

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.

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

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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 ex-

remely 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 retro-

virus, 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.

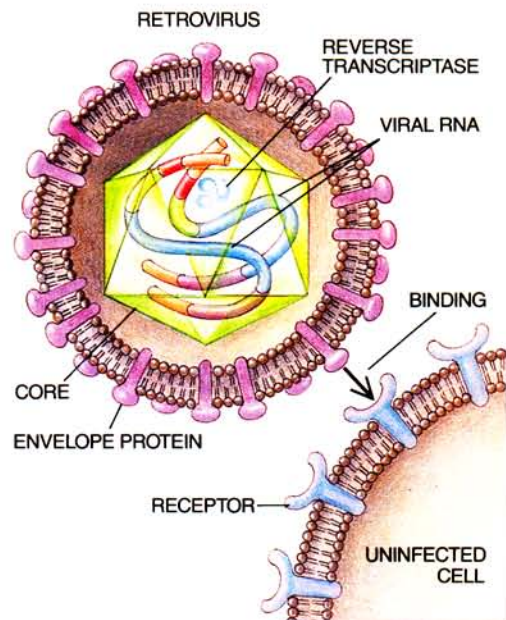
Red blood cells of patients stricken with beta thalassemia are deficient in beta globin, which in healthy individuals combines with alpha globin and iron (heme) to yield hemoglobin. Healthy cells regulate the activity of both genes precisely, ensuring that equal amounts of alpha and beta globin are made. 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 co-workers 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.

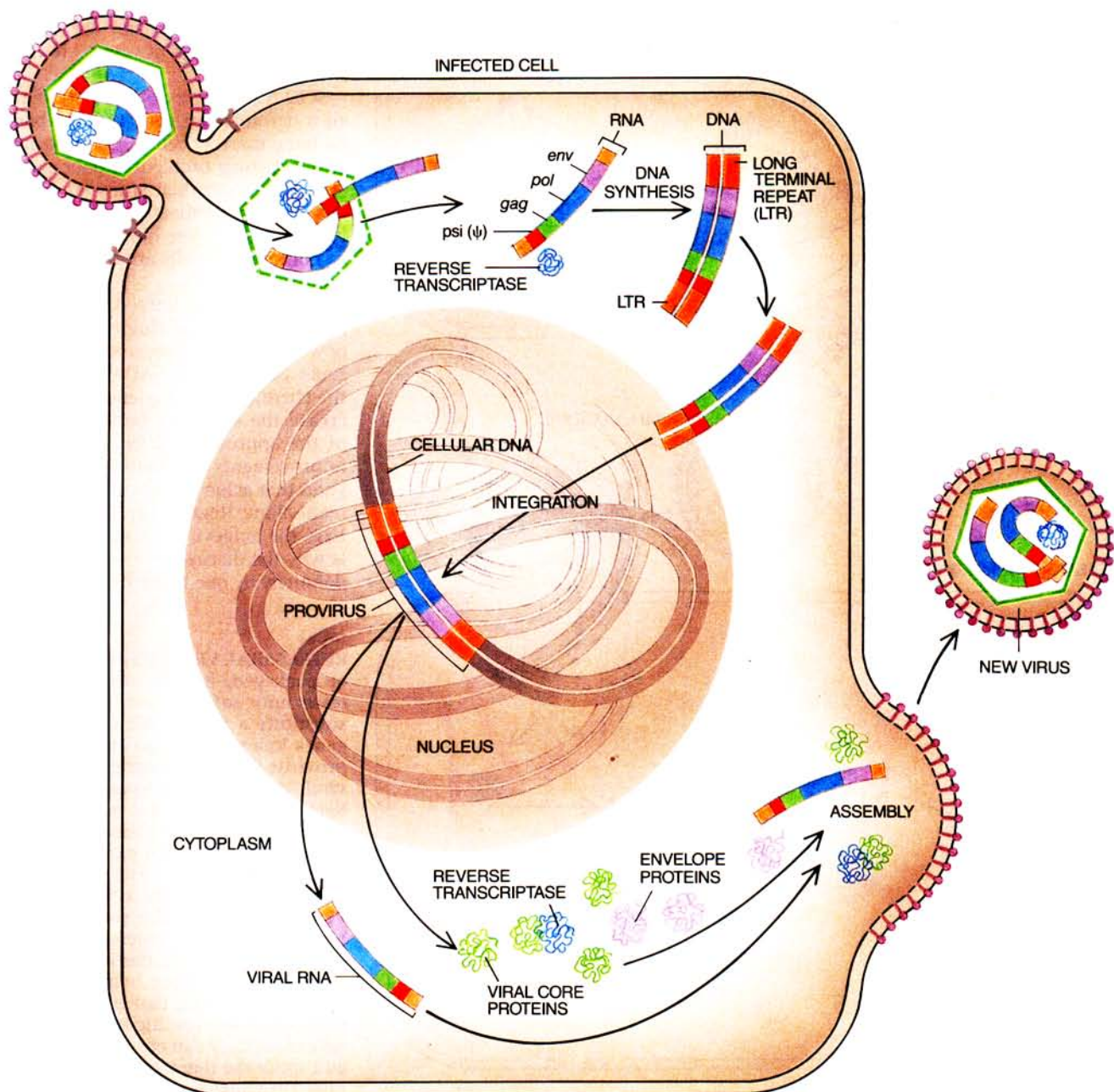
On the other hand, no one has been able to achieve significant levels of globin synthesis in recipient animals. This problem has been a major disappointment, but a discovery by F. G. Grosveld and his colleagues at the National Institute for Medical Research in London offers hope for a solution.

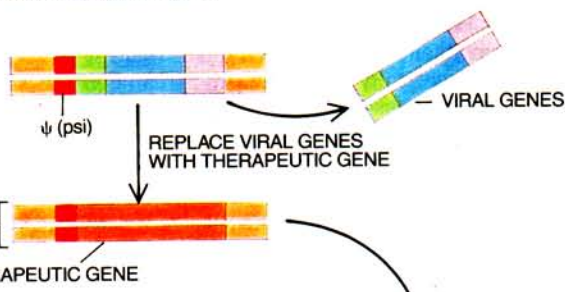
They identified distinct stretches of DNA, thousands of nucleotides apart from the gene itself, that in normal red blood cells dramatically boost the production of globin messenger RNA. Messenger RNA is transcribed, or copied, from DNA and is the template from which protein is made; hence, high levels 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*

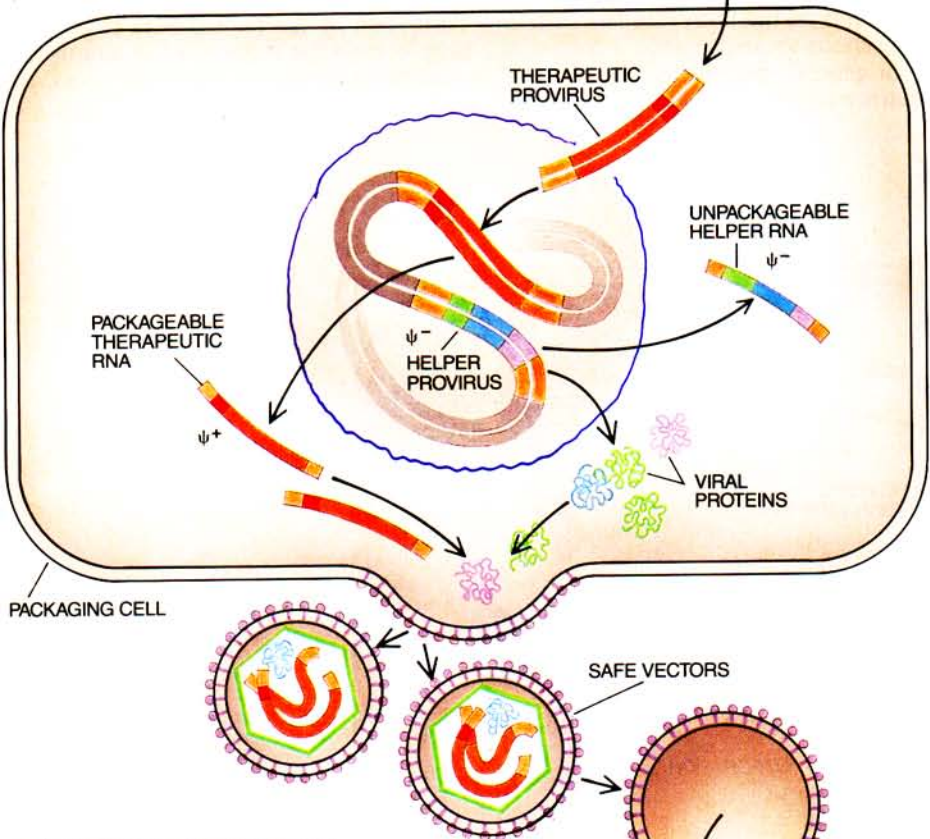
expression of other genes as well. The problem must be resolved before gene therapy based on bone marrow cells can become a reality.

