The Gene Fix

Various gene therapy approaches are already approved for treating blood cancers and a few rare disorders. In the not so distant future, they may become standard care

By Esther Landhuis

THREE DECADES AFTER ITS FIRST, faltering steps in humans, gene therapy is emerging as a treatment option for a small but growing number of diseases. Although the concept faced scientific and ethical uncertainty when it was floated in the 1970s, the foundation of the approach—replacing or fixing a single, disease-causing gene—has proved solid. Researchers have developed different ways to correct or influence the way someone's genes function and used those techniques to create therapies for several blood disorders, as well as degenerative eye and muscle diseases. More than half a dozen such treatments have gained approval in the U.S. in the past five years, and numerous others, aimed at a variety of conditions, are progressing toward clinical trials.

Existing gene therapies rely on two fundamental approaches. The more common approach draws blood from the patient and reprograms specific cells within the laboratory before reinjecting them into the person's body. The other method delivers gene treatments directly into the body, usually to easier-to-reach areas such as the eye. Now the field is beginning to mature and move beyond these initial tactics. Continued advances have made gene delivery safer and more effective, leading to dozens of human trials in new tissues, such as the liver and heart. Other approaches are pushing beyond the original definition of gene therapy, with cutting-edge molecular tools that fix errors within genes rather than replacing or inserting a whole gene.

Yet despite recent progress, gene therapy faces numerous hurdles on the path to wider clinical use—chief among them is how to target specific tissues without triggering an immune response. Broader, long-term challenges include improving both manufacturing efficiency and cost: Gene therapy treatments in the U.S. currently average more than $400,000 per dose. Nevertheless, with so much potential and so many patients in need of new solutions, gene therapy will only continue to grow in both prominence and potency.

EARLY SUCCESS AND SHOCK WAVES

THE CONCEPT UNDERLYING the original gene therapy approaches, some of which are still in use, is fairly straightforward: When a disease results from a missing or dysfunctional gene, deliver a functional copy of the gene into affected cells. That, says Prashant Mali, a bioengineer at the University of California, San Diego, was the "version 1.0 definition of gene therapy."

One of the first attempts came in 1990, when researchers at the National Institutes of Health treated two young girls with severe immunodeficiency caused by a missing enzyme. In that trial, as with many current treatments, the therapeutic genes needed to produce the enzyme hitched a ride into the target cells inside engineered viruses, which had large chunks of their genome stripped out. This rendered the virus unable to replicate while making space for the delivery of the needed human genes. In essence, says Charles Gersbach, director of the Center for Advanced Genomic Technologies at Duke University, the approach capitalized on the virus's ability to infect human cells while "taking advantage of the viral shell as a Trojan horse to deliver therapeutic gene cargo."

The NIH team drew some of the girls' blood to isolate white blood cells, which were then "infected" with the viruses that carried the gene encoding the missing enzyme. Next the team infused the corrected cells into the girls. Each child received about a dozen more infusions over the next 18 to 24 months. The treatment wasn't a cure, but it lessened their symptoms and proved the approach could be used safely. That, in and of itself, was "a major milestone," Gersbach says.

A flurry of new gene therapy trials quickly followed, but in 1999 18-year-old Jesse Gelsinger died when an experimental gene treatment designed to treat his metabolic liver disease sent his immune system into overdrive. A few years later, in 2003, researchers reported that several people treated for immunodeficiency developed leukemias, an unfortunate result of the virus randomly inserting its cargo into cancer-promoting regions of the genome.

Researchers began to think, "'Wait a minute, maybe we don't understand this as well as we thought we did,'" Gersbach says. Gene therapy stalled for the better part of a decade. Clinical trials on hold, researchers turned all their attention back to the lab—studying and tweaking viral vectors, removing additional genes, and treating them with chemicals to make them safer and more effective at reaching target cells.

The renewed focus provided time and space for a better understanding of what worked and what didn't. Today, because of that progress, many gene therapies employ adeno-associated virus (AAV) or retrovirus vectors, each with their own pros and cons, in addition to improved versions of the adenovirus vector from the earliest trials. The genetic cargo delivered by most AAV vectors remains within the cell as separate, free-floating elements rather than stably integrating into the host cell's genome. That

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makes the vectors far less likely than earlier vectors to induce cancer but can make a treatment less durable, depending on how long the therapeutic genes remain in the host cell. On the other hand, because they are small, they can infect a broad range of cells and spread efficiently within tissues. Retroviruses offer different advantages. They can hold larger and more complex genes than AAV vectors. And some, such as lentiviruses, tend to insert themselves into coding regions, the parts of the genome that get translated into proteins. This minimizes cancer risk while conferring longer-lasting benefits than AAV vectors.

