LEADING EDGE

The Future of Gene Therapy

Eventually, gene therapy will become a staple of 21st century medicine. But some experts say society will be better served if medical researchers proceed more slowly and prudently.

BY JACK McCAIN
Senior Contributing Editor

In its current manifestation, gene therapy is an elegant concept crudely executed. That's not an indictment — that's just the way it is for an extraordinarily complicated technology still in its infancy. After all, it has been only 5 years since the concept of gene therapy was convincingly demonstrated to provide, if not a cure, then at least a long-term therapeutic effect for X-linked severe combined immunodeficiency (X-SCID) disease.

(Here, gene therapy is defined as the introduction of genetic material via techniques of molecular biology into somatic cells [in contrast to germ cells] to treat or prevent disease.)

RELATIVELY BRIEF HISTORY

Many people still are under the impression that gene therapy's proof-of-concept was demonstrated as early as 1990. For example, on Jan. 26 of this year, the *Los Angeles Times* wrote that W. French Anderson, MD, was "dubbed 'the father of gene therapy' after a team he led in 1990 cured a hereditary disease of the immune system in a 4-year-old girl." That's not quite the way it happened.

Anderson did indeed gain renown for heading the team that in September 1990 carried out the first gene therapy clinical trial approved for use in a human. The goal of this phase 1 study was to define the safety issues involved. The 4-year-old girl had a genetic disease called adenosine deaminase (ADA) deficiency, which is caused by a defective gene for the enzyme ADA, resulting in SCID. Via a modified retrovirus, normal ADA genes were transferred to T lymphocytes that had been removed from the girl's body and grown in culture. The white cells then were returned to the patient. In January 1991, a 9-year-old girl underwent the same procedure.

Today both patients are alive and doing well, but conventional therapy (pegylated bovine ADA, or PEG-ADA) given before, during, and after their gene therapy confounded the results and makes any claim of "cure" based on the gene therapy problematic. One patient has an ADA level that is 25 percent of normal with the therapeutic gene present in 15 percent of her peripheral blood mononuclear cells. The other has an ADA level that is less than 5 percent of normal, and the presence of the therapeutic gene in the peripheral blood cells is negligible.

PHOTOGRAPH BY ROBERT BURROUGHS

"The concept of fixing a broken gene is now entrenched in medicine," says Theodore Friedmann, MD, director of the University of California–San Diego Program in Gene Therapy. "[It's] a real field, with many ups and downs behind it and surely more ahead. Its technology is evolving, slowly but certainly positively."

Anderson went on to collaborate in 12 of the first 20 gene therapy trials approved in the United States. In an article he wrote about the prospects for gene therapy, he stated, "Indeed, within 20 years, I expect that gene therapy will be used regularly to ameliorate — and even cure — many ailments." More recently, *Time* celebrated the 50th anniversary of Watson and Crick's 1953 discovery of the structure of DNA with a special issue about genetics, again featuring Anderson. Ever the optimist, Anderson looked 50 years ahead and told *Time*, "By 2053, there will be a gene-based treatment for essentially every disease. Cancer, heart disease, and other modern-day scourges will be vastly reduced."

Halfway through Anderson's 20-year window (but barely into his 50-year projection), you can count the number of clearly effective gene transfer therapies in nonexperimental clinical use with your nose. That's right — there's one. And you have to go halfway around the world to get it.

In October 2003, in China, Gendicine became the world's first gene therapy approved for commercial production. According to newspaper accounts, patients from around the world have been traveling there to receive this treatment for head and neck squamous cell carcinoma (HNSCC). The question is how long will Gendicine remain the world's only licensed gene therapy?

Probably, not very long — the Chinese approach is conceptually identical to one that was developed earlier in the United States but has been moving more slowly toward licensing — a pace that means gene therapy will not come into regular use anytime soon.

