Transcriptomic Analysis Identifies Disease Severity and Therapeutic Response in Psoriasis

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Abnormalities in gene expression profiles characterize patients with inflammatory skin diseases, including psoriasis, and changes may reflect the action of specific therapeutic agents. To examine this, gene expression analysis of psoriatic skin was assessed by Gene Set Variation Analysis using informative gene modules, and longitudinal data were analyzed to assess the impact of various treatments. Ridge penalized logistic regression was employed to derive a transcriptomic score. Psoriatic lesional skin exhibited perturbations in gene expression profiles at baseline, with enrichment of signatures for neutrophils, keratinocytes, IFN, IL-12 complex, IL-1 cytokines, TNF, and T helper 17. Treatment with a variety of agents reduced lesional gene expression abnormalities to those in nonlesional skin. Specific gene expression abnormalities at baseline identified clinical responders to each treatment. Changes in gene expression over time were less pronounced in nonlesional skin and lesional skin in clinical nonresponders. The combined transcriptomic scores showed significant positive correlations with PASI scores in clinical responders over time. Overall, gene expression abnormalities characterize the severity of psoriatic skin lesions, can be used to predict responsiveness to individual treatments, and revert toward those of nonlesional skin with effective therapy. Therefore, gene expression analysis can be useful to support management of patients with psoriasis.

Keywords: Bioinformatics, Drug reactions, Inflammatory skin diseases, Psoriasis

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INTRODUCTION

Psoriasis is a chronic inflammatory disease predominantly involving the skin and joints that affects 2−3% of the worldwide population (Kaushik and Lebwohl, 2019). The pathogenesis of psoriasis is thought to involve the inappropriate activation of T cells and, specifically, T helper 17 cells, with subsequent keratinocyte hyperplasia and dermal infiltration of inflammatory cells (Zhou et al, 2022). Increased proliferation of keratinocytes is driven by many inflammatory cytokines, including IL-17, IL-12, IL-23, and TNF. As a result, numerous biologics targeting these cytokines have been shown to be beneficial and approved for the treatment of psoriasis (Mosca et al, 2021; Jeon et al, 2017; Yost and Gudjonsson, 2009; Tobin and Kirby, 2005). For example, the TNF inhibitor etanercept (ETN), the IL-12/IL-23 inhibitor ustekinumab (UST), the IL-17 inhibitors secukinumab (SEC) and brodalumab (BROD), and the IL-23 inhibitor guselkumab (GUS) have all be successfully employed in the treatment of patients with plaque psoriasis (Bertelsen et al, 2020; Brodmerkel et al, 2019; Sofen et al, 2014; Tomalin et al, 2020). The Jak1/TYK2 inhibitor brepocitinib (BREPO) (PF-06700841) has also been tested because of its capacity to inhibit signaling by numerous cytokines, thereby blocking several pathways thought to be involved in psoriasis pathogenesis (Mease et al, 2023). Although existing treatments of psoriasis are effective, few studies have compared clinical responses to these agents, and there is minimal information on whether pretreatment characteristics can predict responsiveness to individual therapies. Moreover, the longitudinal effects of these treatments have not been compared.

Historically, effectiveness of a treatment for psoriasis has been determined by clinical assessment of the disease, specifically, changes in PASI (Fredriksson and Pettersson, 1978). However, interobserver variability and differences in clinical manifestations of psoriasis limit the ability of PASI to measure disease severity accurately (Klein 2004). Hence, it is important to consider other means to assess the degree of abnormality in psoriatic skin and how treatment affects these biologic perturbations. One such means is to evaluate changes in gene expression using various methods of transcriptomic analysis. Indeed, previous studies have compared clinical benefits after treatment with various cytokine antagonists (Lebwohl et al, 2015; Sawyer et al, 2018; Warren et al, 2020). However, there remains a need to compare the gene expression changes caused by a single agent with the changes caused by others and to discern dose-dependent modulation of cellular and molecular abnormalities in psoriasis. In addition, it would be useful to understand molecular differences between clinical responders and nonresponders

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Abbreviations: BREPO, brepocitinib; BROD, brodalumab; ETN, etanercept; GSVA, Gene Set Variation Analysis; GUS, guselkumab; LS, lesional; NL, nonlesional; PsoriaCIS, Psoriasis Cell and Immune Score; SEC, secukinumab; UST, ustekinumab

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as well as to predict responsiveness on the basis of baseline molecular profiles of patients with psoriasis.

In this study, we compared longitudinal gene expression data derived from lesional (LS) and nonlesional (NL) skin biopsies of patients with psoriasis treated with various therapeutic agents. We employed Gene Set Variation Analysis (GSVA), which is a nonparametric, unbiased approach to identify sample-level gene expression changes among individuals with heterogeneous disease presentations. By utilizing informative gene sets (modules) that capture both immune cells, inflammatory pathways, metabolic processes, and resident skin cell biology as input, GSVA could provide a comprehensive assessment of the transcriptomic perturbations of each individual sample and thus could provide additional information beyond that from approaches, such as gene set enrichment analysis, which are more appropriate for determining differences between 2 homogeneous sample groups (Martínez et al, 2022). A combined transcriptomic severity score, Psoriasis Cell and Immune Score (PsoriaCIS), was devised to estimate the degree of abnormality in psoriatic skin, and its correlation with PASI was assessed. The results suggest that transcriptomic analysis can provide useful support for management of psoriasis.

RESULTS

Longitudinal analysis reveals treatment-dependent changes in gene expression profiles of psoriatic skin

We initially examined the effect of various therapeutic agents on the gene expression profiles of psoriatic LS skin over time by assessing the change in GSVA scores of 48 previously reported gene signatures representing immune/tissue cells, metabolic pathways, and inflammatory processes (Martínez et al, 2022) (Figure 1a and Table 1 and Supplementary Table S1). Therapeutic agents were selected on the basis of the availability of publicly available, longitudinal transcriptome data. At baseline, the majority of patients showed typical molecular hallmarks of psoriasis, including enrichment of neutrophil, keratinocyte, IFN, IL-12 complex, IL-23 complex, IL-1 cytokine, T cell, TNF, and T helper 17 signatures. Placebo-treated patients displayed minimal changes in their PASI scores or molecular profiles over the course of treatment. In contrast, patients treated with high-dose BREPO (denoted as BREPO-HI; 100 mg) exhibited a progressive decrease in the transcripts representing inflammatory cells and processes. Similar patterns of changes in gene signatures were also observed in high-dose UST (denoted as UST-HI; 90 mg) and low-dose BROD (denoted as BROD-LO; 140 mg)–treated patients over time (Figure 1a). Gene signatures indicative of skin tissue status, such as melanocytes and endothelial cells, were increased in patients treated with active drug.

To evaluate the differences in the magnitude of gene enrichment over time, we carried out repeated-measures ANOVA on GSVA scores from LS skin (Figure 1b and Supplementary Figure S1a–g). For BREPO-HI and UST-HI, changes in neutrophil, keratinocyte, T-cell IL-23, and proteasome gene signatures were significantly decreased at weeks 4 and 12, respectively, compared with those at

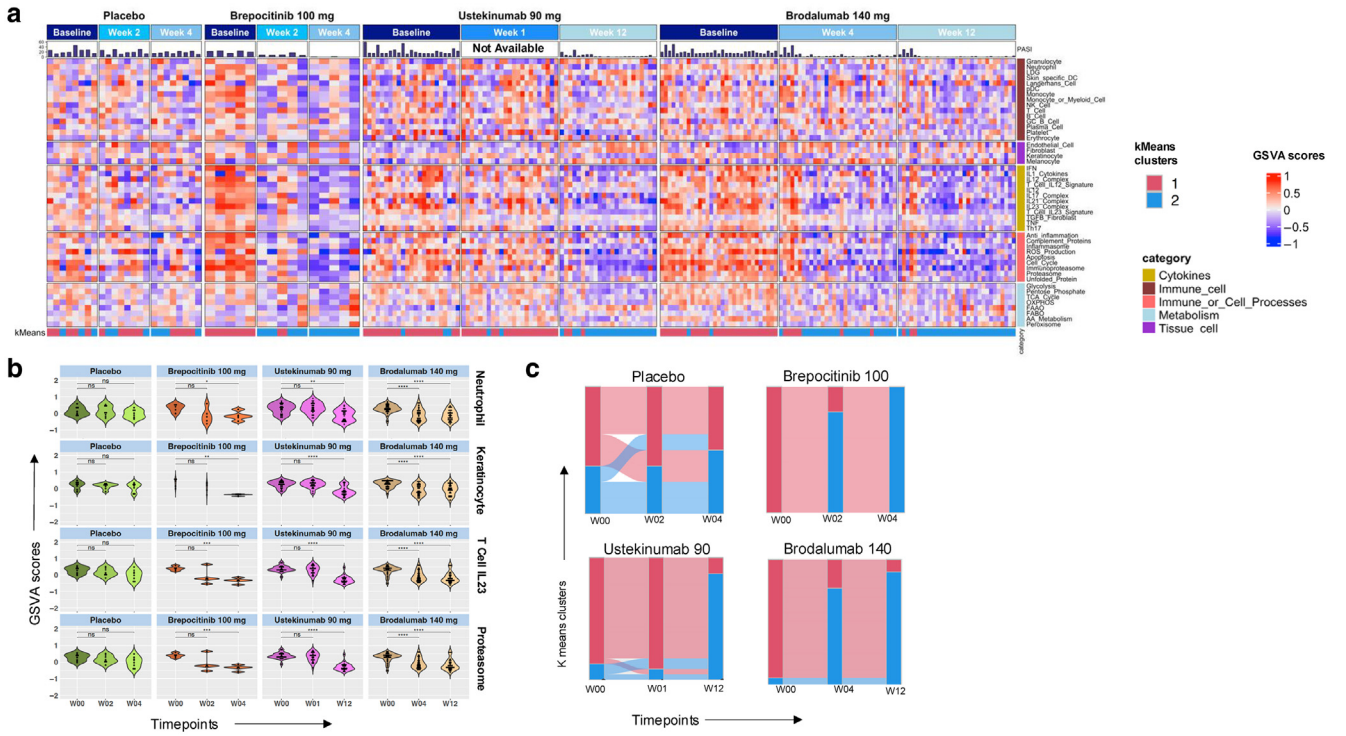


Figure 1. Longitudinal changes in molecular profiles of psoriatic lesional skin treated with various therapeutic agents. (**a**) Heatmap of GSVA scores of lesional skin from placebo (n = 8), 100 mg brepocitinib (n = 5), 90 mg ustekinumab (n = 23), and 140 mg brodalumab (n = 31) –treated patients. PASI scores displayed at the top of the heat map as bar graphs. Samples belonging to 2 K-means clusters (red and blue) are labeled at the bottom of the heat map. A total of 48 gene signatures are grouped into major categories as immune cell, tissue cell, cytokines, immune cell processes, and metabolism. (**b**) Violin plots of neutrophil, keratinocytes, T-cell IL-23, and proteasome signatures showing significant differences between time points. Dots signify individual patient GSVA scores for each treatment group, and violin silhouettes signify the distribution of patient scores. *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$. (**c**) Alluvial plots showing movement of individual patients between k-means clusters (red and blue) at each time point. GSVA, Gene Set Variation Analysis.

Table 1. Table of Psoriasis Gene Expression Datasets

Group	GEO Dataset	Platform	Drug	Cohort	Baseline	Week 1	Week 2	Week 4	Week 12	Total Patients	R	NR
A	GSE136757	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	Placebo	LS	8	NA	8	8	NA	8	—	8
				NL	8							
			30 mg brepocitinib	LS	7		7	7		7	4	3
				NL	7							
			100 mg brepocitinib	LS	5		5	5		5	5	—
				NL	5							
B	GSE117468	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	Placebo	LS	9	NA	NA	9	9	9	2	7
				NL	9				9			
			140 mg brodalumab	LS	31			31	31	31	26	5
				NL	31				31			
			210 mg brodalumab	LS	21			21	21	21	18	3
				NL	21				21			
C	GSE51440	[HT_HG-U133_Plus_PM] Affymetrix HT HG-U133+ PM Array Plate	Guselkumab	LS	5	5	NA	NA	5	5	—	—
				NL	5							
	GSE117239	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	50 mg etanercept	LS	25	25	NA	NA	25	25	18	7
				NL	25							
			45 mg ustekinumab	LS	15	15	NA	NA	15	15	14	1
				NL	15							
			90 mg ustekinumab	LS	23	23	NA	NA	23	23	15	8
				NL	23							

GEO Dataset	Platform	Drug	Cohort	Baseline	Day 4	Day 14 (Week 2)	Day 42 (Week 6)	Day 84 (Week 12)	Total Patients
GSE137218	[Clariom_D_Human] Affymetrix Human Clariom D Assay [transcript (gene) version]	300 mg secukinumab	LS	13	13	13	13	13	13

Abbreviations: GEO, Gene Expression Omnibus; LS, lesional; NA, not applicable; NL, nonlesional; NR, nonresponder; R, responder.

baseline but were not significantly changed at week 1 or 2. For BROD-LO, these signatures were significantly decreased at weeks 4 and 12 compared with those at baseline (Figure 1b).

As an unbiased method to assess the degree to which there were global changes in the gene expression profiles of individual samples with treatment, we clustered the patient GSVA scores for the 48 signatures using K-means clustering, which provides an unsupervised, scalable approach to finding underlying patterns within large datasets, and we have shown it to be more effective at producing stable and distinct patient subsets than other clustering algorithms (Hubbard et al, 2023). On the basis of both elbow and silhouette methods, K = 2 was the optimal number of clusters in each group, and these were designated as red and blue (Supplementary Figure S2 and Supplementary Table S2). Overall, the red cluster was characterized by increased inflammatory disease signatures (severe disease), whereas the blue cluster displayed decreased cytokine or immune cell signatures (mild disease). The transition of patients between these 2 clusters was visualized with alluvial plots (Figure 1c and Supplementary Table S2). More than 90% of baseline samples were in the red cluster. Treatment with placebo had little impact on cluster membership of the samples. In contrast, all active therapies caused many samples to transition from the red (severe) to the blue (mild) cluster, although the timing of this conversion varied by drug.

Transcriptome profiles from LS skin of patients treated with different doses of BREPO (30 mg; BREPO-LO), UST (45 mg; UST-LO), and BROD (210 mg; BROD-HI) as well as additional therapeutic agents, including GUS and ETN, were also analyzed (Figure 2a and Supplementary Figure S1a–g). Similar differences in gene signatures, including decreased inflammatory signature and increased skin tissue signature enrichment, were noted. Significant changes in placebo-treated patients were not seen. An additional study of patients treated over 12 weeks with the IL-17 inhibitor SEC was also analyzed as a validation dataset for longitudinal gene expression changes after treatment with another IL-17 inhibitor, BROD (Figure 2a). Inflammatory gene signatures remained high at day 4 after the initiation of treatment, and changes in LS skin were only apparent at week 2 of treatment with SEC. By week 12, changes to GSVA scores with SEC treatment were similar to those of BROD. As previously observed, patients with baseline K-means cluster membership in the red cluster indicative of severe disease largely transitioned into the blue, mild disease cluster with active treatment but not after treatment with placebo (Figure 2b). These results demonstrate the utility of gene expression analysis to identify perturbations in LS psoriatic skin and the amelioration of these abnormalities with treatment.

