



Immunophenotyping signatures — all in the HTG EdgeSeq Reveal software.

Tumors are composed of malignant cells embedded in a complex microenvironment that is made up of a variety of cell types, including proliferating tumor cells, blood vessels, tumor stroma, and infiltrating immune cells. The composition of the tumor microenvironment (TME), especially the density of immune and stromal cells, has been shown to influence tumor progression and treatment outcomes. To address this, twenty-three signatures were developed to measure the relative cell abundance of immune and stroma cell types using gene expression data from the HTG EdgeSeq™ Precision Immuno-Oncology Panel (PIP). These signatures are part of the HTG EdgeSeq Reveal Oncology Signature family and are intended to build on the existing Immune, Stroma and TME signatures.

- **Development and verification utilized RNA-Seq and HTG EdgeSeq data** from over 1,000 formalin-fixed, paraffin-embedded (FFPE) tissue samples
- **xCell algorithm** (*Aran et al., 2017*) was used to generate reference scores for 23 immune and stroma cell types
- **HTG EdgeSeq Reveal Immunophenotyping scores** were orthogonally compared to multiple commonly used inflammation IHC markers in an independent cohort of over 60 samples
- **Precision evaluation** of the 23 immunophenotyping scores through interclass correlation coefficients and variance component analysis
- **Can be applied to existing and new data** using proprietary HTG EdgeSeq Reveal software

HTG EdgeSeq Reveal Immunophenotyping Signatures

Immune Cells

T-lymphocytes

| | |
|-----------------------------|-----------------------------|
| CD4 T-cells | CD4 memory T-cells |
| CD4 effector memory T-cells | CD8 T-cells |
| CD8 central memory T-cells | CD8 effector memory T-cells |
| Regulatory T-cells | T-helper type 1 cells |
| Natural Killer T-cells | |

B-lymphocytes

| | |
|--------------|---------------|
| B-cells | Naive B-cells |
| Plasma Cells | |

Mononuclear Phagocytes

| | |
|-----------------|---------------|
| Monocytes | Macrophages |
| Macrophage M1 | Macrophage M2 |
| Dendritic Cells | |

Granulocytes

| | |
|------------|-------------|
| Mast Cells | Neutrophils |
|------------|-------------|

Stromal Cells

| | |
|------------------|-------------------|
| Adipocytes | Endothelial Cells |
| Epithelial Cells | Fibroblasts |

Signatures Comparison and Implementation

- **Comparison to reference xCell scores:** Correlations between the reference xCell signature and the predicted scores from the test data set were evaluated for all signatures. HTG Immunophenotyping Signature scores show strong correlation to the xCell reference signatures for all 23 cell types, with Lin's concordance correlation coefficient values (Lc) ranging from 0.66 to 0.9 (*data not shown*).
- **Comparison to IHC staining:** Correlations between the IHC and HTG Immunophenotyping Signature scores for CD8 and CD4 T-cells across 61 non-small cell lung cancer FFPE samples were plotted (*Figure 1 top*). HTG Immunophenotyping Signature scores show strong correlation to immunohistochemistry (IHC) with Pearson correlation coefficients of 0.77 and 0.81 for CD4 and CD8, respectively.
- **Comparison to spiked-in cells:** Correlation between HTG Immunophenotyping Signature scores and the predicted cell density were plotted and analyzed by calculating the Pearson correlation coefficient for each cell type. HTG Immunophenotyping Signature scores show strong correlation to IHC with Pearson correlation coefficients of 0.98 and 0.97 for CD4 and CD8, respectively.
- **Software Signature Implementation:** The twenty-three immunophenotyping signatures are applied to HTG EdgeSeq PIP data using the HTG EdgeSeq Reveal software. The HTG EdgeSeq Reveal software is a fully integrated web-based data analysis software suite that can analyze data quality and generate publication quality figures. New features include downloadable excel file containing signature scores for all samples, heatmaps and radar charts.

Comparison of Immunophenotyping Signature Scores

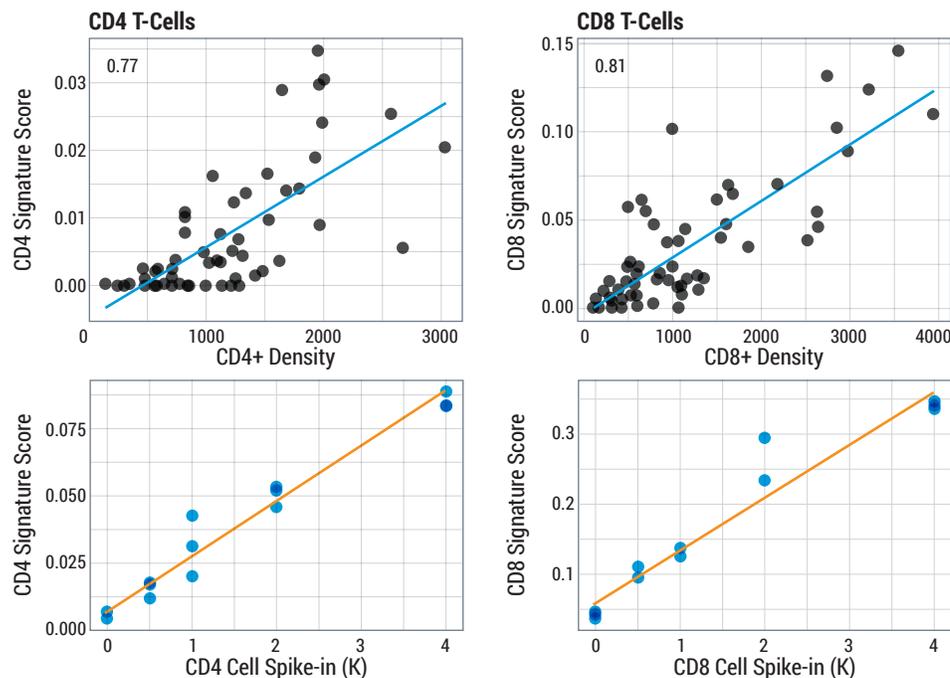


Figure 1: Two examples of comparisons of the Reveal Signature scores to IHC and to spiked-in cells. Correlation between signature outputs and IHC stains for CD4 and CD8 T-cells (top). Correlation between signature outputs and the amount of immune or stromal cells spiked in for CD4 and CD8 cells (bottom). The Pearson correlation coefficient is shown in the upper left corner for each cell type. The orange line represents the estimated linear regression line.

References: Aran D, et al. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol.* 2017. 18:220

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