GENE THERAPY FOR PROSTATE CANCER: WHERE ARE WE NOW?

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ABSTRACT

Purpose: The ability to recombine specifically and alter DNA sequences followed by techniques to transfer these sequences or even whole genes into normal and diseased cells has revolutionized medical research and ushered the clinicians of today into the age of gene therapy. We provide urologists a review of relevant background information, outline current treatment strategies and clinical trials, and delineate current challenges facing the field of gene therapy for advanced prostate cancer.

Materials and Methods: We comprehensively reviewed the literature, including PubMed and recent abstract proceedings from national meetings, relevant to gene therapy and advanced prostate cancer. We selected for review literature representative of the principal scientific background for current gene therapy strategies and National Institutes of Health Recombinant DNA Advisory Committee approved clinical trials.

Results: Current prostate cancer gene therapy strategies include correcting aberrant gene expression, exploiting programmed cell death pathways, targeting critical cell biological functions, introducing toxic or cell lytic suicide genes, enhancing the immune system antitumor response and combining treatment with conventional cytotoxic chemotherapy or radiation therapy.

Conclusions: Many challenges lie ahead for gene therapy, including improving DNA transfer efficiency to cells locally and at distant sites, enhancing levels of gene expression and overcoming immune responses that limit the time that genes are expressed. Nevertheless, despite these current challenges it is almost certain that gene therapy will be part of the urological armamentarium against prostate cancer in this century.

KEY WORDS: prostatic neoplasms, prostate, immunotherapy, gene therapy, DNA

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer death in American men today. An estimated 179,300 new cases of prostate cancer and 37,000 deaths were predicted for 1999.1 The risk of prostate cancer increases steeply with age and continues to increase by a projected 3% to 4% yearly in older men as fewer die of cardiovascular disease.2 Despite concerns that increasing the early detection of prostate cancer by widespread serum prostate specific antigen (PSA) testing may lead to many more cases of small indolent cancers treated unnecessarily, no change was noted in the proportion of such cases at many large medical centers from 1983 to 1996.3 The majority of patients carefully selected for treating clinically localized disease with radical prostatectomy continue to harbor pathologically more advanced disease. Badalament et al evaluated 4 large prostatectomy series with a total of 5,661 patients and reported that 55% had extracapsular disease at surgery.4 The finding of locally advanced prostate cancer, defined as extracapsular extension, increases the likelihood of positive surgical margins at radical prostatectomy and portends a poor prognosis. Repeat examination of the role of radical prostatectomy as monotherapy for extracapsular disease indicates that it is really not curative since 10 and 15-year disease-free survival after radical prostatectomy is only 12% to 60% and 20% to 28%, respectively. Particularly local recurrence rates are as high as 41% 5 years after radical prostatectomy.5 Hence, radical prostatectomy only is not curative in the majority of patients with significant extracapsular disease.

Although it was well established that testosterone deprivation only rarely cures prostate cancer,6 neoadjuvant hormone deprivation was recently administered in an attempt to improve the probability of local cancer control by downsizing or down staging locally advanced prostate cancer before radical prostatectomy. Early studies indicated a decrease in the positive surgical margin rate7 but no changes were noted in the rate of PSA recurrence in a retrospective analysis8 or in prospective randomized studies with 2 years of followup.9,10 Thus, neoadjuvant hormone deprivation has not been shown to change the prostate cancer tumor progression or survival rate.9–12

External beam radiation treatment only also has a high local failure rate in advanced prostate cancer. Zagars et al reported increasing serum PSA after external beam radiation in 17% and in as many as 60% of patients with PSA less and greater than 40 ng./ml., respectively.13 Overall Holzman et al observed a 53% local recurrence rate 8 years after external beam radiation.14 The reported disease-free survival rate for stage T3 disease is 64% at 5, 10% to 35% at 10 and 15% to 18% at 15 years.15,16 When a serum PSA criterion is used, the biochemical failure rate exceeds 90% at 10 years for stage T3 prostate cancer.16 Similarly in a series from Stanford University greater than 60% of patients had increasing serum PSA, indicating cancer progression, at a mean followup of 17 months after definitive external beam radiation treatment,17 including more than 90% with a biopsy positive for prostate cancer.18 In addition, although the true clinical significance remains to be determined, the grade of recurrent prostate cancer after radiation therapy has been shown to be higher than that of the original cancer.19,20