Assessment of the magnitude of change in skin abnormalities resulting from treatment

To assess the magnitude of change in each molecular feature and its statistical significance, we calculated Hedges' g effect sizes on GSVA scores of psoriatic LS skin samples (Figures 3 and 4). Because we did not have data from the same time points for each agent, we grouped samples on the basis of time points assessed (Supplementary Figure S3). Group A contained samples of patients with psoriasis treated with either placebo or BREPO**;** group B contained placebo or BROD-treated patients with psoriasis; group C contained samples of GUS-, ETN-, or UST-treated patients with psoriasis.

Initially, we examined the effect size of each molecular feature for each group of therapeutic agents on LS skin over time compared with that at baseline. In group A, LS skin of all BREPO-treated patients at week 4 was characterized by

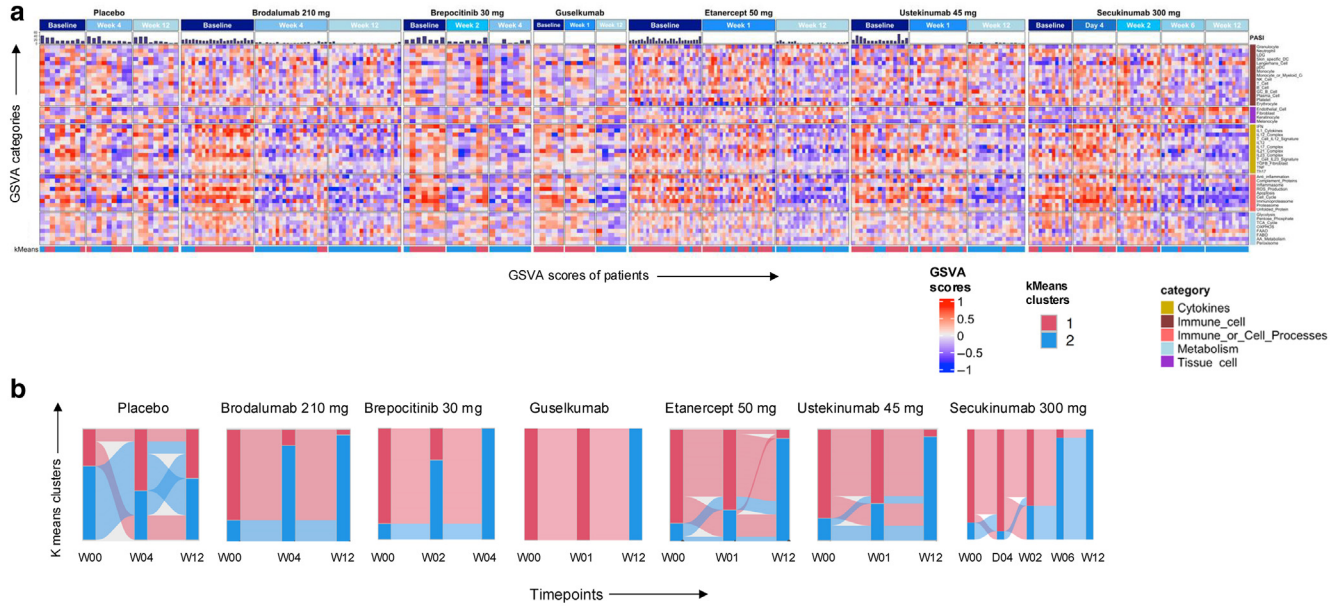


Figure 2. Molecular differences in psoriatic lesional skin over time treated with additional therapeutic agents. (**a**) Heatmap of GSVA scores of placebo (n = 9), 210 mg brodalumab (n = 21), 30 mg brepocitinib (n = 7), guselkumab (n = 5), 50 mg etanercept (n = 25), 45 mg ustekinumab (n = 15), and secukinumab (n = 13) –treated patients. (**b**) Alluvial plots illustrating patients' movement between the red and blue K-means clusters over time. GSVA, Gene Set Variation Analysis.

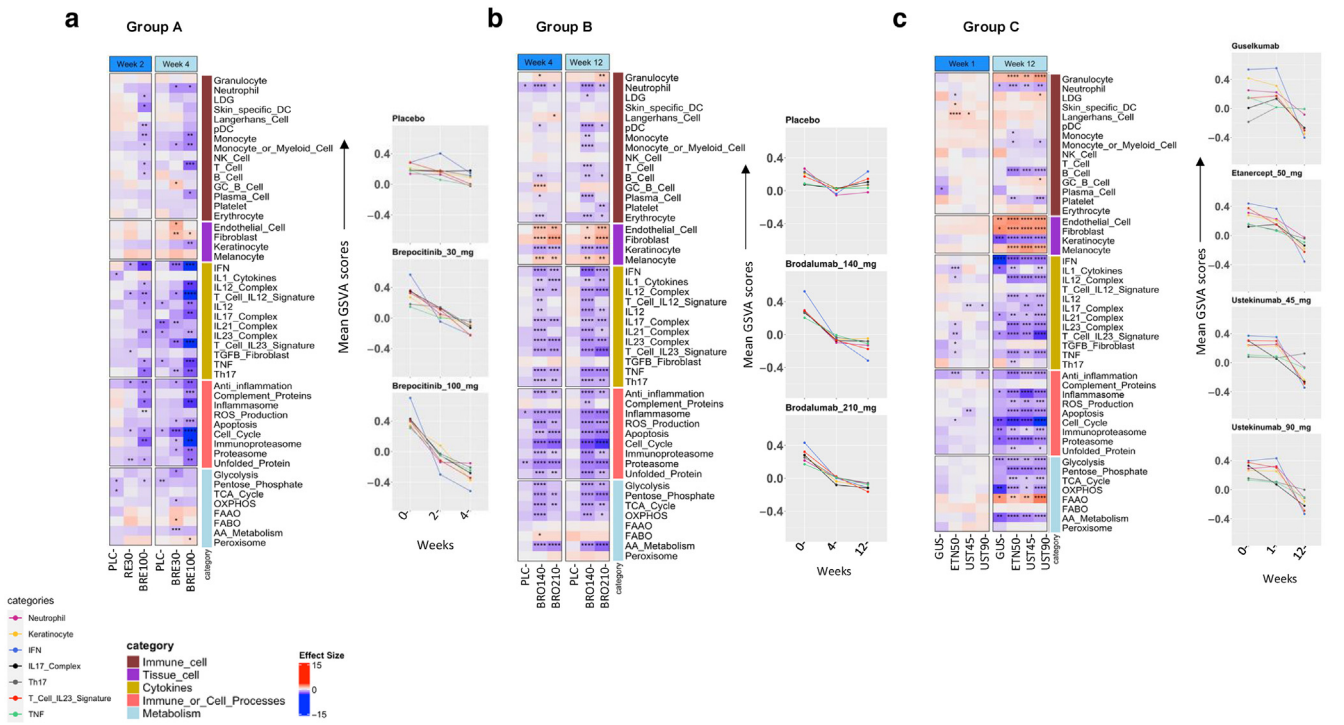


Figure 3. Changes in gene expression profiles of psoriatic skin in response to various therapeutic agents. GSVA using 48 informative gene modules was used to evaluate the molecular response of lesional psoriatic skin to various therapeutic agents over time. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) between baseline (week 0) and the designated time point. Red indicates an increase in effect size, and blue indicates a decrease. The asterisks indicate the *P*-value of the change in each module. Welch's *t*-test was used. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. The line graphs show the mean GSVA values over time for 7 gene modules that exhibited change over time. (**a**) Placebo and brepocitinib (30 and 100 mg) at weeks 2 and 4. (**b**) Placebo and brodalumab (140 and 210 mg) at weeks 4 and 12. (**c**) Data for guselkumab (50 mg), etanercept (50 mg), and ustekinumab (45 and 90 mg) after 1 and 12 weeks. GSVA, Gene Set Variation Analysis.

increases in the fibroblast signature and decreases in the neutrophil and T-cell *IL23* gene signatures (Figure 3a). BREPO-HI− but not BREPO-LO−treated patients showed significant decreases in inflammatory gene signatures after just 2 weeks of treatment indicative of a dose−response effect. A decrease in the keratinocyte gene signature was noted by week 4 in BREPO-HI−treated patients, suggesting that the effect on inflammation preceded the improvement in the keratinocyte signal (Figure 3a). In group B, BROD-treated patients showed a decrease in the neutrophil gene signature and an increase in the endothelial cell, fibroblast, and melanocyte gene signatures at 4 and 12 weeks (Figure 3b). Decreases in keratinocytes and most of the gene signatures representative of cytokines and immune cell processes were seen in both groups at weeks 4 and 12. In group C, there was minimal response on the basis of change in gene expression signatures after 1-week treatment with GUS, ETN, or UST (Figure 3c). However, by 12 weeks of treatment, there was significant enrichment of granulocyte, endothelial cell, fibroblast, melanocyte, and fatty acid alpha oxidation and decreases of neutrophil, keratinocyte, IFN, inflammasome, cell cycle, amino acid metabolism, and glycolysis gene signatures. A significant decrease in the *IL17* complex gene signature was observed only in the UST-treated patients. Gene signatures representative of the *IL12* complex, *IL23* complex, and the T-cell IL 23 complex were downregulated in the ETN-treated patients by week 1 and in GUS- or UST-treated patients by week 12. Changes in gene expression with placebo treatment were largely not significant (Figure 3a and b).

Treatment decreases LS gene expression levels to those noted in NL skin

Next, to evaluate whether various therapeutic agents restored abnormalities in psoriatic LS skin to the level of NL skin, we compared transcriptomic profiles of treated LS skin at various time points with those of baseline NL skin from the same patient (Figure 4a−c and Supplementary Figure S4). In all 3 groups, baseline psoriatic LS skin was characterized by increases in the majority of proinflammatory cytokine gene signatures, cell cycle, and inflammasome genes as well as signatures of neutrophils and keratinocytes compared with baseline NL skin. BREPO-HI−treated psoriatic LS skin showed significant decreases in most of the inflammatory gene signatures, including *IFN*, *IL12*, and T-cell *IL23*, and monocyte and skin gene signatures such as keratinocyte, to levels below those seen in NL skin by week 4 (Figure 4a and Supplementary Figure S4). In BROD-treated patients, inflammatory gene signatures such as plasmacytoid dendritic cell, T cell, plasma cell, IFN, and IL-12 were less enriched at week 4, and by week 12, they were mostly downregulated to levels observed in NL skin (Figure 4c). By week 12, ETN- and UST-treated patients exhibited decreases in neutrophil and T-cell *IL12* gene signatures, and an increase in the melanocyte signature was observed to levels approximating those noted in NL skin

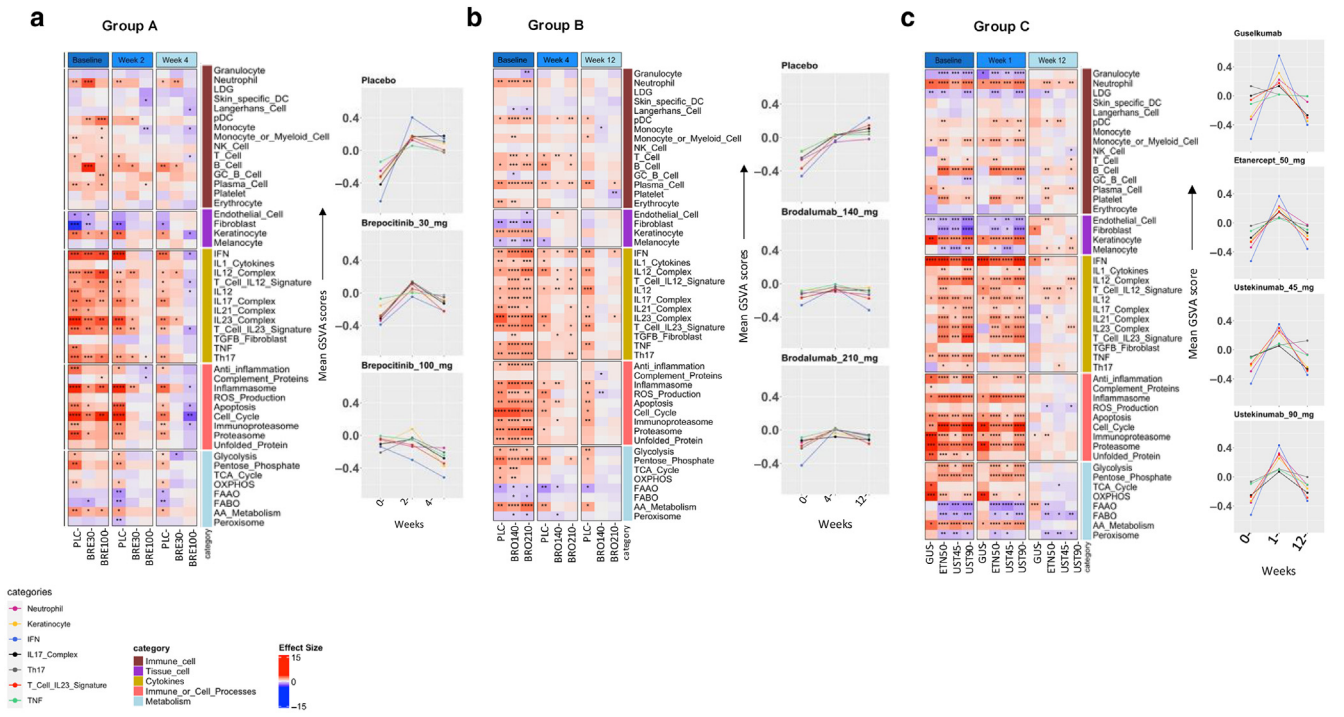


Figure 4. Changes in psoriatic lesional skin compared with those in baseline nonlesional skin in response to various therapeutic agents. GSVA using 48 informative gene modules was used to evaluate whether various therapeutic agents restored psoriatic lesional skin to that expressed by nonlesional skin over time. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) between baseline (week 0) and the designated time point. Red indicates an increase in effect size, and blue indicates a decrease. The asterisks indicate the *P*-value of the change in each module. Welch's *t*-test was used. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. The line graphs show the mean GSVA values over time for 7 gene modules that exhibited change. (**a**) Placebo and brepocitinib (30 and 100 mg) at weeks 2 and 4. (**b**) Placebo and brodalumab (140 and 210 mg) at weeks 4 and 12. (**c**) Data for guselkumab (50 mg), etanercept (50 mg), and ustekinumab (45 or 90 mg) after 1 and 12 weeks. GSVA, Gene Set Variation Analysis.

