Validation and Characterization of Six Distinct SLE Molecular Subtypes Using Two Large Patient Cohorts and Multi-Omics Platforms

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INTRODUCTION

- Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organ systems, complicating clinical trial design. Identifying biomarkers associated with distinct clinical features may enable more targeted drug development.
- The transcriptional dysregulation of key immune pathways in whole blood of SLE patients is well established[1] suggesting the ability to identify patient molecular subtypes based on blood transcriptome[2].
- However, the characterization of such subtypes remains limited due to the use of small patient cohorts, few clinical features, and limited data modalities.
- This gap limits our understanding of the SLE molecular subtypes and our ability to use them to inform treatment strategies and clinical trial design.

AIM

To further characterize SLE molecular subtypes using the ILLUMINATE clinical trials (NCT01205438 and NCT01196091)[3] and a large observational research cohort (Genuity Science, Ireland), leveraging a comprehensive dataset that includes clinical, transcriptomic, proteomic, and genomic information.

METHOD

Gene Expression

- Enrichment scores for representative SLE modules (Plasma cell/Cell cycle, Neutrophil/Inflammation, T cell, NK cell, Interferon, Mitochondrion, Platelet, B cell/Plasma cell, Inositol metabolism) were computed using GSVA in gene expression datasets from 1,760 patients in the ILLUMINATE studies (whole blood microarray) and 812 patients in the Genuity Science cohort (PBMC RNAseq)

Clustering & Modeling

- Consensus clustering was performed using the R package M3C in ILLUMINATE. Optimal number of clusters selected based on stability metrics (consensus index CDF, entropy, RSCI, Monte Carlo permutations p-value. A random forest model was then trained with the R package caret, with the dataset split into 80% for training and 20% for testing, followed by an evaluation of model performance. The model was used to map cluster assignments to the Genuity Sciences cohort.

Comparative Analysis

- Clinical and molecular differences between clusters were examined, including disease activity (SLEDAI), serological activity (anti-dsDNA and complement levels), serum proteomics, and demographics factors such as sex and age, using appropriate statistical tests.

RESULTS

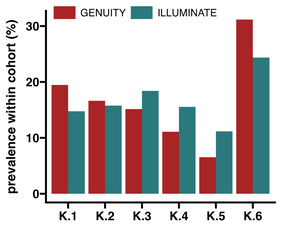

Fig. 1. Six distinct clusters (K.1 – K.6) were identified in the ILLUMINATE cohort and then mapped to the Genuity Sciences cohort (with at least half of patients having at least 50% probability of being classified to their respective cluster) . Prevalence of cluster membership was similar between the two study cohorts, ranging from 5 – 30% of patients in the Genuity Sciences cohort.

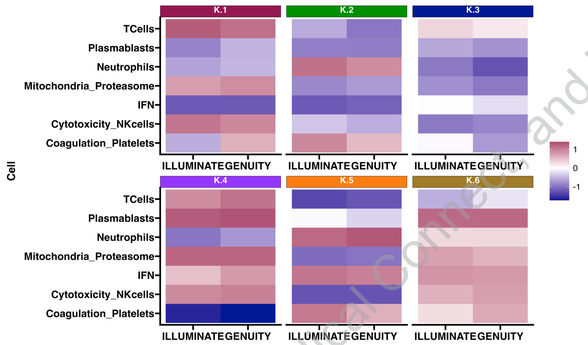

Fig. 2. The transcriptomic profiles across the 6 clusters of patients were consistent between the two cohorts, with IFN (high in Clusters 4-6) and neutrophil/coagulation (high in Clusters 2, 5, and 6) modules being primary drivers of differentiation.

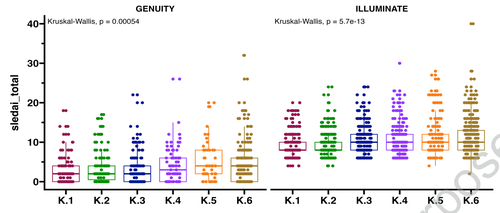

Fig. 3. Disease activity (SLEDAI) was within and across clusters, with lower levels in Clusters 1 and 2 compared to higher levels in Clusters 4, 5, and 6.

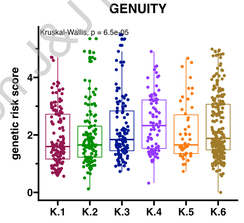

Fig. 4. A genetic risk score derived from 43 susceptibility loci [4] was highest in Cluster 4 (available in Genuity Sciences cohort only)

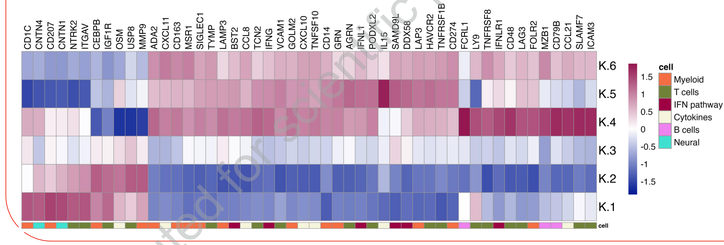

Fig. 5. Olink-HT serum profiles (available in Genuity Science cohort only) exhibited unique protein abundance patterns between the clusters and consistent with whole blood transcriptomic data: IFN pathway proteins are highest in Clusters 4, 5, and 6 but lowest in Clusters 1 and 2; B cell soluble receptors and LAG3 are higher in Cluster 4; and PADI4 and OSM are higher in Cluster 2.

