apremilast treatment was interrupted due to the event. The investigator considered the cellulitis as not suspected to be related to apremilast. The event was resolved on Study Day 95. No relevant medical history was reported.

Case 5: A 49-year-old white man reported a serious AE of moderate cellulitis on Study Day 7. Apremilast treatment was interrupted due to the event. The investigator considered the cellulitis as not suspected to be related to apremilast. The event was resolved on Study Day 14. A relevant medical history of cellulitis in 1995 was reported, as well as a nonserious AE of moderate cellulitis on Study Day –40.

Case 6: A 58-year-old black man reported a nonserious AE of moderate cellulitis on Study Day 100 (16 days after switching to apremilast from placebo). On Study Day 105, the cellulitis was considered a serious AE and apremilast treatment was interrupted. The cellulitis was downgraded to a nonserious event on Study Day 111. The investigator considered the cellulitis as suspected to be related to apremilast. The cellulitis was resolved on Study Day 152. A relevant medical history of type 2 diabetes mellitus was reported.

Biopsy Substudy Supplementary Methods, Statistical Analysis, and Results

Biopsy Substudy Patients

Twenty-one adult patients (11 male, 10 female; mean age 40.4 years; mean baseline EASI 26.3) were enrolled in the biopsy substudy. Twenty-one pretreatment (Week 0) lesional and nonlesional (>1 cm from active lesions) biopsies were obtained; 7 allocated to placebo (2 male, 5 female, mean age 38.7 years, mean baseline EASI 24.4), 7 allocated to apremilast 30 mg BID (4 male, 3 female, mean age 28.7 years, mean baseline EASI 27.0), and 7 allocated to apremilast 40 mg BID (5 male, 2 female, mean age 53.7 years, mean baseline EASI 27.6). See **Table S1** for details.

Immunohistochemistry

Immunohistochemistry (IHC) was performed using purified anti-human monoclonal antibodies (see **Table S3**). For IHC, biotin-labeled horse anti-mouse secondary antibodies (Vector Laboratories) were used to detect the primary antibodies, and the staining signal was developed with chromogen 3-amino-9-ethylcarbazole (AEC, Sigma-Aldrich). For cell counts, positive cells per millimeter were manually quantified using computer-assisted image analysis software (ImageJ 1.42; National Institutes of Health, Bethesda, MD).

Quantitative Real-Time PCR and Gene Array Analysis

RNA was extracted and Affymetrix Human U133Plus 2.0 gene arrays (Affymetrix, Santa Clara, CA) were used as previously described (Tintle et al.). We used human HGU133Plus2.0 GeneChip probe gene arrays (Affymetrix) containing approximately 54,000 probe sets to assess expression of more than 47,000 transcripts. Total RNA was extracted from tissues frozen in

liquid nitrogen by the Qiagen RNeasy Mini Kit (Valencia, CA). DNA was removed with on-column DNase digestion by the Qiagen RNase-free DNase Set. Total RNA (50 ng) was reverse transcribed and amplified with Ovation Whole Blood Solution from NuGen (San Carlos, CA). The labeled target was fragmented and hybridized to probe gene arrays, using Encore Biotin Module from NuGen (San Carlos, CA). The chips were washed, stained with streptavidin-phycoerythrin, and scanned (HP GeneArray Scanner; Hewlett-Packard Company, Palo Alto, CA). On each chip, the human housekeeping genes β -actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as controls. Suite 5.0 software normalized the expression level values using these controls.

RT-PCR Analysis

The TaqMan real time (RT)-PCR was performed with EZ-PCR Core Reagents (Life Technologies), according to the manufacturer's directions. Human acidic ribosomal protein (hARP), a housekeeping gene, was used to normalize each sample and each gene. The primer sequences used were generated with the Primer Express algorithm (Applied Biosystems, Foster City, CA), version 1.0, by using published genetic sequences (National Center for Biotechnology Information–PubMed), as follows: hARP forward CGCTGCTGAACATGCTCAA, hARP reverse TGTCGAACACCTGCTGGATG, and hARP probe 6-FAM-TCCCCCTTCTCCTTTGGGCTGG-TAMRA (Gene Bank accession no. NM-001002). Primers and probes are listed in **Table S4**. The data were analyzed and quantified using the software provided with the Applied Biosystems PRISM 7700 (Sequence Detection Systems, version 1.7).

Statistical Analyses for Biopsy Substudy

For the biopsy substudy, statistical analysis for gene expression was performed using R-language (www.R-project.org) and packages available through Bioconductor (www.bioconductor.org).

