5648: Profiling the Immune Landscape of Bladder Tumors with Single-Cell Spatial Phenotyping

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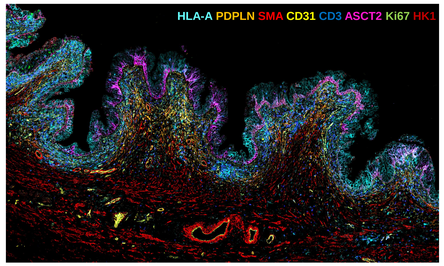
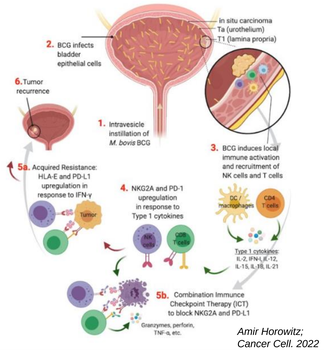
1. Non-Muscle Invasive Bladder Cancer

With 500,000 worldwide cases diagnosed annually, bladder cancer is the 5th most common cancer in the United States. Bacillus Calmette–Guérin (BCG) has been used to treat non-muscle-invasive bladder cancer (NMIBC) for more than 30 years and it remains one of the most successful biotherapies for this cancer type. Despite the success of BCG treatment, the mechanism of its therapeutic effect is still under investigation. We have developed a Spatial Phenotyping application for comprehensive analysis of Human NMIBC tissues and to study potential immune responses that underlie the therapeutic effect of BCG therapy.





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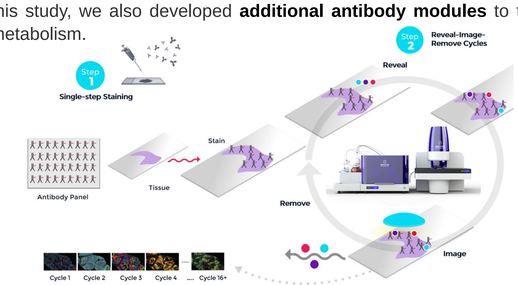
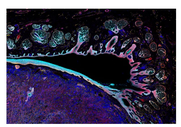
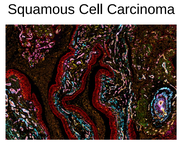
2. High-Plex Spatial Phenotyping & PhenoCode Discovery Panels

The **Phenocycler® Fusion** platform affords high-throughput spatial readouts across whole tissue sections (shown on bottom right are FFPE tissue examples). Here, we present ultrahigh-plex single-cell Spatial Phenotyping of whole-slide FFPE human BCG pretreated and post-treated NMIBC tissues with 57 protein biomarkers. In doing so, we deployed the commercially available **PhenoCode™ Discovery Panels** encompassing markers for identification of *immune cell profiling, activation, proliferation and tissue architecture*. As part of this study, we also developed **additional antibody modules** to target NK cells and cellular metabolism.

