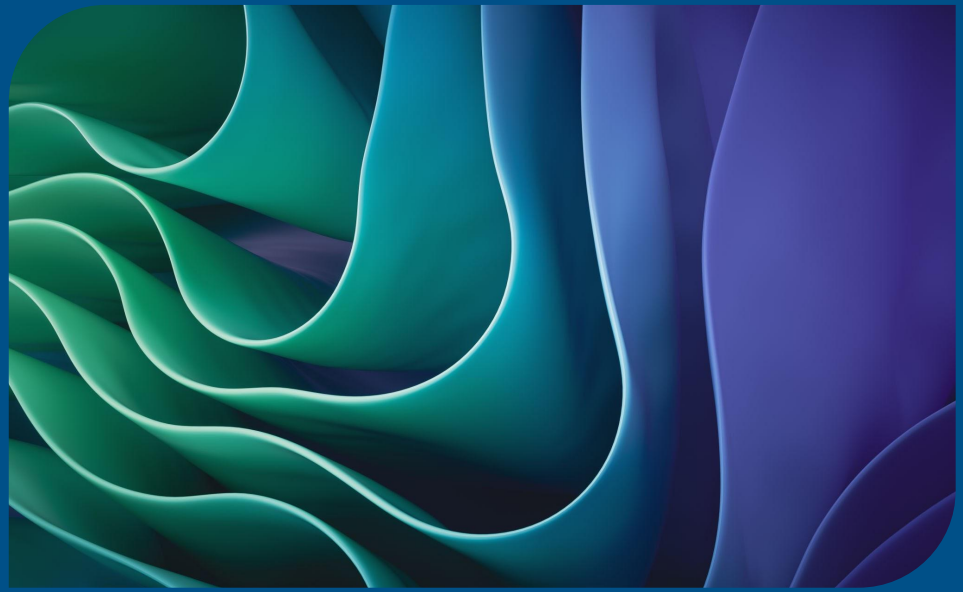
Single-nucleus RNA-sequencing of Merkel Cell Carcinoma Reveals Transcriptional Signatures Associated with Immunotherapy Response

Faith Jean, BS[1]
Maximilian Haist, MD[2]
Magdalena Matusiak, PhD[2]

[1]Meharry Medical College, School of Medicine, Nashville, TN
[2]Stanford University-Department of Pathology, Stanford, CA



Disclosure Statement

I have no relevant financial relationships to disclose.

Background

Rare, aggressive skin cancer

- Driven by Merkel Cell polyomavirus or UV light exposure
- High metastatic potential

Treatment Revolution

- Immune checkpoint inhibitors (PD-1/PD-L1 inhibitors)
- Response rates: 50-70% in advanced disease

The Challenge

- Significant variability in patient responses

Objectives

Primary Goal: Identify and distinguish cell types and their differential enrichment associated with response to immunotherapy treatments (avelumab, nivolumab, and ipilimumab)

Focus on T Cell Signatures

- Primary eliminators of cancer cells upon checkpoint blockade
- Key players in immunotherapy response mechanisms

