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GT News

Researchers fear emotional ban on lentiviral vectors

by Jeffrey L. Fox

Some researchers who are developing lentivirus-based gene therapies fear that, despite assurances from officials at the US **Food and Drug Administration** (FDA, Rockville, MD, USA), policy decisions about clinical uses of lentivirus-based vectors will depend more on emotional responses from the public than on assessments of the safety data. Although no candidate lentivirus-based vectors have yet been submitted for FDA evaluation, the agency has certainly not ruled out the use of re-engineered, de-toxified human immunodeficiency viruses (HIV) as gene therapy vectors, officials say.

Despite the safety features that are incorporated into these lentivirus vectors, researchers and regulators are far from certain that the public will welcome such engineered viruses as a means for delivering genes into relatives or friends and accept them as safe for clinical uses. "We may not necessarily be able to convince people that these vectors are safe at this point," says **Scott McIvor** of the **University of Minnesota** (Minneapolis, MN, USA) and a member of the **NIH Recombinant DNA Advisory Committee.**

Anne Pilaro of the FDA Center for Biologics Evaluation and Research, who was one of several participants discussing safety features of lentivirus vector candidates during March at the second in a series of gene therapy policy conferences to be sponsored by the **National Institutes of Health** (NIH, Bethesda, MD, USA). "But the issues are on the table," said FDA's Pilaro, adding, "The first guy out there will have a lot of work to do to prove the safety of these vectors." The underlying message here is twofold. The FDA will be extra vigilant with its examination of safety data for these vectors. And, simply because the lentiviral vectors are new technology, several scientific boundaries will need to be crossed to ensure safety.

The primary safety concern, Pilaro says, is that lentivirus vectors that have had toxic components either radically modified or deleted might still generate replication-competent viruses. Nonetheless, FDA does not currently require that gene therapy recipients be monitored for the appearance of replication-competent recombinant viruses that arise from use of non-lentiviruses, officials say.

Other safety concerns include the development of latent infections in non-target cells and tissues, inadvertent germ-line transfers of genetic materials by such vectors, inappropriate levels or persistence of gene expression, and the potential for producing mutations in host genes where the vector inserts.

"The worst fear is that a helper-independent recombinant virus forms," says **Inder Verma** of the **Salk Institute** (La Jolla, CA, USA) in agreement with FDA's Pilaro. Despite this and other safety concerns, he adds, a good deal of developmental effort has been invested in modifying HIV to serve as a gene vector. Moreover, other modified lentiviruses show potential as gene vectors for mammalian cells, point out **Alan Kingsman**, chief executive officer of **Oxford bioMedica** (Oxford, UK) and **John Olsen** of the **University of North Carolina** (Chapel Hill, NC, USA). These potential benefits of lentiviral vectors include the ability to deliver DNA to non-dividing cells, integrate the DNA with the target cell genome with long lasting expression, and there is considerable experience in growing and handling them.

One way to assess the safety of HIV-derived, stripped-down, lentivirus vectors is to compare them to HIV-based vaccines intended to protect humans against AIDS and to the model for that protection provided by attenuated versions of live simian immunodeficiency virus (SIV), says **Michael Wyand**, president and chief scientific officer of **GTC Laboratories** (Worcester, MA, USA). SIV produces an AIDS-like syndrome in non-human primates such as rhesus monkeys and macaques.

Unlike lentiviruses, which cannot replicate without helper virus, attenuated SIV that is introduced as a vaccine into rhesus monkeys is fully replication competent, according to Wyand. "This represents a 'worst-case scenario' for delivering lentivirus vectors because the SIV vaccine is very much intact compared to the vectors, which retain very few of the original HIV genetic components," he says.

When several progressively attenuated versions of SIV, each missing an ascending number of genes, are tested in monkeys, the infections produce progressively fewer pathogenic effects, Wyand says. These experiments are based on results obtained by **Ronald Desrosiers** and his colleagues at the **New England Regional Primate Center** (Southboro, MA, USA) and the **Harvard Medical School** (Boston, MA, USA). When five key DNA sequences are deleted from the virus, the infections lead to no viral replication and induce no immune response. The five sequences are the vpr, vpx, vif, and nef genes, which cause the virulent effects of HIV, and the LTRs (long terminal repeats), which allow the virus's genome to integrate with the target cell genome, essential to the virus for replication.

