Exploring the cell surface – expression and polarization of immune-related markers in Merkel Cell Carcinoma and infiltrating immune cells

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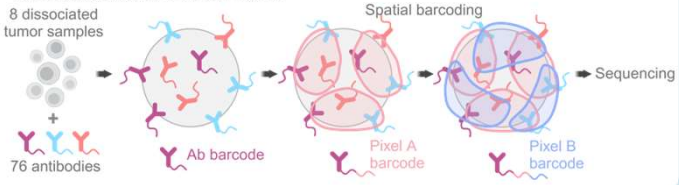
Introduction

- **Merkel cell carcinoma** (MCC) is an aggressive and immunogenic skin cancer.
- **Immune checkpoint inhibition** (ICI) works by blocking immuno-suppressive cellular interactions and is an effective therapy for about half of metastatic MCC patients.
- The **cell surface** is the interface of tumor-immune interactions.
- We employed **molecular pixelation** (MPX) to describe immune-related surface marker abundance, polarization, and colocalization at single-cell resolution on tumor and infiltrating immune cells.

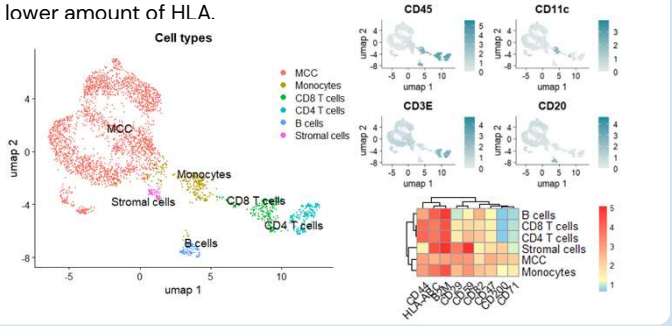
Method: Molecular Pixelation

MPX employs **DNA-barcoded antibodies** to label proteins on the cell surface. Proximal antibodies are then hybridized to DNA pixels adding **location-specific barcodes**. Sequencing of these extended barcodes then provides a mapping of all markers on each individual cell surface.

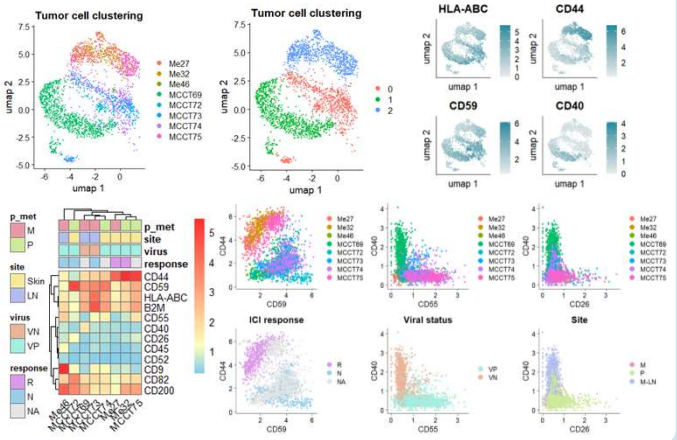


Results

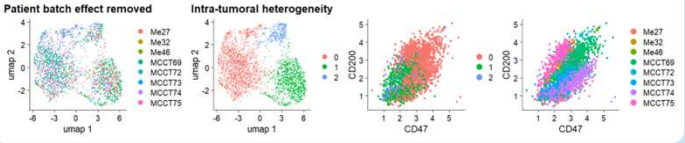
Tumor and infiltrating immune cells were captured. MCC cells express: **CD44, CD59, CD29, CD82, CD47, CD200, CD71** and a lower amount of HLA.



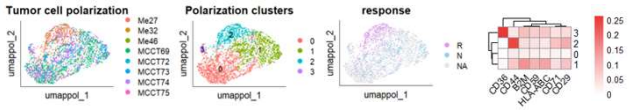
Inter-tumoral heterogeneity was mainly characterized by CD44 and CD59 expression, which correlates to ICI-response status.



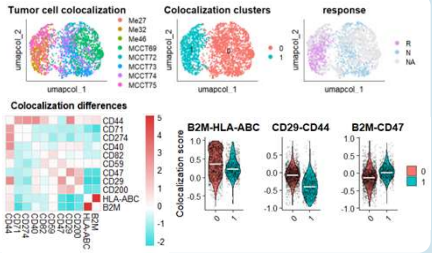
Intra-tumoral heterogeneity reflects the level of expression of other MCC markers, such as CD200 or CD47.

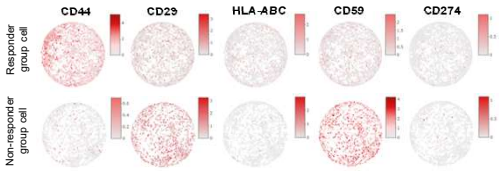


Polarized tumor cells clustered into distinct groups. High **CD44 polarization** was associated with ICI response.



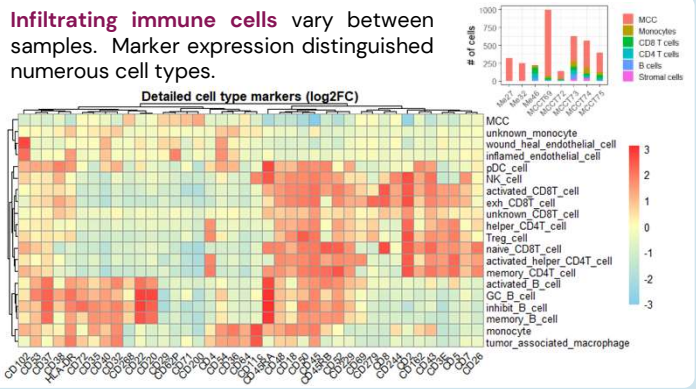
Clusters of CD29, CD47, and CD200 or CD44 alone localize away from other markers. These patterns are associated with ICI-response status and reflect polarization states.



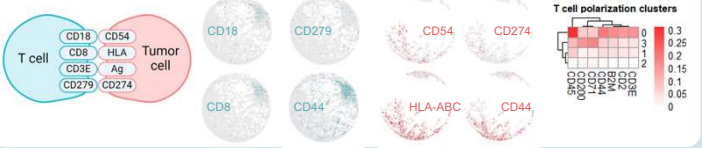


3D visualization of tumor cells. Low CD274 is present on most tumor cells.

Infiltrating immune cells vary between samples. Marker expression distinguished numerous cell types.



A subset of CD8⁺ T cells and tumor cells polarizes consistently with **tumor cell killing**. CD44 is present in the immune synapse. T cells also display an alternative non-killing polarization.



Conclusion

- Tumor cells express several **targetable surface markers** implicated in immune suppression.
- **CD44** and **CD59** expression, polarization, and colocalization were associated with ICI-response status.
- Polarization consistent with tumor killing by T cells shows that CD44 is present at the supposed **immune synapse**.

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