Gene therapy's twisted path to success

Despite the arrival of the first commercialized cancer treatment, the road to generalized therapeutics remains hazardous.

BY MARK S. LESNEY

After years of promising results, coupled to some tragic consequences, gene therapy has finally reached its first commercial milestone with the licensing and marketing in China of a new cancer treatment. Although this commercialization breakthrough is likely to be only the first of many, gene therapy (especially for diseases other than cancer) is not proving the panacea that many researchers hoped it would be. Rather than becoming an easily generalized therapy for a host of different conditions, gene therapy looks likely to achieve success only when it is most individualized—to disease, to vector, and to patient. In fact, the road to success seems to be changing, with some signposts pointing to relatively generalized cancer therapeutics and others, in the more "traditional" gene therapy arena, to the unique targeting of particular inherited diseases.

The first to market

Unexpected by many, the first commercialized gene therapy is the result of Chinese entrepreneurship, expanded for production with the use of cGMP-compliant reactor technology developed in the West. The product, Gendicine (projected to cost $360/injection), is a new treatment for head and neck squamous cell carcinoma—a highly lethal cancer that strikes some 300,000 people yearly in China.

Zhaohui Peng, who worked for 10 years in the United States on gene therapy projects, is now chief of the medicine department at the China National Center of Biotechnology Development and founder and CEO of Shenzen SiBiono Gene Technologies Co. Ltd. He intends to submit an English version of his research, presented in December in a Chinese medical journal, to an interna-

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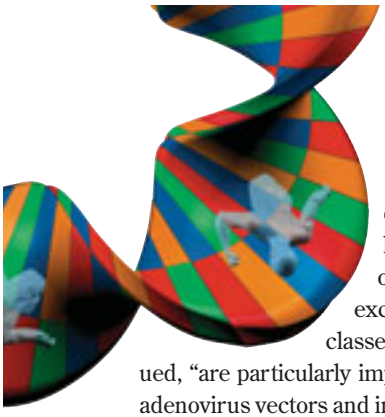
tional publication this year (*1*). SiBiono was founded in 1998 as China's first gene therapy company, and Peng helped to draft China's policies regulating gene therapy product development (*2*).

Whether or not the product will be an unalloyed success, it is not a radical approach. The therapy relies on the use of an adenovirus vector (serotype 5) delivery system expressing the *p53* tumor suppressor gene. In humans, the *p53* gene product is a phosphorylated protein that acts as a transcriptional activator important in regulating cell growth. Its tumor suppression activity is due to its ability to trigger apoptosis by signaling cytotoxic T cells to destroy aberrant cells. Many cancers contain defective *p53* genes or insufficient *p53* gene product to induce an apoptotic response. Gene therapies based on *p53* have been and are currently the subject of numerous clinical trials in the United States and Europe, which are testing its efficacy against various human cancers. Many believe that if there is to be a "magic bullet" against multiple forms of cancer, *p53*-based therapies are the most promising approach. In a parallel attempt to develop a cancer vaccine, isolated DNA or engineered peptides are being used to trigger an immune response against tumors containing mutant *p53*.

In the Chinese study, Gendicine was tested in clinical trials for late-stage head and neck squamous cell carcinoma—a low-survival-rate disease for which there are no U.S. FDA-approved chemotherapeutic treatments available. A total of 120 patients in a combined Phase II/III trial were given single weekly injections of Gendicine for 8 weeks. According to reports provided by SiBiono's equipment supplier, New Brunswick Scientific, "Sixty-four percent of patients' tumors experienced complete regression, and 32% experienced partial regression." In combination with chemo- and radiotherapy, Gendicine also "improved treatment efficacy more than threefold, while, over more than three years of follow-up, no patient relapsed" (*1*). The only side effects detailed were self-limited grade I or II fevers.

Initially, the engineered vector was produced using roller bottles and parallel-plate reactor systems. Commercial production uses a cGMP-compliant packed-bed perfusion bioreactor from New Brunswick Scientific capable of producing 2×10^{15} virus particles in a 14-L autoclavable container. The Chinese State Food and Drug Administration licensed the drug for use in China as a combination treatment with radiotherapy for the cancer on October 16, 2003. SiBiono is conducting further clinical trials of Gendicine in combination with more-traditional therapies for various other cancers, including, the company says, "skin, lung, stomach, intestinal, esophageal, bladder, ovarian, cervical, and breast cancers," with evidence of success such that it "hopes to apply for Chinese approval for some of these indications in 2005" (*2*).

Significant patenting issues may arise around the use of *p53* and adenovirus vectors for delivering the gene. Companies are jockeying for position in this highly promising area of biomedicine and rushing to commercial applications in different countries. In December 2003, Introgen Therapeutics announced that "a patent



directed to classes of adenoviral vectors has been awarded to The Board of Regents of The University of Texas System" and is exclusively licensed to the company. These classes of adenoviral vectors, Introgen continued, "are particularly important in the commercial production of adenovirus vectors and in a number of emerging cancer and gene therapy fields."

Cancer candidates

Gene therapy directed toward the augmentation of *p53* is a promising candidate being tested in clinical trials for a variety of cancers. Similar to Gendicine is Introgen's Advexin, another adenovirus vector containing a *p53* gene, which has been tested in more than 500 patients with different cancers in 20 ongoing Phase I, II, and III clinical trials. In a Phase II study of Advexin as a therapeutic against locally advanced breast cancer, the company reported that "90% of patients treated with a combination of Advexin and chemotherapeutics had 'major clinical responses'" (*2*).

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In tests against recurrent squamous cell carcinoma of the head and neck (for which Advexin was given orphan drug status in the United States), patients receiving the optimal test dose showed cessation of tumor growth or actual shrinkage in 73% of the tumors injected.

