The Current Status of Gene Therapy for Prostate Cancer

Mohammad R. Nowroozi, MD, and Louis L. Pisters, MD

As a new treatment approach, gene therapy may affect both local and systemic control of prostate cancer.

Background: Due to limitations of local and systemic therapies for prostate cancer, interest has continued in the development of new treatment modalities. Gene therapy has emerged as a new approach that may prevent or treat disease by using the therapeutic information encoded in DNA sequences. Several institutions are actively experimenting with this approach.

Methods: The authors review the most common genetic alterations in prostate cancer, the principles of gene therapy, and gene delivery including both viral and nonviral vectors. Treatment strategies for both cytoreductive gene therapy as well as corrective gene therapy are described, and the available protocols to date with gene therapy for the treatment of prostate cancer are presented.

Results and Conclusions: More than 150 active protocols are ongoing to evaluate gene therapy in the treatment of cancer, with 13 of these open for patients with prostate cancer. The future of gene therapy as applicable to prostate cancer depends on additional development of vector systems and a better understanding of the genes involved in tumor induction and proliferation. Although gene therapy is clearly in its infancy, it is in an explosive growth phase and holds tremendous promise as a treatment modality for prostate cancer.

Introduction to Gene Therapy

Although the incidence rate and mortality rate of prostate cancer have decreased slightly in the past few years, prostate cancer remains a major health threat to American men. This disease represents the most commonly diagnosed internal malignancy in men and the second leading cause of cancer death among men in the United States. In 1998, an estimated 185,500 men will be diagnosed with prostate cancer, and 39,200 will die of the disease. Biologically, prostate cancer represents a heterogeneous disease entity that exhibits varying degrees of aggressiveness, patterns of metastasis, and response to therapy.

Universal agreement has not been reached as to the best treatment for prostate cancer at any stage. Radical prostatectomy, external-beam radiation therapy, brachytherapy, and cryotherapy can affect local tumor control and are potentially curative in patients with clinically localized disease. In spite of the widespread use of prostate-specific antigen (PSA) in early detection and screening, many cases are not diagnosed until the disease has advanced or metastasized beyond the reach of these local treatment modalities. Hormonal therapy and chemotherapy are the only systemic treatments available at the present time. Unfortunately, progressive disease develops in many patients who undergo these treatments, thus proving them to be noncurative.

Because of the significant limitations of currently used local and systemic therapies for prostate cancer, interest has continued in the development of new treatment modalities. Gene therapy has emerged as an exciting new treatment that may affect both local and systemic control of prostate cancer.

The term gene therapy broadly refers to the transfer of genetic material into human cells and the expression of that material in these cells for a therapeutic purpose. With respect to cancer, the goal of gene therapy is to prevent or treat disease by using the therapeutic information encoded in the DNA sequences.

Evidence suggests that tumor formation is caused by the overexpression of oncogenes or by mutations in suppressor genes in the presence or absence of cancer-causing environmental events. In the first human experiment of gene therapy, Blaese and coworkers successfully transferred the gene for adenosine deaminase into the cells of a 4-year-old girl with severe combined immunodeficiency caused by adenosine deaminase deficiency. The gene therapy dramatically improved her immune system function. Presently, more than 150 active protocols are evaluating gene therapy in the treatment of cancer, with at least 13 of these protocols open for patients with prostate cancer (Table 1).

<table>
<thead>
<tr>
<th>Institution</th>
<th>Principal Investigator(s)</th>
<th>Gene</th>
<th>Vector</th>
<th>Treatment Approach</th>
<th>Delivery</th>
<th>Target Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johns Hopkins Medical Center</td>
<td>Simons</td>
<td>GM-CSF</td>
<td>Retrovirus</td>
<td>Immunotherapy</td>
<td>Subcutaneous</td>
<td>Metastatic PC</td>
</tr>
<tr>
<td>Vanderbilt University Medical Center</td>
<td>Steiner</td>
<td>Antisense c-myc</td>
<td>Retrovirus</td>
<td>Oncogene inhibition</td>
<td>Intraprostatic injection</td>
<td>Advanced PC</td>
</tr>
<tr>
<td>National Naval Medical Center</td>
<td>Chen</td>
<td>PSA gene virus</td>
<td>Vaccinia</td>
<td>Immunotherapy</td>
<td>Intradermal injection</td>
<td>–</td>
</tr>
<tr>
<td>Duke University Center</td>
<td>Paulson</td>
<td>IL-2</td>
<td>Cationic liposome complex</td>
<td>Immunotherapy</td>
<td>Intradermal injection</td>
<td>Locally advanced Medical or metastatic PC</td>
</tr>
</tbody>
</table>