(Figure 4c). The transcriptional profiles of placebo-treated skin was similar across all time points (Figure 4a and b).

Treatment effects on NL skin

Despite a lack of visible symptoms, we previously described abnormalities in gene expression profiles of NL skin at baseline that were distinct from both LS and healthy skin (Martínez et al, 2022). To study the effect of treatment on NL skin, we compared GSVA scores after 12 weeks of treatment with those of paired NL skin at baseline (Figure 5a). In BROD-LO–treated patients, NL skin at week 12 displayed decreased inflammatory signatures, such as neutrophil, IFN, IL-21 complex, and T-cell IL-23, compared with NL skin at baseline. Significant decreases in metabolic signatures, such as pentose phosphate, oxidative phosphorylation, and amino acid metabolism, were noted in NL skin of UST-treated patients. Despite the treatment effect on NL skin, treated LS skin at week 12 showed increased IFN, T-cell IL-12, and immunoproteasome gene signatures compared with NL skin at week 12 in BROD-treated patients and increased T-cell IL-12, unfolded protein, pentose phosphate, and amino acid metabolism gene signatures in UST-treated patients (Figure 5b). Placebo treatment had minimal effects on NL skin. These results suggest that effective treatment may have selective effects on both LS and NL psoriatic skin.

Enumerating the degree of abnormality in gene expression profiles with the PsoriaCIS

Next, we developed a scoring system to enumerate the degree of abnormality in psoriatic skin gene expression profiles (Figure 6a). Inputs for the PsoriaCIS were concatenated GSVA scores from baseline LS and NL skin samples from all datasets (Table 1 and Supplementary Table S1). Logistic regression with ridge penalty was employed to generate coefficients for expression of each gene module, and the PsoriaCIS for each patient was calculated (Figure 6a and b and Supplementary Table S3). Then, baseline LS psoriasis samples were assessed by k-means clustering to subset patients on the basis of pretreatment GSVA scores (Figure 6c). As a result, 4 subsets of patients with varying degrees of severity were noted, and these differences were reflected in the PsoriaCIS (Figure 6d). However, the molecular severity of patients grouped on the basis of GSVA scores was not reflected in the clinical PASI scores that were comparable in all subsets. Moreover, PASI scores did not correlate with PsoriaCIS at baseline (Figure 6e).

To explore the relationship between PsoriaCIS and clinical disease activity in greater detail, we assessed treatment-related differences in PASI and PsoriaCIS over time. Both PASI and PsoriaCIS significantly decreased in parallel as active treatment progressed, whereas no change in placebo-treated patients was observed (Figure 7a and Supplementary Table S3). Furthermore, PsoriaCIS and PASI showed positive

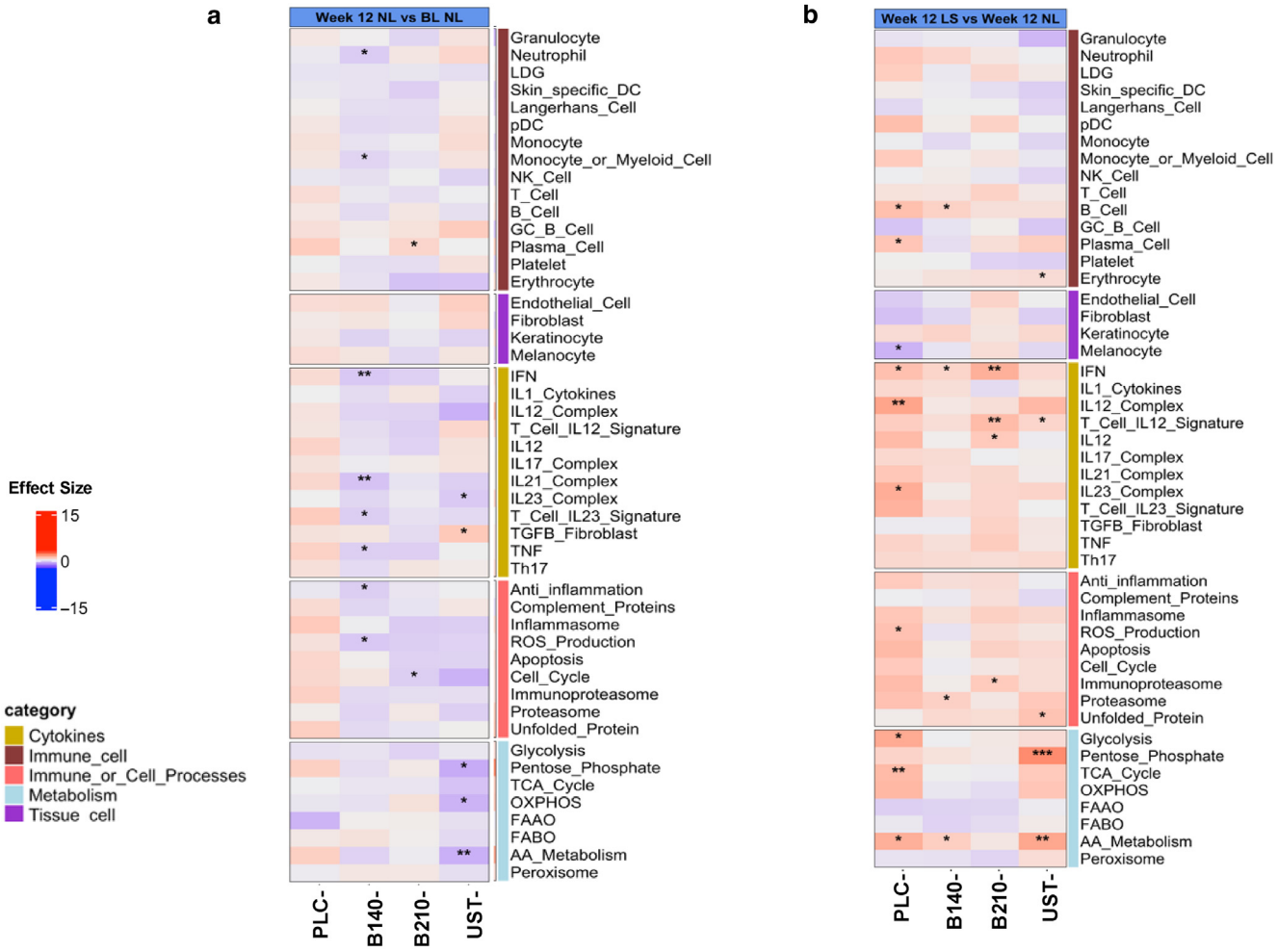


Figure 5. Changes in molecular features of nonlesional psoriatic skin after 12 weeks of treatment. GSVA with 48 informative gene modules was employed to evaluate the impact of treatment on nonlesional psoriatic skin. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) (**a**) between week 12 nonlesional and baseline nonlesional and (**b**) between week 12 lesional and week 12 nonlesional. Red indicates and increase in effect size, and blue indicates a decrease. The asterisks indicate the *P*-value of the change in each module. Welch's *t*-test was used. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. GSVA, Gene Set Variation Analysis.

and significant correlations in all patients receiving active treatment (Figure 7b and Supplementary Table S3). We also looked for correlations between PASI and individual gene signatures, such as that for keratinocytes and neutrophils that are hallmarks of psoriatic gene expression abnormalities. Significant correlations were noted between PASI and the GSVA scores of keratinocytes, neutrophils, and the IFN gene signature but not for erythrocytes, each of which was included in PsoriaCIS (Supplementary Figure S5a–c). In general, however, the correlation coefficients were not as high, and the *P*-values were less significant for the individual gene signatures than PsoriaCIS. Furthermore, variability in the correlations of individual signatures with PASI and PsoriaCIS was reflective of the contribution of each signature to disease and the impact of each therapeutic agent. In particular, correlations with the keratinocyte, neutrophil, and IFN gene signatures were significant with all active treatments, whereas there was only a significant positive correlation with the erythrocyte gene signature and BROD and a negative correlation with the erythrocyte gene signature and ETN and UST (Supplementary Figure S5a–c). Thus, PsoriaCIS provides additional baseline information that captures changes in immune gene signatures over time with treatment and can be used in parallel with PASI scores to identify the molecular determinants of disease status and the differential impact of each therapeutic agent.

Similarities and differences in the molecular profiles of clinical responders and nonresponders to various therapeutic agents

In each treatment group, a small percentage of patients failed to meet criteria to be considered clinical responders as defined by a 75% decrease in baseline PASI score (PASI75) (Supplementary Table S1). Therefore, baseline molecular differences in LS skin of responders and nonresponders were assessed. To account for patient heterogeneity and the lack of sufficient numbers of nonresponders in some datasets to enable a direct comparison, baseline GSVA scores of LS skin were normalized to those of paired NL skin, and effect sizes compared with NL skin were calculated separately for clinical responders and nonresponders. LS skin of responders to either dose of BROD showed elevated transcripts of

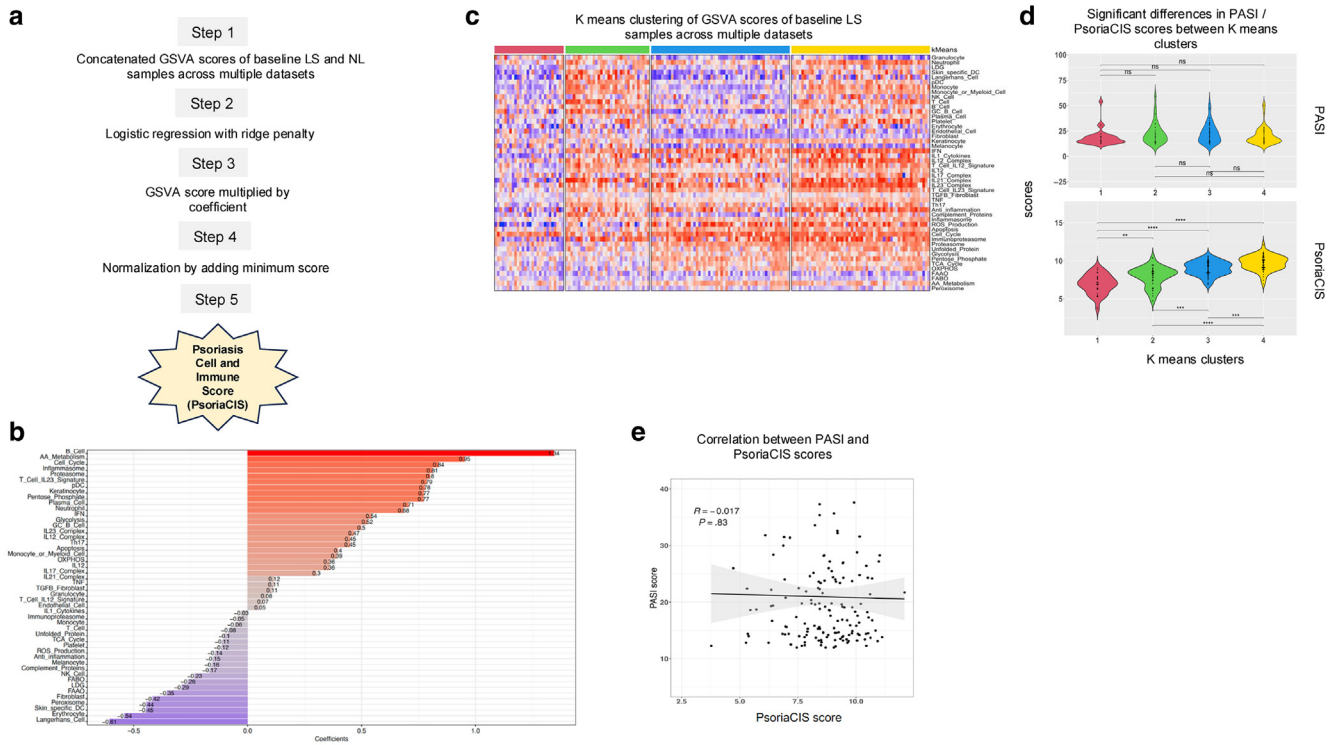


Figure 6. Development of the PsoriaCIS. (**a**) Flow diagram depicting the analysis steps used for development of PsoriaCIS. (**b**) Logistic regression with ridge penalty was carried out between lesional and nonlesional samples at baseline, and its coefficients are visualized as bar graphs. Red > 0, and blue < 0. (**c**) Heatmap of GSVA scores of baseline lesional samples of patients with psoriasis divided into 4 K-means clusters. (**d**) Violin plots of PASI and PsoriaCIS scores of the patients in 4 K-means clusters displaying the pattern of change. (**e**) Scatter plot showing Pearson correlation between PASI and PsoriaCIS for patients in **c**. GSVA, Gene Set Variation Analysis; PsoriaCIS, Psoriasis Cell and Immune Score.

neutrophils, keratinocytes, IL-12, and amino acid metabolism and decreased expression of fibroblast transcripts compared with paired NL skin at baseline (Figure 8a). This was not observed in the LS skin of nonresponders to either dose of BROD. For BREPO-LO, numerous inflammatory gene signatures were increased in LS skin of responders but not in nonresponders, including monocyte/myeloid cell, IFN, T-cell IL-12, IL-17 complex, T-cell IL-23, inflammasome, and cell cycle. Distinct molecular features characterizing LS skin of both responders and nonresponders to ETN and UST were not observed (Figure 8a). When LS skin was compared with NL skin from all treatments combined, IL-17 complex, plasmacytoid dendritic cell, T cell, ROS production, peroxisome, and Langerhans cell gene signatures were more significantly elevated in responders than in nonresponders, regardless of active treatment group (Figure 8b).

Response status was then assessed for patients within each subset as defined by k-means clustering of GSVA scores (Figure 6c) to determine whether baseline differences in global molecular severity would predict response to treatment (Figure 9a). Membership within any 1 subset was not predictive of subsequent response to treatment and the progression of PASI scores, and PsoriaCIS was similar for all 4 subsets, regardless of responder status (Figures 6d and 9b). However, the difference in PsoriaCIS between subsets was greater in responders than in nonresponders, whereas PASI scores remained relatively consistent between baseline subsets (Figure 9c). Thus, responsiveness appeared to relate to baseline expression of specific gene modules (Figure 8) and not the baseline subset membership or PsoriaCIS (Figure 9).

