The tumor microenvironment is a complex tissue that is the site of ongoing interactions between the tumor cells, infiltrating immune cells, and the surrounding stromal cells. Characterization of the transcriptional patterns within the tumor microenvironment can lead to insights about the dynamic interplay that occurs as tumors are evolving strategies to evade immune-mediated detection and clearance. Understanding the prevalence of various evasion strategies within a given tumor type and across tumors and may accelerate development of novel therapeutics for defined patient populations.

The PanCancer IO360 panel was designed for research use on the nCounter platform and enables digital profiling of 770 genes that shape the tumor-immune interface. In addition to providing gene-level information, the panel also condenses genes measuring similar biology into signatures that provide robust characterization of a given pathway, thus enabling greater insights to be gained from fewer samples. The PanCancer IO360 panel is built around the Tumor Inflammation Signature (TIS), a signature which measures the presence of a suppressed adaptive immune response within the tumor, and which can be used to enrich for patient response to immune checkpoint inhibitors. The other signatures and gene content within the panel build upon the TIS to provide more nuanced characterization of the immune response within the tumor and mechanisms of evasion. In summary, the PanCancer IO 360 panel enables deep biological insight into tumor immunity that will increase our understanding of fundamental biology and accelerate the path to novel therapeutics discovery and development.

**Tumor Immunogenicity**

**Antigen processing machinery expression loss** – APM loss – This signature measures the extent to which any of several key MHC genes have atypically low expression conditional on total MHC Class I gene expression. Score values below 2 are expected from tumors with intact antigen presenting machinery.

- Antigen presentation via MHC class I in tumor cells is a major mechanism for immune recognition of tumors.
- Mutation or loss of expression of key class I MHC genes has been observed to confer resistance to immunotherapy.

**Mismatch repair expression loss** – MMR loss – This signature measures the expression levels of several key mismatch repair genes. Mismatch repair deficiency often results when one of these genes has significant expression loss.

- Mismatch repair deficient tumors have high mutation rates due to the loss of important DNA-repair mechanisms. MMR loss is associated with better immune recognition in certain types of tumors. High scores in this signature indicate MMR deficiency.
- Mismatch repair deficiency and microsatellite instability (MSI) predict response to immune checkpoint blockade.
**Tumor Sensitivity to Immune Attack**

*Apoptosis* – Apoptosis - This signature captures genes associated with apoptotic processes, specifically with genes involved in mitochondrial membrane integrity. It includes both pro- and anti-apoptotic genes. A high score in this signature indicates intact apoptotic machinery.

*Tumor proliferation* – Proliferation – This signature measures genes involved in tumor proliferation.
- A highly proliferative tumor can overcome an immune response if replication exceeds immune mediated detection and elimination.

*JAK-STAT pathway expression loss* – JAK-STAT loss – This signature measures loss of genes associated with JAK-STAT signaling.
- JAK-STAT loss has been identified as a mechanism of acquired resistance to immune checkpoint blockade

**Inhibitory Tumor Mechanisms**

*B7-H3 gene expression* – B7-H3 – B7-H3 (CD276) is a negative regulator of T cell activity that is expressed on both tumor and immune cells.
- High B7-H3 gene expression is negatively correlated with response to therapy.

*IDO1 gene expression* – IDO1 – Indoleamine 2,3-dioxygenase 1 (IDO1) is expressed by tumor, immune, and stromal cells and is the rate limiting enzyme of tryptophan catabolism. By catalyzing the degradation of tryptophan, which is necessary for cytolytic T cell proliferation and activity, IDO1 inhibits anti-tumor immune responses.
- High levels of IDO1 gene expression indicate adaptive immune resistance.

*PD-L1 gene expression* – PD-L1 – Program cell death ligand-1 (PD-L1, CD274) is a ligand for PD-1 and negative regulator of T cell activity that is expressed on both tumor and immune cells.
- High levels of PD-L1 biologically indicate the presence of adaptive immune resistance and have been associated with response to anti-PD-1/PD-L1 blockade.

*TGF-beta gene expression* – TGF-beta – TGFβ (TGFBI) is a pleiotropic cytokine which inhibits anti-tumor immune activity and promotes tumor growth and survival.

**Stromal Factors**

*Endothelial cells* – This signature measures genes associated with vascular tissue and angiogenesis.
- Angiogenesis is important for nutrient trafficking to the tumor and proper oxygenation for tumor growth. Tumor angiogenesis forms leaky inefficient vessels that can reduce efficiency of lymphocyte trafficking to tumors.
- Changes in endothelial cell frequency can help identify drug mechanism of action.

*Stromal Tissue Abundance* – Stroma – This signature measures stromal components in the tumor microenvironment.
- The tumor stroma is the collection of non-cancerous and non-immune tissue components surrounding the tumor. Stroma can act as a physical barrier that excludes immune cells from the tumor, preventing effective anti-tumor immunity even when tumor-associated antigens have induced immune cell priming and activation. These cells can also secrete important signals to the tumor, affecting tumor biology and response to the immune system.

