Gene therapy

Hope in a cautionary tale

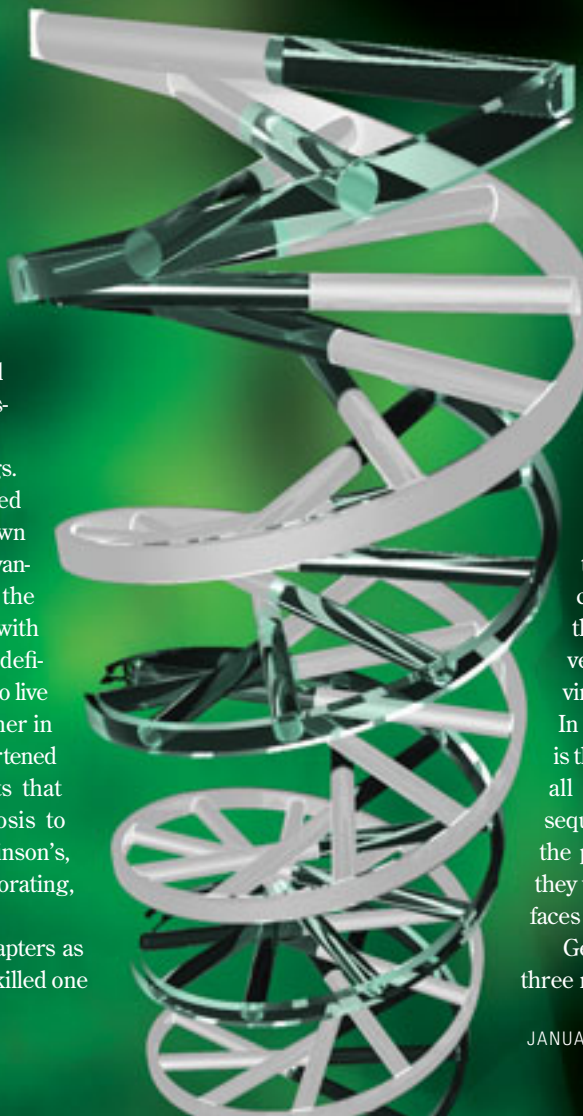
Faced with disasters, mixed successes, and failed models, the story of gene therapy is being rewritten with new strategies and new vectors.

BY MARK S. LESNEY

Not so long ago, in the early 1990s, gene therapy changed from science fantasy to viable technique with the work of cancer researcher W. French Anderson. Since then, it has captured the imaginations and raised the hopes of clinicians around the world faced with the stark reality of a host of tragic genetic diseases and cancers for which there has been neither cure nor, in many cases, even amelioration. It has proved a cautionary tale of the rocky and frustrating road toward success.

There have been some happy endings. Gene therapy, especially when combined with traditional chemotherapy, has shown itself capable of causing lethal tumors to vanish. It has restored to functional levels the immune systems of children suffering with a variety of severe combined immunodeficiency diseases (SCIDs), allowing them to live normally rather than being held prisoner in sterile bubbles throughout tragically shortened lives. It has provided tantalizing hints that other genetic evils, from cystic fibrosis to Huntington's, from Alzheimer's to Parkinson's, might have effective, or at least ameliorating, treatments in the near future.

But there have been nightmare chapters as well. In clinical trials, gene therapy has killed one youth and caused cancer in a three-year-old boy. And it has proved disappointing time and again in a large number of clinical studies where it has done absolutely nothing, or failed to outperform standard therapies, despite near-miraculous cures achieved in animal models.



Blame the messenger

So what exactly is going wrong with gene therapy?

Most researchers blame current problems on difficulties with the vectors. The messengers intended to deliver healthy genes into patients have instead betrayed the goals of therapy, causing allergic reactions and cancers or simply failing to perform in the clinch. This is not surprising. The vectors chosen have been adapted from viruses known to cause human disease. In fact, their ability to infect human cells is their most useful characteristic, and for all the disarming of pathogenic gene sequences, it is still difficult to eliminate the problems caused by the very things they were selected for—binding to cell surfaces or integration into the chromosomes.

Gene therapy has been conducted using three main virus vectors: retroviruses, ade-

noviruses, and adenovirus-associated viruses (AAVs). Retroviruses infect the cell naturally and incorporate the gene of interest directly into the human chromosomes. Adenoviruses are modified from a variety of human and animal pathogens. And AAVs are nonpathogenic to humans and cause a lower immune response, providing a transient burst of therapeutic protein upon infection. Other viral vectors include the lentiviruses and the poxviruses. In addition to viral vectors, the two main biophysical methods used are liposomes and naked DNA (see box, "Looking to liposomes") (*1*).

