Spatial analysis of immuno oncology markers in the Tumor microenvironment of solid tumor samples using GeoMx™ Digital Spatial Profiler (DSP) and MultiOmyx™ Hyperplexed Immunofluorescence (IF)

Lakshmi Chandramohan • Qingyan Au • Nickolas Attanasio • Harry Nunns • Jane Marr • Jiong Fei • Vivek Reddy • Brigitte Lovell • Erinn Parnell • Máté Levente Nagy • Tricia Peters
NeoGenomics Laboratories

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Introduction

In recent years, novel immunotherapy strategies have shown remarkable results in treating various advanced cancers. However, the efficacy of immunotherapeutics has been dictated by the tumor microenvironment (TME), immune tumor infiltrates, immune checkpoint protein expression, and molecular tumor profiles leading to widely variable clinical outcomes and responses in patients. The dynamic and spatially heterogeneous TME is made up of malignant and nonmalignant cells such as endothelial cells, cancer-associated fibroblasts, immune cells, adipose cells, and neuroendocrine cells in addition to vascular and lymphatic networks and the extracellular matrix. The local milieu as well as complex tumor-TME interactions vary widely between tumor of the same type and also within the same tumor and therefore highlights the need for enhanced analysis of tissue-immune content and their spatial context for predicting response and biomarker discovery.

In this study, we applied a multi-faceted, highly multiplexed tissue analysis approach to quantitate and better characterize the spatial arrangement of key immuno oncology protein markers in a pan-cancer cohort of up to 35 FFPE samples from breast, head and neck, prostate, non-small cell lung cancer (NSCLC), endometrial and colorectal indications using NanoString GeoMx™ Digital Spatial Profiler (DSP) and MultiOmyx™ Hyperplexed Immunofluorescence (IF) assay.

For DSP analysis, each sample was profiled for up to 52 protein biomarkers included on NanoString Human Immuno oncology (IO) protein panels. Selection of regions of interest will be guided by H&E staining, fluorescent markers (CD45, PanCk and Syto 13). Protein profiling of tumor and TME regions was achieved by segmenting by PanCk+/PanCk- following by collection of indexed oligonucleotides and digital counting using NanoString nCounter system. Data normalization using endogenous controls across the ROIs were performed to understand the spatial distribution of these IO biomarkers within the TME.

An adjacent section of each tumor sample was stained and analyzed using MultiOmyx technology. MultiOmyx is a proprietary IF platform for the visualization and characterization of up to 60 protein biomarkers in a single FFPE section. Using the MultiOmyx IF assay in combination with proprietary algorithms, we studied the expression and spatial distribution of 14 immuno oncology (IO) markers to characterize different subtypes of immune cells and immune checkpoint inhibitors within the TME in the FFPE tumor samples.

Combination of MultiOmyx IF with NanoString DSP provides a complementary and powerful solution to study the IO markers within the TME. Using the DSP nCounter counts along with MultiOmyx cell classification and spatial analysis results, the researchers can simultaneously profile the high-plex protein expression, characterize the immunophenotypes and visualize the spatial distribution of tumor infiltrating immune cells at single-cell resolution within the TME.

Overview of Study Workflow

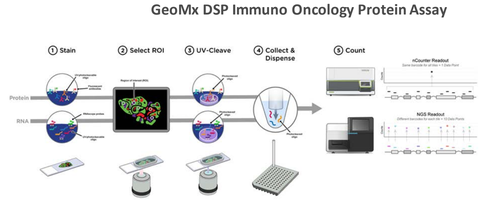

Table 1. GeoMx DSP panel

GeoMx Immuno Oncology Protein panels					
Immune Cell Profiling Core					
PD-1	PD-L1	CD45	Beta-2-microglobulin	PD-1	GZMB
HLA-DR	SMA	CD8	CD20	CD68	PanCk
CD4	Ki-67	Fibronectin	CD56	CD11c	CTLA4
CD3	HKG: Histone H3, S6, GAPDH		NEG: Ms IgG1,Ms IgG2a,Rb IgG		
IO Drug Target Module			**Immune Activation Status Module**		
4-1BB	LAG3	B7-H3	CD127	CD25	PD-L2
OX40L	Tim-3	STING	CD80	ICOS	CD44
VISTA	ARG1	IDO1	CD40	CD27	
GITR					
Immune Cell Typing Module			**Pan-Tumor Module**		
CD45RO	FAP-alpha	FOXP3	EpCAM	MART1	NY-ESO-1
CD34	CD66b	CD14	PTEN	S100B	Bcl-2
CD163			Her2	PR	ER-alpha

Table 2. MultiOmyx panel

14-Marker Panel		
1	CTLA4	LAG-3
2	PanCK	CD56
3	CD8	FOXP3
4	CD3	PD-L1
5	CD4	PD-1
6	CD45	CD45RO
7	CD68	PD-L2

