Gene Therapy

Treatment of disease by introducing healthy genes into the body is becoming feasible. But the therapy will not reach its full potential until the genes can be coaxed to work throughout life

by Inder M. Verma

One infant in every hundred is born with a serious genetic defect. Usually the damage becomes evident in childhood. All too often, it gives rise to physical or mental abnormalities, pain and early death. Of the more than 4,000 known inherited disorders, most lack fully effective therapies.

It is no wonder, then, that scientists have long imagined curing heritable ills by introducing healthy genes into patients. Advances in recombinant DNA technology, which have made possible the isolation of many genes, and new insights into gene regulation are beginning to make this once impossible notion seem feasible.

Indeed, the first federally approved clinical trial of a gene therapy for a genetic disease began this past September. R. Michael Blaese, W. French Anderson and their colleagues at the National Institutes of Health (NIH) are introducing the gene for the enzyme adenosine deaminase (ADA) into children suffering from a rare condition known as severe combined immunodeficiency (SCID). Derangement of this gene debilitates the immune system and is responsible for about 25 percent of all cases of SCID.

The approach of the NIH group requires repeated treatments throughout life, and so it is not a cure. Still, the trial could represent the start of a new era in medicine. The current pace of research suggests that by the turn of the next century clinical trials of gene therapies may be under way for any of a number of diseases—inherited and otherwise.

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Genes can be transferred either into germ cells (sperm, eggs or early embryos) or somatic cells (those not destined to become sperm or eggs). Yet germ-line therapy is not an option for the foreseeable future, in part because the new genes would be passed from generation to generation, a prospect that raises profound ethical concerns.

For instance, should therapy be applied simply to improve one's offspring, not only to prevent an inherited disease? Who would be empowered to decide? Is society willing to risk introducing changes into the gene pool that may ultimately prove detrimental to the species? Do we have the right to tamper with human evolution? The prospect of somatic cell therapy is less troubling, mainly because it would affect only the treated patient.

The most promising candidates for somatic cell therapy are disorders caused by impairment of a single gene that has been isolated and cloned and so is available for transplant. These diseases should be simpler to correct than those caused by multiple genes or by such global disturbances as the loss or addition of whole chromosomes. (Normally, human cells carry one set of 23 chromosomes inherited from the mother and a corresponding set from the father. Every chromosome consists of a long stretch of DNA and includes thousands of genes.)

In the ideal world, the diseases would be cured for life by one treatment, with no side effects. And gene insertion into a chromosome in a target somatic cell would be site specific: in what is called homologous recombination, the healthy, or "therapeutic," gene would exactly replace the damaged copy. Targeted insertion increases the probability that a therapeutic gene will function correctly. It also reduces the likelihood that random insertion will activate a quiescent oncogene (a cancer inducer) or inactivate a cancer suppressor.

In reality, investigators have found it extremely difficult to control the fate of DNA introduced into cells. For every gene spliced into the correct place, more than 1,000 fit randomly into the genome (the total DNA in a cell). Work by Mario R. Capecchi of the University of Utah suggests that the obstacles to site-specific gene delivery are great but surmountable. Meanwhile many laboratories, including my own at the Salk Institute in La Jolla, Calif., are concentrating on developing gene augmentation therapy, in which a healthy gene replaces the product of a missing or defective gene but does not physically replace the flawed DNA itself.

Augmentation can be helpful when a genetic derangement results in little or no production of a protein. (Each gene encodes, or carries instructions for, a single protein.) Low production occurs when mutations hamper the activity of both the maternal and paternal copies of a gene or when a hobbled gene is inherited on a male's only X chromosome. (The cells of males carry one X and one Y chromosome; those of females carry two X chromosomes.)

On the other hand, augmentation therapy might not be of much help when a mutation yields overproduction of a protein or the synthesis of a destructive substance, as is the case in sickle cell anemia. To correct those kinds of disturbances, therapy would often have to include delivery of both a healthy gene and one capable of inactivating the mutated version.

For now, most scientists interested in gene augmentation are planning to remove cells from patients, introduce a therapeutic gene and return the altered cells to the subject. Some day, however, physicians may directly inject patients with genes linked to substances that will deliver those genes to specific target cells.

Fortunately, genetic flaws do not necessarily have to be corrected in all of

the body's trillions of cells in order for therapy to work. First, even though every somatic cell in an individual carries identical chromosomes, certain genes function only in a single cell type. Treatment, then, could focus only on that type. Second, even when a genetic defect results in insufficient synthesis of a protein made in virtually every cell, many cells compensate for the loss. For instance, a flaw in the ADA gene affects most somatic cells to a degree but is devastating only to some constituents of the immune system.

Nontargeted delivery of genes into cells can be accomplished by chemical or physical means (transfection) or by viruses (transduction). In chemical approaches, one mixes many copies of DNA carrying the healthy gene with a charged substance—typically calcium phosphate, DEAE-dextran or certain lipids. Then the mixture is essentially dumped onto recipient cells. The chemicals disturb the cell membrane and transport the DNA into the interior.

The procedure is simple, but the efficiency of gene delivery is dismal. Usually only one cell in 1,000 to 100,000 integrates the gene of interest into its genome. A physician would have to obtain an impossible number of cells from patients to guarantee the appropriate alteration of the millions required for therapy.

I should point out that integration is not always crucial to gene expression (production of the encoded protein). Still, a gene that is integrated is likely to last longer in the cell. Further, it should replicate whenever the rest of the DNA does, as when a cell prepares to divide. The therapeutic gene would thus be inherited by the daughter cells and by their daughters and so on, thereby ensuring a supply of the product throughout a patient's life.

Physical methods include microinjection with a fine glass pipette and electroporation (the exposure of cells to an electric shock). The shock renders cells permeable to DNA in the surrounding medium, but it can also severely damage them. Microinjection can be extremely efficient; perhaps one cell in five takes up the foreign gene permanently. Yet because only a single cell can be injected at a time, this tedious, labor-intensive approach is not suitable for therapeutic purposes.

The final strategy capitalizes on the native ability of viruses to enter cells, bringing their own genetic material with them. Many of these organisms have now been engineered to serve as vectors, or delivery vehicles, for gene transfer. Viruses can be grouped according to whether their genetic material is DNA or RNA. The two substances have important chemical differences, although both are built from units known as nucleotides and both include regulatory codes in addition to those specifying the sequences of amino acids in proteins.

Many DNA viruses that can accept foreign genetic material turn out to be severely limited in the number of nucleotides they can accommodate and in the range of cells they infect. Certain other DNA viruses are roomier but have so far proved unusable for var-

STERILE BUBBLE protected a boy named David, who suffered in the 1970s from severe combined immunodeficiency, or SCID, an inherited disorder in which the immune system is profoundly impaired. SCID patients have better options today and may have more in the future: the first gene therapy approved for clinical trial aims to ease a form of the disorder.

ious reasons. Moreover, DNA viruses often do not splice their genetic material into the chromosomes of the cells they infect.

As is true of the DNA viruses, most RNA viruses are unsuitable for gene therapy, mainly because RNA, which cannot integrate into the DNA of human cells, is degraded rapidly. Varieties known as retroviruses are an exception. They actually convert their RNA to DNA in infected cells and insinuate the DNA into a chromosome. The integrated DNA then directs the synthesis of viral proteins. Retroviruses can entertain more foreign genetic material than some DNA viruses. They can also infect a broad spectrum of species and cell types.

For these reasons, retroviruses are the most promising gene-delivery systems studied thus far. Indeed, unless specified, all approaches to gene transfer discussed in the balance of this article are based on these vectors.

Retroviruses are, of course, not without obvious drawbacks. For instance, they can merge their DNA into a chromosome only in cells capable of actively dividing. Yet many cells do not normally divide—among them, mature neurons—and so they are not readily amenable to being genetically altered by retroviral vectors.

More disturbing is the possibility that retroviruses can cause cancer. The risk is extremely low for the species that have been considered as vectors, but it increases if the viruses are allowed to multiply in the body and spread from cell to cell. Consequently, a major challenge has been devising ways to stop the vectors from reproducing.

The efforts of several laboratories have together yielded at least one technique that seems to work well [*see illustration on page 72*]. The organisms produced by that method have a normal outer coat and contain all of the virus's proteins. The retroviral RNA, however, includes no instructions for synthesizing viral proteins. The therapeutic gene takes the place of those missing instructions.

The coat enables the viruses to enter cells and deliver the viral contents to the cell's cytoplasm. Then viral enzymes convert the RNA to DNA and help to fit that DNA into the genome of the host cell. But that is the end of the line for the virus.

Under normal circumstances, integrated retroviral DNA—called the provirus—would direct the synthesis of viral proteins and RNA, which would then assemble into clones of the original virus. In contrast, the altered retrovirus, bereft of instructions for making viral proteins, produces no progeny. The virus essentially disappears from the cell, leaving behind only the foreign gene and nucleotide sequences that now serve merely to facilitate the expression of the gene.

