## Extensive alopecia areata is reversed by IL-12/IL-23p40 cytokine antagonism

*To the Editor:*

Alopecia areata (AA) is a prevalent (approximately 1.7% lifetime risk) disease.[1] Although it has a large effect on patients' quality of life and poses a large economic burden, treatment options for patients with AA are limited.[2,3] For more extensive alopecia forms, such as total scalp (totalis) or body (universalis) AA, for which spontaneous regrowth is rare,[1] immunosuppressants (systemic corticosteroids, cyclosporine A, and Janus kinase inhibitors) have shown some efficacy but are associated with side effects that preclude long-term use.[2,3] Furthermore, hair loss recurs shortly after cessation of treatment.[3] Cytokines driving hair loss are not well understood,[1] hindering the targeted therapeutic development seen with other skin diseases.[4]

Our recent study in a well-characterized group of 27 patients with AA associated the AA signature with robust $T_H2$ and IL-23p19 and IL-23/IL-12p40 activation in addition to the $T_H1$ skewing that has been the previous focus.[5,6] This provides a rationale for exploring cytokine-targeted therapeutics in patients with AA, which are approved or tested for psoriasis or atopic dermatitis.[4] Our data show a large and significant increase in IL-12/IL-23p40 cytokine levels in scalp from patients with lesional AA versus normal scalp.[5] Ustekinumab, an IL-12/IL-23p40 blocker that is US Food and Drug Administration approved for psoriasis, induces high-grade improvement in 70% to 80% of patients with psoriasis.[4] Here we show that ustekinumab has impressive ability to improve hair growth in patients with extensive AA.