On the other hand, the number and variety of clinical trials of gene therapy are such that Anderson's 50-year projection could pan out. As of December 2004, 667 human gene transfer clinical protocols had been submitted for review by the National Institute of Health's Recombinant DNA Advisory Committee (RAC)[1] and the U.S. Food and Drug Administration

[1] Since 1974, the RAC has reviewed technology involving recombinant DNA, including clinical trials involving human gene transfer if direct or indirect NIH funding is provided. Since 1997, the U.S. Food and Drug Administration has been the principal regulator and overseer of gene therapy trials, while the Recombinant Advisory DNA Committee has promoted public awareness and understanding of issues surrounding gene therapy. Yet, the RAC's function goes beyond education, taking on powerful and public scientific, ethics, and policy advisory oversight. The RAC sends its recommendations to the FDA and local committees, and while those RAC comments formally are recommendations only, they carry great influence on the final actions taken by the local review committees.

(through its Center for Biologics Evaluation and Research). Of these, 617 are for therapeutic purposes (see table on page 56) — as opposed to marking and nontherapeutic purposes — but only a handful have advanced to phase 3. Most are phase 1 trials whose purpose is to demonstrate safety. Of the 59 protocols submitted in 2004, the majority originated in academia, but 37 percent had a corporate sponsor (sometimes working in collaboration with a nonprofit group).

MULTITUDE OF TARGETS

With all the hoopla surrounding the Human Genome Project, it's understandable that people would entertain high hopes for the advancement of gene therapy. The human genome is now known to contain some 25,000 genes, including about 22,000 protein-encoding genes that express about 100,000 proteins. But unresolved questions abound regarding what these genes and proteins actually do and how, when, where, and in what sequence. As answers emerge, gene therapy could evolve in ways that will provide numerous benefits to patients and without deleterious side effects.

Talking about gene therapy as though it were a single entity, though, isn't very helpful. As explained by David A. Sanders, PhD, associate professor of biological sciences at Purdue University, gene therapy falls into three groups:

- Replacing a defective or maladaptive gene that's responsible for some monogenic disease (e.g., cystic fibrosis or sickle cell anemia)
- Altering or killing an aberrant cell (e.g., infected by HIV or cancerous)
- Inducing production of a therapeutic protein (e.g., treating hepatitis C by promoting secretion of interferon by other cells)

Initially, gene therapy focused on the first group, but most current research focuses on the other two. Whatever the application, numerous hurdles stand in the way of developing a successful gene therapy, such as:

- Identifying an appropriate target for gene therapy
- Getting a therapeutic transgene into the right cells (and only those cells) in the right amount
- Delivering the transgene with a vector that doesn't trigger an immune response or, in the case of certain viral vectors, revert to a pathogenic form
- Providing the appropriate regulatory elements for turning the gene on and off at the correct time
- Keeping the transgene in the target cell long enough for it to do its job
- Keeping the transgene from causing damage elsewhere (e.g., spurring development of neoplasms or autoimmune disease, which could happen if the transgene expresses a protein new to the patient's body

Aside from the 1,500 or so diseases known to be caused by a single defective gene, most involve multiple genes, so potential targets for gene therapy abound. Possible therapies aren't restricted to the naturally occurring genes and gene products in the human genome. Fusion genes and nonhuman genes also are being investigated. An example of a fusion gene is an investigational agent being developed by Targeted Genetics, based in Seattle, Wash. This agent fuses the gene for the Fc fragment of human immunoglobulin G with another gene for the soluble p75 receptor for tumor necrosis factor, producing the same molecule provided by etanercept (Enbrel). A phase 1 trial comprising patients who have rheumatoid arthritis was initiated in March 2004.

SKY-HIGH EXPECTATIONS

In the view of Theodore Friedmann, MD, who has been deeply involved in the study of gene therapy for three decades — essentially its entire modern history — hyperbole surrounding early claims had the effect of unrealistically heightening expectations that gene therapy would emerge quickly as a component of health care. Friedmann is a professor of pediatrics at the University of California–San Diego, and director of the UCSD Program in Gene Therapy. A medical ethicist and geneticist, he also has served on the RAC and was its chairman until last year.

