Spatial Profiling of the Immune Landscape of Solid Tumor Treated with Low Dose Radiation and Immunotherapy Using High Plex RNA Profiling with the GeoMx® Platform

Summary

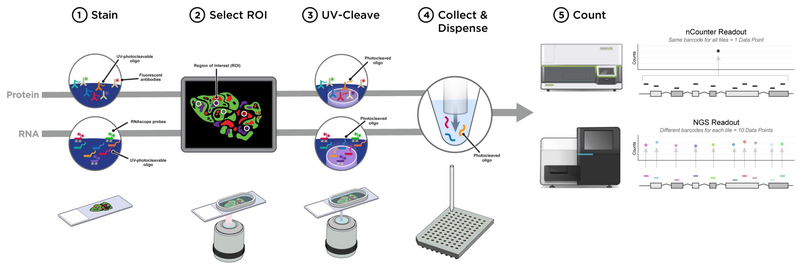
A challenge in the development of immunotherapies for the treatment of cancer is their low response rates for the majority of patients, and the underlying mechanisms are still under investigation. One treatment strategy that holds promise is the use of induction therapy to alter cancer cells or the tumor microenvironment to render them more permissive to immune checkpoint blockade. For example, the use of low dose radiotherapy may induce immunogenic cell death of the cancer cells with the release of residual tumor antigens, and restructure the tumor microenvironment by enhancing immune cell infiltration (. This hypothesis is being explored in a phase I clinical trial studying the combination of nivolumab plus ipilimumab associated with low-dose radiation in patients with advanced, TIL-negative solid tumors ("RAC-T" Radiation, Aspirin, Cyclophosphamide, Nivolumab, and Ipilimumab).

In order to characterize mechanisms of immune evasion at work prior to the start of immunotherapy, and changes induced in response to treatment, translational research efforts have focused on profiling the tumor and microenvironment prior to and during the treatment. In this study, we leverage high plex spatially-resolved RNA profiling on the NanoString GeoMx™ platform to characterize changes in the cellular and molecular landscape of the TME associated with inductive low-dose radiotherapy + low-dose chemotherapy in solid tumors. We identify changes in gene expression and immune signatures in specific cell populations which are associated with positive response to radiation-induced sensitivity to checkpoint inhibitor therapy. We also define aspects of the pre-treatment TME that are associated with favorable response to this induction strategy. These results provide useful insights into the mechanisms underlying immunogenic induction treatment regimens and may inform the design of future biomarkers that could be used to identify patients who would benefit from low-dose radiation induction. The spatial transcriptomics approach enabled by GeoMx is applicable to future studies aimed at dissecting complex microenvironments.

GeoMx™ DSP High-Plex RNA Workflow

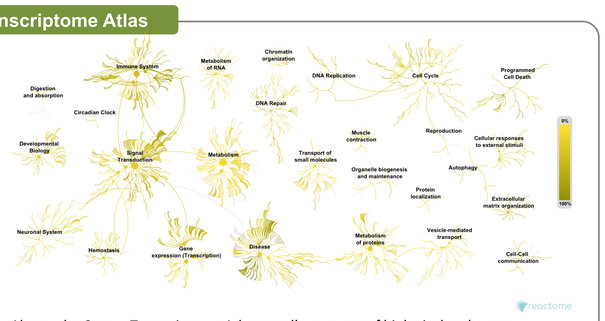
GeoMx™ Digital Spatial Profiler
Your GPS for Immuno-Oncology

The GeoMx DSP machine and chemistry allows for flexible interrogation of tissues. By specifying user-defined ROIs and reading out high-plex reagents the DSP workflow provides flexibility to specifically interrogate relevant regions in precious clinical sample.



GeoMx™ Cancer Transcriptome Atlas

The **Cancer Transcriptome Atlas (CTA)** contains over 1800 genes, representing comprehensive coverage of the tumor, immune system, and microenvironment in cancer.



Above: the Cancer Transcriptome Atlas panel's coverage of biological pathways

RACIN Study

Study Population

Patients with locally advanced or metastatic incurable solid tumors that are Tumor Infiltrating Lymphocytes (TIL)-negative

Hypothesis

Low doses of a DNA-damaging agent and radiation will initiate proinflammatory cell death and prime immune responses in cold tumors.

Treatment

Combination Therapy:
- Low dose cyclophosphamide
- Low dose ionizing radiation

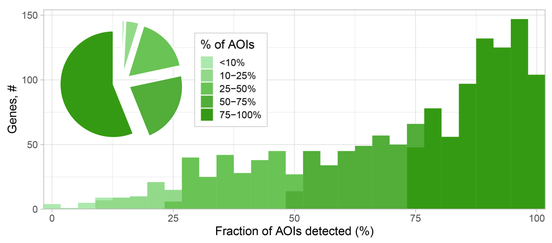
Endpoints

- Safety
- Tolerability
- Objective Response (Exploratory)

NCT03728179

The Cancer Transcriptome Atlas robustly detects genes within this study

The pre-release assay used as part of this study was designed to measure the expression of 1400+ genes. Shown right is the number of regions of interest in which a given gene was detected above the limit of detection. More than half of the panel was detected above background in more than 75% of ROIs.