Table 1. – Current Prostate Cancer Gene Therapy Protocols in the United States
Genetic Alterations in Prostate Cancer

Tumorigenesis is a multistep process that involves initiation, proliferation, loss of contact inhibition, invasion, and metastasis of the cancer cell. It is likely that the pathway leading to malignancy undoubtedly involves many complex genetic and epigenetic influences, such as cell-cycle regulation, angiogenesis, immunoreactivity, and cell adhesion. In fact, no single gene defect has been consistently implicated in the development of any type of cancer; rather, this process has been shown to involve many different mutations.  

A number of genetic changes have been documented in prostate cancer, including allelic loss, point mutations, and changes in DNA methylation pattern. The most consistent changes are allelic loss events, with the majority of tumors examined showing loss of alleles from at least one chromosomal arm. The short arm of chromosome 8 and the long arm of chromosome 16 appear to be the most frequent regions of loss, suggesting the presence of novel tumor suppressor genes.

A summary of the genetic alterations occurring in prostate cancer is presented in Table 2.

Table 2. Common Genetic Alterations in Prostate Cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect on Tumorigenesis</th>
<th>Normal Gene Function</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ras</td>
<td>Oncogene</td>
<td>Regulates DNA replication through cell cycle</td>
<td>Mutated in only 2%-5% of prostate cancers in US men</td>
</tr>
<tr>
<td>bcl-2</td>
<td>Oncogene</td>
<td>Interfaces with the p53 pathway and inhibits apoptosis</td>
<td>Elevated in most androgen-independent and advanced prostate cancers</td>
</tr>
<tr>
<td>c-myc</td>
<td>Oncogene</td>
<td>DNA regulatory protein involved in DNA repair and cell proliferation</td>
<td>May be involved in prostate cancer progression</td>
</tr>
<tr>
<td>GST-P1</td>
<td>Suppressor gene</td>
<td>Detoxifies potential carcinogens</td>
<td>Most common genomic alteration in prostate cancer: found in over 98% of cases</td>
</tr>
</tbody>
</table>
Alterations in gene function and expression also are involved in the emergence of androgen-independent disease. Androgen deprivation causes the loss of prostate cancer cells by means of the cell-death process referred to as apoptosis. Some 70% to 80% of patients experience at least partial remission after hormonal therapy, but treatment failure and tumor recurrence are almost inevitable. Several months or years after treatment, prostate cancer eventually progresses to hormone-refractory status. Several potential mechanisms for the development of androgen-independent disease have been described, including activation of epithelial, fibroblast, or other growth-factor pathways; alteration of genes such as ras; mxi1 (a negative regulator of the c-myc proto-oncogene); mutations; overexpression of the bel-2 oncoprotein (suppressor of apoptosis); amplification of c-myc; loss or mutation of tumor suppressor genes Rb and p53; and loss of metastasis-suppressor genes such as E-cad (cadherin) and kai1 in prostate cancer cell lines. After failing hormonal therapy, most prostate cancers remain incurable with conventional chemotherapy.

Principles of Gene Therapy

When considering gene therapy as a treatment approach for prostate cancer, the treatment team must decide what genes to insert, how to deliver the genes, and how to express the therapeutic genes at the site of cancer. Two major categories of gene therapy are cytoreductive gene therapy and corrective gene therapy. Cytoreductive gene therapy includes treatment strategies designed to selectively destroy malignant cells either directly (eg, toxic genes) or indirectly (eg, genes that stimulate immune responses). Corrective gene therapy involves replacing or inactivating defective genes in preneoplastic or neoplastic cells with genes that can slow or reverse the loss of growth-control mechanisms (eg, tumor suppressor genes). These therapeutic genetic modifications can be performed either ex vivo or in vivo, depending on the strategy.

