Integrative spatially-resolved, high-plex digital profiling enables characterization of complex immune biology in the tumor microenvironment of mesothelioma

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Recommended Citation
Ballesteros-Merino, Carmen; Widmaier, Moritz; Church, Sarah; Herz, Thomas; Budco, Alexei; Medrikova, Das; Kanchev, Ivan; White, Andrew; Hinefeld, Douglas; Jensen, Shawn; Handi, John; Sanborn, Rachel; Bifulco, Carlo; Warren, Sarah; Beechem, Joseph; and Fox, Bernard ... "Integrative spatially-resolved, high-plex digital profiling enables characterization of complex immune biology in the tumor microenvironment of mesothelioma" (2018). Society for Immunotherapy of Cancer 2018 Annual Meeting Posters. 15. https://digitalcommons.psjhealth.org/sitc2018/15
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Background
Malignant mesothelioma is an aggressive cancer with poor prognosis and few effective therapies. Since mesothelioma is derived from the mesothelium of the lung, we hypothesize that immune cells in the tumor microenvironment (TME) may behave differently than other solid tumors. Our previous characterization of mesothelioma samples using multi-plexed immunofluorescence did not reveal specific-immune phenotypes associated with improved patient survival. Here we describe the novel combination of two technologies to spatially characterize the interface between mesothelioma cells, stroma and immune cells in the mesothelioma TME in a high-plex capacity.

Methods
Forty-four FFPE mesothelioma tumors were characterized by Definiens’ Immuno-Oncology Profiling (IOP) and NanoString’s GeoMx™ Digital Spatial Profiling. Stainings and image analysis on three alternating sequential sections were provided by Definiens’ IOP Panel (CD8/PD-1/FOXP3, CD68/PD-L1/CD3, Granzyme B). The resulting IHC multiplex data was used to select T cell and macrophage enriched regions of interest (ROIs) with specific co-expression patterns and tumor environments (tumor, stroma, tumor center, invasive margin) for high-plex analysis on DSP. Once ROIs were selected, DSP was performed on the sequential section. Each slide was stained with a cocktail of pan-cytokeratin, CD3, CD68 and DNA fluorescent markers and a 38 protein cocktail of antibodies conjugated to a UV-phocleavable DNA barcodes. For downstream quantitation on the NanoString nCounter® platform those barcodes where released by UV excitation of the co-registered ROIs in the DSP fluorescent sections.

Results

Patients with Mesothelioma have Distinct Immune Phenotypes

- Good correlation between Definiens and DSP analysis of T cell and macrophage markers confirms the chosen approach (Figure 1).
- IHC and DSP markers cluster by cell type including macrophages, T cells, B-cells, Tumor and other immune markers (Figure 2)
- Expression of Ki67 is associated with decreased OS and CD20 is associated with longer OS (Figure 3).
- On a marker co-localization level, we found that high CD68 density was tightly correlated to PD-L1 expression (Figure 4).
- Mesothelioma samples clustered into four categories: PD-L1/CD68, less CD68 with CD3/PD-L1, CD3/CD68 and CD3 dominate (Figure 4).
- Further analysis of CD3 dominate samples express many T cell activation markers and very few suppressive markers (Figure 4).
- STING and VISTA were highly expressed across mesotheliomas.

Conclusions
- This data set demonstrates how integration of two novel high-plex spatial analysis techniques separates distinct immune mechanisms in mesothelioma TME.
- We hypothesize that analysis mesotheliomas may guide the development of combination immunotherapy trials that will be effective against this incurable disease.