Study Design

Pre- and post-treatment core needle biopsies

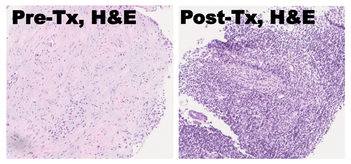
Sample	Tumor Histology	Biopsy Location	ROIs Included	
			Pre	Post
OXE2	Colon	Lung	8	7
1EEY	Prostate	Lymph Node	9	9
02F5	Ovary	Lymph Node	6	11
19F7	Gallbladder	Liver	8	8
OXUL	Prostate	Lymph Node	6	4

ROI Selection Strategy

Regions were selected for profiling based on H&E staining **(A)**, the visualization markers listed in the table below, and tissue morphology. Importantly, lymph node tissue was not profiled, as it may have confounded the analysis. **(B)** An immunofluorescent image of a region of the pre-treatment biopsy from Patient C. Two tumor-rich regions are present, however the top region is associated with lymph tissue and was therefore not included in the analysis. Regions with low cell abundance or necrosis were also excluded.

Vis. Marker	Target
Syto13	DNA
PanCK	Tumor cells
CD3e	CD3+ T cells
CD68	Macrophages

A



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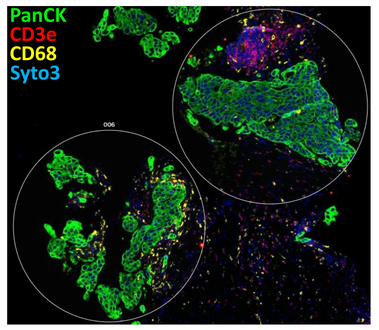
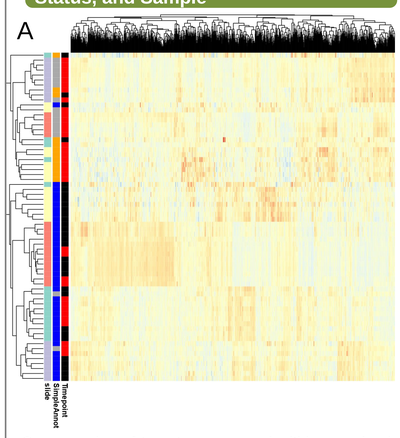


ROIs Cluster by Tissue Type, Treatment Status, and Sample

A



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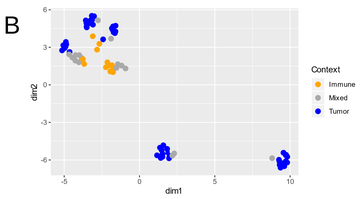

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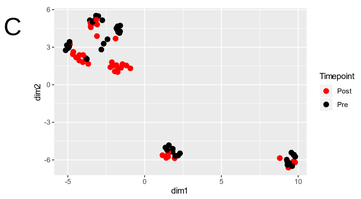

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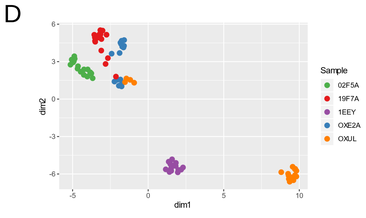
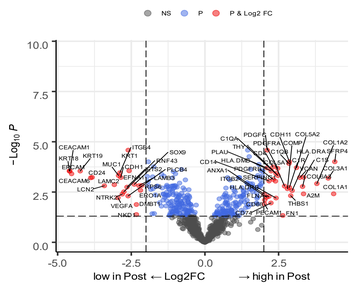
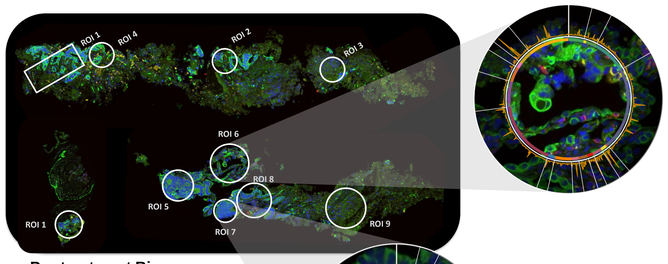


Coexpression of targets across and within samples is evaluated by **(A)** hierarchical clustering heatmap of all genes passing LOD filter, scaled by column. **(B)** Dimensionality reduction by UMAP, each point is an ROI and colored by the type of cells predominantly contained within the region (Immune, Tumor, or Mixed). **(C and D)** Identical UMAP as in (B), but points are colored by treatment status (C) or patient (D).

