

Cancer ImmuneNet Biomarker Profiling Assay



CELLECTA

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Introduction

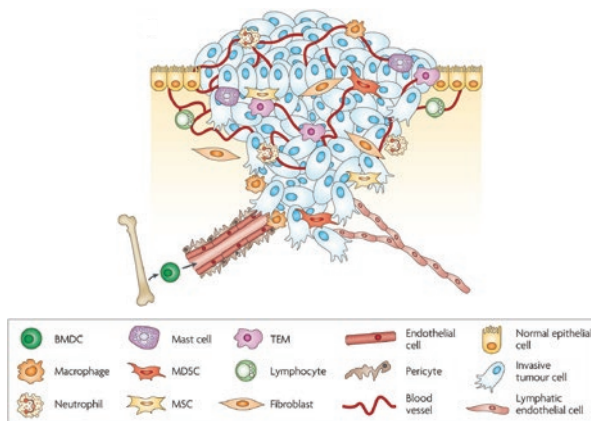


Figure 1. Tumor Microenvironment. Adapted from Joyce, J. A., & Pollard, J. W. (2009). Microenvironmental regulation of metastasis. *Nature Reviews. Cancer*, 9(4), 239–252.

Background:

- Link between the immune infiltrate and several human carcinoma types and prognosis and/or response to therapy
- Increasing evidence suggests that the number, type, and location of tumor-infiltrating lymphocytes in primary tumors harbor prognostic value, and this has led to the development of a “tumor immunoscore/immune” index

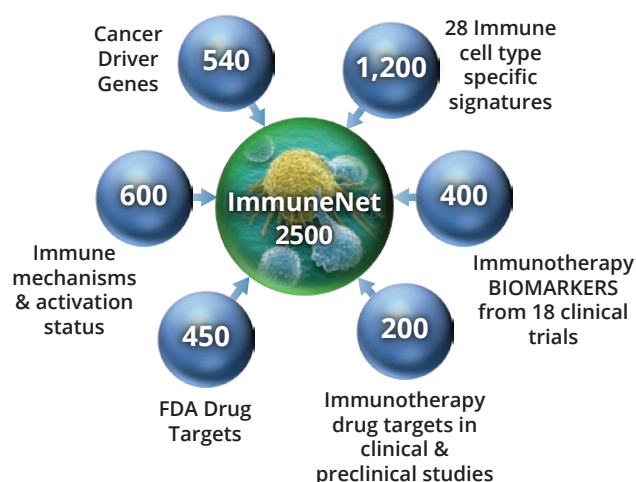
Aims:

- Develop comprehensive expression assay for analysis of cellular composition
- Molecular profiling of key immune-related genes, including drug targets, known biomarkers, and immune mechanisms
- Unbiased discovery of most informative biomarkers that can be analyzed by conventional IHC/FACS assays

What is ImmuneNet?

- Detect cellular composition of immune / stromal / cancer cells in tumor microenvironment
- Identify immunity status and immunoediting mechanisms
- Discover novel biomarkers for immunotherapy
- Profiling of immunotherapy targets and all FDA-approved drug targets

ImmuneNet Assay Gene Composition



The ImmuneNet 2500 panel includes a comprehensive set of 2500 genes specific for detection and profiling of different types of immune cells including T cells, B cells, fibroblasts, stromal and endothelial cells in the tumor microenvironment, activated immune cells suggestive of adaptive and innate immunity, immunity-related genes from 16 predictive and prognostic core gene signatures that have been validated in recent chemo- and immunotherapy clinical trials across several tumor types, including melanoma, colorectal, breast, and lung cancers.