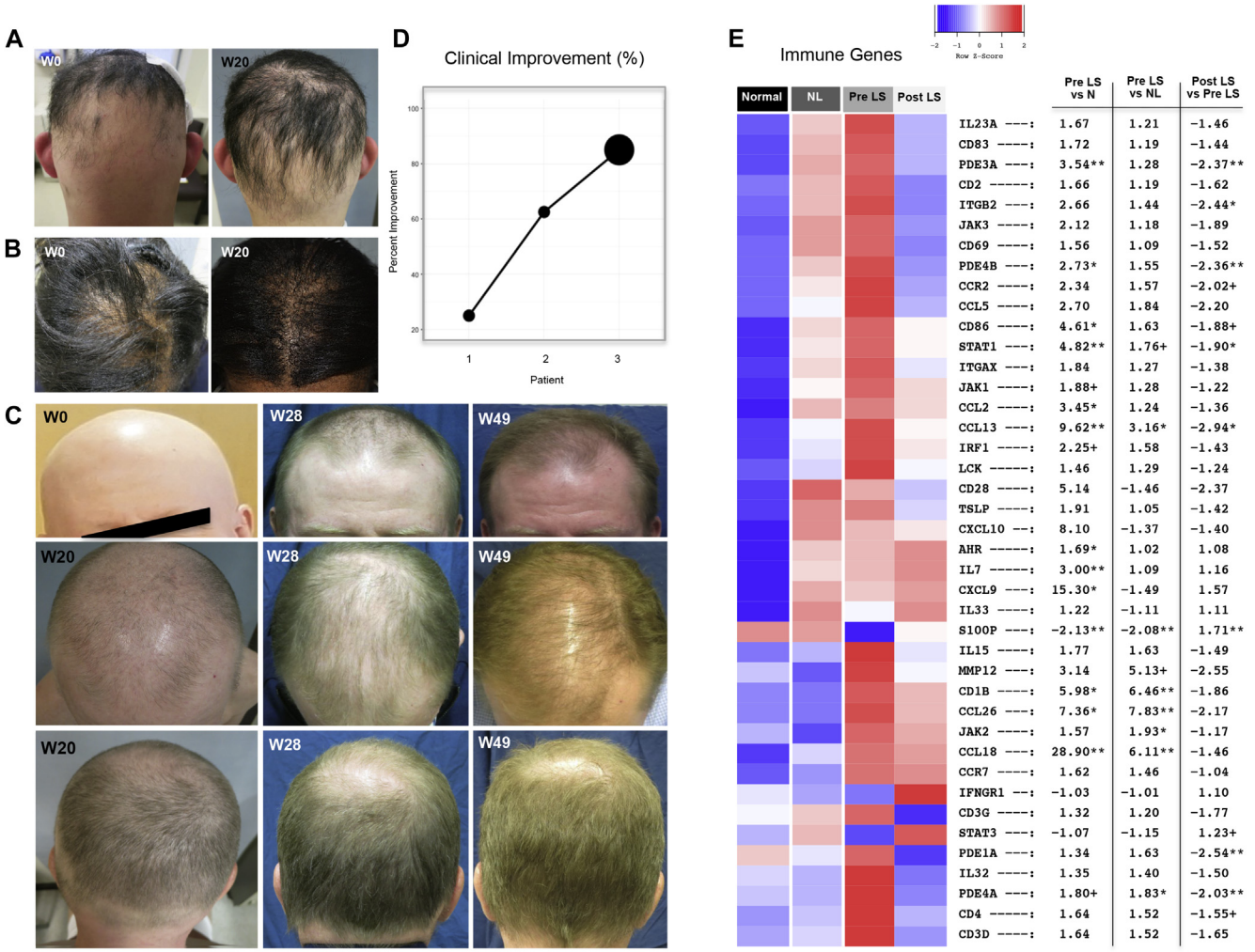


**FIG 1. A** and **B,** Clinical pretreatment and posttreatment (week 20) images for patients 1 (Fig 1, *A*) and 2 (Fig 1, *B*). **C,** For patient 3 with alopecia universalis, clinical photographs show gradual improvement at weeks 20 and 28 and full hair regrowth at week 49. **D,** Clinical improvement (in percentages). *Circles* are proportional to baseline hair loss. **E,** Heat map of microarray expressions of an immune gene subset in normal scalp *(N)*, nonlesional scalp *(NL)*, and lesional scalp before *(Pre LS)* and after *(Post LS)* treatment. *Red* represents upregulated and *blue* represents downregulated expression. Fold changes are shown. $+P < .1$, $*P < .05$, and $**P < .001$.