It is also notable that changes in gene expression patterns over time were less marked in patients who were clinical nonresponders (Supplementary Figures S6 and S7). In all 3 groups of therapeutic agents (Supplementary Figure S3), clinical responders but not nonresponders showed significant decreases in inflammatory gene signatures after 2 or 4 or 12 weeks of treatment. Clinical responders also showed a significant correlation between PASI and PsoriaCIS that was not apparent in clinical nonresponders (Supplementary Figure S8 and Supplementary Table S3). Thus, gene expression–based classification of psoriatic skin effectively captures disease severity and clinical response.

DISCUSSION

To date, a number of targeted systemic therapies have achieved success in the management of psoriasis, but associated research has primarily focused on safety and clinical efficacy, and there remains a gap in understanding the molecular dynamics of disease development and/or response to treatment. To address this, we have carried out a comprehensive transcriptomic analysis of skin biopsies from patients with psoriasis after treatment with multiple therapeutic agents (BROD, ETN, UST , GUS, BREPO, and SEC). As a result, we have identified nuanced differences in the impact of each agent on longitudinal gene expression profiles and developed a gene expression–based score (PsoriaCIS) that serves as a

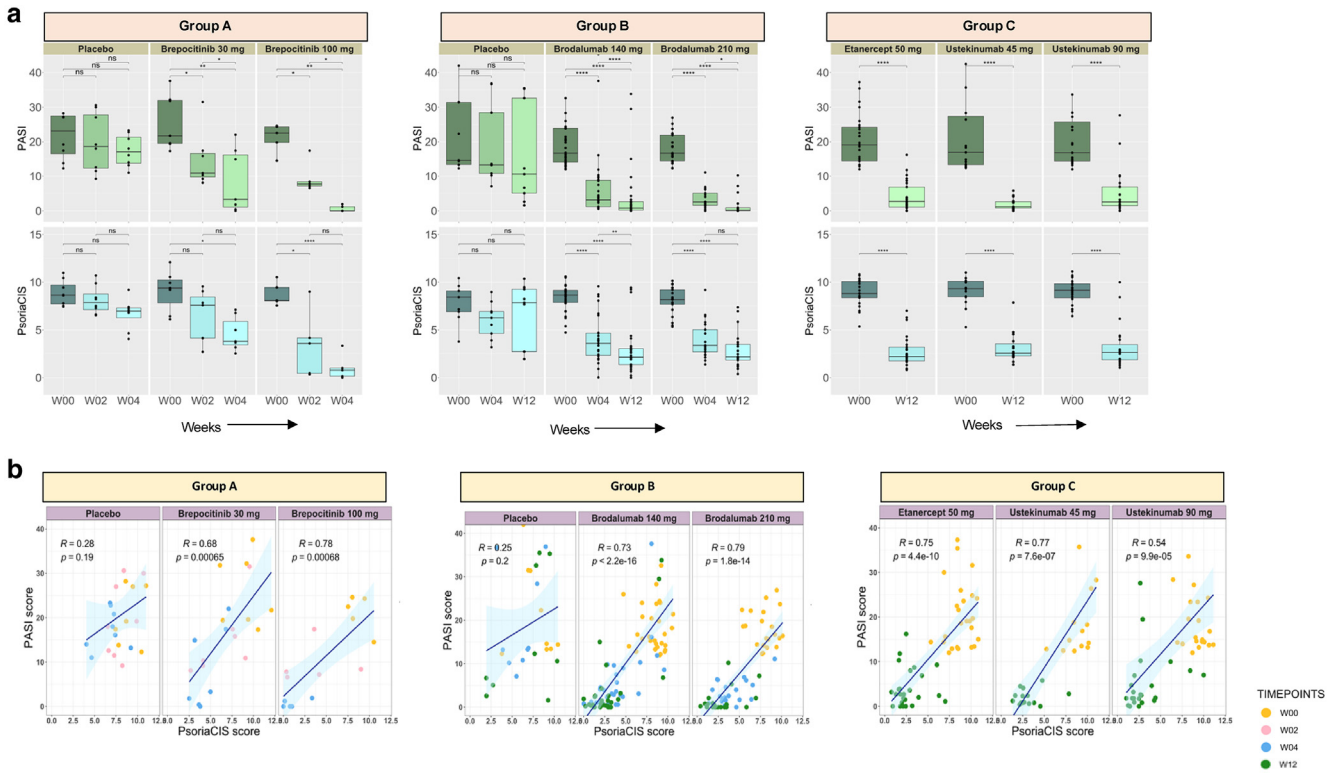


Figure 7. Comparison of PsoriaCIS with PASI. (**a**) Box plots of PASI (top) and PsoriaCIS scores (bottom) across various time points for groups A, B, and C patients with psoriasis. Repeated measures of ANOVA were used to calculate significant differences between time points**. (b)** Scatter plots showing Pearson correlations between PASI and PsoriaCIS across multiple time points. Each time point is represented by the dot color. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$, ns is not significant; PsoriaCIS, Psoriasis Cell and Immune Score.

molecular readout of disease severity and treatment efficacy. This patient stratification approach based on molecular profiling provides insight into the magnitude of the dysregulated inflammatory pathways and resident skin cell abnormalities in individual patients with psoriasis. PsoriaCIS, therefore, has the potential to contribute additional information to improve disease management beyond clinical evaluation alone.

In this study, we demonstrated that GSVA scores representing enrichment of skin-specific gene signatures reflected baseline abnormalities in inflammatory/tissue cell types and cytokines that currently available standard-of-care treatments for psoriasis were effective at mitigating over time. To delineate similarities between molecular profiles at various time points, we used K-means clustering as an unbiased approach to classify patients into endotypes on the basis of molecular severity at baseline and evaluated the ability of each therapeutic agent to revert patients to a less severe endotype. Both statistical and clustering analysis demonstrated similarities in molecular profiles of patients with psoriasis treated with each agent, but despite the overall similarity, expression of different signatures at baseline associated with responsiveness to specific agents. Importantly, each therapeutic agent acted as expected on the basis of its molecular target, such that treatment with BREPO reduced IFN and inflammatory cytokine gene signatures (Mease et al, 2023), BROD and SEC reduced the IL-17 complex signature (Bertelsen et al, 2020; Tomalin et al, 2020), UST had the greatest impact on the IL-12 and IL-23 signatures, and ETA significantly reduced the *TNF* gene signature (Brodmerkel et al, 2019). Overall, GUS (Sofen et al, 2014) appeared to be the least effective at reducing inflammatory gene expression by week 12 compared with other agents. We also noted that enrichment of several cellular signatures, including granulocytes, endothelial cells, fibroblasts, melanocytes, and fatty acid alpha oxidation, increased to a greater extent in treated LS skin of ETN- or UST-treated patients than in BREPO- or BROD-treated patients. This result suggests that treatment with ETN or UST and inhibition of TNF or IL-12/IL-23 has a greater impact on reversal of resident skin cell dysregulation than IL-17 inhibition with BROD. However, we cannot make a direct comparison with BREPO treatment because we did not have data available at the 12-week time point. Further studies are needed to understand the differential effects of inhibiting inflammatory cytokines on tissue biology of psoriatic skin lesions in greater detail.

In addition, we studied molecular abnormalities in response to varying doses of either UST, BROD, or BREPO. For BREPO, patients were given either 30 mg (BREPO-LO) or 100 mg (BREPO-HI) over 4 weeks, after which, we observed a dose-dependent effect on gene expression profiles. Notably, this appears to conflict with phase IIa/IIb clinical trials in which 30 and 60 mg doses were used, and the 30 mg dose was thought to be more effective at reducing PASI scores over the course of 12-week and 16-week studies, respectively (Forman et al, 2020; Mease et al, 2023). This result suggests that future studies are warranted to assess the long-term

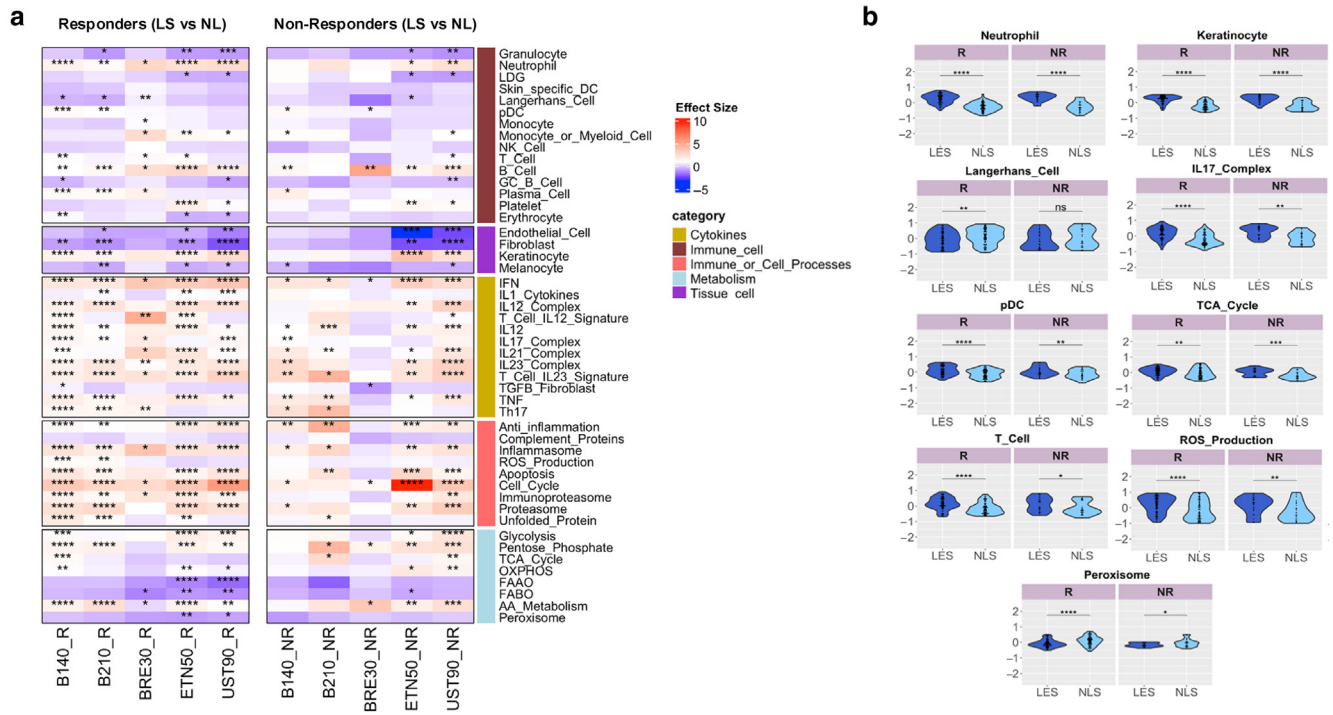
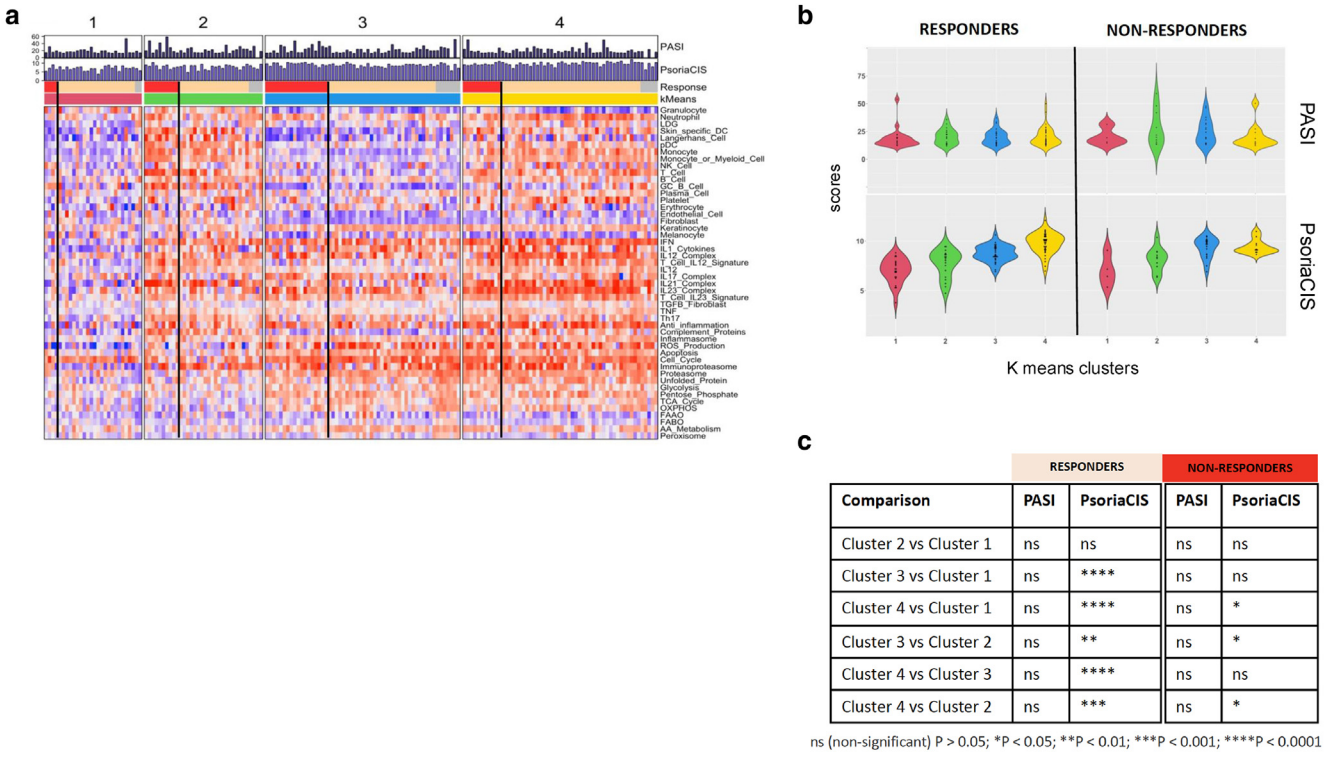


Figure 8. Baseline molecular differences in lesional skin compared with those in nonlesional skin among clinical responders and nonresponders. (**a**) GSVA using 48 informative gene modules was used to evaluate baseline molecular differences in psoriatic lesional skin compared with those in nonlesional skin separately for responders (left) and nonresponders (right) to 140 or 210 mg brodalumab, 30 mg brepocitinib, 50 mg etanercept, and 90 mg ustekinumab. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) between baseline lesional and nonlesional samples. Red indicates an increase in effect size, and blue indicates a decrease. (**b**) Violin plots of 9 gene modules displaying significant differences between cumulative GSVA scores of lesional and nonlesional samples separately for responders and nonresponders. The asterisks in heatmap and violin plots indicate the *P*-value of the change in each module. Welch's *t*-test was used. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001. GSVA, Gene Set Variation Analysis; LES, lesional; NL, nonlesional.

impact of higher doses of BREPO, which may be more effective at reducing inflammatory signatures of psoriasis. Conversely, different doses of UST and BROD did not significantly differ in their effect on gene expression. Interestingly, the Food and Drug Administration–recommended dosages (210 mg for BROD and 90 mg for UST) (Menter et al, 2019) were the high doses in both of these studies, suggesting that the standard treatment regimens for BROD and UST could be adjusted in clinical use. Together, these analyses underscore the dynamic nature of treatment response and emphasize the importance of considering both treatment duration and dosage in therapeutic interventions.

