Overview of MultiOmyx™ Technology Workflow

Background

Immune checkpoint therapies target immune regulatory pathways to enhance anti-tumor immune response. These therapies have contributed to important clinical advances and are a promising approach to combat cancer. Development of effective immune checkpoint therapies requires an understanding of the host immune response within the tumor microenvironment. Clarient Diagnostic Services, Inc., has developed a multiplexed Tumor Infiltrating Lymphocytes (TIL) panel consisting of 12 key cancer immune markers: CD3, CD4, CD8, CD20, CD200, CD68, CD38, CD45RO, CD56, CD68, HLA-DR, and natural killer cells (CD3-CD56+) and macrophages (CD68+). The TIL panel and CD8+ cytotoxic T cells, T regulatory (CD3+CD4+FoxP3+), and T helper (CD3+CD4+) cells in the tumor microenvironment were evaluated in melanoma, lung, colorectal, prostate, and breast cancer.

Figure 1. MultiOmyx IF multiplexing scheme from a single tissue section. Cy3 and Cy5 fluorescent antibodies were applied to a slide, followed by white slide imaging. The dye was chemically inactivated, enabling a second round of staining with another fluorescent antibody. The process is repeated multiple times from a single slide until all biomarkers of interest are multiplexed.

Biomarker IF

- CD3+CD4+
- CD3+CD8+PD1+
- CD3+CD68+
- CD3-CD56+
- CD3+CD8+CD45RO+
- CD3+CD20+
- CD3+CD4+CTLA4
- CD3+CD4+PD1
- CD3+CD4+CD45RO
- CD3+CD4+FoxP3+
- CD3+CD4+
- CD3-CD20+
- CD3-CD68+
- CD3-CD56+

Figure 2. MultiOmyx IF data analysis workflow with representative TIL cell phenotypic profiles. Each biomarker was evaluated individually on each sample. A. Co-expression data table. B. Coexpression Phenotype. C. Co-expression phenotypes algorithmically classified. The summarized Table shows the co-expression of immune markers in different epithelial cell types. TIL panel and individual biomarker color codes are indicated above for different combinations of coexpressions.

TIL Panel BioMarkers

- L1, PanCK, CD3+CD4+
- CD3+CD8+PD1+
- CD3+CD68+
- CD3-CD56+
- CD3+CD20+PD-L1+
- CD3+CD4+CTLA4
- CD3+CD4+PD1
- CD3+CD4+CD45RO
- CD3+CD4+FoxP3+
- CD3+CD4+
- CD3-CD20+
- CD3-CD68+
- CD3-CD56+

Figure 3. Overview of TIL panel composition phenotypes. MultiOmyx™ removes overlap (ART) for use images of individual biomarkers and immune markers. The color expression in each cell type is determined by a combination of the immune marker expression. The color expression is represented by an arrow and an individual biomarker color code is indicated above for different combinations of coexpressions.

Conclusion

MultiOmyx TIL Panel was utilized to profile immune response in the tumor microenvironment within solid tumors including breast cancer, lung cancer, colorectal cancer, prostate cancer, and melanoma. The results shown in figure 4 revealed two distinct immunophenotypes. High TIL (Prostate & Breast), and Low TIL (Colorectal). The high TIL samples showed enhanced T cell population within the tumor and in the periluminal regions. CD8+ cytotoxic T cells, CD4+ helper T cells and HLA-DR+ macrophages. The low TIL sample showed reduced population of T cells and cells expressing different immune phenotypes. In the lung sample shown, PD-L1 is expressed primarily in the tumor while in the breast sample, PD-L1 expression is primarily in the macrophages. Immunophenotyping analysis offered by the MultiOmyx™ TIL panel was utilized unambiguously to identify TIL phenotypes. TIL expressing cells and concordant relationship between immune cells and immune cells in the tumor.

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