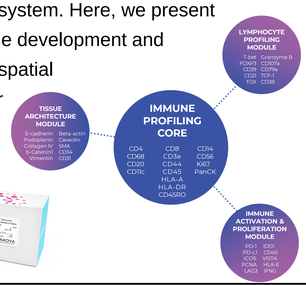
4649: Design and Validation of Ultrahigh-plex Discovery Panels for Immuno-oncology and Oncology

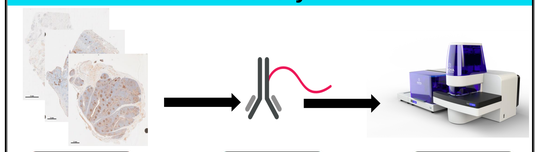
Yan He[1], Shannon Berry[1], Olive Shang[1], Michael McLane[1], Nicholas Ihley[1], Dmytro Klymyshyn[1], Rose Chiang[1], Bassem B. Cheikh[1] and Yi Zheng[1]
[1]Akoya Biosciences, Marlborough, Massachusetts, USA

1. Introduction

Ultrahigh-plex single-cell spatial phenotyping is revolutionizing cancer research through deeper interrogation of cellular and protein-level co-expression, localization, and arrangements within cancer tissues. To enable in-depth characterization of the tumor microenvironment (TME), we have developed and validated pre-optimized antibody panels on cancer tissues to reveal key cell types and cellular architecture using the PhenoCycler®-Fusion system. Here, we present our methodology for the development and validation of multiplex spatial phenotyping panels for oncology and immuno-oncology.