Thus, surgical, radiation or hormonal therapy as monotherapy or in combination does not appear to be adequate to control locally clinical or pathalogical stage T3 prostate cancer, which ultimately leads to morbidity, distant metastasis and decreased survival. Because androgen independent prostate cancer cells eventually lead to death, successful strategies to modify the biological behavior of these cells may potentially have the most significant clinical impact. Clearly other novel treatment approaches to advanced or recurrent disease are desperately needed to achieve long-term local control and particularly to develop effective systemic therapy for metastatic prostate cancer.

The concept that cancer is a genetic disease is based on observations that normal gene expression is frequently altered. Aberrant gene expression may develop due to various reasons, including inherited or acquired gene gain, loss or mutation as cells become malignant. Furthermore, gene expression may be affected at the RNA and protein levels. This altered gene expression may affect normal cellular functions, leading to the phenotypic and characteristic changes associated with malignancy, such as increased proliferation, invasion, the achievement of androgen independence and the ability to metastasize. Therefore, it is conceivable that resuming normal gene expression at 1 or more of these points in the pathway to fatal disease may reverse, arrest or delay this progression. Recombinant DNA technology involving the ability to recombine specifically and alter DNA sequences followed by techniques to transfer these specific sequences or even whole genes into normal and diseased cells has revolutionized medical research and ushered clinicians today into an age of medicine directed at the molecular level. Modifying endogenous gene expression (genes originally in the cell chromosomes) and introducing exogenous genes (genes inserted into the cell that are then expressed in addition to the endogenous genes) into living humans, particularly by viruses, are now possible and currently under way in several clinical trials for prostate cancer. This new type of medicine is called gene therapy. We provide practicing urologists with a review of relevant background information, outline current treatment strategies and clinical trials available for patients, and delineate current challenges facing the field of gene therapy for advanced prostate cancer.

**GENE TRANSFER IN HUMANS FOR THE TREATMENT OF DISEASE**

Gene therapy is a new frontier in the treatment of human disease that represents the pinnacle of applied genetic research. The feasibility of using genes as pharmacological agents for targeting inherited and acquired diseases was only realized within the last 5 to 10 years. It is only 36 years since the genetic code was deciphered, and only within the last 2 decades have scientists manipulated and transferred foreign genetic material into a cell, subsequently altering its phenotypic and functional characteristics. Broadly, genes may be transferred for gene therapy by ex vivo or in vivo techniques (fig. 1). Ex vivo gene therapy introduces foreign genes in the laboratory into cells harvested from the body. The genetically modified cells are then administered back into the patient. For in vivo gene therapy foreign genes are introduced directly into cells while the cells remain within patients. The initial human gene transfer trial in 1989 involved ex vivo gene therapy techniques to replace the defective hemoglobin gene with a normal gene in a patient with thalassemia. The initial in vivo gene therapy trial was done in 1992 and by 1994 more in vivo than ex vivo gene transfer trials were approved by the Food and Drug Administration, and the National Institutes of Health (NIH) Recombinant DNA Advisory Committee.