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

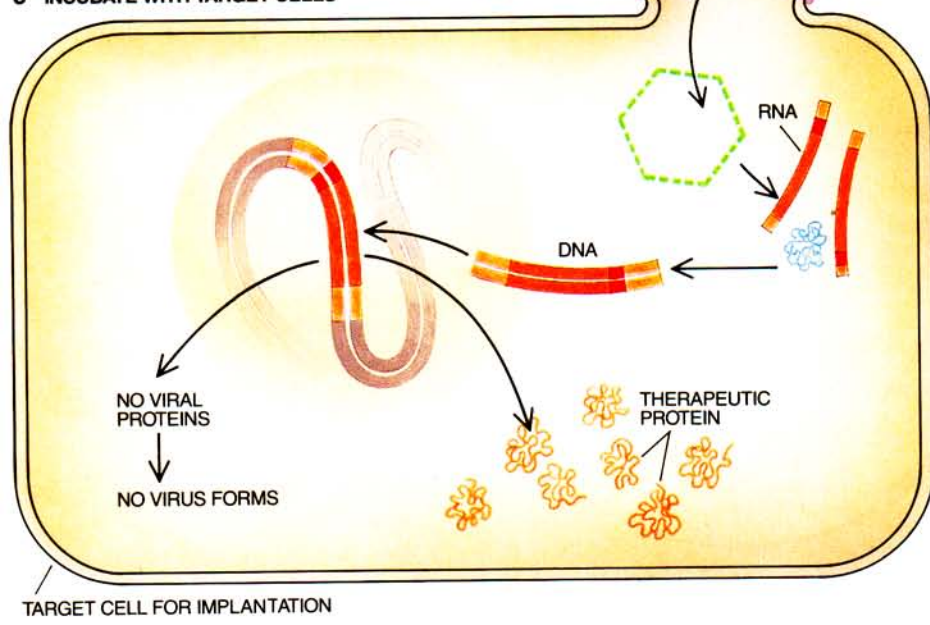




b INSERT INTO PACKAGING CELL



c INCUBATE WITH TARGET CELLS



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.

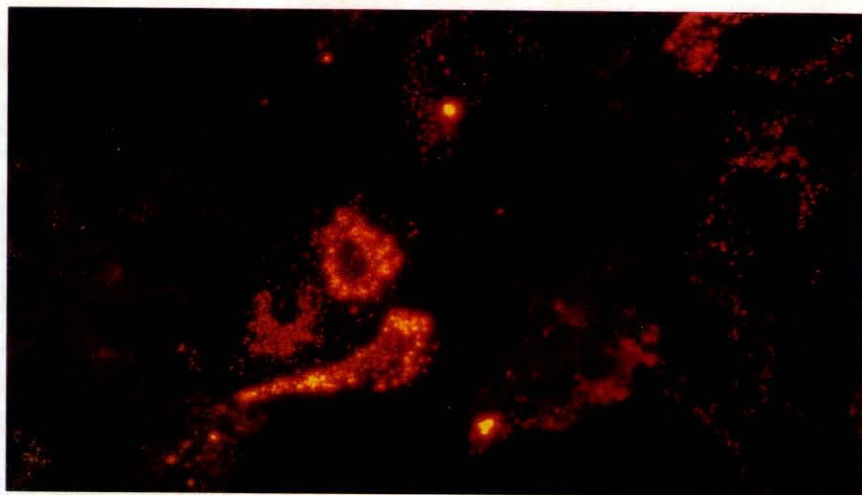
Genetic alteration of lymphocytes or bone marrow cells aims to correct defects in those same cells or their progeny. Skin cells, in contrast, are being studied for quite a different purpose: the synthesis and secretion of proteins that are normally made in one cell type but are ferried in blood plasma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



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.

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 hyper-

cholesterolemia, 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.

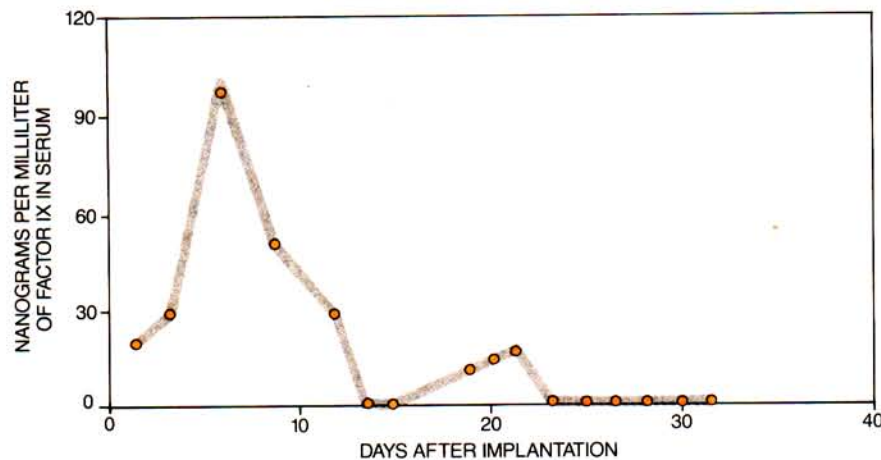
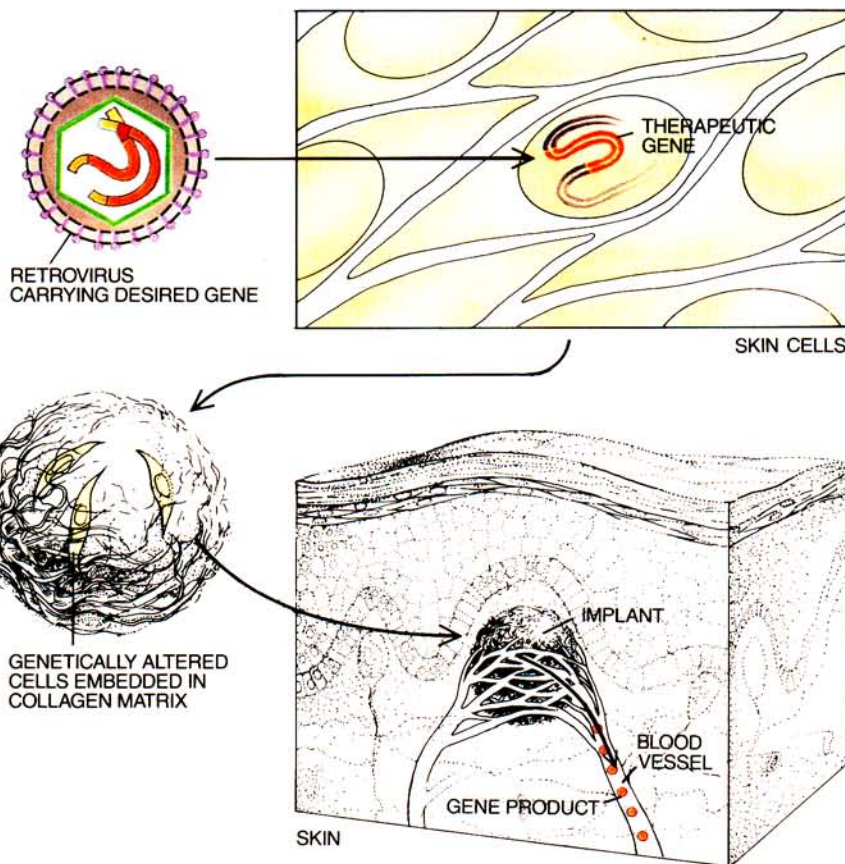
Although bone marrow, skin and liver cells are receiving the most attention, other types are also being considered. For instance, retroviruses can carry genes for secretory products into endothelial cells, which line the arteries. These cells have more intimate contact with the blood than do fibroblasts, 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-TRANS-DUCED STEM CELLS. Elaine A. Dzierzak, Thalia Papayannopoulou and Richard C. Mulligan in *Nature*, Vol. 331, No. 6151, pages 35-41; January 7, 1988.
- DISRUPTION OF THE PROTO-ONCOGENE *INT-2* IN MOUSE EMBRYO-DERIVED STEM CELLS: A GENERAL STRATEGY FOR TARGETING MUTATIONS TO NON-SELECTABLE GENES. Suzanne L. Mansour, Kirk R. Thomas and Mario R. Capecchi in *Nature*, Vol. 336, No. 6197, pages 348-352; November 24, 1988.
- AN ALTERNATIVE APPROACH TO SOMATIC CELL GENE THERAPY. D. St. Louis and I. M. Verma in *Proceedings of the National Academy of Sciences*, Vol. 85, pages 3150-3153; 1988.
- HUMAN GENE THERAPY. Eve K. Nichols and the Institute of Medicine, National Academy of Sciences, Staff. Harvard University Press, 1988.
- PROGRESS TOWARD HUMAN GENE THERAPY. Theodore Friedmann in *Science*, Vol. 244, No. 4910, pages 1275-1281; June 16, 1989.

