TRANSCRIPTIONAL REMODELING DURING CAR-T EXPANSION: CLUSTER-BASED COMPARATIVE GENE EXPRESSION ANALYSIS

Nupur Das[1], Prashant Mehta[2], Kusum Gupta[1], Swati Pabbi[3], Rahul Katharia[3], Pravas C Mishra[2], Monu Harsana[1], Soni Morya[1]

1. Department of Pathology, Amrita Institute of Medical Sciences and Research Centre, Faridabad, India
2. Department of Medical Oncology, Amrita Institute of Medical Sciences and Research Centre, Faridabad, India
3. Department of Transfusion Medicine, Amrita Institute of Medical Sciences and Research

INTRODUCTION

- CAR-T cell therapy induces profound and dynamic changes in the immune landscape following infusion.
- While the kinetics of CAR-T expansion and persistence are well recognized, the transcriptional programs underlying these changes remain incompletely characterized.
- Understanding the transcriptional evolution of immune cells during different stages of CAR-T therapy may therefore provide important insights into the molecular mechanisms governing in vivo CAR-T expansion, functional differentiation, and broader immune reconstitution.

AIM

- To characterize the dynamic transcriptional evolution of immune cells during CAR-T therapy by comparing immune-related gene-expression profiles between leukapheresis samples obtained before CAR-T manufacturing and samples collected during peak CAR-T expansion.
- A cluster-based comparative transcriptomic approach was used to identify distinct patterns of gene expression and immune pathways associated with the transition from the pre-manufacturing state to peak CAR-T expansion

METHOD

- This is a prospective study conducted at Amrita Institute of Medical Sciences and Research Centre, Faridabad, India over a period of 1 year (January 2025 – January 2026).
- A total of 20 samples from different phases of CAR-T expansion (leukapheresis vs peak expansion) from six different patients receiving indigenous CAR-T were studied. Transcriptomic profiling was performed using the NanoString nCounter® immuno-oncology 750-gene panel. Unsupervised cluster analysis was done to identify differentially regulated immune transcriptional programs followed by cluster enrichment analyses..

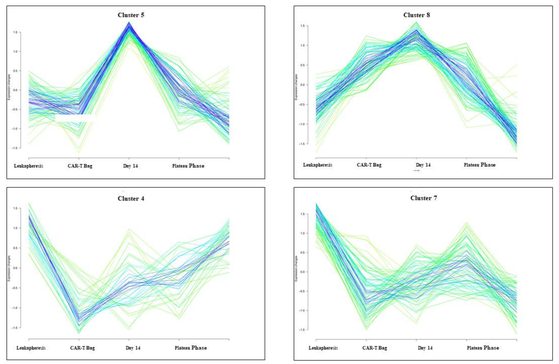
CONCLUSIONS

- Cluster-based transcriptomic profiling demonstrated profound immune remodeling during CAR-T expansion.
- These findings highlight the utility of longitudinal transcriptomic profiling in identifying biologically relevant immune states during CAR-T therapy.
- This may serve as a potential tool for monitoring expansion kinetics, persistence and early exhaustion signatures.
- Integrative cluster-based immune profiling could further aid in developing predictive biomarkers for treatment response, toxicity, and long-term outcomes.

RESULTS

- Unsupervised clustering analysis identified 10 transcriptionally distinct gene clusters comprising 41–77 genes per cluster.
- Clusters 5 and 8 demonstrated significant upregulation during peak expansion, whereas Clusters 4 and 7 were enriched in leukapheresis samples.
- Cluster 5 represented a dominant cytotoxic effector and inflammatory module characterized by upregulation of genes associated with immune activation and trafficking (GZMK, GZMA, IFNG, FASLG, CCR5, CXCR6, TNFRSF9 and multiple TCR variable-region genes).
- Concurrent expression of checkpoint-associated molecules (LAG3, PDCD1 and TOX) suggested emergence of adaptive exhaustion signaling during sustained activation.
- Cluster 8 was enriched for genes involved in T-cell receptor signaling, cytokine responsiveness, and metabolic reprogramming (CD3E, LCK, NFATC1, IL2, IL2RG, STAT1, HAVCR2) and mitochondrial oxidative phosphorylation-associated genes (ATP5MF, COX6B1, NDUFA1/2, CPT1B).
- Leukapheresis-associated Clusters 4 and 7 demonstrated enrichment of innate immune, antigen presentation, and homeostatic programs.
- Cluster 4 contained myeloid and immune-regulatory genes (CD33, CD209, SOCS5, and CDKN1A). Cluster 7 demonstrated NK-cell and antigen presentation signatures (KLRD1, KLRF1, HLA-DRA, IL7 and IL15).

Cluster	Temporal association	Dominant biological program	Representative genes	Key interpretation
5	**Peak expansion**	Cytotoxic effector function, inflammation, immune activation/trafficking	**GZMK, GZMA, IFNG, FASLG, CCR5, CXCR6, TNFRSF9**, TCR V-region genes	Dominant activated cytotoxic T-cell program with concurrent **LAG3, PDCD1 and TOX**, suggesting adaptive exhaustion signaling during sustained activation
8	**Peak expansion**	TCR signaling, cytokine responsiveness, metabolic reprogramming	**CD3E, LCK, NFATC1, IL2, IL2RG, STAT1, HAVCR2**; **ATP5MF, COX6B1, NDUFA1/2, CPT1B**	Activated T-cell signaling coupled with **mitochondrial oxidative phosphorylation and metabolic adaptation**
4	**Leukapheresis**	Innate immune, myeloid and immune-regulatory/homeostatic programs	**CD33, CD209, SOCS5, CDKN1A**	Reflects a predominantly **innate/myeloid and regulatory immune milieu** before CAR-T expansion
7	**Leukapheresis**	NK-cell function, antigen presentation and lymphocyte homeostasis	**KLRD1, KLRF1, HLA-DRA, IL7, IL15**	Enrichment of **NK-cell, antigen-presentation and homeostatic cytokine programs**



Integrated Cluster gene and Reactome Pathway analysis).

CONTACT INFORMATION

nupurdas@fbd.amrita.edu

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