Linking single-cell spatial multiomics of TCR repertoires using CosMx SMI with live T cell function using the Beacon to characterize immune cell dynamics in tumors

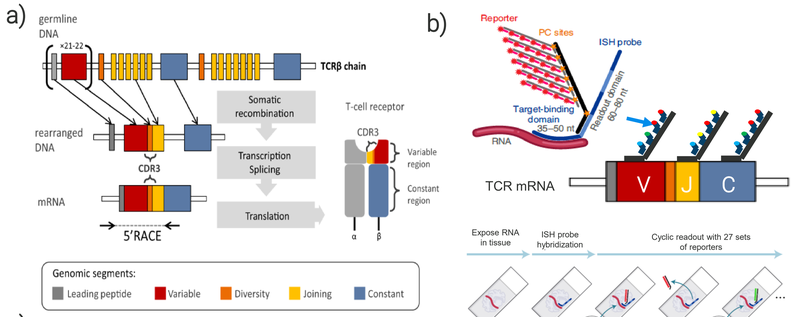
Margaret L. Hoang[1], Rachel Liu[1], Dan McGuire[1], Ashley Heck[1], Kim Young[1], Megan Vanderberg[1], Claire Williams[1], Martin Shelton[1], Prajan Divakar[1], Patrick Danaher[1], Erin Piazza[1], Joseph Beechem[1]

[1]Bruker Spatial Biology, Seattle, Washington, USA

Introduction

Understanding how immune cells interact with tumor cells and the surrounding microenvironment is critical for predicting immunotherapy response. High-resolution spatial mapping of tumor-infiltrating lymphocytes (TILs) enables direct visualization of these interactions within tumor architecture. Using the CosMx® Spatial Molecular Imaging (SMI) platform, we integrate T-cell receptor (TCR) repertoire profiling with Whole Transcriptome (WTX) and immuno-oncology protein readouts to characterize immune phenotypes in FFPE tumor tissues. This approach captures TIL clonotypes and functional states, including activation, exhaustion, and exclusion, in situ, providing a detailed view of immune niches and their relationship to tumor and stromal features. Together, data highlight spatially resolved immune dynamics that inform biomarker discovery and therapeutic response.

TCR diversity and CosMx TCR detection



c)

Chain Type	Constant Chains	Variable Segments	Joining Segments
Alpha	TRAC	TRAV_ 40 probes	TRAJ_ 46 probes
Beta	TRBC1/2	TRBV_ 40 probes	TRBJ_ 4 probes
Gamma	TRGC1/2/ TARP	TRGV_ 5 probes	TRGJ_ 4 probes
Delta	TRDC	TRDV_ 3 probes	TRDJ_ 4 probes

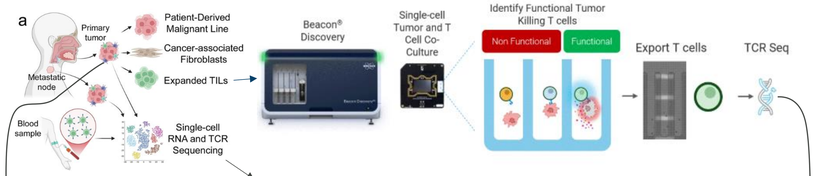
TCR panel includes 100 Negative probes & 6 TCR positive controls
Probes for constant chains in WTx

Fig. 1 Each T-cell expresses a single, unique TCR generated through genomic recombination during development (a). TCRs are made by cutting and pasting individual segments together- V (Variable), J (Joining), and C (Constant) are joined for alpha chains; V, D (Diversity), J, and C for beta chains (a). The CosMx TCR assay detects these segments (panel content in c) alongside the whole transcriptome using barcoded probes and several rounds of reporter binding and imaging (b). Cells are segmented using protein markers and decoded RNA signals are assigned at single-cell resolution.