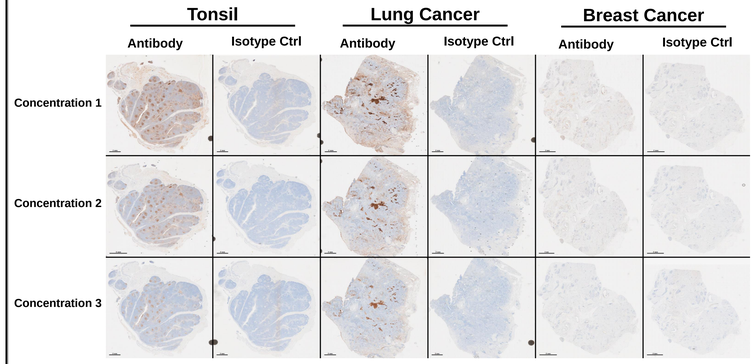


2. Design and Validation of PhenoCode Discovery Panel Modules

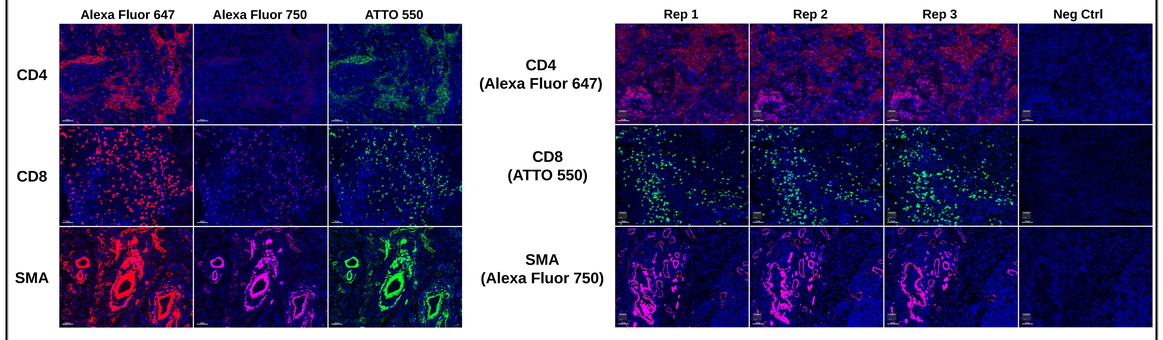


We designed panel modules such that each module answers specific biological questions about the TME and can be multiplexed together for a more comprehensive view of the structure and function of the tumor and TME. The resulting PhenoCode™ Discovery Panel Modules include markers that identify key immune cell types, immune activations and checkpoints, mediators of proliferation, and other hallmarks of cancer. Each antibody in the panel module was conjugated to an oligo barcode and stained on human formalin-fixed paraffin-embedded (FFPE) tissue. The slide was imaged on a PhenoCycler-Fusion platform, where dye-labeled oligo reporters (complementary to the barcodes) are hybridized to the barcode to visualize the antibody. The results were compared to serial sections run with a 3,3'-Diaminobenzidine (DAB) chromogenic immunohistochemistry assay to ensure specificity of the antibody was retained. Antibody concentration, exposure time, and corresponding dye were further optimized, verified, and validated as a whole module using tonsil and cancer tissues based on image analysis and intensity analysis, to achieve an ultrahigh-plex detection.

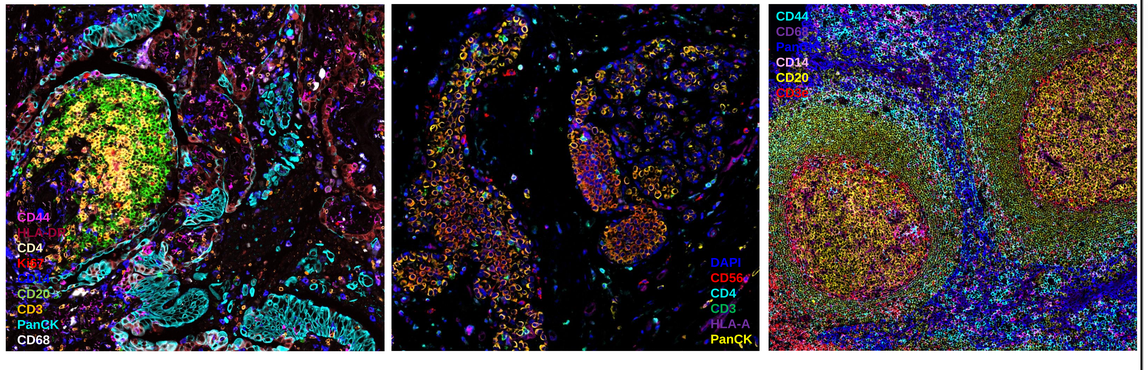
3. From Single Antibody Screen to Panel Module Validation



3.1 Single antibody screening and validation. Representative image of DAB staining (left) of single antibody testing in tonsil, lung cancer, and breast cancer at three different concentrations with its corresponding isotype control. Matched DAB and PhenoCycler images (right) demonstrated that barcode-conjugated antibody showed similar staining pattern as unconjugated antibody.