Although retroviruses can infect many cell types, only certain target cells can be considered for genetic manipulation. The cells must be strong enough to withstand handling and capable of being removed from the body and returned with reasonable ease. In addition, they should be long-lived, surviving for months or years or preferably for the patient's entire life. Because bone marrow, skin and liver cells best meet these criteria, diseases that can be treated by manipulating these cells are among the most promising candidates for gene therapy.

The cells of the bone marrow, where blood is produced, can in theory be exploited to correct disorders caused by genetic flaws in red blood cells or in white blood cells (which are important in immunity). SCID caused by an ADA deficiency is but one of several inherited conditions affecting the immune cells; another is leukocyte adhesion deficiency, which involves the poor mobilization of white blood cells and leads to recurrent infections. Among the diseases associated with impaired red blood cells are the thalassemias, which reflect impairments in the genes encoding subunits of the hemoglobin molecule—the oxygen carrier in red blood cells.

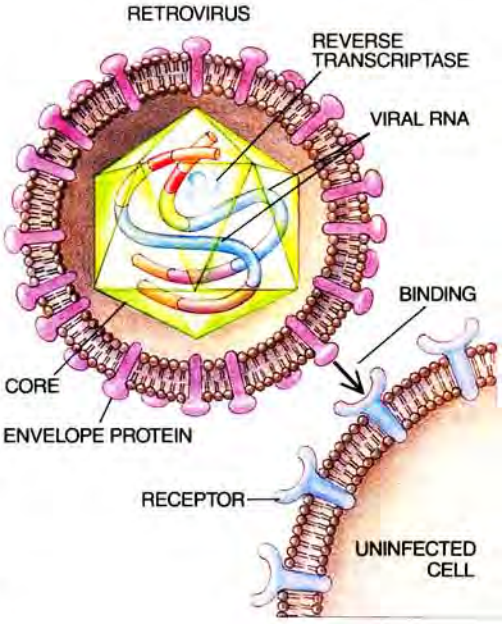
Beta thalassemia was once expected to be the first disorder treated with gene therapy. Its history illustrates some of the problems that have beset the effort to develop gene therapy in general and therapy based on bone marrow cells in particular.

Red blood cells of patients stricken with beta thalassemia are deficient in beta globin, which in healthy individuals combines with alpha globin and iron (heme) to yield hemoglobin. Healthy cells regulate the activity of both genes precisely, ensuring that equal amounts of alpha and beta globin are made. The lack of beta globin gives rise not only to a deficit in hemoglobin production but also to a relative excess of alpha globin. This excess, in turn, hastens cell death and can cause severe anemia. Usually patients succumb to the disease by age 20, after years of pain and suffering.

This disease and other inherited blood disorders could probably be treated efficiently by delivering healthy genes to stem cells, the subset of cells in the marrow that gives rise to the full spectrum of blood cells and replaces dead cells throughout a person's life. Stable introduction of a desired gene into a stem cell could guarantee the production of normal blood cells for as long as a patient lives.

Sadly, human stem cells are far from abundant and are virtually impossible to isolate. Researchers have therefore been forced to resort to a less efficient strategy: infecting enormous numbers of bone marrow cells with a therapeutic retrovirus in the hope that enough stem cells will be infected.

Studies of beta globin have supplied much of the evidence showing that the approach has at least some merit.



LIFE CYCLE of a retrovirus begins when the virus binds to (*above*) and enters (*right*) a cell and injects its genetic material (RNA) and proteins into the cytoplasm. Typical retroviral RNA includes three coding regions: *gag* (*green*), *pol* (*blue*) and *env* (*purple*), specifying, respectively, proteins of the viral core, the enzyme reverse transcriptase and constituents of the coat. It also has three noncoding domains—two at the tips (*light orange*) and another called psi, ψ (*red*). In the cytoplasm, reverse transcriptase converts the RNA into DNA, whose lengthened terminal domains, called long-terminal repeats (*dark orange*), influence the activity of viral genes and facilitate insertion of viral DNA into cellular DNA. The ensconced DNA (the provirus) directs the synthesis of viral proteins and RNA. The proteins then enclose the RNA, forming viral particles that bud from the cell.

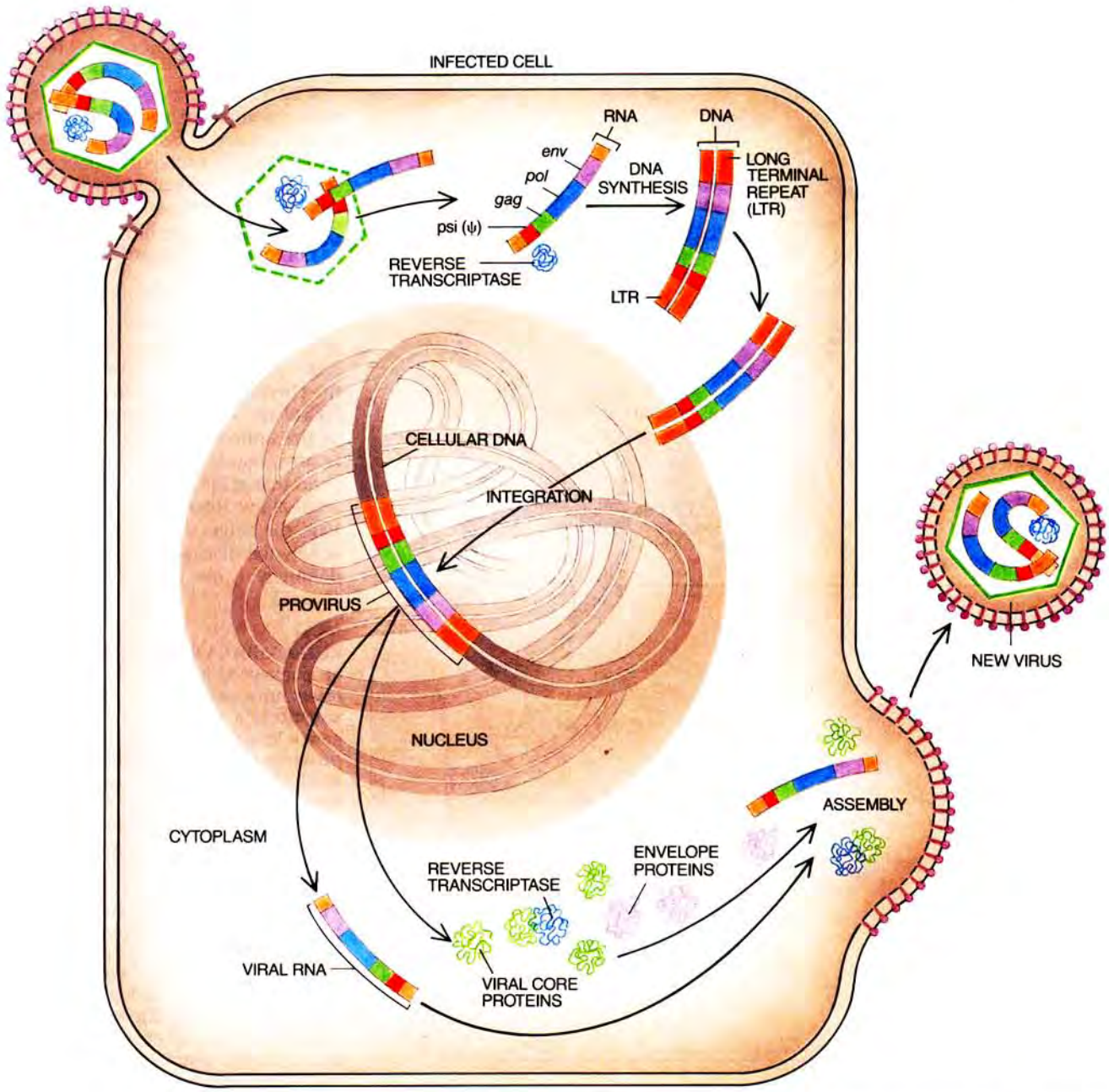
For instance, several laboratories have shown that a human beta globin gene inserted into mouse bone marrow cells by retroviral vectors stays in the cells. And Richard C. Mulligan and his coworkers at the Whitehead Institute for Biomedical Research in Cambridge, Mass., have further shown that the human gene is expressed when such cells are implanted in mice.