Gene therapy begins with the identification and characterization of human genes. Genes consist of specific DNA sequences whose copying or transcription into messenger (m) RNA followed by conversion or translation into a protein is controlled by adjacent sequences of DNA called promoters. These genes must be isolated or cloned from their surrounding DNA into smaller, workable lengths. Cloning is generally performed by creating complementary (c) DNA from the gene mRNA and inserting it into another fragment of DNA, usually a piece of circular bacterial DNA also known as a plasmid. The plasmid containing the inserted DNA is then commonly referred to as a plasmid vector. The vector may then be replicated in greater quantities by bacteria to provide a large enough quantity of cDNA for sequencing. It also serves as a vehicle for transferring the gene into a target cell. Since each cell in the body contains the complete genetic information necessary to construct the whole body, the genes transcribed into RNA and translated into protein for a particular cell are highly regulated by combined factors within the cell, including the gene promoters. In addition, regions known as enhancers that markedly increase the level of gene expression may function only in the presence of other cell specific proteins. These factors provide the basis of how certain cells make specific proteins characteristic of those cells, such as PSA in the prostate, although the cells contain all of the information needed to make any protein in the body. For example, highly prostate specific promoters are only actively expressed in prostate cells and not in other cell types, such as those of the lung or breast. These tissue specific promoters or

**FIG. 1.** Prostate cancer gene therapy delivery is broadly categorized as using ex vivo or in vivo techniques to transfer therapeutic genes. In vivo techniques treat tumor cells in situ. Ex vivo treated tumor cells may be used for subcutaneous (s. c.) vaccination or treated effector cells may be infused intravenously (i. v.) as adoptive immunotherapy. GM-CSF, granulocyte macrophage colony stimulating factor.
enhancers may be exploited by designing gene therapy strategies, so that the transfer and expression of genes may be directed to specific cell types, avoiding potential toxicity due to undesirable therapeutic gene expression in adjacent or distant tissue.

A VECTOR IS THE VEHICLE THAT MOVES GENES INTO CELLS

Gene therapy for human disease may be likened to classic pharmacology, in which the administered drug is the gene product encoded by the transferred DNA sequence and the syringe the vector containing its gene with its promoter. Vector technology for delivering genes is also rapidly evolving. The original methods of introducing genes into cells were inefficient and crude. Practically speaking only ex vivo gene therapy was previously considered. However, newer methods have been developed that enable in vivo gene therapy to become a reality. Generally the 2 vector categories used to transfer genes into cells are the nonviral and viral based vector systems. The Appendix shows the features and limitations of some common vector systems. Generally nonviral vectors may carry large pieces of DNA and are easily mass produced but tend to have a low gene transfer rate and uncertain toxicity. Viral based vector systems take advantage of the genetic composition of viruses, which already have the necessary tools to shuttle foreign DNA into cells and in some cases facilitate the incorporation of viral DNA into host cell DNA for optimal gene expression. Viruses produce disease by introducing their genes into a cell and then using the cell machinery to produce more viruses. Viruses for gene therapy are usually genetically altered, so that they do not independently reproduce and, therefore, are not pathogenic. Through DNA recombination techniques viral genes that encode for virus replication are replaced with the cDNA of the therapeutic gene (fig. 2, A). The resulting replication deficient, recombinant virus acts essentially as a molecular syringe for injecting therapeutic genes into target cells (fig. 2, B).22–24

Each virus type has different properties that may be therapeutically exploited. For example, retroviruses have the ability to integrate the therapeutic gene into the genome of dividing target cells. Therefore, the transferred gene becomes an inherent component of cells and may result in a permanent change in cell characteristics. This approach may be ideal for corrective gene therapy when the intent is to correct an underlying gene mutation of each cell. However, a limitation of retroviral vectors is that genes may only be introduced into dividing cells, which may be problematic since prostate cancer tends to have a low proliferative index, meaning that prostate cancer cells do not generally grow and divide rapidly. In contrast, adenoviral vectors may transfer genes into dividing and nondividing cells but therapeutic gene expression is only transient. Thus, this vector may not be satisfactory when the intent is to express permanently the therapeutic gene as part of the target cell genome. On the other hand, transient gene expression of a toxic gene product may provide a built-in safety feature.22 In addition, viral and generally nonviral based vector systems cannot target specific cell types. This limitation is being addressed by a number of strategies, including incorporating tissue specific expression elements such as promoters, or through tumor cell targeting using surface protein antigens or antibodies that localize the vector to more specific cell types. Although some features of each vector system may be exploited for gene therapy, to our knowledge no vector system is currently ideal. However, waiting for the perfect vector before performing preliminary gene therapy trials would be similar to waiting for the perfect car engine before designing a car. The ultimate goal of gene therapy is to develop the ability to administer vectors systematically, providing highly efficient tissue specific gene replacement or expression. Vector technology is rapidly evolving and the vectors of the future are expected to be different from those used today. Nonetheless, in the meantime it is paramount that our understanding of the basic science of gene therapy and vector technology continue in parallel.