Gene Delivery

One of the rate-limiting factors affecting gene therapy is the development of a safe, reliable vector that can insert the desired gene into the target. Vectors are engineered DNA or RNA sequences into which a therapeutic gene can be inserted. The therapeutic gene generally is positioned adjacent to a promoter sequence for RNA polymerase. From this position, messenger RNA (mRNA) of the therapeutic gene is expressed within the cell. Promoter sequences regulate gene expression downstream and thus can serve as critical pharmacologic targets to modulate gene function. Most viral promoter sequences are, in fact, the endogenous long-terminal repeat sequences. The ability of a vector to successfully deliver the gene of interest into a large number of target cells is defined as gene transfer efficiency. For instance, if 1 in 3 treated cells takes up the vector successfully, then the gene transfer efficiency is 33%. The overall efficacy of the vector is also determined by the degree of gene transduction achieved. Delivery methods are categorized as viral (eg, retrovirus, adenovirus, and adenov-associated virus [AAV]) and nonviral (plasmid DNA, liposome DNA). The advantages and disadvantages of specific types of vectors are summarized in Table 3.

### Table 3. Vectors for Gene Therapy

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrovirus</td>
<td>Easy to produce, efficient transfer, small genome, biology well understood, nontoxic to host cells, high-efficiency genomic integration, stable expression</td>
<td>Targets only dividing cells, risk of replication, carries small DNA sequences only, low transduction efficiency, integration with potential oncogenesis, poor in vivo delivery</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Highly efficient transfer, targets nondividing cells, nontoxic to host cell, high transduction efficiency, immunogenicity</td>
<td>Possible host immune reaction risk of replication, carries small DNA sequences only, low potential oncogenesis, no integration, transient expression</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Less likely to produce immune reactions, targets nondividing cells, nonpathogenic in humans, efficient transfer, good in vivo delivery, integrates into genome</td>
<td>Small capacity, immunogenic, not well studied, risk of replication</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>High titer, large insert size</td>
<td>Antivector immunity, toxicity</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Large insert size</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Plasmid DNA</td>
<td>No size limitation</td>
<td>Low efficiency</td>
</tr>
<tr>
<td></td>
<td>Easy to produce, safety features,</td>
<td></td>
</tr>
</tbody>
</table>

CAM1 = cell adhesion molecules 1
GST = glutathione-s-transferase gene P1
Nonviral Vectors

Nonviral vectors offer several advantages over viral vectors with respect to safety and ease of production. Rigorous tests are not required to validate the absence of replication-competent viruses, which saves both time and money. Another advantage is that nonviral vectors can deliver larger pieces of DNA than viral vectors. However, there are some limitations to traditional methods of plasmid transfection. Several limitations are specific to the use of plasmid transfection in prostate cancer.

Researchers have encountered some difficulties when attempting to passage prostate cancer cells in vitro, and these difficulties make the practical application of plasmid transfection virtually impossible to reproduce. Furthermore, when plasmid transfection is carried out, overall transfection efficiency is very low. With systemic administration, naked DNA plasmids are rapidly cleared from the bloodstream, and DNA degradation occurs within 5 minutes. The stability and transformation efficiency of naked DNA is enhanced by coating plasmids with lipids (liposome-DNA). In this form of nonviral vector delivery, a liposome shell surrounds the plasmid, protecting the DNA from degradation after systemic administration to the host. In addition, the lipid envelope can fuse with tumor-cell membranes, resulting in direct delivery of the therapeutic gene to the cytoplasm of the cell. Plasmid transfection also is not likely to produce immune response and decrease in efficiency because of the presence of blocking antibodies. Because the majority of liposome gene complexes are rapidly cleared from the circulating bloodstream by the liver, systemic therapy cannot be efficiently administered. It is possible that intratumoral injection of liposomes may be able to bypass this intrahepatic clearance of the gene and therefore improve transfection efficiency.

Liposome vectors show significant promise; however, more studies are needed to determine their safety and efficacy in vivo before widespread use.