The GCRMA algorithm was used to obtain expression values, which were analyzed with a linear mixed-effect model and the R *limma* package, as previously described.²⁷ All 21 patients were included in statistical analyses, including 15 paired baseline–Week 12 samples from patients who completed the full treatment course.

For gene arrays, quality control (QC) was performed using standard QC metrics and R Package Quality Control. Images were scrutinized for spatial artifacts using Harshlight (Suarez-Farinas et al.). Expression values were obtained using the GCRMA algorithm (Wu et al.). Data were $\log_2$ -transformed before analysis with a linear mixed-effect model, with time and treatment as fixed factors, and a random effect for each patient. Fold changes (FCH) between comparisons of interest were estimated and hypothesis testing was conducted using contrasts under general framework for linear models in R's *limma* package. *Limma* uses an empirical Bayes moderation of standard errors toward prior variances, which has been fitted using an intensity-dependent trend (Ritchie et al.). *P* values from the moderated (paired) *t* test were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure. Genes with FCH >2 and false discovery rate (FDR) <0.05 were considered differentially expressed. Hierarchical clustering of samples/conditions was performed with Euclidean distance and a McQuitty agglomeration algorithm.

For RT-PCR data, Ct values were normalized to housekeeping gene hARP. Data were log₂-transformed before analysis with the linear mixed-effect model. IHC data, measured as counts, were also analyzed with the linear mixed effect model.

Pharmacodynamic Findings, Gene Array, and RT-PCR Analysis

1. Among 21 patients enrolled in and included in the analysis of the biopsy substudy, demographics and baseline clinical characteristics, including EASI, were similar between patient groups (mean age 40.4 years; baseline EASI score 26.3), except for age, which was significantly higher in the apremilast 40 mg BID group (mean age [years]: placebo, 38.7; apremilast 30 mg BID, 28.7; apremilast 40 mg BID, 53.7; $P=0.006$ between all groups in analysis of variance) (**Table S1**).
2. In the substudy patients, after 12 weeks of treatment, there was no significant improvement in EASI score in any treatment group; however, EASI improved by 26.5% in the apremilast 40 mg BID group, trending toward significance ($P<0.1$; **Table S2**).
3. Gene array analysis showed that, overall, the apremilast 40 mg BID group had the highest baseline dysregulation in lesional skin (**Figure S2a**, shown as more intense red and blue colors in baseline lesional skin). At Week 12, in the apremilast 40 mg BID group, decreases in general inflammation/T-cell activation markers (MMP12, JAK3, and ICOS), Th17/Th22-related markers (PI3, S100As, IL-22, CXCL1, and IL-19), Th1/interferon-related (MX1, OASL), and Th2-attracting chemokines (CCL17) were observed (**Table S5**). Similar changes in these genes, but of lesser magnitude, were observed in the placebo group (**Table S5**).
4. In a representative subset of immune genes, the apremilast 40 mg BID group showed the most severe baseline dysregulation (**Figure S2a**). At Week 12, the apremilast 40 mg BID

group showed reductions in the Th17/Th22 genes and related markers including IL-22 and IL-19 that trended toward significance ($P < 0.1$ for both). Other general inflammatory markers were decreased by apremilast 40 mg BID (MMP12) and Th17/Th22 markers (IL-17, S100A12) and by placebo (S100A9, CXCL1-2, STAT3, but without statistical significance). The apremilast 30 mg BID group did not demonstrate substantial changes in immune gene expression from baseline at Week 12 (**Figure S2b**).

5. When comparing treatment effects of apremilast vs. placebo, apremilast 40 mg BID demonstrated more substantial declines in expression of Th17/Th22-associated markers (S100A12, IL-19, IL-22), with no notable changes in other immune axes.
6. Overall, RT-PCR mRNA changes mirrored gene array findings. Results are shown in **Figure S2a** and **Figure S2b**. Additional immune and terminal differentiation markers are presented in **Figure S3**. At baseline, the apremilast 40 mg BID group had the highest dysregulation, as shown by the more intense red color. In both the apremilast 40 mg BID and placebo groups, the immune dysregulation at Week 12 resolved; no significant changes were observed in the apremilast 30 mg BID group. Consistent with gene array data, with apremilast 40 mg BID, Th17 (IL-17A, IL-12B/IL-12/23p40) and Th17/Th22-associated markers (IL-22, S100A7/S100A8, S100A12) were significantly reduced, while K16 mRNA showed the highest, most significant decreases ($P < 0.05$ for all; **Figure S2a**). The greater improvement in IL-22 related genes is consistent with the greater improvement in hyperplasia seen with apremilast 40 mg BID. K16 mRNA showed the highest, most significant decreases in apremilast 40 mg BID, but was also reduced with placebo. Significant or major reductions were also observed in Th2-associated cytokines IL-13, IL-31, and CCL17 in both the apremilast 40 mg BID and placebo groups. In the

apremilast 30 mg BID group, no significant changes in tissue mRNA were observed, mirroring IHC and gene array findings.