GENE THERAPY FOR PROSTATE CANCER

Prostate cancer is a particularly suitable malignancy to study and use in gene therapy trials because of several features. Prostate cancer is common and there is no cure in the majority of patients diagnosed with advanced disease. The prostate gland produces several known gene products, such as PSA, prostate secretory protein, prostatic acid phosphatase and human glandular kallikrein, and hundreds of others that are not yet characterized or have no obvious function. Each of these may potentially be exploited for vector targeting or gene vaccine immunization.25, 26 Moreover, prostate specific promoters and other enhancers that direct the transcription of these prostate unique proteins may also be incorporated into vectors to direct the prostate specific expression of therapeutic genes.23 The prostate gland does not serve any critical life sustaining function, which obviates the need to distinguish normal from cancerous prostate tissue for targeted gene therapy. The prostate gland is also easily accessible by transurethral, transperineal and transrectal approaches for the intratumoral administration of gene therapy. After gene therapy the prostate may be easily evaluated by transrectal ultrasound, digital rectal examination and other standard radiological imaging, such as magnetic resonance imaging (MRI) and computerized tomography. The pattern of prostate cancer spread is also predictable, since it commonly metastasizes to the pelvic lymph nodes and then to the axial skeleton. Therefore, this pattern of metastasis warrants the development of gene therapy strategies to purge bone marrow of malignant cells or develop vectors that have tropism (attraction or affinity) for bone.24, 27, 28 Although prostate cancer gene therapy is controversial, it may readily be followed by serum PSA testing. It may not be a single absolute end point by which to make treatment decisions but it may serve as a surrogate marker of prostate cancer progression.29, 30

As mentioned, prostate cancer also poses some unique and interesting challenges for current gene therapy technology. Because less than 5% of cells are actively dividing at any time within a tumor, the mean doubling time of prostate cancer is usually estimated as greater than 150 days.31 Due to this low proliferative index vectors capable of providing high gene transfer rates independent of cell division are required for treating prostate cancer. Another challenge is that prostate cancer is heterogeneous, not only among but also within individuals. For example, the underlying genetic mutations

![Fig. 2. Adenoviral vector development. A, therapeutic gene cDNA under regulatory control of promoter sequence is substituted into viral DNA, replacing genes controlling virus replication. B, using appropriate helper cells replication deficient adenoviruses carrying therapeutic gene may be produced for gene therapy.](image-url)
that dictate the phenotype of the primary tumor may not be identical to those responsible for metastasis.21, 32 Thus, appropriate corrective gene therapy in an individual may not necessarily be effective for another patient or even for another lesion in the same individual.

**Prostate targeted delivery of gene therapy**

Current technology cannot achieve the ultimate goal of in vivo systemic administration of a vector that results in adequate tissue specific expression of the therapeutic gene in 100% of target cells without other specific or systemic toxicity. Innovative gene therapy approaches being sought to circumvent the limitations of current vectors include more effective delivery routes, tumor targeting, the incorporation of tissue specific promoters and/or enhancers into vectors and increasing cell death by enhancing the phenomenon known as the bystander effect.

