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BRIEF COMMUNICATION

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Differential RNA Transcriptome in Ustekinumab Responders vs Non-Responders

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Despite numerous available psoriasis treatments, no "one size fits all'' regimen provides complete disease control without side effects, logistical obstacles, and/or expense. Despite increasingly efficacious drugs, only 20-25% of patients treated with biologic therapies achieve completely clear skin (PASI 100) and even fewer achieve this if they have experienced failures of multiple biologics.[1,2]

The balance of cytokines and chemokines produced in psoriatic lesions- exemplified by Interleukin-12 (IL-12) and IL-23- is implicated in disease phenotypic heterogeneity and lesion severity.[3,4] In humans, IL-12 is a heterodimer of the IL-12p40 subunit with the IL-12p35 subunit, which signals through the IL-12 receptor (IL-12R) leading to Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) activation and phosphorylation of signal transducer and activator of transcription (STAT) family molecules.[5] IL-23 is composed of the IL-23p19 subunit and the common IL-12p40 subunit, which signals through IL-23R also activating JAK and STAT signals.[6] Combined, the fascinating interplay between IL-12 and IL-23 accounts for activation of TNFα, IFNγ and IL-17 through activation of myeloid and T helper (Th) specific pathways, now common targets for numerous biologic agents.

However, psoriasis patients often experience a counterintuitive failure to respond to p40 blockade of IL-12 and IL-23 via ustekinumab, yet successfully respond to p19 blockade of the IL-23 pathway.[7] This observation is not limited to psoriasis as Crohn's Disease (CD) patients exhibit similar discordant outcomes. Although in CD some headway has been made to predict patient response to IL-12 and IL-23 blockade by ustekinumab, validated biomarkers are still lacking, and a clear gap in understanding the mechanism(s) responsible for such heterogeneous responses persists.[8]

Our lab has previously reported distinct psoriasis patient endotypes associated with unique clinical phenotypes and responses to treatment based on whole blood transcriptome analyses of psoriasis and psoriatic arthritis (PsA) patients.[9] This analysis identified a general psoriasis signature featuring upregulated interferons that trended toward an increase with age. Other psoriatic endotypes were identified using proinflammatory signals that clustered patients by increasing PASI score, gender and ethnicity.[10] We hypothesize that discovery of differentially expressed gene (DEG) signatures will inform more individualized care and that an integrated 'omic and clinical approach may identify classifiers of psoriasis and PsA patients that may be used to individualize their treatment.

In the current report, we utilized a similar whole blood transcriptome analysis to investigate the intersection of psoriasis endotypes and treatment response. Based on a cohort of psoriasis patients at our institution who failed to improve on ustekinumab, we performed RNA transcriptome analysis of six self-reported Responders (R) and four self-reported Non-Responders (NR) to examine DEGs between the groups that may contribute to treatment response. Patients were of similar age yet mostly male with only one non-white patient. Ustekinumab responders exhibit elevated cardiovascular risk scores, although differences with non-responders did not reach statistical significance (Figure 1). Gene set variation analysis (GSVA) using the Molecular Signatures Database (MSigDB) hallmark pathways to identified genes and associated annotations.

Heat map visualization of the top 50 DEGs by *P* value suggests strong separation between ustekinumab R and NR patients by hierarchical clustering (Figure 2). Each identified gene has significant power to discern groups, suggesting that they may be good sentinels or biologically relevant to the pharmacological mechanism of action associated with ustekinumab. Furthermore, intragroup similarity for NR is also high, such that an inadequate response may be especially uniform across patients. There are roughly equal numbers of upregulated (shades of red) versus downregulated genes (shades of purple), with some variability

FIGURE 1. Clinical summary of psoriasis cohort including anti-p40 responders versus non-responders.

