

Targeting DNA

After 20 years of high-profile failure, gene therapy is finally well on its way to clinical approval.

By Jef Akst | June 1, 2012

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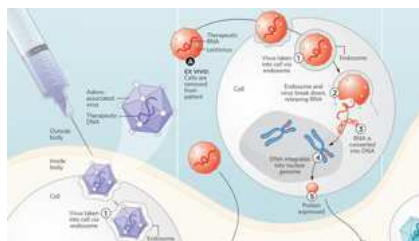
Gettyimages, Cristian Baig (target and eye); istockphoto.com, pial hovik (DNA hoop)

The concept is simple: if a mutated gene is causing a problem, replace or supplement it with a new, accurate copy. In theory, such a strategy could not just treat, but cure countless human genetic diseases. In practice, however, developing safe and effective gene therapies has not been easy. Even when identifying a disorder's genetic basis is fairly straightforward, finding the appropriate delivery vector to target the diseased tissues in the body, while avoiding unintended consequences, has challenged would-be gene therapists for more than 20 years. But more and more researchers are convinced that the technique is on the brink of becoming a common medical practice.

"It's an incredibly exciting time for the field," says researcher and medical oncologist David Kirn, founder, president, and chief medical officer at Jennerex, Inc., a San Francisco-based biopharmaceuticals company that develops and commercializes oncolytic drugs. In the last year alone, he says, major breakthroughs have been published for the use of gene therapy in patients with hemophilia, solid tumors, and leukemia, not to mention the dozens of trials yielding positive results for gene therapies to treat various types of blindness. "It's just remarkable," he says. "These decades of work are suddenly really paying off."

Fits and starts

It hasn't always been such high times for gene therapy, however. The field was booming in its early days, with approvals for gene therapy clinical trials rising exponentially from the first one in 1989 to 116 in 1999. But that year, gene therapy trial participant Jessie Gelsinger, a relatively healthy 18-year-old who had an unusually mild form of liver disease caused by mutations in a gene on the X chromosome, died 4 days after receiving an injection of an adenovirus carrying an unmutated copy of the gene meant to correct his condition. The viral vector apparently triggered a massive immune response that caused multiple organ failure and brain death.



Infographic: Delivering New Genes

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Credit: Lucy Reading-Ikkanda

But in the years that followed, 5 of the 20 trial patients developed a leukemia-like disease—an effect that was traced to the retroviral vector used to deliver the corrective gene to bone marrow cells *ex vivo*. The vector had inappropriately inserted the gene into the babies' genomes close to a proto-oncogene

Clearly gene therapy is going to be a therapeutic pathway forward for a whole range of diseases.

—Katherine High, University of Pennsylvania School of Medicine

involved in white blood cell proliferation, activating the gene and triggering a flood of T cells. After the second child fell ill, the US Food and Drug Administration suspended 30 US trials using the same retrovirus, or about 15 percent of the 200 gene therapy trials under way at that time—a move the agency called a precautionary measure. Of the five patients that developed leukemia, one died; the rest are in remission.

Events like these had “a big negative impact in the field,” recalls molecular cell biologist Mien-Chie Hung of the University of Texas MD Anderson Cancer Center. Interest in gene therapy started to wane, and treatments that might have been expected to hit the market years ago are still plugging through the clinical trial process.

But things are looking up. Just last year, for example, researchers published long-term survival data for two UK gene therapy trials for SCID: the original London trial for X-linked SCID (SCID-X1) and a second trial for adenosine deaminase (ADA) SCID.[1. H.B. Gaspar et al., “Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency,” *Sci Transl Med*, 3:97ra79, 2011.][2. H.B. Gaspar et al., “Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction,” *Sci Transl Med*, 3:97ra80, 2011.] In both trials, the researchers had extracted the patients’ bone marrow, inserted a functioning copy of the disease-causing gene, and infused the altered cells back into the patients. The impressive bottom line: up to 9 years after treatment, 14 of the 16 children treated have had their immune systems restored and have been able to live relatively normal lives free of any bubbles. “These kids were living in bubbles with a life expectancy of less than 20 years; they had no quality of life,” says Kirn. “And now many of them are essentially cured. I mean, it’s a medical miracle.”

