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Multiplexed Immuno-Profiling of the Colorectal Carcinoma Microenvironment Using Archival Human Tissue

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Colorectal carcinoma remains a major cause of cancer-related morbidity and mortality. Clinical therapeutics targeting the tumor immune microenvironment have been employed with remarkable success in subset of tumor types, including some colorectal carcinomas. However, there are limited options to interrogate the immune cell components of the tumor microenvironment that might best predict response to these therapies. We therefore sought to develop a quantitative and high-throughput method for profiling the T cell populations composing the colorectal tumor immune microenvironment.

To this end, we have developed a multiplexed immunofluorescence (mIF) assay relying upon antibodies targeting five immune cell markers (CD3, CD4, CD8, FOXP3 and CD45RO) known to be important in tumor immunity. In addition, a pan-cytokeratin antibody enables identification of epithelial/tumor cells, and a nuclear counterstain (DAPI) aids in cell enumeration. This multiplexed approach allows for simultaneous evaluation of all seven markers in a single section of formalin-fixed paraffin-embedded tumor tissue. Each marker was first separately validated by standard immunohistochemistry performed on samples of lymph node and normal and neoplastic colon tissue. Antibodies were then tested in a single-plex immunofluorescence format and subsequently optimized in an iterative fashion to ensure equivalent performance in the full multiplexed protocol. The final assay has been successfully applied to both whole slide sections of tumor and tissue microarrays (TMAs) (see Figures 1 and 2).

Slides stained using the multiplex panel are imaged using an automated, whole slide scanning platform with calibrated quantification of imaging regions to generate a precise measure of cell density for any defined population per square mm of tissue. Combinatorial marker analysis allows for sensitive detection and quantification of multiple T cell populations, including all T cells ($CD3^+$), cytotoxic T cells ($CD3^+CD8^+CD4^-$), T helper cells ($CD3^+CD4^+CD8^-$), regulatory T cells ($CD3^+CD8^+FOXP3^+$), and memory T cells ($CD3^+CD45RO^+$). Additionally, under pathologist supervision, a machine learning algorithm is employed to automatically segment tissue into regions of tumor epithelium and surrounding stroma. Quantification of immune cells within separate tissue compartments will allow enumeration of true tumor-infiltrating lymphocytes, periglandular immune cells within the stroma surrounding tumor glands/nests, and the immune microenvironment at the leading invasive edge of the tumor compared to the microenvironment within the center of the tumor.

This multiplexed, integrated analysis platform will be used to profile T cell populations composing the immune microenvironment of a cohort of over 1000 colorectal cancer cases from the Nurses' Health Study and Health Professionals Follow-up Study. This dataset has accumulated clinical, prognostic epidemiological, and tumor molecular data, including whole exome sequencing and tumor neoantigen load. Overall, this