**Delivery approaches.** Theoretically the treatment of metastatic disease may require the systemic delivery of gene vectors intravenously. However, in locally advanced, stages T3 and T4 prostate cancer various delivery strategies may help to target gene therapy vectors to the prostate. Lu et al evaluated the effectiveness of an adenoviral vector containing a β-galactosidase reporter gene (a gene whose expression may be detected but does not have significant physiological effects) delivered to the canine prostate by intravenous, intrarterial and intraprostatic administration routes.33 The intra-arterial approach was performed by cannulating the internal iliac artery and advancing the catheter into the inferior vesicle and prostatic arteries. The transduction efficiency of adenovirus β-galactosidase was assessed by X-galactosidase staining of prostate tissue frozen sections, colorimetric β-galactosidase assay and polymerase chain reaction. Intra-prostatic administration of the adenoviral vector resulted in the highest gene transfer rate by far with the least systemic adenoviral dissemination.34 This study provided direct support for clinical intratumoral prostatic injections of gene therapy vectors under ultrasound guidance via the transrectal or transperineal approach in prostate cancer gene therapy trials.35 Further optimization of local gene therapy delivery and gene expression may ultimately be achieved by using various matrices for the vector, such as a gelatin sponge matrix as recently reported by Siemens et al.35

**Tumor targeting.** The identification of cellular pathways or cell surface antigens that are unique or may be exploited for developing drugs or other tumor targeting strategies has been a major pursuit of oncological research for several decades. Using large computerized databases of gene expression36, 37 it is now more feasible to develop intentionally monoclonal antibodies toward known specific antigens that are more likely to be selective for particular tumor cell types and may act as a means of localizing immunotoxins or other conjugates.38, 39 Refining this concept to yet another level, immunotoxins such as Pseudomonas exotoxin or cytotoxic agents such as doxorubicin, may now be targeted by peptide conjugates, which bind to specific antigens or cell receptors such as those for fibroblast growth factors or luteinizing hormone releasing hormone.40 Via such tumor targeting mechanisms systemic toxicity may be minimized while maximizing delivery to the tumor cell targets.

**Tissue specific promoters.** Viral vectors have the ability to bind to and transfer their genes to any mammalian cells expressing the appropriate receptor for the specific virus. Dannull et al demonstrated that a high dose of vectors to any normal tissue which is not tissue specific may have systemic toxicity.41 A strategy for viral and other vectors to target theoretically only prostate cells is the incorporation of a prostate tissue specific promoter and/or enhancer. Therefore, although potentially incorporated into other cell types, expression of the therapeutic gene would be limited to prostate cells since only they would contain the appropriate complement of transcription factors to activate the prostate specific promoter. However, this result depends on tight regulation of the prostate tissue specific promoter-gene vector expression system, so that it would not be expressed at a low level in some tissue, or under changing or leaky conditions that would result in the inadvertent expression of the therapeutic gene in unintended tissue. Unfortunately the prostate promoters currently used in gene therapy are leaky to some degree. What remains to be determined is whether this low level of expression in non-prostate tissue is clinically relevant.

Prostate epithelial cell promoters used for gene therapy include PSA,43–46 probasin,47 mouse mammary tumor virus48–50 and prostate specific membrane antigen.51 The PSA promoter is most commonly used in vector constructs.43, 44 A theoretical concern is that the PSA promoter requires that each androgen receptor and circulating androgen be active. However, the majority of patients with advanced and hormone refractory prostate cancer also undergo androgen deprivation therapy, which may compromise activation of the PSA promoter. To address this potential obstacle Gotoh et al characterized the 5,837 bp PSA promoter as more active than the short 631 bp PSA promoter in an androgen depleted environment and in androgen insensitive cells in vitro.52 Another strategy was modifying a prostate tissue specific promoter, so that activation by another hormone occurred while the tissue specificity of a promoter such as probasin was preserved. Zhang et al developed a retroviral construct containing a glucocorticoid responsive element upstream of the androgen responsive element, which enabled activation of the probasin promoter with dexamethasone.47 Similarly Rodriguez et al demonstrated that the androgen sensitive probasin promoter may also be activated by phenylbutyrate in the absence of androgen.53 Thus, gene therapy using construct type prostate specific promoters may be administered in advanced prostate cancer that is also managed by androgen deprivation therapy.