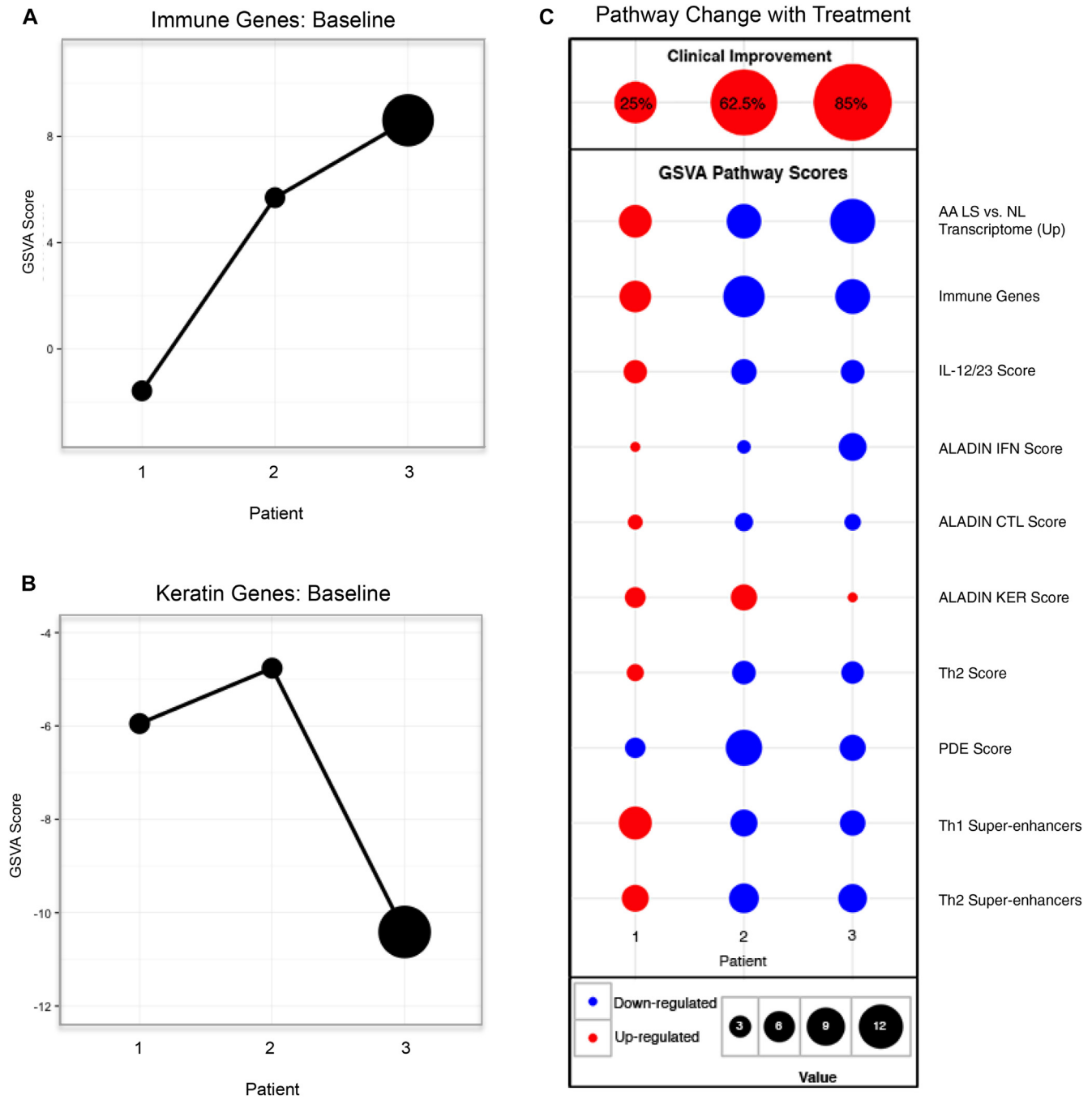


**FIG 2.** **A** and **B,** GSVA scores for each patient's sample *(1-3)* at baseline for immune and keratin gene sets. *Circles* indicate proportional baseline involvement. **C,** Clinical improvement and GSVA score lesional scalp changes by patient with treatment for key alopecia pathways, including the AA transcriptome; ALADIN IFN, CTL, and KER scores (representing interferon/$T_H1$, T-cell, and keratin genes, respectively); and $T_H2$, PDE, and $T_H1$ and $T_H2$ superenhancer gene subsets. *Circles* are proportional to hair regrowth, and *GSVA circles* are proportional to GSVA score changes from baseline. *Red* indicates upregulation, and *blue* indicates downregulation. *LS,* Lesional; *NL,* nonlesional.

We evaluated hair regrowth in 3 patients with AA at 20 weeks after treatment with 3 subcutaneous doses of 90 mg of ustekinumab given at weeks 0, 4, and 16. We chose this dose to allow for maximal efficacy.[4] Clinical presentation was evaluated based on percentage hair loss, which included 2 patients with 40% scalp involvement and 1 patient with alopecia universalis (100%). Demographic and clinical characteristics are presented in Table E1 in this article's Online Repository at www.jacionline.org and Fig 1, *A-C*, respectively. One patient continued receiving 90 mg of ustekinumab every 12 weeks, with pictures available until week 49 (Fig 1, *C*). Patients did not use any additional topical or systemic treatments.

All patients provided written informed consent for skin biopsies and photographs under an institutional review board–approved protocol. We obtained clinical photographs and skin biopsy specimens of lesional and nonlesional scalp from each patient at baseline and week 20.

HGU133plus2.0 (Affymetrix, Santa Clara, Calif) microarrays were used to measure gene expression. Preprocessing and statistical analysis of microarray data were conducted with R software (R-project.org) and Bioconductor. Harshlight was used for quality control of images, and expressions were obtained by using GCRMA. Genes with SDs of greater than 0.1 and expression of greater than 3 in at least 3 samples were kept for further analysis. Mixed-effect models were used to assess changes with treatment. *P* values from the moderated (paired) *t* test were adjusted for multiplicity by using the Benjamini-Hochberg approach.