**Inhibitory Metabolism**

*Glycolysis* – Glycolysis - This signature measures genes participating in energy consumption.
- Up-regulated glycolysis and corresponding increased glucose consumption is nearly universal in tumors. Glycolysis may inhibit effective immune responses by depriving immune cells of glucose in the tumor microenvironment and changing other molecules present in the tumor microenvironment that signal to the immune system.

*Hypoxia* – Hypoxia - This signature measured genes associated with reduced oxygenation in the tumor.
- Hypoxia can induce expression of many cancer promoting processes (e.g. invasion, motility, metabolic reprogramming) and can promote resistance to immune cell-mediated cytolyis and reduced cytolytic activity in NK and CD8+ T cells.
Anti-Tumor Immune Activity

Tumor Inflammation Signature – TIS – TIS measures the abundance of a peripherally suppressed adaptive immune response within the tumor.

• This signature was developed in the context of anti-PD-1 treatment and trained to predict response to anti-PD1 therapy (pembrolizumab). It consists of genes related to Interferon-gamma signaling, antigen presentation, natural killer and T cells and inhibitory pathways. It also includes normalization genes that have been selected to give consistent expression levels across most tissue or tumor types.

• This signature is useful for predicting response to anti-PD1 therapy and determining hot and cold immune status across multiple cancer types.

Cytotoxicity – Cytotoxicity - This signature measures the molecules used by natural killer (NK) and CD8+ T cells to mount a cytolytic attack on tumor cells.

• Cytotoxic cells, both NK and CD8+ T cells, use a number of molecules, including perforin, granzymes and granulysin to penetrate and kill infection cells and tumors. Cytotoxic activity is the mechanism by which the immune system most effectively kills tumor cells.

• This signature is useful for determining immune hot and cold immune tumor status and the presence of functionally active NK and T cells in the tumor.

Interferon gamma signaling – IFN gamma – This signature tracks the canonical response to IFN gamma, including the most universal components of that response.

• Interferon gamma is a critical component of a natural killer cell, CD8+ and Th1 CD4+ T cell-mediated adaptive anti-tumor immune response. IFNγ induces macrophage and NK cell activation, increased antigen presentation, and induces gene transcription patterns that can lead to immune cell recruitment to the tumor.

• Interferon gamma signaling expression is associated with response to anti-PD1/L1 therapy.

Interferon Signaling Response - IFN downstream – This gene signature reflects activation of a broader set of interferon signaling pathways.

• Type I and II Interferons are both implicated in tumor immune responses and regulate anti-tumor activity. Malignant and immune cells in the tumor microenvironment produce type 1 interferents, which have been shown to play a role in immunosurveillance.

• Interferon signaling is associated with improved patient outcome.

Lymphoid compartment activity – Lymphoid – This signature measures a broad set of genes involved in the functioning of lymphoid cells, including genes quantifying T cell abundance, B cell abundance, NK cell abundance, cytotoxic activity, interferon gamma signaling, JAK-STAT signaling, and T-cell co-stimulatory and co-inhibitory molecules.

• This lymphoid signature gives an overview of immune cells and factors in the tumor microenvironment.

• This signature captures a broad look at the lymphoid immune status of a tumor.

• High lymphoid score is associated with improved patient outcome.

MHC class II antigen presentation – MH2 – This signature measures the major human leukocyte antigens (HLA) involved in MHC Class II antigen presentation.

• Professional antigen presenting cells (dendritic cells, macrophages and B cells) use the class II MHC to present extracellular antigens to CD4+ T cells. Activation of CD4+ T cells induces expression of cytokines that can promote cytotoxic T cell activation and effective anti-tumor adaptive immune responses.

• Presence of MHC Class II molecules is associated with improved patient outcome.

Myeloid compartment activity – Myeloid – This signature measures key marker and effector genes of myeloid lineage immune cells.

• Myeloid cells regulate both anti- and pro-tumor activities.

• This signature captures a broad look at the myeloid immune status of a tumor, including general myeloid markers (CD14), toll-like receptors, colony-stimulating factors/receptors, signal regulatory protein (SIRPs) and competitive local change carrier (CLECs) genes involved in monocyte, macrophage and dendritic cell biology.

Inhibitory Immune Signaling

ARG1 gene expression – ARGI – Arginase-1 (ARG1) is expressed by myeloid cells and catalyzes the conversion of arginine to ornithine and urea, which suppresses T cell responses by preventing proliferation.

CTLA4 gene expression – CTLA4 – Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is a checkpoint molecule that inhibits T cell priming by competitively binding CD80/86 to prevent co-stimulation of via CD28. CTLA4 is the target for multiple immune checkpoint blockade therapies, including ipilimumab.

IL10 gene expression – IL10 – Interleukin-10 (IL10) is a pleiotropic cytokine expressed predominately by monocytes that impacts multiple aspects of the tumor immune response, including antigen presentation, T cell activation, and cytokine production.
Inhibitory Immune Signaling (cont.)