A limitation of prostate tissue specific promoters is that the level of expression or activity tends to be less than that of other nonspecific or viral promoters.33 There tends to be an inverse correlation of promoter activity with tissue specificity. In a canine prostate model PSA, probasin and mouse mammary tumor virus promoters were prostate specific but had 10 to 100-fold less activity than the Rous sarcoma virus promoter in vivo.33 To help circumvent this problem Pang et al cloned the mutated PSA promoter PCPSA from a patient with prostate cancer and elevated serum PSA.46 The PCPSA promoter had 50-fold greater activity than the standard PSA promoter. Similarly upstream regulatory sequences of the PSA promoter called prostate specific enhancer sequences were cloned from normal and cancerous prostate tissue. In vitro prostate specific enhancer sequences increased PSA promoter activity 72-fold when isolated from normal prostate compared with 1,000-fold when cloned from prostate cancer tissue.44 Recently Dannull et al incorporated the 822 bp prostate specific enhancer and 611 bp PSA promoter into an E1 deleted adenoviral vector.43 Unfortunately intratumoral injection of this vector into various subcutaneous human prostate SCID mouse xenografts resulted in less robust PSA promoter activity than that in vitro. Activity remained markedly less than that of the cytomegalovirus (CMV) viral promoter in the same system.

An alternate tactic involves gene therapy to treat the supporting bone stromal cells in an effort to eradicate prostate epithelial cells metastatic to the bone. Using the osteocalcin promoter, which is active in bone stroma, including osteoblasts, the prostate cancer gene therapy may be directed toward bony metastatic sites. Ko et al reported that osteosarcoma tumors are inhibited after intratumoral injection of an adenoviral vector composed of the osteocalcin promoter controlling thymidine kinase followed by systemic ganciclovir admin-
Moreover, the osteocalcin promoter is active in spontaneous canine prostate cancer bone metastasis, implying that this strategy may be useful for targeting bone metastasis in humans. Combined PSA enhancers, and other prostate specific epithelial and stromal promoters are currently under intense investigation. Whether any of these promoter combinations are ultimately effective for the systemic treatment of prostate cancer with the required level of promoter activity remains to be determined.

**Bystander effect.** A phenomenon after the application of gene therapy in preclinical models is called the bystander effect, which denotes that more cells are destroyed or biologically altered after gene therapy than those predicted by gene transfer (transduction) only. This result is fortuitous in that no single vector system is currently available that may be applied clinically to transfer therapeutic genes into 100% of target cells. Thus, the ability to affect more cells than just those transfected makes gene therapy more clinically feasible. Several hypotheses attempt to explain the bystander effect mechanism, such as intratumoral cell-to-cell transfer of the therapeutic gene, gene product or gene activated toxic prodrug through cellular vesicles, endocytosis or diffusion through gap junctions and cell channels. Alternatively the therapeutic gene or vector antigens may induce an intense immune response that contributes to cell kill. This immune response appears to be natural killer cell mediated. In addition, the bystander effect may be associated with tissue ischemia resulting from endovascular injury secondary to toxic gene product or a nonspecific immunological response. Although to our knowledge the exact mechanism is not known, the bystander effect is a clinical entity that may help to compensate partially for the inefficient in vivo gene transfer limitations of currently available vectors.

**GENE THERAPY STRATEGIES TO COMBAT PROSTATE CANCER**

Gene therapy strategies are rapidly evolving as new gene targets, better vectors and improved gene expression systems become available. The initial approach involving the transfer of genes into cells growing outside of the body in tissue culture is classified as ex vivo gene therapy (fig. 1). Primarily because of new technological advances, including viral based vectors, in vivo gene therapy is also possible. Innovative gene therapy strategies currently used for treating prostate cancer include enhancing the immune system antitumor response, correcting specific aberrant gene expression, exploiting programmed cell death pathways, introducing toxic or cell lytic suicide genes and combined strategies in conjunction with conventional cytotoxic chemotherapy or radiation therapy.