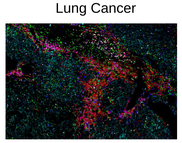




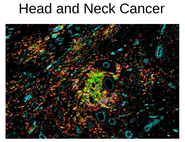
Squamous Cell Carcinoma



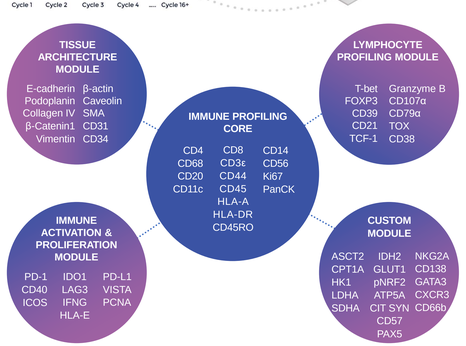
Lung Cancer



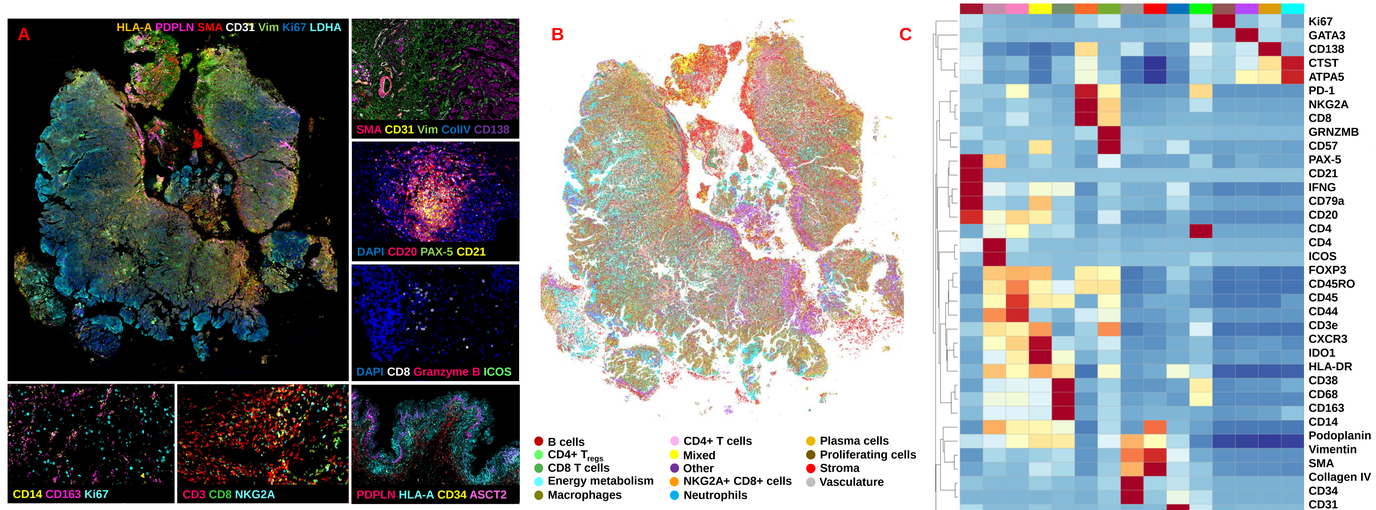
Head and Neck Cancer



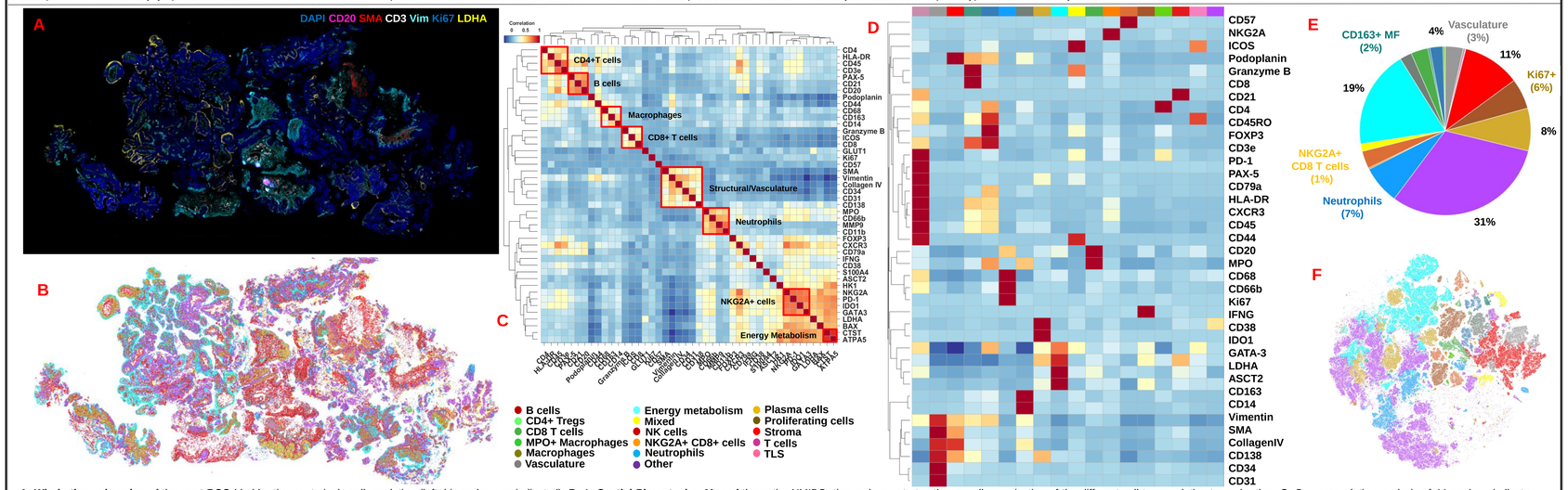
Renal Cell Carcinoma



3. High-Plex Single-Cell Spatial Phenotyping of Human FFPE NMIBC



A. Spatial Phenotyping of whole human NMIBC BCG pre-treated tissue sections at single-cell resolution (left; biomarkers as indicated). **B.** A **Spatial Phenotyping Map** of the entire NMIBC tissue recapitulated the overall organization of different cell phenotypes relative to each other. **C.** The clustering dendrogram indicates cell phenotypes colored rectangles in the top row. **D. Pie chart** of cell-type fractions for NMIBC tumor with infiltrating immune cells colored by cell type. The NMIBC tumor exhibited high levels of proliferation and angiogenesis as evidenced by Ki67 and CD31/CD34 staining, respectively. CD163$^+$ macrophages were present in both tertiary lymphoid structures and tumor stroma. NKG2A expression was associated with CD8$^+$ T cells in all tested NMIBC pre-treated tumors. **E.** The **tSNE plot** shows distinct phenotype clusters sorted by color.



A. Whole tissue imaging of the **post-BCG** bladder tissue at single-cell resolution (left; biomarkers as indicated). **B.** A **Spatial Phenotyping Map** of the entire NMIBC tissue demonstrates the overall organization of the different cell types relative to each other. **C.** Cross-correlation analysis of 44 markers indicates immune cell populations as well as cell lineages and functional niches with vasculature/structural signatures. **D.** In this example 770800 cells were clustered to yield a minimum of 17 distinct cell phenotypes. **E.** The **pie chart** (bottom right) summarize proportions of cell phenotypes in BCG post-treated tissue and indicate distinct expansion of certain cell types; post-treated tissue exhibited a remarkable infiltration of neutrophils (7:1; post treated : pre-treated sample); both infiltrated NKG2A+ CD8+ T cells and tumor associated macrophages (CD163 $^+$) – two characteristic markers associated with poor prognosis – were significantly decreased in BCG post-treated tissue compared with the pre-treated sample (3:1 for CD163 $^+$ and 6:1 for NKG2A$^+$ CD8 $^+$). **F.** The **tSNE plot** shows distinct phenotype clusters sorted by color.

4. Conclusions

We developed a comprehensive **PhenoCode™ Discovery Panels** for in-depth analysis of the TiME of the BCG pre-treated and post-treated non-muscle invasive bladder tumors. The results revealed that post-treated BCG tissues were found to have less infiltrated NKG2A$^+$ CD8 T cells, CD163$^+$ macrophages and Ki67$^+$ proliferating cells compared to the BCG non-treated tissues. Notably, post-treated tissues also exhibited a remarkable infiltration (7:1) of neutrophils compared to the pre-treated samples. With the power of single cell spatial biology, we are defining novel functional interactions as new correlates of BCG resistance and identifying pathways that can be exploited for next-gen immunotherapies.

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