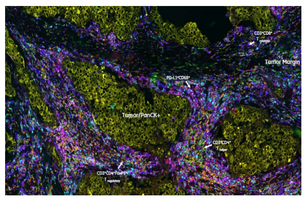

Figure 1. Assay Workflow. For NanoString DSP, slides were stained with a cocktail of 52 antibodies. ROI selections in two locations, Tumor and TME were guided by staining with fluorescent markers (CD45, PanCK, Syto 13). Per slide, 3 ROIs of 300 µM diameter circle were selected, and segmented by PanCk+/PanCk-.Photocleaved oligos were collected and counted using NanoString nCounter per manufacturer protocols. An adjacent slide for each sample was analyzed by MultiOmyx IF assay. For MultiOmyx IF study, slides were prepared and stained using MultiOmyx multiplexing IF staining protocol. For each round of staining, conjugated fluorescent antibodies were applied to the slide, followed by imaging acquisition of stained slides. The dye was erased, enabling a second round of staining with another pair of fluorescent antibodies.

Key Findings

In this study, NanoString technologies GeoMx DSP and MultiOmyx, both platforms offered by NeoGenomics Laboratories were utilized to perform expression profiling and immune cells phenotyping in breast, head and neck, prostate, lung, endometrial and colorectal cancer samples.

- Correlation coefficient was calculated between GeoMx DSP counts and the positive cell densities measured by MultiOmyx IF assay for the 10 markers that showed enough positivity in this study (>100 positive cells per ROI). Direct correlation was observed for 8 out of 10 markers.
- This integrated analysis by both technologies provides comprehensive understanding of the immune landscape in oncology FFPE tissues.
  - GeoMx DSP provides a high multiplexing, non-destructive, spatial approach to overall expression profiling of the 52 IO markers from FFPE samples tested in the study.
  - MultiOmyx is a robust approach for immunophenotyping and characterizing the co-expression/co-localizing of the biomarkers at single cell level.

