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Prioritizing Safety in CAR-T Therapy: Patient Monitoring with Cerba Research's Testing Portfolio

by

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"Most of these products use retroviral vectors to produce CAR-T cells. The vectors are needed for the long-term stable expression of CAR and have proven to be safe in clinical use," says Jérôme Sallette, Chief Scientific Officer at Cerba Research.

"Since the vectors integrate into the genome itself, one must understand not only the exact integration site within the genome, but also the quantity of copies integrated into cells. The importance of this is underscored by recent reports[1,2] on using genome sequencing in CAR-T trials."

It is also theoretically possible for a vector to recombine with other viruses, restoring their ability to replicate. Therefore, their replication-competency must be monitored in patients undergoing CAR-T therapy. In response, Cerba Research has formulated comprehensive solutions to support pharma and biopharma companies addressing these specific regulatory demands.

Rigorous Testing Required

Sallette stresses that monitoring patient safety in CAR-T clinical trials typically involves rigorous testing and assessments, which starts with product safety during the manufacturing process. Cerba Research provides solutions focused on long-term follow-up of patients in CAR-T trials as well as other CGT trials.

Key elements include monitoring the replication-competency of viral vectors and accurately identifying the integration of viral vectors in two ways: specifying the location of integration and quantification of CAR copies within the cells; and evaluating their persistence by enumerating and immunophenotyping them in the patient's blood, DNA, or cerebrospinal fluid (CSF). All of these solutions can be applied in both the manufacturing process and clinical trials.

"We have a unique suite of services combining cutting-edge flow cytometry with ultrasensitive molecular and genomic services," Sallette says. "These methods can be combined with our off-the-shelf cytokine and chemokine assays. One core strength is that our team of scientific experts can tailor each assay according to the sponsor's unique requirements in close collaboration with the sponsor."

Long-Term Monitoring Is Essential

Karthikeyan Devaraju, Senior Scientist for Cell and Gene Therapy at Cerba Research, stresses the importance given by regulatory agencies to monitor both CAR-T cells and the link to retroviral vectors integrated into the genome. As long as CAR-T cells are alive, these CAR+ cells help to eliminate tumor cells from patients. That requires following up the CAR-T cells in patients immediately post-therapy and for up to 15 years after administration.

Stable expression is one of the reasons why retroviral vectors are used. They have been used safely for 20 years, however, there are two theoretical concerns around CAR-T cells harboring them in the genome: random integration and the number of copies that are integrated into the genome.

To address these issues, regulators have created a list of assays to check patient safety by monitoring CAR-T cells. The first is replication-competent virus testing. The viral vectors used for the therapy are replication-incompetent, i.e., they cannot divide. The viral vectors have undergone multiple changes to ensure safe clinical use.

The screening starts with the raw materials used for production and in the cells used to

manufacture the plasmid to make the viral vectors, as well as during CAR-T cell production. Before and after CAR-T administration to patients, the same tests must be done repeatedly at a regular interval. To ensure against the remote chance that these vectors recombine with viruses, Cerba Research screens them at the recommended stages of clinical trials.

“Having ensured that it's replication-incompetent, we have to confirm that the vector is safe, meaning that the number of integration copies in a given cell must be verified,” Devaraju says. “The FDA and other regulatory agencies prescribe a safety limit, especially on how many copies per cell are allowed, so that it doesn’t induce any toxicity.”

To measure such low copy numbers, the second approach to monitor safety and efficacy, sensitive genomic methods like digital droplet polymerase chain reaction (ddPCR) are used. With ddPCR, the DNA sample in a PCR can be divided into (currently) anything up to 20,000 droplets smaller than 1 nanoliter. The PCR in each droplet measures very low levels of vector integration to the cell genome.

The third approach is about precisely mapping where the vectors are integrated into the genome, called viral integration sites (VIS). This requires more sensitive sequencing, with unbiased and targeted methods. Devaraju likens it to finding the proverbial needle in a haystack.

