AGBT 2026, POSTER #104

Whole Transcriptome multi-omic subcellular imaging in space AND time: bringing spatial biology to life!

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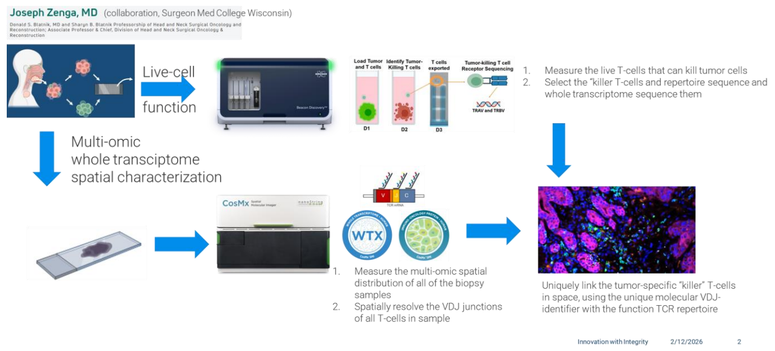
GOAL: establish an experimental strategy to "connect" live-cell functional assays with high-plex spatial biology. To achieve this capability, we have developed an experimental approach to bridge-the-gap between live-cell functional assays using single-cell optofluidic Beacon Discovery™ technology (Bruker Cellular Analysis) combined with CosMx® whole-transcriptome Imaging (Bruker Spatial Biology).

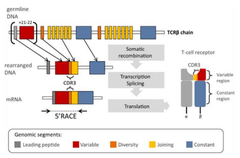
EXPERIMENTAL APPROACH: From cancer excisional biopsies, directly measure live dissociated T-cells ability to kill primary tumor cells & isolate the killer-T-cells (via Beacon Discovery) and perform scRNA-seq and TCR-seq on those T-cells. This establishes a unique killer T-cell *molecular identifier.* From same biopsy material (adjacent section, FFPE), perform CosMx whole transcriptome (with T-cell repertoire spike-in capability) to spatially resolve the identical *molecular identifier* measured by Beacon Technology, thereby directly linking spatial-resolution with live-cell based killer-functionality.

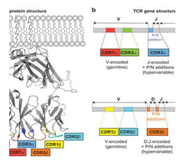
Direct from the operating room to: live T-cell functional imaging with TCR-seq. Adjacent section, spatial whole transcriptome with TCR-seq "matching"



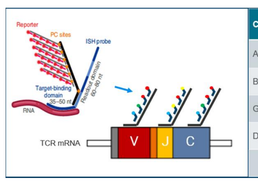
Finding killer T-cells in Space:

To "find" the killer T-cells, we need to develop a WTX panel "spike-in" that will spatially resolve T-cell clonality, and uniquely map back all T-cells back to the exact molecular identifier, "TCRseq-match", of the T-cells that functionally kill





Densely measure all the VDJ junctions of all spatially resolved T-cells



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

New TCR-spike-in panel can measure Tcell clonal expansion and uniquely map Back to T-cells that can kill tumo cells

How to "find" the tumor-killing T-cells using CosMx whole transcriptome plus "TCRseq-Spatial Match" spike-in panel

- 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)

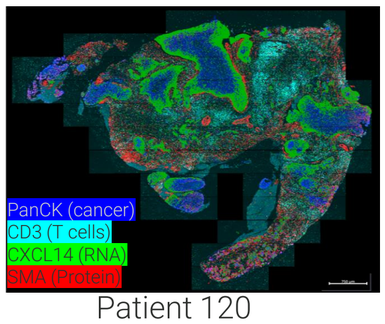
Data generating process for the TCR counts

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

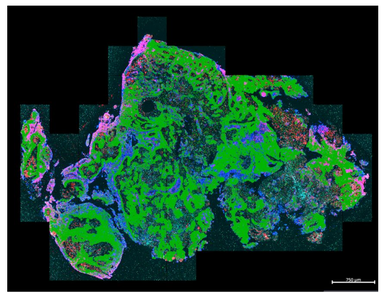
Counts from TCR genes for that clonotype ($g \in t$) are expressed 'above background for that cell'

