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

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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 active studies worldwide, spanning 100+ pharmaceutical, biotechnology and specialized academic cancer 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 understand the factors influencing efficacy and safety in patients. In large part, we know this challenge is multiplied by highly variable input materials used 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 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 8 leading centers in the field of CAR-T therapy and is designed for use across the entirety of the CAR-T work flow, enabling uniform and robust profiling of leukapheresis, manufactured product and post-infusion CAR-T cells. The customizable, 780-gene expression panel incorporates content to measure 8 essential components of CAR-T cell biology including T-cell activation, metabolism, 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.

NanoString Technology & nCounter Workflow

1. Hybridization → 2. Purification → 3. Digital Imaging

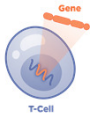
Feature	Specification
Number of Targets	Up to 800 targets
Sample Input-Standard (No amplification required)	25-300 ng
Sample Types	PBMC, FFPE-derived RNA, total RNA, fragmented RNA, cell lysate, sorted cells, whole blood/plasma, synovial fluid
Customizable	Add up to 30 unique genes with Panel-Plus and up to 10 custom protein targets
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Advanced Analysis Software (RUO)

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 codesets, 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 codeset and the codeset hybridizes to the mRNA target in solution. Purification is carried out robotically which removes excess codeset and immobilizes the codeset/RNA complexes in the nCounter cartridge for data collection. CCD 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 use the included nSolver Software for convenient data analysis.

CAR-T : Challenges with the Living Drug

CAR-T Development

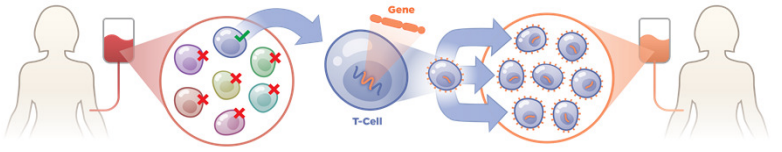


Key Questions and Challenges:
- Selection of Antigen Target
- Selection of Mechanism of Antigen Recognition
- Selection of Construct Design
- Selection of Cellular Product

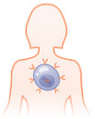
Chimeric antigen receptor T cells (CAR-T) represent the forefront of immunotherapeutic innovation, but along with the momentum come many challenges. Autologous CAR-T cells are generated from naïve peripheral T cells harvested from each individual patient and transferred back into their donor post-transduction and expansion. Development, manufacturing and treatment using CAR-T cells is inherently complex and personalized, and represents a diverse range of challenges whereby each step in the process may potentially impact the efficiency of the process, the quality of the product and the ultimate efficacy of the treatment. Ongoing research has defined a number of facets that make up a successful CAR T-based intervention: the selection of the correct host cell population and antigenic target, the ideal construct design leading to correct activation of the cell upon binding its cognate antigen, and the inherent "stem-ness" present in the transformed population allowing for rapid expansion and persistence in the patient. Many of these facets are part of a multi-step manufacturing process that today is evaluated using a myriad of analytical methods and biological assays that are stretched to deliver the accuracy and reproducibility to adequately characterize the CAR-T cells. To address these challenges and begin the movement to a more standardized molecular approach, NanoString has created the CAR-T Characterization Gene Expression Panel that takes into consideration the many questions that need to be addressed, identifies the most meaningful biology and captures these into what we define as the 8 Essential Components of CAR-T Characterization. The NanoString platform and panel will enable the field to in rapid fashion characterize on a molecular level the CAR-T product throughout its life cycle – from initial leukapheresis, to in-vitro expansion, from infusion into the patient to monitoring longitudinal immune activity over time.

CAR-T Manufacturing

1. Leukapheresis → 2. Selection & Activation → 3. Gene Tranfer → 4. Cell Expansion → 5. Infusion



Post-Infusion



Key Questions and Challenges:
- Immune-responsiveness
- CAR-T cell expansion
- CAR-T cell persistence
- On-target/Off-tumor oncogenicity
- Toxicity: CRS, Neurotoxicity
- B cell Aplasia

Key Publications Used in Panel Development

Activation	**Courtney, Lo, and Weiss.** "TCR Signaling: Mechanisms of Initiation and Propagation". Trends Biochem Sci. 2017.
Cell Types	**Danaher et al.** "Gene expression markers of Tumor Infiltrating Lymphocytes". JITC. 2017.
Exhaustion	**McKinney et al.** "T-cell exhaustion, co-stimulation, and clinical outcome in autoimmunity and infection". Nature. 2015.
Metabolism	**Kishton, Sukumar, and Restifo.** "Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy". Cell Met Rev. 2017.
	Buck, O'Sullivan, and Pearce. "T cell metabolism drives immunity". JEM. 2015.
Persistence	**Scarfo and Maus.** "Current Approaches to increase CAR T cell potency in solid tumors: targeting the tumor microenvironment". JITC. 2017.
Phenotype	**Vargas and Apetoh.** "The Secrets of T Cell Polarization". Oncoimmunology. 2018.
TCR Diversity	**Zhang et al.** "A New Approach to Simultaneously Quantify Both TCR a- and b-Chain Diversity after Adoptive Immunotherapy". CCR. 2012.
Toxicity	**Rossi et al.** "Preinfusion Polyfunctional anti-CD19 chimeric antigen receptor T cells associate with clinical outcomes in NHL". Blood. 2018.

