

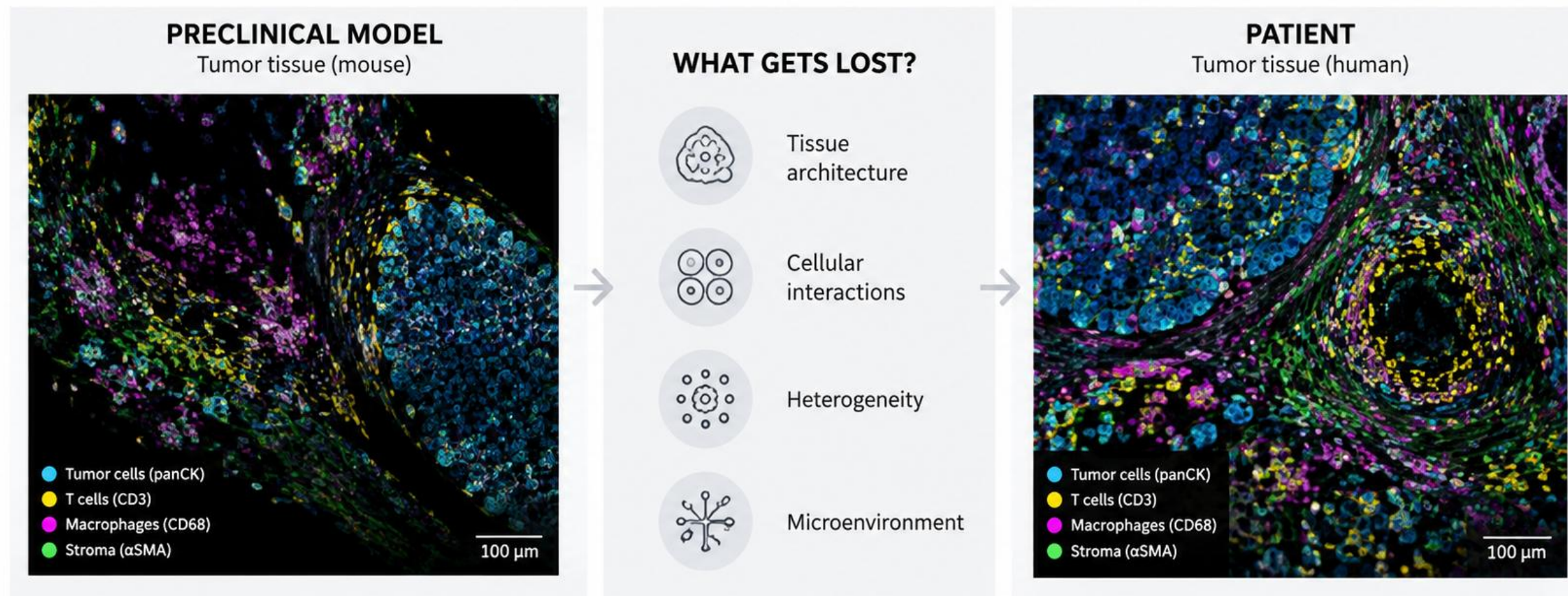
From Spatial Biomarkers to Development Decisions

Practical Applications Across Preclinical and Clinical Drug Development

Corinne Ramos, PhD — Aliri

The Translational Gap Is a Context Gap

Spatial analysis tests whether the mechanism occurs in the relevant compartment, cell population and microenvironment.

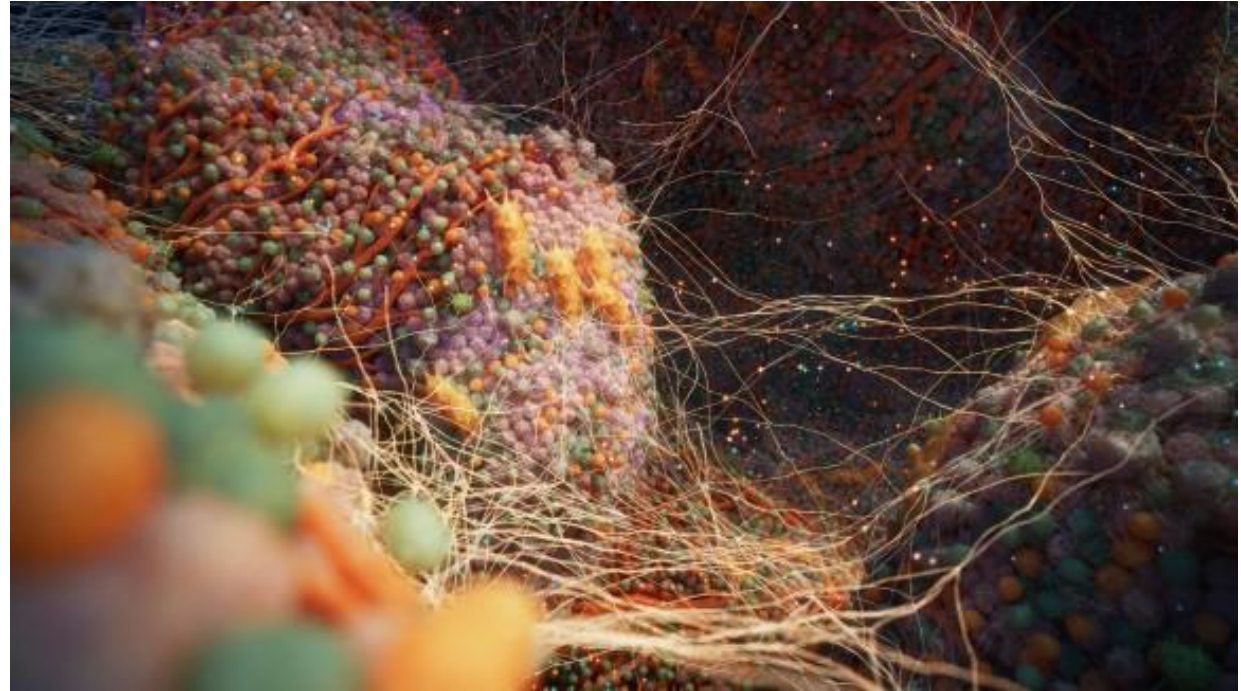


Mechanism can be correct, but its context can change

Why Spatial? Biology Happens in Context

Spatial biology maps cells and their molecules *in situ* within intact tissue linking identity and function to precise location and nearby neighbors.

