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Cytokine responses in non-lesional psoriatic skin as clinical predictor to anti-TNF agents

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Author contributions

J.E.G. initiated the study. J.E.G. and L.C.T. designed and directed the analysis. B.R., Y.H., J.J.V., J.M.K., and J.E.G. contributed to the sample collection and coordination. L.C.T., M.T.P., S.C., and Z.H. implemented the statistical and computational analysis. N.N.M. and E.M. provided support for the biological interpretation of the findings. S.S. conducted the immunohistochemistry and USP18 experiments. B.P.W. and S.G. collected the keratinocytes, and M.K.S. conducted the cytokine stimulations. A.C.B., X.X., R.U., C.Z., and J.F. provided the support for all the bench experiments including RNA-seq library preparation. S.W. contributed to the independent psoriasis RNA-seq dataset. L.C.T. and J.E.G. drafted the manuscript, and every author has reviewed the work.

Conflict of interest

The other authors report no conflicts of interests.

Abstract

Background—A major issue with the current management of psoriasis is our inability to predict treatment response.

Objective—Our aim was to evaluate the ability to use baseline molecular expression profiling to assess treatment outcome for psoriatic patients.

Methods—We conducted a longitudinal study of 46 patients with chronic plaque psoriasis treated with anti-TNF agent etanercept, and molecular profiles were assessed in over 200 RNA-seq samples.

Results—We demonstrated correlation between clinical response and molecular changes during the course of the treatment, particularly for genes responding to IL-17A/TNF in keratinocytes. Intriguingly, baseline gene expressions in non-lesional, but not lesional skin, were the best marker of treatment response at week 12. We identified *USP18*, a known regulator of IFN responses, as positively correlated with PASI improvement ($p=9.8\times10^{-4}$) and demonstrate its role in regulating IFN/TNF responses in keratinocytes. Consistently, cytokine gene signatures enriched in baseline non-lesional skin expression profiles had strong correlations with PASI improvement. Using this information, we developed a statistical model for predicting PASI 75 (i.e. 75% of PASI improvement) at week 12, achieving AUC=0.75 and up to 80% accurate PASI75 prediction among the top predicted responders.

Conclusion—Our results illustrate feasibility of assessing drug response in psoriasis utilizing non-lesional skin and implicate involvement of IFN regulators in anti-TNF responses.

Capsule summary

Enrichment of cytokine signatures in non-lesional psoriatic skin, prior to treatment, exhibits associations with future PASI improvement, and we illustrate the feasibility of integrating cytokine signals with transcriptomic data for drug response prediction in psoriasis.

Keywords

psoriasis; etanercept; cytokine response; PASI; drug response prediction

INTRODUCTION

Psoriasis is an immune-mediated condition that affects the skin and joints of >100 million individuals worldwide. Patients with psoriasis have elevated tumor necrosis factor (TNF) in both lesional skin and blood (1, 2), and different agents inhibiting tumor necrosis factor (adalimumab, infliximab, golimumab, etanercept, certolizumab pegol) have been developed (3–7), and approved to treat moderate-to-severe psoriasis, making it one of the most commonly used biologic classes to treat psoriasis, and also recommended by expert for pregnancy/pediatric populations as well as patients with other comorbidities including psoriatic arthritis, cardiac disorders, and Crohn's disease (8, 9).

Etanercept was amongst the first anti-TNF drugs approved to treat psoriasis. It is a fusion protein composed of two extracellular domains of the TNF receptor-2 fused to the human

IgG1 Fc region. Etanercept binds and neutralizes soluble TNF (10) and lymphotoxin (11). Studies have illustrated its efficacy in reducing TNF expression in both non-lesional and lesional psoriatic skin (12). Furthermore, TNF inhibition has been shown to associate with reduced Th17 responses (13). Prior studies have demonstrated decreased Th17 immune response among clinical responders to etanercept (14). A double-blind study with 672 patients demonstrated that etanercept increases the proportion of patients achieving PASI75 (≥75% improvement of psoriasis severity index from baseline) within 12 weeks of treatment (3); however, similar to other anti-TNF agents, patient outcomes for etanercept treatment vary, with PASI75 responses ranging from 23% to 60% depending on the study (3, 15–18). Furthermore, the mechanisms involved in the difference in patient responses remain unclear and are incompletely explained by psoriasis susceptibility genes (19, 20). Providing assessment of drug responses prior to treatment may enhance treatment efficiency, limit the risk of unnecessary drug exposures, and reduce the economic burden for patients and society.

Precision medicine aims to identify which patients will respond best to a specific therapeutic approach for a disease (21–25). High-throughput advancements in the field of genomics have facilitated biomarker research and discovery endeavors, making precision medicine a more feasible approach to patient care. However, applications to complex skin conditions including psoriasis are still limited, despite available and extensive transcriptomic studies (20, 26–31). Here, we demonstrate that by applying statistical modeling using RNA-seq along with *in vitro* cytokine response genomic data to a cohort of etanercept-treated psoriatic patients, we can provide effective risk assessment for drug response. We demonstrate gene expression in healthy appearing non-lesional psoriatic skin at baseline is most predictive of therapeutic responses. Specifically, by using an integrative approach, we illustrate the enrichment of IFN and TNF signatures, and show how this prior information can be used in an analytical framework for predicting drug response.

METHODS

Patient cohort

Patients with moderate-to-severe chronic plaque psoriasis (sPGA ≥ 2) for longer than 6 months were enrolled for an open-label 50mg biweekly etanercept treatment for three months. Study protocols were approved by the University of Michigan IRB, and were carried out in accordance with Good Clinical Practice requirements and the Declaration of Helsinki. Informed consent was acquired from all participants. Patients were evaluated at baseline, and biopsy obtained under local anesthesia (lidocaine 1:10,000 epinephrine) from both non-lesional and lesional skin at baseline, and from lesional skin only at weeks 2, 6 and 12. Patients had full clinical assessment at 2, 6 and 12 weeks by a dermatologist and body-surface area, physician-global assessment and Psoriasis Area and Severity Index were recorded. The study was registered on clinicaltrials.gov (NCT01971346).