	Stelara response		
	Adequate (n=6)	Inadequate (n=4)	*p* †,‡
Demographics			
Age (median [IQR])	47.00 [33.50, 71.00]	40.50 [30.75, 53.25]	0.52
Male/Female (%)	5/1 (83.3/16.7)	4/0 (100.0/0.0)	1.00
White/Black (%)	6/0 (100.0/0.0)	3/1 (75.0/25.0)	0.40
Psoriasis			
Psoriasis (%)	6 (100.0)	4 (100.0)	NA
No PsA/PsA (%)	5/1 (83.3/16.7)	3/1 (75.0/25.0)	1.00
PASI (median [IQR])	12.20 [10.18, 19.25]	4.60 [1.80, 8.18]	0.09
PASI < 10/PASI ≥ 10 (%)	2/4 (33.3/66.7)	3/1 (75.0/25.0)	0.52
Tremfya response adequate/inadequate/NA (%)	2/0/4 (33.3/0.0/66.7)	2/0/2 (50.0/0.0/50.0)	NA
No systemic/Systemic (%)	2/4 (33.3/66.7)	1/3 (25.0/75.0)	1.00
Systemic failures (median [IQR])	2.00 [1.25, 2.75]	4.00 [2.00, 6.00]	0.38
ASCVD			
10-yr Framingham HCE risk (median [IQR])	14.62 [5.51, 20.05]	10.40 [4.43, 21.88]	0.83
10-yr ACC/AHA ASCVD risk (median [IQR])	26.52 [18.35, 41.94]	19.62 [14.55, 24.69]	0.56
No self-reported CVD/Self-reported CVD (%)	6/0 (100.0/0.0)	3/1 (75.0/25.0)	0.40
Systolic blood pressure (median [IQR])	138.00 [126.75, 145.50]	131.00 [130.00, 131.50]	0.39
Flow Cytometry			
hsCRP (median [IQR])	2.60 [1.57, 3.63]	7.10 [4.80, 8.35]	0.29
RDW (median [IQR])	12.80 [12.62, 13.35]	12.90 [12.73, 12.93]	0.83
MCV (median [IQR])	96.00 [92.25, 99.00]	92.00 [90.75, 93.50]	0.33
MPV (median [IQR])	11.25 [10.90, 12.13]	11.00 [9.97, 12.10]	0.59
Monocyte Doublet % (median [IQR])	0.10 [0.08, 0.11]	0.06 [0.05, 0.08]	0.14
Intermediate Monocyte % (median [IQR])	3.06 [2.60, 5.38]	4.41 [3.92, 6.06]	0.20
Monocyte Platelet Aggregate % (median [IQR])	5.58 [1.77, 10.73]	14.80 [9.59, 17.60]	0.16

† Non-parametric Kruskal-Wallis test; ‡ Fisher's Exact test

FIGURE 2. Heat map containing the top 50 DEGs in ustekinumab responders versus non-responders contrast. Heat maps were z-score transformed with higher gene expression in red and lower gene expression in purple ($P \leq 0.05$). Strong grouping of patients by biologic response and roughly equal partitioning of genes into upregulated and downregulated subsets is observed.