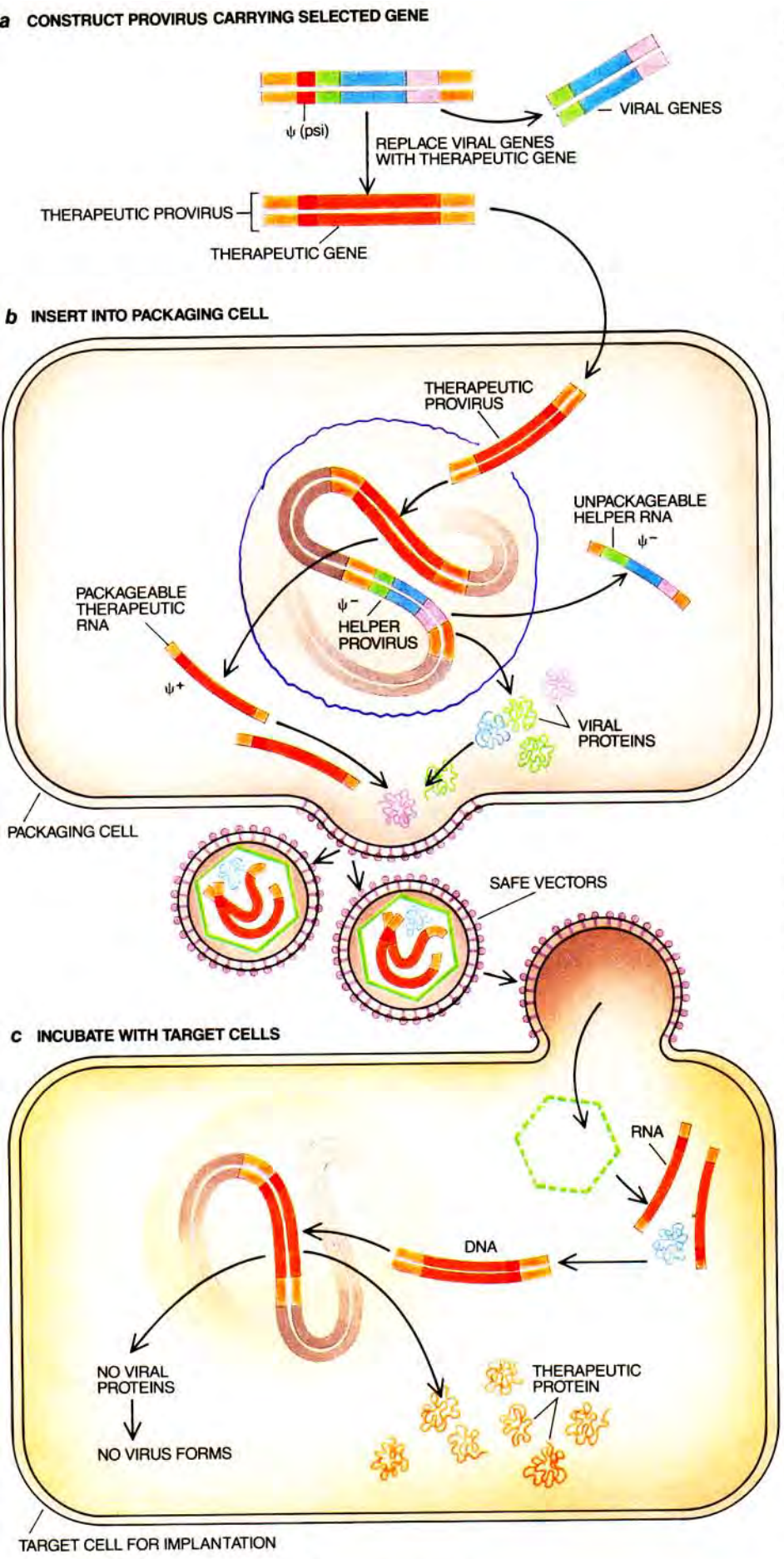
On the other hand, no one has been able to achieve significant levels of globin synthesis in recipient animals. This problem has been a major disappointment, but a discovery by F. G. Grosveld and his colleagues at the National Institute for Medical Research in London offers hope for a solution.

They identified distinct stretches of DNA, thousands of nucleotides apart from the gene itself, that in normal red blood cells dramatically boost the production of globin messenger RNA. Messenger RNA is transcribed, or copied, from DNA and is the template from which protein is made; hence, high levels of a messenger RNA indicate that the encoded protein is being produced in abundance. It seems reasonable to think that linking globin-specific enhancers to a globin gene in a retroviral vector might enhance globin synthesis in the body. Studies of this hypothesis are in progress.

In general, genetically altered bone marrow cells have yielded poor in vivo expression of other genes as well. The problem must be resolved before gene therapy based on bone marrow cells can become a reality.

Along with an acceptable level of gene expression, one would hope for long-term activity. Recent findings relating to globin indicate that achieving prolonged expression of genes inserted in bone marrow may be less problematic than attaining high levels of protein synthesis. For instance, Chung L. Li and V. J. Dwarki in my laboratory have produced sustained, albeit weak, expression of the human beta globin gene in mice for at least a five-month study period—the equivalent of 15 to 20 years in a human being. The alpha





globin gene remained functional for at least 10 months.

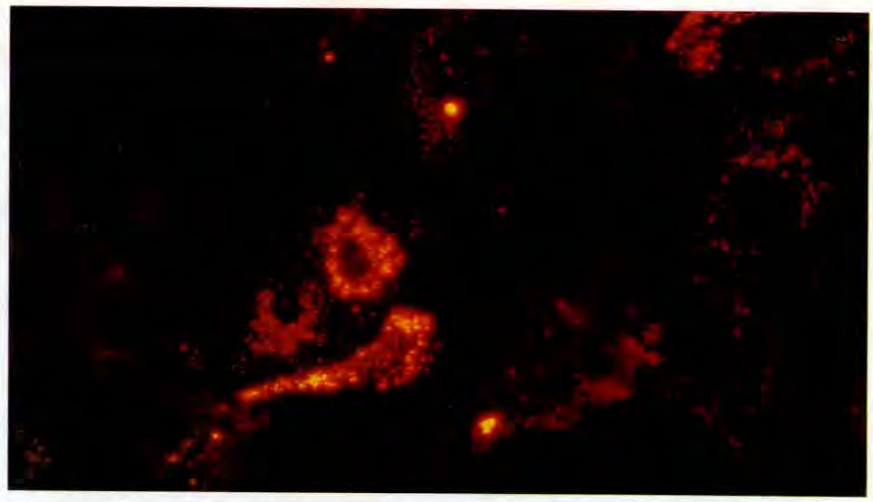
Other findings emerging from the work on beta thalassemia highlight the complexity introduced when correction of a disease requires precisely regulated expression of a therapeutic gene. For many disorders, including SCID, simply producing some amount of a missing protein is better than none. The same is not true for thalassemia. Because a relative excess of either alpha or beta globin can damage cells, the activity of a therapeutic globin gene must exactly mimic that of a normal version. Unfortunately, the mechanisms that control the activity of genes are understood only imperfectly—both for the beta globin gene and for most others. Discoveries are made constantly, however, and are helping improve the design of vectors for gene therapy.

SCID researchers at the NIH have taken a detour from gene therapy based on bone marrow cells, in part because of the ongoing problem of poor expression. Patients in their study are treated with a select subset of circulating *T* lymphocytes, white blood cells crucial to immunity. *T* cells are devastated by a lack of ADA.

The retrovirally altered lymphocytes are infused into children who are now being helped somewhat by injections of PEG-ADA—ADA mixed with the chemical polyethylene glycol to increase the enzyme's half-life. Success of the approach will be measured by improvements in immune function beyond that achieved by enzyme replacement alone. Regrettably, *T* cells do not have the longevity of stem cells, which is why the disease cannot be cured indefinitely by one treatment.

RETROVIRAL VECTORS are assembled, or packaged, in cells designed to release only safe vectors. Investigators substitute a therapeutic gene for viral genes in a provirus (*a*) and insert that provirus into a packaging cell (*b*). The viral DNA directs the synthesis of viral RNA but, lacking viral genes, cannot give rise to the proteins needed to package the RNA into particles for delivery to other cells. The missing proteins are supplied by a "helper" provirus from which the psi region has been deleted. Psi is crucial to the inclusion of RNA in viral particles; without it, no virus carrying helper RNA can form. The particles that escape the cell, then, carry therapeutic RNA and no viral genes. They can enter other cells (*c*) and splice the therapeutic gene into cellular DNA, but they cannot reproduce.

The availability of nongenetic treatments for SCID (including bone marrow transplantation) raises the general question of whether subjecting patients to highly experimental gene therapies is justified when alternatives exist. The prevailing opinion holds that such experimentation is acceptable if the risks are demonstrably low and if, on the one hand, a gene therapy promises to be significantly more helpful than existing approaches or, on the other, patients are ineligible for the established treatments. In the case of SCID, for example, not all patients have access to bone marrow from a tissue-compatible donor.



LIVER CELLS from rabbits genetically deficient in the receptor for low-density lipoprotein (LDL) began to make the missing receptor (*bright regions*) after being altered to carry the receptor gene. The finding raises the possibility that a similar genetic disorder leading to excess serum cholesterol in humans might one day be treatable by gene therapy. James M. Wilson of the Howard Hughes Medical Institute Research Laboratories at the University of Michigan at Ann Arbor and J. Roy-Chowdhury of the Albert Einstein College of Medicine made the photomicrograph.

Genetic alteration of lymphocytes or bone marrow cells aims to correct defects in those same cells or their progeny. Skin cells, in contrast, are being studied for quite a different purpose: the synthesis and secretion of proteins that are normally made in one cell type but are ferried in blood plasma for use by other cells.

In principle, implants of skin cells could correct many disorders. These conditions might include hemophilia (caused by a lack of blood-clotting factors made in the liver) and diseases caused by insufficient production of particular hormones (for example, growth hormone). Certain disorders caused by deficient production of widely made proteins would also be candidates, if the tissues most affected by the deficiency could take up replacement proteins from the blood.