Methodology

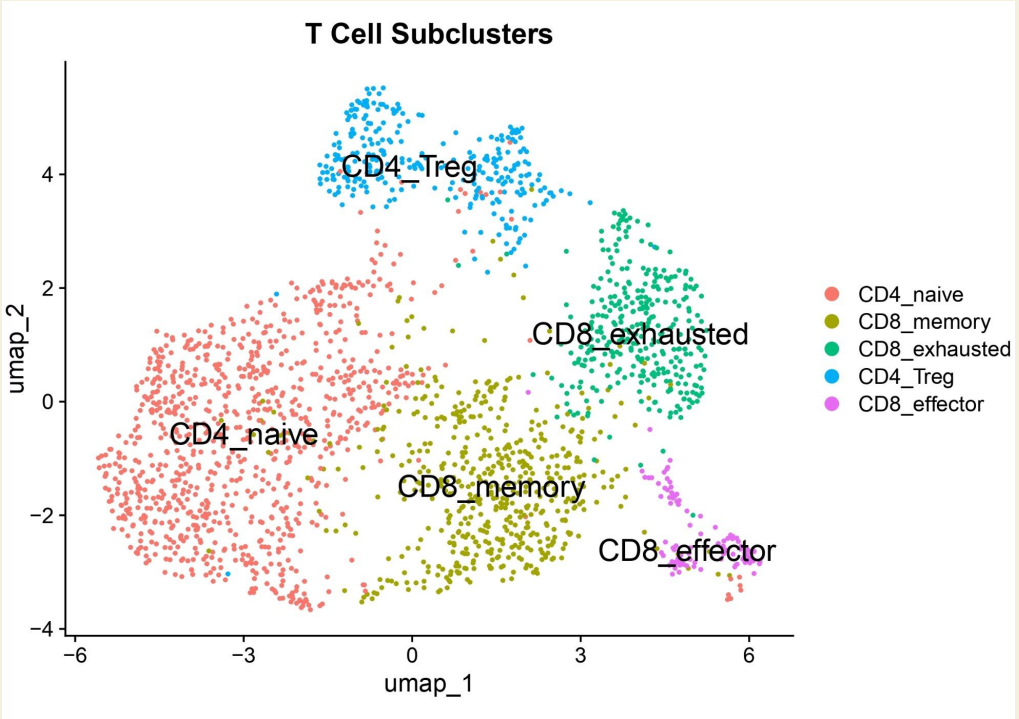
Single-nucleus RNA Sequencing Approach

- **Patient Cohort:** 10 clinical patients
- **Data Processing:** Standard normalization and scaling
- **Clustering Strategy:**
  - Louvain algorithm on principal components
  - Identified transcriptionally similar cell populations
- **T Cell Identification:** Canonical T cell markers
- **Comparative Analysis:**
  - Differential composition between responders vs. non-responders
  - Proportional distribution of each subcluster within response groups

T Cell Subpopulations Identified

The five subpopulations identified were:

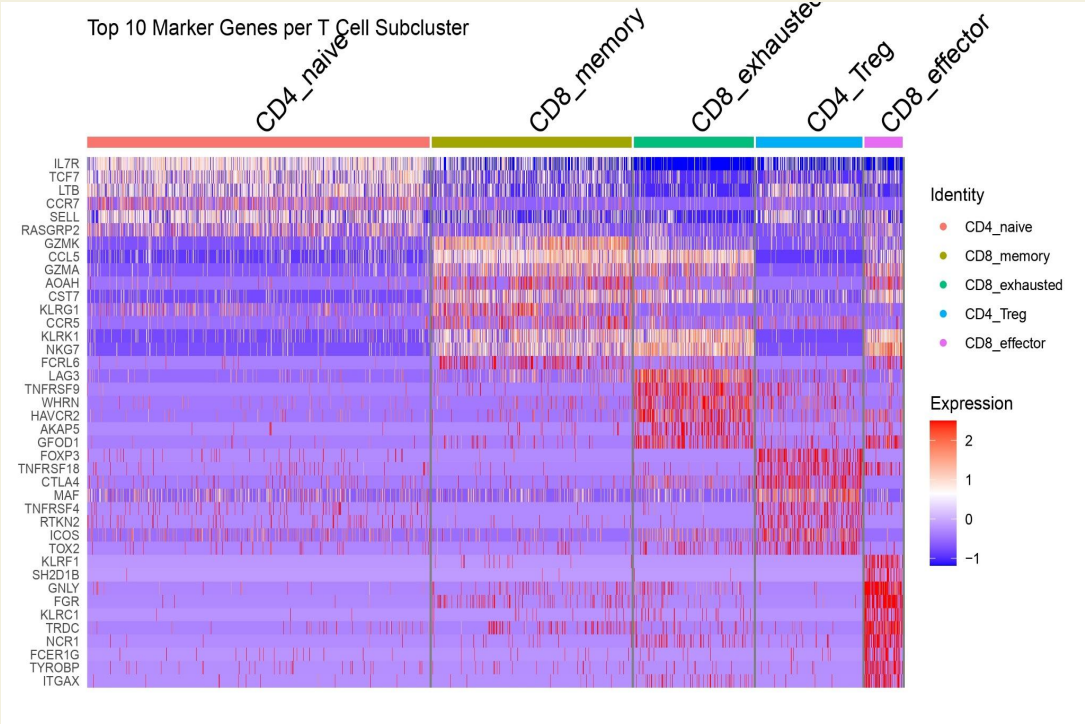
1. Naïve CD4 T cells
2. Memory CD8 T cells
3. Exhausted CD8 T cells
4. Regulatory CD4 T cells
5. Effector CD8 T cells



Molecular Signatures

These gene signatures were used to annotate the five subclusters:

1. Naïve CD4 T cells: IL7R, TCF7, CCR7, SELL
2. Memory CD8 T cells: GZMK, CCL5, GZMA
3. Exhausted CD8 T cells: LAG3, HAVCR2, TOX2
4. Regulatory CD4 T cells: FOXP3, TNFRSF18, CTLA4
5. Effector CD8 T cells: KLRF1, GNLY, FCER1G