CRISPR Cas-9 Coupled With Oxford Nanopore’s Technology

“We have developed a very sensitive approach targeting long-read sequencing, coupled with the CRISPR Cas-9 system using Oxford Nanopore’s technology,” Devaraju says. “We have reliable data so there’s a robust and time-efficient approach without the pitfalls associated with other sequencing methods.”

Oxford Nanopore’s technology, Devaraju adds, is not just more accurate than ever before, it also has long-read sequencing capability, enabling it to address difficult-to-sequence parts of the genome. There is no need for PCR amplification like in short-read sequencing. “Thus, it is targeted, long-read sequencing and unbiased.

All three things are really needed to ensure that we don’t miss any VIS.” Targeted sequencing approaches, especially for viral vector integration sites, are crucial to understand where the viral vectors are integrated, and this could be used for making them more detectable than with other methods. “There are some technical challenges, but with CRISPR-Cas these challenges are overcome,” Devaraju says.

Flow Cytometry

Nithianandan Selliah, Global Director of Flow Cytometry at Cerba Research, adds that monitoring CAR-T cells in patients is critical to knowing that they actually have these cells in their circulation to achieve a therapeutic effect. Flow cytometry, he says, “is a very powerful

technology that we use to monitor and characterize CAR-T cells".

Put simply, the higher the number of CAR-T cells, the likelier it is that they are responding to and killing cancer cells and that the therapy is working. If they are not present as expected, clinicians can re-evaluate the therapy. The number of CAR-T cells at different time points also provides valuable data about their persistence.

"The phenotype of the CAR-T is important for persistence, which can mean that patients might be cured of the cancer," Selliah adds. "Additional characterization of these cells' activation markers and memory phenotypic markers will provide more data about what happens to them once inside the patients. Certain memory subsets could indicate that the CAR-T cell can stay inside for longer, which is important for the response to reemerging cancer cells."

All of these data can be collected relatively rapidly using flow cytometry assays. Cerba Research has introduced high parameter spectral flow cytometry, which provides more information on the cells, enabling users to characterize many different markers. This is also useful with limited sample volumes, such as in the CSF or bone marrow aspirates.

Complementary Options

Selliah notes that complex technologies such as flow cytometry and next-generation sequencing (NGS) can be complementary for certain applications, including CAR-T cell monitoring. The main limitation on the flow cytometry assay is its sensitivity and this is where NGS comes in.

"Molecular methods such as ddPCR and/or NGS are more sensitive and can also detect the presence of CAR-T cells with high sensitivity, but they also take longer to produce the data. Ideally, we would use high-throughput screening with flow cytometry to identify certain samples or phenotypes, then potentially complement with NGS solutions," he says.

In terms of the FDA's concern about certain tumors, transcriptome, and immune repertoire sequencing (IRS) provide additional data on the T cells. IRS allows the characterization of any emerging clonal T cell subset post-CAR-T therapy.

"The challenge with flow cytometry is that identifying T-cell lymphoma in CAR-T cell patients is difficult because there are no specific markers for T-cell lymphoma," Selliah says, "but we could look at T-cell clonality with flow cytometry."

New assays with TRBC-1 (T-cell Receptor Beta Chain-1) can provide some indication of clonality for the T-cells within these patients, he adds. This can be combined with sequencing approaches like IRS to assess potential lymphoma.

Devaraju concurs, noting that IRS monitors B-cell as well as the T-cell receptors that are the

primary immune cells within the body. These are key to understanding if there is any immune reaction to CAR therapies as well as for clonality of these cells.

NGS also applies to genomics and diagnostics for detecting cancer-causing mutations. This is also applied for minimum residual disease analysis for various cancers because many panels are available especially for multiple myeloma, acute myeloid lymphoma, and chronic lymphoid leukemia. NGS and VIS by targeted sequencing have added value in allogenic cell therapies because it plays a critical role to verify and certify the clones that will be used subsequently for CAR therapy product manufacturing.

