

# Tumor microenvironment is a powerful companion diagnostic for cancer immunotherapy applications

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Currently approved checkpoint inhibitors are antibodies that block the function of three key proteins expressed on the surface of T cells: CTLA-4, PD-1 and PD-L1.

Under normal conditions, these proteins function as brakes to prevent immune-related toxicity from arising because of persistent T cell activity. Cancer hijacks this essential function of immune homeostasis to protect itself from immune-mediated elimination [1, 2]. By expressing high levels of PD-L1, tumor cells engage PD-1 receptors on T cells, suppressing their anti-tumor activity and escaping T cell-mediated killing. By blocking PD-1 and PD-L1 signaling, the checkpoint inhibitors remove the brakes on T cells imposed by the tumor and enhance their anti-tumor activity [3].

The use of these inhibitors in the clinic has provided a significant, durable response to a subset of patients resulting in improved overall survival – and in many instances – a cure [4, 5]. However, a significant unmet need in cancer immunotherapy is to expand the use of these novel therapies to more patients. Therefore, the pressing need now is to discover biomarkers for selecting patients who will experience maximal benefit from treatment, without experiencing immune-related toxicity.

## Tumor microenvironment modulates response to checkpoint inhibitors

Although the mechanism by which checkpoint blockades activate T cells is relatively well known, there are significant gaps in our understanding of factors that regulate tumor clearance. It is now recognized that the presence of T cells in the tumor microenvironment is necessary, but not sufficient for response. On the other hand, the anti-tumor response of T cells is modulated by other tumor intrinsic and extrinsic factors that together determine the durability of response.

For example, many clinical studies have shown that tumors with a large number of mutations in the coding sequence (tumor mutation burden) are more responsive to checkpoint inhibitors, such as mismatch repair-defective colorectal cancer [6, 7]. Other tumor extrinsic factors that blunt checkpoint inhibitor response include:

- Presence of activated stroma
- High angiogenic burden
- Lack of pro-inflammatory cytokines such as interferon-gamma (IFN- $\gamma$ )
- Tumor necrosis factor-alpha (TNF- $\alpha$ )
- Presence of immune suppressive cells such as T-regulatory cells (Treg)
- Alternatively activated macrophages (M2-type)
- Myeloid-derived suppressor cells (MDSCs) [8-10]

Finally, defects in antigen presentation machinery of tumor cells that render them invisible to the host immune system can lead to a lack of response to the checkpoint inhibitors. Taken together, an array of tumor-derived and host-derived factors interact among themselves to create a microenvironment that is permissive for evoking an anti-tumor response. Therefore, analysis of the tumor microenvironment becomes critical for selecting patients who will benefit from checkpoint blockade therapies.

## Methods to analyze the tumor microenvironment

Tumor microenvironment is a complex mixture of cells that interact with each other constantly to create an ecosystem that resembles more of a wound rather than normal tissue [8, 10, 11]. Rapid advances in genomic and proteomic technologies have enabled better characterization of the microenvironment, capturing

molecular and biochemical details not possible by traditional immunohistochemical methods. However, immunohistochemistry provides spatial distribution of cells in the tumor, which is lost when bulk tumors are analyzed by genomic and proteomic methods.

For example, immunohistochemical methods can only show whether T cells are present in close proximity to the tumor cells, or present at the margin of the tumors – strong anti-tumor response is observed when T cells are in close proximity to the tumor cells [12]. Although powerful, immunohistochemistry cannot capture the whole array of features that render tumors sensitive to checkpoint blockade. Lack of adequate tumor tissue, cost of labor, low throughput workflow and turnaround time are some of the limitations that make immunohistochemistry unsuitable for use in selecting patients for cancer immunotherapy applications.

Genomics, on the other hand, is not restricted by any of the limitations mentioned above. Low input tissue requirements, robust high throughput workflow driven by automation in sample preparation, and sequencing keep cost lower and turnaround times shorter, which is ideal for complex biomarker-driven patient selection strategies. Sequencing all expressed genes in a tumor (whole transcriptome analysis) captures a large number of features associated with all the different cell types present in the tumor microenvironment [13]. The challenge is to deconvolute the complex mixture of information and extract relevant biomarkers, which will predict response of the tumor to checkpoint blockade.

One approach is to create gene expression signatures specific to immune cell types, stromal cells and other cell types present in the tumor microenvironment and apply these signatures to estimate their absence, or presence, in a specific tumor. A deeper level of analysis would be to assess the functional phenotype of specific immune cells – for example, to determine whether the T cells are functionally active or exhausted. The anti-tumor response is closely linked to the activation state of T cells [14, 15].

Recently, the technique of single-cell sequencing has provided an unprecedented level of resolution to seek deeper insights into the tumor microenvironment [16, 17]. Single-cell sequencing enables accurate quantification of various cell types present in the tumor, including a list of genes transcribed by individual cells. For the first time, one can identify IFN- $\gamma$ -producing cell types from cells that produce

immune suppressive factors. The current methodologies of single-cell sequencing require live cells and therefore are not readily applicable in clinical settings.

Researchers should take an integrated approach to combine discoveries from the single-cell sequencing space and deconstruct the complex tumor microenvironment from bulk transcriptome data. This approach provides detailed cellular and molecular features of the tumor microenvironment, which can be used as a CDx (companion diagnostics to select patients who will benefit from checkpoint inhibitor treatment) and provide guidance to combination therapies, specifically vaccines and other cancer immunotherapy drugs. ■

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