We investigated the molecular differences between clinical responders and nonresponders, with particular focus on baseline characteristics, emphasizing the importance of examining foundational molecular features. Responders to BROD and BREPO-LO exhibited molecular profiles distinct from those of nonresponders to the same agent at baseline that reflected increased inflammation and likely served as prerequisites to more effective treatment outcomes. Specifically, LS skin of responders to treatment with BROD was enriched for neutrophil, IL-12 cytokine, and keratinocyte gene signatures and de-enriched for fibroblasts, whereas responders to BREPO treatment were enriched for monocyte/myeloid cell and multiple inflammatory cytokine gene signatures. Conversely, responders and nonresponders to ETN or UST-HI did not exhibit clear distinctions in molecular profiles at baseline, and instead, responders to these agents exhibited a greater magnitude of change in signatures that were also affected but to a lesser degree in nonresponders. Examining baseline molecular features holds promise for predicting treatment response, but further longitudinal studies after treatment over time are important to establish molecular dynamics and to determine how clinical response is correlated to molecular fluctuations.

We developed a method, PsoriaCIS, to assess molecular abnormality in psoriatic skin, demonstrating its potential to enhance traditional evaluations of the disease beyond information provided by the clinical PASI score. To evaluate and compare clinical (PASI) and molecular (PsoriaCIS) scores at baseline, we identified 4 distinct endotypes of patients with psoriasis on the basis of molecular abnormalities. Interestingly, whereas molecular scores reflected the heterogeneity among baseline LS biopsies and the differences in inflammatory signatures between endotypes, clinical scores were not different among the endotypes. This could indicate that the molecular scores identify clinically unimportant pathways. However, the finding that molecular pathways observed to be elevated reflected inflammatory signatures known to be involved in psoriasis pathogenesis would tend to discount this contention. Rather, we propose that the greater amount of information available through gene expression analysis as opposed to clinical evaluation is more sensitive than PASI scores alone and may be better equipped to



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	RESPONDERS		NON-RESPONDERS	
Comparison	**PASI**	**PsoriaCIS**	**PASI**	**PsoriaCIS**
Cluster 2 vs Cluster 1	ns	ns	ns	ns
Cluster 3 vs Cluster 1	ns	****	ns	ns
Cluster 4 vs Cluster 1	ns	****	ns	*
Cluster 3 vs Cluster 2	ns	**	ns	*
Cluster 4 vs Cluster 3	ns	****	ns	ns
Cluster 4 vs Cluster 2	ns	***	ns	*

ns (non-significant) P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

Figure 9. Baseline molecular heterogeneity in lesional skin of clinical responders and nonresoponders. GSVA using 48 informative modules was carried out using baseline lesional skin samples only. (**a**) Heatmap of GSVA scores grouped into 4 K-means clusters (red, green, blue, and yellow). PASI and PsoriaCIS scores are illustrated as bar graphs at the top. Responders and nonresponders are labeled in beige and red color. (**b**) Violin plots of PASI and PsoriaCIS scores of the patients in 4 K-means clusters displaying the pattern of change. (**c**) Table showing statistical differences (*P*-values) between PASI and PsoriaCIS scores across different clusters in responders and nonresponders. GSVA, Gene Set Variation Analysis; PsoriaCIS, Psoriasis Cell and Immune Score.

identify subtle variations in immune profiles and disease status of patients with psoriasis. In contrast to baseline, significant correlations were observed between clinical and molecular scores at later time points in patients who responded to treatment with active drug but not in nonresponders or in those treated with placebo. Thus, molecular markers alone or in combination with clinical scores have the potential to capture current disease state and response to treatment more effectively and, with additional validation, could facilitate improved patient stratification and development of tailored treatments for patients with psoriasis. It is also notable that individual components of PsoriaCIS, such as the keratinocyte and neutrophil signatures, also correlated with PASI scores in patients receiving active treatment. However, in general, the individual signatures exhibited less significant correlations and, in some cases, variable correlations with PASI, supporting the conclusion that the composite PsoriaCIS gave a more complete view of the numerous molecular abnormalities in psoriatic skin and also captured a more nuanced view of the response to specific treatments.

The limitations of this study are largely related to the longitudinal gene expression studies of patients with psoriasis treated with different therapeutic agents that were available for analysis. One such limitation was the relatively low sample sizes of certain datasets, including those from the BREPO (GSE136757) and GUS (GSE51440) studies as well as the placebo-treated control subjects, which may have introduced inadvertent biases into the analysis. Having a small sample size to work with potentially decreases the diversity of the patient pool in terms of demographic distribution and, thus, could increase the risk that the results will not serve as a true representation of the general population. The discrepancies in sample size between treatments could also impact interpretation of the comparative efficacy of each therapeutic agent in improving the molecular profiles of patients with psoriasis. Furthermore, limited sample sizes precluded a direct comparison of responders and nonresponders for certain datasets. Despite these concerns, the impact of the various agents was relatively consistent within the treatment groups, and longitudinal data were often available to confirm results. However, further studies that include larger and more diverse sample populations that are balanced between treatment arms are needed to refine our findings. The included studies also differed in the microarray platforms they employed because the 4 main studies (the treatments in groups A–C) utilized a gene chip different from that of the SEC validation study. However, although the overall number of assessed transcripts varies between these platforms, we have found that by processing and running GSVA separately for each dataset to compare enrichment of gene sets rather than differences in expression of individual genes, we are able to mitigate platform differences (Martínez et al, 2022). Importantly, the vast majority of preselected genes employed as GSVA gene sets was detected by both array platforms, further mitigating this potential problem. Finally, the varying clinical study designs for each agent prevented direct

comparisons of all therapies at every time point. In particular, the BREPO study only extended through 4 weeks, whereas all other studies extended to 12 weeks. Thus, to confirm these results, more comprehensive longitudinal studies with uniform time points for each therapeutic agent are warranted.

We have developed PsoriaCIS as a molecular tool to assess the degree of immune pathology in patients with psoriasis and demonstrated the ability of PsoriaCIS to convey changes to the molecular profiles of patients with effective therapy. However, because this initial implementation of PsoriaCIS was generated using currently available datasets, as with any biomarker, additional prospective studies are needed before it can be used to support the treatment of psoriasis. Despite these limitations, the results of this study provide a comprehensive analysis of psoriasis treatment effects on gene expression and offers numerous potential avenues, including a molecular scoring system, for improving the management of patients with psoriasis. As a clinical biomarker, PsoriaCIS has the potential to support clinical care of psoriasis by aiding in the selection of the most effective therapeutic agent for each individual and to monitor the effects of treatment over time. Thus, by incorporating additional studies and therapeutic agents, PsoriaCIS could be used alongside current methodologies to ensure that each patient is given the optimal treatment regimen over time.

MATERIALS AND METHODS

Datasets

This study utilized 6 publicly available microarray datasets (GSE51440, GSE117239, GSE117468, GSE136757, GSE137218, and GSE137220) from Gene Expression Omnibus. These datasets included longitudinal gene expression data from patients with psoriasis, obtained from LS and NL skin biopsies at baseline and various time points during various drug treatments (Table 1). The treatment regimens varied, with patients receiving either placebo or 50 mg of ETN, 45 or 90 mg of UST, 140 or 210 mg of BROD, 30 or 100 mg of BREPO, GUS, or 300 mg of SEC. In the case of SEC, paired blood gene expression data from skin-biopsied patients were also analyzed.

Data preprocessing and grouping

CEL files were downloaded from Gene Expression Omnibus and were normalized using GeneChip Robust Multiarray Average. Normalization and sample processing were carried out independently for each dataset, and for studies with a placebo treatment arm, these samples were included with the dataset from which they originated. For analytical simplicity, we organized samples into 3 distinct groups (groups A, B, and C) on the basis of the time points assessed (Supplementary Figure S3).

GSVA

GSVA is a nonparametric, unsupervised method that calculates sample-wise gene set enrichment scores as a function of genes inside and outside the gene set (Hänzelmann et al, 2013). The GSVA algorithm was implemented using the R Bioconductor open-source package gsva (version 1.40). Input genes were filtered, and only those with interquartile range > 0 were considered. To assess the effects of each drug, GSVA was conducted using LS and NL samples at baseline as well as LS samples at various time points. Previously identified gene signatures for skin (Martínez et al, 2022) were used as gene sets for GSVA. In brief, these gene sets were carefully curated to include genes that are measured consistently across most microarray platforms, thereby enhancing the comparability of the results. Although platform-specific variability can influence which genes contribute to the enrichment score in a given dataset, we observed minimal variation in gene representation across the platforms utilized in our analysis. The vast majority (>89%) of all preselected genes contributing to the GSVA scores were consistently detected across the different platforms, resulting in highly comparable enrichment scores across datasets. To study baseline disparities distinguishing responders from nonresponders, GSVA was specifically performed using baseline samples. Moreover, to examine treated NL skin, GSVA was extended to include LS and NL samples at week 12 only for the GSE117468 dataset.

Statistical analysis

Statistical differences of GSVA scores between various groups were calculated using Welch's *t*-test for unpaired samples, paired *t*-test for paired samples, or repeated measures of ANOVA for paired longitudinal samples. Bonferroni correction for *P*-values was applied for repeated measures of ANOVA. The magnitude of difference (effect size) was estimated using Hedge's g as calculated below:

$$g = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{(n_1-1)*s_1^2+(n_2-1)*s_2^2}{n_1+n_2-2}}}$$

where

$\overline{x_1}$ and $\overline{x_2}$ = cohort 1 mean and cohort 2 mean, respectively

n_1 and n_2 = cohort 1 size and cohort 2 size, respectively

s_1^2 and s_2^2 = cohort 1 variance and cohort 2 varaince, respectively

Cohort 1 and cohort 2 could be either LS sample at a particular time point or LS/NL sample at baseline. All the statistical analysis was carried out in using effectSize (version 0.8.1) and stats (version 3.6.2) library in R. For visualization of GSVA scores and effect sizes, ComplexHeatmap library was used, and for the violin plots, ggplot2 library in R was used.

Logistic regression and PsoriaCIS

Logistic regression with ridge penalty was carried out on GSVA scores of LS and NL samples at baseline across various drugs. Ridge penalization helped in coefficient shrinkage and in prevention of multicollinearity. Ridge penalized coefficients were then used to derive PsoriaCIS for each patient. Briefly, to calculate PsoriaCIS for each patient, GSVA scores were multiplied by coefficients and summed to generate a raw score. Raw scores were then normalized by adding a minimum score. PsoriaCIS scores approximately ranged from 0 to 12. PsoriaCIS scores were correlated to PASI using Pearson correlation, and statistical differences between PASI scores or PsoriaCIS scores across various time points were estimated using repeated measures of ANOVA with Bonferroni correction for *P*-values.

K-means clustering and alluvial plots

K-means clustering, an unsupervised algorithm, was used to cluster GSVA scores of patients with psoriasis at various time points for each drug separately. Elbow and silhouette methods were used to choose optimal number of clusters. Alluvial diagrams helped in

understanding assignment of patients at each time point into various K-means clusters.

ETHICS STATEMENT

All human datasets analyzed in the manuscript are publicly available and were subject to institutional approval at the institutions from which they were generated. All dataset accessions are available in Table 1.

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at GSE136757, GSE117468, GSE51440, GSE117239, and GSE137218 hosted at the Gene Expression Omnibus (Bertelsen et al, 2020; Brodmerkel et al, 2019; Page et al, 2020; Sofen et al, 2014; Tomalin et al, 2020).

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: SS, KK, PL; Data Curation: SS, PB; Formal Analysis: SS; Funding Acquisition: AG, PL; Investigation: SS, KK, AD; Methodology: SS, KK, PB; Supervision: AG, PL; Validation: SS, KK, PB; Visualization: SS, AD; Writing - Original Draft Preparation: SS; Writing - Review and Editing: SS, KK, AD, PL

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMS)

The authors did not use AI/LLM in any part of the research process and/or manuscript preparation.

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.xjidi.2024.100333.

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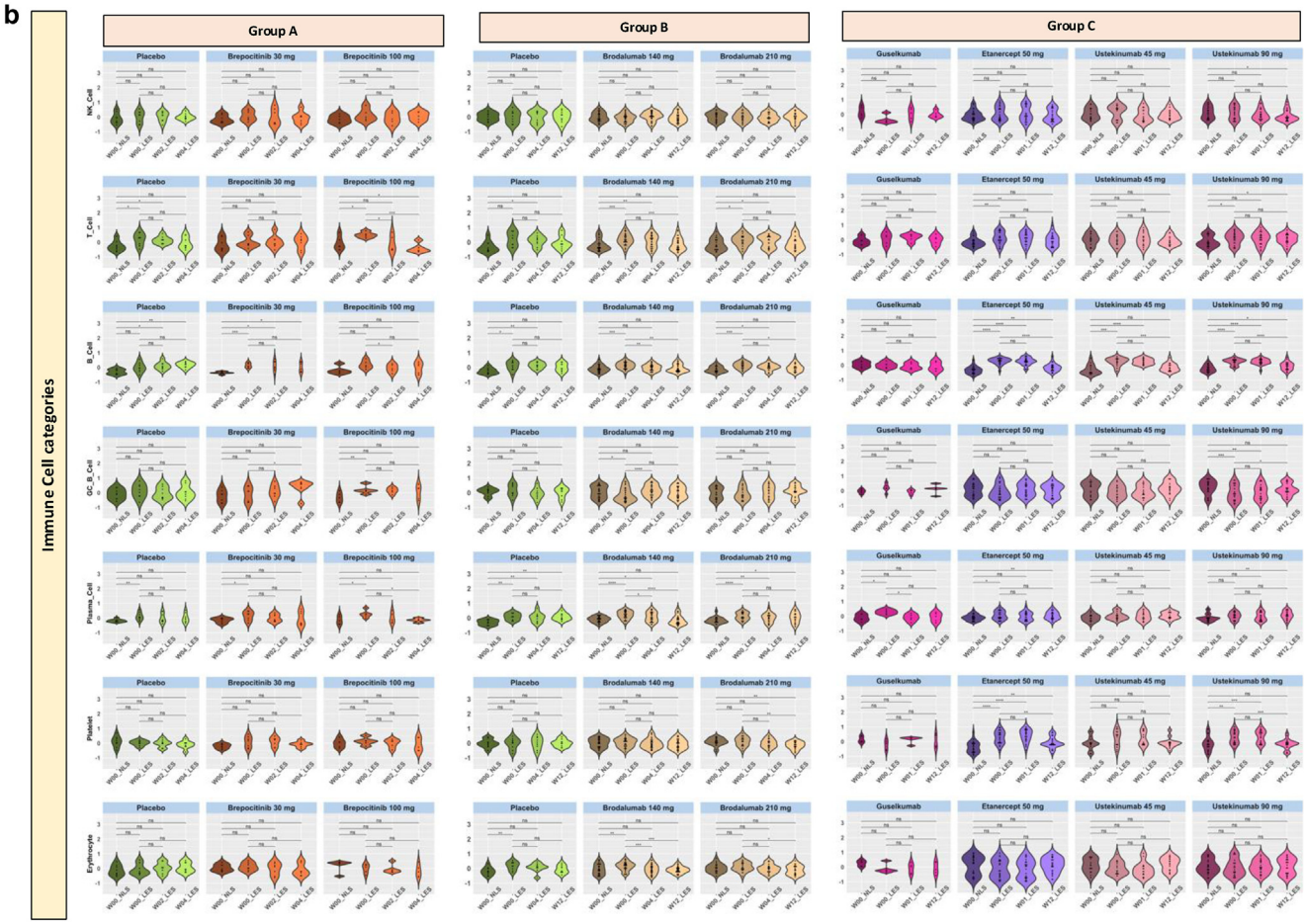
Supplementary Figure S1. Line graphs displaying the changes in mean GSVA scores over time. Line graphs of 48 gene signatures are visualized in 5 groups (immune cell, cytokines, metabolism, tissue cells, and immune cell processes). Each line graph has baseline NL, LS, and LS time point (either week 1 or week 2 or week 4 or week 12) based on group A, B, or C as defined in Supplementary Figure S3. GSVA, Gene Set Variation Analysis; LS, lesional; NL, nonlesional.



