

Cancer Gene Therapy Steadily Advances

By Vicki Brower

This is the first of a two-part series on gene therapy.

Since 1999, when gene therapy research was blemished with the death of Jesse Gelsinger, the first publicly identified person to die in a gene therapy trial, the field has been slowly advancing. Positive phase III trial results with a first-generation gene therapy for cancer, known as Advexin, were announced this spring along with other earlier-stage studies. Researchers in the field hope that these advances will help restore respect to a treatment modality that, alone and in combination with chemotherapy and radiation, shows promise.

Advexin targets the cancer suppressor gene p53, and although this single-gene approach is finally having success—in August, Advexin was accepted for review by the European Medicines Agency based on the phase III trial results that showed that certain patients responded well to Advexin—two decades of research has led many scientists to believe that replacing one gene may not be the best strategy for a complex, multigenic disease such as cancer. Researchers are now developing other gene therapy strategies to treat cancer. For example, Introgen, the Houston-based biotechnology company that developed Advexin, has seven other gene therapies in development that reflect a newer approach: focusing on both therapeutic genes and genes to produce a systemic immune reaction against micrometastases. But despite these advances, all these therapies are still in the preliminary stages of clinical testing.

Like the early history of monoclonal antibodies, gene therapy was a victim of early hype and hope. Over the years, the press devoted more attention to cases of death and illness caused by the experimental treatments than to the field's slow, steady progress.

Although gene therapy has produced no cures for cancer, many researchers think that the approach can succeed where small molecules and proteins have failed, said Thomas Kipps, M.D., Ph.D., professor of

medicine at the University of California at San Diego. Scientists are now investigating the role of gene therapy as part of a combination treatment to treat primary tumors and metastases and as a chemo- and radiation sensitizer.

Gene therapy was initially conceived as a treatment for single-gene diseases, such as cystic fibrosis and hemophilia. In such cases, the therapy involves introducing a normal gene into a patient's cells to replace, repair, or turn off an abnormal gene. Though genes can be delivered into cells without a carrier or vector—known as “naked DNA” gene therapy—a virus, lipid, or polymer is more commonly used as a carrier to transfer genes into cells.

Treating cancer successfully, however, may require an additional step: Besides

Although gene therapy has produced no cures for cancer, many researchers think that the approach can succeed where small molecules and proteins have failed.

targeting abnormal genes with healthy ones, scientists are also introducing genes for cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin 2, to stimulate the body's immune system to fight cancer systemically. Challenges to successful gene transfer include identifying the right vector to deliver genes only where they are needed and extending the expression of the transferred gene so that it can do its job before the immune system eradicates it.

Multiple Challenges

For many years, scientists have been rethinking their approach to the emerging challenges of gene therapy: determining which

genes are likely to be most effective to treat tumors and metastases and whether local or systemic administration is best, discovering and optimizing new vectors best suited to the type of tissue being targeted, improving gene expression, and reducing toxicity.

Much effort has been directed toward developing better nonviral vectors and improving safety of viral vectors; about 70% of gene transfer treatments to date are viral. Viral vectors have the advantage of millions of years of evolution, during which time they “learned” to incorporate themselves into host DNA to survive. But there are many safety issues associated with their use. Viruses administered even locally have been detected in remote, nontargeted tissues, such as reproductive organs. Improperly disabled viruses can trigger exaggerated immune reactions in patients with an underlying disease, which was determined to be the cause of Jesse Gelsinger's death. The viruses can also insert themselves into the wrong sites, which has caused cases of leukemia in children being treated for severe combined immunodeficiency disease.

Another weakness of viral vectors is that, like viruses, a patient's immune system can disable them. The good news is that new viral vectors are being engineered to remain in the body longer, produce longer-term gene expression, and have better safety profiles. Newer modified viral vectors include measles, vaccinia, coronaviruses, and vesicular stomatitis virus. Another strategy to enable viral vectors to avoid immune detection is to disguise them by using polyethylene glycol “stealth” coatings, enabling both carrier and gene to remain in circulation longer before the body destroys them.