What Cloning Means



The recently debuted technology for cloning is usually discussed as a means of creating genetic copies of whole adult individuals. This is far from its only use, however. Cloning could be combined with other biotechnologies, either to achieve more novel goals or to improve on previous methods. Although the technique is still in its infancy, and needs to be studied and developed much further, educated musings about cloning's ability to inform gene therapy are already being brought to the table. An area that might particularly

benefit is germ-line gene therapy—genetic modifications that could correct a problem for future generations. “I think cloning is going to be used as a tool that will make gene therapy work,” comments Lee Silver, a molecular biologist at Princeton University and an expert on reproductive technologies. “For the first time, germ-line gene therapy becomes realistic.”

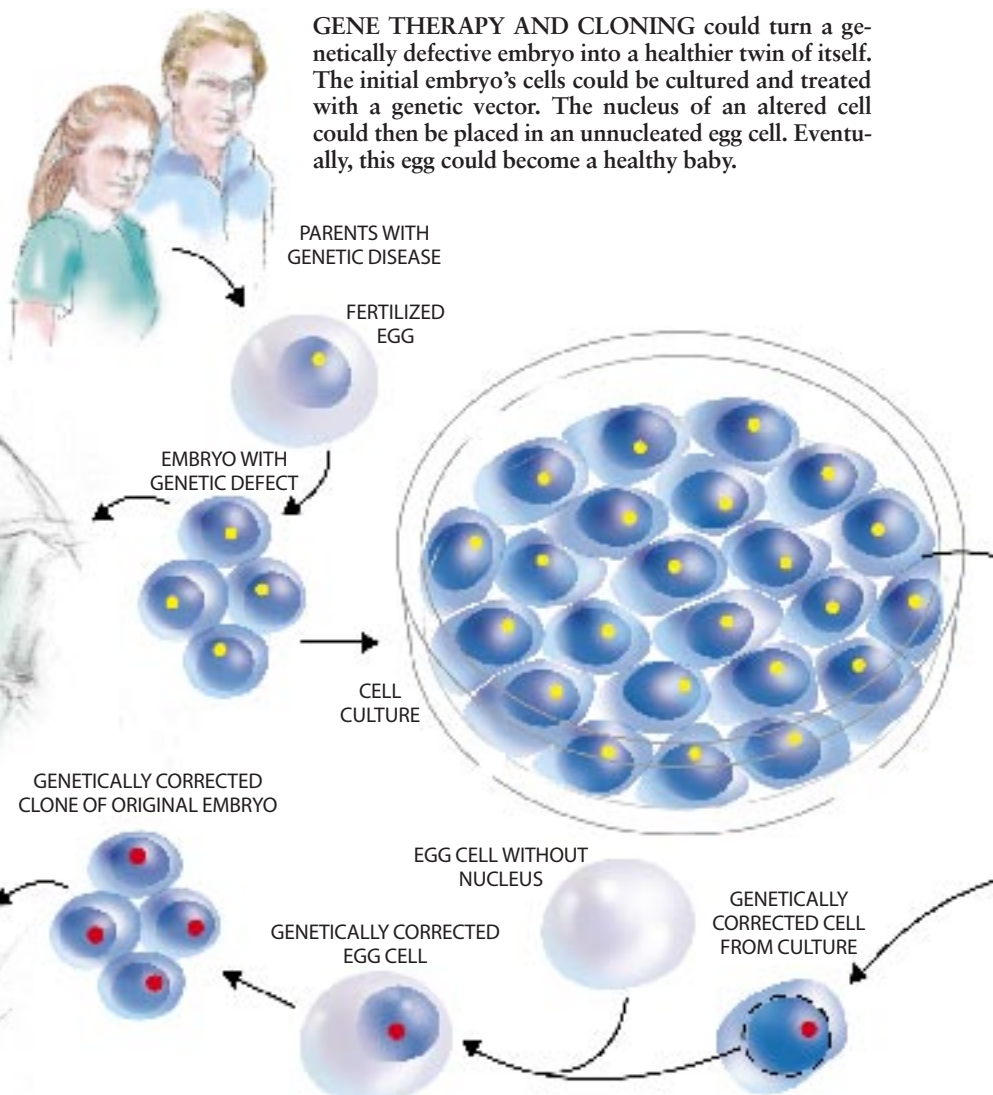
Germ-line therapy, which is not yet being studied in humans, could ideally prevent deadly or debilitating disorders such as sickle cell anemia or cystic fibrosis. Such diseases are typically transmitted silently from generation to generation by people carrying one copy of a defective gene; the disease be-

comes manifest when two carriers have a child who inherits two copies.

Today prenatal genetic testing can reveal whether a fetus or embryo is affected with many of these conditions. The parents then have the option of aborting and rolling the genetic dice again with another pregnancy. In some cases, however, the dice are guaranteed to come up snake eyes. “If both parents are sickle cell diseased,” Silver says, “then all of their embryos will also carry the disease. You can’t select, because there are no good embryos.” But gene therapy, aided and abetted by cloning, could theoretically correct the condition for their children, and all subsequent progeny as well.

The recipe would begin with a fertilized egg growing, in the laboratory, into

GENE THERAPY AND CLONING could turn a genetically defective embryo into a healthier twin of itself. The initial embryo's cells could be cultured and treated with a genetic vector. The nucleus of an altered cell could then be placed in an unnucleated egg cell. Eventually, this egg could become a healthy baby.



for Gene Therapy

Further Readings on Gene Therapy

OBSTACLES TO THERAPY

GENE THERAPY FOR HUMAN GENETIC DISEASE. Theodore Friedmann and Richard Roblin in *Science*, Vol. 175, pages 949–955; March 3, 1972.

HUMAN SOMATIC GENE THERAPY: PROGRESS AND PROBLEMS. M. K. Brenner in *Journal of Internal Medicine*, Vol. 237, No. 3, pages 229–239; March 1995.

RECOMBINANT DNA RESEARCH. Agency: National Institutes of Health. *Federal Register*, Vol. 61, No. 131, pages 35774–35777; Monday, July 8, 1996.

NONVIRAL GENE DELIVERY

DIRECT GENE TRANSFER INTO MOUSE MUSCLE IN VIVO. Jon A. Wolff, Robert W. Malone, Phillip Williams, Wang Chong, Gyula Acsadi, Agnes Jani and Philip L. Felgner in *Science*, Vol. 247, pages 1465–1468; March 23, 1990.

DIRECT GENE TRANSFER FOR IMMUNOTHERAPY. G. J. Nabel and P. L. Felgner in *Trends in Biotechnology*, Vol. 11, No. 5, pages 211–215; May 1993.

DNA VACCINES. J. J. Donnelly, J. B. Ulmer, J. W. Shiver and M. A. Liu in *Annual Review of Immunology*, Vol. 15, pages 617–648; 1997.