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

Two patients: same disease diagnosis = same therapies, but totally wrong on both fronts

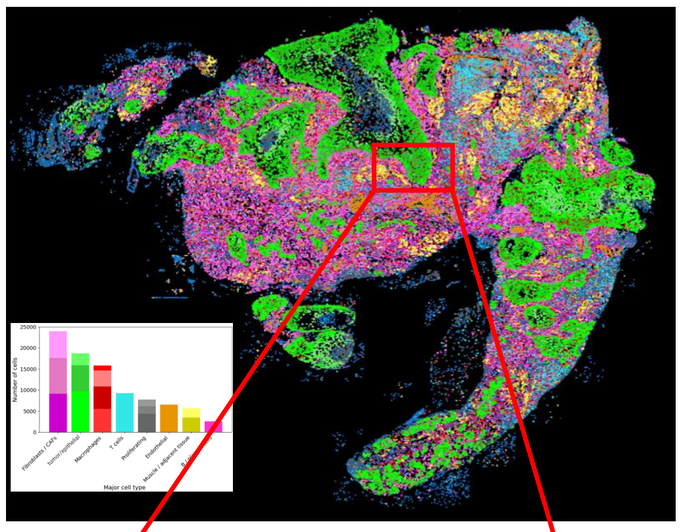


Patient 120
226,117 cells
31,837 T-Cells

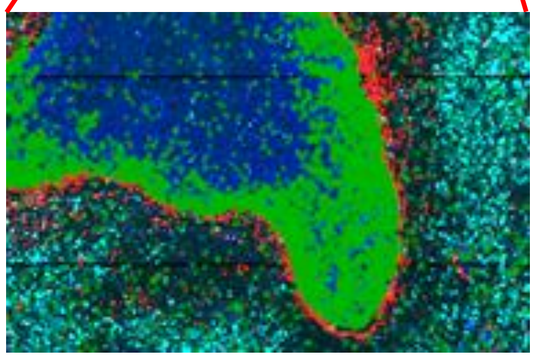


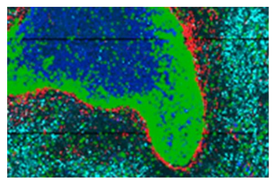
Patient 137
165, 817 cells
6500 T-Cells

Patient 120 (left) has 5X the number of T-cells, but NO "killer" T-cells as determined by live-cell Beacon assay. Why?

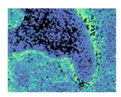


T-cells (light blue), are absolutely excluded from the tumor nests (green). Why?

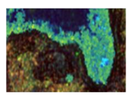




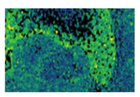
Protein-layer of data, show an incredible smooth-muscle-actin "wall" (red) around the complete tumor nest



RNA-layer of data (1): Extra-Cellular Matrix rearchitecturing



RNA-layer of data (2): Interferon Signalling pathways



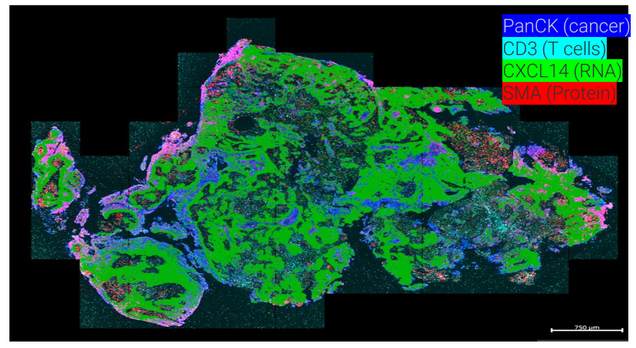
RNA-layer of data (3): non-productive antigen presentation by macrophages