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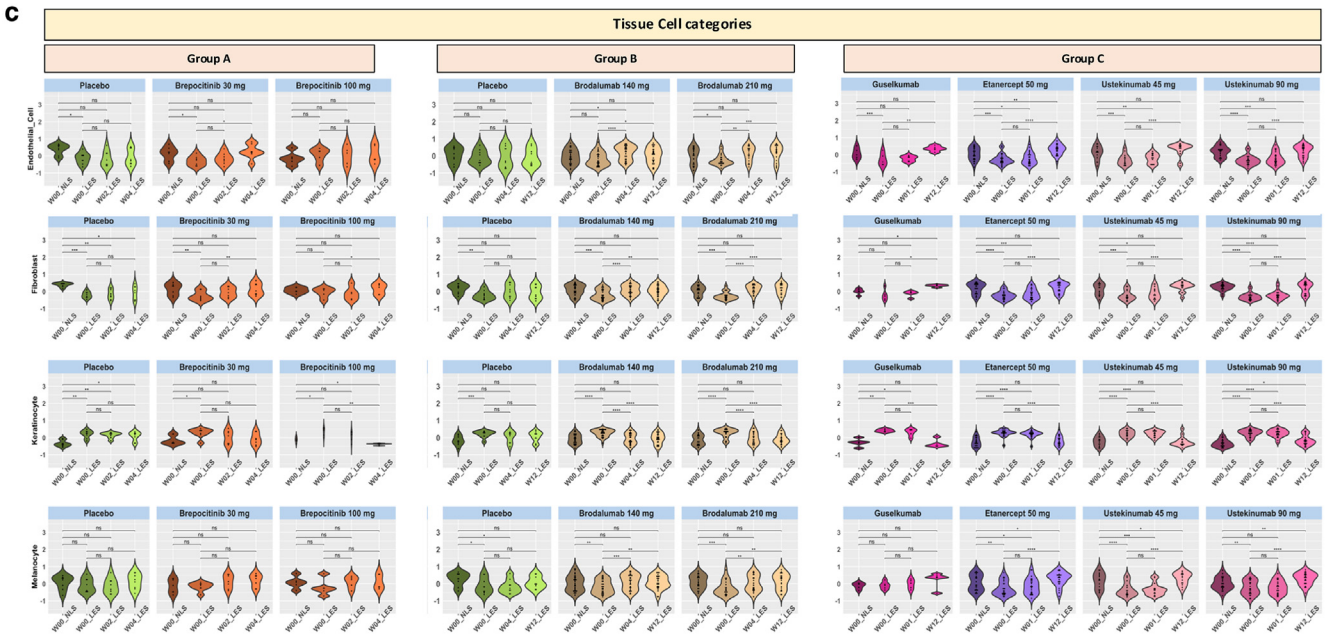

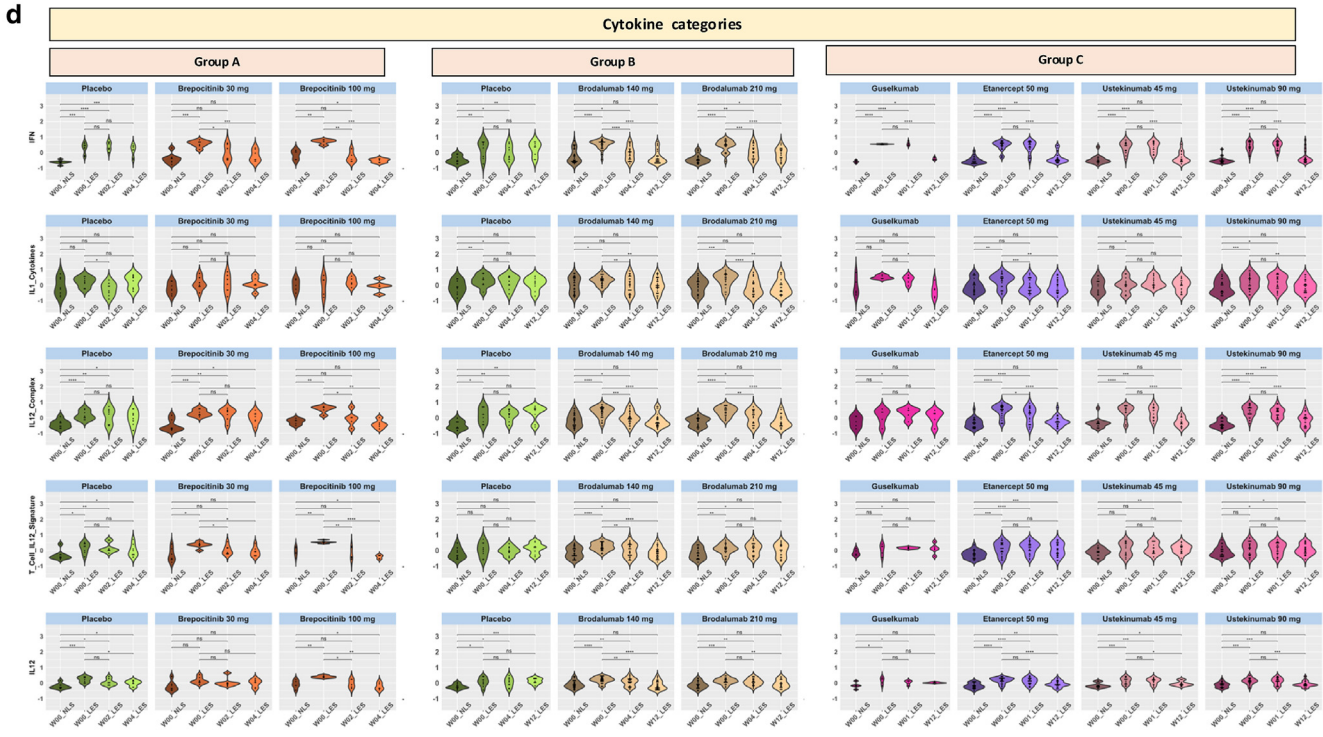

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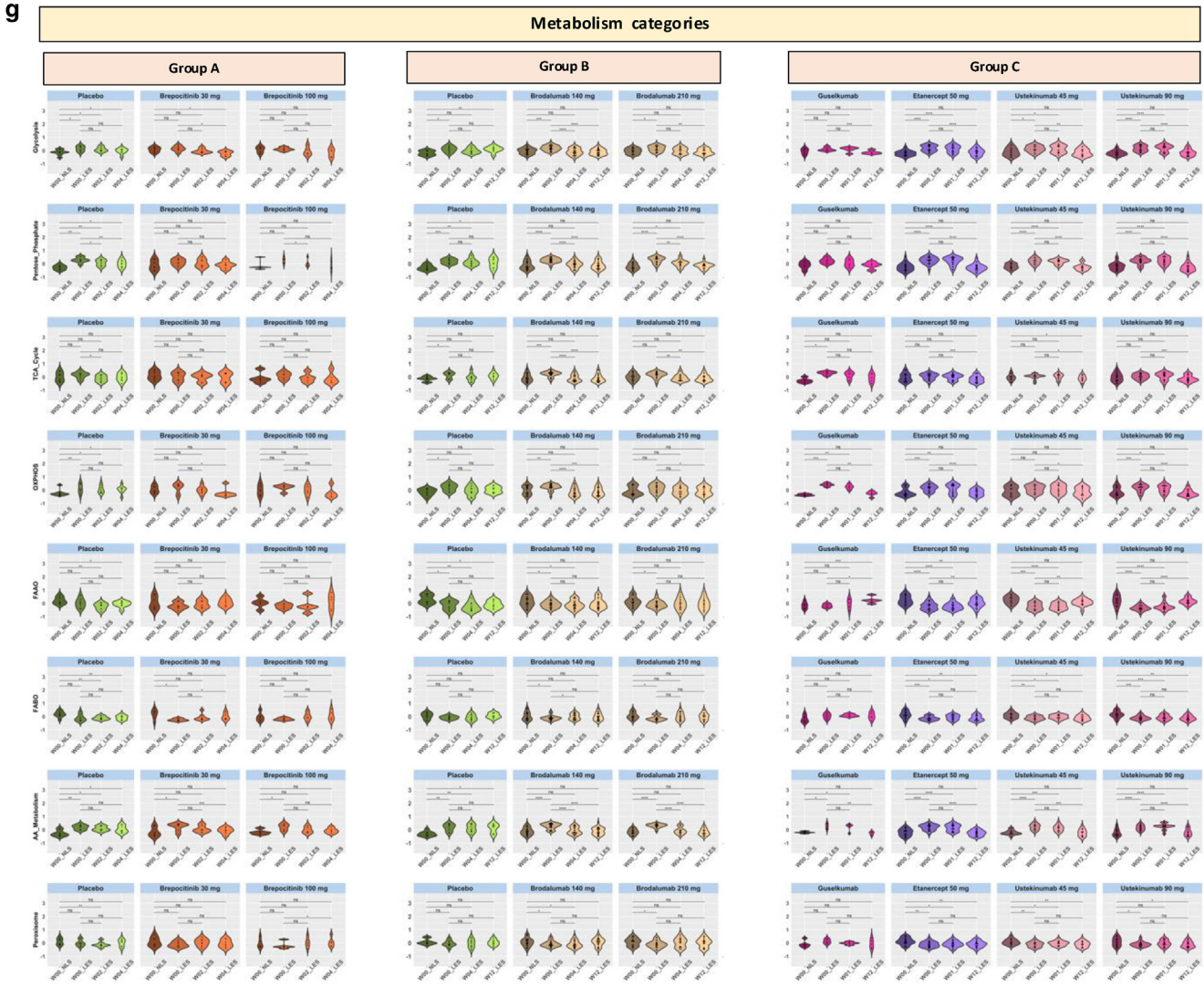

