InfiltrateID™

Quantification of the Immunocellular Content in the Tumor Microenvironment

The emergence and success of immuno-oncology drugs in the treatment of several cancer types has transformed clinical practice. However, many patients do not respond to these drugs, compelling researchers and clinicians to broaden their search for biomarkers that can better predict therapy response. Historically, the identification of single, tumor-specific alterations has successfully served to determine the likelihood of beneficial response to targeted, precision oncology therapies. In contrast, response to immunotherapies, such as immune checkpoint modulators (ICMs), are largely dependent on both the tumor's immunophenotype and its mutational profile, rendering the ability to analyze both tumor- and immune-related analytes within the tumor microenvironment (TME), a primary focus for oncology researchers today. Recent studies of current clinical biomarkers, such as tumor mutational burden (TMB), PD-L1 expression, and microsatellite instability (MSI) status have demonstrated suboptimal performance in separating responders from non-responders, exemplifying the need for additional biomarkers with improved predictive power.

Despite our understanding of the critical role that immune cells have to play in modern cancer treatment, traditional methods still lack the ability to accurately profile the presence of these cells within the TME of tumors. The gold-standard approach, immunohistochemistry (IHC), is burdened by its limited throughput, the difficulty associated with profiling multiple analytes simultaneously, and its laborious analytical process. Alternatively, flow cytometry requires a large amount of starting material and is typically incompatible with formalin-fixed paraffin-embedded (FFPE) samples – the most commonly used technique for storing and preserving clinical tumor samples. For these reasons, Personalis has developed InfiltrateID, a sophisticated analytical module that quantitates the presence of eight immune cell populations in a tumor sample. This module leverages the augmented gene expression data derived from the NeXT Transcriptome™ and is one of many analytical outputs of ImmunoID NeXT™, Personalis' flagship immunogenomics platform, facilitating the simultaneous characterization of both the tumor and the immune microenvironment from a single tumor specimen.

The Personalis Approach

InfiltrateID utilizes the single-sample gene set enrichment analysis (ssGSEA) approach to compute transcriptome-based enrichment scores for eight distinct immune cell types (**Table 1**) from a single tumor sample, quantifying the abundance of those populations within the TME of that sample. For this purpose, an in-house proprietary cell type-specific signature for the eight distinct immune cell types were created using NeXT Transcriptome gene expression data derived from purified immune cell populations. Each signature consists of genes curated based on a strict selection criterion, requiring consistent expression and low expression variability in each of the eight immune cell types.

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Table 1: Immune Cell Populations Profiled by InfiltrateID and Their Respective Roles and Relevance in Cancer

Immune Cell Type	Roles and Relevance in Cancer
B-cells	B-cells are the primary drivers of the humoral immune response; generating B-cell receptors (BCRs) and antibodies which enable the host to mount an immune response against a wide range of antigens/pathogens (Sharanov et al., 2020).
Dendritic cells – Conventional (cDCs)	In the context of the TME, cDCs can present antigens derived from tumor cells to T-cell receptors (TCRs) to stimulate an adaptive immune response. Presence of cDCs in the TME is typically associated with better prognosis (Böttcher et al., 2018).
Dendritic cells – Plasmacytoid (pDCs)	pDCs are best known for their regulatory effects, including the rapid and large production of type I interferons. Functional impairment of pDCs have been implicated in creating an immunosuppressive TME in cancers (Koucky et al., 2019).
Macrophages	Circulating monocytes are recruited to the TME by chemotaxis and a subset of them can differentiate into tumor-associated macrophages (TAMs). TAMs play a prominent role in the formation of an immunosuppressive TME by producing chemokines and cytokines (Lin et al., 2019).
NK cells	NK cells are effector immune cells that are capable of direct cell-killing. The elevated presence of NK cells in the TME of solid tumors is generally considered an indication of good prognosis (Habif et al., 2019).
T-cells – Cytotoxic (CD8+)	The pre-existence of elevated numbers of tumor-infiltrating lymphocytes (TILs) (specifically, CD8+ T-cells) has been associated with improved prognostic effects and has also correlated with beneficial response to ICM therapy in many solid tumor types including melanoma, colorectal, and triple-negative breast cancer (Maimela et al., 2019).
T-cells – Helper (CD4+)	CD4+ T-cells play a significant role in mediating the immune response via the secretion of specific cytokines and subsequent activation and expansion of CD8+ T-cell and antibody production by B-cells (Lai et al., 2011).
T-cells – Regulatory (Tregs)	Tregs are a subset of CD4+ T-cells known for their immunosuppressive influence, mediated by mechanisms including production of immunosuppressive cytokines such as IL-10 and TGFβ and the conversion of ATP into adenosine (Li et al., 2020). Unsurprisingly, tumor infiltration of Tregs is associated with poor prognosis in many cancer types including melanoma, NSCLC, gastric, and ovarian (Kim et al., 2020).