Gene Set Variation Analysis (GSVA), a method that produces a score of activity for a set of genes or pathways for each sample,[7] was performed by averaging *z* scores of expression values over all genes in a given pathway. IL-12/IL-23 (individual IL-12/IL-23 pathway genes are listed in Table E2 in this article's Online Repository at www.jacionline.org), $T_H2$, and phosphodiesterase (PDE) scores were selected by using pathway-specific genes differentially expressed in our recent AA report.[5]

Clinical pictures and biopsy specimens were taken at baseline and at a week 20 follow-up (≥1 cm from the scar from baseline biopsy). All 3 ustekinumab-treated patients exhibited varying degrees of hair regrowth after 20 weeks, with no reported adverse events during or after treatment (Fig 1, *A-D*, and see Table E1). Patient 3 had a history of AD, and the other patients had no associated comorbidities. Percentage hair regrowth was highest in the patient with the greatest involvement (alopecia universalis; Fig 1, *C* and *D*, and see Table E1) and shortest duration of disease (2 years; see Table E1). This patient showed the best improvement, with regrowth of all body hair, eyebrows, and 85% of the scalp at week 20 and further improvement and full regrowth of scalp hair at week 49 (except a frontal scalp area that could be attributed to androgenetic alopecia; Fig 1, *C*, and see Table E1). Although the other 2 patients began with similar (40%) scalp involvement, the patient with shorter disease duration (8 years) had better improvement (Fig 1, *A* and *B*, and see Table E1).

We recently associated more extensive AA with higher activation of polar cytokines (IFN-γ, IL-13, and IL-23) and related mediators, as well as increased dysregulation of hair keratins.[5] Thus in these 3 patients we aimed to evaluate the active involvement of different immune pathways, as well as hair keratins at baseline, and assess the extent of their respective modulations with treatment. RNA expressions of immune (Fig 1, *E*) and hair keratin (see Fig E1 in this article's Online Repository at www.jacionline.org) gene subsets[5] from lesional scalp of the 3 patients with AA and nonlesional scalp of the 2 patients with AA before and after (week 20) ustekinumab treatment are presented as heat maps. A full list of modified genes is available in Table E3 in this article's Online Repository at www.jacionline.org. Normal scalp biopsy specimens from 3 healthy volunteers were also included for appropriate comparisons.

Marked increases in levels of inflammatory markers were observed in pretreatment lesional scalp biopsy specimens from patients with AA compared with those in pretreatment nonlesional and normal scalp biopsy specimens, as visualized by bright red colors, with decreased expression at week 20 (transition to blue colors; Fig 1, *E*). Although both $T_H2$ (*CCL26*, *CCL18*, and *CCL13*) and interferon/$T_H1$-related (signal transducer and activator of transcription 1 *[STAT1]*, *CXCL10*, and *CXCL9*) genes were significantly upregulated at baseline, $T_H2$ axis genes showed consistent suppression with treatment (significant for *CCL13*), whereas expression of only some (*STAT1* and *CXCL10*) interferon/$T_H1$ genes were reduced. As we have recently shown,[5] markers of general inflammation (matrix metallopeptidase 12 *[MMP12]*), dendritic cells (*CD1B*, *CD86*, and *ITGAX/CD11C*), T cells (*CD4* and *CD28*), and IL-23A (*IL23p19*) were increased at baseline and decreased after treatment. Similarly, levels of PDE markers (ie, *PDE4*, which hydrolyzes cyclic AMP, an intracellular second messenger controlling inflammatory mediators) were markedly upregulated at baseline, with significant reductions after treatment (Fig 1, *E*).

Because we and others have shown major suppression of hair keratins in scalp biopsy specimens from patients with AA,[5,6] particularly in those with more extensive and/or more inflammatory AA,[5] we also evaluated regulation of hair keratins with treatment. Overall, as can be seen in a heat map (see Fig E1), expression of hair keratins and keratin-associated proteins was decreased in lesional scalp at baseline (dark blue) and increased after treatment (lighter blue). Using GSVA, we also examined individual patients' expressions of immune and keratin gene sets at baseline (Fig 2, *A* and *B*) and changes with treatment in expressions of several gene sets recently associated with AA (Fig 2, *C*).[5,6]