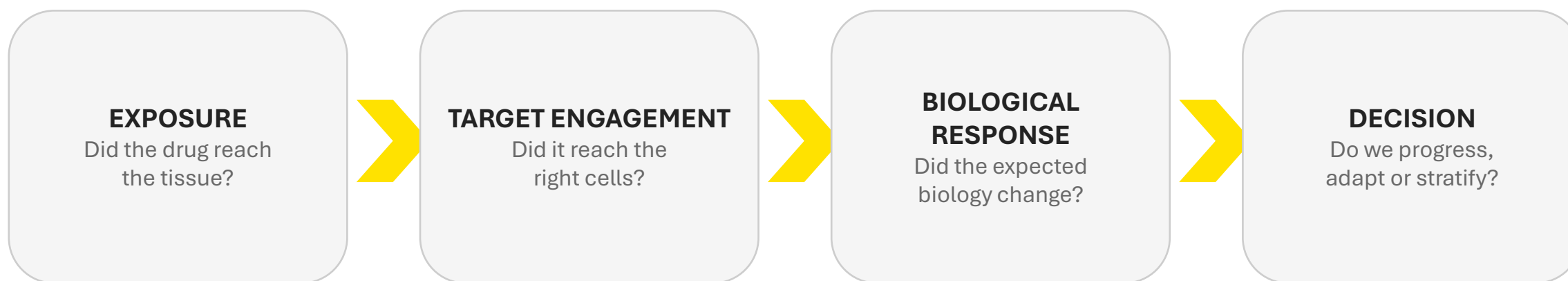
- What it is: Analysis of cells within intact tissue with architecture preserved.
- What it measures: RNA/protein at single-cell resolution *in situ*.
- Key feature: Maintains tissue architecture: regions, gradients, and boundaries.
- Quantify who the cells are, where they are, and what they are doing (state/signaling) in intact tissue.



Tissue biology is organized: cells interact across boundaries, gradients and neighborhoods.

From Measurement to Decision

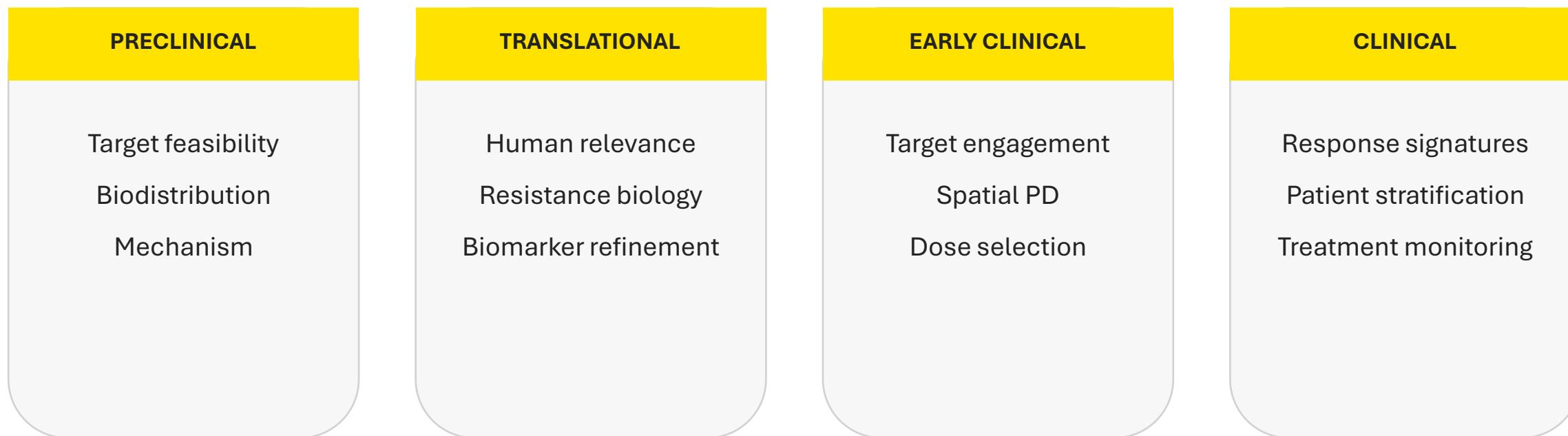
The value of a spatial biomarker is not the image — it is the decision it enables.



Spatial context converts concentration and expression into mechanistic evidence.

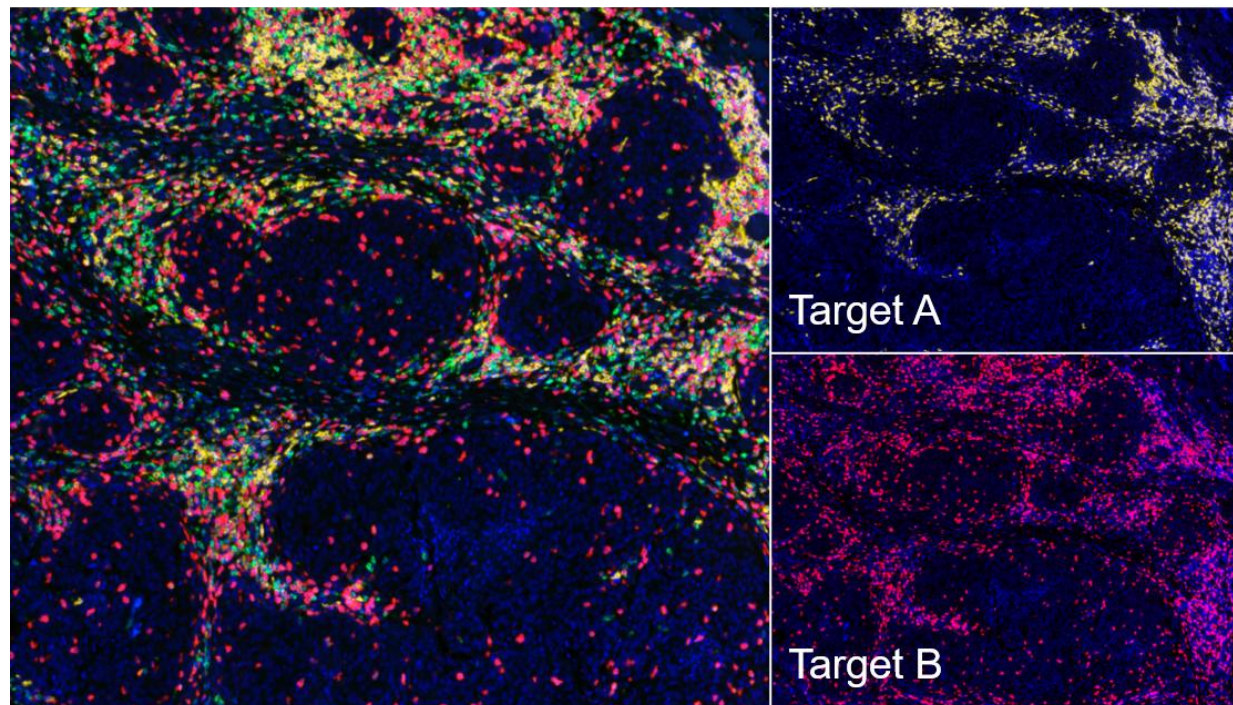
Spatial Biomarkers Across Drug Development