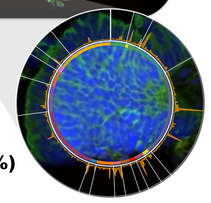
Differential Expression Analysis

Sample OXE2
Best Objective Response: SD (9%)

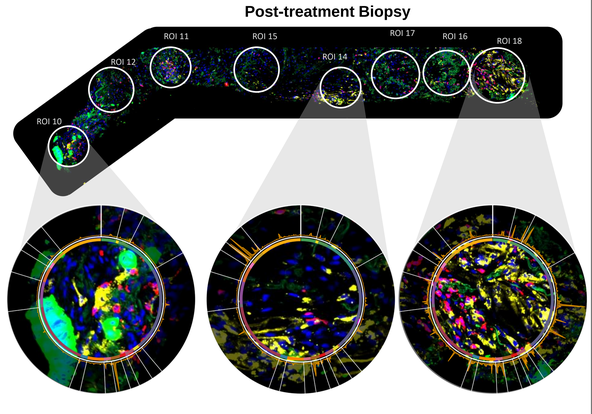




Pre-treatment Biopsy

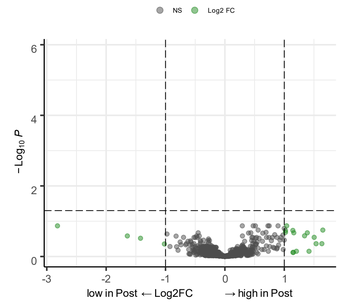


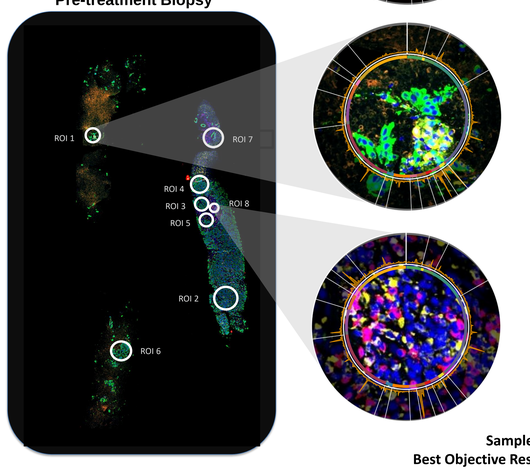
Post-treatment Biopsy



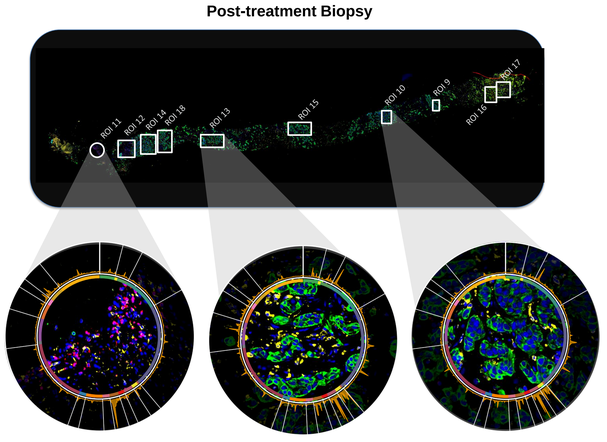
Sample 19F7
Best Objective Response: PR (-68%)

Pre-treatment Biopsy

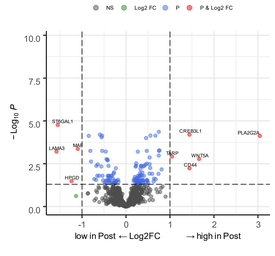




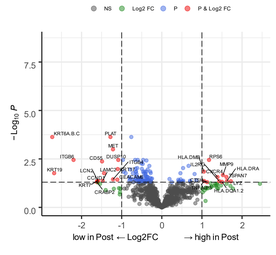
Post-treatment Biopsy



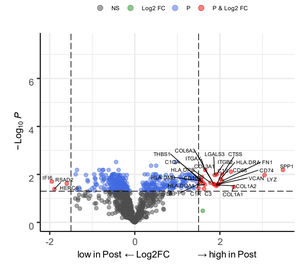
Sample 1EEY
Best Objective Response: SD (-14%)



Sample 02F5
Best Objective Response: SD (-15%)



Sample OXUL
Best Objective Response: SD (9%)



Differential expression of targets across space and pre- and post- treatment. The small cohort size and study design of matched pre- and post treatment biopsy preferences interpretation of data at the level of the individual patient. ROIs were pooled and differential expression was calculated between the pre and post treatment samples. Surprisingly, there was seemingly inverse correlation between amount of differential expression and objective response. Further examination of the pretreatment biopsies revealed that the sample from the patient that experienced the PR (19F7) had a preexisting immune response within the tumor. In contrast, the two patient who experienced the largest increase in tumor volume (OXE2 and OXUL) had the most differentially expressed genes, suggesting that the therapy was sufficient to induce an anti-tumor immune response. Spatial variation in target expression was also evaluated through the use of circle plots to identify gene clusters most differentially expressed between AOIs. Significantly different gene expression was observed, even for AOIs in close proximity.

Conclusions

Spatial profiling of tumor biopsies from patient samples can be used to characterize the abundance and quality of the immune response pre-existing in tumors and in response to therapeutic pressure. The CTA assay was used to generate expression data on 1400+ genes from a single 5 µm core biopsies. This in depth profiling strategy allows collection of a large body of expression data from minimal sample input. In addition, this study revealed changes in the immune contexture of tumors in response to immuno-stimulating therapies used in the RACIN trial, which may someday be correlated to efficacy and or used in biomarker discovery.

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Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact us at TAP@nanostring.com.

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