Treating Metastases

Some researchers are focusing on gene therapy as a better approach to treating metastases. At the recent annual meeting

of the American Society of Clinical Oncology, researchers at Epeius Biotechnologies in San Marino, Calif., reported the results of a trial testing a tumor-targeted gene transfer treatment, Rexin-G, which showed antitumor activity in several metastatic cancers.

Rexin-G consists of three components: a modified cell-cycle control gene, cyclin G; a retroviral vector that infects only dividing cells and does not elicit the production of neutralizing antibodies; and a tumor-targeting guidance system made of a small peptide that homes in and binds to exposed areas of collagen present in the extracellular matrix of tumors and metastases.

“While the drug has a short half-life, the vector with its genetic payload accumulates in cancer lesions within minutes, delivering its tumor-targeting construct to cancer cells and their blood supply so that only transient gene expression is needed,” said Erlinda M. Gordon, M.D., medical director of Epeius. Developed by Gordon and Frederick Hall, Ph.D., Epeius’ president and CEO, Rexin-G received marketing approval in the Philippines in December 2007 for all chemotherapy-resistant solid tumors, making it the world’s second commercially approved gene therapy. It is now designated as an orphan drug in the U.S. for osteosarcoma, soft-tissue sarcoma, and pancreatic cancer.

Interim results from a phase I/II trial of chemotherapy-resistant pancreatic cancer patients who received 4 weeks of increasing doses of Rexin-G two to three times a week showed that the treatment yielded reductions in a biomarker that has been associated with pancreatic cancer progression. There was no dose-limiting toxicity. Similar results were seen in metastatic breast cancer and sarcoma patients.

Three previous phase I/II studies published in the *International Journal of Oncology*

demonstrated the functional activity of Rexin-G—blocking the cell cycle, preventing the cancer cells from undergoing cell division, and thus causing cancer cells and associated blood vessels to die via apoptosis. Active as a single therapeutic agent in many chemotherapy-resistant tumor types, Rexin-G showed a lack of systemic toxicity, a reduction of tumor burden, and enhanced quality of life.

Epeius has developed a second tumor-targeted agent, Reximmune-C, designed as an immune stimulant, or vaccine, to be used alone or in combination with Rexin-G. Reximmune-C incorporates the GM-CSF gene in place of the cyclin G1 gene to activate the patient’s own immune cells in the area of the residual tumors to prevent recurrence. Preclinical studies and phase I testing of the combination in several cancers showed greater cancer cell death than

“Gene replacement infects only a minority of cells, which is improved by a mild bystander effect, but the efficacy of this approach is limited.”

that achieved with Rexin-G alone, along with an abundance of tumor-infiltrating lymphocytes in the dying tumors and metastases, which are associated with prolonged patient survival. Ongoing research by Gordon and Hall (to be published in September in the *International Journal of Cancer*) shows that this combination of gene therapy kills cancer cells, thereby exposing new tumor antigens, while promoting a local immune response. In a newer version of Reximmune-C, the scientists at Epeius have designed an “off switch” as an additional safeguard—a herpes simplex–thymidine kinase suicide gene, which can be deactivated by the administration of the antiviral drug gancyclovir to precisely control just how long the GM-CSF gene will be expressed by the targeted cancer cells, that is, inside the tumors themselves.

Exploiting Viral Attraction

Different viruses have a natural attraction, or tropism, to certain cell types, for example, herpes simplex to neurons and adeno-

viruses to lymph glands. Researchers have long exploited the natural affinity of certain viruses to particular tissues. Using an adenovirus, Lily Wu, M.D., Ph.D., and colleagues at University of California, Los Angeles recently described in *Nature Medicine* the feasibility of using gene transfer for sentinel lymph node diagnosis. They developed several prostate-specific adenoviral vectors; injected them into cancerous prostate glands; and, using positron emission tomography imaging, observed the rapid accumulation of virus in regional lymph nodes.