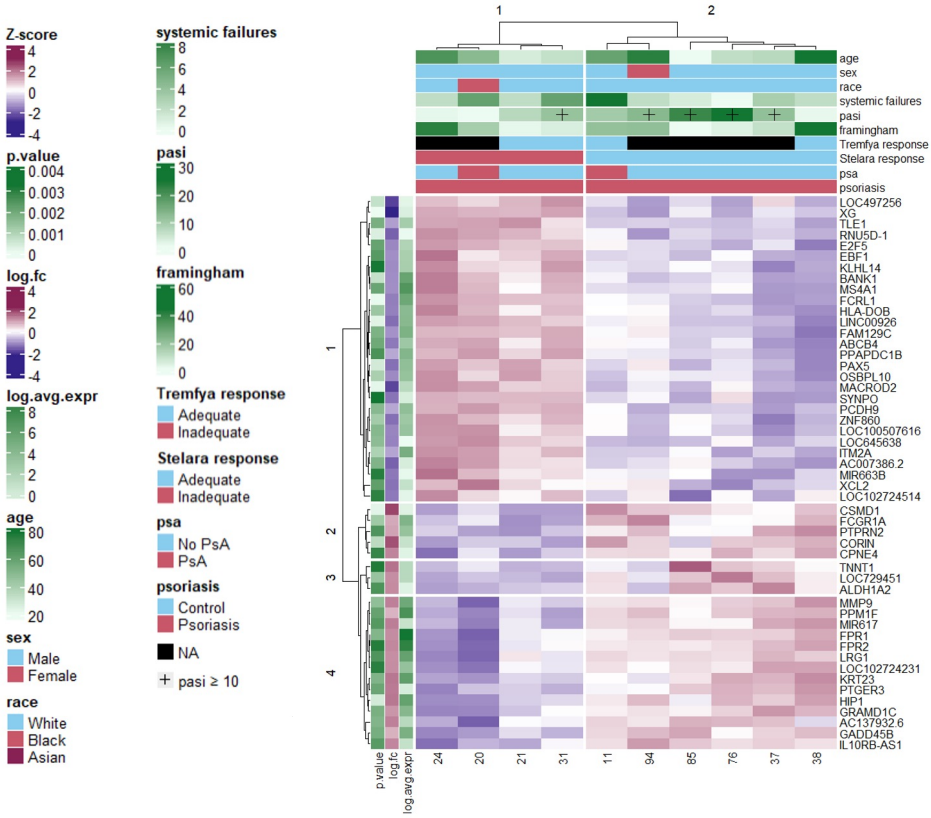
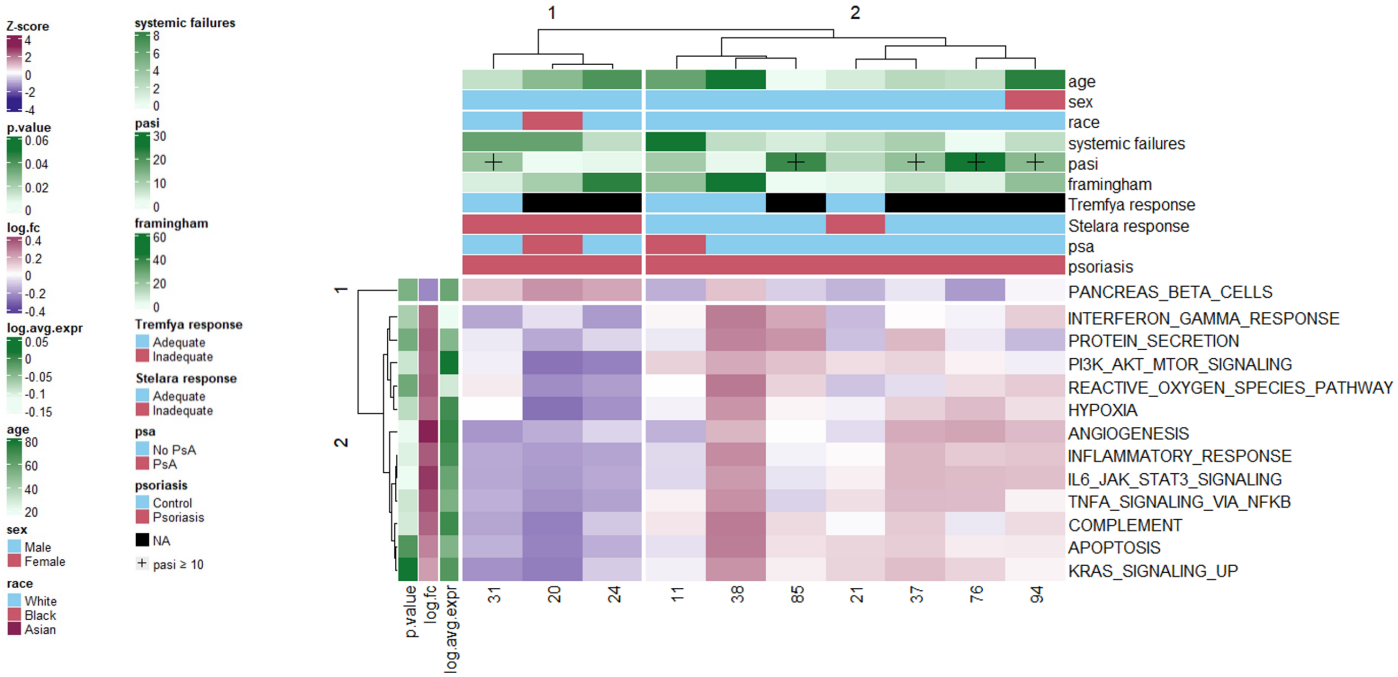


FIGURE 3. Heatmap of differentially expressed pathways between ustekinumab responders versus non-responders shows a common psoriatic signal and increased variability between patients relative to differentially expressed genes.



in expression of the upregulated genes in ustekinumab responders. Thus, even with a small cohort of self-reported data, large differences in transcriptome signal separating R from NR are evident.

Note the strong signature of pathways known to be associated with psoriasis pathogenesis including interferon response, TNF-alpha, and inflammation shown in Figure 3. It follows that these pathways would show themselves as significantly affected by ustekinumab treatment or at baseline in patients with psoriasis and suggests that different specific genes within psoriasis pathways influence pathways and drive pharmacologic response to ustekinumab. Interestingly, confirmation of our previously observed monocyte-driven PI3K-MTor signaling pathway may play a role in patients' responses to ustekinumab.[11]

Unsupervised learning of differential gene expression demonstrates a strong transcriptome signal separating ustekinumab R from NR patients (Figure 4a). In this case, R cluster separately from NR in a multidimensional scaling plot (Figure 4b). Distances on the plot approximate the typical log2 fold changes of the top DEGs between the samples (ie, leading-log-fold-change). Thus, the closer two objects appear within an MDS plot, the greater the similarity between the two.

