Integrating bulk and spatial profiling technologies for the discovery of RNA and protein biomarkers in muscle invasive bladder cancer

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Introduction
Muscle-invasive bladder cancer (MIBC) is an aggressive disease with limited therapeutic options. Although immunotherapies are approved for MIBC, the ~40% of patients fail to respond, suggesting the existence of complementary immune evasion mechanisms underscoring the need for comprehensive immune profiling of patient tumor samples. The PPARγ pathway constitutes a tumor-intrinsic mechanism underlying immune evasion in MIBC. Immune cell infiltration is controlled by activated PPARγ, which in turn inhibits expression/secretion of inflammatory cytokines. Clinical data sets indicate that PPARγ expression is anti-immunotherapies.

Background & Study Design
PPARγ mediates immune exclusion in bladder cancer

Key Study Objectives
- Technical evaluation of bulk vs spatial genomics for assessing tumor microenvironment and defining MIBC molecular subtypes
- Define mechanisms of immune evasion in PPARγ/MIBC
- Evaluate the role of TMB (tumor mutation burden) and tumor somatic mutations on immune evasion and PPARγ expression
- Establish a solution for comprehensive immune profiling in clinical samples where tissue is limiting

Spatial organization of immune cells
GeoMx™ enables spatially resolved mRNA and protein quantitation

Fig 2: Nanostring GeoMx™ technology complements bulk genomics and IHC analyses. A) Overview of DSP Protein and ISH context and analysis methodology. B) Micromirrors define tumor and TME areas of illumination based on IF staining. C) Examples DSP IF images of PPARγ, TLX, TGFβ and PANDA, RAL3, TIS11 MIBC tumors. D) IHC illustrating tumor exclusion phenotype in PPARγ+ tumors and inverse correlation with tumor TMB.1

Quantification of immune exclusion phenotype in PPARγ+mRNA tumors

Fig 3: Immune markers are excluded from PPARγ+mRNA tumor compartment. A) Volcano plot illustrating immune exclusion. B) CD8 box plot illustrates quantification T cell exclusion into stroma. C) Volcano plot of differentially expressed genes in the tumor and stroma comparing PPARγ+mRNA vs PPARγ-

Tumor and stromal signaling
Detection and classification of molecular subtypes

Fig 5 Molecular subtyping is defined using GeoMx™ ISH platform. A) Tumor expression of GATA3, one of several subtype-associated genes, shown by patient sorted by average tumor expression. B) Heatmap of genes defining molecular subtypes measured by DSP ISH within tumor A08b. C) ROC analysis for DSP ISH defining luminal and basal molecular subtypes.

Signal pathways in the tumor and TME

Fig 6: WNT signaling is enriched in stromal compartment of PPARγ+mRNA tumors. A) Volcano plots of differentially expressed genes in tumor vs stroma of PPARγ+mRNA tumors and PPARγ-mRNA tumors. B) Pathway diagram of differentially expressed genes from between tumor and stroma.

Summary
- Bulk genomics profiling masks stromal expression patterns and mechanisms associated with immune exclusion in MIBC
- GeoMx™ DSP technology is compatible with limiting clinical sample and provides high-plex comprehensive data with just two FFPE slides
- High tumoral PPARγ expression is associated with a “immune cold” microenvironment and restriction of immune cells to the stromal compartment
- PD-L1 expression is anti-correlated with PPARγ expression suggesting mutually exclusive immune evasion mechanisms in luminal vs basal subtypes of MIBC
- Spatial Genomics uncovers potential novel strategies for targeting tumor-stromal interactions leading to metastasis, immune evasion and tumor progression