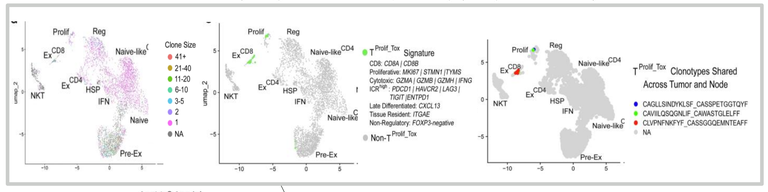
Linking live T-cell function to spatial context in head and neck cancer via CosMx multiomics and TCR repertoire profiling

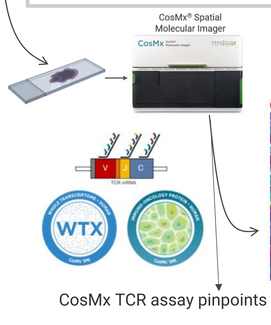
Joseph Zenga, MD
Donald S. Blatnik, MD and Sharyn B. Blatnik Professorship of Head and Neck Surgical Oncology and Reconstruction; Associate Professor & Chief, Division of Head and Neck Surgical Oncology & Reconstruction

Live, patient-derived T-cells that can kill tumor cells identified via Beacon platform and TCR sequenced

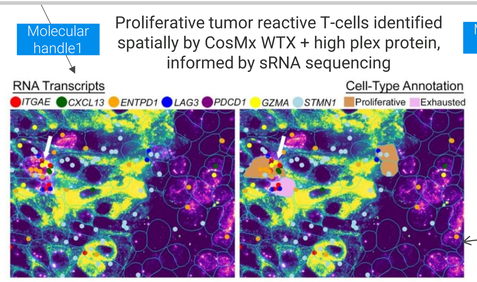


Gene expression signatures for proliferative tumor reactive T-cells (T^{Prolif_Tox}; tumor killing) identified by single-cell RNA sequencing (Zenga et al. 2025)

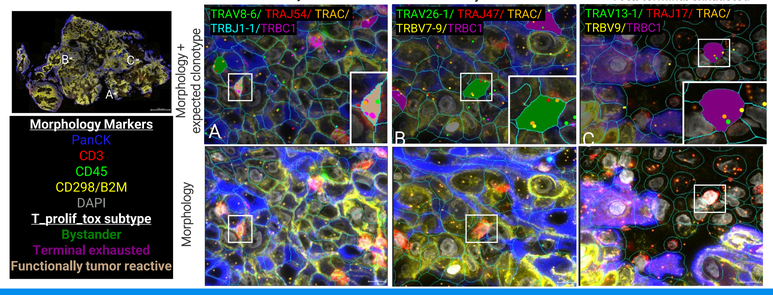




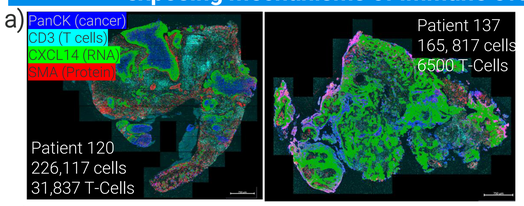
Proliferative tumor reactive T-cells identified spatially by CosMx WTX + high plex protein, informed by sRNA sequencing



CosMx TCR assay pinpoints functionally tumor reactive T-cells with known clonotypes from TCR seq in space



CosMx WTX + TCR + protein finds tumor-killing T-cells in space, exposing mechanisms of immune evasion by tumors



b) TCR clonotype calling algorithm

- The data we have
- y_c := observed counts for cell c across V,J TCR genes
- b_c := estimated background rate using TCR Negative Controls
- n_c := total counts for cell c (across all RNA gene targets, not just TCR's).
  Higher total counts typically imply better sensitivity as well as more background counts
- t := clonotype (shared based on abundance data in all_clones.xlsx + 1 'empty' no-clone clonotype)

Data generating process for the TCR counts

- $P(y_c \mid T = t) = \prod_{g \in t} Pois(n_c(\theta_g + b_c)) \prod_{g \notin t} Pois(n_c b_c)$

Counts from TCR genes for that clonotype ($g \in t$) are expressed 'above background for that cell'

Counts from non-clonotype TCR genes ($g \notin t$) can only be observed from background