Fibroblasts, a constituent of the dermis (the lower layer of the skin), are best suited for therapy, which would involve implanting the altered cells back into the dermis. They are accessible and strong and able to multiply in the laboratory. Furthermore, they can secrete substances into the blood and would be easy to remove if necessary.

My laboratory has extensively studied the value of skin fibroblasts for treating the form of hemophilia caused by a lack of the liver product known as clotting factor IX. Our results underscore the great therapeutic potential of such cells.

In one of our studies, for instance, A. Dusty Miller, now at the Fred Hutchison Cancer Research Center in Seattle, collaborating with George G. Brownlee and Don S. Anson of the University of Oxford, showed that fibroblasts could be induced to synthesize and secrete factor IX, even though they do not typically make that protein. (Whether the same will be true for all foreign proteins remains to be seen.) Furthermore, when Daniel C. St. Louis, Jonathan H. Axelrod and Raphael Scharfmann in my group used retroviruses to insert the human factor IX gene into fibroblasts and implanted the cells in the dermis of mice, the implants became highly vascularized and released the factor into the blood.

This study not only demonstrated that expression of factor IX in animals was possible, it also taught us an important lesson. About 15 days after the cells were implanted, the human factor disappeared from the blood of the mice. The recipients, it turned out, had mounted an immune response against the foreign human protein. The moral: gene therapy will probably be most successful in patients who make at least a small amount of a deficient protein; otherwise the immune system may become aroused against the product of an inserted gene.

We have also found some evidence to suggest that, unlike the bone marrow cells studied to date, fibroblasts may be able to produce enough of a selected product to correct disease. Extrapolation from data in mice indicates that an implant the size of a quarter should make enough protein to alleviate a factor IX deficiency in a human. In collaboration with Kenneth M. Brinkhous of the University of North Carolina at Chapel Hill, we expect to study the ability of fibroblast implants to correct hemophilia in dogs. If those experiments are successful, trials in humans would be justified.

Genetically altered fibroblasts might also be implanted in the brain to correct disorders in neurons. The brain is notoriously hard to treat because many drugs that circulate in the blood are barred from the brain. Moreover, neurons cannot be removed for direct genetic alteration without consequence to the brain. Fibroblasts could in theory be engineered to secrete proteins for diffusion into nerve cells.

Preliminary results are encouraging. Fred Gage of the University of California at San Diego has shown that implants engineered to secrete nerve growth factor could stimulate neuronal growth in the rat brain. The regeneration occurred in the kinds of neurons whose decay is associated with memory loss in Alzheimer's disease, although the role of the factor in that disease has not been established. Similarly, implants that make levodopa (L-dopa), a precursor of the neurotransmitter dopamine, are under study in animal models of Parkinson's disease. No one knows exactly what causes Parkinson's, but a deficiency of dopamine seems to play a part. Exactly how long fibroblast implants can survive in the skin or brain is still being investigated.

Compared with bone marrow and skin cells, liver cells are a newcomer to the field of gene therapy. They could become important for the treatment of any number of genetic diseases caused by malfunctioning liver cells. Recently, for instance, Mulligan of the Whitehead Institute and James M. Wilson, then also at the institute, and, separately, Theodore Friedmann and his colleagues at San Diego succeeded in delivering the gene for

the low-density lipoprotein (LDL) receptor to liver cells and inducing them to make biologically active receptors in the laboratory. The cells came from Watanabe rabbits, which are genetically deficient in the LDL receptor—as are humans afflicted with familial hypercholesterolemia, a condition that can lead to heart attacks.

The feasibility of directly injecting live Watanabe rabbits with complexes of the receptor gene and a protein that homes to the liver has also been studied. (Direct injection in humans would, of course, avoid surgery to remove liver cells.) The encoded protein was detected in the body but, as was also true in the cell-culture study, was made only transiently. Longevity may yet be improved; investigation of liver cells is still in its infancy.

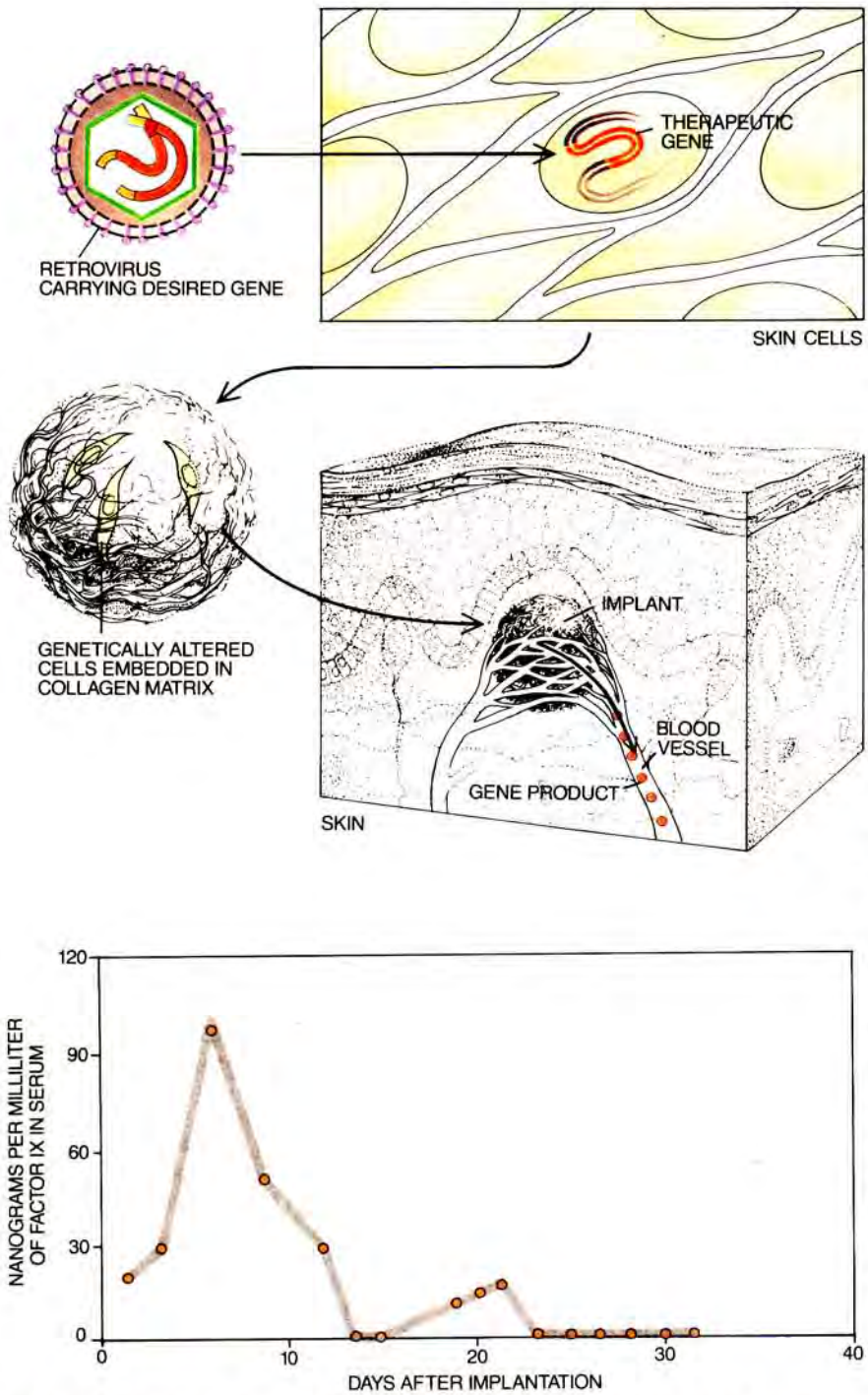
Although bone marrow, skin and liver cells are receiving the most attention, other types are also being considered. For instance, retroviruses can carry genes for secretory products into endothelial cells, which line the arteries. These cells have more intimate contact with the blood than do fibroblasts, and so they might deliver the products more quickly.

Researchers are also considering injecting a healthy gene encoding dystrophin (a structural component of muscle) directly into muscles of mice that have acquired a disorder akin to Duchenne's muscular dystrophy. There is reason to hope the genes will be expressed; other genes injected into muscles in live animals gave rise to proteins for several months, even though the DNA was not integrated into chromosomes. It may also be possible to treat cystic fibrosis, an inherited lung disorder, by packaging healthy genes in retroviruses that would be inhaled in an aerosol spray.

Gene therapy does not have to be limited to repairing the effects of malfunctioning genes. It can also add novel properties to cells to enhance their ability to combat disease.

For instance, Steven A. Rosenberg and his colleagues at the National Cancer Institute have demonstrated that lymphocytes taken from a patient's tumor and cultured with interleukin-2 (a *T* cell activator) can shrink some cancers. They now hope to increase the cancer-fighting powers of those tumor-infiltrating lymphocytes, or TILs, by inserting a gene encoding tumor necrosis factor, a potent immune-system molecule. The factor, which has anticancer activity, is not ordinarily made in *T* cells. Clinical trials are expected to begin soon [see "Adoptive Immunotherapy for Cancer," by Steven A. Rosenberg; SCIENTIFIC AMERICAN, May].