That the initial human gene transfer protocol was hailed a success, Friedmann says, is a "perfect example of the confluence of exaggerated expectations and wishful thinking. Everyone wanted it to work." But, he adds, it was unfair to patients and the public that the heightened expectations generated by some of the scientists and their institutions, the media, and others

served to raise false hope in many patients with many kinds of disease. "Hope is necessary, but knowingly making undeliverable promises and raising false hope is cruel," Friedmann says. "The delusion of a cure contributed to crashing disappointment later."

With their early expectations dashed, people concluded that gene therapy might be another biotech bust. Friedmann thinks the perception of failure was also fueled by widely publicized setbacks being portrayed as "disasters" — which he says were regarded as disasters only because expectations were so high to start with.

Friedmann sees gene therapy today at a point comparable to the early days of organ transplantation, when successes were scarce and failures frequent. Even the first clear success of gene therapy, he notes, has been muted by the emergence of three cases of leukemia (including one death) among the 18 children who were treated. The outcome of these cases is that gene transfer therapy is now reserved for patients who have had unsuccessful attempts at bone marrow transplantation or for whom this approach is not feasible.

"These children received very effective treatment, but at a very high cost," Friedmann says, "and if additional cases of leukemia develop, we'll have a greater problem." But he adds, that doesn't mean that we should not push ahead with the field.

Human gene transfer protocols, United States and worldwide, 1988-2005

	U.S. total	%	World total*	%
Cancer	**436**	**65**	**675**	**66**
Immunotherapy/*in vivo* transduction	159			
Immunotherapy/*in vitro* transduction	129			
Pro-drug/HSV-TK and ganciclovir	45			
Tumor suppressor gene	38			
Vector-directed cell lysis	28			
Other therapeutic approaches	37			
Monogenic Diseases	**60**	**9**	**93**	**9**
Cystic fibrosis	23			
Severe combined immunodeficiency (SCID)	6			
Hemophilia	5			
Fanconi anemia	4			
Other monogenic diseases	22			
Cardiovascular disease	**55**	**8**	**85**	**8**
Peripheral artery disease	29			
Coronary artery disease	21			
Other cardiovascular disease	5			
Infectious disease	**42**	**6**	**68**	**7**
Human immunodeficiency virus	39			
Other viral diseases	3			
Central Nervous System Diseases (Alzheimer's disease, Parkinson's disease, epilepsy)	**5**	**<1**	**5**	**<1**
Other Diseases & Disorders (arthritis, autoimmune disease, bone fracture, cubital tunnel syndrome, erectile dysfunction, eye disorders, intractable pain, peripheral neuropathy, ulcer)	**19**	**3**	**26**	**3**
Marking and Nontherapeutic Uses	**50**	**7**	**68**	**7**
Total gene transfer protocols	**667**		**1,020**	

HSV-TK=herpes simplex thymidine kinase.
* Includes United States.