RNA-seq processing—We generated 50bp single-ended reads from 210 RNA-seq samples from 46 patients. For each sequence file, we conducted adapter trimming (32), and aligned reads to human genome hg19 (33). Only uniquely mapped reads were used for

expression level quantification (34). We removed 1 patient due to drop out, and 2 patients that did not have baseline (week 0) biopsy samples, leaving 43 patients and 206 RNA-seq samples for the subsequent analysis. One patient's last visit was conducted on week 15, and we grouped the results to the week 12 data for all other patients when conducting the analysis. We were able to detect 28,182 genes with on average ≥1 read/sample, and we applied DEseq2 for read normalization (35). Principal component analysis was conducted using all genes after applying inverse normalization for the DESeq2 normalized data.

Associating RNA-seq expression with clinical response—For each gene expression profile from baseline non-lesional or lesional skin samples, we correlated against change in PASI, BSA, and sPGA in each of the three follow-up visits. We adjusted the age, sex, and baseline BMI, and evaluated both percent (%) as well as absolute (i.e. delta) disease improvement referencing the week 0 values. False Discovery Rate (FDR) ≤10% was declared significant for association. Differential expression analyzes (i.e. comparing non-lesional vs lesional skin at baseline; comparing lesional skin at baseline versus subsequent visit) were conducted using DESeq2 negative binomial distribution, and FDR≤5% and $|\log_2\text{Fold Change}| \geq 1$ were used as criteria to declare significant genes.

Comparison against cytokine signatures in keratinocytes—The procedures have been described previously (36). Briefly, we obtained 50 normal human keratinocytes from 50 different healthy adults. Keratinocytes were grown in 12 well plate in 154 CF medium (Thermo Fisher #M154CF500) with human keratinocyte growth supplement (Thermo Fisher #S0015). Keratinocytes were grown to confluency at which time the complete medium (with supplements) was replaced by basal 154 CF medium (without supplements). Cells were stimulated with cytokines (IL-4, IL-13, IFN-α, IFN-γ, TNF-α, IL-17A, R&D Systems) individually at 10 ng/ml concentration. After 8hrs cells were harvested and RNA was isolated using RNeasy Plus Mini kit (Qiagen # 74136). RNA was analyzed by RNA Nano Chips (Agilent Technologies) and sequenced (37). We extracted the top 1,000 genes with their baseline expression profiles showing the strongest correlations with future absolute PASI improvement in each of the three follow-up visits, and used hypergeometric test to compare against cytokine signatures to understand their molecular basis.

Predicting drug response—We assigned each patient from our cohort a TNF or IFN score using their baseline expression profiles of non-lesional skin: for each gene i induced by TNF/IFN in keratinocytes, we first computed the relative expression of that gene in patient p by referencing the median value across samples: $r^{pi}=g^{pi}/median(g^i)$, where g is the expression value after DESeq2 normalization; the TNF or IFN score for patient p was then defined as the upper quartile of the r^p value across all genes induced by TNF or IFN, respectively(36, 38). To model the week 12 PASI response, we utilized the baseline non-lesional skin expression profiles for the genes induced by TNF/Type I IFN in keratinocytes. We used principal components to reduce the data dimension and applied logistic regression to model the drug responses at week 12 using the PASI75 criteria, using leave-one-out to ensure the model robustness (i.e. principal component analysis and regression modeling performed only on the training data). To assess the potential clinical implication, we measured the precision (proportion of true positives among the predicted

PASI75) and recall (proportion of actual PASI 75 predicted) as functions of the proportion of top samples predicted from our model.

Immunohistochemistry

Formalin fixed, paraffin-embedded tissue slides obtained from patients with psoriasis and normal controls were heated for 30 min at 60°C, rehydrated, and epitope retrieved with Tris-EDTA, pH6. Slides were blocked, incubated with primary antibody UPS18 (1:100, LS-B1182–50, Lifespan Bioscience) overnight at 4°C. Slides were then washed with PBS and incubated with biotinylated secondary antibody (biotinylated goat anti-rabbit IgG Antibody, Vector Laboratories BA1000) for 30 min in room temperature, and then incubated with fluorochrome-conjugated streptavidin for 10 min in room temperature. Slides were prepared in mounting medium with 4',6-diamidino-2-phenylindole (DAPI) (VECTASHIELD Antifade Mounting Medium with DAPI, H-1200, VECTOR). Images were acquired using Zeiss Axioskop 2 microscope and analyzed by SPOT software 5.1. Images presented are representative of at least three experiments.

RNAi -depletion, generation of USP18 overexpressing N/TERTs, RNA extraction, qRT-PCR

Upon reaching semi-confluence, the culture medium of keratinocytes was changed by Accell Delivery Media (B-005000, Dharmacon) with 1 μM Accell siRNA targeting *USP18* (E-004236–00-0005, Dharmacon). After 48h, cells were stimulated with IFN-α (10ng/ml, R&D Systems; I4276), TNF (10ng/ml, R&D Systems: 210-TA), IL-17A (20ng/ml, R&D Systems; 7955-IL), IFN-γ (10ng/ml, R&D Systems: 285-IF) for another 24h. RNA was isolated from cells using Qiagen RNeasy plus kit (74136). QRT-PCR was performed on a 7900HT Fast Real-time PCR system (Applied Biosystems) with TaqMan Universal PCR Master Mix (ThermoFisher 4304437). N/TERT keratinocytes stably overexpressing USP18 were generated using 4D-Nucleofector X Unit (Lonza Cologne, Germany). Cells were prepared using standard protocol for Normal Human Epidermal Keratinocyte X Unit kit (4D Nucleofector Solution, supplement and 100μL single nucleocuvette) obtained from Lonza. For each electroporation, 5μg pCMV6-AC-GFP USP18 plasmid (Origene, Rockville, Maryland, USA) was used. Unit X program used was DS-138 for stable keratinocytes. Following transfection, keratinocytes were grown in a 12-well plate using fully supplemented Keratinocyte-SFM medium, penicillin streptomycin and 500μg/mL G418 (Geneticin by Thermo Fisher Scientific (Waltham, Massachusetts, USA)) for selection followed by expansion for approximately 30 days. USP18-GFP overexpression was validated using qRT-PCR and western blotting. Primers (ThermoFisher Scientific) used in this study were: USP18, Hs00276441_m1; IL36G, Hs00219742_m1; DEFB4, Hs00175474_m1; MX1, Hs00895608_m1; OASL, Hs00984387_m1; IRF7, Hs01014809_g1; IFNK, Hs00737883_m1.