A FIELD REAWAKENS

GENE THERAPY GOT A FRESH START in the early 2010s, when researchers in Pennsylvania and Maryland independently reported results from trials for the treatment of leukemia or lymphoma. The experimental therapies trained and turbocharged the patients' immune systems so they could detect and destroy cancer cells. To do this, the scientists had to engineer genes that would equip cells to recognize and kill tumors. They put those genes into retroviral vectors and delivered them to T cells, immune cells that had been isolated from the subjects' blood. When the treated T cells were reinfused, they put the cancer into remission. "Everything was looking really promising again," says Cynthia Dunbar, a physician-scientist who studies blood cell treatments at the National Heart, Lung, and Blood Institute.

The U.S. Food and Drug Administration has since approved several of these T cell treatments, known as chimeric antigen receptor (CAR) T cell therapies, for certain lymphomas and leukemias, as well as multiple myeloma. Because CAR T cell treatments don't address gene dysfunction per se but rather endow T cells with tumor-hunting capabilities, some have debated whether they qualify as gene therapies at all. Methodologically, though, CAR T boosts cell function by using viral vectors to deliver genes—similar to the earliest forays. "What you define as 'gene therapy' is a little bit gray on the edges," Dunbar says.

Another genetic approach that is a bit gray around the categorical edges is known as oligonucleotide therapy. Rather than correcting existing genes, this technique uses short sequences of nucleic acids, or oligonucleotides, to influence how cells translate genes into proteins. One such treatment, nusinersen (Spinraza), binds to intermediary RNA molecules to trick cells into making more of a protein that is missing in people with spinal muscular atrophy.

GENE THERAPY 2.0

IN THE PAST DECADE technological advances have ushered in a new era, and the definition of gene therapy continues to evolve, Mali says. The newest approaches forgo the delivery of healthy genes and instead aim to precisely repair the gene within the cell. When there is a mutation or other error in the genome, Mali says, now the question is, "Could we actually go in and fix it?"

This innovation is fueled by the Nobel Prize–winning discovery of CRISPR-Cas9, an immune defense system in bacteria that detects specific DNA sequences of invading viruses and directs an enzyme to slice up and destroy the viral genome. The system has utility far beyond bacteria: Scientists found they could also use it to make precise cuts within the mammalian genome. In just seven years the technique has moved from in vitro lab experiments in mammalian cells to human trials.

The "cargo" in CRISPR-based therapies is not a piece of DNA but the gene-editing system itself, introduced into cells either by a virus, within a nanoparticle, or on its own as an RNA-protein complex. The therapies can be used ex vivo (outside the body) to alter cells in the lab before returning them to the patient or by sending gene-editing tools directly to affected tissues, where they edit cellular genomes.

Several dozen companies are now developing such CRISPR-based therapies. One early-phase clinical trial employed an ex vivo gene-editing method to treat people with sickle cell disease or with a related blood disorder called beta thalassemia. Those researchers reported results for their first two subjects in January 2021. And in June another company reported the first-ever successful trial of direct gene editing, which used nanoparticles to deliver CRISPR-Cas9 components into liver cells and inactivate a gene implicated in a rare disease called transthyretin amyloidosis.

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Emerging methods have allowed for greater precision and nuance—exchanging individual nucleotides, for example, or temporarily dampening a gene's activity without changing its DNA—giving researchers the latitude to set their sights ever higher. They are working on treatments for neurological diseases, autoimmune disorders and additional cancers. Over the long term they aim to move beyond single-gene disorders to treat conditions caused by interactions of multiple genes, such as cardiovascular disease and chronic pain. With gene treatments successfully alleviating some illnesses, researchers, clinicians and patients are hoping to sustain the progress of the past decade and establish gene therapy as a cornerstone of modern medicine.

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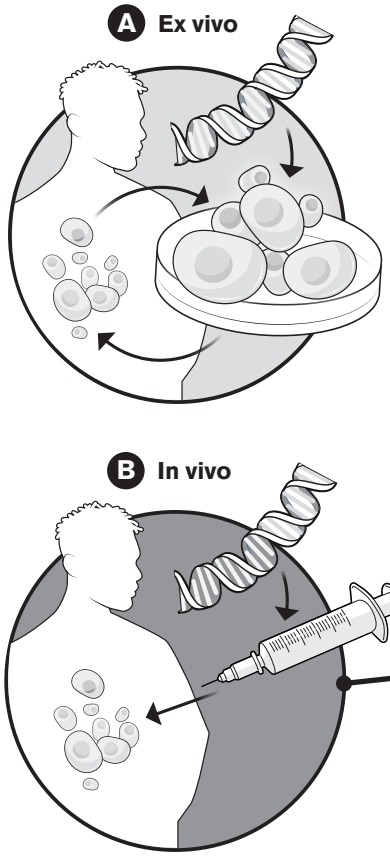
Editing the Book of Life