**Immunotherapy.** Class I major histocompatibility complex protein expression by tumor cells is critical for achieving an effective antitumor immunological response. Alterations in or the loss of class I major histocompatibility complex expression represents a common means by which prostate cancer cells may evade the host immune system. Several approaches have been used to stimulate or augment the body antitumor immune response to circumvent essentially the loss of the critical class I major histocompatibility complex proteins (fig. 1). The general immunological approaches developed for prostate cancer involve autologous or nonautologous gene vaccine therapy using ex vivo gene transfer techniques, direct in vivo intratumoral injection of gene therapy vectors containing cytokine genes, adoptive immunotherapy for treating effector immune cells, such as dendritic cells, tumor infiltrating lymphocytes and cytotoxic T lymphocytes, by ex vivo gene transfer techniques, and cytokine immunotherapy. However, the latter is not gene therapy in the purest sense since patients are treated systemically with purified cytokines (immune system signaling proteins), such as interleukin (IL)-2 or granulocyte macrophage colony stimulating factor.

**Gene vaccine therapy** (fig. 1). Tumor vaccines have been generated using autologous tumor or fibroblast cells harvested from patients by biopsy or surgery, while alternatively nonautologous cells such as tumor cell lines are modified by ex vivo gene therapy with genes that encode for cytokines. In addition, before inoculation the genetically altered cells are irradiated to destroy their replication capacity. Cytotoxic T lymphocytes not only recognize tumor specific antigens present on the surface of these inoculated, irradiated cells, but also are induced by local secretion of the transferred stimulatory cytokines. Activated cytotoxic T lymphocytes expand in number and then target and destroy tumor cells throughout the body that share these antigens on the cell surface. Gene therapy with cytokine genes, IL-2 and granulocyte macrophage colony stimulating factor would theoretically stimulate an antitumor response independent of the class I major histocompatibility complex level by using class II major histocompatibility complex expression and natural killer cell mediated tumor lysis. In contrast, to mediate their immune effects tumor necrosis factor- and interferon-γ depend on class I major histocompatibility complex proteins, which as mentioned are often altered in prostate cancer.

Preclinical studies clearly showed that gene vaccines are not efficacious for a large tumor burden but theoretically may be more useful against micrometastasis after primary tumor debulking. Other clinical obstacles of autologous prostate cancer gene vaccines include the fact that sufficient prostate cancer tissue may not be obtainable, tumor tissue may not be easily cultured, cytokine gene transfer efficiency may be poor and large scale production yields of genetically modified tumor cells may be inadequate.

Gene vaccines with some efficacy against prostate cancer in animal models include the gene transfer of cytokine genes granulocyte macrophage colony stimulating factor, IL-2, interferon-γ, tumor necrosis factor-α and B7. Using the transgenic adenocarcinoma of mouse prostate transgenic model of prostate cancer and derived cell lines, Kwon et al demonstrated that increased expression of B7 by normally tumorigenic cell lines resulted in the complete rejection of cancer cells injected subcutaneously into immune and intact but not into nude immunocompromised mice. MAT-LyLu cells grown in Copenhagen rats that produced an increased level of the cytokines IL-2, granulocyte macrophage colony stimulating factor and interferon-γ were also evaluated. The retroviral delivery of IL-2 or granulocyte macrophage colony stimulating factor inhibited prostate cancer tumors and increased animal survival with up to 30% cured after treatment with granulocyte macrophage colony stimulating factor gene vaccine. Similar results were reported for ex vivo IL-2 liposomal gene vaccine therapy. In contrast, Kawakita et al used canary pox virus for genes IL-2, interferon-γ, tumor necrosis factor-α and B7, and observed that only tumor necrosis factor-α and IL-2 delayed RM1 tumors in C57BL/6 mice.