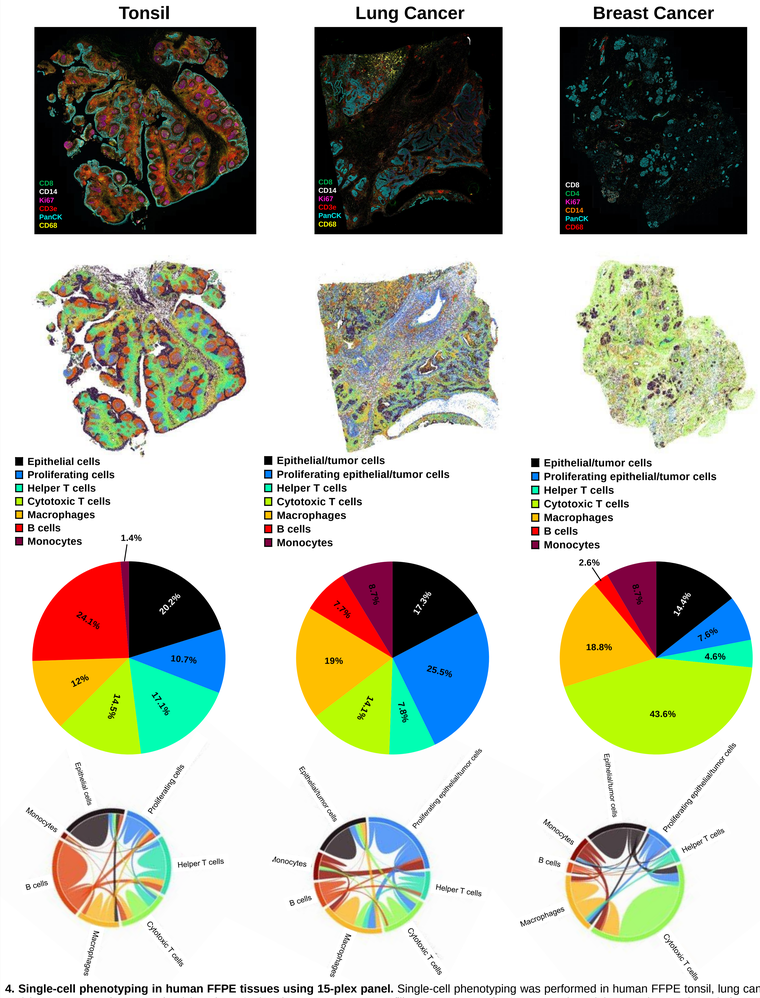


3.2 Development and validation of PhenoCode Discovery Panel Modules. Each antibody in the modules was optimized for its fluorophore selection, exposure time and concentrations to ensure the most efficient experiment running time. Left panel showed three representative antibodies being tested in three channels in lung cancer tissue. The right panel showed the antibodies stained in triplicates plus its negative control.



3.3 Panel module validation in human FFPE tissues. Module performance was assessed by performing multiplex staining in both tonsil and cancer tissues. Representative 15-plex images stained by PhenoCode Discovery Immune Profiling Human Protein Core (biomarkers as indicated) were shown in lung cancer (left), breast cancer (middle) and tonsil (right).

4. Ultrahigh-plex Single-cell Phenotyping of Human FFPE tissues



4. Single-cell phenotyping in human FFPE tissues using 15-plex panel. Single-cell phenotyping was performed in human FFPE tonsil, lung cancer and breast cancer tissues stained by PhenoCode Discovery Immune Profiling Human Protein Core. Panel modules can be used on their own or combined to increase your plex and answer a different biological question. Representative whole-slide images at single-cell resolution of tonsil (left), lung cancer (middle) and breast cancer (right) were shown on the top panel (biomarkers as indicated). Corresponding spatial phenotyping of each FFPE tissue into 7 distinct cell types were demonstrated below the whole-slide images. The pie chart show the abundance of distinct phenotype clusters sorted by color. Interaction chord diagrams reveal the frequency of a specific cell type and interaction between cell types in each tissue sample. Data analysis was performed using the Enable Medicine Cloud Platform.

5. Unlocking the Power of Ultrahigh-Plex Spatial Discovery with Ready-to-Use Combinable Panel Modules

Our study showcases the rigorous development and validation process undertaken to create the PhenoCode Discovery Panel Modules. The results showcase the panels' ability to seamlessly work together enabling thorough interrogation of the tumor and the surrounding tumor microenvironment (TME). Each panel module has been meticulously designed with essential markers to answer key biological questions and can be used on their own or combined to increase plex and gain new insights. The introduction of these optimized modules marks a pivotal point in the evolution of ultrahigh-plex spatial phenotyping by simplifying experimental design and assay setup, allowing researchers to answer important questions in an efficient cost-effective manner. To learn more, download our technical note: akoyabio.com/discovery-validation

Akoya Biosciences, Inc., 100 Campus Drive, 6th Floor, Marlborough 01752, MA USA (855) 896-8401 www.akoyabio.com