Inflammatory chemokines – Inflam chemokines - Inflammatory chemokines recruit both myeloid and lymphoid populations to the tumor microenvironment.

Myeloid-derived inflammatory signaling – Myeloid Inflam – This signature measures gene expression for myeloid lineage cells with pro- and anti-tumor functions.
  • Myeloid cells produce a number of cytokines and chemokines that promote a state of inflammation. Depending on the context, these can promote tumorigenesis or anti-tumor immune responses.

NOS2 gene expression – NOS2 – Nitric Oxide Synthase 2 (NOS2) is induced by IFNγ and regulates expression of nitric oxide, which at low levels can promote tumor growth but at high levels may be cytostatic or cytotoxic to tumor cells.

PD-1 gene expression – PD1 – Program cell death receptor 1 (PD-1, PDCD1, CD279) is expressed predominantly on lymphocytes, it is upregulated upon activation and becomes a negative regulator of activation by preventing proliferation and cytokine secretion. PD-1 expression has been shown to be associated with tumor-specific T cells.

PDL2 gene expression – PDL2 – Program cell death ligand 2 (PD-L2, PDCDLG2, CD273) is a ligand for PD-1 and negative regulator of T cell activity that is expressed on antigen presenting cells.

TIGIT gene expression – TIGIT – T cell immunoreceptor and Ig and ITIMS domains (TIGIT) is an immune checkpoint molecule that suppresses anti-tumor immune activity in CD8+ T cells.

Immune Cell Population Abundance

B cell abundance – B cells – This signature measures the abundance of B cells in the tumor microenvironment.
  • B cells are one of the main components of the humoral immune response. B cells have many functions including developing into plasma cells that secrete antibodies and presenting antigens to T cells. Presence of B cells in the tumor microenvironment have been shown to be associated with tertiary lymphoid structures in some tumor types.
  • This signature measures the presence of B cells in the tumor microenvironment.

Cytotoxic cell abundance – Cytotoxic cells – This signature measures the abundance of cytotoxic cells in the tumor microenvironment.
  • Cytotoxic cells, both NK and CD8+ T cells, use a number of molecules, including perforin, granzymes and killer cell lectin-like receptor (KLRG) family members to recognize, penetrate and kill infection cells and tumors. Cytotoxic activity is the mechanism by which the immune system most effectively kills tumor cells.
  • This signature is useful for determining immune hot and cold immune tumor status and the presence of functionally active NK and T cells in the tumor.

CD45+ cell abundance – CD45 – CD45, Protein tyrosine phosphatase, receptor type, C (PTPRC) is a marker expressed on all immune cells.

CD8+ T cell abundance – CD8 T cells – This signature measures the abundance of CD8+ T cells in the tumor microenvironment.

Dendritic cell abundance – DC – This signature measures the abundance of dendritic cells in the tumor microenvironment.

Exhausted CD8 cell abundance – Exhausted CD8 – This signature measures the abundance of exhausted CD8 cells in the tumor microenvironment.
  • T cells in the tumor microenvironment often become less functional or exhausted after exposure to factors from suppressive immune, stromal and malignant cells. These exhausted T cells lose their ability to eliminate and kill tumors. Exhausted T cells express markers and genes that are associated with reduced anti-tumor activity.
  • This signature looks at the functional activity of T cells in the tumor microenvironment.

Macrophage abundance – Macrophages – This signature measures the abundance of macrophages in the tumor microenvironment.
  • Macrophages can either augment tumor immunity (e.g. by presenting antigen) or suppress tumor immunity (e.g. by releasing immunosuppressive cytokines).

Mast cell abundance – Mast cells – This signature measures the abundance of mast cells in the tumor microenvironment.

Natural Killer cell abundance – NK cells – This signature measures the abundance of NK cells in the tumor microenvironment.
Immune Cell Population Abundance (cont.)

**NK CD56dim cell abundance** – NK CD56 dim cells – This signature measures the abundance of NK cells in the tumor microenvironment. This signature represents the subset of NK cells with the most cytolytic activity.

**Neutrophil abundance** – Neutrophils – This signature measures the abundance of neutrophils in the tumor microenvironment.
- In the context of the tumor, neutrophils tend to have immunosuppressive activity.

**T-cell abundance** – T-cells – This signature measures the abundance of T cells in the tumor microenvironment.

**Th1 cell abundance** – Th1 cells – T-box transcription factor TBX21 (T-bet) is the canonical transcription factor that defines Th1 T cells and is used to measure Th1 cell abundance. Th1 T cells promote anti-tumor immune activity (particularly supporting CD8+ T cell function) by producing IFNγ.

**Treg abundance** – T-regs – Regulatory T cell (Treg) abundance is measured by gene expression of Forkhead box P3 (FOXP3). FOXP3 is the canonical transcription factor that defines the regulatory T cell (Treg) population and is used to measure Treg abundance. Regulatory T cells suppress other T cell activities through a variety of mechanisms.

Selected publications