RESULTS

RNA-seq to profile transcriptomic changes in patients initiated on etanercept therapy

We enrolled 46 psoriatic patients for this study (Figure 1a). Each patient was treated with etanercept, 50mg twice weekly. Prior to initiation (baseline/week 0), demographic (age, sex) and other clinical information including psoriasis area and severity index

(PASI), body surface area (BSA), and static physician's global assessment (sPGA) were collected. Biopsies were performed at baseline on non-lesional and lesional psoriatic skin for transcriptome profiling using RNA-seq, and additional lesional samples were obtained at time of follow-up on week 2, week 6, and week 12, along with clinical assessment. Follow-up data on 42 patients was obtained (i.e. with transcriptomic data from both baseline and at least one of the follow-up visits), and a total of 36 patients completed the study with RNA-seq and clinical data available at both baseline and week 12 visit. A total of 210 RNA-seq experiments were performed.

By using principal component analysis (PCA), we tracked transcriptomic changes over the treatment course (Figure 1b). As expected, PCA separated non-lesional and lesional skin at baseline, but during the treatment period, the largest principal component (i.e. PC1) of lesional skin changed over time such that by week 12 more lesional skin samples overlapped with that of non-lesional skin at baseline, while the PC1 of some lesional skin samples still remained at baseline level, despite improvement in PASI by week 12. Notably, the former group tended to have a lower PASI score than the latter group at week 12 (lower panel in Figure 1b). We further investigated this at the gene expression level and demonstrated how the expression profiles changed during the 12-week treatment (Figure 1c). Similar to the PCA results, there was a sharp contrast between non-lesional and lesional psoriatic skin at baseline, but the contrast became less pronounced as treatment proceeded. Indeed, when comparing gene expression in baseline lesional skin with that from follow-up visits, we observed a gradual increase in the number of differentially expressed transcripts (Supplementary Table 1), and restoration towards gene expression levels in non-lesional skin at baseline (Supplementary Figure 1). Nevertheless, heterogeneity was observed among patients' transcriptomic responses at week 12, concordant with the clinical variations (3, 14).

Expression profiles at baseline non-lesional psoriatic skin are associated with PASI improvement—To examine the associations between baseline expression profiles and clinical presentation, expression level (inverse normalized to ensure robustness) from non-lesional or lesional skin of each gene at week 0 was correlated with the change in PASI, BSA, and sPGA in each of the three follow-up visits. We evaluated both percent (%) as well as absolute (i.e. delta) disease improvement referencing the week 0 values. Surprisingly, we could only identify significant associations for gene profiles in baseline non-lesional skin, rather than lesional skin. When using percent change as measure there were 198 genes with their week 0 expression profiles significantly (False Discovery Rate, FDR≤10%) associated with week 12 PASI improvement; when using absolute change as measure, there were 192 and 391 genes with their baseline expression profiles significantly associated with week 6 and week 12 PASI improvement, respectively. We were not able to reveal any significant results for associations against BSA and sPGA change. Among the significant genes with their baseline expressions associating with PASI improvement at week 12, 105 overlapped between the percent and absolute measures. *USP18*, an ubiquitin specific peptidase, and *KRT2*, a type I cytokeratin, were two prominent examples with expression in non-lesional skin at baseline showing significant association with PASI improvement in follow-up visit (Figure 2): *USP18* was significantly up-regulated in lesional skin at baseline (fold change, FC=2.5; $p=1\times10^{-26}$), and its expression in non-lesional skin at baseline was positively

correlated with absolute PASI improvement at week 12 ($p=9.8\times10^{-4}$; 20% increase from mean expression level had on average 2.3 PASI improvement after adjusting for age, sex, and BMI); *KRT2* was significantly down-regulated in lesional skin at baseline (FC=0.32; $p=1.3\times10^{-6}$) and its expression in non-lesional skin at week 0 was inversely correlated with both the absolute ($p=1.4\times10^{-5}$; 20% decrease from mean expression level has on average 0.99 PASI improvement after adjusting for age, sex, and BMI) and percent ($p=5.4\times10^{-4}$) PASI improvement at week 12. Although their associations with PASI improvement at week 2 and week 6 were not significant, the direction of correlations was consistent (Figure 2a, d); interestingly, these associations were not observed when using baseline expression levels in lesional skin (Figure 2b, e). Furthermore, the expressions of these two genes in lesional skin gradually "normalized" towards the expression levels in non-lesional skin with treatment (Figure 2c, f). These results indicate that both *USP18* and *KRT2* are dysregulated in psoriatic skin but can be "restored" to non-lesional skin levels by etanercept treatment, and their expressions in non-lesional skin prior to treatment correlate with PASI improvement.

To understand the role of *USP18* in TNF responses, we used an independent transcriptomic cohort for psoriasis (36) to validate the up-regulation of *USP18* in lesional skin (Supplementary Figure 2); interestingly, we also observed that its expression was marginally up-regulated in non-lesional skin when compared with healthy control skin (FC=1.5; $p=1.2\times10^{-2}$). *USP18* was also positively correlated with expression of other psoriasis cytokines (*IL23A* and *IL36G*) in lesional skin ($p=6.3\times10^{-5}$ and $p=8.3\times10^{-7}$, respectively). Immunostaining (Figure 3a) confirmed expression of USP18 in the epidermal layer in both non-lesional and lesional psoriatic skin. Knockdown of *USP18* using siRNA (Supplementary Figure 3) was done in keratinocytes to assess the impact on TNF/IFN responses. Effective depletion of *USP18* affected both TNF and IFN response (Figure 3b, c), for instance, *IL36G* mRNA expression was suppressed upon TNF stimulation but *DEFB4* was induced upon IFN-γ stimulation). Similarly, *USP18* knock-down promoted higher expression of IFN-responding genes, including *MX1*, *IFNK*, *OASL*, and *IRF7*. Conversely, *USP18* overexpression decreased type I and type II IFN responses (Figure 3d) but enhanced IL-17A induced effect on *IL36G* and TNF-induced effect on *DEFB4* (Figure 3e). These results suggest that *USP18* shifts the balance between IFN and TNF responses in keratinocytes. As these two signals have been thought to have counter-regulatory roles in psoriasis (39), lower *USP18* might promote higher IFN response and thus lower TNF dependence, and vice versa, agreeing with the positive correlation with PASI improvement we observed during the course of etanercept treatment.