LIPIDIC VECTOR SYSTEMS FOR GENE TRANSFER. R. J. Lee and L. Huang in *Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 14, No. 2, pages 173–206; 1997.

CANCER AND AIDS

GENE TRANSFER AS CANCER THERAPY. Glenn Dranoff and Richard C. Mulligan in *Advances in Immunology*, Vol. 58, pages 417–454; 1995.

STEPS TOWARD GENE THERAPY, 2: CANCER AND AIDS. R. M. Blaese in *Hospital Practice*, Vol. 30, No. 12, pages 37–45; December 15, 1995.

GENE THERAPY STRATEGIES FOR NOVEL CANCER THERAPEUTICS. Maryland E. Rosenfeld and David T. Curiel in *Current Opinion in Oncology*, Vol. 8, No. 1, pages 72–77; January 1996.

NERVOUS SYSTEM DISORDERS

LONG-TERM BEHAVIORAL RECOVERY IN PARKINSONIAN RATS BY AN HSV VECTOR EXPRESSING TYROSINE HYDROXYLASE. M. J. During et al. in *Science*, Vol. 266, pages 1399–1403; November 25, 1994.

USE OF HERPES SIMPLEX VIRUS VECTORS FOR PROTECTION FROM NECROTIC NEURON DEATH. D. Y. Ho et al. in *Viral Vectors: Gene Therapy and Neuroscience Applications*. Edited by M. Kaplitt and A. Loewy. Academic Press, 1995.

GENE TRANSFER TO NEURONS USING HERPES SIMPLEX VIRUS-BASED VECTORS. D. J. Fink, N. A. DeLuca, W. F. Goins and J. C. Glorioso in *Annual Review of Neuroscience*, Vol. 19, pages 245–287; 1996.

a mass of early embryonic tissue. A functioning gene—say, for the blood's oxygen-carrying protein, beta globin, which is mutated in sickle cell anemia—would then be inserted into the embryonic cells by tailored viruses or other vectors. (A marker sequence inserted along with the gene might help identify which cells took up the gene correctly.) The DNA of one of those cells could then be implanted into a new egg cell from the mother, beginning the pregnancy afresh. In effect, this last step replaces the original embryo with a healthier clone of itself.

Germ-line therapy does not require a cloning step, but cloning might make it far easier. Very early stage embryonic cells, if separated, retain the ability to regenerate into whole embryos (indeed, that is how identical twins, triplets and quadruplets arise). Gene therapists could therefore alter the DNA of the embryonic cells and return one to the mother for gestation. The problem is that embryonic cells lose their “pluripotent” capacity after a few cell divisions, so the gene therapists would be forced to work on relatively few cells. The inefficiency of current gene manipulation techniques would consequently undermine many

therapeutic attempts. With cloning, however, the age and number of cells eligible for manipulation is unlimited.

In theory, cloning would allow therapy on cells from a more advanced pregnancy (although this would raise more troubling ethical issues for many parents). In a variation on this theme, the gene therapy might also be conducted on cells from one of the parents. A child cloned from those altered cells would be free of the genetic defect but in other ways a genetic duplicate of its donor parent.

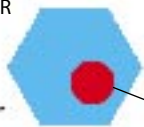
Cloning may remove some of the practical barriers to germ-line gene therapy, but it does not alter the ethical ones. Many researchers, not to mention the general public, are deeply concerned that germ-line techniques could be misapplied toward eugenic goals with authoritarian or even genocidal overtones. So even if cloning does enable the technology, there may not be a sudden rush to perform germ-line gene therapy.

Cloning may also benefit somatic gene therapy as a tool for basic research. By making it easy to obtain large numbers of genetically identical cells for study, cloning should help elucidate how embryonic cells commit to become a particular cell type. “That process of commitment involves shutting off genes that would otherwise have played a role in becoming a liver or a brain,” reflects Jon Gordon, professor of obstetrics, gynecology and reproductive science at the Mount Sinai School of Medicine in New York City. “I think the fact that we can now reverse that gives us hope that we can understand that process better and understand diseases that are based on or manifest as errors in this process, like cancer.” Cloning might therefore help therapists determine which genes they should be aiming to correct in various illnesses. If so, cloning's greatest utility may not be for making more people but for making more people healthy.

—Steve Mirsky and John Rennie

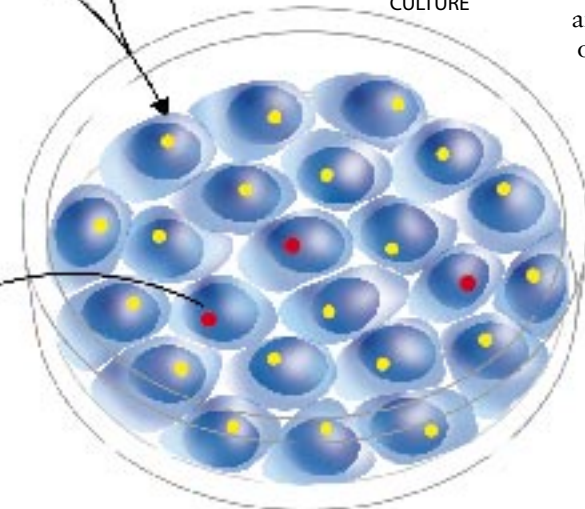
STEVE MIRSKY is *Reuter Fellow in Medical Journalism at Columbia University*. **JOHN RENNIE** is editor in chief of *SCIENTIFIC AMERICAN*.

GENE THERAPY
VECTOR



THERAPEUTIC
GENE

TREATED
CULTURE



Hitting the Genetic OFF Switch

A host of start-ups is speeding development of a new class of drugs that block the action of RNA **By Gary Stix**

In 1996 *Worth* magazine proclaimed that Isis Pharmaceuticals could become the next Microsoft, a prediction that turned out to be a particularly egregious example of hyperbole run amok. To be sure, Isis remains a leader in the gene-blocking technology called antisense. But the road to successful treatments for cancer and other diseases has been littered with disappointments.

During the past few years, a new gene-silencing technology has emerged that may be poised to fulfill the promise that was once trumpeted for antisense. "I've been writing in grants for 25 years that during the next five years I'm going to test this process or that process to see if I can do gene inactivation studies in mammalian cells in culture. And I did them, and they were so awkward and so complicated that you just couldn't apply them generally," says Phillip A. Sharp, director of the McGovern Institute for Brain Research at the Massachusetts Institute of Technology. "Lo and behold, all of the time right there in front of me was a process that I could have used."

Sharp, a co-winner of the 1993 Nobel Prize in Physiology or Medicine, was referring to a series of relatively recent dis-

coveries that cells have a mechanism, dubbed RNA interference (RNAi), which blocks gene expression. It prevents RNA transcripts of genes from giving rise to the proteins those genes encode. This natural method of gene silencing comes into play, for example, when viruses try to commandeer a cell's protein-making machinery to produce viral proteins.

A milestone arrived in 1998, when Andrew Z. Fire, now at the Stanford University School of Medicine, and Craig C. Mello of the University of Massachusetts Medical School identified in worms double-stranded RNAs that acted as the switch to turn off genes in RNAi. And in 2001 Thomas Tuschl, now at the Rockefeller University, found that an abbreviated version of double-stranded RNAs—short interfering RNAs (siRNAs)—could shut off genes in mammalian cells. The number of research papers on RNAi has mushroomed from a dozen-plus in 1998 to multiple hundreds last year. Even if the promise for therapeutics never materializes, it is quite likely that some of the seminal discoveries will garner Nobel Prizes. "This has touched everything we do in biological science, from plants to man," Sharp notes. [See "Censors of the Genome," by Nelson C. Lau and David P. Bartel; *SCIENTIFIC AMERICAN*, August 2003.]

The excitement about siRNAs as drugs relates to how they differ in critical ways from antisense therapeutics. At first glance, siRNAs seem very similar to antisense. An antisense drug consists of an artificially synthesized chain of nucleotides, or genetic building blocks, that binds to a messenger RNA containing a complementary sequence. This binding blocks gene expression. An siRNA also silences genes—and it even uses a complementary RNA, or antisense, strand to do so. Once inside a cell, an siRNA attaches to an aggregate of proteins called an RNA-induced silencing complex (RISC), which retains only the antisense strand. The siRNA-bearing RISC then binds to the targeted messenger RNA and degrades it or prevents it from functioning [*see box on page 100*].