The biological question evolves — the mechanistic thread should remain continuous.



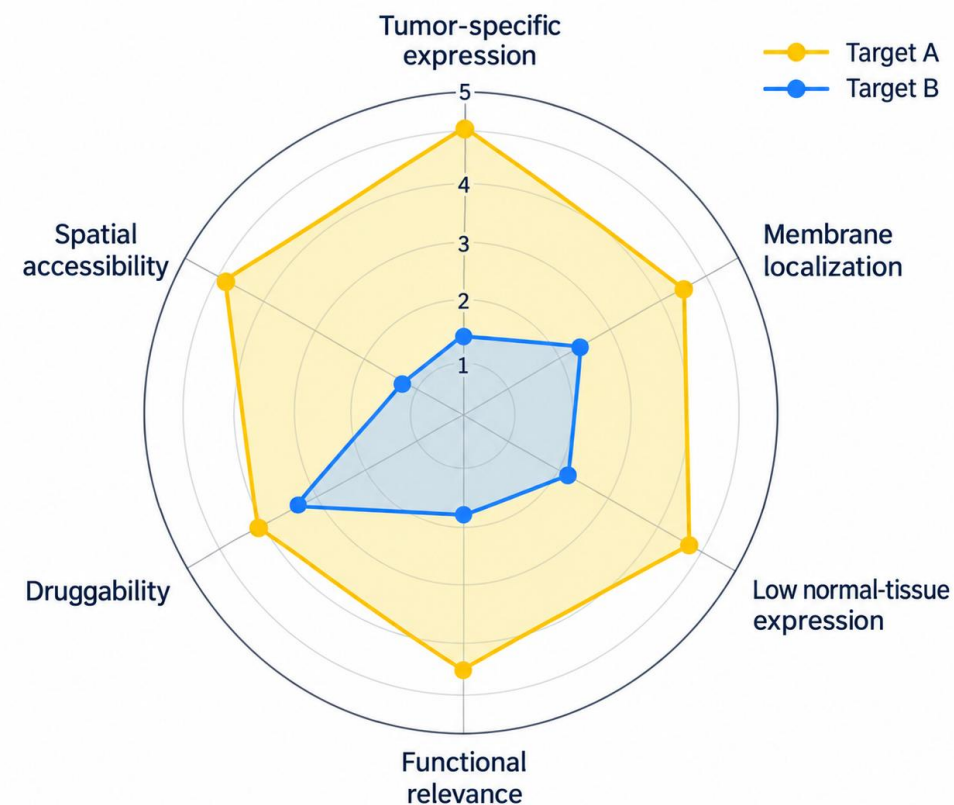
The role of spatial biomarkers evolves with the program, while the biological question remains continuous.

Application 1 — Is the Target in the Right Place?



Representative multiplex IF showing differential target expression and spatial distribution in human CRC