Integrative approach to provide biological and clinical implications using transcriptomic data from non-lesional psoriatic skin—To provide biological and clinical implications for etanercept treatment in psoriasis, we utilized cytokine signatures in keratinocytes obtained from independent RNA-seq transcriptomic data (36, 38). Among the genes showing the strongest differential expression in lesional skin between baseline and subsequent follow-up visits, we observed significant (FDR≤5%) enrichment of IFN, TNF, and IL-17 response genes (Figure 4a), concordant with the drug's TNF inhibitory effect and its associated negative regulation on Th17 response (14, 15). Next, we extracted the top 1,000 genes with their baseline expression profiles showing the strongest correlations

with absolute PASI improvement in each of the three follow-up visits, and compared them against type I IFN, TNF, and IL-17A keratinocyte cytokine signatures to understand their molecular basis. Strikingly, we found significant enrichment for TNF and Type I IFN signatures for week 0 non-lesional skin gene expression profiles which have the strongest correlations with week 6 and week 12 absolute PASI improvement (Figure 4b). Notably, there was no significant enrichment when using week 0 gene expression profiles in lesional skin (Figure 4c). When using percentage PASI improvement as a measure, we observed the same Type I IFN signature enrichment (week 6 for IFN-α and week 12 for IFN-γ), and we also revealed enrichment for IL-17A responses at week 12. It is notable that both *USP18* (Supplementary Figures 3 and 4) and *KRT2* were differentially expressed upon TNF stimulation in keratinocytes, and *USP18* was also upregulated by IFNs.

We assigned each patient from our cohort a TNF or IFN score (see Methods), summarizing the respective cytokine signature loading for the non-lesional skin of that patient at baseline (Figure 5a, b). Among the patients showing higher IFN/TNF scores, we observed a larger proportion for PASI improvement, in terms of both absolute or percentage measures. By using keratinocyte cytokine response signatures as prior information, we computed, for each patient, the principal components of the baseline non-lesional skin expression levels for >2,900 genes that were induced in TNF, IFN-α, or IFN-γ stimulations. We applied logistic regression to these components to model drug responses at week 12 using the PASI 75 criteria, using the leave-one-out cross validation to ensure robustness upon model evaluation, and were able to obtain up to 0.75 in area under the receiver operating characteristic (AUC). To further assess the potential clinical implications, we measured the precision (proportion of true positives among the predicted PASI75) and recall (proportion of actual PASI75 predicted) among the top samples predicted from our model (Figure 5c). For the top 20% of samples exhibiting the top PASI 75 prediction, we achieved up to 80% accuracy, representing the ability to use baseline non-lesional psoriatic skin transcriptome to identify patients that benefit most from etanercept treatment.

DISCUSSION

The use of targeted biologic drugs has revolutionized the treatment of psoriasis. The anti-TNF class includes different agents and has been in use for psoriasis for close to two decades (3). However, biologics have different effectiveness (40), and clinical responses may vary widely across patients (41), despite otherwise highly similar or identical disease. The reasons for this variability have remained unclear until now, and are incompletely explained by the contribution of genetic susceptibility variants (42, 43). Together with the fact that biologic treatments pose a huge economic burden on both patients and society (44, 45), information regarding how each patient's characteristics associate with treatment outcomes is urgently needed.

Previous studies using gene expression microarray data have demonstrated that response to the anti-TNF agent etanercept is accompanied by suppression of Th17 and TNF signaling pathways (14, 46). However, as IL-17 responses are prominent in practically all patients with psoriasis (47), this observation has not led to ways to predict treatment responses. In this study, we used RNA-seq to profile the skin prior to and during the course of etanercept

treatment of psoriasis. Our results unexpectedly demonstrate that baseline expression data from non-lesional skin, rather than lesional psoriatic skin, is a better predictor of clinical response to etanercept.

It has been established in multiple studies that non-lesional skin from psoriatic patients is different than healthy control skin. Noted differences include an increased level of epidermal proliferation (48); lower levels of the epidermal barrier proteins, e.g. filaggrin and loricrin (49), changes in innate immune response and lipid metabolism genes, e.g. *S100A7* and *ELOVL3* (28), and an abnormal epidermal barrier recovery (50). The reason for these changes in non-lesional psoriatic skin is unclear but speculated to reflect a systemic response from increased levels of circulating pro-inflammatory mediators (51), genetic predisposition (52, 53), or a combination of both. This suggests that while expression profiles from non-lesional skin may provide predictive power in PASI improvement, expression profiles of lesional skin in turn provide richer information for understanding pathogenic mechanisms of psoriasis in general, as has been demonstrated in multiple studies (26, 27). Thus, in the chronic inflammatory state of lesional psoriatic skin, where multiple different cytokines act locally, constituting a heterogeneous environment to study context-specific inflammatory response (54), non-lesional skin likely represents a state with refined resolution to decipher subtle variations of individual and likely systemic pro-inflammatory state, thereby enhancing predictions of therapeutic response.

We were able to associate non-lesional expression profiles at baseline with both percent and absolute PASI improvement and using PASI75 to categorize responders versus non-responders. While percent improvement in PASI is a more conventional metric in psoriasis studies, in some scenario it does not distinguish well between patients with different disease severity at baseline. For example, patient with a drop from 40 to 10 in PASI score has the same PASI 75 response as someone dropping from 10 down to 2.5. As the inflammatory load would be expected to be much higher in the first compared to the second patient, using an absolute change in PASI (delta PASI of 30 in the first patient vs. 7.5 in the second) can potentially provide a greater likelihood of detecting a systemic inflammatory signal in non-lesional skin. To determine if this approach leads to a bias towards disease severity, we correlated the PASI75 with baseline PASI score, and did not identify significant changes, suggesting that our observation was not dependent upon disease severity.

