Replication-Competent Virus Testing: A Critical Step in Cell and Gene Therapy Quality Control

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Scientists commonly use lentiviruses and adeno-associated viruses (AAVs) as viral vectors in cell and gene therapy (CGT) production. However, therapies that use these viruses must undergo strict quality control measures, including testing for replication-competent viruses (RCV), to ensure that the treatment is safe. Cell culture–based methods are currently the gold standard for RCV testing, but it can take up to 45 days for results to be returned. Droplet Digital™ PCR (ddPCR™) kits now offer an attractive orthogonal method as they are highly sensitive and return results in just eight hours. These kits can potentially accelerate the development of a new wave of CGTs.

CGT Manufacturing

CGTs have enormous potential to treat and even cure a variety of diseases, from cancers to genetic disorders and immunodeficiencies (Poletti et al. 2021). As such, the field is growing rapidly, with 838 CGT clinical trials currently underway (ClinicalTrials.gov). However, producing these groundbreaking therapies is a complex process.

Manufacturing CGTs involves using viral vectors such as lentiviruses and AAVs that scientists have engineered to carry a gene that can do anything from replacing a defective or missing gene to supercharging immune cells to attack cancer cells. When human cells are transfected ex vivo with the viral vector, the viral vector itself can't replicate, but the cells take up the gene and express it stably. At this point, the cells could be infused back into the individual receiving the treatment. However, manufacturing CGTs comes with risks, so each batch must first undergo strict quality control (QC) testing to ensure its safety and effectiveness. Only then can drugs in development progress toward clinical applications.

Challenges Associated with CGTs

QC testing of CGT products can indicate whether key events during the manufacturing process were executed correctly. Chronologically, scientists first prepare the virus and measure viral titer to determine their virus concentration. They use this information to determine how much virus is needed to infect host cells, a critical step to ensuring safe and accurate dosing of patients.

Next, scientists must determine how many transgene copies will be inserted when the virus infects host cells. If the therapy contained no copies of the gene, it would not be effective, but if it had too many copies, it could lead to a dangerously potent product that could trigger oncogenesis (Zhao et al. 2019). By measuring transgene copy numbers, manufacturers can ensure that individuals receive the proper dose of the therapeutic gene. For example, the U.S. Food and Drug Administration (FDA) recommends that CAR T cells generated using lentiviral vectors contain no more than four transgene copies per cell (Zhao et al. 2017).

Notably, during CGT manufacturing, viral vectors delivering the therapeutic gene can potentially turn into RCVs, which can cause a variety of cancers, including lymphoma, and must be avoided at all costs (Hacein-Bey-Abina et al. 2008). Each vector introduces the potential for RCVs in a slightly different way. For lentiviruses, rare recombination events may transform the virus into an RCV, which can then go on to infect non-target cells. The RCV can also cause insertional mutagenesis when the virus integrates multiple times into the host cell DNA. When using AAV as a viral vector, recombination events may transform this replication-incompetent strain into an RCV, especially in the presence of a replication-competent helper virus used to stimulate replication during the vector manufacturing process (Naso et al. 2017). Any of these scenarios raise significant safety concerns.

It is recommended to test for RCVs at three stages: testing clinical vector lots prior to manufacturing, testing the finished CGT product, and testing the individual after receiving the therapy. When it comes to testing the finished product before lot release,

the U.S. FDA recommends testing sufficient supernatant to ensure a 95% probability of RCV detection if the RCV is present at a concentration of as little as one RCV copy per dose equivalent (U.S. Department of Health and Human Services 2006). While it is possible to test for RCVs using a cell culture-based method, the process is cumbersome. However, since these elements can be tracked by measuring nucleic acids, a more rapid approach is available: ddPCR technology.

ddPCR Technology: A Fast, Sensitive Method for Evaluating CGTs

ddPCR technology is a highly accurate and sensitive tool for the direct quantification of nucleic acid sequences, making it an ideal tool for assessing CGTs to ensure they will be safe and effective. Indeed, studies have already demonstrated that ddPCR technology is suitable for the testing of viral titer and transgene copy number (Lu et al. 2020; Corre et al. 2022), but now, ddPCR kits are also available to test for RCVs.

Any assay to test for RCVs must be sensitive enough to detect them at extremely low levels. While scientists may use quantitative PCR to test for RCVs and get results quickly, the use of a standard curve to estimate nucleic acid concentration in these assays introduces the possibility of human error that raises the assay's limit of detection. Ultimately, this means that qPCR is not necessarily sensitive enough to detect the presence of RCVs with a probability of detection over 95% and is thus not a satisfactory test for assessing CGTs for RCVs before lot release.

However, two fully validated ddPCR-based kits have recently become available that detect RCVs with over 95% probability of detection in under 8 hours. One test for replication-competent lentivirus (RCL) offers 99% specificity and has a limit of detection of 0.35 copies/µl. A separate test for replication-competent AAV (RCAAV) in AAV-based CGTs shows 99.9% specificity, can detect RCVs with a limit of detection of 0.50 copies per µl, and has been validated for AAV serotypes one through ten.

Together, these orthogonal assays meet FDA requirements while providing answers to developers on a timescale that allows the drug development process to proceed safely and more rapidly. Moreover, they offer qualitative and quantitative data on a CGT's RCV status and substantial cost savings compared to cell culture-based methods.

Ensuring Safety Through QC Analysis

Which tools can help developers and manufacturers be confident in their CGT products? ddPCR technology is revolutionizing CGT manufacturing, particularly the measurement of viral titer, transgene copy number, and assays verifying the absence of replication-competent viruses. These highly sensitive techniques improve the standards by which each CGT batch may be deemed safe and effective. Moreover, ddPCR kits to check for RCVs accelerate this vital step from weeks to hours, accelerating production without compromising safety.

As new therapeutics enter clinical trials, ddPCR technology will continue to be vital in advancing them toward regulatory approval. Ultimately, ddPCR-based assays will help prepare these therapeutics to serve as personalized medicine that addresses some of the world's most complex health issues. Ultimately, the CGTs that pass these tests and reach the market may offer individuals a second chance at life.

Visit our website for more information on testing for replication-competent viruses.

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