"A triple deletion [of vpr, vpx, and nef] is safe in 95 percent of the adult population on which it was tested," Wyand says. "And four deletions [also deleting vif] made it 100 percent safe, although neonates still have an immune response to these attenuated viruses." When exposed to the triple deleted vector, neonates showed Aids-like symptoms and an immune response, whereas when the vif gene was also deleted only the immune response was seen.

In addition the attenuated vaccine strains have gained some tendency to invade different cell types, including nerve cells, which is not characteristic of unaltered SIV. Nonetheless, Wyand says, "When I look at vectors, they seem much safer than any of these vaccine strains. There is quite a margin of safety between these vaccine deletions and any of the vectors."

In a radically modified and particularly promising HIV-derived lentivirus vector, which Verma is working on, only 22% of the original viral genome remains, he says. "We use a minimal set of HIV sequences to generate such vectors," says Verma's former colleague, **Luigi Naldini** of **Cell Genesys** (Foster City, CA, USA). Although these so-called second generation vectors lack packaging signals and are missing the two LTRs (long terminal repeat sequences) that are present in the original HIV, the vectors infect both dividing and non-dividing cells. Additionally they can effectively carry genetic material for therapeutic purposes that can integrate into the chromosomes without disrupting the nuclear membranes of such target cells.

Importantly from a safety standpoint, this stripped-down version of HIV "is unlikely to form a replication competent recombinant, and even if it did so, it could not be pathogenic," Naldini says. A newer, further modified version of this vector is "even safer because it is self-inactivating," he adds. "It actually works better than the second generation lentivirus vectors."

To be sure, because some potential recipients of HIV-based vectors may be infected with ordinary HIV, there is some potential for generating novel replication-competent HIV strains, but presumably the important health risk would be the primary not the secondary HIV infection in such cases, Naldini and others contend. If anything, based on *in vitro* tests, the vector may interfere with that primary HIV infection, he points out.

RESEARCH

Research at **Ohio University**, (Athens, OH, USA) done in collaboration with **Progenitor** (Menlo Park, CA, USA) has shown that a non-viral gene expression system, owned by Progenitor, has achieved 60% tumour regression. The non-viral system, T7T7, delivered the herpes simplex virus-thymidine kinase (HSV-TK) gene to tumour cells, which then expressed the gene. This was followed with treatment using the anti-viral drug ganciclovir, resulting in the permanent elimination of 30% of the tumours. The secrets of T7T7 are held in Progenitor's patents, but it is known that it is a self-contained system incorporating the genes of interest, plus control elements and an enzyme component that rapidly initiates high-level protein synthesis.
Human Gene Therapy **9**:729-736

A review article in the *Journal of Molecular Medicine*, discusses the use of electroporation in *in vivo* gene therapy. Electroporation, the use of electrical pulses to open pores in the cell membrane allowing target genes to enter the cell, is currently only used in around 1% of the studies relating to *in vivo* gene therapy. The technology works on a variety of cells and tissues and gene transfer can be done within seconds. Additionally, the amount and size of the DNA is not constrained, and no immune response is associated with the treatment. One of the leading companies in the field, is **Genetronics Biomedical** (San Diego, CA, USA), which has been developing its electroporation technology since 1991, works in conjuction with **Tatsuo Muramatsu**, from the Department of Biological Resources and Environmental Sciences at **Nagoya Universtiy** (Nagoya, Japan), a co-author of the review.
Journal Molecular Medicine **1**:52-62

At the meeting of the **American College of Cardiology** (Atlanta, GA, USA) **Timothy Henry**, assistant professor of medicine at the **University of Minnesota** (Minneapolis, MN, USA), showed data suggesting that *ex vivo* expression of the VEGF gene, to produce VEGF protein, can be used to treat heart disease. In a study involving 15 patients, administered with VEGF by infusion into the coronary arteries via a catheter, the therapeutic protein was shown to promote blood-vessel growth in five patients. Thirteen of the patients reported improved symptoms such as reduced chest pains.
American College of Cardiology meeting 30 March 1998

Research done at the **Institute of Human Virology** (Baltimore, MD, USA), part of the **University of Maryland** (Baltimore) has shown a new way to use gene therapy to help treat HIV infection. **Robert Gallo**, director of the institute, has shown that HIV can infect $CD8^+$ T cells, which were previously thought to be uninfectable. When the $CD8^+$ T cells are stimulated through the T cell receptor complex, $CD4^+$ antigen is expressed on the cell surface making it susceptible to HIV infection. Gallo proposes that a gene inserted into the $CD8^+$ T cell through gene therapy could suppress the expression of the $CD4^+$ antigen and protect the cells.
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