Viral Vectors

Retroviruses and AAVs have an advantage in that they facilitate stable integration and a steady level of expression once the therapeutic gene is transfected and incorporated into the host genome. As the target DNA is replicated, so too is the inserted therapeutic gene embedded in the transferred chromosomal DNA. Thus, transduction via these vectors can produce durable gene expression. In corrective gene therapy, the durability of the replication is essential for maintaining the corrected cell phenotype over the patient’s lifetime. Furthermore, this can be advantageous in tumor vaccine strategies in which a steady level of gene expression may enhance efficacy. In contrast, adenovirus, vaccinia, and liposomal vector transfer are episomal methods: the transferred gene is expressed without actual integration of the gene into the target cell genome. These vectors are not well suited to clinical strategies requiring durable expression of transferred therapeutic genes.

Retroviral gene expression and dissemination depend on the presence of a rapidly proliferating tumor-cell population. In some malignancies, such as prostate cancer, in which the tumor is inherently slow growing, the retroviral approaches may not be effective. The possibility of pathogenic mutagenesis during chromosomal insertion of the vector and difficulty in isolating high titers of retrovirus for clinical use further limit retroviral transfer.

In contrast to retroviruses, adenoviral vectors can facilitate highly efficient transfection of therapeutic genes into cell culture. The adenovirus has several characteristics that make it ideal for use in prostate cancer gene therapy. The adenovirus enters target cells by receptor-mediated endocytosis after binding to integrins or fibronectins, providing significant tropism to cells of epithelial origin. Unlike the retrovirus, the adenovirus does not depend on cell replication for transfer of expression of its genetic material. The safety of adenoviral gene therapy vectors already has been shown in human trials involving cystic fibrosis, ornithine transcarbamylase deficiency and factor IX deficiency. Because the adenovirus can be efficiently delivered into replicating or nonreplicating cells of epithelial origin, it may be ideal for in vivo systemic therapy, provided an effective avenue of delivery to sites of metastatic cancer can be devised.

Genomic integration rates are low; consequently, there is little risk of long-term sequelae with the administration of adenovirus. Short-term expression has obvious limitations for chronic gene replacement, but it may be considered advantageous for the treatment of neoplasias. For example, transient overexpression of p53 in prostate cancer may be sufficient to activate apoptosis in neoplastic cells without the concerns associated with integration of genetic material into normal cells. Induction of antiviral antibodies and high levels of hepatic deposition of circulating virus would make these vectors inefficient for readministration and systemic administration, although it has been reported that the coadministration of adenovirus and immunosuppressive drugs such as cyclosporin A can drastically increase the duration of transgene expression in hepatocytes and hence suppress cellular immunity against adenovirus.

Some AAV vectors appear to allow durable genetic transduction and are stable for potential direct in vivo gene transfer. However, integration of AAV genomes into target cell DNA appears to be less efficient and has been associated with tendency toward inactivations via deletions or rearrangement during gene transfer.

Cytoreductive Gene Therapy

According to the immune surveillance theory of Burnet, the immune system is responsible for eliminating newly transformed cells; therefore, the emergence of a tumor signals the failure of the immune system. One gene therapy approach involves the activation of antitumor immune responses via T-cell killing of cancer cells.

The most thoroughly evaluated form of cytoreductive gene therapy for cancer so far involves stimulation of an antitumor immune response against a malignancy by vaccinating affected patients with genetically modified tumor cells. In the ex vivo vaccine approach, tumor cells are removed at surgery from the patient, grown in cell culture, and transfected with cytokine genes that stimulate an immune response to antigens present on the tumor cell vaccine. The gene-modified tumor vaccine is then reinfused into the patient in an attempt to generate either a local or systemic immune response against the remaining tumor burden in the patient. These immune effector cells activated at the vaccination site may include T cells, B cells, natural killer cells, and antigen-presenting cells such as dendritic cells and macrophages. In theory, the B-cell or T-cell arm activated by vaccination can then circulate systemically and eradicate or slow the growth of distant micrometastatic cells that share antigens with the genetically engineered vaccine cell.

