Molecular Characterization for CAR-T Cell Therapy: A Step Toward Standardization with the nCounter® CAR-T Characterization Panel

Abstract

CAR-T cell therapy has produced significant advancements in the treatment of hematological malignancies and an explosion of research aimed at development of CAR-T therapies for solid tumors. This great momentum has fueled over 600 clinical trials worldwide, spanning 100+ pharmaceutical, biotechnology and specialized academic centers with a focus on CAR-T therapy and even more investigating next generation approaches. Despite this activity, the field recognizes that a number of significant challenges remain, as we have yet to fully realize the significant benefits influencing efficacy and safety in patients. In large part, we know this challenge is multiplicative by highly variable input materials and as part of the manufacturing process both from the patient and the vector, as well as the complex biology behind producing a living drug that persists in patients potentially even years after treatment.

To address these challenges and further support the need for standardized approaches, Nanostring has developed a new gene expression panel for use with the nCounter® platform for the molecular characterization of CAR-T cells in research, development and manufacturing including both pre and post infusion monitoring. The nCounter CAR-T Characterization Panel was created in collaboration with leading centers in the field of CAR-T therapy and is designed for use across the entirety of the CAR-T workflow, enabling uniform and standardized quantitation of whole-cell and secreted protein and post infusion CAR-T cells. The customizable, 786 gene expression panel incorporates content to measure 6 essential components of CAR-T biology including T-cell activation, memory, exhaustion, and TCR receptor diversity with optional customization for measuring transgene expression with NanoString’s Protein Barcoding Service or gene expression probes.

The nCounter CAR-T Characterization panel leverages the robustness, ease of workflow and rapid time to results of the nCounter platform and aims to provide a standardized set of biomarker discovery tools for the community to both enable and advance the field of CAR-T therapy.

Key Publications Used in Panel Development

**Activation**

**Cell Types**

**Molecular**

**PERSISTENCE**

**T Cell Diversity**

**Key Questions and Challenges:**
- Selection of Antigen Target
- Selection of Mechanism of Antigen Recognition
- Selection of Construct Design
- Selection of Cellular Product

**Data Analysis**
- NanoString's nCounter Analysis System performs a highly multiplexed, digital quantification of up to 800 genes in a single reaction. This is achieved via reporter code sets, which are color-coded "barcodes" specific for each gene. Workflow consists of three major steps: 1) Hybridization, 2) Purification, and 3) Digital imaging. In the hybridization step, sample material is mixed with code set and the code sets hybridize to the mRNA complexes in the nCounter cartridge for data collection. cQD capture technology is used for data collection and digital images are processed and reporter probe counts are tabulated for data analysis using NanoString's® nSolver® software and advanced analysis modules.

- **Boost Productivity:** Intuitive workflow with only fifteen minutes of hands-on time from sample to data. Separate Digital Analyzer and Prep Station units help eliminate bottlenecks in sample processing and data collection.
- **Detect Small Fold Changes:** Eliminate cDNA synthesis, amplification, and library prep so you experience less technical variation in your assay and reduce the need for experimental replicates.
- **Simplify Analysis:** No need for a specialized Bioinformatician. Results generated as direct counts and reported in a standard CSV file that can be imported into your favorite application or the included nSolver® Software for convenient data analysis.