Unlike the antisense drugs that have been under development for the past 15 years, siRNAs do not disrupt only a single messenger RNA. They act as catalysts, doing the same job over and over, one explanation for their apparent potency. "They are 100- to 1,000-fold more effective than antisense," says Judy Lieberman, a senior investigator at the CBR Institute for Biomedical Research in Boston and one of the first researchers to show the therapeutic potential of the technique in animals.

Already almost 100 companies are

involved in RNAi; nearly half supply the chemicals and technology needed to perform experiments, and the others are biotechnology or pharmaceutical companies doing commercial research with RNAi, according to Kewal K. Jain, chief executive officer of Jain PharmaBiotech, a Basel, Switzerland, market research firm. “All of this has happened within the last two or three years,” Jain says.

A small fraction of these companies have dedicated themselves to producing therapies using siRNAs. As soon as Tuschl’s paper documenting siRNAs in mammalian cells was published, the venture-capital community sprang into action. “It was worth it to make a bet realizing in vivo efficacy was not guaranteed,” says Christoph H. Westphal, one of the

Paul R. Schimmel, a professor of molecular biology and chemistry at the Scripps Research Institute, and the founder of several biotechnology companies before this one, insisted on the name Alnylam, an Arabic word meaning “string of pearls” that is also the designation for the middle star of Orion’s belt. Schimmel made the case, over the protests of others, that the name—pronounced “al-NIGH-lam”—was difficult to pronounce but impossible to forget. Barry Greene, the company’s chief operating officer, furnishes a simpler explanation: “The URL was open,” he joked at a recent investors’ conference.

The founders constituted an all-star scientific advisory team, and some also filled slots on the board of directors. But

scientific smarts, would determine who would thrive or falter as drug development and clinical trials got under way. “We were very focused and running very hard,” he remembers. “We recognized that if we weren’t first, others would grab it from us.”

The fledgling Alnylam even bought the German firm Ribopharma to get a hold of a key patent. The stir created by RNAi—tagged by *Science* magazine as “breakthrough of the year” in 2002—helped to bring in venture money. The total take thus far has reached about \$85 million, including a somewhat disappointing initial public offering in the spring, and provides enough to keep Alnylam going for another two years, until the first drug makes it through the preliminary safety phase of clinical trials.

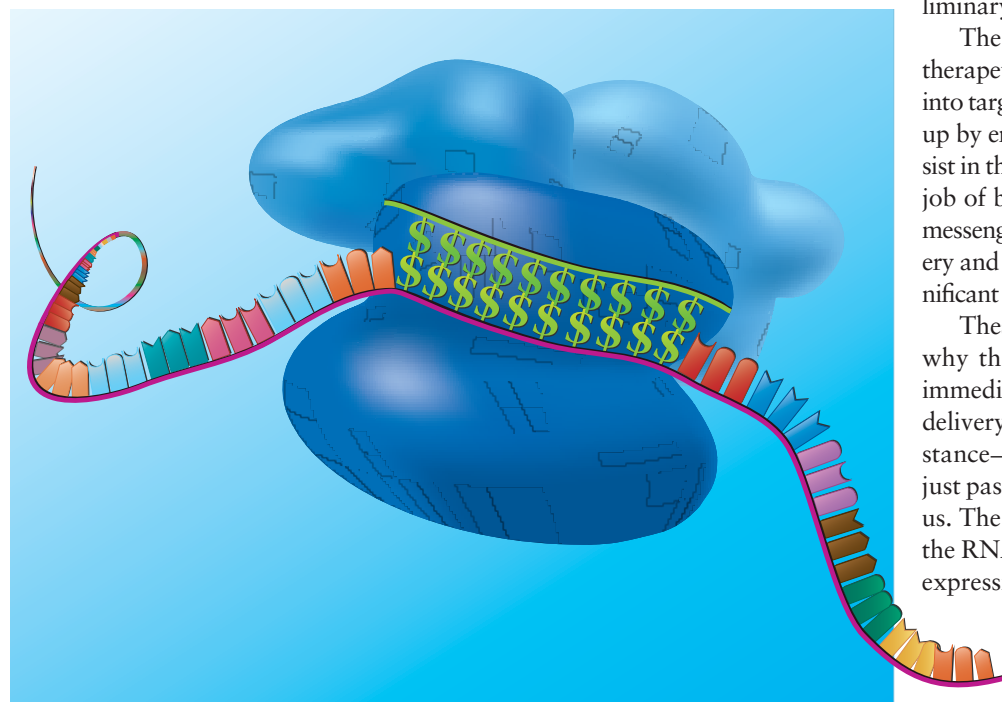
The success or failure of RNAi as a therapeutic will hinge on getting the drug into target cells without its being chopped up by enzymes. The drug must then persist in the cell long enough to carry out its job of binding to and inhibiting specific messenger RNAs. The challenge of delivery and stabilization has also posed a significant hurdle for the success of antisense.

These difficulties serve as one reason why the newly formed Alnylam team immediately discarded one approach to delivery: using a vector—a virus, for instance—to ferry a stretch of DNA, not just past the cell wall, but into the nucleus. The gene would then go on to make the RNA that would interfere with gene expression. “In my mind, nothing about

RNAi solves the problem of gene therapy,” Maraganore notes, referring to the downsides of using viruses to deliver

the drug to the right location and the unwanted side effects that they sometimes provoke. Consequently, short interfering RNAs are synthesized in the laboratory from a soup of nucleotides until they form double-stranded molecules that have 21 nucleotide pairs. Some other companies, such as Benitec in Australia, are still pursuing a gene therapy approach [see table on page 101].

Key expertise and intellectual property to accomplish this task came from an unlikely source. Alnylam had hired away



BINDING A MESSENGER RNA (long strand) to complementary RNA and an aggregate of proteins (blob) could potentially become a lucrative new approach to drug development.

founders of Alnylam in Cambridge, Mass., and a general partner with Polaris Venture Partners. Many of the early innovators in RNAi technology, including Tuschl, Sharp and David P. Bartel of M.I.T., among others, got together to form Alnylam Pharmaceuticals in 2002. Sharp, a founder of biotech giant Biogen, brought together this banner group after conversations with more established companies failed to generate sufficient interest.

when John M. Maraganore, the company’s first permanent chief executive, a transplant from Millennium Pharmaceuticals, hired the people who actually would be entrusted with the task of making siRNA drugs, he did not at first seek out postdoctoral students of these research heavyweights. “Five people were focused on intellectual property and one on science,” Maraganore says. A bulletproof patent estate, as much as

from Isis an executive, Muthiah Manoharan, to become vice president of drug discovery. Maraganore called Isis president Stanley T. Crooke last summer and reassured him that Alnylam still wished to be on good terms with the antisense manufacturer. A few months later a dialogue between the two companies resulted in an agreement in which Alnylam would pay \$5 million to license Isis's ex-

tensive patent portfolio of chemical techniques for delivering and stabilizing RNA. "We will be able to take advantage of the 10-plus years of development of chemistry used in antisense," Maraganore says. In turn, Isis invested \$10 million in Alnylam, giving it a 5 percent equity stake in the company and a stream of royalties and fees once siRNA products hit the marketplace. It will also get

the rights to make some siRNA drugs. The development trajectory for siRNA recapitulates the path that antisense has taken. The only antisense drug approved to date is Isis's Vitravene, intended to treat an eye disease once prevalent in AIDS patients. The drug is injected directly into the eye, concentrating the compound at its target while impeding it from producing adverse side effects in