In more preliminary work, another group is trying to induce various cell types to produce CD4, a molecule found on *T* cells depleted by the AIDS virus. The virus enters the cells after a protein in its coat binds with CD4. A flood of CD4 molecules in the blood might serve as a decoy to keep the virus from interacting with the cells. Many other creative ideas for applying gene therapy are also being discussed, including coaxing endothelial cells to secrete factors that would pre-



SKIN CELLS carrying an inserted gene can be embedded in a collagen matrix and implanted in the dermis to deliver the gene's product to the blood (*top*). In one early experiment, skin fibroblasts containing the human gene for factor IX, a protein normally secreted by the liver to aid in blood clotting, became well vascularized in mice and secreted the human factor for approximately two weeks (*graph*). Much longer release of foreign proteins has now been achieved by fibroblast implants.

DISORDER	INCIDENCE	NORMAL PRODUCT OF DEFECTIVE GENE	TARGET CELLS	STATUS
Hemoglobinopathies (thalassemias)	1 in 600 in certain ethnic groups	Constituents of hemoglobin	Bone marrow cells (which give rise to circulating blood)	Globin production in animals receiving gene needs to be improved
Severe combined immunodeficiency (SCID)	Rare	Adenosine deaminase (ADA) in about a quarter of SCID patients	Bone marrow cells or *T* lymphocytes	Clinical trial of lymphocyte therapy for ADA deficiency is under way
Hemophilia A Hemophilia B	1 in 10,000 males 1 in 30,000 males	Blood-clotting factor VIII Blood-clotting factor IX	Liver cells or fibroblasts	Good chance for clinical trials (with fibroblasts) in next five years
Familial hypercholesterolemia	1 in 500	Liver receptor for low-density lipoprotein (LDL)	Liver cells	Animal studies are in early stages
Inherited emphysema	1 in 3,500	$Alpha_1$-antitrypsin (liver product that protects lungs from enzymatic degradation)	Lung or liver cells	Work is very preliminary
Cystic fibrosis	1 in 2,500 Caucasians	Substance important for keeping air tubes in lungs free of mucus	Lung cells	Aerosol delivery of gene directly to lungs is a theoretical possibility
Duchenne's muscular dystrophy	1 in 10,000 males	Dystrophin (structural component of muscle)	Muscle cells (particularly embryonic ones that develop into muscle fibers)	Work is preliminary. Nondystrophin genes injected into muscle have directed synthesis of the encoded proteins
Lysosomal storage diseases	1 in 1,500 acquires some form	Enzymes that degrade complex molecules in intracellular compartments known as lysosomes	Vary, depending on disorder	Most diseases would require delivery of gene into brain cells (a difficult task) as well as into other cell types

POTENTIAL CANDIDATES for the earliest gene therapies will be disorders caused by defects in a single gene that has been cloned. In general, physicians will remove cells from a patient, insert a healthy gene and return the cells to the body.

vent blood clots from forming in a patient's arteries after heart surgery.

The idea of introducing genes to correct heritable and other disorders is nothing less than revolutionary. Perhaps that is one reason why the field has progressed somewhat more slowly than was once expected. Modern creatures are the products of millions of years of evolution. One cannot expect that the initial stabs at inserting genes into cells will yield normal, stable expression easily.

Yet to cure diseases, investigators must find ways to ensure that therapeutic genes are expressed well and persistently in the body. Continually emerging clues, such as the importance of including particular enhancers with some genes in retroviral vectors, are beginning to point the way. Also needed are better methods for returning genetically altered cells (such as liver cells) to the body, ways of extending the survival of implanted cells, and techniques for isolating human stem cells (to replace the bone marrow cells now being studied).

At the same time, the safety of retroviral vectors must be confirmed in extensive studies of both small and large animals, and efforts to incorporate added safeguards should continue. In spite of the advent of retroviral vectors that cannot replicate, there is still a chance they could cause cancer. Efforts to develop alternatives to retroviral vectors should be pursued further as well, as should research into site-specific gene delivery.

The goal of curing genetic diseases for life with a single, safe treatment is unquestionably worth the effort being put into it, but I must end with the reminder that gene therapy cannot correct all human disease. Most human afflictions are not genetic. They are environmental, caused by microbial infections that spread because of poor sanitation, polluted drinking water, malnutrition and other factors that are outside the scope of genetic engineering. Those diseases, too, deserve increased study.

FURTHER READING

LINEAGE-SPECIFIC EXPRESSION OF A HUMAN β-GLOBIN GENE IN MURINE BONE MARROW TRANSPLANT RECIPIENTS RECONSTITUTED WITH RETROVIRUS-TRANSDUCED STEM CELLS. Elaine A. Dzierzak, Thalia Papayannopoulou and Richard C. Mulligan in *Nature*, Vol. 331, No. 6151, pages 35–41; January 7, 1988.

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Gene Therapy's Second Act

A decade and a half after a series of tragic setbacks led to critical reevaluations, scientists say gene therapy is ready to enter the clinic

By Ricki Lewis | Mar 1, 2014

Kotryna Zukauskaite

Gene therapy may finally be living up to its early promise. In the past six years the experimental procedure for placing healthy genes wherever they are needed in the body has restored sight in about 40 people with a hereditary form of blindness. Doctors have seen unprecedented results among another 120-plus patients with various cancers of the blood—several of whom remain free of malignancy three years after treatment. Researchers have also used gene therapy to enable a few men with hemophilia, a sometimes fatal bleeding disorder, to go longer without dangerous incidents or the need for high doses of clotting drugs.

The positive results are even more impressive considering that the field of gene therapy essentially ground to a halt 15 years ago, following the untimely death of Jesse Gelsinger, a teenager with a rare digestive disorder. Gelsinger's immune system reacted to the gene treatment he received by launching a counterattack of unexpected ferocity that killed him. Gene therapy's preliminary successes in the 1990s, it turns out, had fueled unreasonably high expectations among doctors and researchers—and perhaps a bit of hubris.

This and other setbacks forced scientists to rethink some of their approaches, as well as to be more realistic about gene therapy's feasibility for treating various conditions in people. Investigators curbed their hopes and returned to basic research. They examined potentially fatal side effects such as those experienced by Gelsinger and learned how to avoid them. And they paid more attention to explaining the risks and benefits to volunteers and their families.

The turning point, in the view of many observers, came six years ago, when doctors treated then eight-year-old Corey Haas for a degenerative eye disorder that caused his sight to deteriorate. The gene therapy they used allowed the defective retina of Haas's left eye to make a protein that his body could not otherwise produce. Within four days he took a trip to the zoo and found, to his delight and astonishment, that he could see the sun and a hot-air balloon. Three years later he underwent the same treatment in his right eye. Now Haas sees well enough to go turkey hunting with his grandfather.

Although gene therapy is still not available in hospitals and clinics, that is likely to change in the next decade. Europe approved its first gene treatment, for a rare

but extremely painful disorder called familial lipoprotein lipase deficiency, in 2012. At the end of 2013 the National Institutes of Health removed some of the regulatory speed bumps that the agency now considers unnecessary. The first U.S. approval of a commercial gene treatment, some industry watchers predict, may come in 2016. Gene therapy, after its lost decade, is at last beginning to fulfill its destiny as a revolutionary medical treatment.

Heartbreak

The early failures of gene therapy highlight how difficult it is to establish a safe and efficient means of delivering genes to the target tissue. Too often the safest delivery systems were not very effective, and some of the most effective systems turned out not to be very safe, setting off either an overwhelming immune reaction, as in Gelsinger's case, or the development of leukemia, as in other instances.

To understand what triggered these side effects and to figure out how to lessen the risks of their occurrence, scientists focused on the most common delivery system for gene therapy: engineering a virus to act as a kind of microscopic injection gun.

For starters, researchers remove some of the virus's own genes to create room for the healthy genes that they want to deliver to a patient. (This step also has the added benefit of preventing the virus from making copies of itself once inside the body, which increases the chances of an immune reaction.) Then the customized viruses are injected into that person, where they insert the new genes into various places in cells, depending on the type of virus being used.

By the time Gelsinger volunteered for a clinical trial, the delivery system of choice consisted of adenoviruses, which in their natural state can cause mild upper respiratory infections in people. Scientists at the University of Pennsylvania determined that the best chance for success was to inject the viruses into the liver, where the cells that normally make the digestive enzyme Gelsinger was missing are located. They packaged a working copy of the gene for that enzyme into stripped-down adenoviruses. Then they injected one trillion of these viruses—each with their custom payload—directly into Gelsinger's liver.

Once in Gelsinger's body, however, some of the viruses took a tragic detour. They entered the liver cells as planned, but they also infected huge numbers of macrophages, the large wandering cells that serve as sentries for the immune system, and the dendritic cells that announce an invasion. The immune system responded by destroying each infected cell, a violent process that ultimately ravaged Gelsinger's body from the inside out.