T-cells can't get in

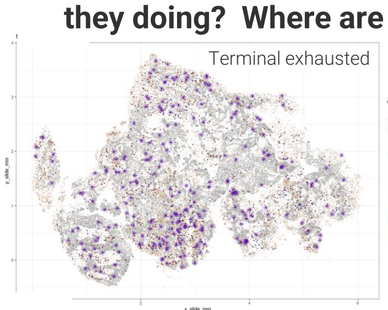
If T-cells get "close" they get totally "turned-off" by these mechanisms

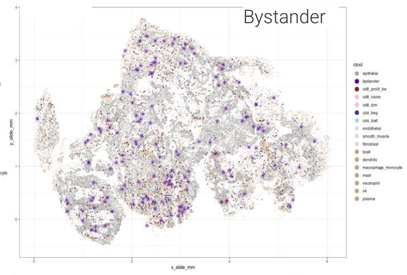
High-plex Protein same-slide, subcellularly layered-onto every protein-coding pathways (mRNA) in WTX: the mechanism of immune evasion is clear

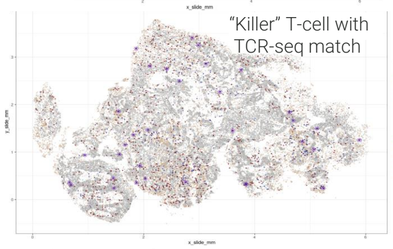
Patient 137 has good T-cell infiltrate, AND proven T-cells that CAN kill tumor cells, why doesn't this patient get better?



Where are all the T-cells in this tumor, and what are they doing? Where are the Killer-T-cells?





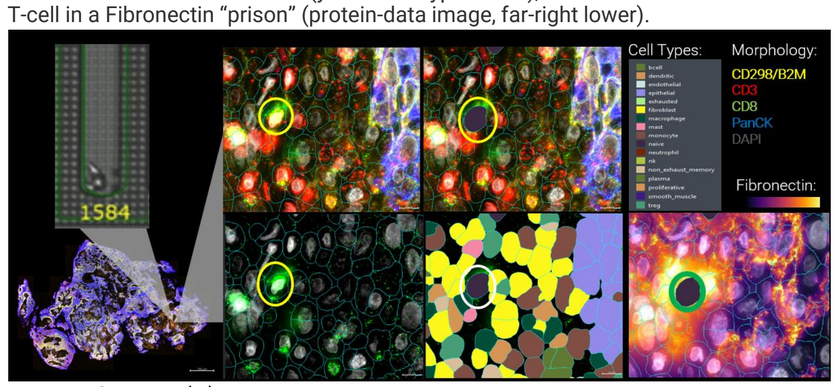


Only 0.5% of T-cells in tumor (33/6500) can actually "kill"! And those that can, actually "can't"…

Cell Type	Cell Number
Total Cells in Biopsy	160,000
Total T-cells in Biopsy	6500
T-Regs	1375
T-Naïve	1130
T-memory	1009
T-exhausted	1523
T-proliferative	1463
T-functional-killing (as measured)	33

Example of a single "killer" T-cell clone measured "live" then measured with "TCRseq-Match" in space.

Measured killer T-cell active in well #1584 of Beacon (left below), followed by exact clonotype match location in tissue (far left lower). Single cell expanded images: T-killer cell is surrounded by Cancer Associated Fibroblasts (yellow cell-type below), and the CAFs have encased that T-cell in a Fibronectin "prison" (protein-data image, far-right lower).



Conclusion(s):

(1) For the first time, a method has been developed that allows live cell functional measurements of surgical biopsies (specifically => can this T-cell kill a primary tumor cell, and if yes, what is the T-cell repertoire) followed by CosMx whole-transcriptome multi-omic profiling with T-cell repertoire VDJ "TCRseq spatial matching" spike-in panel to locate killer T-cells in spatial context

(2) The complete-nature of whole transcriptome spatial imaging allows ALL of the pathways of immune evasion to be directly measured (with many new discoveries)

3. The Protein-Layer of information is absolutely crucial, as protein-based physical-boundaries play a key role in immune exclusion (both macroscopic in panel 2, and single-cell-based panel 3)

Scan here to download or learn more

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