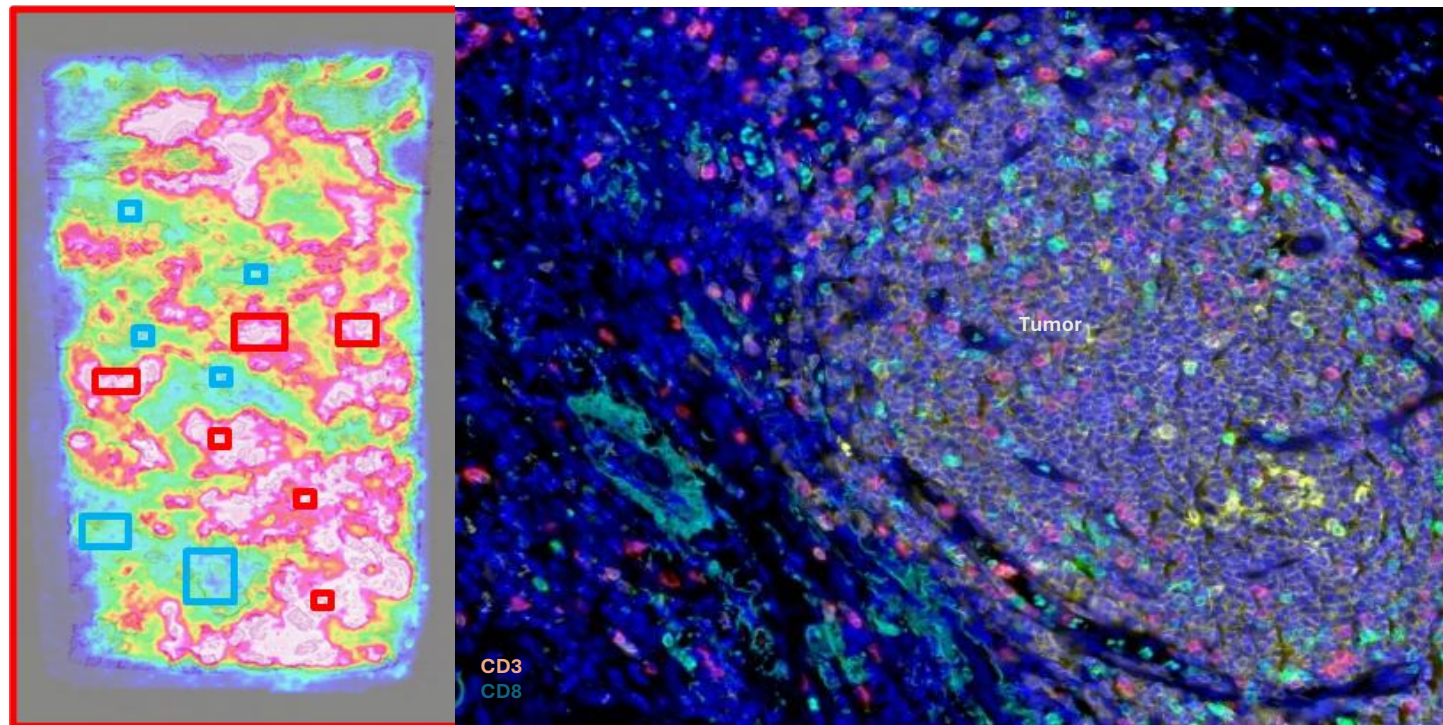
ADC Target Scoring Comparison



Decision: is this target truly druggable in tissue?

Target Heterogeneity Changes the Development Question

- Uniform expression supports broad target coverage.
- Heterogeneous expression creates target-negative niches and variable payload exposure.
- Spatially inaccessible target can remain biologically irrelevant despite high average expression.



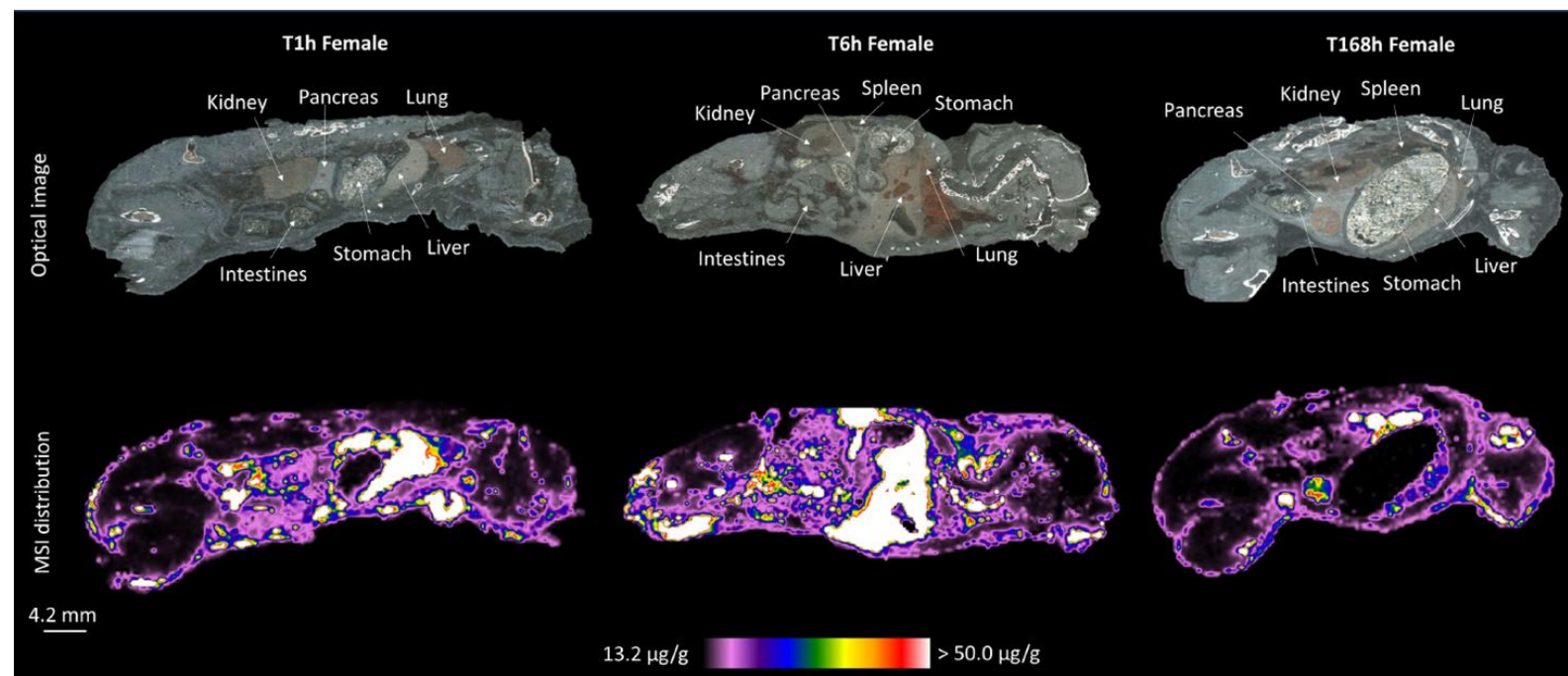
Decision → Do we progress the target, adapt the modality or define a patient-selection strategy?

Application 2 — Does the Drug Reach the Intended Tissue?

Plasma exposure cannot establish whether drug reaches — and persists in — the tissue compartment where the mechanism must occur.

- Quantify tissue exposure and regional distribution.
- Identify retention, penetration and potential off-target accumulation.

Tissue PK reveals distribution and retention than plasma PK cannot

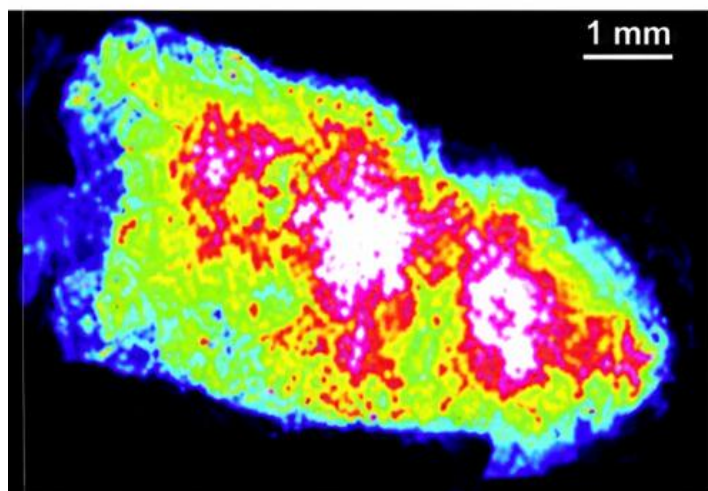


Decision → Is tissue exposure sufficient to support the intended mechanism and dose?

