Background Pancreatic ductal adenocarcinoma (PDAC) often fails to respond to immune therapies due to various factors, including the role of epithelial to mesenchymal transition (EMT) plasticity in conferring broad resistance to diverse therapies.1-6 However, the relationship between cancer cell heterogeneity and the tumor immune microenvironment remains unclear. To address this, we utilized single nucleic RNA-seq and spatial transcriptomics to uncover the landscape of these cell-cell interactions in human PDAC,7 which we applied to specimens from two immunotherapy trials.

The first trial is based on TGF-beta being a major driver of EMT.7-11 Losartan, an indirect TGF-beta inhibitor,12 13 showed promise with combination chemotherapy FOLFIRINOX in an initial clinical trial.14 A randomized multi-institutional clinical trial of FOLFIRINOX +/- losartan +/- FOLFIRINOX +/- losartan +/- losartan +/- FOLFIRINOX +/- nivolumab (anti-PD1) for PDAC has been completed (NCT03563248).

The second trial focused on exploring the abscopal effect induced by radiation therapy when combined with immune checkpoint inhibition (anti-PD-L1 + anti-CTLA4) in PDAC tumors.15 A pilot trial demonstrated a 29% disease control rate with combined nivolumab + ipilimumab and radiation therapy.16 A follow-up Phase II single-arm study evaluating this combination in metastatic PDAC is completed (NCT04361162).

**Methods** Using the NanoString GeoMx Digital Spatial Profiler, we selected multiple regions of interest in formalin-fixed paraffin-embedded (FFPE) human PDAC specimens. Immunofluorescent antibody-guided isolation of RNA and protein from cancer cells (pan-cytokeratin), cancer-associated fibroblasts (alpha-SMA), and immune cells (CD45) were performed. Utilizing the whole transcriptome assay (WTA; 18,000+ protein-coding genes) and a new IO Proteome Atlas (IPA; 500+ plex proteins), we ventured to understand the relationship between tumor cells and the surrounding microenvironment.

**Results** PDAC cells, CAFs, and immune cells were successfully characterized using NanoString GeoMx in clinical trial specimens. Analysis revealed associations between cancer cell plasticity, TGF-beta signaling, and PDAC cell states (Epithelial and Mesenchymal). These differences were observed between Arm 1 (FOLFIRINOX) and Arm 2 (FOLFIRINOX+losartan) in resected neoadjuvant-treated PDAC tumors (figure 1). Immune deconvolution analysis identified variations in immune infiltrates, showing an anti-correlation between macrophages and T-cells (figure 2).

**Conclusions** Spatial transcriptomics and proteomics reveal insights into the spatial relationship between PDAC tumor cell EMT plasticity, CAFs, and immune infiltrates. This enables the discovery of novel immune response biomarkers and potential therapeutic avenues to target tumor and microenvironment interactions.

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**Trial Registration** DF/HCC protocol 18-179: Losartan and Nivolumab in Combination With FOLFIRINOX and SBRT in Localized Pancreatic Cancer. NCT03563248

**DF/HCC** protocol 19-587: Nivolumab + Ipilimumab + Radiation in MSS Pancreatic Cancer NCT04361162

**REFERENCES**


**Ethics Approval** All studies presented were approved by the Dana-Farber/Harvard Cancer Center IRB protocols 18-179 and 19-587.
Abstract 655 Figure 1  Gene expression heatmap of epithelial (Classical) and mesenchymal (Basal like) genes in tumor cells from patients on the FOLFIRINOX +/- losartan +/- nivolumab trial.

Abstract 655 Figure 2  Immune cell de-convolution from patients on the losartan trial demonstrating relative anti-correlation of T-cells and macrophages across arms

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