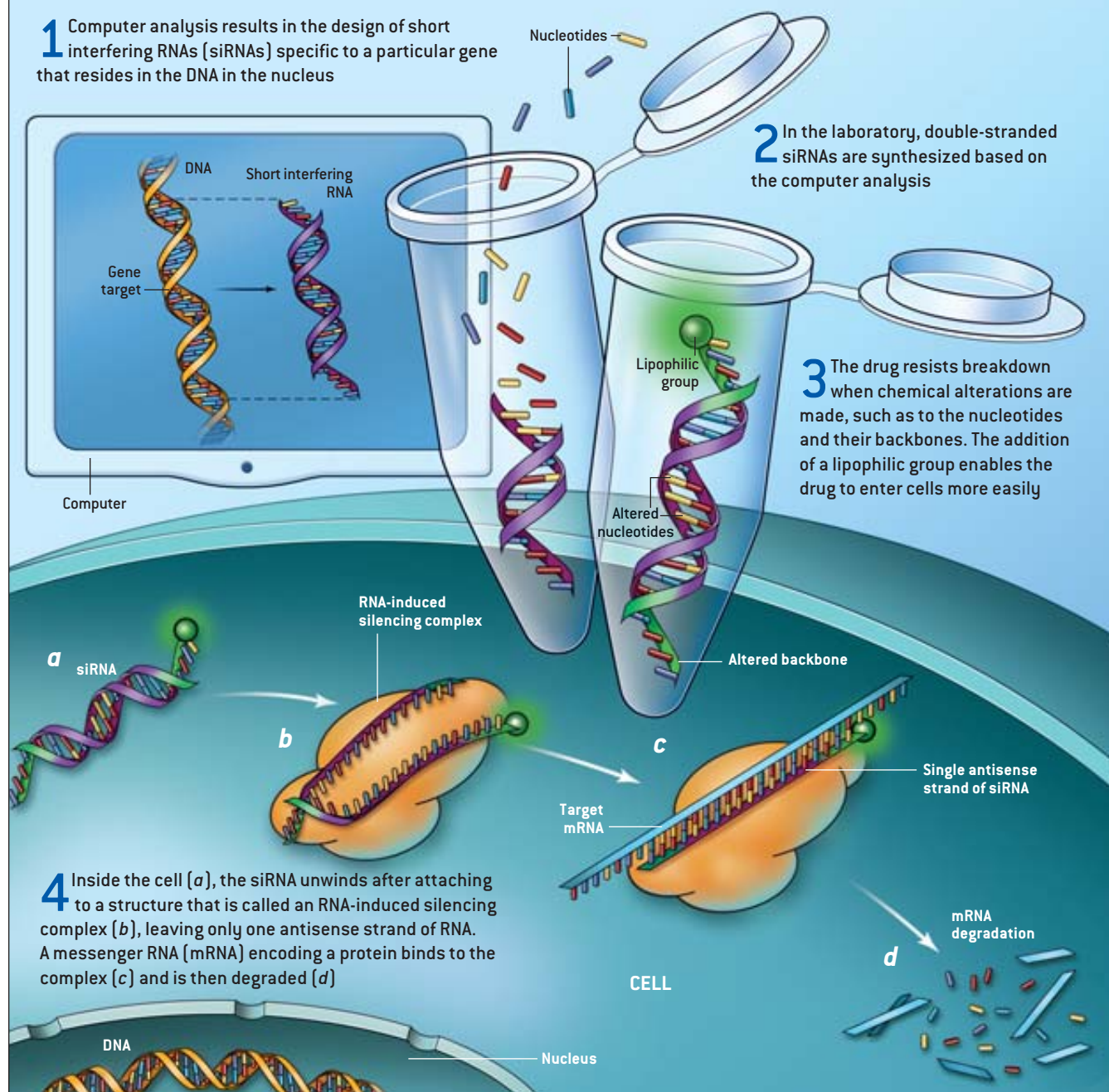
MAKING A GENETIC SILENCER

1 Computer analysis results in the design of short interfering RNAs (siRNAs) specific to a particular gene that resides in the DNA in the nucleus

2 In the laboratory, double-stranded siRNAs are synthesized based on the computer analysis

3 The drug resists breakdown when chemical alterations are made, such as to the nucleotides and their backbones. The addition of a lipophilic group enables the drug to enter cells more easily

4 Inside the cell (a), the siRNA unwinds after attaching to a structure that is called an RNA-induced silencing complex (b), leaving only one antisense strand of RNA. A messenger RNA (mRNA) encoding a protein binds to the complex (c) and is then degraded (d)



BIOTECHS DEVELOPING DRUGS BASED ON RNAi

COMPANY	THERAPIES
Acuity Pharmaceuticals Philadelphia	Age-related macular degeneration and diabetic retinopathy
Alnylam Pharmaceuticals Cambridge, Mass.	Age-related macular degeneration, Parkinson's disease and, over the longer term, cancer, metabolic and autoimmune diseases
atugen Berlin	Cancers and metabolic diseases, for systemic applications, and ocular and skin diseases, for topical delivery
Benitec Queensland, Australia	Hepatitis C and, over the longer term, cancer, autoimmune and viral diseases using a gene therapy approach
CytRx Los Angeles	Amyotrophic lateral sclerosis, cytomegalovirus, obesity and type 2 diabetes
Intradigm Rockville, Md.	Cancer
Nucleonics Horsham, Pa.	Hepatitis B and C and, over the long run, cancer, inflammatory and viral diseases using a gene therapy approach
Sirna Therapeutics Boulder, Colo.	Macular degeneration, hepatitis C and, later, cancer, metabolic, inflammatory, dermatological and central nervous system diseases

other parts of the body. But the market for the drug virtually disappeared as other treatments for AIDS became available and prevented most cases of the cytomegalovirus retinitis infection.

Short RNAs may eventually be delivered to the bloodstream to treat systemic diseases. But, reprising the antisense experience, the first petition to begin a clinical trial was filed in August by Acuity Pharmaceuticals, a Philadelphia company that will attempt to treat age-related macular degeneration by intraocular injection. Other companies, including Alnylam, will follow suit with their own drug trials for macular degeneration. One of these filings will bring Alnylam head to head with the other drug developer that stands a chance of becoming a leader in this emerging market niche.

A Boulder, Colo., company called Sirna Therapeutics was expected to submit an application to the FDA for a macular degeneration drug in early September, perhaps half a year or more before Alnylam. Unlike Alnylam, Sirna is no start-up. It is a reincarnation of another firm, Ribozyme Pharmaceuticals, which for a decade staked its fate on a different type of RNA-related drug. Ribozymes are RNAs acting as catalytic enzymes that, in principle, can cut up messenger RNA and prevent a protein from being

produced. But, as with antisense, the potency of ribozymes came into question. A drug to combat hepatitis C caused a monkey to go blind, probably because of the massive doses injected. And another drug did not seem to slow growth of tumors in patients with advanced breast cancer. At the time, Howard W. Robin, a new chief executive, who had managed development of drugs like Betaseron for multiple sclerosis at Berlex Laboratories, was faced with a decision about whether to close shop. The company had only \$2 million in cash left and risked being delisted from the Nasdaq.

The darkest days coincided with Tuschl's publication about RNAi in mammalian cells. Instead of turning out the lights, Ribozyme Pharmaceuticals became Sirna Therapeutics. Sirna rejiggered the chemical techniques it had used to deliver and stabilize ribozymes and adapted them to siRNAs. Robin claims that single doses of its siRNAs have remained active inside cells of live animals for up to 22 days. The revamping succeeded in attracting \$72 million in new investment

during an 18-month period. Besides macular degeneration, the company has programs in hepatitis, oncology and Huntington's disease, among others. "It's not often that you take the skills and intellectual property from a technology that's not working very well and transfer it to the hottest area of biology," Robin says.

Sirna has filed for 90 patents that Robin believes cover the most attractive drug prospects. Patent fights may loom as the technology gets nearer the marketplace. "If you look at our competitors, we believe almost everything they're doing violates our patents," Robin proclaims. But Maraganore begs to differ: "We have a bit of a toll road for anyone doing therapeutics." Any dispute is not likely to emerge until siRNA drugs are much closer to approval. In the meantime, investigators will be closely watching whether siRNAs produce unwanted immune responses or shut down genes they are supposed to leave intact.

For the time being, optimism about RNAi reigns. "If you do with RNAi in man what you do in cell culture, you have the most unbelievably powerful technology for making pharmaceuticals," Maraganore says. "It's the dream of medicine to do selective and efficient gene silencing," Robin asserts. But Isis's Crooke, tempered by the failure of trials for a few of his company's antisense drugs, has a slightly different perspective: "Any time you think something is magic, you're going to get in trouble. [RNAi] is a complicated system with lots of interesting nuances that mechanistically should lead to some unexpected effects as well as those you desire."