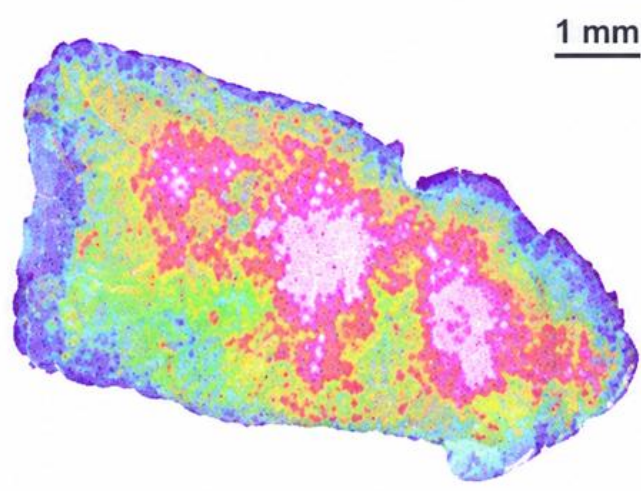
Exposure Is Not the Same as Delivery

A measurable tissue concentration does not prove delivery to target-positive cells.

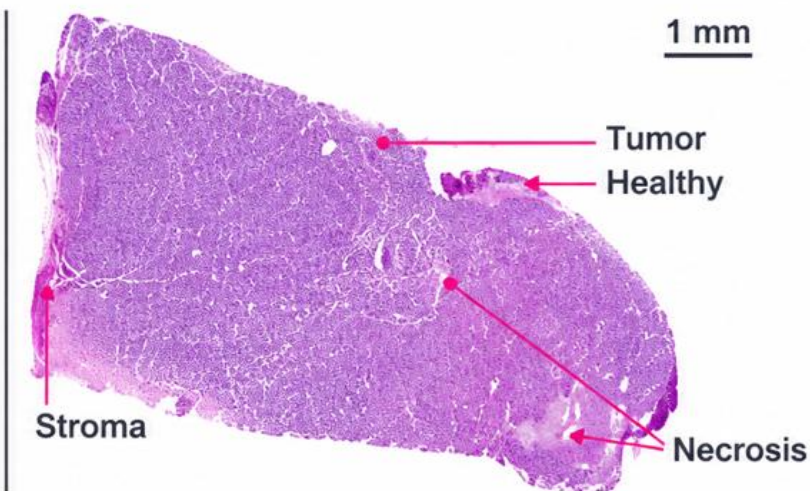
ADC payload



Overlay with histology



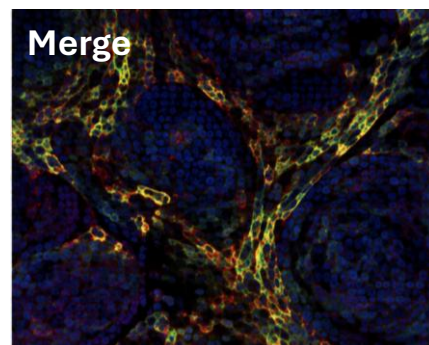
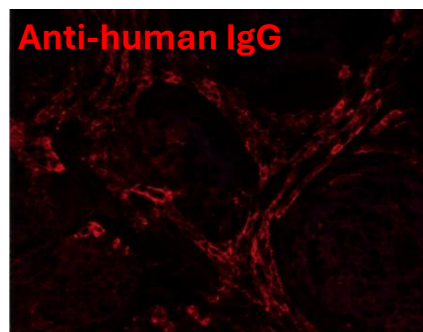
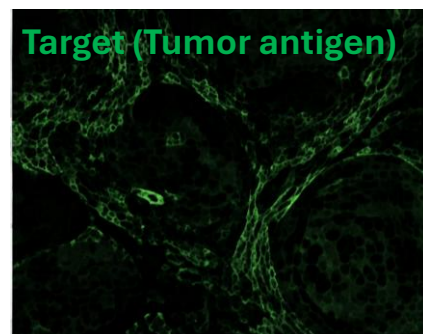
H&E (histology)



- Co-localize drug and target within intact tissue.
- Distinguish vascular or stromal trapping from productive tumor-cell delivery.
- Resolve heterogeneous penetration that bulk tissue PK cannot detect.

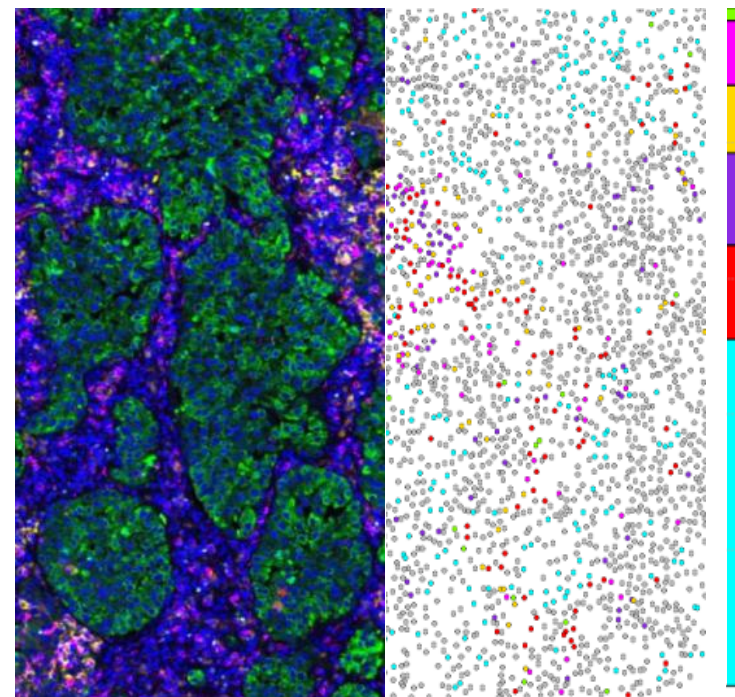
Decision → Did the drug reach the right cells at a biologically meaningful level?

Application 3 — Did We Engage the Mechanism?

