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Spatially-mapped quantitative single-cell characterization of PD-L1-related RNA microenvironments in triple-negative breast cancer

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Abstract

Triple-negative breast cancer (TNBC) is an aggressive disease with limited therapeutic options. PD-L1-targeted checkpoint blockade has shown efficacy in a subset of patients, but therapeutic response prediction's immune and other biomarker correlates are not well understood. NanoString's new Spatial Molecular Imaging (CosMx ™ SMI) platform is designed to generate high-plex, single-cell quantitative RNA profiles with preservation of spatial context. In this study, we used SMI technology to analyze the single single-cell spatial transcriptomic landscape of TNBC. Specifically, our initial goal was to identify and map PD-L1+ cells (verified with clinical FDA-approved PD-L1 companion assays) within user-defined fields of view, to characterize differences between PD-L1+ and PD-L1- immune and/or epithelial cells, and to elucidate molecular features associated with interactions between such cells and neighboring cells within the tumor microenvironments. FFPE tissue biopsies taken at diagnosis or disease progression were profiled using SMI with a 1000-plex human immuno-oncology RNA panel. The results of these studies provide fundamental new insight into the immune architecture of TNBC tumors, and such insight into the spatial immune landscape of TNBC may inform a rational approach to (combinatorial) immunotherapy in patients with TNBC.

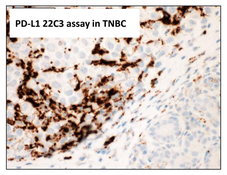
Study aim and Sample information

Study aims:

- Determine impact of spatial context on immune cell transcriptomes in TNBC
- Determine impact of spatial context on neoplastic and immune cell PD-L1-associated transcriptomic profiles in TNBC
- Comparison of transcriptomic profiling in TNBC using CosMX ™ and GeoMx® CTA platforms

Study materials:

- 5 µm sections from well-characterized Mayo TNBC FFPE tissue micro-array (composed of 200+ early-stage treatment naive tumors) with clinical PD-L1 22 C3 status
- CosMx ™ 1000 plex human immuno-oncology RNA panel

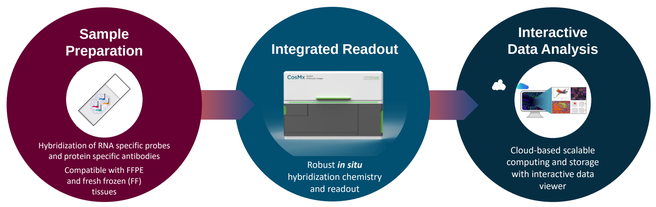


TNBC cores on a slide

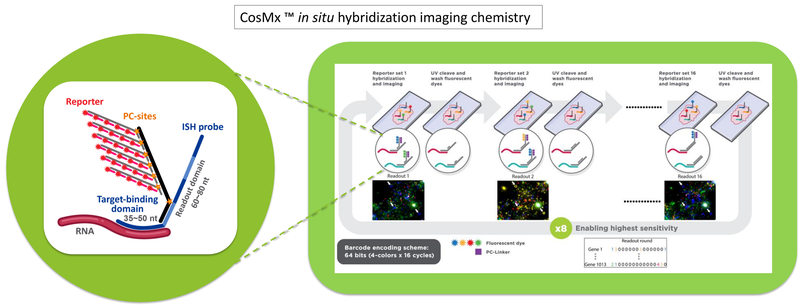
CosMx ™ and GeoMx® assays

CosMx ™ assay

CosMx™ SMI is a single instrument solution for subcellular spatial analysis



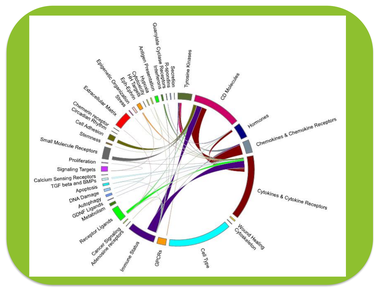
CosMx ™ *in situ* hybridization imaging chemistry