RNAi has become a preeminent research tool in a remarkably short time. But its potential as a genetically based pharmaceutical will not become clear for several years, when the first clinical trials prove whether a simple injection is capable of shutting down the effects of a disease-causing gene. SA

MORE TO EXPLORE

The RNAi Revolution. Carl D. Novina and Phillip A. Sharp in *Nature*, Vol. 430, pages 161–164; July 8, 2004.

The Silent Revolution: RNA Interference as Basic Biology, Research Tool and Therapeutic. Derek M. Dykxhoorn and Judy Lieberman in *Annual Review of Medicine*, Vol. 56 (in press).

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Immune systems of 'bubble babies' restored by gene therapy, UCLA researchers find

By **Kim Irwin** | September 11, 2012

UCLA stem cell researchers have found that a gene therapy regimen can safely restore immune systems to children with so-called "bubble boy" disease, a life-threatening condition that if left untreated can be fatal within one to two years.

In the 11-year study, researchers were able to test two therapy regimens for 10 children with ADA-deficient severe combined immunodeficiency (SCID), which has come to be known as "bubble boy" disease because some of its victims have been forced to live in sterile environments.

During that time, the researchers refined their approach to include a light dose of chemotherapy to help remove many of the blood stem cells in the bone marrow that were not creating the enzyme adenosine deaminase (ADA), which is critical for the production and survival of healthy white blood cells, said study senior Dr. Donald Kohn, a member of the [Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA](#).

The refined gene therapy and chemotherapy regimen proved superior to the other method tested in the study, restoring immune function to three of the six children who received it, said Kohn, who is also a professor of pediatrics and of microbiology, immunology and molecular genetics in UCLA Life Sciences Division. An even further-refined regimen using a different type of virus delivery system will be studied in the next phase of the study, which already has enrolled eight of the 10 patients needed.

The study appears Sept. 11 in the advance online issue of the peer-reviewed journal *Blood*.

"We were very happy that in the human trials we were able to see a benefit in the patients after we modified the protocol," Kohn said. "Doctors treating ADA-deficient SCID have had too few options for too long, and we hope this will provide them with an efficient and effective treatment for this devastating disease."

Children born with SCID, an inherited immunodeficiency, are generally diagnosed at about 6 months old. They are extremely vulnerable to infectious diseases and don't grow well. Chronic diarrhea, ear infections, recurrent pneumonia and profuse oral candidiasis commonly occur in these children. SCID occurs in about one of every 100,000 births.

Currently, the only treatment for ADA-deficient SCID calls for injecting patients twice a week with the necessary enzyme, Kohn said, a lifelong process that is very expensive and often doesn't return the immune system to optimal levels. These patients also can undergo bone marrow transplants from matched siblings, but matches can be very rare.

About 15 percent of all SCID patients are ADA-deficient. Kohn and his team used a virus delivery system that he had developed in his lab in the 1990s to restore the gene that produces the missing enzyme necessary for a healthy immune system. To date, about 40 children with SCID have received gene therapy in clinical trials around the world, Kohn said.

Two slightly different viral vectors were tested in the study, each modified to deliver healthy ADA genes into the bone marrow cells of the patients so the needed enzyme could be produced and make up for the cells that don't have the gene. Four of the 10 patients in the study remained on their enzyme replacement therapy during the gene therapy study. There were no side effects, but their immune systems were not sufficiently restored, Kohn said.

In the next six patients, the enzyme therapy was stopped, and a small dose of chemotherapy was given before starting the gene therapy to deplete the ADA-deficient stem cells in their bone marrow. Of those patients, half had their immune systems restored. The human findings confirmed another study, also published recently in *Blood* by Kohn and UCLA colleague Dr. Denise Carbonaro-Sarracino, which tested the techniques in parallel, using a mouse model of ADA-deficient SCID.

One of Kohn's clinical trial patients enrolled in the first study was a baby boy diagnosed with ADA-deficient SCID at age 10 months. The boy had multiple infections, pneumonia and persistent diarrhea and was not able to gain weight. He received the enzyme replacement treatment for three to four months but did not improve and joined the gene therapy study in 2008. Today, that boy, who lives with his family in Arizona, is a thriving 5-year-old.

"You would never know he had been so sick," Kohn said. "It's a very promising response."

The boy's younger sister, also born with ADA-deficient SCID, was diagnosed at 4 months of age and is enrolled in the second phase of the study. She's also doing well, Kohn said. In fact, it appears that children who are diagnosed and treated younger seem to do better.

The study was funded by the Doris Duke Charitable Foundation, the National Heart, Lung and Blood Institute at the National Institutes of Health and the U.S. Food and Drug Administration's Orphan Product Development award (1P50 HL54850 and RO1 FD003005).

The Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research: UCLA's stem cell center was launched in 2005 with a UCLA commitment of \$20 million over five years. A \$20 million gift from the Eli and Edythe Broad Foundation in 2007 resulted in the renaming of the center. With more than 200 members, the Broad Stem Cell Research Center is committed to a multidisciplinary, integrated collaboration among scientific, academic and medical disciplines for the purpose of understanding adult and human embryonic stem cells. The center supports innovation, excellence and the highest ethical standards focused on stem cell research with the intent of facilitating basic scientific inquiry directed toward future clinical applications to treat disease. The center is a collaboration of the David Geffen School of Medicine at UCLA, UCLA's Jonsson Cancer Center, the UCLA Henry Samueli School of Engineering and Applied Science and the UCLA College of Letters and Science.

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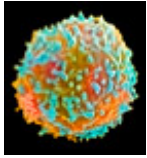
Gene Therapy Cures Adult Leukemia

CLL Tumors 'Blown Away' in 2 of 3 Patients Given Experimental Treatment

By [Daniel J. DeNoon](#)

Reviewed by [Laura J. Martin, MD](#)

WebMD Health News



Aug. 10, 2011 -- Two of three patients dying of chronic lymphocytic [leukemia](#) (CLL) appear cured and a third is in partial remission after infusions of genetically engineered T cells.

The treatment success came in a pilot study that was only meant to find out whether the treatment was safe, and to determine the right dose to use in later studies. But the therapy worked vastly better than University of Pennsylvania researchers David L. Porter, MD, Carl H. June, MD, and colleagues had dared to hope.

"Our results were absolutely dramatic. It is tremendously exciting," Porter tells WebMD. "These kinds of outcomes don't come around very often. We are really hopeful that we can now translate this into treatment for much larger numbers of patients and apply this technique to other diseases and to many more patients."

Excitement is spreading as oncologists learn about the findings. "I think it is a big deal," says Jacques Galipeau, MD, professor of hematology and medical oncology at Emory University Winship Cancer Center. Galipeau was not involved in the Porter study.

"Here's this guy, the handwriting is on the wall, any hematologist will tell you he is a goner -- this guy was essentially cured," Galipeau tells WebMD. "These genetically engineered cells did what everyone in the field has tried to do for 20 years. The man probably had kilograms of disease in his body, and the cells mopped it up completely."

The treatment uses a form of white [blood](#) cells called T cells harvested from each patient. A manmade virus-like vector is used to transfer special molecules to the T cells. One of the molecules, CD19, makes the T cells attack B lymphocytes -- the cells that become cancerous in CLL.

All this has been done before. These genetically engineered cells are called chimeric antigen receptor (CAR) T cells. They kill cancer in the test tube. But in humans, they die away before they do much damage to tumors.

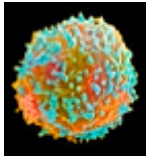
What's new about the current treatment is the addition of a special signaling molecule called 4-1BB. This signal does several things: it gives CAR T cells more potent anti-tumor activity, and it somehow allows

the cells to persist and multiply in patients' bodies. Moreover, the signal does not call down the deadly all-out immune attack -- the feared "cytokine storm" -- that can do more harm than good.

This may be why relatively small infusions of the CAR T cells had such a profound effect. Each of the cells killed thousands of cancer cells and destroyed more than 2 pounds of tumor in each patient.

"Within three weeks, the tumors had been blown away, in a way that was much more violent than we ever expected," June says in a news release. "It worked much better than we thought it would."

CLL Patient Describes CAR T-Cell Treatment



The treatment was not a walk in the park for patients. One of the three patients became so ill from the treatment that steroids were needed to relieve his symptoms. The steroid rescue may be why this patient had only a partial remission.