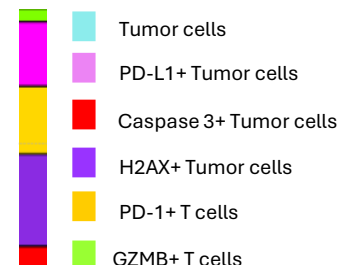


- ✓ Confirms ADC binding in real tissue
- ✓ Increases confidence in mechanism-of-action
- ✓ Justifies early dose range selection

Single cell spatial phenotyping

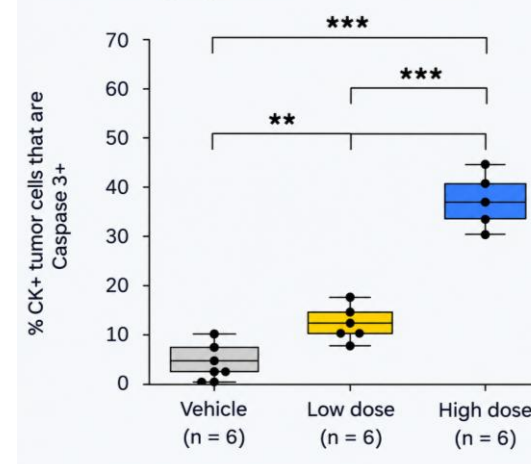


Quantification



- ✓ Validates that payload induces expected biology
- ✓ Supports selection of biologically effective dose
- ✓ Strengthens mechanistic PK/PD relationships

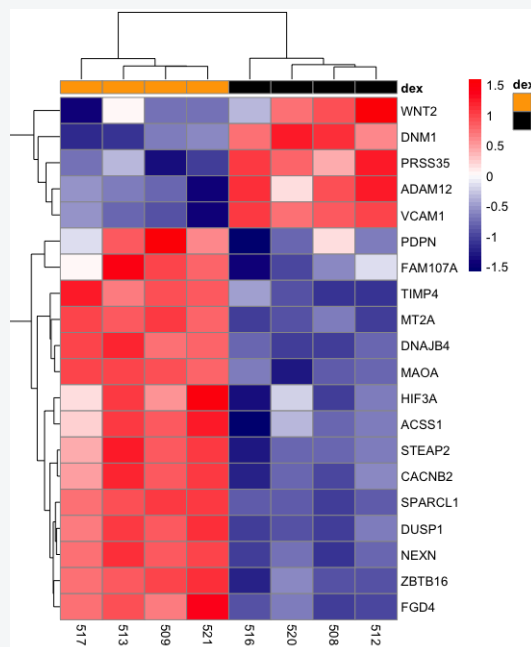
Tumor Apoptosis



Decision → Does target engagement translate into the expected pharmacodynamic effect in the relevant cells?

Spatial PD Reveals What Bulk Measurements Miss

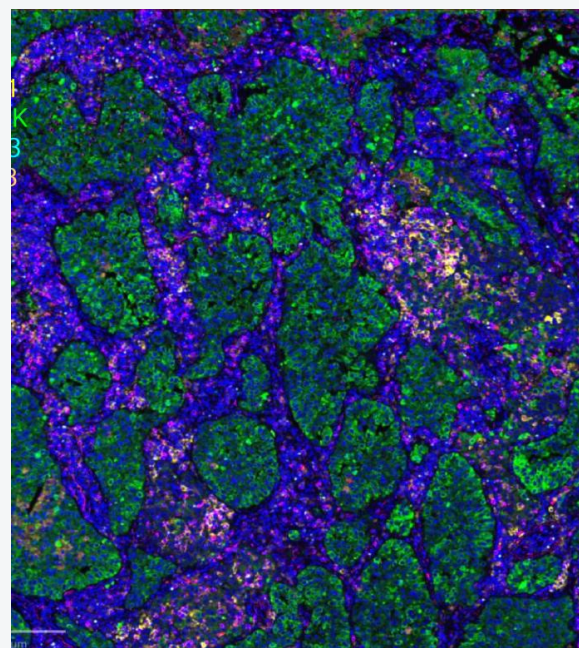
BULK READOUT



Expression change

Useful for detecting a signal, but the tissue average cannot tell which cells changed or where the biology occurred.