Many other gene therapy trials are currently underway—and yielding positive results—for numerous other diseases, including various forms of hereditary blindness, HIV, hemophilia, neurodegenerative diseases, and a variety of cancers. Though no gene therapies have yet received FDA approval, nearly 2,000 clinical trials have been initiated in the last 5 years alone, according to clinicaltrials.gov, many with seemingly miraculous results and—thanks to improved vectors and techniques—none of the devastating side effects that plagued the field in its earlier days.

“There is a lot of exciting information coming out right now,” says Howard Hughes Medical Institute investigator Katherine High of the University of Pennsylvania School of Medicine and the Children’s Hospital of Philadelphia, who coauthored a report published last December of a successful gene therapy for hemophilia B.[3. A.C. Nathwani et al., “Adenovirus-associated virus vector-mediated gene transfer in hemophilia B,” *N Engl J Med*, 365:2357-65, 2011.] Loading up an adenovirus-associated virus with a gene encoding a functional version of the clotting agent known as factor IX, researchers in the United Kingdom infused the vector into six men with severe hemophilia B. A single treatment was enough to increase clotting factor IX to levels that, while still well below normal, enabled sufficient clotting to allow four of the patients to discontinue factor IX replacement therapy altogether, and the other two to receive factor IX injections less frequently. “To me, clearly [gene therapy] is going to be a therapeutic pathway forward for a whole range of diseases,” predicts High.

An eye on gene therapy

Nowhere has gene therapy made more of a splash than in blindness research. Because the eye is an immune-privileged site, injecting viruses is unlikely to result in the sorts of immune complications that killed Gelsinger in 1999. Indeed, there are some 23 completed and ongoing clinical trials for various types of blindness disorders, and no serious side effects have been reported.

“It’ll be a few years” before these therapies reach the market, says University of Florida molecular virologist William Hauswirth. But the results so far are “bordering on spectacular as far as improving vision in the patients,” he adds.

We’re entering a golden age here of genetic therapies and viral therapies.

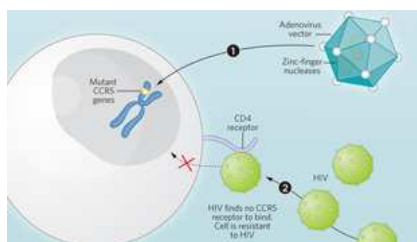
—David Kim, Jennerex, Inc.

In a recent, 3-year follow up on 15 patients with Leber congenital amaurosis (LCA), a degenerative retinal disease that causes childhood blindness, Hauswirth and his colleagues found that within the area of the retina they are targeting for treatment, “patients have gained light sensitivity from as little as to 200-fold to as much as 60,000-fold,” he says. Twelve of the patients have also demonstrated significant improvement in visual acuity, reading an extra three lines lower on an eye chart, and in 13 of the patients, their pupils constricted when exposed to light as much as 100-fold dimmer, a more objective measure of light sensitivity.[4. S.G. Jacobson et al., “Gene therapy for Leber congenital amaurosis caused by RPE65 mutations,” *Arch Ophthalmol*, 130:9-24, 2012.]

This particular group of patients was treated with a modified adeno-associated virus (AAV) carrying the gene *RPE65*, which helps metabolize a form of vitamin A that allows rods and cones to function. The modified virus was injected behind the eye, directly under the retina, where the corrective gene entered some 15 to 20 percent of cells in the retinal pigment epithelium (RPE), the nourishing cell layer just beneath the retinal visual cells.

The AAV is a popular choice for gene therapies now in development. It is a nonpathogenic virus that usually elicits no noticeable immune response, and does not integrate into the host genome, meaning there is little risk of triggering disease-causing mutations, via the activation of an oncogene, for example. The virus simply delivers the gene to the cell’s nucleus, where it forms small circles of DNA called episomes that can be expressed under the control of promoters, also delivered by the virus. “AAV is by far the most successful vector for many applications in disease so far,” says Hauswirth.