"Those engineered T cells don't hug the cells to death. They release an array of substances, nasty things that have evolved to clear virus- infected cells from your body," Galipeau says. "But now they are using this to melt down a couple of pounds worth of tumor burden, you will get some side effects."

One of the patients, whose case is reported in the *New England Journal of Medicine*, described his experience in a University of Pennsylvania news release. The patient chose not to identify himself by name, although he discloses that he has a scientific background. He was diagnosed with CLL at age 50; 13 years later his treatment was failing. Facing a bone-marrow transplant, he jumped at the chance to enter Porter's clinical trial of CAR T cells.

"It took less than two minutes to infuse the cells and I felt fine afterward. However, that fine feeling changed dramatically less than two weeks later when I woke up one morning with chills and a fever," he says. "I was sure the war was on. I was sure the CLL cells were dying."

A week later the patient was still in the hospital when Porter brought him the news that the CLL cells had disappeared from his blood.

"It was working and I was winning," the patient says. "It was another week later that I got the news that my bone marrow was completely free of detectable disease. It has been almost a year since I entered the clinical trial. I'm healthy and still in remission."

Is he cured? Doctors hate to declare a cure until patients have been cancer-free for at least five years. But there are signs the CAR-T cells persist in patients' immune memory, ready to mop up any CLL cells that reappear.

And there's a big downside. The CAR T cells that fight CLL also kill off normal B lymphocytes. These are the cells that the body needs to make infection-fighting antibodies.

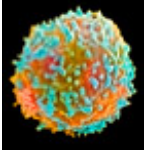
As long as the CAR T cells persist -- which may be for the rest of patients' lives -- patients will require regular infusions of immune globulin.

Hope for Cancer Patients, but Treatment Years Away

CLL is the second most common form of adult leukemia. In the U.S. there are about 15,000 new cases and about 4,400 deaths each year.

Cure is possible, but it requires a risky bone marrow transplant. About 20% of patients don't survive this treatment -- and even when they do, there's only a 50-50 chance of a cure.

Hope for Cancer Patients, but Treatment Years Away continued...



CAR T cells appear to be a much better option. But the amazing success now reported came very early in the development of this new treatment. Only a few of the thousands of CLL patients facing death will be able to enter the still-small clinical trials testing CAR T cells.

"The distressing thing is the need will far, far, far outweigh any slots in clinical trials," Galipeau says.

But Porter says his team is energized by the early success and is pushing forward as quickly as possible. Even so, a lot of work remains to be done.

"We've treated only a very small numbers of patients," Porter says. "So part of the goal is to see these results in more people, see that the results are sustained, and that it is safe over time. We need to find the appropriate dose and to make incremental modifications. And now we have shown activity, we can try and apply it earlier in the course of the disease. We have reason to think treating patients sooner may be even safer and more effective.

Although the CAR T cells in the study were designed to fight CLL, there's good reason to hope they can be effective in other forms of cancer. The catch is that it can work only on tumor cells that carry markers flagging them for destruction. Normal cells that carry the same markers will also be destroyed.

Many cancers are known to carry such markers, and there's hope of finding more.

"We have a clinical trial at the University of Pennsylvania with an anti-mesothelin molecule [which marks [mesothelioma](#), ovarian, and pancreatic tumors]," Porter says. "There are other trials around the country trying to target [renal](#) cell carcinoma [[kidney](#) cancer] and myeloma [[skin cancer](#)]. We are hoping to identify other tumor targets, particularly in other leukemias, to adapt this technology."

Porter, June, and colleagues report their findings in the Aug. 10 early online versions of two major journals: *The New England Journal of Medicine* and in *Science Translational Medicine*.

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SOURCES:

News releases, University of Pennsylvania.

Kalos, M. *Science Translational Medicine*, published online, Aug. 10, 2011.

Porter, D.L. *The New England Journal of Medicine*, published online, Aug. 10, 2011.

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TOP NEWS

Gene therapy proves effective for hemophilia B

Sat, Dec 10 22:11 PM EST

By Deena Beasley

SAN DIEGO (Reuters) - A single treatment with gene therapy, an experimental technique for fixing faulty genes, has been shown to boost output of a vital blood clotting factor, possibly offering a long-term solution for people with hemophilia B.

Researchers said the same technology was also being studied as a treatment for hemophilia A, the far more common type of the inherited bleeding disorder.

"It is a technique for potentially permanently curing patients," said Dr. Charles Abrams, American Society of Hematology secretary and associate chief of hematology/oncology at the University of Pennsylvania in Philadelphia.

Both safety and efficacy have held back the field of gene therapy. One experiment cured two French boys with a rare immune disorder but gave them leukemia in 2002, and an Arizona teenager died in a 1999 gene therapy experiment.

The approach used by researchers at the University College London Cancer Institute and St. Jude Children's Research Hospital in Memphis, Tennessee, involved the use of a novel viral "vector," designed to target the liver specifically.

The strategy involves replacing the defective gene that causes the bleeding disorder with a correct version delivered via the virus to the patient's liver cells - the only cells in the body capable of producing certain clotting factors missing or deficient in people with hemophilia.

The factors are numbered using Roman numerals. The two main forms of the disease are hemophilia A, caused by a lack of clotting factor VIII, and hemophilia B, caused by a lack of clotting factor IX.

Researchers have so far treated six men with severe hemophilia B who were producing clotting factor IX at less than 1 percent of normal levels. The general goal of current treatment with recombinant factor IX is to achieve factor levels greater than 1 percent of normal.

Four of the six trial participants have stopped routine treatment and remain free of spontaneous bleeding. The other two have increased the interval between factor infusions to once every 10 days to two weeks from two to three times a week, said Dr. Andrew Davidoff, chairman of the department of surgery at St. Jude's and co-author of the study.

HIGH COST FOR CURRENT TREATMENT

Frequent treatments with manufactured factor IX, known as recombinant factor concentrates, can cost hundreds of thousands of dollars a year, making hemophilia a tempting target for gene therapy.

The trial "is truly a landmark study," Dr. Katherine Ponder, hematology and oncology professor at Washington University in St. Louis, said in a *New England Journal of Medicine* editorial.

"If further studies determine that this approach is safe, it may replace the cumbersome and expensive protein therapy currently used for patients with hemophilia B," she wrote.

The trial results were published in the *NEJM* and reported on Saturday at a meeting of the American Society of Hematology in San Diego.

The six trial subjects were broken into three groups with each group receiving a different concentration of new genes.

Factor IX levels in the first subject have remained at 2 percent for nearly two years, while the two patients treated with the highest dose have seen FIX levels rise to between 3 and 12 percent, researchers said.

One high-dose subject developed elevated levels of transaminases, an indicator of possible liver damage, and another had a slight increase in liver enzymes. Both cases were resolved with steroids, the researchers said.

Plans are to treat more patients with the highest dose used so far, and if research continues to succeed, the treatment could be widely available "in the next five years or so," said Dr. Amit Nathwani, co-lead study author of the Department of Hematology at UCL Cancer Institute in London.

He also said the team was working to use the technique for treating hemophilia A.

ISI Group analyst Mark Schoenebaum said the gene therapy could pose big competition for companies such as Biogen Idec that are producing recombinant factor concentrates.

"This clearly presents a curveball to our (and much of Wall Street's) assumptions around the future of the hemophilia market," he said in an email to investors.

The analyst said estimated sales of the hemophilia factors accounted for between \$10 and \$17 of his \$125 price target for shares of Biogen, which closed at \$112.95 on Friday.

People with hemophilia bleed more following trauma than people without the disease, and those with severe disease may bleed spontaneously. Since the gene is carried on the X chromosome, hemophilia is almost exclusively a disease of men.

But women can pass the gene to their offspring.

Hemophilia has often been called the "Royal Disease" since it was carried by Britain's Queen Victoria and affected many of the royal families of Europe.

Hemophilia B is much less common than hemophilia A. About one in five hemophilia patients has hemophilia B, according to the National Institutes of Health.

The global market for Factor VIII products is about \$5 billion, while the market for Factor IX is worth about \$1 billion.

Worldwide, about one in 5,000 men is born with hemophilia A and 1 in 25,000 men is born with hemophilia B each year.

(Reporting by Deena Beasley; Editing by Peter Cooney)

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