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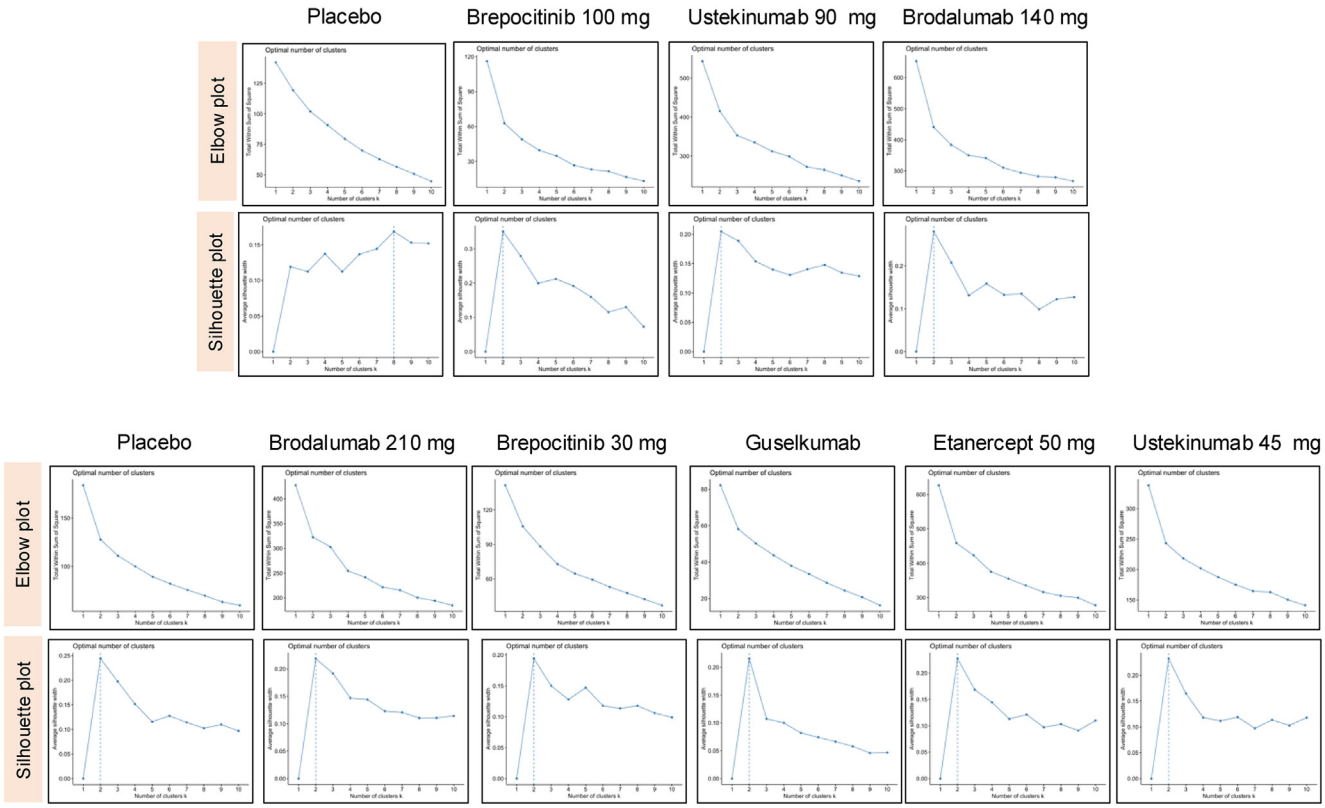
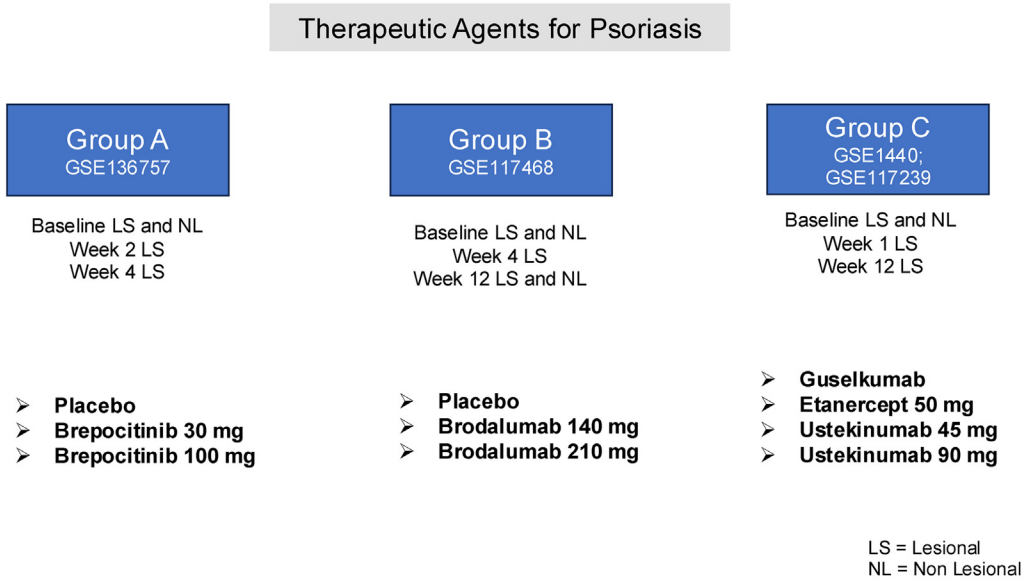
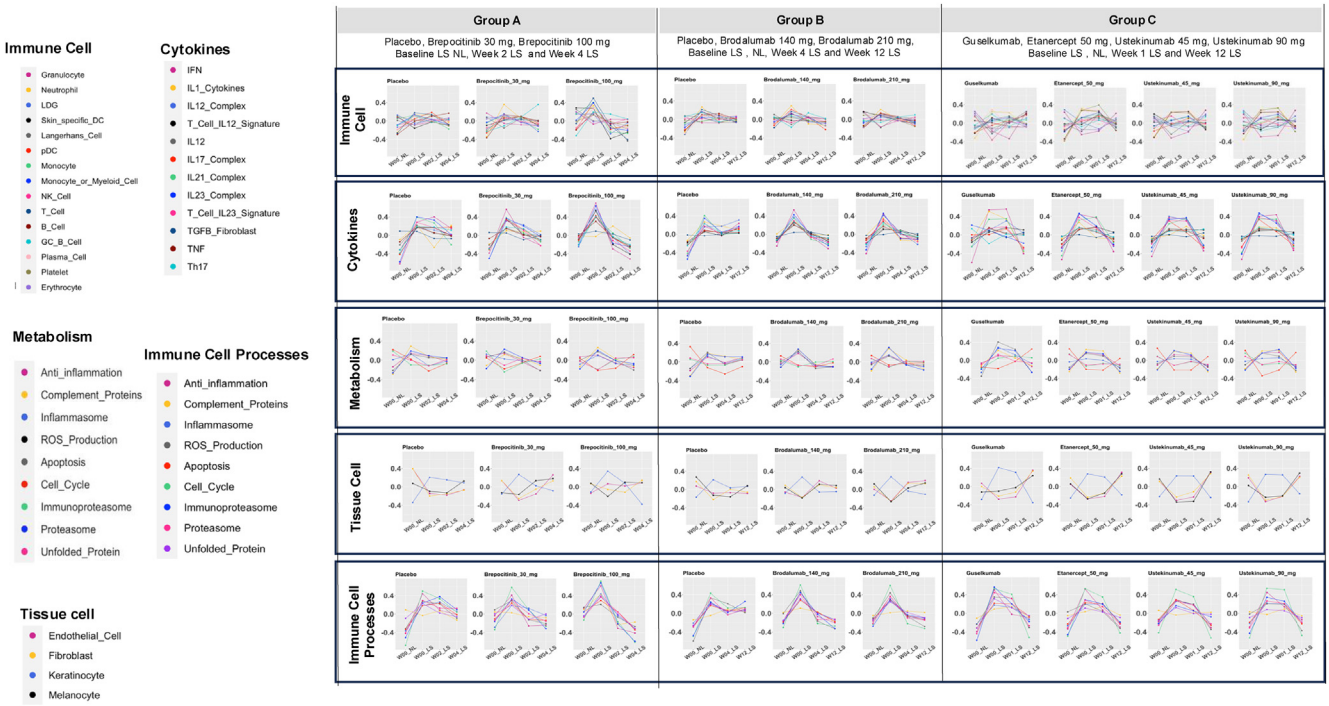


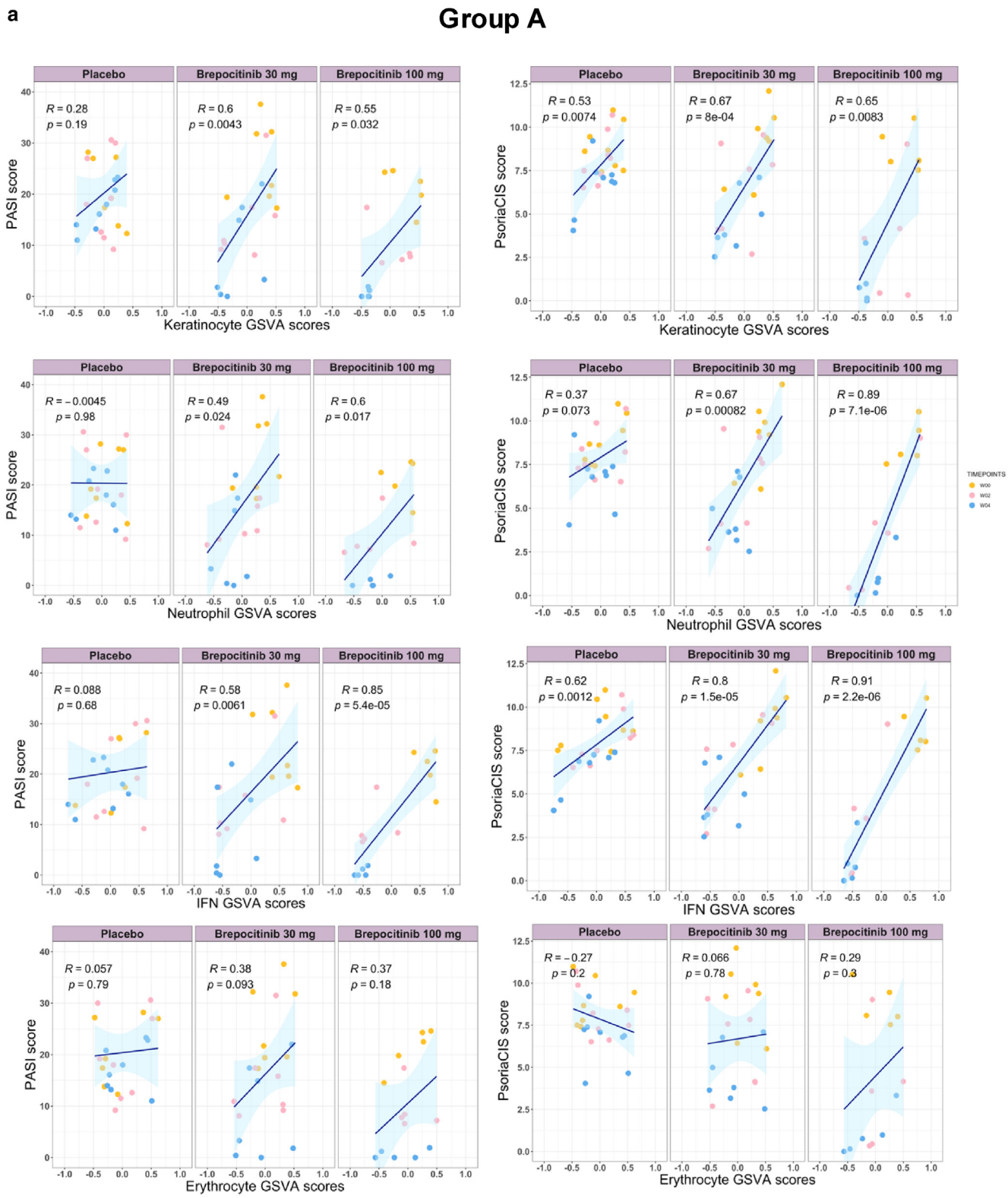
Supplementary Figure S2. Elbow and silhouette plots showing optimal number of clusters for K means. The elbow and silhouette plots for each therapeutic agent are organized as heatmaps of Figure 1 and Supplementary Figure S1. Elbow plot (top) displaying WSSs on y-axis and number of clusters K on x-axis and silhouette plot (bottom) displaying silhouette score on y-axis and number of clusters on x-axis. The optimal number of clusters (K = 2) aligns with the highest silhouette score for each therapeutic agent. WSS, within-cluster sum of square.

Supplementary Figure S3. Schematic overview and grouping of psoriasis datasets used in this study. Group A contains baseline LS and NL and week 2 LS and week 4 LS skin biopsies of patients with psoriasis treated with either placebo or brepocitinib (30 or 100 mg). Group B contains baseline LS and NL, week 4 LS, week 12 LS, and NL skin biopsies of patients with psoriasis treated with either placebo or brodalumab (140 or 210 mg). Group C contains baseline LS and NL, week 1 LS, and week 12 LS skin biopsies of patients with psoriasis treated with either guselkumab, etanercept (50 mg), or ustekinumab (45 or 90 mg). LS, lesional; NL, nonlesional.

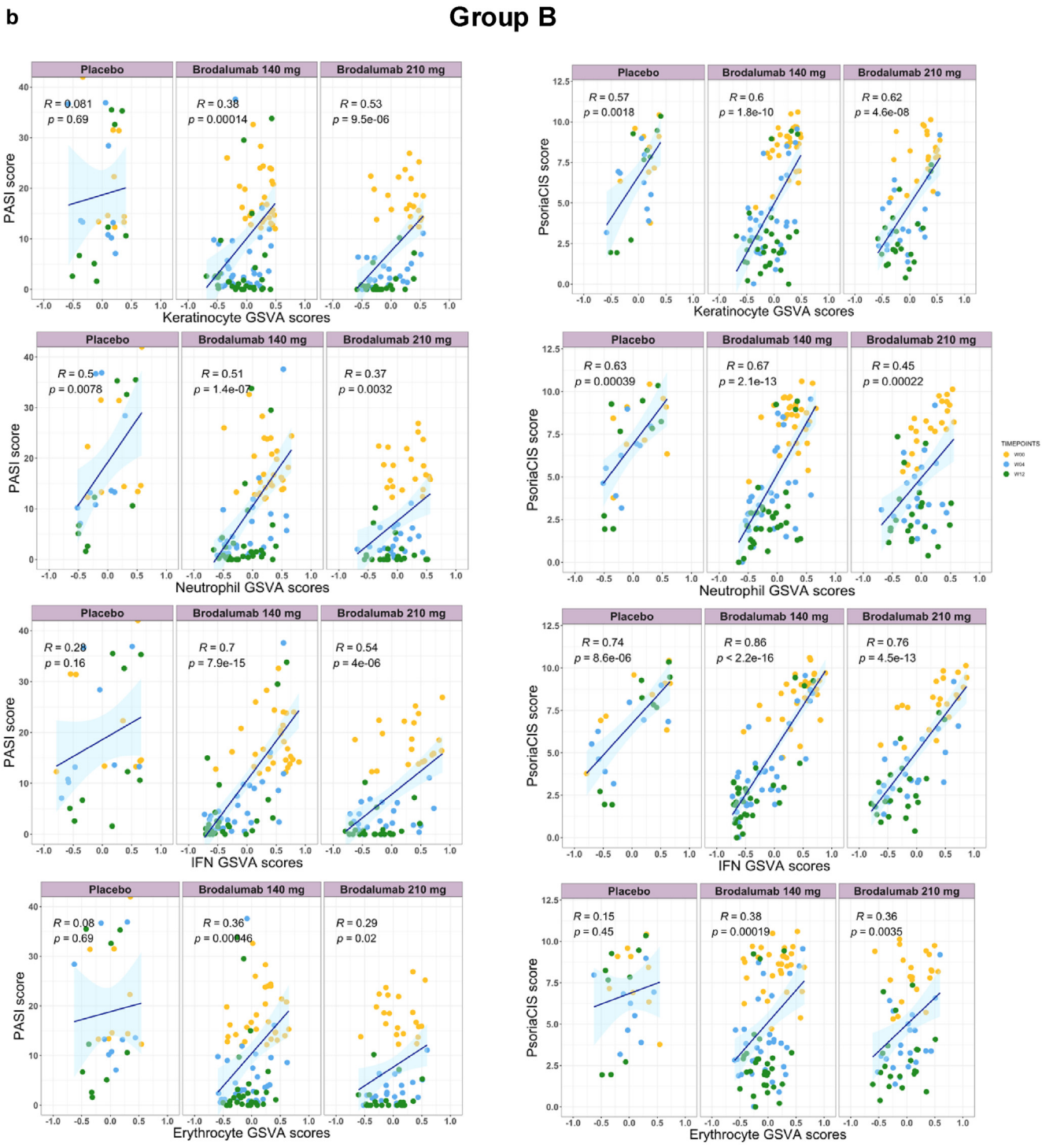




Supplementary Figure S4. Line graphs displaying the changes in mean GSVA scores over time. Line graphs of 48 gene signatures are visualized in 5 groups (immune cell, cytokines, metabolism, tissue cells, and immune cell processes). Each line graph has baseline NL, LS, and LS time point (either week 1 or week 2 or week 4 or week 12) based on group A, B, or C. GSVA, Gene Set Variation Analysis; LS, lesional; NL, nonlesional.



Supplementary Figure S5. Correlation between GSVA scores and PASI or PsoriaCIS. Scatter plots showing Pearson correlations between keratinocyte, neutrophil, IFN, and erythrocyte GSVA scores across multiple time points with PASI (left) or PsoriaCIS (right) for group A, B, and C patients. *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$. GSVA, Gene Set Variation Analysis; ns, not significant; PsoriaCIS, Psoriasis Cell and Immune Score.