Expression Profiling using NanoString GeoMx DSP

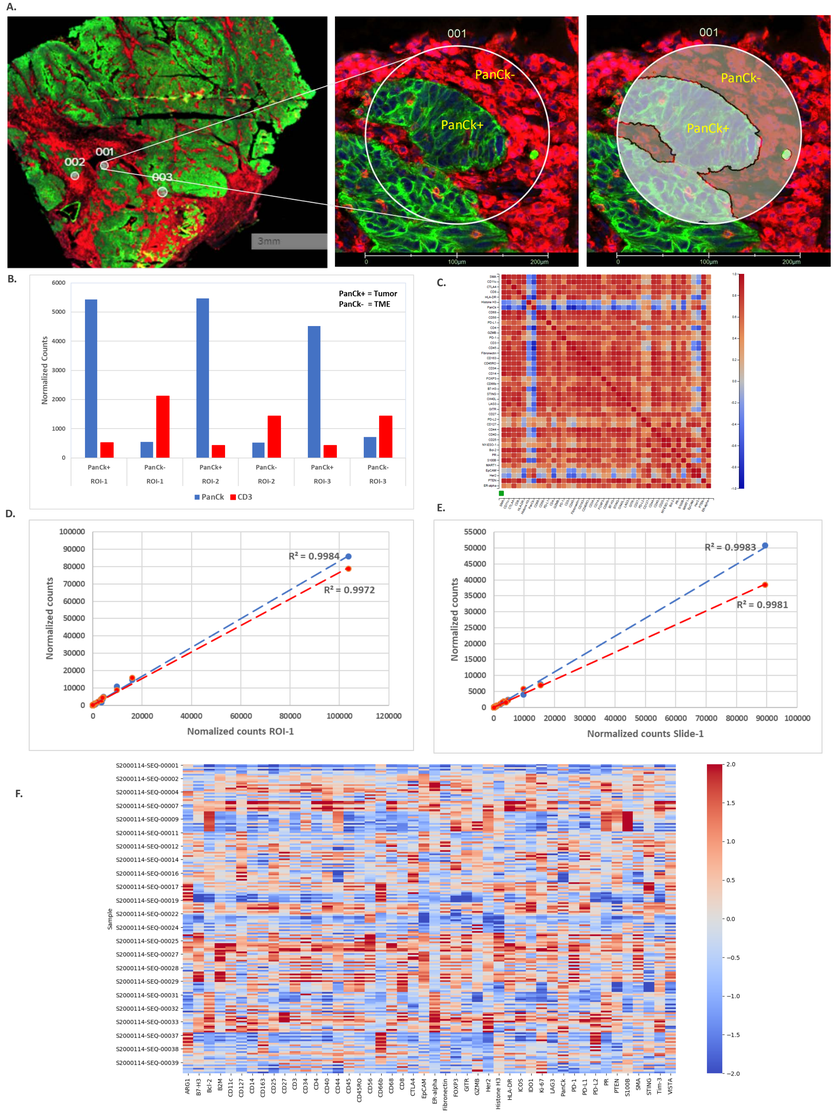

Figure 2. Analysis of biomarker profiling using DSP in FFPE samples. A. A representative DSP image showing ROI selection and segmentation using PanCk. B. Normalized counts of PanCk and CD3 in PanCk+ (Tumor) and PanCk-(TME) regions. C. Reproducibility of DSP biomarker expression between when tested using different analysts. D and E. Repeatability of DSP biomarker expression between ROIs within a sample (D) and between serial sections of the sample (E). F. Heatmap of the biomarker density results (for a subset of biomarkers) in the all tissue areas.

Immunoprofiling using MultiOmyx Immunofluorescence Assay

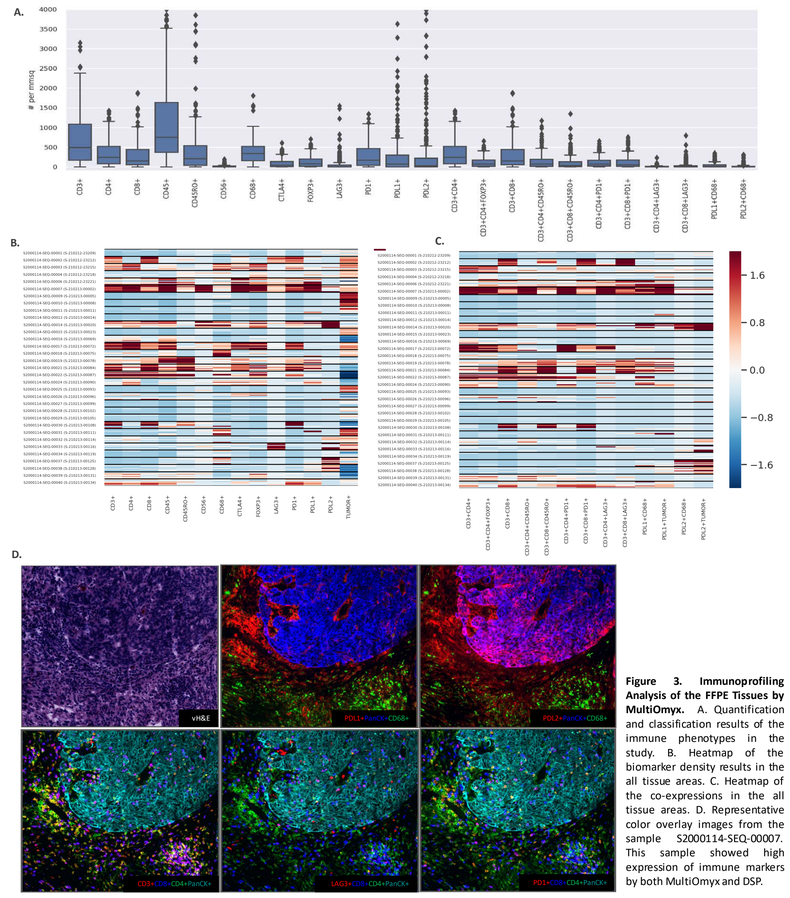

Figure 3. Immunoprofiling Analysis of the FFPE Tissues by MultiOmyx. A. Quantification and classification results of the immune phenotypes in the study. B. Heatmap of the biomarker density results in the all tissue areas. C. Heatmap of the co-expressions in the all tissue areas. D. Representative color overlay images from the sample S2000114-SEQ-00007. This sample showed high expression of immune markers by both MultiOmyx and DSP.

Correlation Study

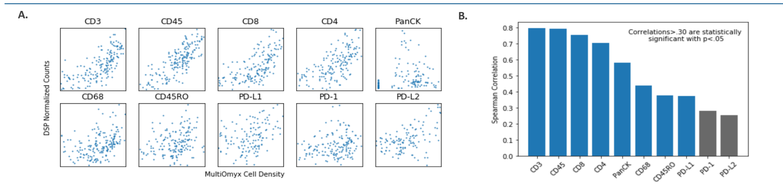

Figure 4. Correlation Between NanoString DSP and MultiOmyx IF. A. Scatter plots showed the correlation between NanoString DSP counts and the densities of positive cells measured b MultiOmyx IF assay. B. Correlation coefficient was calculated for the 10 markers with enough positivity (>100 positive cells per ROI) in this study. Direct correlation was observed for 8 markers.

Lakshmi.chandramohan@neogenomics.com