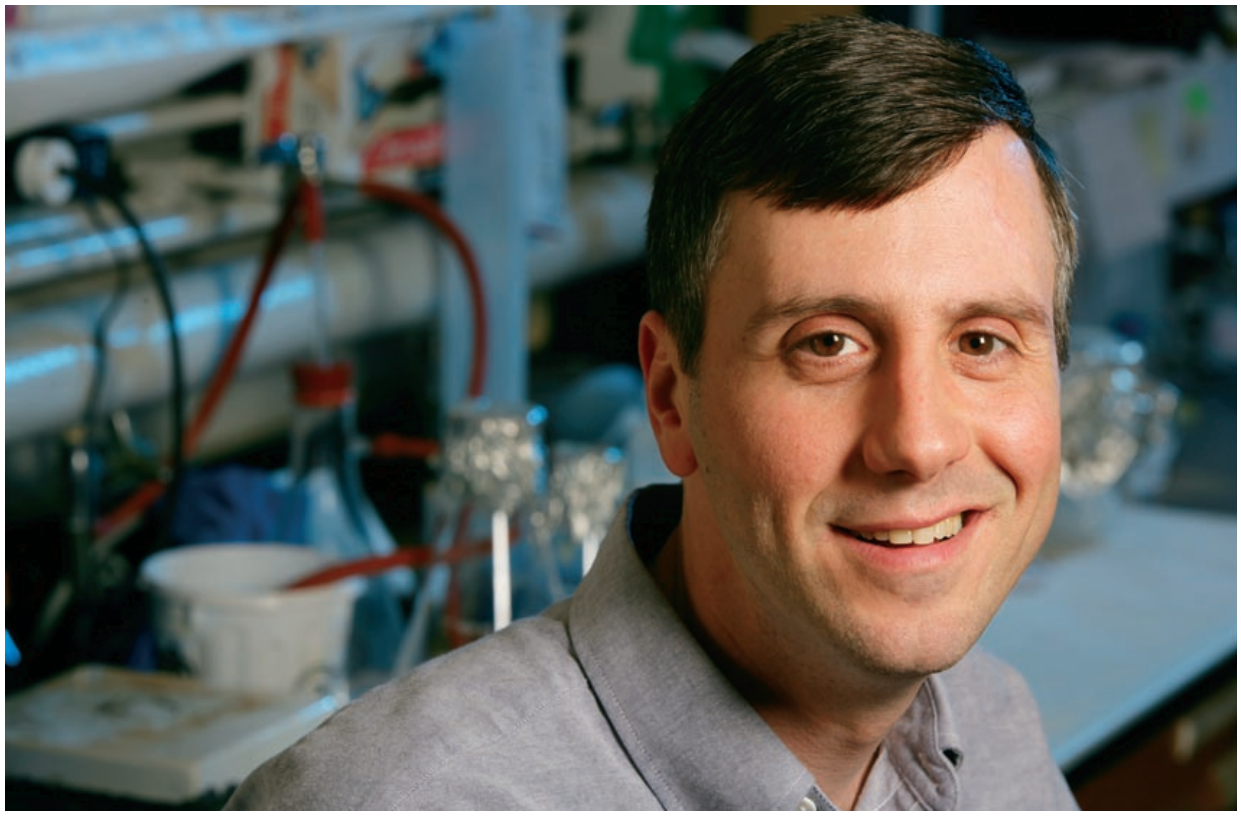
SOURCES: RECOMBINANT DNA ADVISORY COMMITTEE.
Available at: «http://www4.od.nih.gov/oba/rac/PROTOCOL.pdf»;
GENE THERAPY CLINICAL TRIALS WORLDWIDE.
Available at: «http://www.wiley.co.uk/genmed/clinical».

WHERE THE PATIENTS ARE

At first it was thought that gene therapy would focus on monogenic diseases — hereditary diseases such as SCID, hemophilia, or cystic fibrosis — that stem from a single defective gene. The thinking was that such diseases could be ameliorated, if not cured, by providing the patient with a properly functioning gene. Thus far, the initial expectations have not been fulfilled. Moreover, as gene therapy has evolved, it has drifted away from monogenic diseases and toward diseases like cancer.

This makes sense given that can-

PHOTOGRAPH BY SHAWN SPENCE



"Whatever the problems with certain drugs might be, they begin and end in a single generation," says David A. Sanders, PhD, associate professor of biological sciences at Purdue University. In contrast, he points out, gene therapy has the potential to affect a patient for a lifetime, which mandates lifelong observation.

cer is where the patients are and probably will be. Which is also where the money will be. According to the American Cancer Society, cancer has become the leading killer of Americans under the age of 85, surpassing cardiovascular disease. For demographic reasons, it is logical that CVD also would attract the attention of gene therapy researchers — and it has, along with incurable conditions such as Alzheimer's disease and Parkinson's disease.