ImmuneNet Assay Workflow

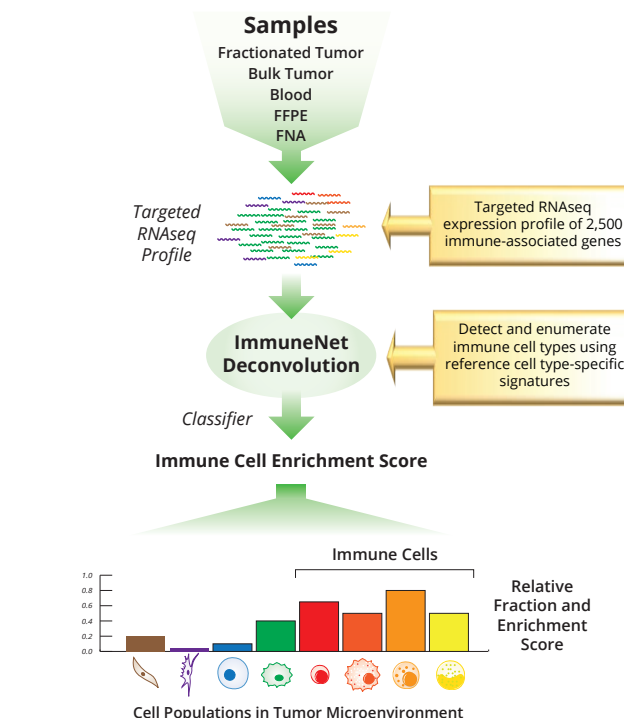


Figure 2. Summary of the ImmuneNet 2500 pipeline and application to infiltrating tumor immune cell deconvolution. ImmuneNet uses a novel, multiplex, quantitative, targeted RNAseq assay and classifier algorithm to measure the absolute digital expression for ~2500 genes and accurately resolve relative fractions of diverse immune cell types from complex tissues.

Development of Classifier Model for Different Immune Cell Types in Tumor Microenvironment

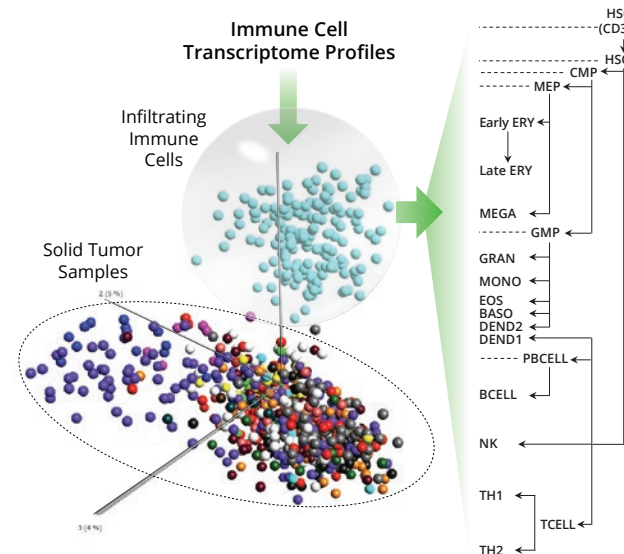


Figure 3. Cluster Analysis with ImmuneNet 2500: Model reference gene expression data by principal component analysis using ImmuneNet differentially expressed (DE) genes successfully distinguishes hematopoietic cell phenotypes from bulk tissues and tumor cells. Diagram to right describes major cell types detected by the assay.

Development of Functionally Validated RT-PCR Primers

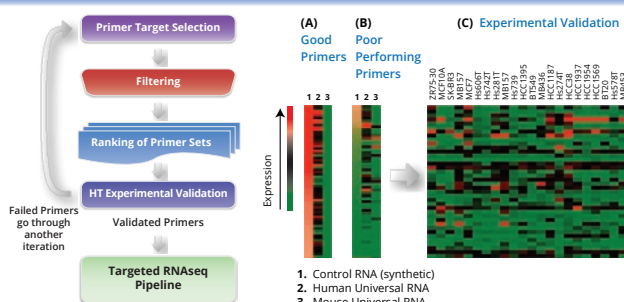
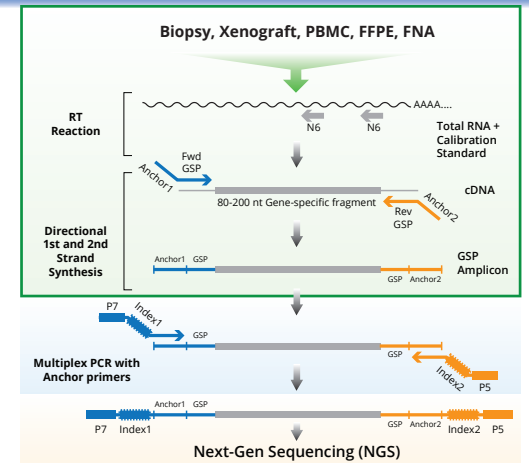


Figure 4. Testing Primer Efficiency. Primers designed for the select panel of genes are functionally validated to be compatible with our high-throughput, multiplex protocol. Synthetic RNA control (Lane 1) and Human Universal RNA control (Lane 2) help demonstrate that the primers work poorly in Fig 4A and efficiently as in Fig 4B. Mouse Universal RNA control (Lane 3) denotes cross-reactivity. Validated Primers with greater efficiency are selected and are subsequently used in the Targeted RNA Seq assay. 24 cancer model cell lines were profiled against a select panel of genes (125). Red denotes upregulated genes and green denotes low expression in the heat-map presented (Fig. 4C).

