GENE THERAPY

Lentiviral vectors, two decades later

A deadly virus became an effective gene delivery tool

By **Luigi Naldini,[1] Didier Trono,[2] Inder M. Verma[3]**

In the mid-1990s, several years after a variety of viral vectors started being used for gene transfer into cells, tissues, and in some cases humans, it became clear that there were considerable limitations (*1*). For applications requiring a stable genetic modification that could lead to sustained gene expression in cells and their progeny, a delivery vehicle was needed that could transduce foreign cargo into dividing and nondividing cells, without causing immuno- or genotoxicity. A decade earlier, human immunodeficiency virus (HIV) had been identified as the cause of AIDS, and rapid studies of its biology led to the idea that this genus of retrovirus—lentiviruses—could be optimized for gene therapy.

Lentiviruses, in contrast to other types of retroviruses used to develop vectors, had the ability to infect, replicate, and integrate in some nondividing cells, such as macrophages (*2*). However, there were substantial challenges in developing gene therapy vectors from HIV. Paramount among them was the pathogenic nature of HIV, which was not fully elucidated and for which no effective treatment was available at that time. In addition, the restricted tropism of HIV to some types of lymphocytes and myeloid cells would limit the usefulness of a vector derived from HIV to the targeting of only a few cell types. The complexity of its genome, comprising many more regulatory genes than simple gammaretroviruses, posed new challenges for vector design.

THE MILESTONE EXPERIMENT

In 1994, one of us (L.N.) joined the other's (I.M.V.'s) laboratory at the Salk Institute as a visiting scientist to explore the possibility of targeting retroviral vectors to specific cell types. Two floors above, another one of us (D.T.) was working independently on the role of HIV proteins in infection and replication, with a particular interest in how the provirus was imported into the host cell nucleus. We started a collaboration to see whether specific genomic sequences from HIV could be inserted into a gammaretroviral vector [Moloney mouse leukemia virus (MLV)] to impart the ability of transducing nondividing cells. The quick fix did not work, so we decided to use HIV itself as a vector, even though we anticipated that the idea of using derivatives of a lethal pathogen to deliver genes in people would raise a lot of eyebrows and disbelief.

To broaden the very limited range of cellular targets accessible through the HIV envelope protein, we replaced it with the more promiscuous envelope glycoprotein (G) of vesicular stomatitis virus, which afforded an additional advantage. The stability of G protein allowed one to concentrate the viral particles released into the culture media from "producer" cells (or "packaging" cells) by ultracentrifugation. This afforded an infectious titer that was hundred-folds higher than previously achieved. In 1996, these concentrated first generation lentiviral vectors could transduce neurons in the rat brain, a paradigmatic example of nondividing cells (see the image). This milestone experiment was made possible by advanced in vivo imaging developed in the next-door laboratory of Fred "Rusty" Gage (*3*). Indeed, targeted neurons could be made to carry stably integrated foreign transgenes in their genome.

> ***"...the idea of using...a lethal pathogen to deliver genes in people would raise a lot of eyebrows and disbelief."***