“Sequencing has a complementary role to flow cytometry and is highly sensitive,” Devaraju says. “It also applies to other immune cell therapies, like CAR-NK (natural killer) cells, CAR macrophages, CAR dendritic cells, and other types of CAR cells, as well as TCR-T therapies and onco-gene therapies.”

NGS can also expand into other areas, he continues. “For example, using IRS or other sensitive molecular approaches, we can identify tumor-infiltrating lymphocytes (TILs), especially in solid tumors. This opens up opportunities for the discovery of new biomarkers, and new targets for designing CAR and TCR-T therapies. Coupled with flow cytometry and other molecular methods, it can help in broadening the monitoring of current CAR therapies, especially in identifying new biomarkers to develop new targets for difficult-to-treat cancers.”

What Is On The Horizon?

Sallette says that Cerba Research is constantly innovating and investigating new technologies and modalities for patient safety in clinical trials. For instance, it recently introduced a new Aviti™ sequencer by Element Biosciences in its genomics platform, improving quality standards and spatial omics (Nanostring® GeoMx®) to decipher the tumor environment. More cutting-edge technologies are in the pipeline.

“But innovation is not just about science and testing,” he adds. “It covers the whole value chain from a sample to a new health care value proposition. For example, we are now flying drones to develop faster and more flexible logistical solutions.”

Logistics are particularly essential in this arena. “Preserving the sample via a logistic chain and white-glove preanalytical procedures are crucial for the integrity of the sample. This is not just about transporting tubes from one location to another but making sure that preanalytical procedures are fitted for delivering accurate results, especially since we are working all around the world.”

Artificial intelligence (AI) is becoming a routine tool for improving both science and processes at Cerba Research. “And it is only the beginning,” says Sallette. “We really believe that all those

innovations, not taken individually but combined in a smart way by smart people, can revolutionize the lives of our patients. This is a very exciting time, especially in genomic research and medicine."

For safety monitoring in CAR-T trials, he adds, "it is essential that various cutting-edge technologies are available under one roof, with all the equipment but even more important, all the experts. If you want to be successful in this arena, it is important to have the full range of technologies and experts working together, and this is what we have at Cerba Research."

Corporate Biography: Cerba Research

Cerba Research is part of Cerba Healthcare, which handles 50 million patients every year. It is a global patient diagnostics and clinical trial organization that provides comprehensive services to support clinical trials and research in the pharmaceutical, biotechnology, and medical device industries worldwide.

As a strategic partner, Cerba Research offers expertise in areas such as clinical trial management, data management, biostatistics, regulatory affairs, logistics, and central and specialty laboratory services. It also plays a crucial role in helping customers to navigate and execute various aspects of clinical research, from study design to regulatory submission, including patient safety monitoring in CAR-T therapy.

For more information see *www.cerbaresearch.com* or download our whitepaper: *Prioritizing Safety in CAR-T Therapy: Patient Monitoring with Cerba Research's Testing Portfolio."*

References:

1. Verdun N, Marks P. Secondary Cancers after Chimeric Antigen Receptor T-Cell Therapy. New Eng. J. Med. 2024 Feb 15;390(7):584-586. doi: 10.1056/NEJMp2400209. Epub 2024 Jan 24. PMID: 38265704.

2. Michael D. Jain, Bachisio Ziccheddu, Caroline A. Coughlin, Rawan Faramand, Anthony J. Griswold, Kayla M. Reid, Meghan Menges, Yonghong Zhang, Ling Cen, Xuefeng Wang, Mohammad Hussaini, Ola Landgren, Marco L. Davila, Jonathan H. Schatz, Frederick L. Locke, Francesco Maura; Whole-genome sequencing reveals complex genomic features underlying anti-CD19 CAR T-cell treatment failures in lymphoma. Blood 2022; 140 (5): 491–503. doi: https://doi.org/10.1182/blood.2021015008