In another example in the United States, the Eastern Cooperative Oncology Group, in cooperation with the National Cancer Institute, is initiating Phase I pilot studies using adenovirus *p53* gene therapy and radiotherapy for non-small-cell lung cancer (*3*).

And *p53* is not the whole story for cancer gene therapy. Other genes are involved in cancer prevention and protection, and gene mutations can be addressed using gene therapy as well. Some of these approaches include investigating alternative tumor suppressor genes to *p53*. A variety of genes that code for cytokines show promise, including the interleukin-2 (*IL-2*) and *IL-12* genes, which are used to produce growth factors for killer T cells. In terms of cancer cell "poisoning", gene therapy is being used for the controlled delivery of cytotoxic compounds such as tumor necrosis factor (TNF) to tumor cells, and it also is being studied for the controlled transfer of drug sensitivity to tumor cells. An example of the latter is the use of the herpes thymidine kinase (*TK*) gene. When the *TK* gene is inserted into a tumor cell, the cell becomes sensitive to the antiviral compound ganciclovir, which is comparatively harmless to nontransformed human cells.

"Traditional" gene therapy

But gene therapy is not just about cancer. If there is a "traditional" gene therapy, it is the kind whose goal has been to alleviate a wide variety of genetic disorders caused by inherited systemic dysfunctional genes. Gene therapy is under investigation for a wide variety of conditions, including hemophilia (factor IX gene), Gaucher's and Fabry diseases (glucocerebrosidase and ceramidtrihexosidase genes, respectively), cystic fibrosis (*pGT-1* gene), Alzheimer's disease (human nerve growth factor gene), leukocyte adherence deficiency disease (leukocyte integrin *CD18* gene), and the severe combined immunodeficiency diseases (SCIDs; adenosine deaminase gene for ADA-SCID and IL-2 receptor gamma chain gene for X-linked SCID) (*3*).

For all the promising research in gene therapy, it is the failures and adverse effects of traditional gene therapy that loom largest in the minds of regulators and the public. The death of Jesse Gelsinger in 1998 from an allergic response to the adenovirus vector used in an early clinical trial to develop a liver-directed gene therapy for partial ornithine transcarbamylase deficiency was the first tragedy associated with gene therapy. According to researchers Andrea Amalfitano and colleagues at the Duke University Medical Center, the typical first-generation adenoviral gene therapy vectors that contain a deleted E1 region induce strong immune responses to both the vector backbone and the inserted transgene, which results in "substantial liver toxicity". They noted that the more foreign the transgene, the stronger the toxicity. Their research showed that in mouse test systems, a combined $E1^{-}$, $E2b^{-}$ adenovirus vector could transfer even a highly immunogenic foreign gene without triggering a toxic response in the liver (*4*). Such research is among a host of studies being done to "defang" toxic vectors as well as to expand the repertoire of potential vectors.

The tragic response of Gelsinger to treatment with adenovirus was followed only a few years later by evidence that even a successful application of a different vector—retrovirus-based gene therapy—could prove dangerous to patients. Even though 10 of 11 children treated for X-linked SCID showed clinical improvement, including the production of functional T cells and antibodies to vaccinations against childhood diseases, two patients fell victim to a rare form of T-cell leukemia. Researchers have become convinced that the cancer was caused by the insertion of the therapeutic gene into a known leukemia-associated gene on the host chromosomes that allowed for cancer to be triggered (*5*). These unintended consequences led to temporary moratoriums followed by intense scrutiny of these and similar retroviral trials.

Such vector-related problems have, of course, led to extensive research in other vectoring systems, including liposomes, cation-mediated DNA transfer, electroporation, and adeno-associated viruses, as well as to improvements in the existing adeno- and retroviral vectors.

And more . . .

Gene therapy is being studied not only for inherited genetic diseases but also, more recently, for tissue repair. Chronic wounds can be caused by many conditions, including diseases such as diabetes, as well as by physical pressure, as in bedsores. These wounds not only cause significant pain but also raise the risk of infection in mil-

lions of patients each year.

People with diabetes, for example, can develop peripheral vascular disease and peripheral neuropathy that prevent them from feeling pressure, such as from shoes, that can wound the skin. Such wounds may heal slowly or not at all because of complications from the disease.

The U.S. National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is examining gene-activated matrix (GAM) technology as a method of placing a therapeutic gene contained in a structural matrix into such wounds. To this end, NIAMS is initiating a study to evaluate the safety and clinical utility of topical applications of GAM5—a matrix formed from a bovine type I collagen gel containing the gene for platelet-derived growth factor B (PDGF-B; previously approved for use in treating diabetic ulcers) in an adenoviral vector. "This formulation allows for the migration of wound repair cells into the structural matrix, where they encounter the viral vector and subsequently produce the therapeutic protein within the local wound environment" (*6*).

In another area—infectious diseases—gene therapy is being examined as an alternative or adjunct to anti-retrovirus drug therapeutics in the treatment of AIDS. A Phase II trial sponsored by Johnson & Johnson Research Pty. Ltd. is being initiated to evaluate the safety and efficacy of autologous CD34+ hematopoietic progenitor cells transduced with an anti-HIV-1 ribozyme in patients with HIV-1 infection (*3*).

A road ever onward . . .

Gene therapy is a technology in transition. For lethal cancers and crippling or fatal inherited disorders, researchers and patients believe the promise of gene therapy far outweighs its perils. However, the road is fraught with difficulties and potential dangers, with each disease and its gene-paired therapeutic existing in a complex web of interaction, including such factors as patient physiology and vector choice. But after nearly 14 years of promise since the first gene therapy experiments were performed in 1990, and despite gene-therapy-induced death and disease, the licensing and marketing of the first commercial product of this technology mark a milestone of hope for doctors, patients, and investors alike.

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