SPATIAL READOUT

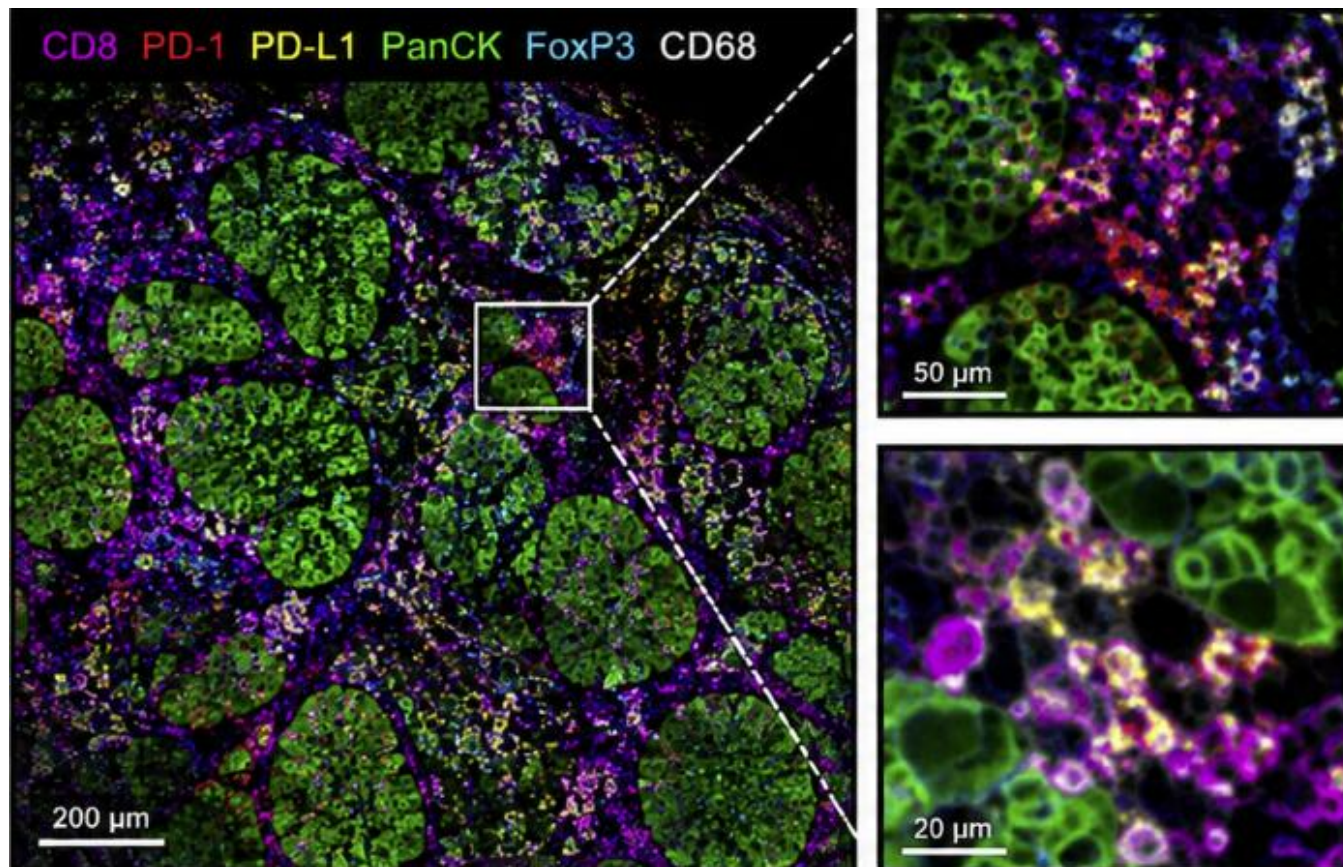


Context + mechanism

Preserves tumor, immune and stromal architecture to quantify cell state, infiltration, proximity and exclusion.

Decision → Is the observed molecular change occurring in the cells and compartment required by the mechanism?

Application 4 — How Does Treatment Reshape the Microenvironment?



Mechanistic spatial endpoints

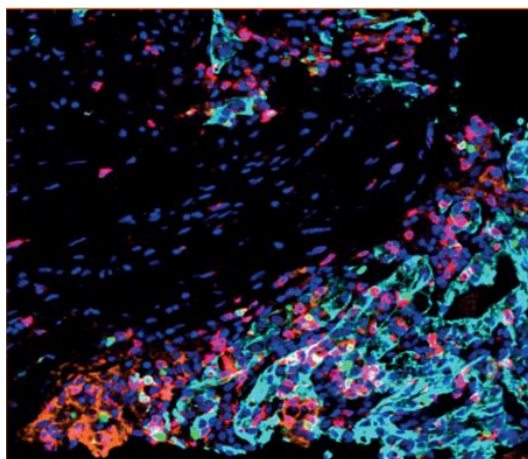
- Immune-cell infiltration into tumor compartments
- Tumor-immune proximity and cell-cell interactions
- Changes in suppressive or activated immune phenotypes
- Stromal organization and immune-exclusion boundaries

A treatment-induced change is most informative when it occurs in the compartment predicted by the mechanism of action.

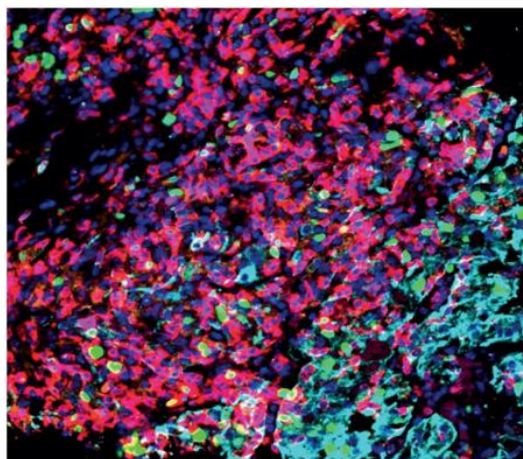
Decision → Is the therapy producing the expected pharmacodynamic remodeling in tissue?

Spatial Relationships Can Separate Responders from Non-Responders

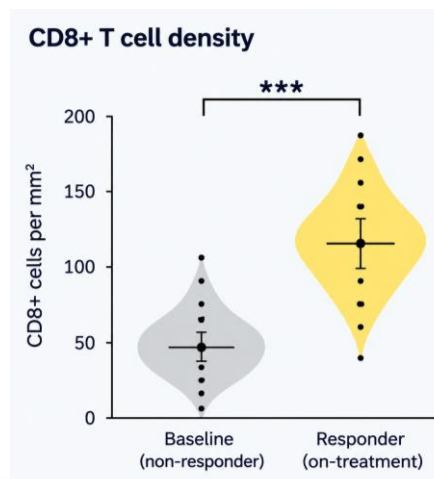
Baseline



Responder



CK/CD8 DAPI



- ✓ Identifies drivers of response and resistance
- ✓ Guides combination strategies
- ✓ Informs indication selection or expansion

Stratification hypothesis

Immune-hot

- High CD8 infiltration
- T cell activation
- Higher likelihood of response

→ **Single agent**

Intermediate

- Mixed immune/stroma
- Potential benefit from combinations

→ **Combination strategy**

Immune-cold

- Low immune infiltration
- Stromal barriers
- Higher risk of resistance

→ **Alternative approach**

TME remodeling

CD8 infiltration • macrophages • stroma • cytokine gradients

Stratification hypothesis

Decision → Can spatial architecture support stratification, combination strategy or indication selection?