References

- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl* 1980;92:44-7.
- Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;43:e47.
- Suarez-Farinas M, Pellegrino M, Wittkowski KM, Magnasco MO. Harshlight: a "corrective make-up" program for microarray chips. *BMC Bioinformatics* 2005;6:294.
- Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583-93.e1-4.
- Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F, Spencer F. A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 2004;99:909-17.

Table S1. Baseline Demographics and Disease Characteristics

	Placebo n=7	Apremilast 30 mg BID n=7	Apremilast 40 mg BID n=7	ANOVA P value
Age, mean (SD), years	38.7 (16.2)	28.7 (8.3)	53.7 (12.1)	0.006
Male, n (%)	2 (28.6)	4 (57.1)	5 (71.4)	0.263
Race, n (%)				
Asian	3 (42.9)	3 (42.9)	0	0.154
African American	1 (14.3)	0	3 (42.9)	
Caucasian	3 (42.9)	4 (57.1)	4 (57.1)	
AD duration, mean (SD), years	23.7 (20.6)	22.8 (13.6)	39.4 (25.3)	0.257
EASI, mean (SD)	24.4 (11.6)	27.0 (12.8)	27.6 (9.2)	0.858
IgE (log ₂), mean (SD)	12.3 (1.6)	12.3 (2.9)	11.8 (4.3)	0.943

AD=atopic dermatitis, EASI=Eczema Area and Severity Index, IgE=Immunoglobulin E.

Table S2. Clinical Response to Treatment for Completers

	Placebo n=5	Apremilast 30 mg BID n=4	Apremilast 40 mg BID n=6
Week 0 EASI, mean (SD)	27 (13.1)	26.5 (12.6)	26 (9.0)
Week 12 EASI, mean (SD)	18.4 (10.9)	24.75 (19.5)	26 (9.0)
<i>P</i> value	0.165	0.780	0.059
EASI score, mean (SD) change, %	30.3 (26.9)	16.05 (46.3)	26.5 (29.9)

EASI=Eczema Area and Severity Index.

P values indicate comparisons between Week 0 and Week 12 EASI.

Table S3. Antibodies Used for Immunohistochemical Analyses

Antibody	Vendor	Clone*	Isotype	Dilution
Keratin 16	AbD Serotec (Bio-Rad)	LL025	IgG1	1:100
Ki67	Santa Cruz	MIB-1	IgG1	1:100

*Antibodies used in this experiment were mouse monoclonal antibodies, unless stated otherwise.

Table S4. Primers and Probes Used for RT-PCR

Gene Symbol	Sequence	
RPLP0/hARP	Forward	CGCTGCTGAACATGCTCAA
	Reverse	TGTCGAACACCTGCTGGATG
	Probe	6-FAM-TCCCCCTTCTCCTTTGGGCTGG-2TAMRA
Assay ID		
CCL17	Hs00171074_m1	
CCL18	Hs00268113_m1	
CCL22	Hs01574247_m1	
FLG	Hs00856927_g1	
IFNg	Hs00989291_m1	
IL-13	Hs00174379_m1	
IL-17A	Hs00174383_m1	
IL-1B	Hs01555410_m1	
IL-22	Hs01574154_m1	
IL-31	Hs01098710_m1	
IL-5	Hs00174200_m1	
IL-12B	Hs01011518_m1	
IL-23p19	Hs00900828_g1	
IL-23p40	Hs01011518_m1	
KRT16	Hs00373910_g1	
LOR	Hs01894962_s1	
PPL	Hs00160312_m1	
S100A12	Hs00942835_g1	

S100A7	Hs01923188_u1
S100A8	Hs01923188_u1

Table S5. Differentially Expressed Genes and Effect With Treatment

Please see the Excel file available for download with this article for Table S5.

Figure S1. Patient Disposition (CONSORT Flow Diagram)

The chart shows the numbers (%) of patients who were randomized and completed the placebo-controlled and active treatment phases, and the numbers (%) of patients who discontinued the trial during each phase, along with reasons for discontinuation.

