

PHENOTYPING THE TUMOR MICROENVIRONMENT

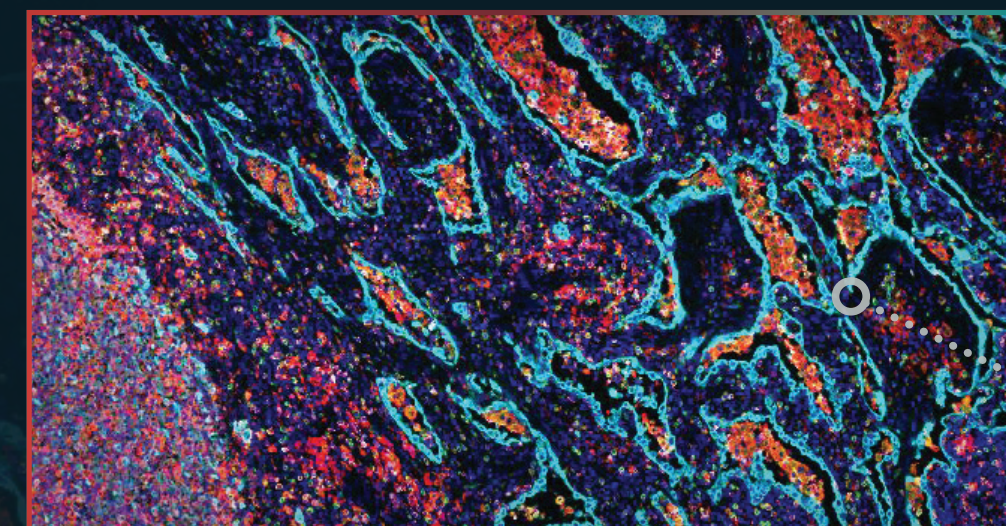
WITH MULTIPLEX IMMUNOHISTOCHEMISTRY

The tumor microenvironment (TME) is a complex and dynamic landscape. Cells within the TME interact to either stifle or promote cancer progression. Immune cells make up much of this environment, where they detect and destroy cancer cells. Cancer cells can evade the immune response by suppressing immune cell activity and recruiting additional cells that dampen the anti-cancer response.



Researchers analyze the TME to find novel cell phenotypes and disease mechanisms, develop targeted therapies, study drug efficacy, and plan patient treatments. Identifying biomarkers provides crucial information on tumor and immune cell status and their spatial arrangement. Traditional TME analysis methods, such as flow cytometry, destroy tissue morphology to obtain single cell suspensions. New immunohistochemistry (IHC) techniques stain tissue sections for multiple biomarkers concurrently and allow researchers to image an entire slide. These methods are non-destructive and can be combined with H&E staining to precisely locate cells of interest in the tissue section, identify immune cell infiltration, and detect cell interactions.

MAJOR PLAYERS IN THE TUMOR MICROENVIRONMENT



1 ANTI-TUMOR T CELLS

T cells play multiple roles in the tumor microenvironment depending on their phenotype. Memory T cells are primed against tumor antigens and cytotoxic T cells directly destroy cancer cells by releasing lytic substances. T cells may become exhausted due to antigen over-stimulation or through immunosuppressive cell effects.

- **Memory T cells • Cytotoxic T cells**
- **Proliferating T cells**
- **Exhausted T cells**

2 IMMUNOSUPPRESSIVE T CELLS

Regulatory T cells (Tregs), a subclass of T helper cells, in the TME suppress the anti-tumor immune response. Other subsets of T cells may also promote cancer progression.

- **Treg cells**
- **Double positive T cells**

3 ANTIGEN-PRESENTING CELLS (APCs)

APCs initiate and regulate anti-cancer immunity, often by activating T cells. However, within the TME, some APCs may stimulate Tregs and help tumor cells avoid the immune response.

- **Dendritic cells • Macrophages • B cells**

4 TUMOR EVASION AND IMMUNE INFILTRATION

Cancerous cells often have immune evasion mechanisms that block T cell effector functions. Immune cells may also promote cancer progression by acting against the anti-cancer immune response.

- **PD-L1-upregulated cancer cells**
- **Tumor-associated macrophages (TAMs)**

5 MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs)

MDSCs expand in response to chronic infections and cancer, inhibit anti-cancer immune cells, and stimulate Tregs.