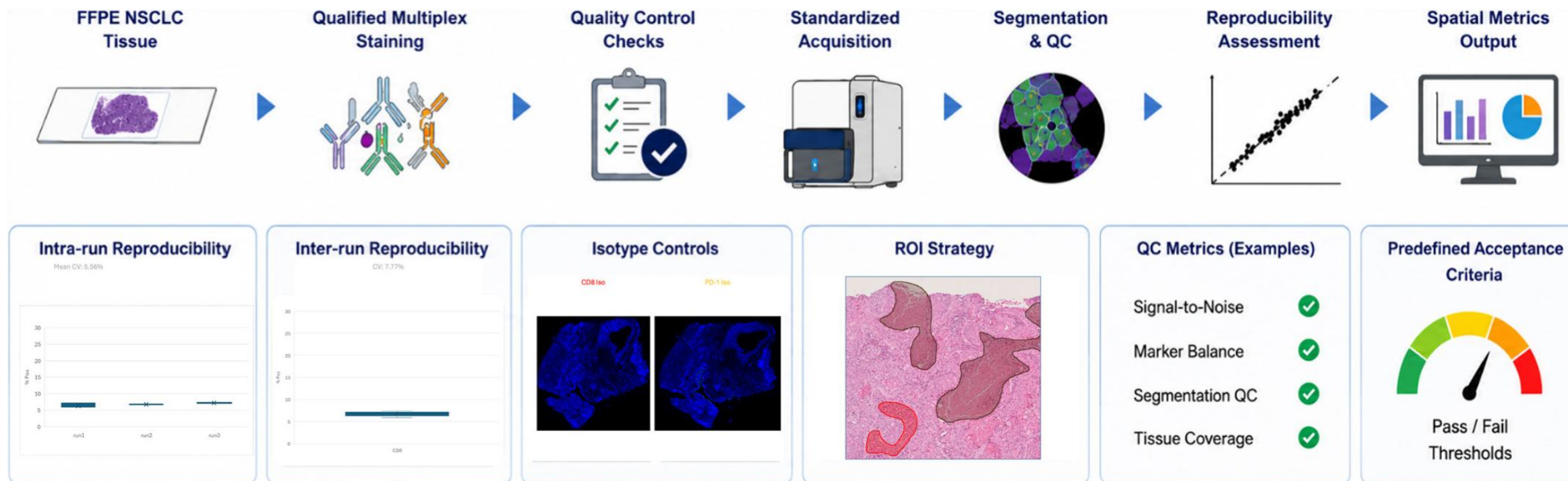
From Discovery Biology to a Clinical Biomarker

Clinical translation is a process of reducing complexity without losing the biology that predicts outcome.



Reducing Complexity. Preserving Biology. Enabling Clinical Decisions

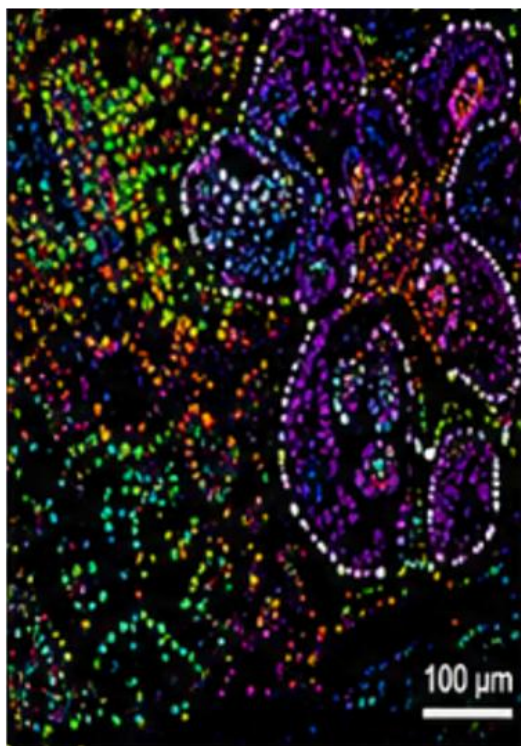
Clinical Spatial Biomarkers Must Be Fit for Purpose



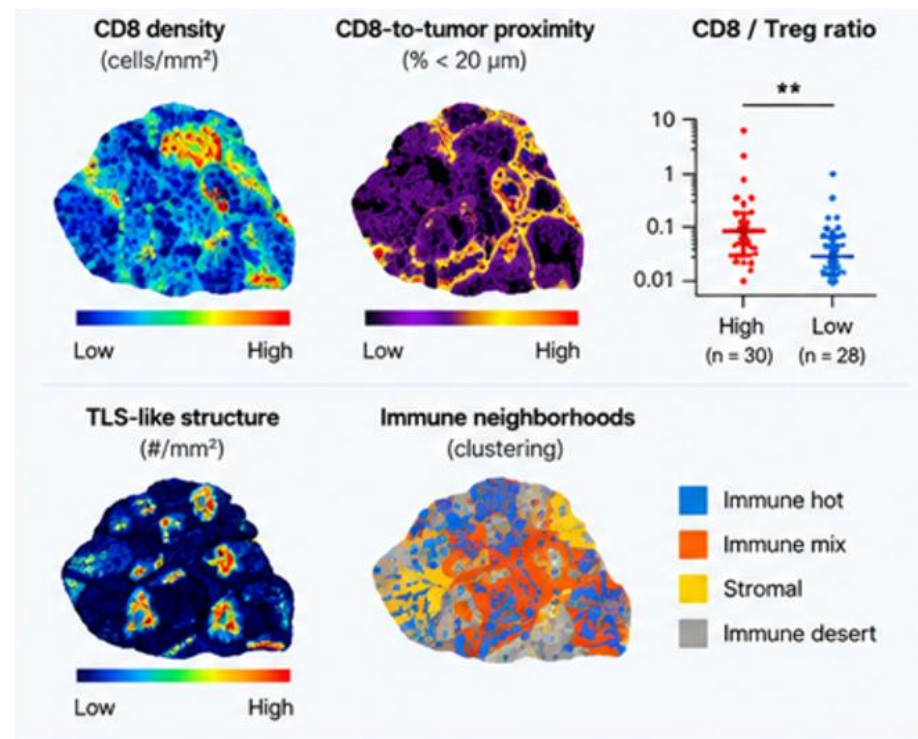
Decision → Is the endpoint robust enough to support dose, stratification or response decisions in a clinical study?

Spatial Biomarkers Create Decision Points

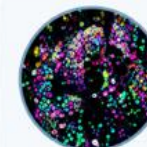
Multiplex tissue



Quantitative spatial metrics

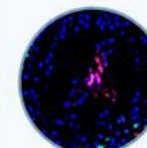


Biological interpretation



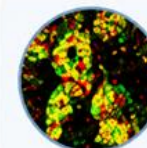
Inflamed

- High CD8, organized structures
- Immune active



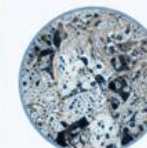
Excluded

- Immune cells present
- Limited tumor access



Suppressive

- High suppressive markers
- (low CD8, high Treg, etc.)



Immune desert

- Low immune infiltrate
- Stromal dense

Take-Home: Measure What Changes the Decision

- 1. Spatial context turns expression into biological relevance.**
- 2. Mechanistic biomarkers connect exposure to response.**
- 3. The best spatial endpoint is the one that changes a development decision.**

From spatial biomarkers → to mechanistic confidence → to better development decisions