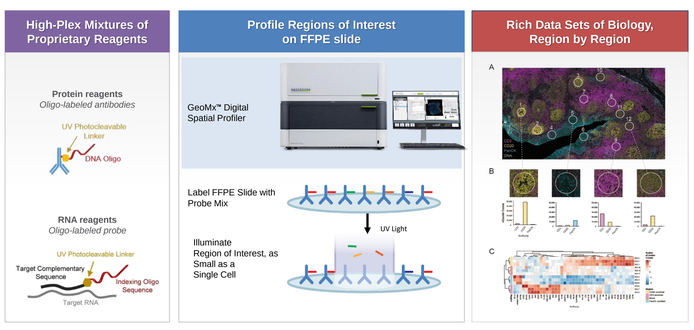
Human Universal Cell Characterization Panel

1000-Plex RNA Panel for Cell Typing and Cell-Cell Interaction Studies

Applications	# of genes
Cell typing and mapping	243 genes
Cell state and function	269 genes
Cell-cell interaction (ligand-receptor)	435 genes
Hormone activities	46 genes



GeoMx® assay

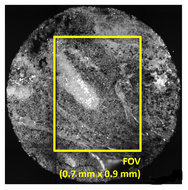


CosMx ™ molecular cell type classification of TNBC

Dataset metrics overview

Data summary	
Tissue type	FFPE TNBC TMA
Panel	960 gene targets
Number of FOVs for final analysis	75
Total tissue area analyzed (XY plane)	133 mm^2
Number of total cells	245,487
Number of cells passed QC	238,515
% of cells passed QC	97%
Transcripts detected	91,596,217
% of transcripts assigned to cell	91%
Mean transcripts per cell	339
Mean negative counts per cell	1.27

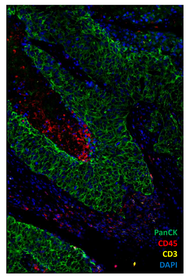
1. ROI selection



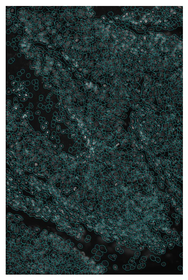
FOV: Field of View

2. Target imaging

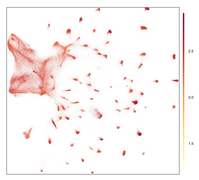
3. Morphology imaging



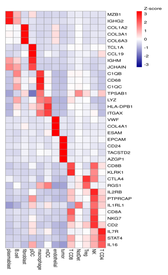
4. Cell segmentation



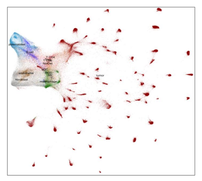
5. Individual cell expression profiling and clustering



6. Cell cluster characterization



7. Cell type determination



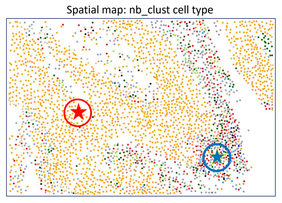
8. Mapping cell types to individual cells

Spatial maps of nb_clust cell types for FOVs

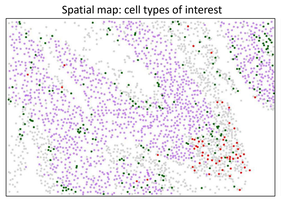
CosMx enables spatial transcriptomics at single cell resolution