The ferocity of the immune response took investigators by surprise. None of the 17 volunteers who had previously undergone treatment for the same disorder had exhibited such severe side effects. Researchers knew that adenoviruses could provoke an immune response, but apart from a study of a slightly different reengineered virus in which a monkey died, they did not realize how explosive the reactions could be. “Humans are much more heterogeneous than colonies of animals,” says James Wilson of the University of Pennsylvania, who developed the viral delivery system used in the clinical trial in which Gelsinger had participated. “What we saw in that trial was one individual out of 18 who had a very exaggerated host response.” In hindsight, it seemed that it would have been wiser to inject fewer—billions rather than one trillion—gene-bearing viruses into his body. The researchers were also criticized for not informing Gelsinger and his family about the monkey's death so that they could make up their own minds about whether it was an unrelated event.

Gelsinger's death was not the only gene therapy tragedy. Soon after, treatment for another disorder—called severe combined immunodeficiency X1, or SCID-X1—triggered five cases of leukemia, including one death, in 20 children. Once again the gene delivery system turned out to be at fault. In this instance, however, the microscopic injection gun in question consisted of a retrovirus, a kind of virus that inserts its genetic payload directly into the DNA of a cell. The exact placement of the therapeutic genes is a bit haphazard, however, and the retrovirus sometimes inserted its payload into an oncogene—a gene that can cause cancer under certain circumstances.

Rethinking the Technology

Given the propensity of adenoviruses to provoke lethal immune reactions and of retroviruses to trigger cancer, investigators began paying more attention to other

viruses to see if they offered better results. They soon focused on two more widely suitable entrants.

The first new delivery system, adeno-associated virus (AAV), does not make people sick (although most of us have been infected by it at one time or another). Because it is so common, it is unlikely to cause extreme immune reactions. This virus has another feature that should also help minimize side effects: it is available in several varieties, or serotypes, that favor specific types of cells or tissues. For example, AAV2 works well in the eye, whereas AAV8 prefers the liver, and AAV9 slips into heart and brain tissue. Researchers can choose the best AAV for a specific body part, decreasing the number of individual viruses that need to be injected and thus minimizing the chances of an overwhelming immune response or other unwanted reaction. Plus, AAV deposits its genetic payload outside the chromosomes, so it cannot accidentally cause cancer by interfering with oncogenes.

Adeno-associated virus was first used in a clinical trial in 1996, on cystic fibrosis. Since then, 11 serotypes have been identified, and their parts have been mixed and matched to engineer hundreds of seemingly safe and selective delivery tools. Current studies are evaluating AAV-borne gene therapy for several brain diseases, including Parkinson's and Alzheimer's, and for hemophilia, muscular dystrophy, heart failure and blindness.

The second, rather more surprising new gene vector is a stripped-down version of HIV—the virus that causes AIDS. Once you look beyond HIV's reputation as a killer, its advantages for gene therapy emerge. As a member of the *Lentivirus* genus of retroviruses, it evades the immune system and—crucial for a retrovirus—does not typically disturb oncogenes.
After the genes that make HIV lethal are removed, the viral packaging that remains “has a large capacity,” says Stuart Naylor, formerly chief scientific officer at Oxford Biomedica in England, which is pursuing “gene-based medicines” for eye diseases. Unlike the smaller AAV, “it's great for installing multiple genes or big, chunky genes,” he says. “There's no toxicity and no adverse immune reaction.” Stripped-down lentiviruses are now being used in a number of clinical trials, including treatments for adrenoleukodystrophy—the disease featured in

the 1992 movie *Lorenzo's Oil*. To date, a few of the boys who have received this treatment have become healthy enough to return to school.
Although clinical trials using AAV and HIV are on the rise, researchers have also redirected or modified the older viral delivery systems so that they can be used in limited circumstances. For example, non-HIV retroviruses are now genetically edited so that they inactivate themselves before they can trigger leukemia.

Even adenovirus, which caused Gelsinger's death, is still in clinical trails as a gene therapy vector. Investigators restrict its use to parts of the body where it is unlikely to cause an immune response. One promising application is to treat “dry mouth” in patients undergoing radiation for head and neck cancer, which damages the salivary glands, located just under the surface of the inside of the cheek.

The nih is running a small clinical trial that involves inserting a gene that creates channels for water into the glands. Because the glands are small and contained, and the experimental design calls for 1,000-fold fewer viruses than were used on Gelsinger, the chances of an immune overreaction are reduced. In addition, viruses that do not hit their target cells should wind up in a patient's drool, either swallowed or spit out, with little chance of irking the immune system. Since 2006, six of 11 treated patients have been shown to produce significantly more saliva. Bruce Baum, a dentist and biochemist who led the research before he retired, calls the results “cautiously encouraging.”

New Targets

Emboldened by these successes, medical researchers have moved beyond treating hereditary diseases to trying to reverse genetic damage that naturally occurs over the course of a lifetime.

Scientists at the University of Pennsylvania, for example, are using gene therapy to tackle a common childhood cancer known as acute lymphoblastic leukemia (ALL).

Although most children with ALL respond to standard chemotherapy, about 20 percent do not. Researchers are turning to gene therapy to turbocharge these children's immune cells to seek out and destroy the recalcitrant cancer cells.

The experimental approach is particularly complex and is based on so-called chimeric antigen receptor (CAR) technology. Like the chimera of Greek mythology that is made up of different animals, a chimeric antigen receptor consists of two molecules from the immune system that are not normally found together. Some immune cells, known as T cells, are then outfitted with these chimeric antigen receptors, which allow the cells to target proteins that are found in greater numbers on a leukemia cell. The fully armed and deployed T cell then destroys the cancer cell. The first test subjects were adults with chronic leukemia, who responded favorably. The next attempt, with a child, exceeded the researchers' wildest dreams.

Emily Whitehead was five in May 2010, when she was diagnosed with leukemia. Two rounds of chemotherapy did not work. In the spring of 2012 “she was given a [third] chemotherapy dose that would have killed an adult, and she still had lesions in her kidneys, liver and spleen,” says Bruce Levine, one of Whitehead's doctors. The girl was days from death.

Doctors took a sample of Whitehead's blood and isolated some of her T cells. They then injected the sample with lentiviruses that had been outfitted with the appropriate genes. After a rocky start, which fortunately responded to treatment, Whitehead quickly improved. Three weeks after treatment, a quarter of the T cells in her bone marrow bore the genetic correction. Her T cells began homing in on the cancer cells, which soon vanished. “In April she had been bald,” Levine recalls. “By August she went to her first day of second grade.”

Although Whitehead's modified cells might not last forever—in which case doctors can repeat the treatment—this beautiful girl with shaggy brown hair has been free of cancer for about two years. And she is not alone. By late 2013 several groups of researchers reported that they had used the CAR technique on more than 120 patients, for Whitehead's form of leukemia and three other blood cancers. Five adults and 19 of 22 children have achieved remission, meaning that they are currently cancer-free.

Into the Clinic

With safer viral delivery systems in hand, gene therapy specialists are now tackling the greatest challenge that any new drug faces: earning the approval of

the U.S. Food and Drug Administration. This daunting step requires so-called phase III clinical trials, which are designed to assess efficacy in a larger group of volunteer patients and typically take one to five years to complete (the time varies widely). As of the end of 2013, about 5 percent of approximately 2,000 clinical trials for gene therapy had reached phase III. One of the furthest along is aimed at Leber congenital amaurosis—the condition that was robbing Haas of his sight. So far several dozen patients have had corrective genes inserted into both eyes and are now able to see the world.

China was the first country to approve a gene treatment, in 2004, for head and neck cancer. In 2012 Europe approved a gene therapy–based drug called Glybera to treat familial lipoprotein lipase deficiency. Working copies of the mutant gene wrapped in AAV are injected into the leg muscles. Netherlands-based company UniQure is in early talks with the fda about approval in the U.S. One potential stumbling block: the price tag for a single curative dose is $1.6 million, but that cost may come down as researchers develop more efficient procedures.

As with many medical technologies, the decades-long path to successful gene therapy has been circuitous and is far from complete. But as gene therapy accumulates more success stories such as Corey Haas and Emily Whitehead, it is moving closer to becoming a mainstream medical treatment for some disorders and a promising new option for others.

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Ricki Lewis is a science writer with a Ph.D. in genetics. She is author of several textbooks, many magazine articles and the book *The Forever Fix: Gene Therapy and the Boy Who Saved It* (St. Martin's Press, 2012).

MORE TO EXPLORE

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Is the Gene-Editing Revolution Finally Here?

A DNA-editing technique based on bacterial “memories” could revolutionize medicine. But some worry it could get out of control

By Margaret Knox | Nov 18, 2014

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Ben Voldman

The age of genetic engineering began in the 1970s, when Paul Berg spliced DNA from a bacterial virus into a monkey virus and Herbert W. Boyer and Stanley N. Cohen created organisms in which introduced genes remained active for generations. By the late 1970s Boyer's company, Genentech, was churning out

insulin for diabetics using*Escherichia coli* modified to contain a synthetic human gene. And in laboratories around the country, researchers were using transgenic mice to study disease.
These triumphs changed the course of medicine. But the early methods had two big limitations: they were imprecise and hard to scale. Researchers overcame the first limit in the 1990s by designing proteins that could snip specific locations of DNA, a big improvement over inserting DNA into cells at random and hoping for a useful mutation. Yet they still had to devise a new protein tailored to every sequence of DNA that they wanted to target—and that was slow, painstaking work.

