Evaluation of the NanoString GeoMx Digital Spatial Profiling (DSP) Technology in Formalin-Fixed Paraffin Embedded Cell Line Mixtures, PBMCs, and NSCLC Tissues

Poster #

Joshua J. Rusbuldt1, Tanesha Cash-Mason1, Shaozhou K. Tian1, Alison VanSchoick2, Yan Liang2, Chandra Rao1, Denis Smirnov1
1Janssen Research & Development LLC, Spring House, Pennsylvania, USA; 2NanoString Technologies Inc, Seattle, Washington, USA

Background
Interrogating the complex nature of tumors requires multiplexed, quantitative analysis of tissues which is challenging using traditional immunohistochemistry (IHC). Several novel approaches (including the NanoString GeoMx DSP technology) were developed to enable multiplexed analysis of tissues. Here we report on a comparative evaluation of the GeoMx DSP platform, including a comparison to IHC.

Methods
Whole-slide assay was assessed with cell line mixtures and PBMCs on a subset of markers from the full panel for preliminary validation (asterisk, *). Cells were characterized by Flow Cytometry and then formalin fixed and paraffinized to slides, deparaffinized (Citrate), and retrieved for antigens (Citrates). Antibody cocktail was applied overnight and whole-slides cleaved with UV excitation the following day to collect oligo-labels.

Full DSP platform was assessed with NSCLC FFPE tumors as compared to IHC for few markers (bold). Serial sections were stained by IHC and H&E and regions of interest were selected based on expression levels. Selected regions of interest (ROIs) were blinded and assayed at NanoString using the full 39plex panel (including the 5 IHC markers) and compared to qualitative IHC scoring.

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Figure 1: Surface protein detection (by Flow Cytometry) highly correlates with NanoString counts in cell mixtures and healthy donor PBMCs. CDM/C1 and Raji cells were profiled by flow cytometry for a subset of markers and these cells were preserved to FFPE slides (top). Markers exclusively positive to either cell line (CDM/C1 [CD4; blue, left] and Raji [CD19; orange, right]) correlate strongly with percent composition (top; r² range between 0.90 and 0.94). Additionally, healthy donor PBMCs were profiled by flow cytometry for a subset of markers and these cells were preserved to FFPE slides (bottom), and phenotypic profile shows good concordance with NanoString observations (bottom; r² of 0.49 and 0.51). NanoString counts were IgG control (background) subtracted and normalized to Histone H3 counts for this whole-slide assay. Black lines indicate composition of cell type contained.

Figure 2: CD4 counts by NanoString GeoMx DSP trend strongly with qualitatively assessed NSCLC ROIs. Representative example (one slide of five) of one GeoMx DSP marker (CD4, one of five markers). ROI’s were selected based on varied expression levels determined by IHC staining of markers of interest (CD3, CD4, and CD68 shown here; CD8 and PD-L1 staining was very low by IHC and similarly by DSP). IHC stained ROIs were qualitatively scored as “high” (orange), “moderate” (yellow), or “low/negative” (blue). Counts were calculated by normalization to internal controls and corrected for cell number. Inset box and whisker plots outline trends of ROIs within each qualitative grouping.

Figure 3: NanoString GeoMx DSP counts trend strongly with qualitatively assessed IHC ROIs across five NSCLC tissue samples. Twelve ROIs each from 5 NSCLC tissue samples were selected based on varied expression levels determined by IHC staining of markers of interest (CD3, CD4, and CD68 shown here; CD8 and PD-L1 staining was very low by IHC and similarly by DSP). IHC stained ROIs were qualitatively scored as “high” (orange), “moderate” (yellow), or “low/negative” (blue). Counts were calculated by normalization to internal controls and corrected for cell number. Inset box and whisker plots outline trends of ROIs within each qualitative grouping.

Figure 4: NanoString DSP counts yield expression maps that trend with composition of the ROI. NanoString DSP counts for the full 39plex panel were applied to the web-based Morpheus application from the Broad Institute to yield heat maps of expression patterns. Representative map for one of the five NSCLC tissue samples. IHC images for each marker from three selected ROIs are depicted (clockwise: tumor section, mucinous cells, and lymph node). Heat maps of expression patterns. Representative map for one of the five NSCLC tissue samples. IHC images for each marker from three selected ROIs are depicted (clockwise: tumor section, mucinous cells, and lymph node). Heat maps of expression patterns. Representative map for one of the five NSCLC tissue samples. IHC images for each marker from three selected ROIs are depicted (clockwise: tumor section, mucinous cells, and lymph node). Heat maps of expression patterns. Representative map for one of the five NSCLC tissue samples. IHC images for each marker from three selected ROIs are depicted (clockwise: tumor section, mucinous cells, and lymph node).

Conclusions
• The NanoString GeoMx DSP platform yielded comparable measurements to those obtained with Flow Cytometry and most importantly IHC for the markers interrogated in parallel.

• The GeoMx DSP technology is successfully able to collect high parametric proteomics data from FFPE tissue samples which can better help to study complex biology of the tumor microenvironment.