Several therapeutic cytokine genes have been studied for use in gene therapy. Sanda et al. and Blades et al. showed a defect in cell-surface expression of class I major histocompatibility complex (MHC) in prostate cancer. Cytokines that are dependent on MHC class I processing for immunostimulatory effects, eg, interferon (IFN)-β, interleukin (IL)-1, and IL-6, are not prime candidates for cytokine gene-therapy approaches. On the other hand, cytokines not dependent on MHC class I antigen processing (IL-2 and granulocyte macrophage-colony stimulating factor [GM-CSF]) may be more suitable for prostate cancer gene therapy. Sanda and coworkers found that therapy using gene-modified, irradiated vaccine cells genetically transduced to secrete GM-CSF prolonged survival in animals with prostate cancer; thus, this approach is feasible and has potential for wide application as a treatment strategy for human prostate cancer.
There are, however, limitations to the ex vivo approach. The efficacy of this tumor vaccine approach depends on establishing reliable, high levels of cytokine expression within the tumor. Transfection of tumor-cell vaccine often requires surgical removal of the primary tumor. Furthermore, the harvesting, in vitro culture, and transfection of autologous cells makes the ex vivo vaccine approach labor intensive, time consuming, and expensive. Another cytokine therapy approach involves the introduction of cytokine genes directly into viruses or packaged segments of DNA that can deliver the cytokine gene directly into the tumor cells. Transfection and genomic incorporation result in the chronic local production of cytokines from within the tumor itself. Both viral vectors and liposomally encoded cytokine gene complexes have been shown to be effective in animal and human studies.7

A second form of cytoreductive gene therapy under clinical development is the transfer of drug-susceptible or “suicide” genes. In this strategy, a gene is transfected into tumor cells that encodes the active site of an enzyme, which converts a nontoxic prodrug form of an antineoplastic drug into a cytotoxic one in transfected tumor cells. The prodrug agent is given intravenously after gene transfer. Herpes simplex virus thymidine kinase (HSV-tk) is a classic suicide gene. Others, such as varicella zoster and E. coli cytosine deaminase, have been used in other models.5

**HSV-tk** converts nontoxic nucleoside analogues such as ganciclovir (GCV) into phosphorylated compounds that act as chain terminators of DNA synthesis.9 GCV is safe when given systemically as an chemotherapeutic antivirus and is a high-affinity substrate for HSV-tk. The introduction of the HSV-tk gene has been achieved via adenoviral or retroviral vectors. One of the potential advantages of HSV-tk therapy is that it demonstrates the “bystander effect,” which is the percentage of cells killed exceeds the percentage of cells originally transduced. There are several explanations for this phenomenon, including the possibility that toxic metabolites are transferred between juxtaposed cells and the possibility that systemic immune response is induced.27 To date, close cell–cell contact appears necessary for the “bystander” effect to work. Hall and colleagues28 demonstrated that adenovirus-mediated HSV-tk/GCV therapy leads to systemic activity against spontaneous and induced metastasis in an orthotopic mouse model of prostate cancer. Eastham et al29 showed that HSV-tk/GCV cytotoxic gene therapy can inhibit the growth of mouse and human prostate cancer cells in vitro and can interrupt tumor growth of an aggressive mouse prostate cancer cell line in vivo.

A modification of cytoreductive gene therapy involves tissue-specific expression of drug-susceptible genes. Cell toxin vectors are being constructed that contain tissue-specific promoters to restrict expression of the transferred cytotoxic gene. In prostate cancer, this approach has additional promise for the creation of tissuespecific gene therapy because of the recent discovery of a bipartite, PSA enhancer-promoter sequence. This DNA sequence results in high levels of androgen-sensitive, prostate tissue-specific gene expression. In more than 95% of the cases of metastatic prostate cancers, PSA promoter is used to express detectable levels of the PSA protein. When the PSA enhancer-promoter sequence is combined with the HSV-tk, there is potential for prostate cell-specific expression of the transfected gene, with cytotoxic effects limited only to the target tissue.7

An entirely new class of cytoreductive gene therapy vectors can be generated as a consequence of the identification of specific transcription elements: oncolytic, replication-restricted viruses. Minimal enhancer-promoter DNA sequences for PSA have been put into the adenovirus genome to drive the control of viral replication genes.18 The human adenovirus E1B gene encodes a 55-kd protein (E1B 55K) that binds and inactivates p53. Bischoff et al30 showed that a mutant adenovirus that does not produce this viral protein can replicate in and lyse p53-deficient human tumor cells but not cells with functional p53.