Spatial maps of cell type and transcript

Spatial map: nb_clust cell type

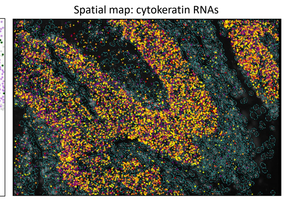


Spatial map: cell types of interest



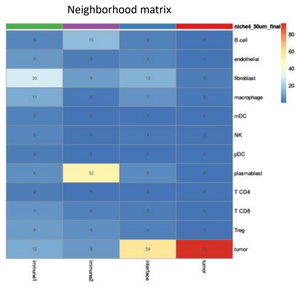
Tumor cell, macrophage, T cell

Spatial map: cytokeratin RNAs

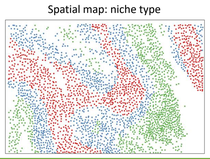


Neighborhood analysis

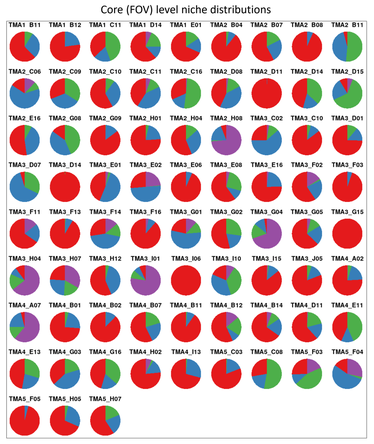
Neighborhood matrix



Spatial map: niche type



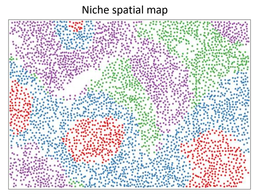
Core (FOV) level niche distributions



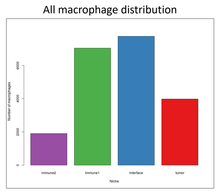
Macrophages in different niches show distinct molecular signatures

CD274(+) macrophages are less abundant in tumor niche

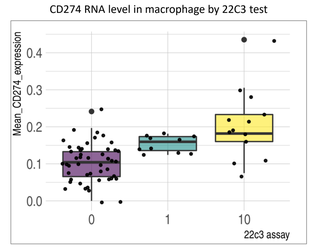
Niche spatial map

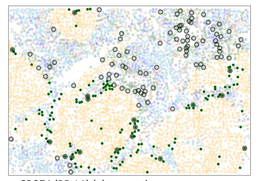


All macrophage distribution



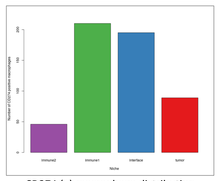
CD274 RNA level in macrophage by 22C3 test





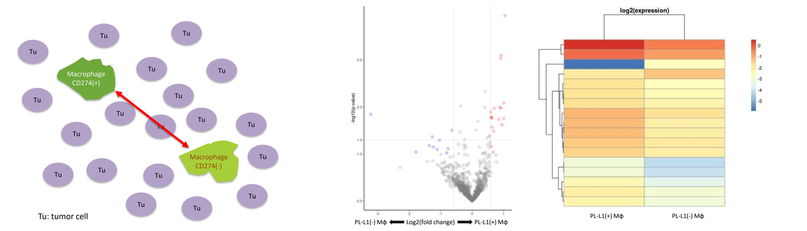
CD274 (PD-L1) (+) macrophage

PD-L1(+) in tumor PD-L1(+) in interface



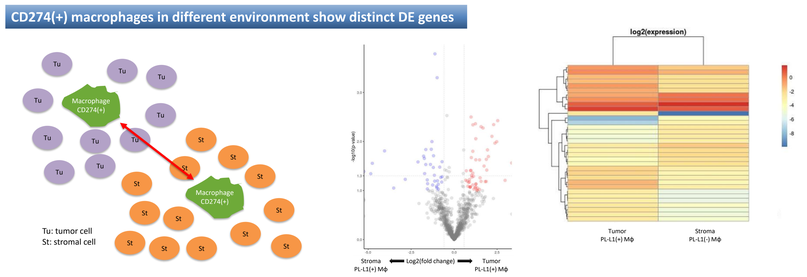
CD274 (+) macrophage distribution

CD274(+) and CD274(-) macrophages show distinct DE genes



DE gene analysis of macrophages by CD274 expression in tumor environment

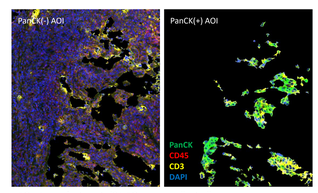
CD274(+) macrophages in different environment show distinct DE genes



