Droplet Digital PCR (ddPCR) Is Well Suited for Quantifying Transgene Copy Number

Abstract

Since they entered clinics in 2017, chimeric antigen receptor (CAR) T cells have transformed leukemia and lymphoma treatment. The United States Food and Drug Administration (FDA) has approved five CAR T-cell therapies so far, and because a majority of cell immunotherapies in development today use CAR T cells, the number of therapeutic options will only increase. One forecast predicts that the CAR T-cell market will grow to $6.1 billion by 2030. In this paper, we discuss why CAR T-cell therapy is so exciting, note some common hurdles in development and manufacturing, and propose Droplet Digital PCR as a solution for some of these issues.

A New Paradigm in Cancer Treatment

CAR T-cell therapy represents a new paradigm in cancer treatment because instead of blasting the body with toxins, it involves engineering a patient's own immune system to attack cancer cells. First, a technician extracts T cells from a patient's blood and transfects the cells with the *CAR* gene, most often using a virus. The *CAR* gene integrates into the genome and transforms the T cells into CAR T cells. The technician then expands the cells in a bioreactor and returns them to the clinic, where a physician transfuses the cells into the patient's bloodstream. Armed with the CAR protein on their surfaces, the CAR T cells seek out cancer cells expressing specific tumor-associated antigens and kill the tumor.

But despite the promise of CAR T cells, developers must work out the kinks in their manufacture. Since CAR T cells are living therapies, manufacturers must take extra steps to ensure the treatments are safe and effective. CAR T-cell development takes place in partnership with nature: technicians generate CAR T cells with the help of viruses and T-cell machinery, and then physicians need to work with each patient's unique physiology to ensure that CAR expression is successful and has the desired therapeutic effect.

The Need for Standardized CAR T-Cell Development

CAR T-cell development has not been standardized. Manufacturers have many cell sources and bioprocesses to choose from and, as a result, the behavior of CAR T cells can vary widely from batch to batch. Consequently, to succeed in clinical trials and reach the market, CAR T-cell developers must implement rigorous quality control measures to monitor their cells during manufacturing.

One significant challenge is ensuring a T cell contains an appropriate number of *CAR* transgenes. The transgenes enter the cells via viruses such as adeno-associated virus or lentivirus, but viral transduction efficiency can vary and different cells may contain different numbers of transgene copies.

The FDA recommends that CAR T cells contain between one and four transgene copies. If a cell does not contain any copies, it will not be effective, and if it contains more than four, the treatment may not be safe. CAR T cells that contain five or more transgene copies can induce a systemic inflammatory response that could cause an adverse response to the treatment. Consequently, developers need to quantify the transgene copy number in CAR T cells precisely.

Unfortunately, the technique most commonly used for this purpose, quantitative PCR (qPCR), is not accurate or precise enough to monitor transgene copy number during CAR T-cell production. The technique requires that technicians estimate copy number based on a standard curve. Generating a standard curve requires technicians to perform time-consuming serial dilutions, a process that is often fraught with human error. This error produces variability, which reduces the method's sensitivity.

Consequently, qPCR cannot be used to determine the success of viral transduction and categorize CAR T cells as safe. In contrast, Droplet Digital PCR is well suited for quantifying transgene copy number. Droplet Digital PCR quantifies nucleic acids directly, without employing a standard curve, giving it the precision and sensitivity to address several challenges during CAR T-cell development.

Ideal Solution for Transgene Quantification

Rather than estimating nucleic acid concentration based on DNA standards, ddPCR technology provides an absolute count of nucleic acid molecules, making the technique more sensitive. A technician loads a 20 µl sample into a cartridge, which gets partitioned into approximately 20,000 nanoliter-sized droplets containing one or a few nucleic acid strands each, effectively creating thousands of individual samples where only some contain the *CAR* transgene. Next, technicians use primers targeted at the *CAR* gene for the independent PCR reactions in each droplet.

DNA amplification takes place only in the droplets that contain the transgene. As the DNA in these droplets multiplies, a reporter gets cleaved from a probe and emits a fluorescent signal. Using a QX200 Droplet Reader or a QX ONE Droplet Digital PCR System, the technician counts the number of fluorescent droplets. This count is used to determine the transgene concentration in the original CAR T-cell batch.

Yaoyao Luo, PhD, and her colleagues at the Huazhong University of Science and Technology in Wuhan, China, put ddPCR Assays to the test using DNA standards as well as samples from patients on CAR T-cell therapy. Among the series of DNA standards, ddPCR technology was more sensitive than qPCR in detecting *CAR* transgenes. Specifically, the researchers' ddPCR Assay detected as few as 3.2 copies/ml while qPCR could not detect the transgene at that concentration.

Compared to qPCR, their ddPCR Assay showed lower intra-assay and inter-assay coefficients of variation, suggesting that the technique is more repeatable and reproducible. Among clinical blood samples, the limit of detection for their ddPCR Assay was five copies per reaction, while that of qPCR was only 20 copies per reaction.

ddPCR Technology Increases CAR T-Cell Development Success

ddPCR technology has proven useful for more than just quantifying *CAR* transgene copy number. It can also be used to detect replication-competent lentiviruses, help optimize viral transduction protocols, measure CAR T-cell persistence in the body, and support several other aspects of development. The technique's ability to quantify nucleic acids with precision and accuracy means its adoption will help CAR T-cell developers satisfy the needs of regulators and succeed in creating more therapies that help more patients.

Visit **bio-rad.com/ddPCR-CART** for more information.

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