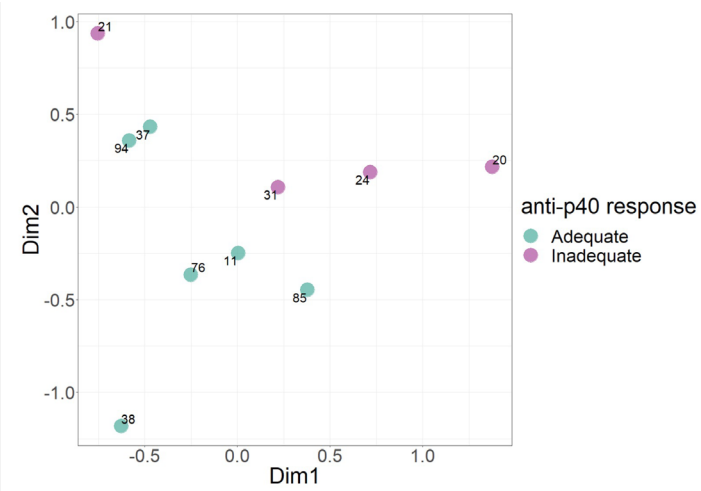
Additionally, ustekinumab responders also exhibited elevated cardiovascular risk scores, although differences with non-responders did not reach statistical significance (Figure 1). Interestingly, significant associations with ustekinumab initiation and major adverse cardiovascular event occurrence in patients with high-level cardiovascular risk has been previously observed.[12] Although Ustekinumab-treated psoriasis patients also exhibited a statistically significant decrease in aortic vascular inflammation (AVI), as well as a reduction in inflammatory biomarkers, and an increase in apolipoprotein B lipoproteins compared with placebo-treated patients, suggesting more analytic endpoints in ustekinumab-treated patients are valuable.[13] With increased statistical power, we may find that patients benefit from co-therapy for relevant comorbidities.

Similarly, ustekinumab responders have higher signals for key psoriasis clinical features such as circulating monocyte doublet percentage, circulating levels of proinflammatory intermediate monocytes, and increased levels for circulating monocyte platelet aggregates.[14] This suggests that there may be a relatively simple path to translational adoption and other more common (eg, CBC) values that could be used to inform the clinical evaluation of ustekinumab response.

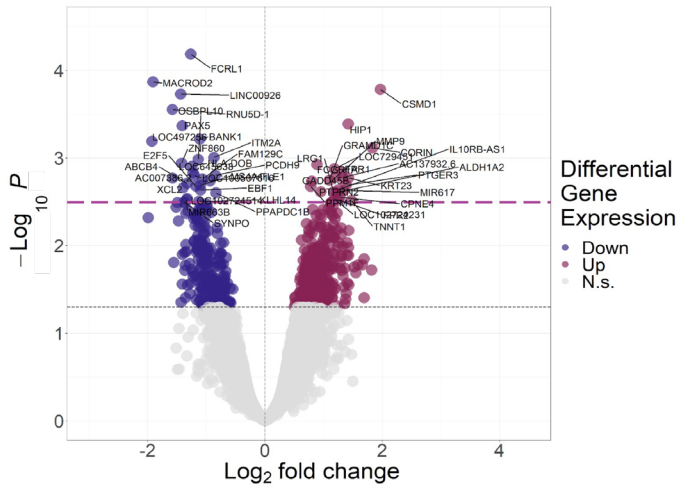
By utilizing patterns of DEGs from whole blood transcriptome analysis to analyze and correlate with patient outcome, we may improve the possibility of reaching skin resolution while reducing the trial-and-error treatment approach often employed. The ability to rule out (or in) specific therapeutics based on predictive efficacy would lead to a more personalized approach for psoriasis treatment.

FIGURE 4. Differential gene expression analysis of psoriasis patients by self-reported response to anti-p40 biologics regardless of anti-p19 response. (A) A multidimensional scaling plot separates ustekinumab responders from non-responders, suggesting that there is a large difference in differential gene expression between the groups. (B) Volcano plot indicating upregulated genes (red) and downregulated genes (blue) in ustekinumab responders versus non-responders contrast (n=357 DEGs, $P<0.05$). The top 50 differentially regulated genes are indicated with annotated genes of interest.

(A)



(B)



DISCLOSURES

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