Rethinking retroviruses

Some 34% of current gene therapy clinical trials involve retroviral vectors (*2*). Because retroviruses insert their DNA into the host chromosome and have been known to cause cancers naturally, there have always been fears of so-called insertional oncogenesis with this technique. Thus, the main benefit of these viruses to a long-term cure of genetic diseases has always been their greatest potential source for harm. In the most recent gene therapy controversy, Alain Fischer and colleagues at the Hospital Necker-Enfants Malades in Paris used the retrovirus murine leukemia virus (MLV) as a vector for treating SCID. One of 11 children in the trial developed a leukemia-like cancer that the researchers determined was attributable to the insertion of the vector into a well-known oncogenic site.

In this particular case, targeting was not an issue, because the transformed cells were specific bone-marrow stem cells that had been removed, modified, and then reinserted into the patient. But a variety of in vivo tissue targeting methods are under investigation, from the use of bifunctional antibodies that bind to both the virus surface and a specific host cell protein to the development of genetically engineered surface glycoproteins containing ligand sequences that bind to receptors on the host cell of interest (*1*). Such directed targeting, although it does not eliminate the problem of insertional mutagenesis or oncogenesis, has the potential to limit the chances of such a problem occurring as well as limit the exposure to specific tissues.

Altering adenoviruses

Adenovirus vectors account for nearly 27% of current gene therapy trials (*2*). These viruses were originally considered ideal as therapeutic vectors because, although the virus efficiently transferred the functional gene into human cells after binding to the common Coxsackie-adenovirus receptor (CAR), it did not integrate into the host chromosome, thereby obviating the fears of insertional oncogenesis. In addition, transient expression was considered without the fear of lingering transgenic proteins staying beyond their welcome. Finally, these viruses also can incorporate large gene constructs (up to 30 kb compared with less than 7.5 kb for retroviruses) for transfer into cells.

But today, adenoviruses bear the stigma of causing Jesse Gelsinger's death from a hyperimmune response against the huge quantity of vector needed in untargeted therapy (many tissues do not contain the CAR receptor, including most tumors). In addition, adenovirus appears to accumulate naturally in the liver, where it causes toxic effects due to allergic responses. Work is being done to modify adenoviruses in a number of ways to make them targetable and to limit or eliminate their interfering immunogenic characteristics. These methods include blockage or removal of the viral fibers that bind to CAR and replacing or covering them with newly engineered sequences that target tissue-specific receptors. Specific targeting has also been accomplished in the laboratory using divalent antibodies that simultaneously bind to the virus particle and a unique receptor on the target cell selected.

Recently, researchers at the University of Washington School of Medicine reported significant results in animal models using dystrophin gene therapy to reverse the ravages of muscular dystrophy, not just prevent them as had previously been demonstrated. To do this, they used a heavily modified adenovirus that had most of its immunogenic components stripped away to allow insertion of a full-length muscle-specific dystrophin gene (*3*). Previously, only microgenes, which were not as effective, could fit within the viral genome limit.

In animal trials, genetically transformed adenovirus has also shown promise of being an oncolytic (cancer-killing) agent. Researchers at Geron Corporation (Menlo Park, CA, *www.geron.com*) put a critical virus replication gene (E1A) under the control of a human telomerase reverse transcriptase gene. This caused the virus to express in and kill only the human cancer cells containing telomerase, leaving the healthy (telomerase-negative) cells unharmed. The telomerase promoter construct is envisioned as being useful for directing other forms of gene therapy expression into cancer tissues as well.

One alternative, adeno-associated viruses (AAV), is considered important for several reasons, especially low immunogenicity. This is critical in treating diseases such as cystic fibrosis (CF), where inflammation of the affected tissue (the lungs) is already a significant problem.

Looking to liposomes

Despite all of the efforts to modify viral vectors, many researchers argue for a more concerted effort to improve the two main biophysical methods that are used: liposomes and naked DNA.

The liposomes are generally untargeted lipid vesicles to transfer the gene of choice into cells. Of special interest is the development of more sophisticated liposome vectors, specifically through addressing the issue of targeting (an ability that liposomes have traditionally lacked). Researchers are attempting to incorporate appropriate receptor-targeting proteins or divalent antibody cofactors.

Naked DNA, by contrast, is taken up by the cells with or without the chemical assistance of poly(ethylene glycol) or polycations, or with the use of physical techniques such as electroporation. Problems of serum aggregation and difficulties with the release of the genetic material into the cell are being addressed through a number of means, from modified surface components to addition of DNA packaging molecules (*1*).