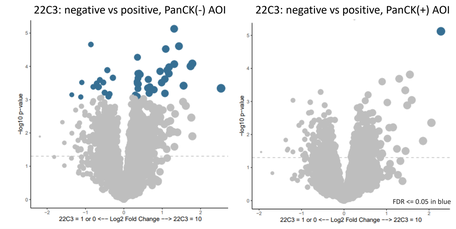
DE gene analysis of CD274(+) macrophages by environments

GeoMx® spatial transcriptomics and cell type deconvolution

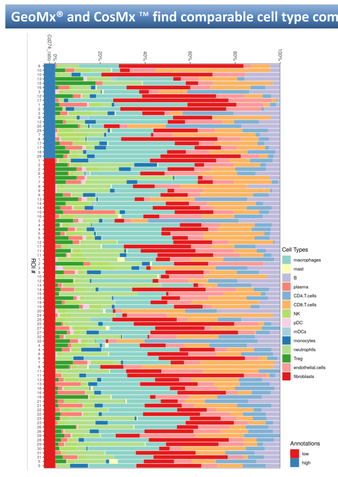
DE analysis of PanCK(+) vs PanCK(-) regions by 22C3 test results



AOI: Area of Interest: (94 AOIs were collected)



GeoMx® and CosMx ™ find comparable cell type compositions



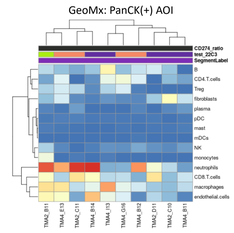
GeoMx cell type deconvolution:

Gene expression patterns are deconvolved based on a training matrix of single-cell sequencing data.

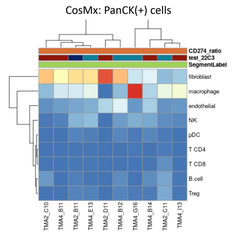
· GeoMx Cancer Transcriptomics Atlas (CTA) panel: >1800 genes

· CTA and CosMx panels share 570 genes

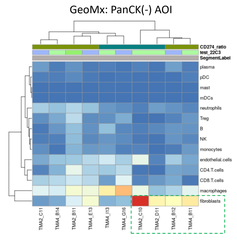
GeoMx: PanCK(+) AOI



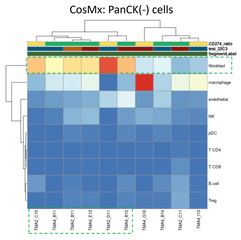
CosMx: PanCK(+) cells



GeoMx: PanCK(-) AOI



CosMx: PanCK(-) cells



Conclusions

- **CosMX ™ is a powerful new digital spatial platform with single-cell resolution of transcriptomic profiles obtained in single 5-micron FFPE tissue sections.**
- **In TNBC, the clinically-relevant spatial niches of PD-L1+(CD274+) macrophages were associated with differential expression of key genes involved in interferon and chemokine signaling.**
- **In TNBC, PD-L1 (CD274) status in macrophages, other immune cells, and neoplastic cells within the intraepithelial tumor niche, impacted differential expression of dozens of genes associated with interferon and chemokine signaling, and clinically actionable immune-based targets.**
- **Using deconvolution of spatial transcriptomic data obtained from GeoMx® in the same TNBC TMA (CTA panel with N=570 overlapping genes with CosMx ™ panel), similar distributions of immune cell types, and differentially expressed immune-related genes were identified in PD-L1+(CD274+) intraepithelial or stromal segments.**

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