Then, two years ago, a small group of researchers working in the labs of Emmanuelle Charpentier at Umeå University in Sweden and of Jennifer Doudna at the University of California, Berkeley, reported the discovery of a genetic mechanism in cells that allows scientists to edit genomes with unprecedented speed and ease. Shortly thereafter, a team of scientists at Harvard University and the Massachusetts Institute of Technology showed that the technique could be used to make multiple changes in a cell's genome, with great precision, all at once.

Already the advance has accelerated the genetic-modification industry in ways that are almost certain to have profound and beneficial effects on the field of genetics and medicine. Scientists can now engineer custom transgenic lab animals in a matter of weeks—saving about a year's worth of work. Researchers are using the technique to explore therapies for diseases as diverse as HIV, Alzheimer's disease and schizophrenia. Yet the technique makes genetic modification so easy and inexpensive that some ethicists are anticipating possible negative consequences.

The technology is called CRISPR,
after *c*lustered, *r*egularly *i*nterspaced, *s*hort *p*alindromic *r*epeats—the genetic mug shots that bacteria use to remember viruses that have attacked them. Scientists have been studying these odd genetic sequences since Japanese researchers discovered them in the late 1980s. But CRISPR's promise as a gene-

editing tool did not become clear until Doudna's and Charpentier's teams figured out how to use a protein called Cas9.

The Power of RNA

Doudna and Charpentier met in 2011, at a scientific conference in San Juan, Puerto Rico. They had a lot in common. Both managed research groups that studied how bacteria defend themselves against viruses. Both had done work confirming that a bacterium identifies attacking viruses by using “memories” of past invaders' DNA to spot those enemies when they reappear.

Shortly after the meeting, Charpentier and Doudna decided to join forces. Charpentier's lab in Umeå was picking up clues that*Streptococcus* bacteria used a single protein, Cas9, as a kind of sword to chop up viruses that breached their cell walls. Doudna put her Berkeley lab on the job of figuring out how Cas9 worked. By one of those quirks of fate that underpin many scientific discoveries, it turned out that Krzysztof Chylinski, a researcher in Charpentier's group, and Martin Jinek, then in Doudna's, had grown up in neighboring towns and spoke the same Polish dialect. “They started speaking by Skype, hit it off, and started to share data and discuss ideas for experiments,” Doudna says. “The project really took off from there.”

Scientists in both labs realized that Cas9 might be useful for genome editing, a type of genetic engineering that uses enzymes as molecular pruning shears. The enzymes, called nucleases, create breaks at specific sites in the double-stranded DNA helix; a cell then repairs the break, sometimes incorporating new genetic material that a scientist has placed in the nucleus. When Doudna and Charpentier began collaborating, the most advanced method available for disabling or altering a gene was to customize an enzyme that could find and cut the desired DNA target. In other words, for every genetic modification, scientists had to tailor a new protein targeted to the right DNA sequence.

But Doudna and Charpentier realized that Cas9, an enzyme that the strep bacterium used in its immunological defense, employed RNA to guide it to the DNA target. Probing for the target, the Cas9-RNA complex would bounce off the DNA, seemingly at random, until it found a promising site. The bouncing turned out to be the Cas9 enzyme searching, each time, for the same short “signal” sequence of DNA; Cas9 would attach to that sequence, pry open the double helix

of the adjacent DNA and see if it matched the RNA guide. Cas9 would make the cut only when the RNA matched the DNA molecules. If that natural RNA-guided system could be harnessed, researchers would not have to construct a new enzyme to reach every target on the genome. Editing might become simpler, cheaper and more efficient.

After months of studying Cas9 together, the transatlantic team had a breakthrough. Doudna recalls the moment vividly: Jinek, then a postdoctoral researcher, had been running tests on Cas9 in the lab, which sits across from the Greek Theatre on a leafy hillside at the edge of the Berkeley campus. He showed up in Doudna's office one day to discuss results, and they mused about something that he had been discussing with Chylinski: in nature—in *Streptococcus* bacteria—Cas9 used not one but two RNA guides to target the right spot in the double helix of an invader's DNA. What if they could streamline those two guides into a single, artificially produced RNA strand without harming its effectiveness as a guide? With only one RNA sequence to modify, the engineering might be sped up tremendously. An RNA guide would be much easier to construct than the binding agents of the old customized enzymes, with their elaborate coding schemes.

"It was one of those moments when you see data, and something clicks," Doudna says. "We realized that we could design those RNA molecules into a single guide. A single protein and a single guide would be a powerful tool. I had chills running down my spine and realized, 'Oh, my gosh, run, don't walk, to the lab. If this works….'"

And work it did, with implications that Doudna, for all her excitement, could never have imagined. When Doudna and Charpentier published the results of their CRISPR-Cas9 research on August 17, 2012, scientists in the field immediately recognized its transformative potential—and a global race was on to test the applications.

Rush to Commercialization

By last year researchers were getting CRISPR-Cas9 to work in the cells of plants and animals much more complex than bacteria, and they were speculating about applications as fantastical as bringing back Neandertals and woolly mammoths.

At Harvard, a team led by geneticist George Church used CRISPR to alter genes in human cells, opening up a whole new world of therapeutic possibilities.
Not surprisingly, money soon began to pour into CRISPR-Cas9 work. A little more than a year ago Doudna teamed up with Church, Feng Zhang of M.I.T. and other researchers to launch Editas Medicine, with $43 million in venture capital and the goal of developing a new class of drugs based on CRISPR. (The company is not yet talking about which diseases it will target first.) In April, CRISPR Therapeutics launched in Basel and London, with investments of $25 million and a similar goal. Therapies from companies like CRISPR Therapeutics and Editas Medicine are still years away. But lab-supply firms are already shipping ready-to-inject CRISPR and made-to-order, CRISPR-altered mice, rats and rabbits to customers around the world.

On a steamy day this past summer I visited SAGE Labs in St. Louis, the first company to license Doudna's CRISPR technology for altering rodents, so I could see for myself how CRISPR works. SAGE ships to about 20 of the top pharmaceuticals companies, along with lots of universities, biotech institutes and foundations. (Horizon Discovery Group, a biotechnology company based in Cambridge, England, which was already barreling into CRISPR production of its own, bought SAGE for $48 million in September.) At SAGE, a set of low office buildings on a cul-de-sac in an industrial complex, scientists receive an online order from a lab in, say, Sacramento, Calif., for 20 *Pink1* knockout rats for research on Parkinson's disease. In a new, $2-million wing of the building, rats with this modification, as well as other CRISPR-modified rodents, live in superclean, climate-controlled cages that are neatly stacked from floor to ceiling. Filling the order is as easy as selecting 20 of the right rats, packing them gently into boxes and airfreighting them to California. The same goes for animals ready-made for research on ills ranging from schizophrenia to pain control.
If a customer needs a rat or mouse that is not in stock, however, the process is different. A SAGE customer who wants to study a link between Parkinson's and a newly suspect gene—or even a specific mutation within a gene—has several options. SAGE scientists can use CRISPR to turn off the targeted gene, to introduce a mutation, or to turn off the gene and insert a human gene in its place. Many diseases, from Parkinson's to cystic fibrosis to AIDS, are affected by multiple genetic variants, and it used to take up to a year to create the complex,

sequential mutations in animals that were needed to study such illnesses. Unlike previous genome-editing techniques, CRISPR allows researchers to make multiple genetic changes to a cell quickly and simultaneously, reducing the time it takes to produce a modified animal to a matter of weeks.

The SAGE employees start this process by making customized DNA from a chemical kit—and RNA to match the DNA. In a petri dish, they mix the RNA and Cas9, which combine into a chemical substance with gene-editing powers: the CRISPR tool. Then they spend about a week testing that tool on animal cells, using what looks like a desktop scanner to run electric currents that shock the CRISPR into the cells. The CRISPR goes to work, cutting the DNA and causing small insertions or deletions. Because CRISPR is not 100 percent efficient, it makes cuts and creates mutations in some cells but not in others. To see how well the CRISPR has performed, the scientists collect the DNA from the cells, pool it, and make copies of the region around the site of the supposed mutation. After processing and analyzing that pooled DNA, they look at the results on a computer monitor. Cut, mutated DNA shows up as a dim band—and the more DNA the CRISPR has cut, the brighter that dim band will be.

Next the process moves to the animal wing, where scientists use CRISPR to churn out genetically modified embryos and create mutant rodents. In one of those labs, I watched biologist Andrew Brown work the magic of CRISPR. Swaddled in surgical gloves and blue paper clothing—robe, overshoes and puffy bonnet—he hunched over a dissecting microscope, sucking at the end of a glass pipette to bring up a rat embryo. He then trundled the embryo across the room to a bigger microscope, flanked by robotic arms, released it into a drop of liquid on a slide and settled onto a stool. With his right hand, he commanded a joystick that moved a hollow glass needle into place against the wall of the embryo.