Identification of *USP18* as a predictor of therapeutic response to etanercept is consistent with the cross-regulation that is known to occur between type I IFNs and TNF (55). Notably, TNF regulates type I IFN production by blocking development of plasmacytoid dendritic cells (pDCs), as well as inhibiting its secretion; in contrast, blocking TNF sustains type I IFN release from pDCs (55). An example of this is so called “paradoxical psoriasis” that arises in the setting of anti-TNF treatment. It is a relatively uncommon reaction occurring in approximately 2–5% of patients with psoriasis and is characterized by accumulation of pDCs and overexpression of type I IFNs (39). Our data suggests another source of cross-regulation between these two cytokines at the level of the keratinocyte, involving the ubiquitin specific peptidase; USP18. Consistent with our findings, *USP18* has been implicated as a negative regulator of IFN signaling (56), and a promoter of TNF responses (57).

Given the cross-regulation of TNF and IFN responses outlined above it is worth noting that we observe both of these pro-inflammatory signals in non-lesional skin (Figure 4). However, these are averaged across our cohort of >36 patients and therefore do not fully reflect the balance on an individual level at baseline between these two signals. Furthermore, while we see suppression of IL-17 responses with effective anti-TNF treatment (13), it is noteworthy that the strength of the IL-17 responses at baseline in lesional or non-lesional skin was not as predictive of etanercept treatment response. IL-17 is a critical component of the inflammatory network in psoriasis, as outlined by both genetic and clinical studies (58, 59). Notably, IL-17 responses synergize with TNF (60), but, interestingly, it can also synergize with IFNs, particularly IFN-γ (61, 62), suggesting that both of these inflammatory circuits can work with IL-17 to maintain disease activity. It is likely that predictors for treatment responses to anti-IL-17 and anti-IL23 agents would be different than that for etanercept. Anti-TNF treatment is prescribed to ~20% of psoriatic patients, particularly those that have concomitant psoriatic arthritis, and in a recent study on prescription patterns associated with biologic therapies for psoriasis from a U.S Medical Records database (63), etanercept was the second most prescribed biologic for psoriasis (second only to adalimumab). As etanercept has been shown to have decreased risk of infection compared to other anti TNF agents (64), the benefit from being able to predict response to a lesser effective biologic, but one with decreased risk of broader immunosuppression and infectious complications, is clear. Future studies are needed evaluate if the biological predictors identified in this study can be used to provide drug response assessment for other anti-TNF agents.

In summary, our data illustrates that transcriptomic data can provide distinguishing power for individuals carrying extreme IFN/TNF burden in non-lesional psoriatic skin. Our findings are important as they show the feasibility of using transcriptomic approaches for drug prediction in psoriasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AUC	area under the receiver operating characteristic
BSA	body surface area

FDR	false discovery rate
PASI	psoriasis area and severity index
PCA	principal component analysis
sPGA	static physician's global assessment

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Key messages

- We illustrated *USP18* association between baseline non-lesional psoriatic skin expression with PASI improvement, and show *USP18* has inhibitory effect on IFN response, while facilitating TNF responses.
- By using cytokine signatures from keratinocytes, we assessed drug response, obtaining up to 0.75 in area under the receiver operating characteristic (AUC).