Since the concept of treating diseases by targeting their underlying genes arose half a century ago, gene therapy research has advanced dramatically. Recently the pace of progress has intensified. In the past five years the U.S. Food and Drug Administration has approved more than half a dozen gene therapy products aimed at several types of cancer and inherited conditions. These treatments work in various ways, such as delivering healthy genes to affected cells or reshaping the activity of existing genes. Some of the newest approaches, which have shown promise in early-stage clinical trials, aim to fix errors in the genome itself. And experts expect the pace of new product approvals will continue to pick up. *—E.L.*

Location

Ex vivo gene therapy involves removing blood, bone marrow or other tissues from a patient, isolating the cells of interest and correcting them in the lab before reinfusing them back into the body **A**. In vivo approaches send therapeutic genes, gene modulators or gene-editing tools directly to cells in affected tissues within the patient's body **B**.

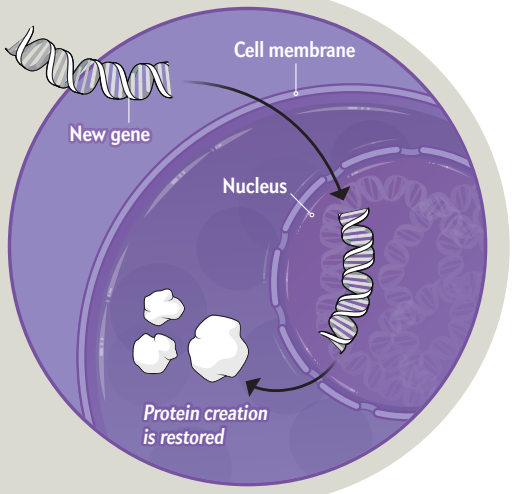


Technique

Gene therapies use various strategies to supply cells with healthy genes, influence gene activity or tweak the genome directly. Each of these methods has advantages and drawbacks, including treatment duration and potential side effects.

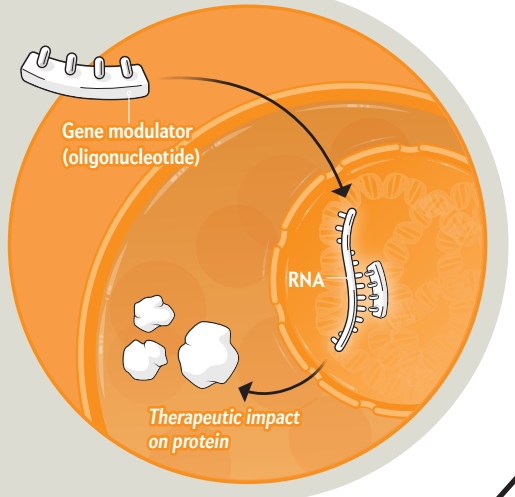
Introduce a New Gene

This approach, the first to be tested in humans, equips affected cells with a working copy of the gene that is missing or malfunctioning in the disease. Whereas this strategy can work for diseases traced to a single genetic glitch, many conditions involve multiple genetic and environmental factors.



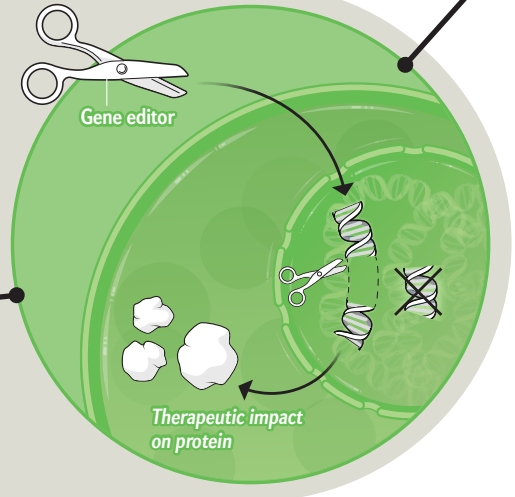
Modulate an Existing Gene's Activity

Other therapies send short sequences of nucleic acids, called oligonucleotides, into affected tissues where they can influence how cells build working proteins from underlying genetic code. Unlike gene replacement or correction, this approach is not permanent, and patients must receive regular infusions for continued benefits.



Edit Gene Directly

These approaches aim to fix errors in specific genes of affected cells. Newer methods use a gene-editing system called CRISPR-Cas9 to make precise changes in the genome.