Key Findings: Responders vs Non-Responders

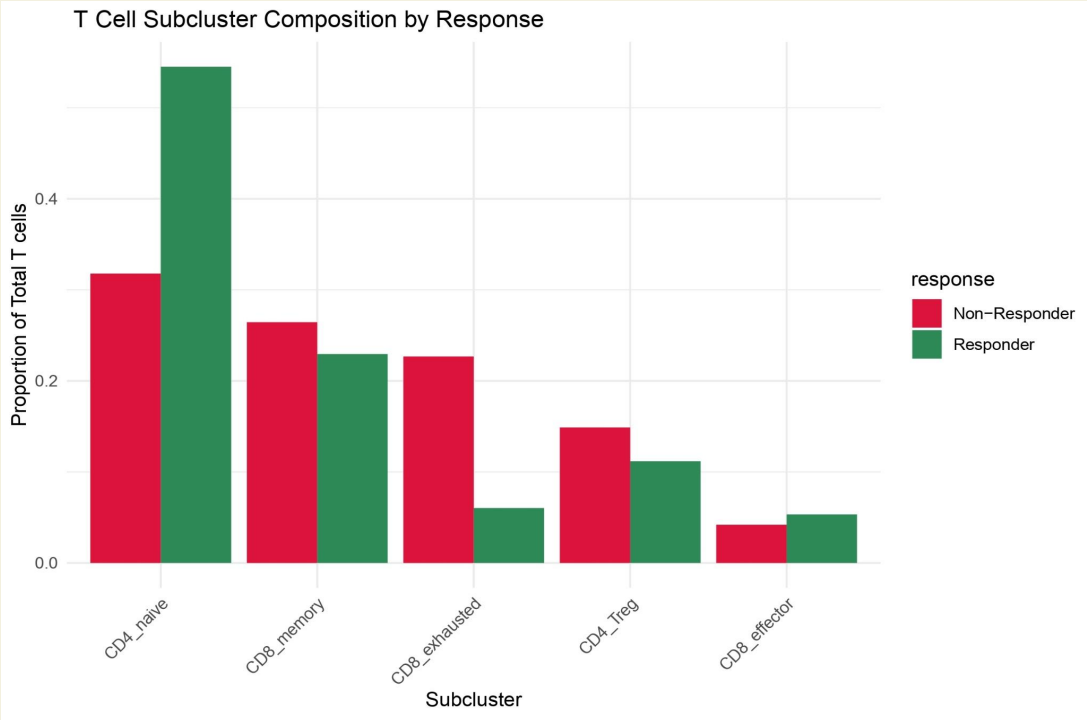
Differential T Cell Enrichment Patterns:

Enriched in Responders (n=5):

- Naïve CD4+ T cells ↑

Enriched in Non-Responders (n=5):

- Exhausted CD8+ T cells ↑
- Regulatory CD4+ T cells (Tregs) ↑



Biological Implications

- **Naïve CD4+ T cells in responders:**
  - Preserved immune potential
  - Better capacity for anti-tumor response
- **Regulatory T cells in non-responders:**
  - Suggests immune system downregulation
  - Correlates with lack of immunotherapy response
- **Exhausted CD8+ T cells in non-responders:**
  - High presence indicates a pre-existing exhausted immune state
  - Unable to mount effective response despite checkpoint blockade

Clinical Significance

Potential Clinical Applications:

- **Biomarker Development**
  - T cell signatures could predict immunotherapy response
- **Therapeutic Implications**
  - Combination therapies for exhausted T cell–dominant tumors
  - Stratify patients for appropriate immunotherapy approaches

Limitations and Future Directions

Current Limitations:

- **Small sample size:** n=5 per group
  - Limited statistical power

Next Steps for Research:

- **Larger validation cohorts**
  - Increase statistical power
  - Confirm preliminary findings

References

Toberer, F., Winkler, J. K., Atienza Fernandez, L., Adams, L., Brobeil, A., Tóth, M., Enk, A. H., Becker, J. C., & Lonsdorf, A. (2025). Inhibitory Immune Checkpoints beyond Programmed Cell Death Ligand 1 in Merkel Cell Carcinoma: Abundant Expression of TIGIT Independent of the Presence of Merkel Cell Polyoma Virus. *Acta dermato-venereologica*, *105*, adv42882. https://doi.org/10.2340/actadv.v105.42882

Haist, M., Matusiak, M., Tan, Y., Zimmer, S., Stege, H., Kempchen, T. N., Mitschke, S., Chu, P., Weidenthaler-Barth, B., Barlow, G. L., Rogall, F., Gonzalez, A. D., Baertsch, M. A., Albertini, S., Gariglio, M., Borgogna, C., Goltsev, Y., Grabbe, S., Hickey, J. W., & Nolan, G. P. (2025). Spatially organized inflammatory myeloid-$CD8^+$ T cell aggregates linked to Merkel-cell Polyomavirus driven Reorganization of the Tumor Microenvironment. *bioRxiv : the preprint server for biology*, 2025.06.06.657162. https://doi.org/10.1101/2025.06.06.657162