An alternative strategy for cytoreductive gene therapy focuses on bone metastasis of prostate cancer. Osteoblastic response to prostate cancer is the hallmark of progression at this metastatic site. Osteocalcin (OC), a noncollagenous bone matrix protein, is expressed in high levels by osteoblasts. Ko et al31 constructed a recombinant adenovirus, AD-OC-tk, which contains the OC promoter that drives the expression of HSV-tk as suicide gene. They showed that the OC promoter mediated high levels of expression in osteoblast cell lines. Treatment with the AD-OC-tk plus prodrg had potential to eradicate osteoblastic cells that may be required to maintain the survival of osseous metastatic tumors in prostate cancer.

**Corrective Gene Therapy**

Many different genetic alterations have been identified in prostate cancer (Table 2). Most of the lesions represent either overexpression of an oncogene or inactivation of a tumor-suppressor gene. Tumor-suppressor genes and antisense oncogenes are used chiefly as reagents for corrective gene therapy.

In metabolic diseases in which a single gene defect has been identified as the cause of the disease state, such as in cystic fibrosis, replacing the defective gene product is a promising treatment approach. However, the main problem in cancer is that there is no single oncogene or tumor-suppressor gene defect.7

Tumor-suppressor genes are a diverse group of genes present in the normal genome. Their inactivation may result in the initiation or the progression of a cancer. The **p53** gene is the most commonly mutated gene in human cancer. The **p53** gene replacement is a particularly attractive therapeutic strategy because in vitro restoration of wild-type **p53** in many tumor cell lines causes growth arrest or apoptosis. The most common genomic alteration in prostate cancer is inactivation of the glutathione-S-transferase P1 gene (GST-P1).32 This inactivation may occur as early as prostatic intraepithelial neoplasia (PIN), and it is identified in over 98% of cases of clinically detected prostate cancer. GST-P1 is an attractive target gene for corrective gene therapy research. Many other suppressor genes also have been identified as potential targets for prostate cancer corrective gene therapy, including **p21**,33 **CAMS**, and **kai1**. The University of Texas M.D. Anderson Cancer Center with the University of Tennessee and the University of California at Los Angeles have begun phase I clinical trials of **p53** gene replacement therapy for prostate cancer patients (Table 1).6

Antisense oligodeoxynucleotides are short synthetic nucleotide sequences formulated to be complementary to specific DNA or RNA sequences.3 They may be delivered to target cells with annealing of the strands and thus can potentially disrupt transcription or translation of target oncogenes.5 The **bcl-2** oncoprotein suppresses apoptosis, and when overexpressed in prostate cancer cells, **bcl-2** makes these cells resistant to a variety of therapeutic agents, including hormonal ablation drugs. Dorai et al41 have synthesized a hammerhead ribozyme against **bcl-2** mRNA and demonstrated efficient cleavage in vitro. Antisense **bcl-2** is only one example of many antisense oligodeoxynucleotides that could be used in corrective gene therapy.

**The p53 Gene -- An Appropriate Gene for Prostate Cancer Gene Therapy**

The **p53** tumor suppressor gene is a 393-amino acid nuclear phosphoprotein that acts as a transcription factor to control expression of proteins involved in the cell cycle.34 The **p53** gene maps to the P arm of chromosome 17 with loss of heterozygosity resulting in expression of a mutant allele.35 It is continuously synthesized and degraded; its levels are low under physiological conditions. When **p53** levels rise, the result is G1 arrest of cell cycle or apoptosis. The levels of **p53** increase after irradiation and other types of cell damage. These increased levels of **p53** in the nucleus arise from an increase in its half-life. Increased levels of **p53** downregulate **bcl-2** (which inhibits apoptosis) and upregulate the expression of specific genes including **p21** and **Bax**.36 **Bax** is a potent promoter of the apoptotic cell death pathway, and **p21** inhibits cyclin-dependent kinases. Because of its functions, **p53** has been called the "guardian of the genome," and its loss has been implicated in tumor progression.34
There is some evidence that p53 might exert some of its antitumor activity through inhibition of angiogenesis as a consequence of an altered production of thrombospondin (bystander effect).\(^{37}\) In addition, the presence of the wild-type p53 gene is speculated to be useful in accelerating induction of apoptosis caused by cytotoxic drugs such as cis-platinum.\(^{18}\) Thus, corrective gene therapy with p53 is viewed by some as having the potential to improve the therapeutic index of available chemotherapeutic drugs.