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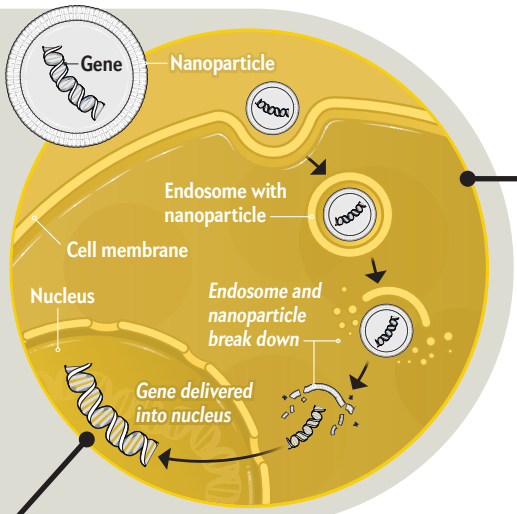
Cargo Delivery Method

Decades of research have honed several methods for carrying either genes, or tools to edit those genes, into target cells. Not only do they have to reach the cell, but they must also evade the immune responses that are often triggered when foreign substances enter the bloodstream.

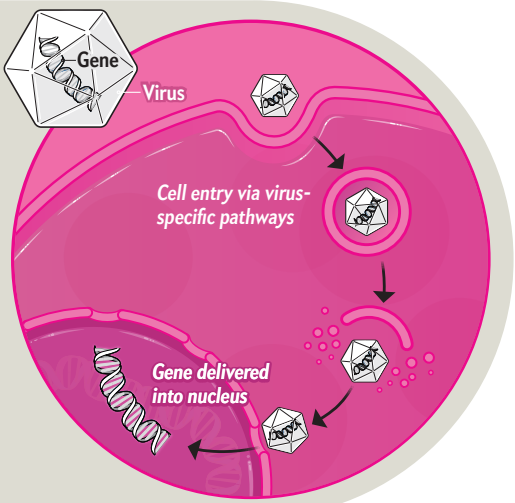
Nanoparticles

These gene therapies use nanoparticles to carry genes or gene-editing tools directly into cells of affected tissues. Nanoparticles can be chemically modified to avoid immune detection and to better target cells.



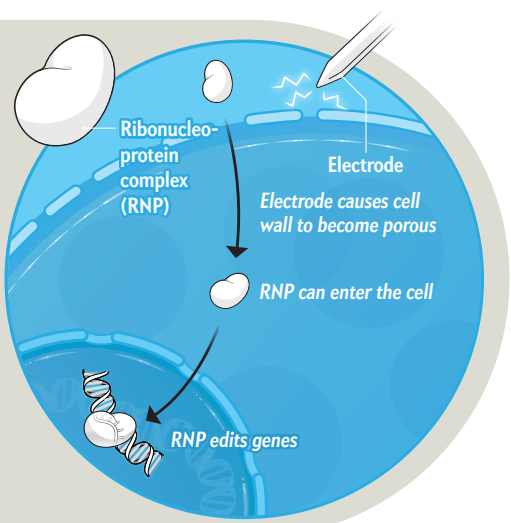
Virus

This approach delivers genes or gene-editing cargo with viruses that researchers have engineered to minimize chances of harmful immune responses and unintended effects on healthy cells.



Other

Clinical trials are testing newer approaches that send gene-editing machinery into cells as complexes of molecules that work together to target and make precise cuts within specific DNA sequences to delete or fix genes.



Examples

In a small study, people with an inherited disease called transthyretin amyloidosis that causes misfolded proteins responded well to an experimental in vivo gene-editing treatment, NTLA-2001 (Intellia Therapeutics/Regeneron), that uses nanoparticles to carry CRISPR-Cas9 into liver cells to inactivate the gene culprit.

A protein called SMN is necessary for motor neuron function, and people with spinal muscular atrophy have a mutation that decreases its production. Spinraza (Ionis Pharmaceuticals/Biogen)—an in vivo gene modulator—coaxes cells into making more SMN protein by boosting its production from a different, unmutated gene.

Leber congenital amaurosis and retinitis pigmentosa are forms of severe vision loss caused by genetic mutations. In certain cases, vision can be restored with Luxturna (Spark Therapeutics), which uses a virus to deliver the healthy gene into retinal cells.

Kymriah (Novartis) is the first approved gene therapy to equip a patient's own immune cells to fight cancer. The approach, known as chimeric antigen receptor (CAR) T cell therapy, involves isolating a patient's T cells and using a virus to equip them with receptors that enable them to recognize and kill certain kinds of tumor cells.

An experimental, ex vivo gene-editing treatment, CTX001 (CRISPR Therapeutics/Vertex Pharmaceuticals), boosted hemoglobin production in blood stem cells of trial participants with sickle cell disease or transfusion-dependent beta thalassemia.

Source: "The Once and Future Gene Therapy," by Karen Bulaklak and Charles Gersbach, in *Nature Communications*, Vol. 11; November 2020 (*reference*); Cynthia Dunbar *National Institutes of Health* (*expert reviewer*)

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