The disadvantage to this strategy is that if the replacement gene is not integrated into the cell’s DNA, it will be lost when the cell divides, as circular DNA is not replicated with the rest of the nuclear genome. But because retinal cells are extremely long-lived, a nonintegrating virus is perfectly suitable for treating eye diseases.



Infographic: Messing with HIV
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Credit: Lucy Reading-Ikkanda

adenoviral vectors, which, like AAVs, are nonintegrating vectors, but, with a 36-kilobase genome, provide much more space for therapeutic DNA. However, because many humans already carry antibodies to adenovirus, the great drawback of this approach is the risk of immunotoxicity, which can disable the therapeutic vector or cause side effects in the host. It was an extreme reaction to an adenoviral vector that killed Jesse Gelsinger. Because adenoviruses are efficient at entering many cell types and delivering the goods, they are targets of intensive research to make them safer for the treatment of cancer, diabetes, HIV, and genetic diseases.

Lentiviruses, a type of RNA retrovirus, also have a sizeable carrying capacity: some 9 kilobases of genetic material. "You only have to delete a few genes to get lentivirus to carry twice as much as AAVs," Hauswirth says. And in contrast to adenoviruses and AAVs, they do integrate into the genome, making them efficient at delivering and establishing stable high levels of transgene expression in both dividing and nondividing cells. While they cannot yet be targeted to integrate at specific sequences, they don't gravitate to oncogenes or growth-related genes as some other retroviral vectors do, and are at the forefront of the race to market.

Oxford BioMedica, for example, is using its integrating lentiviral vector, called LentiVector, to deliver treatments for a variety of eye diseases, including wet age-related macular degeneration, Stargardt disease, Usher syndrome, and corneal graft rejection. All of these therapies are in phase I/II development in partnership with Sanofi, and "the results we have seen in our clinical trials to date have been encouraging," says the company's chief scientific officer, Stuart Naylor. "We believe it is only a matter of time before a gene therapy is approved for the market."

"The field is running at the speed of light," agrees veterinary ophthalmologist and basic vision scientist Gustavo Aguirre of the University of Pennsylvania. "We're beyond anything that we thought in the '90s."

Targeting cancer: pluses and minuses

As with gene therapies for other diseases, the technique's use in treating cancer is gathering steam. Oncolytic viruses that target and destroy tumor cells are being combined with gene therapy techniques to provide tools to jack up those viral vectors with more potent genetic loads. "The concepts have been around for centuries—the fact that viruses can certainly destroy cancer cells," says Jennerex's Kirm. Now, "in addition to replicating and expressing viral genes, we also express therapeutic transgenes and imaging genes."

Jennerex is developing a vaccinia virus vector called JX-594, for example, to deliver genes that activate the epidermal growth factor receptor (EGFR)/Ras pathway in cancer cells, resulting in cell lysis and increased anticancer immunity. In multiple phase I and II trials involving numerous cancer types, including liver, colon, kidney, lung, and melanoma, JX-594 has shrunk tumors and is well-tolerated by patients. Last November, Jennerex researchers announced that advanced liver cancer patients receiving JX-594 in a phase II trial had a 60 percent decreased risk of death after 1 year as compared to controls.

Now gene therapy is actually fulfilling the promise that people said it would have.

—Carl June, Penn Medicine

Another engineered oncolytic virus nearing clinical approval is OncoVEX, developed by Massachusetts-based biotech BioVex, which last year partnered with Amgen in a deal that could be worth \$1 billion. The drug, named last year as one of FierceBiotech's 10 promising late-stage cancer drugs, is a special strain of the *Herpes simplex* type 1 (HSV-1) virus that carries an immune-boosting component. Results of a phase II trial for metastatic melanoma announced in 2009 showed that 26 percent of 50 patients responded to treatment, with 8 completely recovering.^[5] H.L. Kaufman et al., "Local and distant immunity induced by intralésional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma," *Annals of Surgical Oncology*, 17:718-30, 2010.] and the treatment is now in phase III trials. OncoVex has also shown activity against breast and pancreatic cancers in phase I trials.