What is the viability and purity of the leukapheresis product?	What is the cell activation state?	Are the cells expressing the transgene and CAR?	Are the CAR-T cells fit and expanding?	Is there a sustained response?
•Profile cell phenotype •Measure TCR diversity •Quantify contaminating cell types	•Profile signaling pathways •Measure known activation markers	•Quantify transgene and CAR expression with customized probes	•Measure metabolic fitness •Track differentiation status •Profile cell phenotype	•Confirm persistence •Measure overall TCR diversity •Profile cell phenotypes •Monitor exhaustion •Identify potential toxicities

Relevant Biology

Data

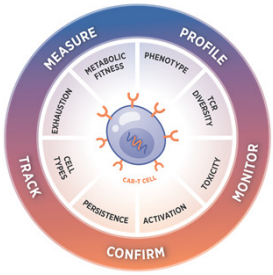
Feedback

Literature Search
- Covering gene content and biology related to T-cell and CAR-T function
- >65 unique sources for pathway information (Database and Literature)

Gene Selection
- > 3800 genes considered and annotated
- 2 published datasets, CAR-T study (Fraietta et al, 2018) and sorted T-cells from Tumors (De Simone et al, 2016)

Expert Input
- CAR-T leaders provide feedback on gene list, overall content and utility.

nCounter CAR-T Characterization Gene Expression Panel



8 Essential Components of the nCounter CAR-T Characterization Panel

Activation	Cell Types	Exhaustion	Metabolism	Persistence	Phenotype	TCR Diversity	Toxicity
TCR activation and its immediate downstream pathways are crucial in understanding CAR-T activity	CAR-T products are impacted by other immune cells	Avoiding CAR-T exhaustion is of great importance to ensuring persistent cells remain active	Metabolic changes are essential in supporting T-cell activity and define underlying cell fitness	Ongoing CAR-T presence is key to an optimal anti-tumor response and preventing relapse	Known phenotypes and associated signaling define T-cell function	TCR diversity can an informative metric for monitoring CAR-T populations over time	Off-target toxicities of treatment are correlated with cytokines and chemokines produced by CAR-T cells
• Chemokine Signaling • Costimulatory Molecules • Interleukin Signaling • TCR signaling • JAK-STAT, MAPK and PI3K Signaling • Myc targets • NFAT • Antigen processing & presentation • T-cell activation markers	• Immune Cell Profiling Content	• T-cell exhaustion markers • Apoptosis • Interactions with Non-Lymphoid Cells	• Glycolysis and glucose import • Mitochondrial biogenesis • Fatty Acid Metabolism • Glutamine metabolism • Circadian Clock • One-carbon metabolism • Oxidative phosphorylation • mTOR • Cell Cycle • Autophagy	• T-cell migration • T-cell profiling	• Notch • Wnt signaling • Th • TGF-beta • Th1, Th17, Th2, Th9, Tscm • Treg • Innate-like T-cells • Vitamin A (RA) Signaling • Naive and Memory Markers	• TCR Content, including variable and constant regions for alpha, beta, gamma, and delta TCR	• NK cell cytotoxicity • NKT Receptors • NF-κB • Type I interferon signaling • Type II interferon signaling • Interleukin signaling • Chemokine signaling
299 Genes	58 Genes	139 Genes	210 Genes	35 Genes	199 Genes	104 Genes	253 Genes

A Step toward Standardization

Surrounding all the excitement around CAR-T cell therapies, many experts are concerned that with the growing number of institutes engaged in CAR-T research and manufacturing and the diversity of CAR constructs, standardization and steps towards better cell product characterization will become critical. Remarks made by FDA Commissioner in May of 2018 specifically highlighted this issue: in contrast to the traditional drug review process where 80 percent of the review is focused on the clinical portion of the process and 20 percent on the product, with cell and gene therapy this general principle is almost completely inverted. This suggests the more challenging questions relate to the product manufacturing and ultimate "biological quality".
NanoString's CAR-T Characterization panel was built to enable the community to begin this discussion, to provide a single practical tool, with rapid turnaround time and high reproducibility instead of different platforms producing non-comparable data for monitoring product characteristics during manufacturing. The use of a standardized panel on the nCounter platform for CAR-T cell product characterization would allow the generation of real time and comparable relevant data between investigators, resulting in sharable knowledge and faster improvement in manufacturing process and ultimate patient treatment.

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