The p53 gene is the most commonly mutated gene in human cancer.\(^{18}\) Early studies have shown that approximately 60% of prostate cancer cell lines have mutations in the p53 gene.\(^{7}\) Although primary prostate tumors have few mutations in p53 gene, specimens from advanced stages of the disease and metastases as well as their cell lines frequently display mutations or deletions at both alleles of the p53 gene.\(^{36}\) Transfection of wild-type p53 into prostate cancer cell lines expressing mutant alleles results in loss of tumorigenic capability.\(^{39}\) Gotoh et al.\(^{39}\) demonstrated that p53 was able to suppress tumorigenicity regardless of the background p53 status of the tumor cells.\(^{40}\)

**Ad-p53 - Intraprostatic Gene Therapy (INGN 201)**

Gene therapy strategies that attack the underlying genetic mechanism of this disease offer promise in improving outcomes. In the protocol described here, investigators at M.D. Anderson Cancer Center and the University of Tennessee propose to study the effect of intraprostatic AD-p53 (INGN 201) injection (Table 1). INGN 201 is a replication-defective adenoviral vector that encodes a wild-type p53 gene driven by a cytomegalovirus (CMV) promoter.

Eligible patients will have clinical stage T1c or T2a with high-grade disease (Gleason grade 8-10) on initial biopsy or clinical stage T2b-T2c with Gleason grade 7 and PSA >10, or clinical stage T3.

Patients with locally advanced prostate cancer who are enrolled in the study undergo baseline magnetic resonance imaging (MRI) and ultrasound of the prostate. One course of p53 therapy is defined as three separate injection procedures separated by two weeks, with reevaluation using transrectal ultrasound and MRI. If these show reduction in the size of measurable lesion, then a second course of p53 gene therapy is performed. If there is no change after the first course from the baseline MRI and transrectal studies, then patients are treated with radical prostatectomy. Those patients completing a second course of p53 gene therapy are again reevaluated and proceed to radical prostatectomy.

**The Technique of AD-p53 Injection**

Using a specially designed needle guide that fits to the transrectal ultrasound probe and positions a needle at a measurable distance above the ultrasound transducer head, 17-gauge needles with stylettes are inserted transperineally into the prostate at the positions shown in Fig 1. The needles are advanced to the apex of the prostate and then the stylettes are withdrawn, which allows an 18-gauge core biopsy to be obtained longitudinally along the axis of the prostate. The stylette is then advanced back through the needle, and the needle is advanced along the tract of the biopsy longitudinally from the apex to the base of the prostate. This allows a longitudinal core biopsy to be obtained at the site of injection. The p53 gene in the adenoviral vector is preloaded in 1-mL plastic "TB" syringes that are then hooked onto the 17-gauge needles and injected into the prostate as shown in Fig 2.

A total of 30 patients will be enrolled in this study. Because the study is ongoing and the results are preliminary, it is not possible to comment on the efficacy of the treatment at this time.

**The Future of Gene Therapy**

The future of gene therapy approaches to prostate cancer or other cancers will depend on further development of vector systems at the basic science level as well as a better understanding of the genes involved in tumor induction and proliferation. In the long term, we will look at the construction of artificial chromosomes that can carry whole clusters of genes with their natural control elements into cells. With this new technology also comes new ethical responsibilities to ensure that these
strategies are safe for patients and staff.3

References


From the Department of Urology, The University of Texas M.D. Anderson Cancer Center, Houston, Tex. Dr Nowroozi is currently at the Department of Urology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Keshavarz Blvd, Tehran, Iran.

Address reprint requests to Louis L. Pisters, MD, at the Department of Urology, Box 110, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

Back to Cancer Control Journal Volume 5 Number 6