Next Generation Targeted RNAseq



- Profiling of up to 10,000 genes in a single tube assay
- Requires 10-100 ng of total RNA from biopsy, blood, FFPE, or FNA samples
- Built-in calibration standards for QC and normalization of expression data

Figure 5. Our novel workflow leverages the power of quantitative PCR (upstream) and Next-Gen Sequencing (downstream read-out). Experimentally validated gene-specific primers are used to amplify selected target regions. Synthetic calibration controls act as an effective QC metric.

Validation of ImmuneNet Assay with TNBC Samples

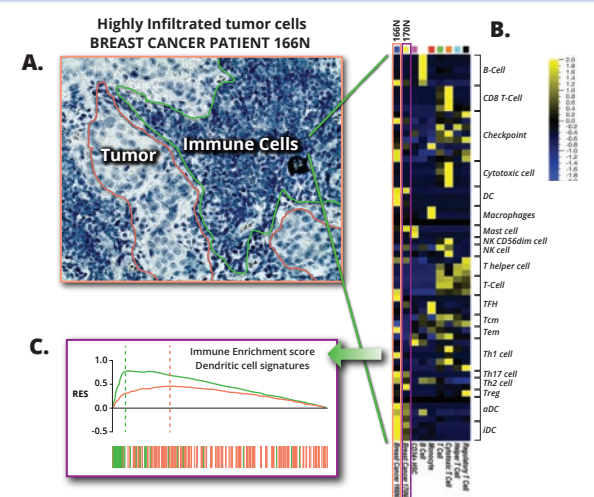
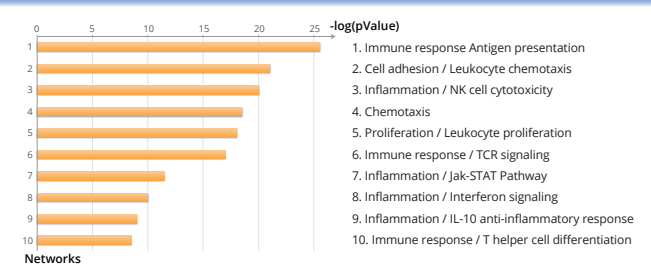


Figure 6. Validation of ImmuneNet 2500 assay with TNBC clinical samples. (A) Tumor immune cell infiltration visualized by H&E staining (40X magnification). (B) Heat map showing differentially expressed genes detected by ImmuneNet assay using immune-infiltrated and control tumor samples. (C) Example immune enrichment score for immune infiltrating dendritic cells.

Signaling Networks Detected by ImmuneNet



Conclusions

We present the novel Cancer ImmuneNet 2500 assay panel, a quantitative, multiplexed, high throughput targeted RNAseq approach that leverages the power of multiplex PCR technologies and NGS and allows you to obtain the transcriptome profile of the tumor microenvironment.

The ImmuneNet Panel includes 2,500 immunity-related genes. In order to develop cell-type specific gene signatures, we developed a non-probabilistic binary linear classifier algorithm to infer the level of infiltrating immune cells in tumor tissues. Our panel can distinguish hematopoietic cell phenotypes from bulk tissues and tumor cells and offers a unique approach to identify tumor-infiltrating cells.

In this study, we present the design and algorithms of our panel that can accurately resolve relative fractions of diverse immune cell types from complex tissues.

Intended Applications:

- The platform is applicable for novel predictive and prognostic biomarker discovery.
- Comprehensive profiling of tumor-associated immune cell composition will provide important insights into cancer immunoediting mechanisms
- Has the potential to provide a new molecular stratification approach applicable to cancer immunotherapy. Currently, a portfolio (Driver-Map™) of such assays are in development to address specific disease areas.