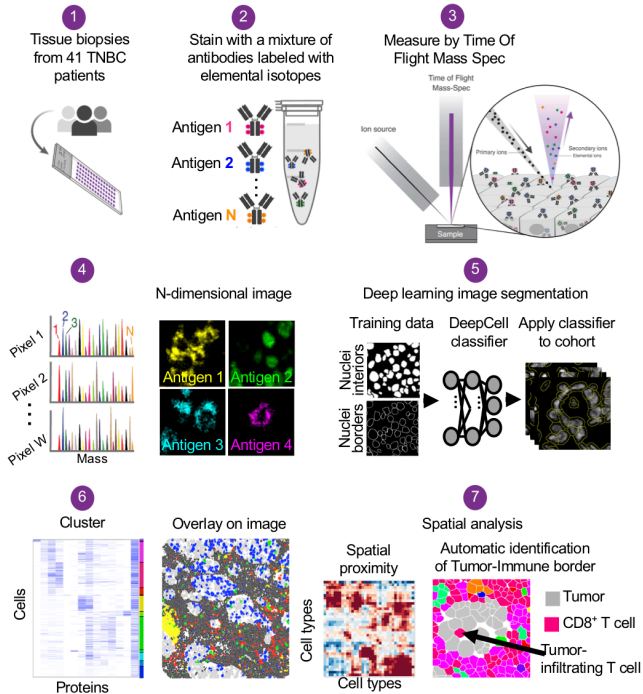# A Structured Tumor Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Imaging

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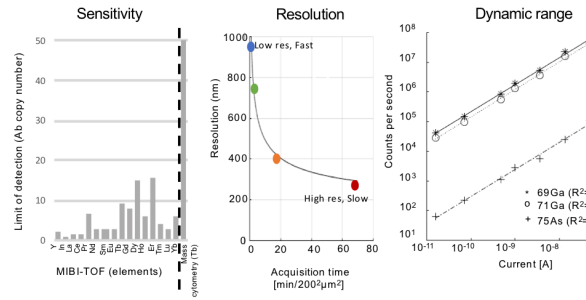1 Department of Pathology, Stanford University, California, USA

Abstract

The immune system is critical in modulating cancer progression, but knowledge of immune composition, phenotype, and interactions with tumor is limited. We used Multiplexed Ion Beam Imaging by Time-of-Flight (MIBI-TOF) to simultaneously quantify in-situ expression of 36 proteins covering identity, function and immune regulation at sub-cellular resolution in 41 triple-negative breast cancer patients. Multi-step processing, including deep-learning-based segmentation, revealed variability in the composition of tumor-immune populations across individuals, reconciled by overall immune infiltration and enriched co-occurrence of immune subpopulations and checkpoint expression. Spatial enrichment analysis showed immune mixed and compartmentalized tumors, coinciding with expression of PD1, PD-L1 and IDO in a cell-type- and location-specific manner. Ordered immune structures along the tumor-immune border were associated with compartmentalization and linked to survival. These data demonstrate organization in the tumor-immune microenvironment that is structured in cellular composition, spatial arrangement, and regulatory-protein expression and provide a framework to apply multiplexed imaging to immune oncology (Keren et al., Cell 2018).
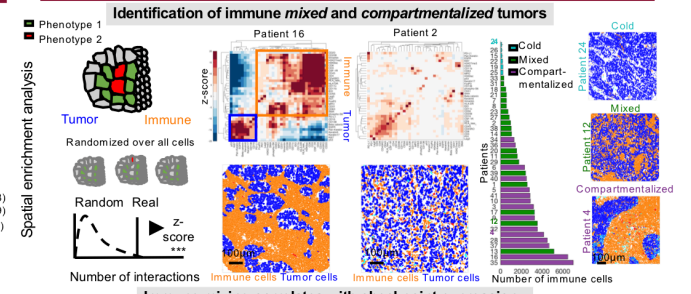
## MIBI Experimental & Analytical Pipeline

1. Tissue biopsies from 41 TNBC patients
2. Stain with a mixture of antibodies labeled with elemental isotopes
3. Measure by Time Of Flight Mass Spec
4. N-dimensional image
5. Deep learning image segmentation
6. Cluster / Overlay on image
7. Spatial analysis

MIBI Analytical Properties

Sensitivity / Resolution / Dynamic range

Structured Immune Composition in TNBC

Tumor-Immune Mixing Correlates to Checkpoint Expression & Survival

Identification of immune *mixed* and *compartmentalized* tumors

Immune mixing correlates with checkpoint expression
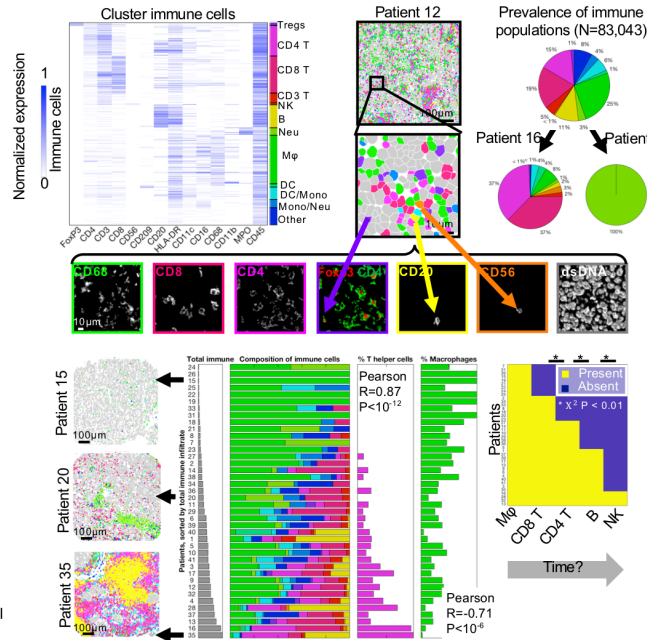
Unique expression on tumor-immune border identifies patient clusters
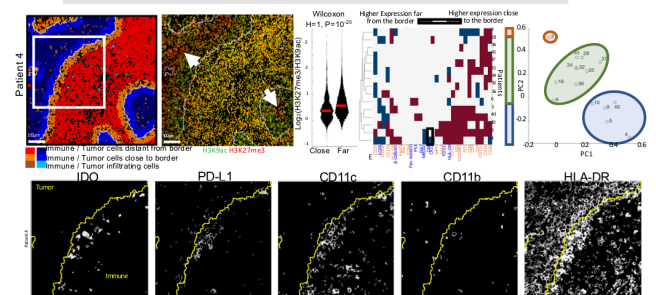
Tumor-immune architecture is correlated with survival