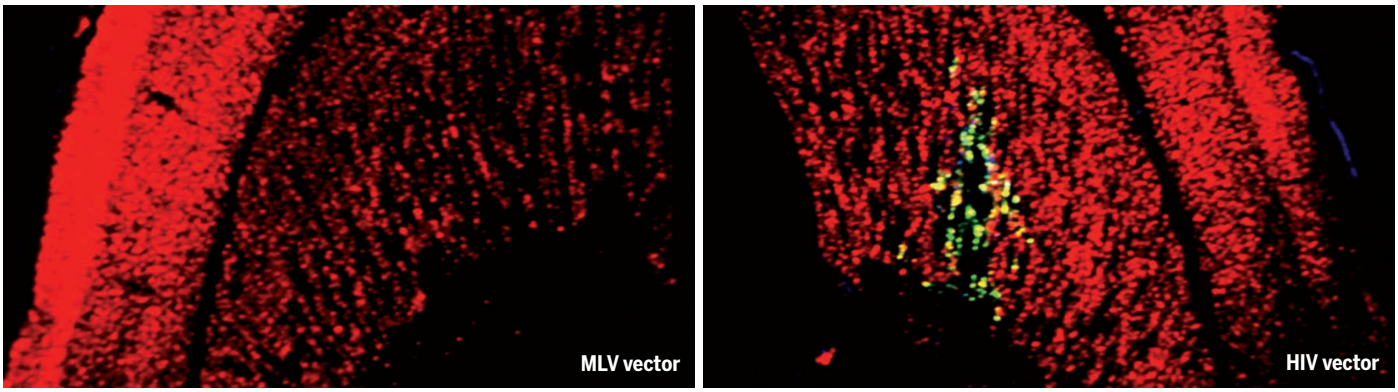
RESEARCH ADVANCES

Soon thereafter, hepatocytes, hematopoietic stem cells (HSCs), and a host of nondividing as well as dividing cells were efficiently transduced by these vectors (*4*). Sequences encoding proteins important for HIV virulence, but with no apparent role in the transduction process per se, were deleted from the packaging construct to generate second (ΔVpr, Vif, Vpu, Nef) (*5*) and third (also ΔTat) (*6*) generation vector systems, thus moving away from the pathogenic features of the parental virus. And to add additional safety features, promoter-enhancer sequences in the long terminal repeats (LTRs) of the integrated transgene (a provirus) were deleted through the so-called self-inactivating (SIN) design, which further minimized the chance of generating replication-competent recombinant lentivirus. This assigned full control of transgene expression to the exogenous promoter inserted in the vector (*6*).

These tools held great promise for the gene therapy of human disorders, notably those for which manipulating HSCs was likely to do the job. These cells could now be more efficiently transduced ex vivo and then reinjected to repopulate the organism with gene-corrected progeny of all hematopoietic lineages. However, it is in the research arena that the potential of lentiviral vectors was immediately exploited. There was an abundance of knowledge accumulated through the study of HIV, and from the past successes and failures of other gene delivery systems. That HIV was also a retrovirus allowed one to capitalize on much of the experience acquired through MLV-based systems, and to easily design vectors that were highly efficient at transducing most cell types, could be exogenously regulated, or could be made tissue-specific using well-established or new methods, such as by exploiting microRNA regulation (*7*). Soon, the lentiviral vector system became a favorite gene delivery tool for experimentalists from many fields over a broad range of research applications. These included transgenesis by instillation of a few nanoliters of concentrated vector in the perivitelline zone of fertilized mouse eggs, a procedure soon extended to species that were out of reach by the more conventional intranuclear injection of plasmid DNA, such as the rat, pig, and marmoset (*8*). Lentiviral vectors could also be used to generate transgenic mice in which the expression of a specific gene was reduced by vector-encoded small-hairpin RNAs (*9*).

HIV-derived vectors also became workhorses for introducing libraries of complementary DNAs, small-hairpin RNAs, and cis-acting regulators into a wide variety of targets, including embryonic stem cells. As such, they have been routinely used for a broad diversity of functional screens for tumorigenesis, cell growth, and senescence, among other cellular states. Lentiviral vectors are

[1]San Raffaele Telethon Institute for Gene Therapy; Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute; Tissue Biology and Gene and Cell Therapy, Vita-Salute San Raffaele University Medical School, Via Olgettina, 58, 20132 Milano, Italy. [2]School of Life Sciences, École Polytechnique Fédérale de Lausanne, SV LVG Station 19, 1015 Lausanne, Switzerland. [3]Laboratory of Genetics, Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. Email: naldini.luigi@hsr.it; didier.trono@epfl.ch; verma@salk.edu



Confocal microscope image of a section of an adult rat brain transduced in vivo with either an MLV or HIV vector that expresses β-galactosidase (β-Gal). The protein only appears on the HIV vector side, visualized (green) by staining with antibodies to β-Gal.

also being used to generate models of human cancers and, by altering the viral envelope protein, can be designed to target specific cell types and generate vaccines. Nonintegrating lentiviral vectors have also been used to transduce dendritic cells with tumor antigens for cancer gene therapy *(10, 11)*.

CLINICAL APPLICATIONS

Clinical testing of lentiviral vectors eventually ensued, initially involving the expression of antiviral transgenes in the lymphocytes of HIV-infected individuals. This was in a setting where the risk of first-in-human administration of HIV-derived vectors was alleviated *(12)*. This paved the way for applications of lentiviral vectors in cancer immunotherapy—notably, to engineer lymphocytes to express tumor-specific T cell receptors or chimeric antigen receptor (CAR) (*13*). The combination of versatile vector design and robust stable expression in T cells has propelled lentiviral vectors to the forefront of this rapidly growing field.

Efficient transduction of HSCs by lentiviral vectors also has led to successful clinical applications. Indeed, earlier clinical trials using gammaretroviral vectors showed a low extent of gene transfer into HSCs, thus limiting utility to those diseases in which gene correction confers a selective growth advantage to the transduced cell progeny and enables functional reconstitution of one or more hematopoietic lineages, even from few corrected progenitors. Some of these trials using gammaretroviral vectors also reported the delayed occurrence of leukemia, driven by vector insertion near oncogenes, in a fraction of treated patients. This increased skepticism about the overall safety of this strategy *(14)*.