And these successful examples are not the outliers. "We're entering a golden age here of genetic therapies and viral therapies," says Kirm.

Getting to this point took some careful planning, however, to avoid the destruction of healthy tissue. "The most important thing is you need a specific target," says MD Anderson's Hung. Most oncolytic viruses are designed to target a receptor or surface protein that is overexpressed on cancer cells, to increase the chance that the viruses enter and kill only diseased tissue. But this, of course, requires that tumors have unique surface antigens. "That's the ideal situation—to find something on tumor cells that's not on normal cells," says hematologist-oncologist David Porter of the University of Pennsylvania Medical Center. "That's not possible in most tumors," he says, which may be part of "the reason this field has been so slow to develop."

So Hung is taking a slightly different approach. Instead of focusing on viruses that selectively target cancer cells, or rigging them to do so, Hung and his colleagues have designed a vector, dubbed VISA, that goes everywhere, but whose package is only activated in cancer cells. "DNA by itself does not cause side effects—every cell has DNA," says Hung. "It [is] the gene product."

The VISA vector is designed to amplify expression of the genes it carries under the control of a promoter that is expressed at higher levels in tumors. "Then whatever DNA we inject into the bloodstream may go anywhere, but will express only in cancer cells," Hung explains. In 2007, the researchers used VISA, equipped with a modified apoptosis promoter, called *BikDD*, to knock down pancreatic cancer in mice.[6. X.M. Xie et al., "Targeted expression of BikDD eradicates pancreatic tumors in noninvasive imaging models," *Cancer Cell*, 12:52-65, 2007.] The protocol is currently being tested for safety in a phase I trial, and Hung and his colleagues are now applying the VISA vector to breast cancer, with promising preclinical results coming out just last year,[7. J.-Y. Lang, "BikDD eliminates breast cancer initiating cells and synergizes with lapatinib for breast cancer treatment," *Cancer Cell*, 20:341-56, 2011.] as well as to lung, ovarian, and liver cancers.

Yet another gene therapy strategy for fighting cancer involves the collection of immune cells from patients, insertion of genetic material that essentially trains the cells to target and kill cancer, and the infusion of those cells back into the patients. "The idea is to somehow modify a person's own T cells so they can now recognize and attack tumor cells that they otherwise aren't able to kill," says Porter. "By definition, if someone has a tumor, their T cells aren't able to kill [it]."

In 2011, Porter, along with Carl June and other colleagues at Penn Medicine, reported that they had engineered a patient's T cells *ex vivo* to target chronic lymphocytic leukemia (CLL) cells, marking the first gene therapy success for advanced cancer. The researchers used a lentivirus to insert a chimeric antigen receptor targeting CD-19—a molecule found on "all CLL cells but only a small subset of normal cells," Porter says—as well as two potent signaling domains, which help the T cells survive longer and activate strongly at the appropriate time. Last August, the researchers published the results of a phase I trial, in which two participants had been in complete remission for up to a year, and a third was showing signs of a strong antitumor response.[8. D.L. Porter et al., "Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia," *N Engl J Med*, 365:725-33, 2011.][9. M. Kalos et al., "T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia," *Sci Transl Med*, 3:95ra73, 2011.] In addition, some of the cells persisted as memory T-cells, primed to attack in the event of a recurrence.

However, researchers had to weigh the fact that the engineered T cells could also attack healthy tissue. CLL is characterized by the abnormal proliferation of B cells, but it's not just the malignant B cells that express CD-19; normal B cells also carry the antigen on their surface. As a result of the lentiviral therapy, patients experienced a loss of B cells down to undetectable levels. "[This] potentially makes them susceptible to infections," Porter notes. But he is not worried. No patients have contracted any unusual infections in a year and a half of follow up, and antibody replacement is always an option. "We think it is possible to live without B cells," he says. But it's an important concern when developing such therapies, he adds. "If you were to target a cell that was also on the lining of your heart or your lungs or your intestines, that wouldn't be safe."