Through the eyepiece of the microscope, the embryo's two pronuclei, one from each rat parent, looked like little craters on the surface of the moon; Brown nudged the cell until a pronucleus spun close to the tip of the needle. He clicked the button of a computer mouse, and the needle squirted a tiny drop of liquid containing CRISPR through the plasma membrane of the cell. The pronucleus swelled like a flower blooming in fast motion. With luck, Brown had created a

mutant cell. SAGE's three technicians repeat the task as many as 300 times a day, four days a week.

When Brown finished injecting his rat embryo, he sucked it into a pipette, deposited it in a petri dish and stored it in a cupboard heated to body temperature. He would eventually inject the modified embryo—and some 30 to 40 others—into a surrogate rat mother. Twenty days later the rat would bear five to 20 pups, and when the pups were 10 days old, SAGE scientists would take tissue samples to see which ones had the modified gene.

“That's the exciting part,” Brown said. “It might be just one of 20 that have the modification. That's what we call our founder animal. When we get to that point, everybody celebrates.” Watching the SAGE scientists making RNA or injecting embryos, it all looked easy—and the same processes are turning out genetically engineered animals at many labs. It is, as SAGE CEO David Smoller put it, gene editing “for the masses.”

Promise and Maybe a Little Peril

As CRISPR charges ahead into commercial use, researchers and entrepreneurs keep imagining new applications for the technology, and some can come across as hubristic. It might be possible to tweak the chromosomal abnormality associated with Down syndrome early in a pregnancy, for example, or to reintroduce susceptibility to herbicides in resistant weeds, or to bring back animal species that have gone extinct. Not surprisingly, some people find it scary. Startled commentators have warned that in our rush to rid the world of malarial mosquitoes, cure Huntington's disease or design better babies, we could create a *Jurassic Park*–ful of harmful new genes.

Consider the idea of using CRISPR to eliminate malarial mosquitoes. It is one thing to vanquish the malarial parasite but quite another to annihilate its vector, says Todd Kuiken, a biosecurity analyst at the Woodrow Wilson International Center for Scholars in Washington, D.C. If the goal is eradicating malaria—which infects 200 million people a year and kills 600,000—Kuiken says that we have to be careful not to cause 10 other problems. “We've got to have an opportunity to ask, ‘Do we really want to do this?’ And if the answer is ‘yes,’ what kinds of systems do we have in place, what kinds of safeguards?”

To their credit, scientists are moving quickly to envision the most realistic dangers of CRISPR technology and to develop responses. In July, when a Harvard team published a paper on CRISPR-powered mosquito elimination, the scientists called for a public discussion and began to suggest technological and regulatory fixes for altered genes gone wild. “CRISPR is happening so incredibly fast,” observes Jeantine Lunshof, a bioethicist on the team. “Many people have not heard of it, but people are using it. That is a new dynamic.” Within Berkeley's Innovative Genomics Initiative, Doudna has been assembling a group designed specifically to discuss the ethical implications of CRISPR applications.

It is hard to imagine ethics concerns smothering the excitement over CRISPR. In June, for example, researchers at M.I.T. reported curing adult mice of tyrosinemia—a rare liver disorder caused by a mutation in an enzyme—by injecting CRISPR directly through their tails. Delivering three RNA guide strands, along with Cas9 and the correct DNA sequence for the mutated gene, they managed to insert the correct gene in about one of every 250 cells in the livers of mice. During the following month, the healthy liver cells thrived, eventually replacing a third of the bad cells, enough to rid the mice of the disease. And in August virologist Kamel Khalili of Temple University and his colleagues reported having used CRISPR to slice the HIV virus, which causes AIDS, out of several human cell lines.

For Khalili, who has labored in the trenches of HIV/AIDS since the dark days of the 1980s, CRISPR is nothing short of revolutionary. Despite huge strides in AIDS treatment, today's medications only control the virus—they do not eradicate it. But by using CRISPR, Khalili's team completely excised the integrated copy of HIV, converting infected cells to uninfected cells. Besides eliminating the virus from an infected cell, CRISPR can also protect an uninfected cell, Khalili says, immunizing it by incorporating a sequence from the attacking virus, just as Doudna and her team observed primitive bacteria doing. You could call it a genetic vaccine. “If you'd asked me two years ago, ‘Can you precisely excise the HIV from a human cell?’ I would have said that's a tall order. Now we've done it,” Khalili says. “That is the ultimate cure.”

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DNA Editing of Human Embryos Alarms Scientists

A call by scientists to halt precision gene editing of DNA in human embryos would allow time to work out safety and ethical issues

nature

By David Cyranoski and Nature magazine | March 13, 2015
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Sperm cell fertilizing an egg.
Credit: Wikimedia Commons

Amid rumors that precision gene-editing techniques have been used to modify the DNA of human embryos, researchers have called for a moratorium on the use of the technology in reproductive cells.

In a Comment published on March 12 in *Nature*, Edward Lanphier, chairman of the Alliance for Regenerative Medicine in Washington DC, and four co-authors call on scientists to agree not to modify human embryos — even for research.

“Such research could be exploited for non-therapeutic modifications. We are concerned that a public outcry about such an ethical breach could hinder a promising area of therapeutic development,” write Lanphier and his colleagues, who include Fyodor Urnov, a pioneer in gene-editing techniques and scientist at Sangamo BioSciences in Richmond, California. Many groups, including Urnov's company, are already using gene-editing tools to develop therapies that correct genetic defects in people (such as by editing white blood cells). They fear that attempts to produce ‘designer babies’ by applying the methods to embryos will create a backlash against all use of the technology.

Known as germline modification, edits to embryos, eggs or sperm are of particular concern because a person created using such cells would have had their genetic make-up changed without consent, and would permanently pass down that change to future generations.

“We need a halt on anything that approaches germline editing in human embryos,” Lanphier, who is also chief executive of Sangamo, told *Nature*’s news team.

But other scientists disagree with that stance. Although there needs to be a wide discussion of the safety and ethics of editing embryos and reproductive cells, they say, the potential to eliminate inherited diseases means that scientists should pursue research.

Related trials

Geneticist Xingxu Huang of ShanghaiTech University in China, for example, is currently seeking permission from his institution’s ethics committee to try genetically modifying discarded human embryos. In February 2014, he reported using a gene-editing technique to modify embryos that developed into live monkeys. Human embryos would not be allowed to develop to full term in his experiments, but the technique “gives lots of potential for its application in humans,” he says.

Besides Huang’s work, gene-editing techniques are also being used by Juan Carlos Izpisua Belmonte, a developmental biologist at the Salk Institute for Biological Studies in La Jolla, California, to eliminate disease-causing mutations from mitochondria, the cell's energy-processing structures. Belmonte's work is on

unfertilized eggs; human eggs with such modified mitochondria could one day be used in *in vitro* fertilization (IVF) procedures to prevent a woman's offspring from inheriting mitochondrial disease.

There are also suspicions that scientists have already created human embryos with edited genomes. Several researchers who do not want to be named told *Nature*'s news team that papers describing such work are being considered for publication.

Scientists who attended a meeting in Napa, California, in January to discuss potential uses of germline gene-editing have written a perspective paper about their concerns for publication in *Science*. Geneticist Dana Carroll of the University of Utah in Salt Lake City, who was at the Napa meeting, says that it will call for discussions of the safety and ethics of using editing techniques on human embryos.

"Germline genome alterations are permanent and heritable, so very, very careful consideration needs to be taken in advance of such applications," Carroll says.

Wide concerns

Germline gene editing is already banned by law in many countries — a 2014 review by Tetsuya Ishii, a bioethicist at Hokkaido University in Sapporo, Japan, found that of 39 countries, 29 have laws or guidelines that ban the practice. But the development of precise gene-editing techniques in recent years has brought fresh urgency to the issue. These techniques use enzymes called nucleases to snip DNA at specific points and then delete or rewrite the genetic information at those locations. The methods are simple enough to be used in a fertility clinic, raising fears that they might be applied in humans before safety concerns have been addressed.

One concern, for example, is that the nucleases could cause mutations at locations other than those targeted. Guanghui Liu, a stem-cell researcher at the Chinese Academy of Sciences Institute of Biophysics in Beijing, collaborated on a study that showed that modifying one gene in stem cells resulted in minimal mutations elsewhere, but he warns that this is only one case.

Every application to use gene-editing technology for a therapy would have to be validated independently as safe and effective, says Jennifer Doudna, a biochemist at the University of California, Berkeley. "It would be necessary to decide, for

each potential application, whether the risks outweigh the possible benefit to a patient. I think this assessment must be made on a case-by-case basis," she says.

Ishii worries about countries such as the United States: there, germline editing is not banned but requires government approval, but such restrictions have a history of being circumvented, as in the case of unproven stem-cell treatments. He is also concerned about China, which prohibits gene-editing of embryos but does not strictly enforce similar rules, as shown by failed attempts to curb the use of ultrasound for sex selection and to stamp out unauthorized stem-cell clinics. China is also where gene-editing techniques in primates have developed fastest. "There are already a lot of dodgy fertility clinics around the world," he says.

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