When evaluating by patient, a higher inflammatory profile and greater suppression of hair keratins at baseline were associated with higher recovery (patient 3, Fig 2 and see Table E1). Indeed, the patient with alopecia universalis with the highest baseline inflammation and lowest expression of hair keratins exhibited the highest regrowth (Figs 1, *C* and *D*, and 2 and see Table E1). Furthermore, the patient with minimal regrowth (patient 1) had the lowest inflammatory status and highest keratin levels at baseline (Figs 1, *A* and *D*, 2). Among the gene sets and pathways included in the GSVA analyses were the recently identified upregulated and immune genes within the AA transcriptome (expression differences between lesional and nonlesional AA scalp),[5] IL-12/IL-23 pathway genes in the AA transcriptome,[5] 3 scores recently proposed[6] to mark AA disease activity (Alopecia Areata Disease Activity Index [ALADIN] IFN, and CTL, and KER scores, representing interferon/$T_H1$, T-cell, and keratin genes, respectively), and $T_H2$ and PDE gene subsets we recently associated with AA.[5] $T_H1$ and $T_H2$ superenhancer gene subsets, representing regulators of genomic expression, were also evaluated.[8]

Fig 2, *C*, is a bubble plot illustrating the changes with treatment in GSVA scores for each pathway by patient. Blue and red represent downregulation and upregulation, respectively, and bubble areas are proportional to pathway score changes. Clinical improvement was associated with reversal of immune abnormalities and increases in hair keratin levels (increased ALADIN KER scores in all 3 patients). The patients with substantial clinical improvement (62.5% and 85% for patients 2 and 3, respectively) showed normalization of the AA genomic profile of upregulated genes,[5] as well as reversal of immune gene dysregulation. Mirroring the reversal in immune genes in patients 2 and 3, there was improvement in IL-12/23 and

ALADIN IFN and CTL scores, as well as $T_H2$, PDE, and $T_H1$ and $T_H2$ superenhancer gene subsets (Fig 2, *C*). Substantial $T_H2$ downregulation was observed in the 2 patients with dramatic clinical improvement, whereas all patients had reduction of PDE gene expression (Fig 2, *C*). Because PDE genes were significantly upregulated at baseline and downregulated with treatment, a PDE score might also provide insights into disease resolution with treatment.

This is the first case series of patients with AA (including a patient with alopecia universalis) that demonstrates hair regrowth with a specific cytokine-targeting strategy. Our recently published study[5] highlighted the cytokine profiles characteristic of lesional and nonlesional AA compared with normal scalp, as well as with the genomic phenotypes of AD and psoriasis. Although that study provides scientific rationale for possible use of anti-$T_H2$ or anti–IL-12/23p40 cytokine–targeting therapeutics in patients with AA, it could not prove the hypothesis that AA is reversible by means of single-cytokine targeting. Our case series is the first report linking specific immune antagonism to clinical and molecular AA reversal. Furthermore, it associates hair restoration with improvement in inflammatory gene expression with a specific cytokine antagonist. Of note, although IL-23 is considered a regulator of IL-17 and IL-22, IL-17– and IL-22–induced genes (eg, CCL20 for IL-17 or the S100As, which are synergistically induced by both cytokines) were not suppressed by ustekinumab treatment. However, IL-23 can have direct effects on keratinocytes,[9] and larger studies are needed to assess whether IL-23 possibly mediates follicular growth in an IL-17/IL-22–independent manner in patients with AA.

Future larger trials are needed that should stratify patients into different AA groups based on involvement and by disease chronicity. It might be that the level of inflammatory signal decreases with longer-standing disease rather than increased involvement,[5] decreasing the chances for regrowth, but this still remains to be determined. Larger studies will also need to define the best biomarkers of therapeutic response.

Our study supports expanding the recently proposed ALADIN scores (considering $T_H1$/interferon, T-cell, and keratin markers) in measuring disease improvement on a molecular level with our $T_H2$ and PDE scores. Future clinical trials with broad and/or specific agents for patients with AA are necessary to evaluate scoring systems for AA activity and reversal. In particular, future larger clinical trials with specific IL-12/23p40, $T_H2$, and PDE antagonists, such as apremilast, should further dissect the molecular pathways underlying AA and bring hope to many patients with this emotionally devastating disease.

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## Altered expression of chemoattractant receptor–homologous molecule expressed on $T_H2$ cells on blood basophils and eosinophils in patients with chronic spontaneous urticaria

*To the Editor:*

Chronic spontaneous urticaria (CSU) has a significant effect on patients' quality of life through symptoms of pruritus and recurrent skin lesions. Biopsies of CSU skin lesions consistently reveal degranulated mast cells and infiltration by leukocytes, such as basophils, eosinophils, and T lymphocytes.[1] A putative role for basophils in patients with CSU is supported by the association of