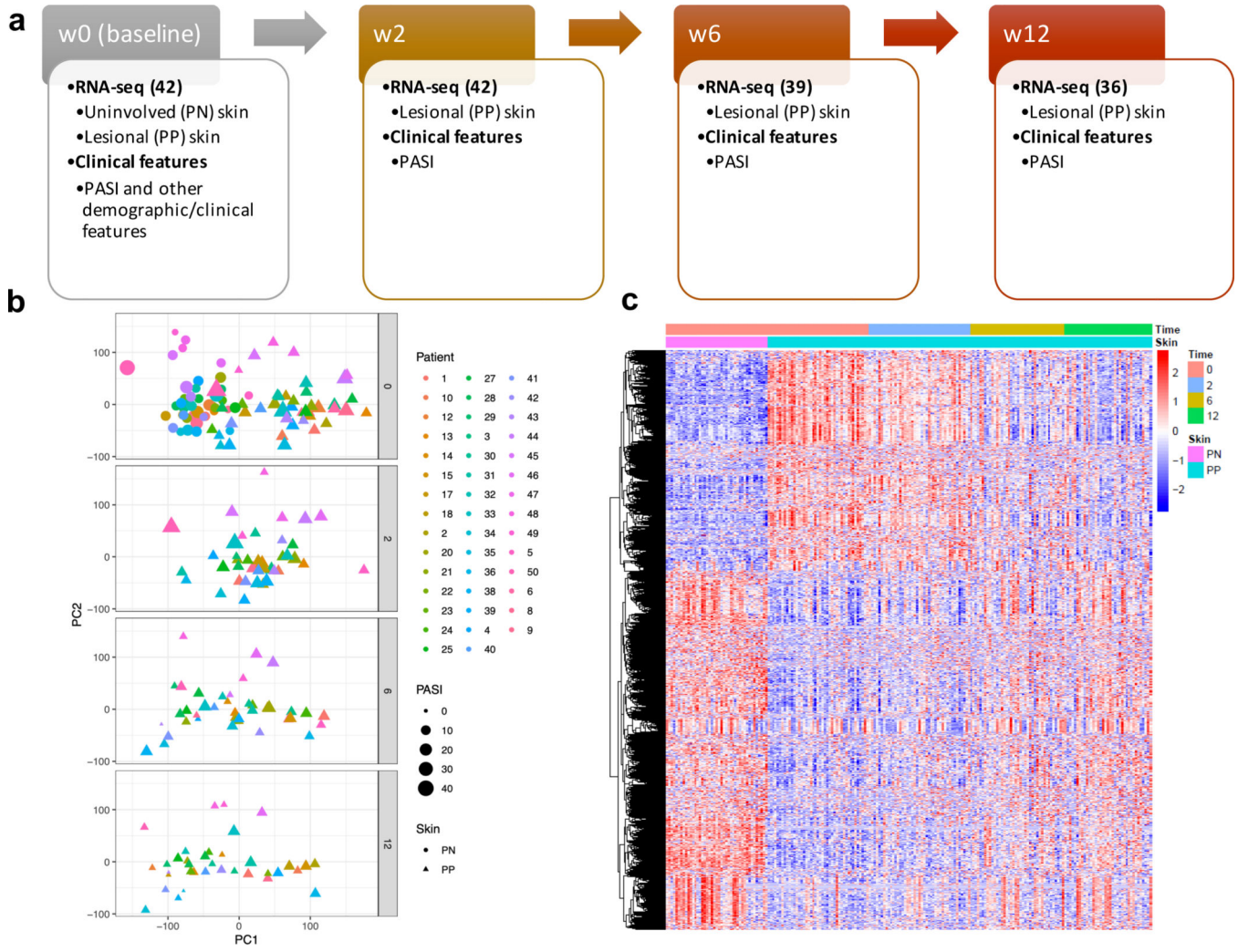

Figure 1. Transcriptome of the longitudinal cohort.

a) Design of the study; b) the top two principal components computed using transcriptomic data for all the RNA-seq samples; c) heatmap illustrating the change in expression profiles across the treatment time course. Note 46 patients were recruited in our cohort, and 42 patients have RNA-seq data from both baseline and at least one of the follow-up visits. The union set of genes that are differentially expressed between baseline lesional skin versus follow-up lesional skin samples was used to construct this heatmap.

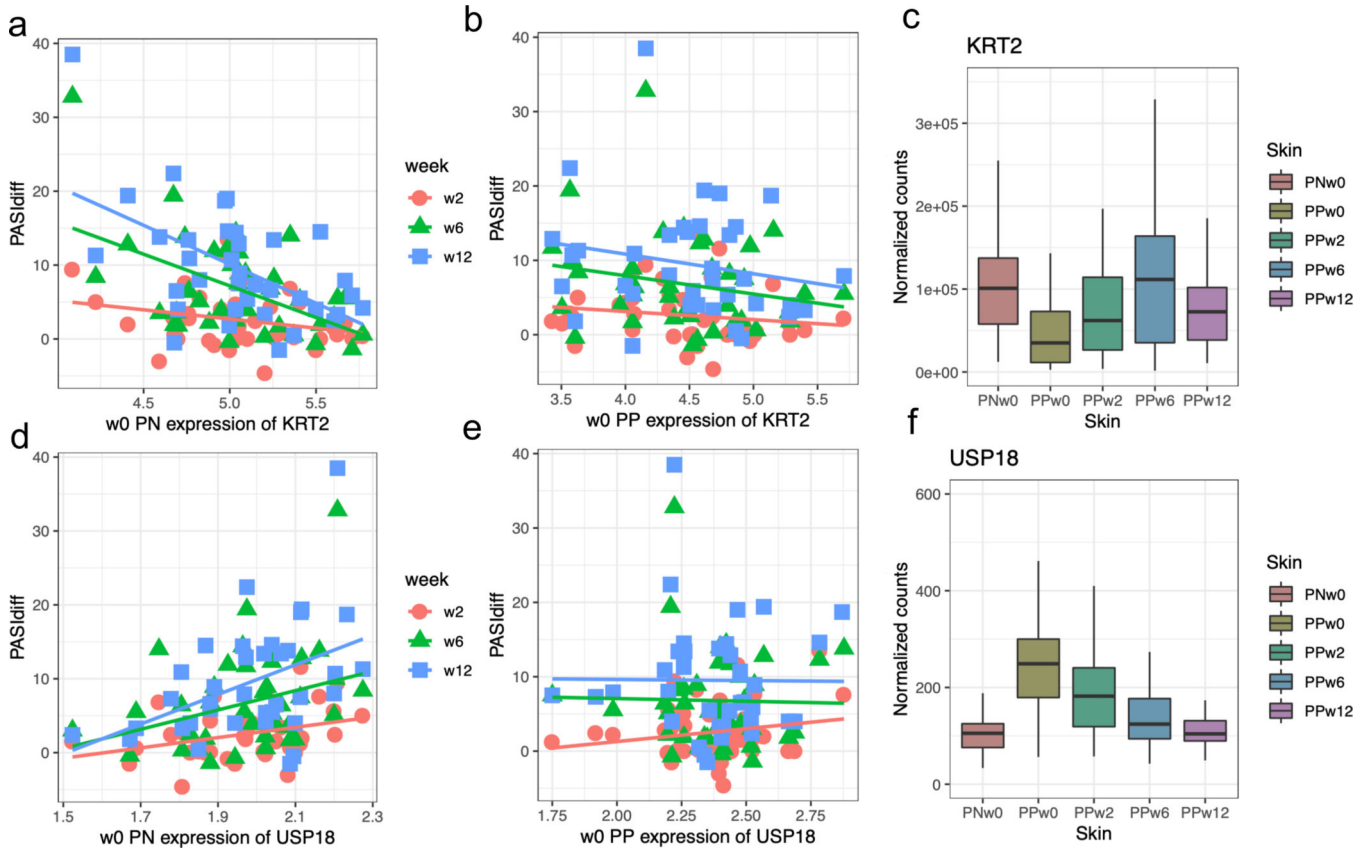

Figure 2. The associations between PASI improvement and baseline expression.

The PASI improvement (y-axis) was plotted against the baseline expressions of *KRT2* (a, b) and *USP18* (d, e) in non-lesional and lesional skin; the boxplots for normalized expression levels of *KRT2* (c) and *USP18* (f) in different skin types and time points during the treatment course are shown in c and f.