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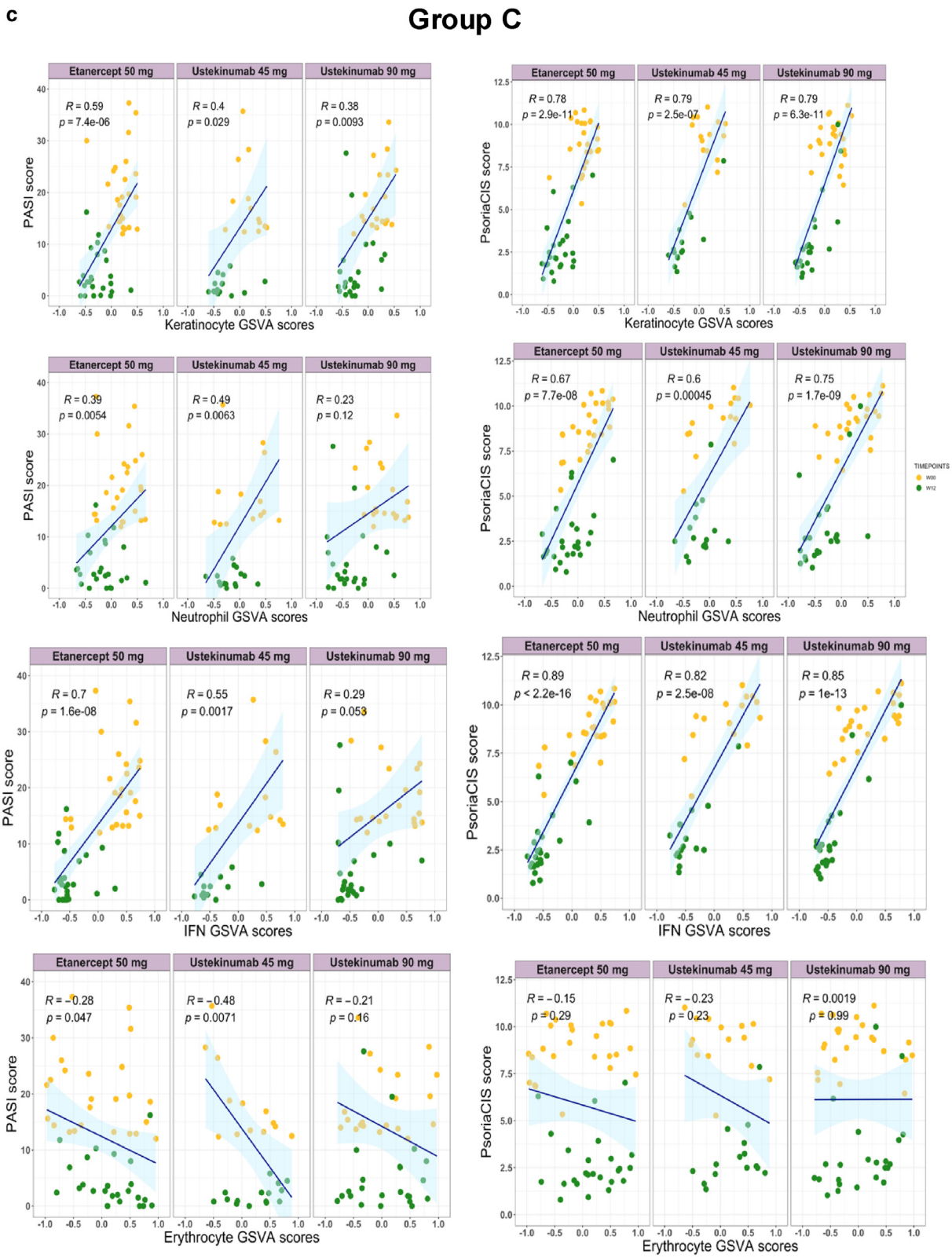
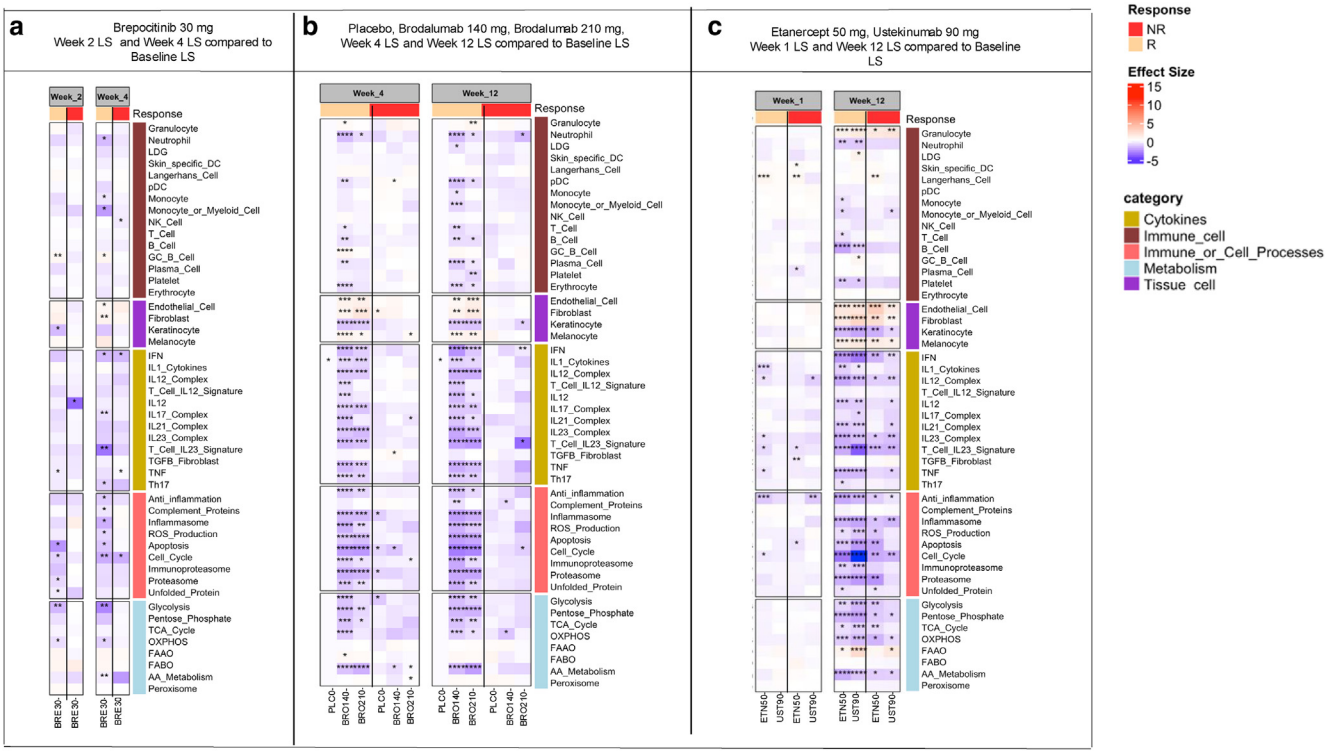
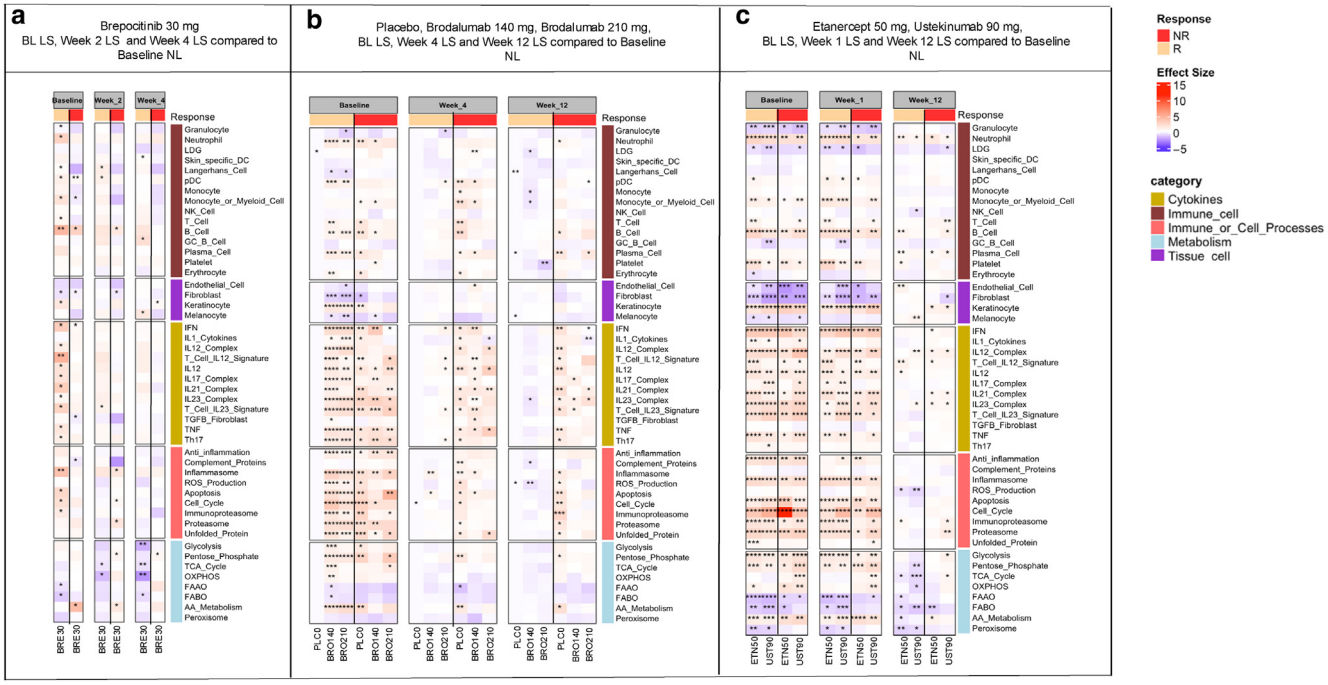


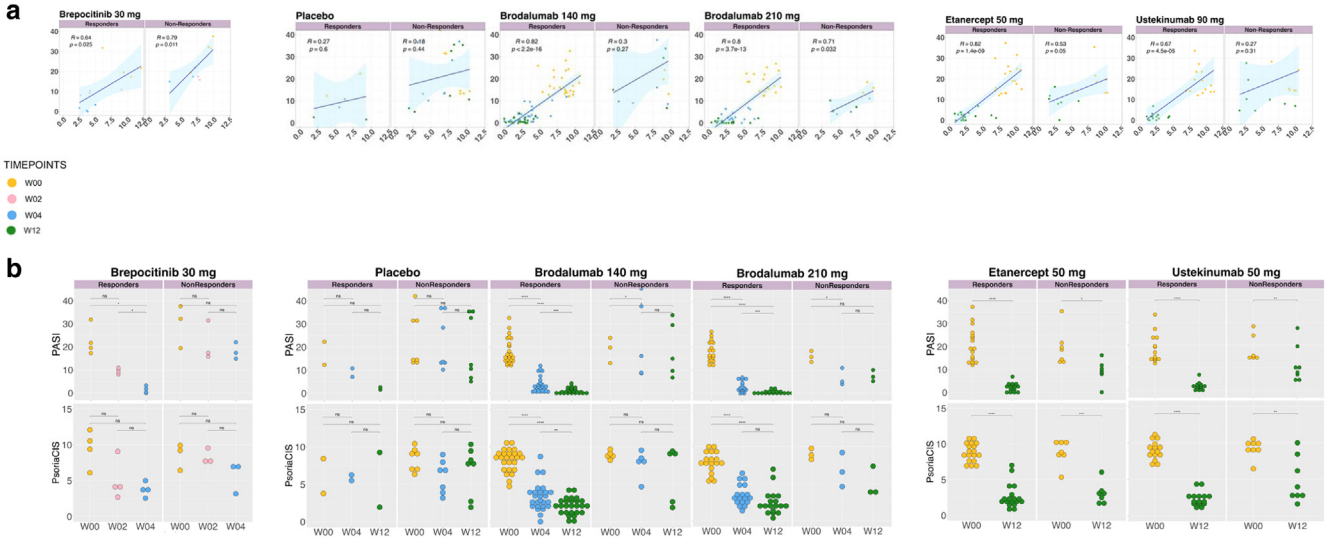
Supplementary Figure S5. Continued.



Supplementary Figure S6. Changes in gene expression profiles of psoriatic skin in response to various therapeutic agents among responders and nonresponders. GSVA using 48 informative gene modules was used to evaluate the molecular response of lesional psoriatic skin to various therapeutic agents over time. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) between baseline lesional (week 0) and the designated time point separately for responders and nonresponders. Red indicates an increase in effect size, and blue indicates a decrease. The asterisks indicate the *P*-value of the change in each module. Welch's *t*-test was used. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. (**a**) Brepocitinib (30 mg) at weeks 2 and 4. (**b**) Placebo and brodalumab (140 and 210 mg) at weeks 4 and 12. (**c**) Data for etanercept (50 mg) and ustekinumab (90 mg) after 1 and 12 weeks. GSVA, Gene Set Variation Analysis; NR, nonresponder; R, responder.



Supplementary Figure S7. Changes in psoriatic lesional skin compared with those in baseline nonlesional skin in response to various therapeutic agents among responders and nonresponders. GSVA using 48 informative gene modules was used to evaluate whether various therapeutic agents restored psoriatic lesional skin to that expressed by nonlesional skin over time. Heatmaps show the magnitude of change in GSVA score of each gene module (Hedges G effect size) between baseline nonlesional (week 0) and the designated time point separately for responders and nonresponders. Red indicates an increase in effect size, and blue indicates a decrease. The asterisks indicate the *P*-value of the change in each module. Welch's *t*-test was used. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. (**a**) Brepocitinib (30 mg) at week 0 and weeks 2 and 4. (**b**) Placebo and brodalumab (140 and 210 mg) at week 0 and weeks 4 and 12. (**c**) Data for etanercept (50 mg) and ustekinumab (90 mg) at week 0 and after 1 and 12 weeks. GSVA, Gene Set Variation Analysis; NR, nonresponder; R, responder.



Supplementary Figure S8. Comparison of PASI and PsoriaCIS in responders and nonresponders. (**a**) Dot plots of PASI (top) and PsoriaCIS (bottom) across various time points for groups A, B, and C patients with psoriasis. Repeated measures of ANOVA or paired *t*-tests were used to calculate significant differences between time points. **(b)** Scatter plots showing Pearson correlation between PASI and PsoriaCIS across multiple time points. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. ns, not significant; PsoriaCIS, Psoriasis Cell and Immune Score.