**Introduction**

- Immunoprofiling has become highly significant in order to understand in depth the immune microenvironment of tumors and their spatial relationships.
- NanoString GeoMx® DSP is a new high-plex technology that allows the assessment of multiple proteins or RNA in a single slide, promoting the identification of targetable biomarkers in both tumor and microenvironment.
- We aim to describe the standard technical overview of the protein panel of this assay in normal tonsil tissue.

**Methods**

- We used a 4μm FFPE section of a reactive tonsil to run a Human Immuno-Oncology Protein Panel (24 antibodies) in GeoMx® DSP assay.
- In the first day, we perform the tissue and reagents preparation, followed by washing, staining, scanning, and region of interest (ROI) selection in the second day.
- Indexing oligos were released by the Sequential UV light exposure of each ROI were and quantified on NanoString’s nCounter® system.
- The data was exported and analyzed. Heat maps and box plots were used to visualize the different levels of protein expression.

**Results**

- A total of 9 regions of interest were selected, passed by quality control, and analyzed.
- After the normalization using the signal background ratio, fifteen biomarkers showed median counts higher than one.
- Among them, we analyzed the different distribution in tonsil compartments of Pancytokeratin, CD3, CD4, CD20, CD45, CD68, Granzyme B, and Ki-67.
- The germinal center areas were characterized by the high expression of CD20 and Ki-67, while the interfollicular areas showed a high concentration of T-cell markers, such as CD3, CD4, and Granzyme B.
- The macrophage marker CD68 had a slightly higher concentration in germinal centers and there was no difference between the concentrations of the pan-immune marker CD45 between germinal center and interfollicular areas.

**Conclusions**

- An adequate protein distribution pattern for typically expressed markers were seen in different compartments of a normal tonsil.
- GeoMx® DSP is a robust and promising high-plex technology for the study of spatial protein immune landscape and can represent a great tool for translational cancer research.

**References**

1) Hernandez et al. Front Oncol, 2022