- **Monocytic MDSCs**
- **Polymorphonuclear MDSCs**

PHENOTYPING THE TME USING INSITUPLEX® TECHNOLOGY

The InSituPlex® IHC technology allows researchers to stain tissues samples with multiple biomarkers for rapid hypothesis testing. With the ability to colocalize multiple markers in the same cell compartment, researchers can identify a multitude of cell phenotypes in the tumor microenvironment with a single U-VUE™ panel (left – U-VUE 8 plex kit: *CD3*, *CD4*, *CD8*, *CD68*, *FoxP3*, *PD-1*, *PD-L1*, *CK*).

Cytotoxic T cell
CD3+ CD8+

Exhausted T cell
CD3+ PD-1+

T helper cell
CD3+ CD4+

CD8 Regulatory T cell
CD3+ CD8+ FoxP3+

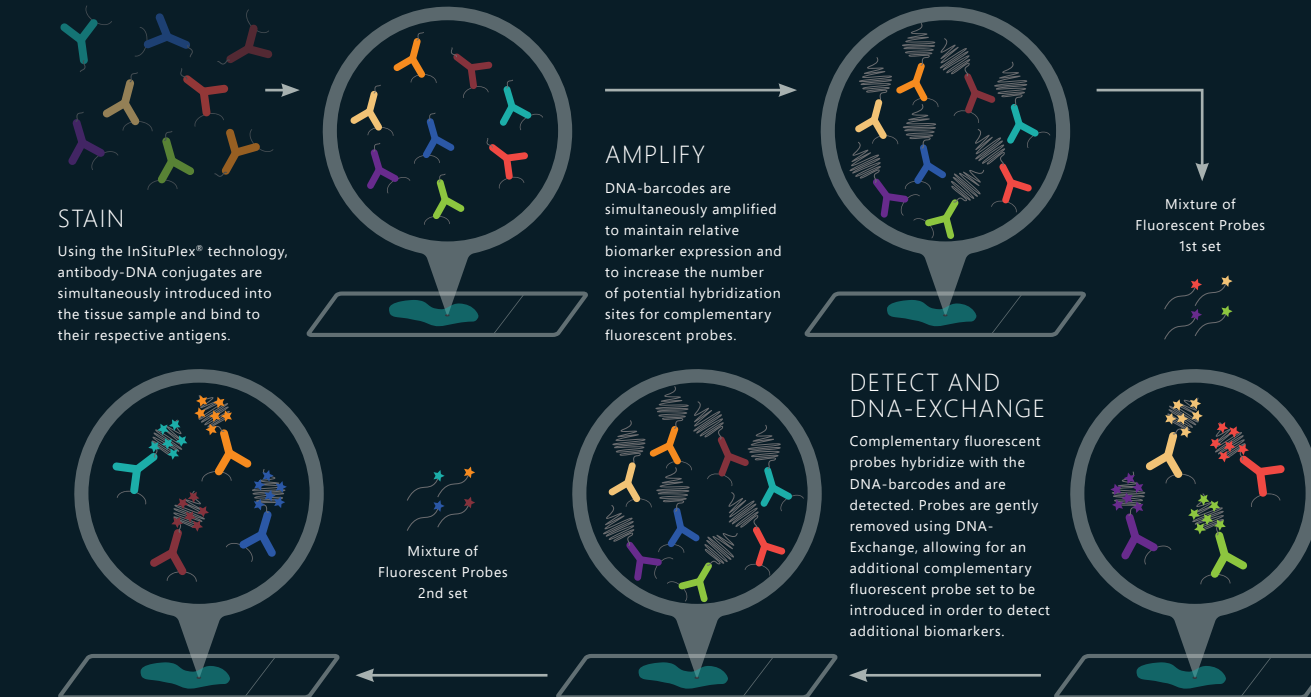
Immunosuppressive macrophage
CD68+ PD-L1

Regulatory T cell
CD3+ CD4+ FoxP3+

Immuno-evasive tumor cell
CK+ PD-L1+

Ultivue products are for research use only; not for use in diagnostic procedures.

INSITUPLEX IHC ASSAY WORKFLOW



SAME-SLIDE IHC AND H&E STAINING

Whole-slide H&E staining performed after InSituPlex® multiplex IHC provides both biomarker multiplexing and tissue morphology. This view of both brightfield and fluorescence gives a more complete view of the TME, including the localization of specific cell types, and facilitates phenotype identification.