Table 1: Demographics

ILLUMINATE	Total	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	p-value
Age, mean (SD)	42 (12)	46 (12)	46 (12)	42 (11)	38 (11)	40 (12)	38 (12)	<0.001
Sex, N (% of cluster)	1,626 (93%)	249 (96%)	254 (92%)	296 (92%)	262 (96%)	178 (91%)	387 (90%)	0.016
Genuity								
Age, mean (SD)	44 (13)	45 (11)	48 (14)	45 (14)	40 (12)	40 (14)	44 (14)	<0.001
Sex, N (% of cluster)	716 (88%)	140 (89%)	120 (89%)	112 (91%)	80 (89%)	46 (87%)	218 (86%)	0.8

- Patients in Clusters 4 – 6 were younger than those in Clusters 1 – 3 in both ILLUMINATE and Genuity Science cohorts

Table 2: Disease Manifestation & immunosuppressants

Genuity, N (% of cluster)	Total	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	p-value
Skin Rash	75 (20%)	7 (11%)	16 (29%)	10 (19%)	10 (21%)	8 (29%)	24 (19%)	0.2
Arthritis	85 (16%)	15 (14%)	10 (13%)	15 (21%)	9 (15%)	5 (15%)	31 (18%)	0.7
Nephritis ever	260 (32%)	38 (24%)	38 (29%)	30 (25%)	31 (35%)	20 (38%)	103 (41%)	0.003
Nephritis at baseline	80 (9.9%)	7 (4.4%)	10 (7.4%)	11 (8.9%)	15 (17%)	7 (13%)	30 (12%)	0.026
Prednisone use	379 (58%)	67 (44%)	56 (67%)	52 (51%)	35 (42%)	29 (81%)	140 (70%)	<0.001
NSIS use	197 (30%)	32 (21%)	30 (35%)	29 (28%)	18 (21%)	19 (51%)	69 (35%)	0.001

- Nephritis tended to be higher in the clusters with high IFN module scores (Clusters 4 -6)
- Prednisone use was highest in Clusters 2, 5, and 6, corresponding to the clusters also highest for neutrophil module scores
- NSIS (non-steroidal immunosuppressant: Azathioprine, Cyclophosphamide, Mycophenolate Mofetil, or Tacrolimus) use highest in Cluster 5, corresponding to the cluster lowest for T/NK module scores

Table 3: Serologic Activity

ILLUMINATE, N (% of cluster)	Total	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	p-value
Anti-dsDNA	1012 (58%)	92 (36%)	121 (44%)	167 (52%)	187 (69%)	139 (71%)	306 (71%)	<0.001
Baseline C3	650 (37%)	51 (20%)	48 (17%)	100 (31%)	142 (52%)	96 (49%)	213 (50%)	<0.001
Baseline C4	384 (22%)	27 (10%)	32 (12%)	51 (16%)	82 (30%)	57 (29%)	135 (32%)	<0.001
Genuity, N (% of cluster)								
Anti-dsDNA	310 (57%)	43 (44%)	44 (52%)	47 (62%)	34 (61%)	25 (63%)	117 (62%)	0.056
Anti-Smith	98 (13%)	11 (8.0%)	8 (6.6%)	11 (9.5%)	19 (23%)	11 (23%)	38 (17%)	<0.001
Anti- Ro	244 (41%)	36 (26%)	17 (24%)	53 (54%)	36 (47%)	12 (36%)	90 (50%)	<0.001
Baseline C3	284 (45%)	45 (41%)	42 (38%)	42 (42%)	29 (48%)	26 (54%)	100 (50%)	0.2

- Strong correlations were observed with serological activity between the two cohorts, measured by anti-dsDNA positivity and suppressed complement levels (highest activity in Cluster 6 and lowest in Cluster 1).
- The Genuity Sciences cohort revealed distinct distributions of other SLE-associated autoantibodies across clusters, namely, anti-Ro and anti-Sm (data not available for ILLUMINATE cohort).

CONCLUSIONS

- Using two large SLE cohorts, we identified and confirmed six molecular subtypes based on whole blood transcriptomes.
- The clusters exhibited significant differences in disease severity, genetic risk scores, serological activity, and proteomic profiles.
- IFN and neutrophil module scores were major differentiators of the clusters and showed correlations with nephritis and prednisone use, respectively.
- These findings provide valuable insights for patient stratification in clinical trials and may enable the development of targeted treatment strategies based on predicted responses to immune-modulating therapies.

Cluster	SLEDAI	Sero-activity	IFN*	PB*	Neutr*
1	↓	↓	↓	↓	↓
2	↓	↓	↓	↓	↑
3	↓	↓↑	↓↑	↓	↓
4	↑	↑	↑	↑	↓
5	↑↑	↑	↑↑	↓↑	↑
6	↑	↑	↑	↑	↓↑

* Module scores for: IFN, interferon; PB, plasmablast; Neutr. Neutrophils

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- All authors are employees of Janssen Research & Development, LLC and may hold stock in Johnson & Johnson.