This strategy is now being applied to diverse cancer types, including mesothelioma (a type of lung cancer), breast cancer, ovarian cancer, and melanoma, all in early-stage trials. With the improved ability to grow T cells in the lab and make better-targeted viral vectors, "the field is really growing," Porter says. "You can now use this technology to target really almost anything you can identify as a unique target."

The gene's the limit

In 2007, Timothy Brown, a 40-year-old American living in Berlin, had a relapse of acute myeloid leukemia and received a bone-marrow transplant to boost his immune function. Because he was also living with HIV, his doctor chose a donor with a mutation in both copies of the *CCR5* gene, which encodes an HIV co-receptor carried on the surface of T cells to which HIV usually must bind in order to enter the cells. People with mutations in both copies of the *CCR5* gene are resistant to HIV infection. A year later, Brown relapsed again, and once again received a stem-cell transplant from the *CCR5*-mutant donor. Finally, he beat his cancer, and as of 2010, his HIV was still at undetectable levels, despite his having discontinued immune-suppressive treatment.[10. G. Hütter et al., "Long-term control of HIV by *CCR5* Delta32/Delta32 stem-cell transplantation," *N Engl J Med*, 360:692-98, 2009.] By most accounts, Brown is the first person to have been cured of an HIV infection.

The impressive result validated Sangamo Biosciences' efforts to design a gene therapy to modify the *CCR5* gene of HIV patients' T cells. This is done *ex vivo*, using an adenovirus vector that delivers its package to T cells carrying the glycoprotein receptor CD4, after which the cells are infused back into the patients. This strategy is different from corrective gene therapies in that it's taking a normal gene and replacing it with a defective copy. In this case, it's important to not just supplement the existing copies of the gene, which would continue to produce normal *CCR5* receptors, but to render the gene defective so the cells present no *CCR5* receptors on their surfaces. To do this, Sangamo researchers are using zinc-finger nucleases, which act as molecular scissors to edit the existing gene.

"What we're doing is . . . actually editing the gene in a way that changes its nucleic acid sequence," says Sangamo CEO Edward Lanphier. "It's quite different from classical gene therapy, where you're just essentially putting in the coding region of a gene."

Preliminary results of these trials, presented at the Conference on Retroviruses and Opportunistic Infections (CROI) last March, have shown that all 21 HIV patients treated with one infusion of their own modified T cells have tolerated the treatment well, and show increased CD4⁺ T cell counts more than a year later. And when six of the patients took a planned 12-week hiatus from antiretroviral treatment, they had lowered HIV viral loads that correlated with the levels of circulating modified CD4⁺ T cells. One patient, who naturally carried one copy of the defective *CCR5* gene, had undetectable viral levels.

The results are promising, says June, who is heading up one of two phase I trials of the therapy, and who hopes that such gene-editing techniques will be applicable to a wide range of diseases. "We're trying to make a good gene bad in the case of the HIV, so that the cells aren't infected," he says, "but you can do the inverse, which is to take a bad gene and fix it." Such genome editing would be permanent, and leaves the gene under the control of natural promoters, making it more therapeutically appealing, says Lanphier. "The downstream biology of changing the endogenous gene is fundamentally different—and quite frankly, from a therapeutic outcome, superior—than conventional gene therapy approaches," he says.

Other novel gene therapy strategies are also in development, such as employing transiently expressed RNA to confer a therapeutic benefit, and using cell- and protein-based delivery systems instead of viral vectors. With these and other techniques, researchers have recently demonstrated successes in treatments for hemophilia, with promising trial results released last December; numerous neurodegenerative diseases, such as Parkinson's and Huntington's; and autoimmune disorders, such as SCID, among others. And importantly, there have been no more safety scares like the ones that rattled the field a decade ago.

"The history of medicine says every new technology starts with a great idea and then requires hard work and optimization," says Kirn. "And I think that's exactly what's happened with gene therapy. Hurdles were identified—and there's always hurdles once you get into a complex human disease situation—and they've been addressed."

"The concepts aren't that much different than they were early on, but the tools are much better," agrees June. "Now [gene therapy] is actually fulfilling the promise that people said it would have