Alameda, Calif.-based Avigen began a phase 1/2 clinical trial of AV201 for the treatment of severe Parkinson's at the end of 2004. This agent uses an adeno-associated virus (AAV) vector to deliver the gene for an enzyme, acetoacetate decarboxylase (AADC), directly into the striatum. The striatum is the section of the brain where movement is controlled via dopamine. As Parkinson's progresses, dopamine levels decrease. Patients are treated with levodopa, which is converted to dopamine by AADC. Eventually, the effectiveness of levodopa diminishes, presumably because the concentration of AADC declines. The idea behind AV201 is that with the enzyme restored, patients once again will respond to levodopa.

To take another example, some gene therapy research is exploring the role of angiogens — molecular mediators that promote the formation of blood vessels during normal cardiac and vascular development. One such angiogen is vascular endothelial growth factor (VEGF). The VEGF gene consists of eight exons that can be spliced in different ways (omitting one or more exons), leading to the synthesis of amino acid sequences of varying lengths (specifically, 121, 165, 189, and 206 amino acids). Using an adenovirus vector, attempts have been made to transfer cDNA for VEGF121 into skeletal muscle as a treatment for peripheral arterial disease and into myocardial tissue as a treatment for severe coronary artery disease.

It was once thought that the receptors for the VEGF group of proteins were restricted to endothelial cells, but they since have been found in cells of nonendothelial origin, in-

cluding tumor cells. That finding points to a characteristic that gene therapy shares with pharmacotherapy: the molecular targets of therapy often are not restricted to cells in the tissue of interest. That puts a premium on developing vectors that deliver transgenes to specific cells and only those cells.

IMPROVING VIRAL VECTORS

Among the numerous vehicles for carrying therapeutic genes to target cells, vectors adapted from viruses stand out because of the ease with which viruses enter cells and then spill out their contents — the viral genes that induce the host to generate the components of new virions. When a therapeutic gene is inserted in place of most of the viral genome, the virion retains its ability to penetrate the target cells while delivering a presumably beneficial payload. The families that have been most often used as vectors are the adenoviruses and retroviruses, but AAV and lentivirus are among other viral vectors increasingly employed in gene therapy experiments. Lentiviruses actually are a genus in the retrovirus family, but they differ from other retroviruses in being able to integrate their genome into the chromosomes of nondividing cells (e.g., brain, peripheral nerves). Other retroviruses can transduce only dividing cells.

According to Sanders, one of Friedmann's major contributions, among many, to the development of gene therapy was showing that a recombinant *pseudotyped* virus could be created to serve as a vector for delivering genetic material to cells. The genome of a pseudotyped virus lacks the coding for one or more of its structural proteins, which confers a safety benefit and other advantages.

In contemporary gene therapy experiments, vesicular stomatitis virus G protein (VSV-G) pseudotyped retroviruses and lentiviruses are commonly used but have several shortcomings, such as being toxic to cells producing virus in culture and targeting primary cells in culture or *in vivo*. In his own work, Sanders has been looking at other viruses that could be used to create pseudotyped viruses, and he says that he has found alphaviruses to be promising. Among the species in this insect-transmitted genus are the Ross River virus, Eastern equine encephalitis virus, Semliki Forest virus, and Venezuelan equine encephalitis virus.

The field of gene therapy is driven largely by medical professionals instead of scientists. Because patients are their primary concern, doctors might be predisposed to trying gene therapy experiments on severely ill patients — even if the science is still a bit ragged.

A protein biochemist by training, Sanders did postdoctoral work with Harvard's Richard Mulligan, PhD, who joins Friedman as another pioneer of gene therapy. Sanders is especially interested in the proteins found on the exterior of viruses, because the way these proteins match up with other proteins embedded in the cell membrane determines whether a virus can enter a given cell. Sanders has designed a new class of pseudotyped viruses, constructing their shell from a variety of alphaviruses and their core from retroviruses and lentiviruses. Injected into the tail vein of a mouse, these pseudotyped viruses are delivered in quantity to the liver. And in the central nervous system, these vectors go specifically to glial cells but not to neurons, in contrast with VSV-G vectors, which enter neurons but not glial cells. Sanders says this property has important implications for the treatment of brain tumors, most of which are of glial origin.