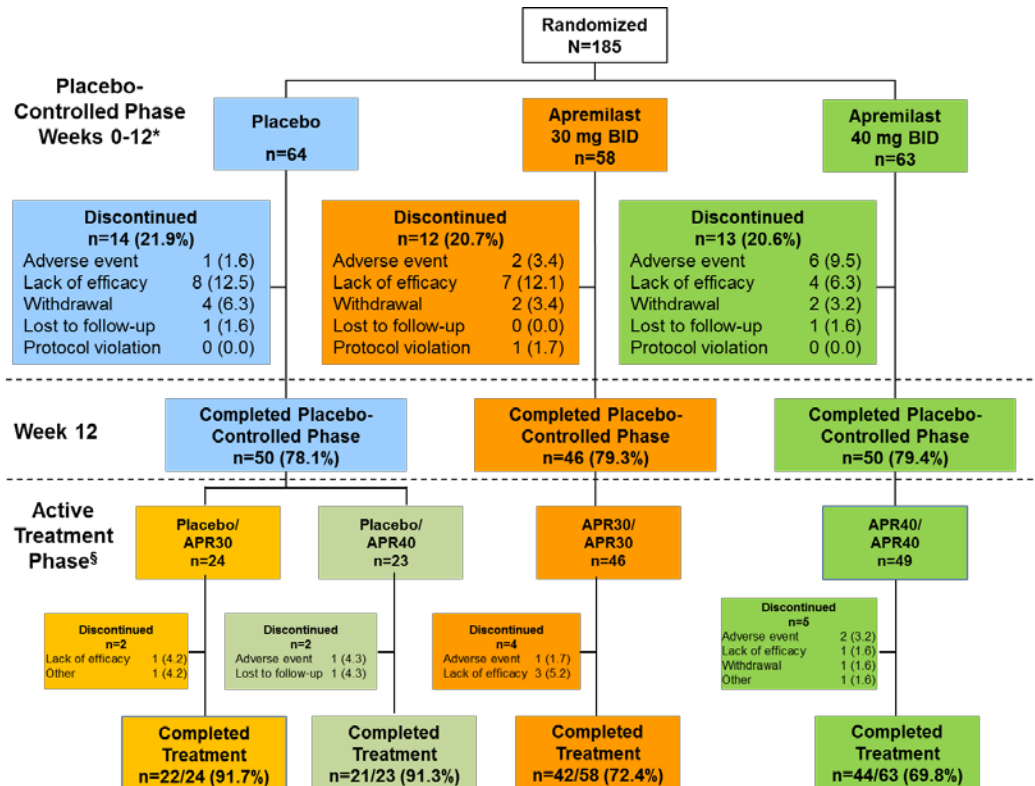


Figure S2a. Gene Array Heatmap of Immune Gene Expression Levels

Heatmap representing mean expression levels of immune genes dysregulated in the atopic dermatitis transcriptome pre- (baseline) and post (Week 12)-treatment. Red indicates gene upregulation and blue indicates gene downregulation. APR30=apremilast 30 mg BID; APR40=apremilast 40 mg BID; LS=lesional; N=normal; NL=nonlesional; PCB=placebo. + $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