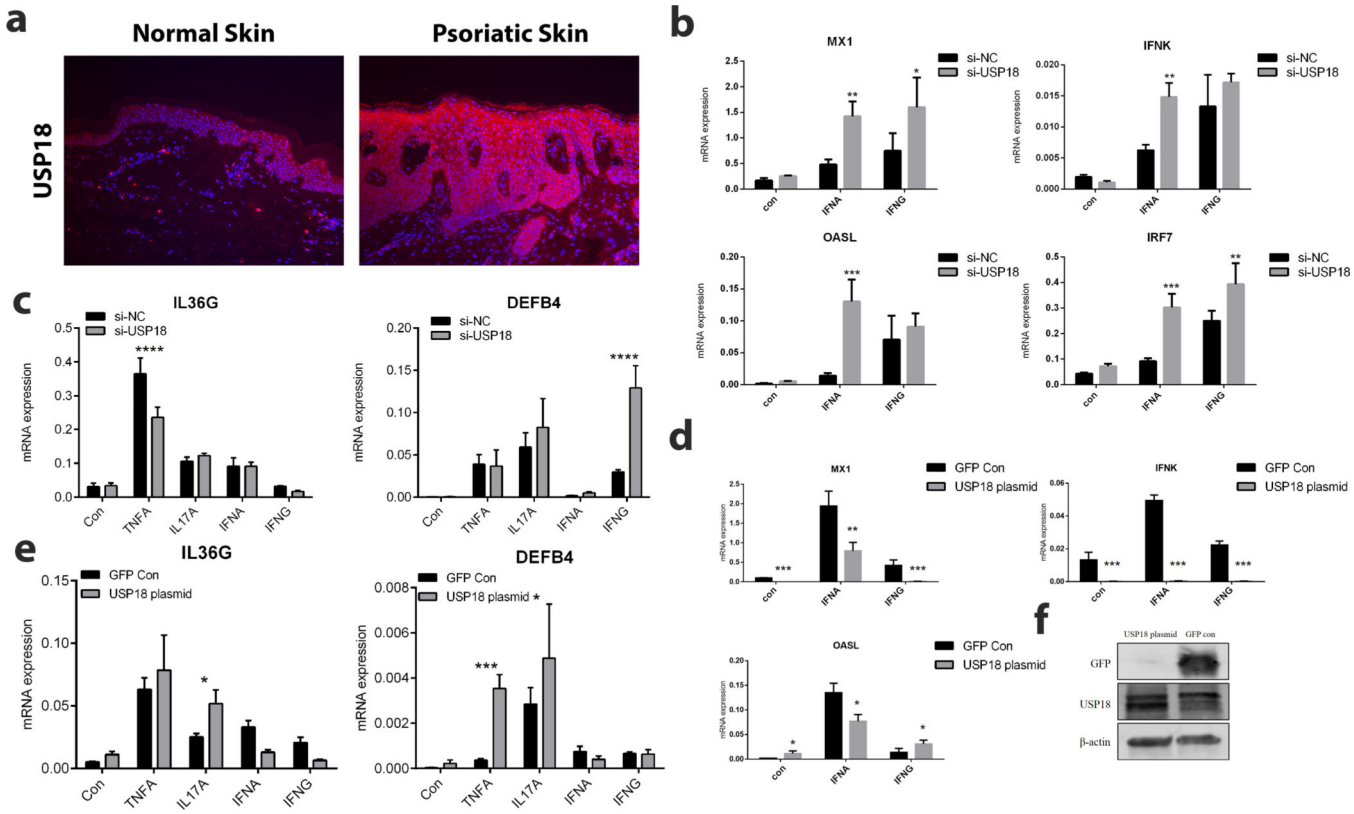

Figure 3. USP18 as a modulator for IFN/TNF response.

a) Immunostaining for USP18 in normal and psoriatic skin; b) effect of depleting *USP18* on type I and type II stimulations (x-axis); c) effect of depleting *USP18* on expression of *IL36G* and *DEFB4* upon TNF, IL-17A, IFN-α and IFN-γ stimulations (x-axis); d) effect of *USP18* overexpression on type I and type II IFN responses; e) effect of *USP18* overexpression on expression of *IL36G* and *DEFB4* upon TNF, IL-17A, IFN-α and IFN-γ stimulations; f) Western blot confirming increased USP18 protein levels in USP18 plasmid transfected cells. Data shown with STDV and are representative of 3 biologic replicates. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