In many cases, delivering a gene to the right cells is not sufficient. The gene also must be brought to the correct portion of the cell. That's because many cells are polarized, having one portion of their plasma membrane exposed to the outside world (the apical membrane) and another exposed to the blood stream (the basolateral membrane). The proteins embedded in the apical membrane differ from those in the basolateral membrane, and a tight junction prevents proteins from passing from one domain to the other. Because different proteins offer a foothold for different viruses, it's important to select a viral vector that's specific for the apical or basolateral membrane of polarized target cells, such as airway epithelial cells in the lung. Influenza viruses enter and leave these cells through the apical membrane, which is exposed to the outside. But a retrovirus like HIV or

murine leukemia would have to enter through the basolateral membrane, which is exposed to the bloodstream in which these viruses find their major target (blood cells).

Sanders explains that a practical consequence of failure to appreciate the difference between apical and basolateral membranes is this: More than a decade ago, the gene responsible for cystic fibrosis was discovered. Great excitement ensued, and the success of gene therapy based on this discovery was eagerly anticipated. But success has been elusive, partly owing to difficulty in getting the therapeutic gene into the target cells. Sanders says that's because researchers used retroviral vectors that approached the epithelial cells from the wrong side (the basolateral side). He says a more promising approach may be to base a vector on a modified Ebola virus shell, which specifically targets airways epithelial cells and can enter through the apical membrane if it's aerosolized. This technique appears to improve gene delivery by two orders of magnitude compared to retroviral vectors, Sanders says.

WRONG DRIVER?

Setting aside the specifics of cystic fibrosis, the greater question focuses on why researchers would use the wrong vector in the first place. In Sanders' view, it's because the field of gene therapy is driven largely by medical professionals instead of scientists. The physician's paramount concern is to help a patient, and if the patient is severely ill, the physician may disregard loose ends or ambiguity in the science supporting a new technology.

"Medical doctors are interested in the individual patient's welfare at all costs," Sanders says. "I would demand that from my own physician or a physician for a member of my family. They are not necessarily the best evaluators of the societal and public health effects of their procedures. When confronted with seriously ill patients for whose condition there is no existing effective treatment they think, 'Let's go ahead and do the experiment anyway. Maybe it will work.'"

Looking at gene therapy from the perspective of public health, Sanders says he's opposed to doing gene therapy experiments too early. "Yes, we did learn some things from the death of Jesse Gelsinger [the 19-year-old who died during a gene therapy experiment at the University of Pennsylvania in 1999], but we didn't have to learn them in *that* way," he says.

As an example of how physicians' haste to help patients can lead researchers in the wrong direction, Sanders cites a key study from W. French Anderson that paved the way for the first human gene therapy experiments. With the concern that a viral vector might acquire the ability to replicate, it was important to demonstrate that replication did not occur if a retrovirus was used as a vector. Toward this end, Anderson and colleagues injected rhesus macaques (a proxy for human subjects) with a replication-competent murine leukemia retrovirus. Because no infection developed in the macaques, the researchers concluded that murine retrovirus probably would not pose an acute health risk in humans. The researchers attributed the lack of infection in the monkeys to neutralization of the retrovirus by complement.

Nonetheless, there was a problem, Sanders says. The presence of complement implies the presence of antibodies against the retrovirus. The question of where those antibodies came from should have

First FDA-approved gene therapy for cancer may be on its way

One frontrunner for the distinction of being the first gene therapy to win approval from the U.S. Food and Drug Administration is Advexin, made by Introgen, of Austin, Texas, and which resembles Gendicine. Both use an adenoviral vector to deliver the p53 tumor suppressor gene and can be used in combination with radiotherapy.