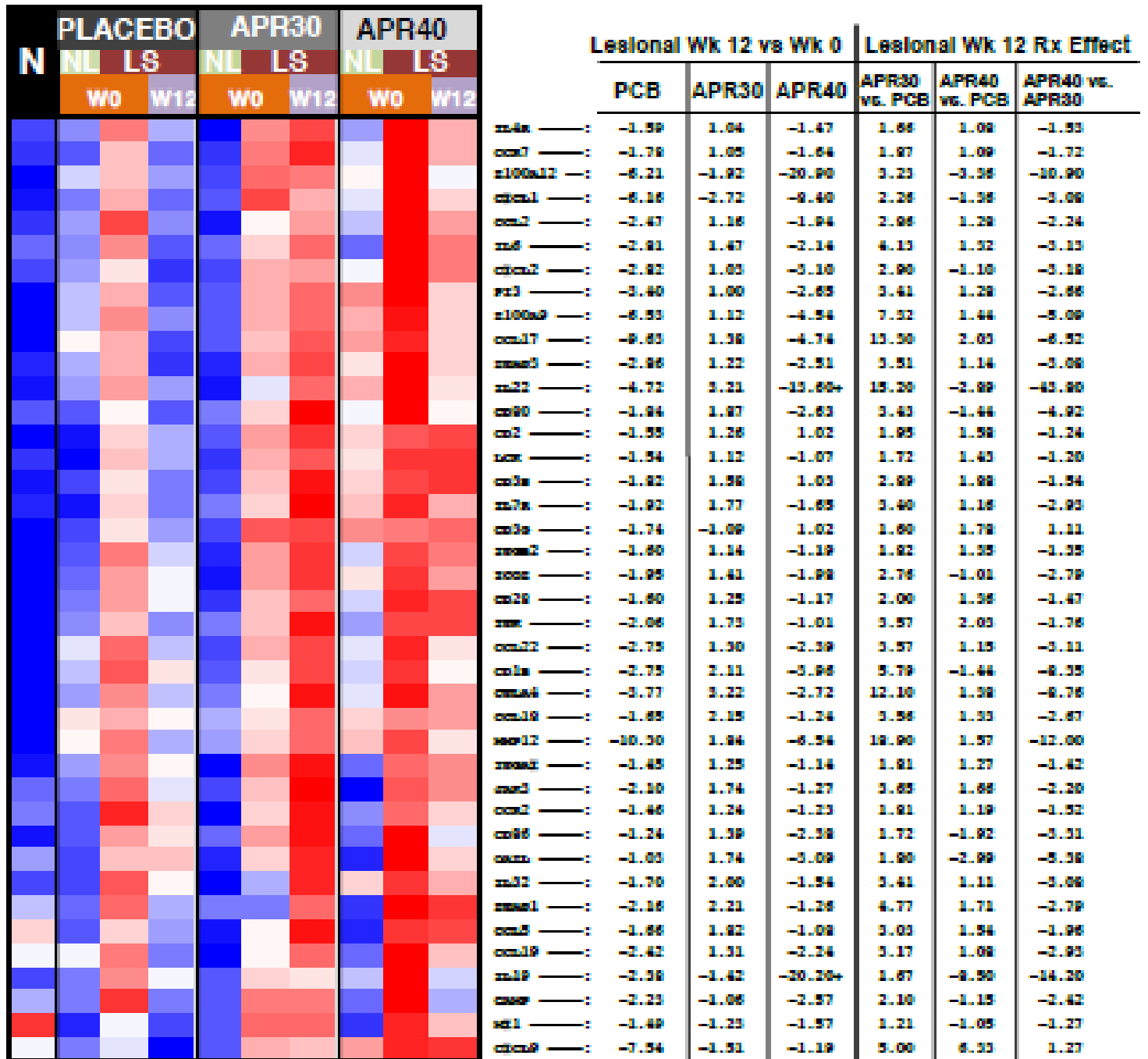


Figure S2b. Percent Improvement in AD Transcriptome

Boxplots show the overall improvement in up- and downregulated genes in the AD genomic profile with placebo and apremilast 40 mg and 30 mg. Light blue dots indicate mean percent improvement, and the light blue bars indicate 1 standard deviation above/below. The black horizontal lines indicate medians, the vertical black lines indicate values that are within 1.5 times the interquartile range of the median, and the top and bottom of the dark green boxes represent the 75th and 25th percentiles, respectively. The black dots indicate outliers. Lines above boxplots represent inter-group comparisons.

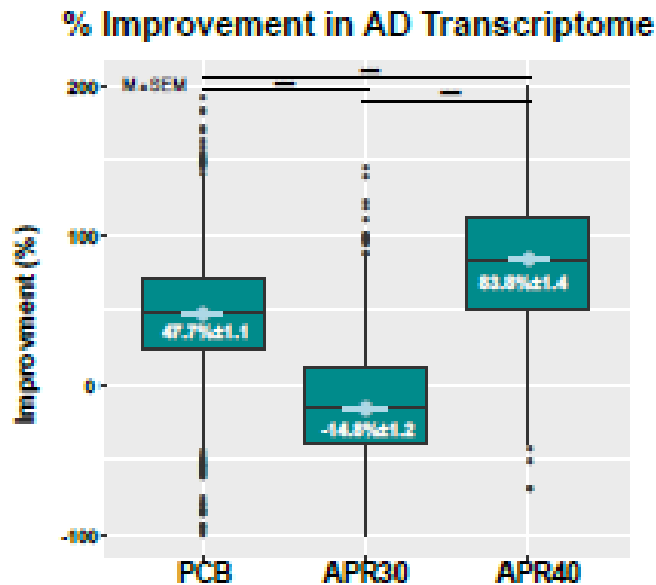


Figure S3. Additional Immune and Barrier Genes Quantified by Real-Time PCR

Line graphs of mRNA expression changes of select immune and barrier genes in lesional and nonlesional atopic dermatitis skin areas treated by drug or placebo. Immune genes are grouped by major inflammatory/regulator pathways. Black stars denote significance in change between nonlesional and lesional skin; top red stars denote significance in change from baseline; bottom red stars denote significance of treatment effect between apremilast 40 mg and placebo. Red or blue numbers denote sample number for lesional or nonlesional skin, respectively. APR30= apremilast 30 mg; APR40= apremilast 40 mg; + $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