At the 16th Annual North American Cystic Fibrosis Conference in October 2002, Targeted Genetics Corporation (*www.targen.com*) presented data from their Phase II clinical trial. They used an aerosol administration technique to deliver an AAV vector, which contained a copy of the CTFR gene that is deficient in CF patients, directly into sinuses. Gene transfer was demonstrated, and patients showed statistically significant improvement in lung function at 30 days after treatment. The company has adapted the vector production system by developing suspension-cultured cell lines engineered to contain the therapeutic gene and AAV packaging genes. The process uses an added helper virus to initiate vector production.

Small interfering RNAs (siRNAs) are poised for clinical trials after promising results in animal models.

Although standard AAV has the fewest potential problems of the available viral vectors in terms of causing a disease response, researchers are still attempting to improve the vectors. Normally AAV infects by binding to the heparan sulfate proteoglycan receptor. As with adenoviruses, researchers are attempting to do more directed targeting, using bispecific antibodies or the genetic modification of viral capsid protein to bind to a variety of receptors, including those for CD34, CXCR4, serpin, integrin, NGR, and fibrinogen. This may work with a variety of cancers (*1*).

Waking Sleeping Beauty

To develop improved viral alternatives, researchers at Stanford University Medical Center are banking on DNA sequences from bacteriophages to provide them with an ideal method for specifically directing and integrating therapeutic genes into human chromosomes. The method avoids the use of a viral vector and the potential for insertional mutagenesis caused by the random insertions done by retro- and lentivirus vectors (*4*). The researchers demonstrated the process by injecting mice with DNA containing the factor IX protein (important in treating some forms of hemophilia) with an attached integration tag sequence from the bacteria along with the gene for bacterial integrase, which causes the tagged sequence to insert. Mice injected with the bacteriophage genes produced 12 times as much therapeutic protein as those lacking the helper sequences.

Similarly, another new method being tested for altering gene therapy is named for the fairy tale *Sleeping Beauty.* It refers to a dormant transposon from fish that has been "awakened" for potential use in human gene therapy (*5*). By incorporating the transposon gene and its insertion sequence with the gene of interest, a different team of Stanford scientists created an adenovirus vector capable of inserting its payload into the host chromosome. They used factor IX protein as well and demonstrated stable integration and production at therapeutic levels in mice over a six-month period.

In other research, small interfering RNAs (siRNAs) are poised for clinical trials after promising results in animal models. Such treatments are an outgrowth of the use of antisense RNA to control gene expression and are being tested for controlling autosomal dominant diseases such as Huntington's (*6*). And in another approach, researchers at the University of North Carolina at Chapel Hill are using gene therapy to mask problems in the normal gene rather than replace it, by using a lentivirus vector to insert a modified small nuclear RNA (snRNA) gene that has sites complementary to a defective splicing site on the pre-mRNA of hemoglobin. The snRNA blocks the aberrant splicing site, preventing it from being used and ensuring that the normal hemoglobin splicing site is used instead (*7*).

Other researchers are pursuing physiological targeting. Although this form of targeting uses the same vectors as normal gene therapy, it changes the promoter sequence on the inserted gene to one that is triggered only in specific cell lines or under specific environmental conditions. Some examples of this currently under study are the use of heat shock elements, the endoplasmic reticulum stress response element, and the hypoxia inducible factor element. With regard to hypoxia, there is growing interest in using the anaerobic bacterium *Clostridium*—which would be not so much a gene vector as a genetically engineered, therapeutic protein vector—to target the inner, oxygen-starved regions of solid tumors.

Ever after?

It is inevitable that the story of gene therapy will continue. Governments, researchers, and a vast number of patients and patient support groups have collectively determined that gene therapy is too important to stop. The risks are real; but in too many cases, the symptoms of the diseases are far worse. Experimentation on the critically ill is not a thing done lightly; the courage of patients giving their last days to studying cures that will probably not yet be effective on them is overwhelming to contemplate. But some might live from their treatments; and later, others may live from the knowledge gained from those treatments that failed. The happy endings promised are needed for a host of diseases that otherwise can only end in death or disability.

Big money is involved as well—life or death for a host of venture-capital biotech companies, not to mention scientific careers that rest upon the promise and growth of gene therapy. Despite its checkered past, the story of genes as drugs is only just beginning.

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Mark S. Lesney is a senior associate editor of *Modern Drug Discovery*. Send your comments or questions regarding this article to mdd@acs.org or to the Editorial Office address on page 3. ■

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