Advexin is being studied in two phase 3 trials that are enrolling patients with recurrent, unresectable head and neck squamous cell carcinoma. In one trial, Advexin monotherapy is being compared to methotrexate monotherapy, the primary outcome being the effect on survival time. The other compares combined chemotherapy plus Advexin versus the same chemotherapy without Advexin, with the primary endpoint being the time to progression.

The FDA has designated Advexin as an orphan drug, so if it's approved, Advexin could win seven years of marketing exclusivity. Advexin also is being studied as a treatment for many other cancers.

given the researchers pause. When another team of researchers did a similar experiment at a later date, they used a recombinant retroviral vector that was contaminated with a replication-competent virus. And, whereas in the first experiment the virus was introduced *in vivo*, in the second the virus was mixed with cells in culture, and the cells were reintroduced into the monkeys. This time, the monkeys developed leukemia.

"There was a huge amount of retroviral replication," Sanders says. The results differed, he explains, because mice and most other mammals possess a sugar, galactose-alpha (1,3)-galactose, as a component of glycoproteins on cell surfaces, but Old World monkeys (and humans, along with the great apes) lack this sugar. Yet, they have ample antibodies against it — from 1 to 3 percent — because alpha-galactose is found on the coat of many viruses that infect these animals. When the antibodies recognize a virus sporting this sugar, the complement cascade is triggered. That's what happened in the first experiment, creating the illusion of safety. In the second experiment, with the transduction taking place in a cell culture instead of *in vivo*, there was no possibility of an immune response. As a result, the viruses produced in culture lacked the sugar and went undetected and replicated, causing leukemia when the cells were returned to the macaques.

ECOLOGY OF GENE THERAPY

Sanders likens gene therapy to the development of transgenic crops. As he sees it, the major issue with transgenic plants isn't their potential to affect human health, as many critics fear, but rather the possibility that genes introduced into crops in the hope of improving agriculture might find their way into other plants with detrimental results for the whole environment. He thinks similar ecological issues apply to gene therapy. If someone receives a treatment with a recombinant virus that has the potential to replicate, can untreated individuals also be receiving gene therapy? And if DNA enters the germ line as a result of gene therapy, the effects could be felt by future generations.

If society supports widespread gene therapy directed at disease prevention — in addition to treating existing disease — Sanders wonders, who will determine how and what resources should be allocated toward that end?

The societal implications of gene therapy are so profound that a cautious, step-by-step approach is warranted, Sanders says. "Gene therapy will become a component of 21st century medicine. There's no reason it can't work. But huge questions remain to be resolved. The history of mankind tells us that whenever you have new technology, you have problems. But by now we should be intelligent enough to anticipate the problems that might be associated with gene therapy."

What are some of these problems? For starters, should patients who have received gene therapy be allowed to donate blood? Probably not, in Sanders's opinion, given how little we know at this point about long-term outcomes in gene therapy. Then there's the important political question of whether the contemporary pharmaceutical industry should serve as the model for the development of gene therapy. Sanders observes that the big money generated by small molecules and biologics is for treating chronic diseases, not for preventing disease. If society supports widespread gene therapy directed at disease prevention in addition to treating existing disease, who will determine how and what resources should be allocated toward that end?

Given the genetic basis for most diseases, instead of contemplating the future of *gene* therapy, it might be equally interesting to wonder about the future of gene therapy in the context of *drug* therapy. Right now, whether the disease is cancer or CVD, gene therapy investigations for the most part are focused on developing new treatments for high-risk patients with severe illness — patients beyond the point where conventional treatment is effective. Eventually, however, conventional treatments and gene therapies will overlap. Thorny ethical and political issues will have to be addressed, but, over the long term, the future of drug therapy could be gene therapy. BH

Based in Durham, Conn., Jack McCain is a freelance medical writer and editor. He holds degrees from Allegheny College and Wesleyan University.