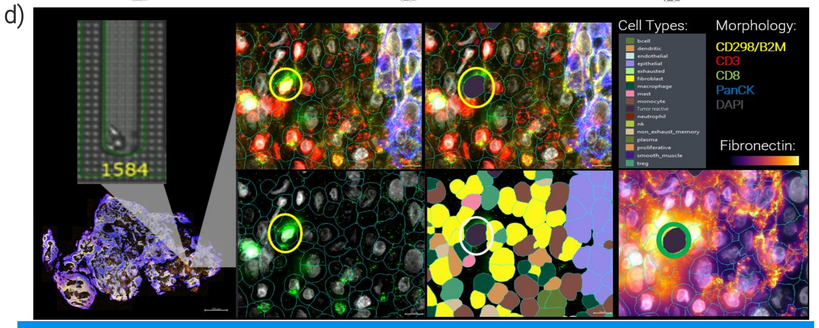
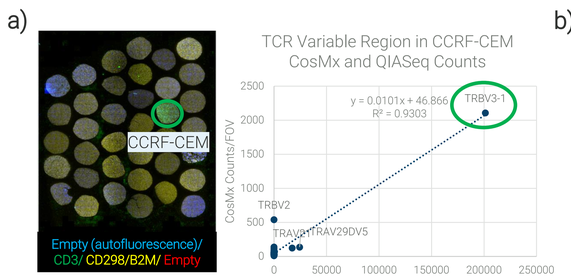


Fig 3. (a) Distinct immune escape phenotypes revealed by CosMx in two head and neck cancer patients. P137 shows immune infiltration with T-cell exhaustion, whereas P120 exhibits abundant T-cells that fail to penetrate the tumor due to differences in ECM barriers and/or chemokine patterns. (b) Spatial algorithm for calling TCR clonotypes based on colocalization of VDJ segments measured by the CosMx TCR assay. (c) Spatial mapping of T-cell subtypes based on clonotypes identified by TCR-seq in P137. (d) Functionally tumor-reactive T-cells identified by Beacon platform infiltrate malignant regions but are frequently isolated and suppressed by nearby Tregs and fibronectin.

CosMx TCR assay is specific in cell lines and FFPE tissue



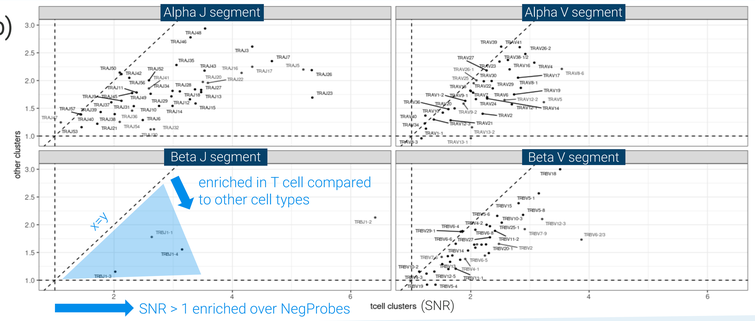


Fig. 2. a) CosMx TCR panel measurements show strong concordance with RNAseq data in the CCRF-CEM T-cell line and detect the expected enrichment of the TRBV3-1 segment, supporting the specificity of the TCR assay. B) TCR probe signal is enriched in T-cells relative to other cell types and background across head and neck cancer FFPE tissue, confirming the specificity of TCR panel targets in FFPE tissue context.

Conclusion

- CosMx SMI enables single-cell, spatial TCR segment and clonotype identification with integrated WTX and protein in FFPE tumors.
- Spatial TCR mapping Identifies T-cells shown to functionally kill tumors in their spatial context
- Combined WTX, high-plex protein, and TCR profiling offers a comprehensive view of the tumor microenvironment surrounding functional T-cells, exposing mechanisms of immune evasion and guiding patient specific therapeutic strategies

References:
Zenga, J., Awan, M.J., Frei, A. *et al.* Radiation therapy results in preferential tumor antigen-specific lymphodepletion in head and neck cancer. *Nat Commun* 16, 5660 (2025). https://doi.org/10.1038/s41467-025-60827-w

Scan here to download or learn more