Quantification Concordance with Immune Cell Mixtures

To assess the quantification accuracy of InfiltrateID, a set of pre-defined mixtures combining four purified immune cell populations in various proportions was created. The abundance of each immune cell type in these mixtures were quantified with flow cytometry and was then compared with the Infiltrate ID ssGSEA scores (**Figure 1**). Strong concordance in the results were observed, suggesting that the ssGSEA scores correlated well with the underlying relative abundance of different immune cells in a given sample.

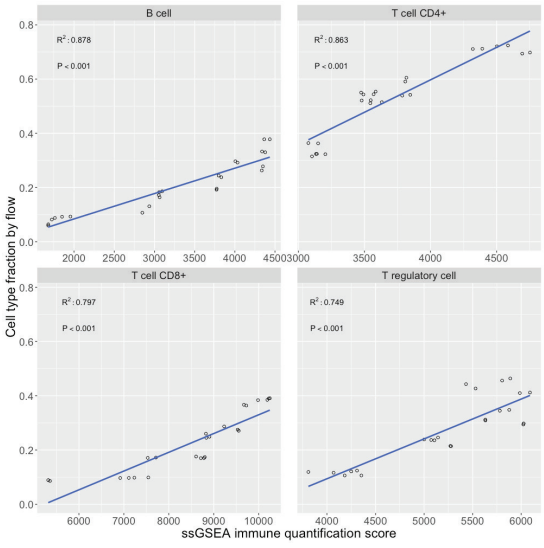

Figure 1: Comparison of InfiltrateID ssGSEA scores with immune cell fractions (B cells, CD4+ T cells, CD8+ T cells, Treg cells) evaluated with flow cytometry for immune cell mixtures.

Quantification Concordance with Tumor FFPE Samples

The quantification accuracy of the ssGSEA scoring approach was evaluated on a set of colorectal and lung cancer FFPE samples. Good concordance was observed between the immunohistochemistry (IHC)-based cell type fraction calculation and the ssGSEA scores for these samples (**Figure 2**). These results suggest that InfiltrateID ssGSEA scores can be reliably used to assess the underlying Immunocellular content within the TME of tumor FFPE samples.

InfiltrateID enables delineation of the underlying Immunocellular profile in a given tumor sample, providing novel information that may help stratify cohorts of patients with similar profiles. The ssGSEA scores can be computed for any individual sample independently, giving the flexibility to examine data from any single patient within a sample cohort even before the entire cohort dataset is available. Furthermore, while other enrichment scoring methods require a large number of samples to produce stable comparable scores, the ssGSEA scoring method provides the ability to compare and contrast the differences in the underlying immunocellular content for both small and large sample cohorts.

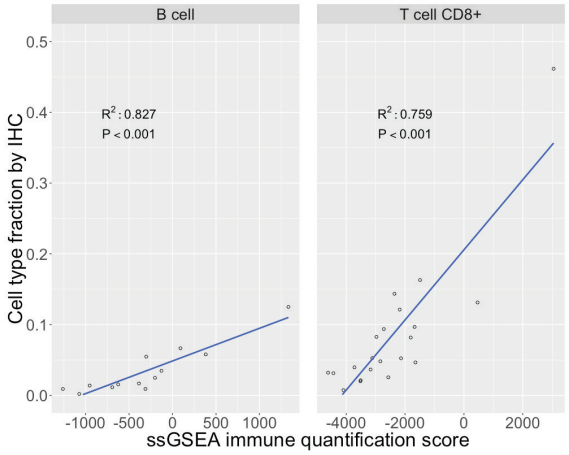

Figure 2: Comparison of InfiltrateID ssGSEA scores with immune cell fractions (B cells, CD8+ T cells) evaluated with IHC for tumor FFPE samples.

The complex nature of resistance to immunotherapy, as well as potential toxicities associated with treatment, underscores the need for biomarkers that can accurately predict therapeutic response and improve clinical outcomes. The Personalis NeXT Platform® provides an entire suite of analytical outputs to help address this increasing need for effective biomarkers to help advance precision immunotherapy discovery efforts, taking into account both tumor-specific and immune-specific characteristics for improving the predictive strength of biomarkers and thereby facilitating effective therapeutic stratification.

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