A rapid alternative to standard safety tests for lentiviral vectors

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A new, publicly available test to assess the safety of cell therapy products altered by lentivirus generates results within a few hours, potentially hastening the pace at which viral immunotherapies move into clinical trial. Current assays required by the U.S. Food and Drug Administration take about six weeks to complete. The rapid test, which does not have a significant risk of false positives, is also a fraction of the cost of the standard approach. The work appears September 21 in the journal *Molecular Therapy—Methods & Clinical Development*.

"A lot of people avoid this technology for rapid assay because it has this potential for false positives, but I think we've proven that it's very possible to have high sensitivity and high accuracy with this test," says senior author David DiGiusto, Executive Director of Stem Cells and Cellular Therapeutics Operations at the Stanford University School of Medicine. "This method is pretty straightforward, and any lab using lentiviral vectors should consider running it on their lentiviral transduced cell products to document the lack of detectable replication-competent viruses."

Lentiviruses are a type of retrovirus commonly used by companies to introduce new genes into therapeutic CAR-T cells (chimeric antigen receptor T cells), which are introduced back into patients to fight cancer. These viruses are typically engineered to be safe, but if still actively replicating in a patient they have the potential to cause cancer. Early iterations of first-generation retroviral vectors have been associated with leukemia, but that risk has been brought to near-zero with third-

generation lentiviruses. As a precaution, agencies such as the FDA do require testing for all cells altered by lentiviral vectors to ensure no replication-competent virus is present.

The rapid test developed by DiGiusto and colleagues looks for a viral envelope marker in a sample of engineered cells that shouldn't be there if there is no viral replication present. One advantage is that this can be done in the lab when the cultures are freshly prepared with some molecular reagents and a person working for a couple of hours. The standard procedure required by the FDA waits 3-4 weeks to check if viral cultures grow, and samples need to be frozen and shipped to an outside testing site.

"I think with good scientific practices and good technical staff, this could be set up in people's labs in general," DiGiusto says. "We're publishing our work so that people in early-stage trials can have access to a highly qualified test."

The challenge of making the rapid test was to ensure that the assay had specificity and sensitivity to the viral markers. False positives are still possible, but only for about 1 in 100 samples, and those can then be confirmed by running the sample through the standard procedure. DiGiusto's group has partnered with Stanford Medicine's Crystal Mackall, a cancer immunotherapy expert, and is using the rapid test in phase 1 clinical investigations. The procedure may also be helpful in other areas where viral modification is necessary, and those uses are being explored as well.

More information: *Molecular Therapy—Methods & Clinical Development*, Skrdlant et al., Detection of Replication Competent Lentivirus Using a qPCR Assay for VSV-G. www.cell.com/molecular-therapy … 2329-0501(17)30098-0 , DOI: 10.1016/j.omtm.2017.09.001

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