Background and Results

Background: An intriguing phenomenon in many solid tumors is the de novo lymphoid neogenesis of tertiary lymphoid structures (TLS). However, it remains unclear whether spontaneous TLS restricts or promotes tumor progression and more studies are required to define the role of TLS. One interesting question is the pathological and functional connection of myeloid-derived suppressor cells (MDSCs), known inhibitors of T-cell-mediated anti-tumor immunity, and TLS.

Methods: To perform a spatial analysis of MDSCs and other immune cell phenotypes in the bladder tumor microenvironment we used MultiOmyx™, a multiplexed immunofluorescence (FI) assay utilizing a pair of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining. Using a 14-marker panel and proprietary cell segmentation and classification algorithms developed at NeoGenomics, we have analyzed the presence of TLS (positive for CD20, CD3, and PNAd), followed by a spatial analysis of MDSCs, T cell subtypes, and M1/M2 type tumor-associated macrophages (TAMs) in relation to the TLS in 25 FFPE samples from patients with bladder cancer.

Results: As expected we found a significantly higher density of B cells and T cells present inside the TLS compared to either near or far from the TLS, while the density of tumor associated macrophages (TAMs), which are not associated with TLS, was the same in all regions. To test our initial hypothesis that MDSCs repel TLS in bladder cancer, we quantitated the presence of MDSCs and found a linear decrease related to their proximity to the TLS, with a 30% lower density in the region near the TLS, and a 48% decrease in the regions far from the TLS. This pattern was consistent for both M-MDSCs and G-MDSCs, as well as for MDSCs expressing CXCR2, a chemokine receptor which has been shown to regulate the migration of MDSCs.

Conclusion: The finding of the co-abundance of MDSCs and T/B cells following the distance from TLS suggests that the TLS may attract MDSCs through cell-cell interaction, a finding with potential important implications for bladder cancer immunotherapy.

MultiOmyx Multiplexing and Biomarker Panel

Key Findings

- We found a significantly higher density of B cells and T cells present inside the TLS compared to either near or far from the TLS, but no difference in TAM density.
- When quantitating MDSCs we found a linear decrease related to their proximity to the TLS, with a 33% lower density in the region near the TLS, and a 48% decrease in the regions far from the TLS.
- This study demonstrates that blocking MDSCs may promote T cell infiltration and tumor immunity within the TLS, potentially leading to increased T cell proliferation and tumor-infiltration.