“This [approach] could also be used to image lymph node metastases for breast cancer and melanoma, for which sentinel lymph nodes are also mapped to detect the first stage of cancer dissemination,” Wu said. “Less invasive than surgery, gene transfer with an appropriate gene could be used to treat any micrometastases to prevent the watershed event of cancer moving from lymph node into bone.” For treating lymph nodes, longer gene expression would be necessary, which is problematic in immune-competent patients, whose immune systems quickly disable adenoviral vectors. One way of extending transgene expression would be to give a low dose of rapamycin, which extends transgene expression and has the benefit of inhibiting cancer cell growth by targeting an important signaling pathway, mTOR.

Nonviral vectors, including polymers, liposomes (lipid bubbles that resemble cell membranes), stem cells, and artificial chromosomes may be safer than some viral vectors because they do not cause the same immune reactions, but the downside to liposomes can be inefficient entry of DNA into the cell’s nucleus.

Overall, cancer gene therapy is moving away from the Advexin model of local replacement of a nonfunctional gene with a functional one toward more systemic approaches, said Steven Albelda, M.D., professor of medicine at the University of Pennsylvania. “Gene replacement infects



Steven Albelda, M.D.

only a minority of cells, which is improved by a mild bystander effect, but the efficacy of this approach is limited.” Efforts to mobilize an immune system attack on cancer by transferring only cytokine genes, such as GM-CSF and interleukin 2, have had limited success. Two more recent trends in cancer gene therapy are new approaches to immunotherapy and oncolytic (replicating, cancer killing) viruses.

Retooling Immunotherapies

A pioneer in cancer immunotherapy, Steven Rosenberg M.D., Ph.D., chief of surgery at NCI, had long focused on the use of T-cell transfer with the aid of cytokines to treat metastatic melanoma with some success. Moving to gene therapy was a natural extension of that work. Rosenberg genetically engineered the lymphocytes of 17 patients with certain T-cell receptors by using a retrovirus, enabling cells to recognize tumor cell surface proteins and kill cancer cells. The experiment, called adoptive cell transfer,

was hailed as the first successful use of gene therapy to treat cancer. Two patients experienced regression and are disease free more than 3 years later. “The advantage here is that we are not dependent on obtaining natural antitumor lymphocytes from the patients,” Rosenberg said. “We can create the cells we need in the laboratory.”

Rosenberg recently identified additional T-cell receptors that recognize common epithelial cell cancers such as breast, ovarian, and colon cancer and is conducting clinical trials using gene therapy to treat these common cancers.

UCSD’s Kipps is taking a different approach to treat chronic lymphocytic leukemia, which has not only several well-known mechanisms to evade immune detection but also several chronic lymphocytic leukemia-specific antigens that can be targeted, Kipps said. He infected patients’ CD40 B cells with its ligand, CD154, by using an adenoviral vector, which enhances antigen-specific immune recognition by autologous T cells.

Preliminary studies at UCSD Moores Cancer Center produced immune responses in 10 of 11 patients treated with one dose. Recently, Kipps developed an improved human version of CD154, called ISF35, now licensed to Memgen in Dallas, and began a phase II trial in July with 12 patients.

With so many different approaches to fighting cancer with gene therapy, researchers in this field are again feeling the optimism that they felt years ago. “The initial burst of excitement over gene therapy hit inevitable hurdles, but we’ve found that if we stay with it, we are learning many ways to improve treatments and improve safety,” said Jennerex CEO David Kirn. “We are starting to reap the benefits now of the work done earlier—learning which genes to choose, how to improve expression, and amplify their cancer-killing abilities by adding other approaches, such as cancer vaccines and chemotherapy.”

© Oxford University Press 2008. DOI: 10.1093/jnci/djn335