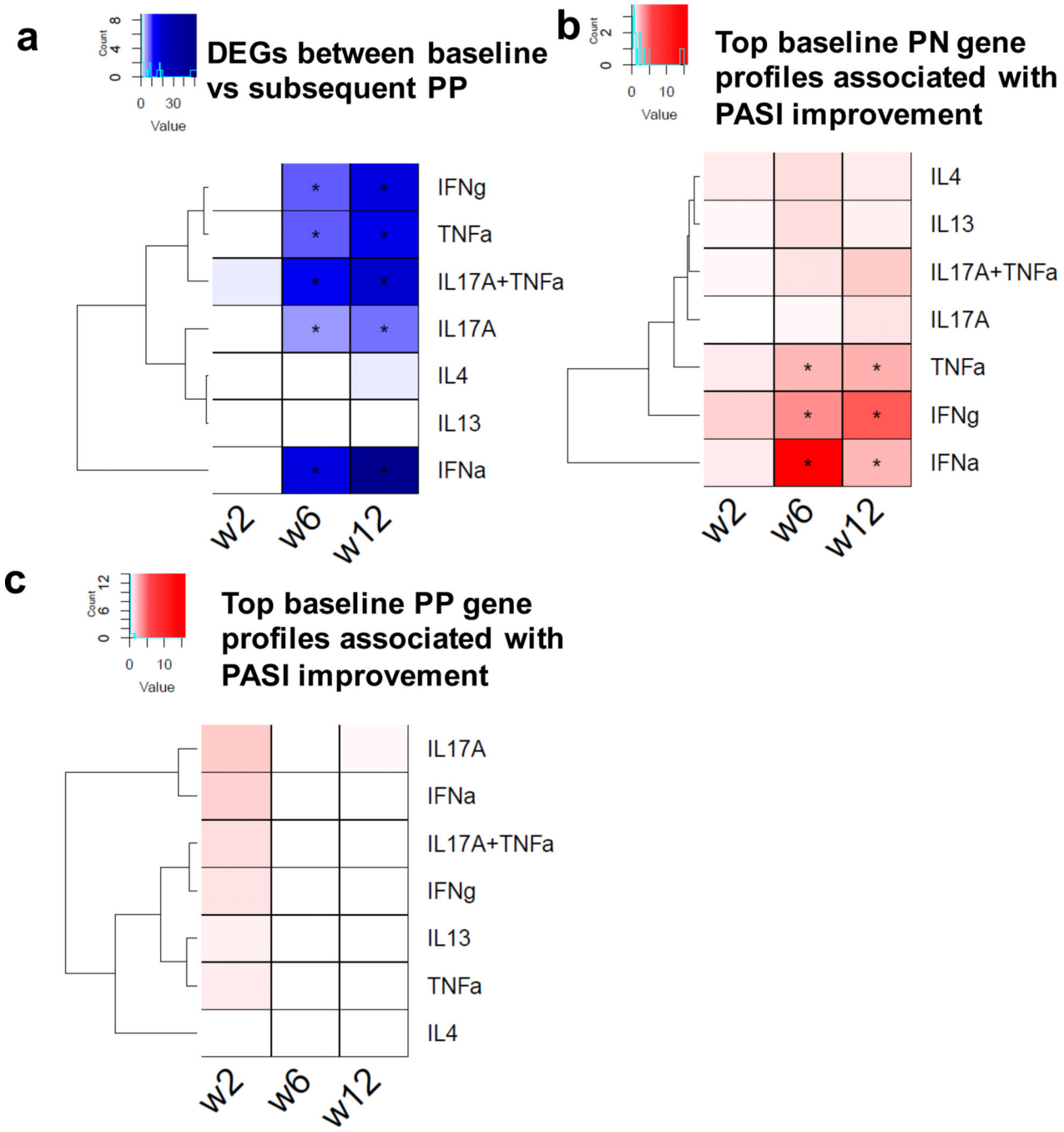

Figure 4. Enrichment of different cytokine signatures in different association comparisons.

a) Enrichment of the signatures among genes showing strongest differential expression in lesional skin between baseline versus follow-up; b,c) enrichment of the signatures among expression profiles at baseline in non-lesional psoriatic skin (b) or lesional skin (c) having strongest association with follow-up PASI improvement.

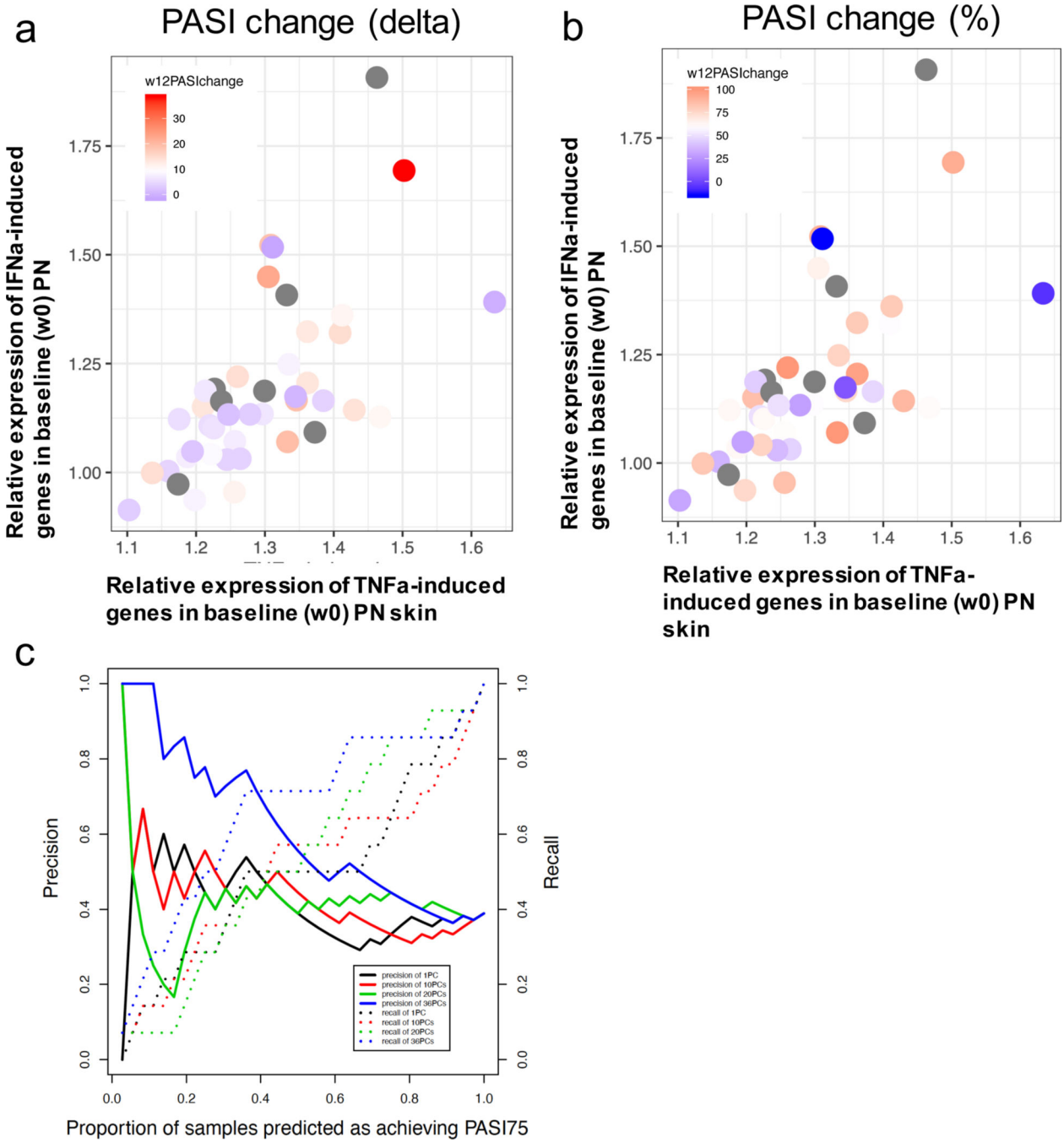

Figure 5. Assessment of PASI response in week 12 using baseline non-lesional skin expression profiles.

a,b) Each patient's TNF score at baseline non-lesional psoriatic skin (x-axis) was plotted against the patient's IFN score, and the color scheme (grey color represents no follow-up data) represents the PASI improvement by week 12 using either absolute (a) or percent (b) measure; c) the precision (solid line) and recall (dotted line) were plotted against the top proportion of samples predicted to achieve PASI75 by week 12.