By contrast, substantial levels of stable HSC gene transfer have been obtained in clinical trials of lentiviral-based HSC gene therapy, achieving up to 80% success. Many children suffering from rare fatal disorders such as X-linked adrenoleukodystrophy (ALD) (*15*), metachromatic leukodystrophy (*16*), Wiskott-Aldrich syndrome (*17*), and a growing number of other severe combined immunodeficiencies have been treated with lentiviral vectors that provide the deficient gene product. These patients are reported to enjoy substantial benefits to this day, 9 years after the first ALD patient was treated. More recently, lentiviral vectors have been used to treat patients with hemoglobinopathies such as thalassemias and sickle cell disease. These later developments were spearheaded by seminal work exploiting the biology of HIV to load the vector with complex regulatory sequences from the hemoglobin locus. This modification ensured robust, erythroid-specific expression of the globin transgene (*18, 19*). In all of these trials, engraftment of the transduced HSCs required preparative myeloablation, similar to when transplanting HSCs from healthy donors. In HSC gene therapy, however, the infused cells are the patient's own cells; thus, there is no risk of immune complications such as rejection or graft-versus-host disease, which are major causes of morbidity in conventional transplants. Sustained expression of the transgene in the reconstituted hematopoiesis is observed for many years. Because the vector integrates quasi-randomly genome wide, each transduced cell acquires a unique genetic marker (the junction between the vector LTR and the genomic sequence at the insertion site) that can be used to track its progeny in vivo (*20*). The clonal composition of the hematopoietic graft can thus be monitored in the treated patients. To date, these studies have revealed that lentiviral vectors exhibit far less genotoxicity than observed with gammaretroviral vectors (*15-19*), consistent with preclinical predictions from experimental models (*21*). Moreover, these studies now provide the first glimpse of HSC activity in living humans at the clonal level. Whereas long-term follow-up data are still limited, the number of patients treated is rapidly growing given the increasing number of active studies and trial sites.

Lentiviral vectors are also being tried for the gene therapy of various other genetic and acquired diseases, by administration into the eye or brain, or intravenously to reach the liver. However, it is for stem cells, including induced pluripotent stem cells, that they are uniquely attractive, because of their ability to integrate into the host genome. At the dawn of the genome-editing era, lentiviral vectors (whether integrase defective or competent) continue to serve scientists by providing effective delivery of the template for targeted gene repair and for the expression of engineered nucleases (*22*), including Cas9 and clustered regularly interspaced short palindromic repeat (CRISPR) guide RNAs.

It is immensely rewarding and a testimony to the transformative power of science that 20 years after the original work, and thanks to the contribution of many other scientists and clinicians, a deadly virus that has plagued humankind and claimed millions of lives has been turned into a beneficial tool that now helps to relieve the suffering of people affected by debilitating maladies. ■

REFERENCES

1. I. M. Verma, *Sci. Am.* **262**, 68 (November 1990).
2. J. B. Weinberg *et al.*, *J. Exp. Med.* **174**, 1477 (1991).
3. L. Naldini *et al.*, *Science* **272**, 263 (1996).
4. R. Zufferey, D. Nagy, R. J. Mandel, L. Naldini, D. Trono, *Nat. Biotechnol.* **15**, 871 (1997).
5. T. Dull *et al.*, *J. Virol.* **72**, 8463 (1998).
6. H. Miyoshi, K. A. Smith, D. E. Mosier, I. M. Verma, B. E. Torbett, *Science* **283**, 682 (1999).
7. B. D. Brown *et al.*, *Nat. Biotechnol.* **25**, 1457 (2007).
8. A. Pfeifer, *Curr. Gene Ther.* **6**, 535 (2006).
9. G. Tiscornia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1844 (2003)
10. L. Yang *et al.*, *Nat. Biotechnol.* **26**, 326 (2008).
11. K. Wanisch, R. J. Yáñez-Muñoz, *Mol. Ther.* **17**, 1316 (2009).
12. P. Tebas *et al.*, *Blood* **121**, 1524 (2013).
13. M. V. Maus *et al.*, *Annu. Rev. Immunol.* **32**, 189 (2014).
14. L. Naldini *et al.*, *Nat. Rev. Genet.* **12**, 301 (2011).
15. N. Cartier *et al.*, *Science* **326**, 818 (2009).
16. A. Biffi *et al.*, *Science* **341**, 1233158 (2013).
17. A. Aiuti *et al.*, *Science* **341**, 1233151 (2013).
18. C. May *et al.*, *Nature* **406**, 82 (2000).
19. R. Pawliuk *et al.*, *Science* **294**, 2368 (2001).
20. A. R. Schroder *et al.*, *Cell* **110**, 521 (2002).
21. E. Montini *et al.*, *Nat. Biotechnol.* **24**, 687 (2006).
22. A. Lombardo *et al.*, *Nat. Biotechnol.* **25**, 1298 (2007).

10.1126/science.aah6192

PHOTOS: COURTESY OF U. BLÖMER AND F. GAGE

Lentiviral vectors, two decades later
Luigi Naldini, Didier Trono and Inder M. Verma (September 8, 2016)
Science **353** (6304), 1101-1102. [doi: 10.1126/science.aah6192]

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.