Another approach alters prostate cancer cells or immune cells by the ex vivo gene transfer of genes encoding tumor specific antigens. When inoculated, these modified prostate cells recruit immune effector cells capable of eliciting a wide array of immunological antitumor responses, sensitizing the host immune system against these newly introduced prostate tumor specific antigens. Tumor specific antigens that appear to have relative prostate cancer specificity include PSA, prostate specific membrane antigen, GAGE-T, PAGE, TAG-72, and the prostate mucin antigens mucin-1 and mucin-2. Lubaroff et al demonstrated that a PSA producing adenoviral vector induced potent antitumor immunity in vivo mediated by cytotoxic T lymphocytes and humoral immune responses. Overall by producing cytokines or presenting tumor specific antigens cancer gene vaccines
aim to enhance the induction of T cell immunity for eradicating prostate cancer micrometastasis.

Direct in vivo intratumoral injection of gene therapy vectors containing cytokine genes (fig. 1). This immunotherapy approach treats tumor cells more directly by injecting vectors containing cytokine genes intratumorally. In animal models of prostate cancer Naitoh et al noted that the intratumoral injection of liposome and adenoviral vectors containing IL-2 gene expression systems resulted in the activation of specific T cell antitumor responses.62 Similarly Sanford et al intratumorally injected adenoviral vectors containing IL-12 for treating primary prostate cancer tumors in mice, which significantly decreased the number of lung metastases.63 The molecular mechanism may include the stimulation of T and natural killer cells, induction of interferon-γ and upregulation of fas expression.62, 64 Phase I clinical trials of IL-2 gene transfer vectors for the intratumoral injection of prostate cancer are nearing completion.91

Adaptive immunotherapy (fig. 1). Effector immune cells, including dendritic cells, tumor infiltrating lymphocytes and cytotoxic T lymphocytes, are genetically modified by ex vivo gene transfer techniques. This form of therapy for prostate cancer is still in its infancy. The difficulty of this approach in prostate cancer is in selectively obtaining specific effector cell types from patients, ex vivo amplification of the effector cells and ex vivo gene transfer of specific biological modifiers, such as cytokine genes, back into patients. Thus, immunotherapy may ultimately be effective against micrometastatic disease but greater metastatic or primary tumor volume would most likely require some other nonimmunological therapeutic intervention. Another concern is the theoretical possibility that the autologous or nonautologous cell production of a low level of tumor associated antigens would paradoxically induce immune tolerance, which would suppress the host immunity against prostate cancer. In addition, generally tumor antigens are also weak inducers of the immune system. Therefore, only well designed clinical trials of gene immunotherapy may answer these critical questions.

Corrective gene therapy (fig. 3, A). Corrective gene therapy seeks to replace inherited or acquired defective genes that are important for the normal growth regulation of the cell cycle. The molecular components of the cell cycle include proto-oncogenes, tumor suppressor genes and growth factors with their respective receptors. Similar to most tumor cell types, individual prostate cancer cells represent an accumulation of 1 or more gene mutations or other genetic events. Therefore, it is unlikely that correcting a single gene alteration would have a significant biological consequence on the cancer cell phenotype. Furthermore, this problem is confounded by the fact that current vector technology cannot typically achieve stable in vivo expression of target genes in 100% of prostate cancer tumor cells, as mentioned. Nevertheless, replacing or correcting single gene alterations with various vectors and genes altered the malignant phenotype in several preclinical studies and in some cases eradicated prostate tumors.84, 92–95 These observations are consistent with models in which some genetic mutations were more critical for cellular control than others.84, 94, 96 They imply that additional phenomena, such as the bystander effect in suicide gene therapy,54, 97 may also have a necessary or complementary role. In the more likely scenario that an association of several genetic events responsible for clinically significant prostatic adenocarcinoma is identified delivery techniques for replacing 1 or more genes simultaneously or sequentially may ultimately be required for effective gene therapy.

Most corrective gene therapy strategies involve retroviral or adenoviral vectors administered by intratumoral injection. Prostate cancer preclinical studies were reported in which an assortment of tumor suppressor genes were replaced, including AdCMVp53,96, 98–100 retroviral LXS N BRCA-1,34 AdCMVp21,96, 99 and AdCMV CAM1.101 Another critical cell cycle component, cell cycle dependent kinase inhibitor p16, is commonly changed in prostate cancer.102, 103 Inactivation of p16 is common in the majority of human prostate cancer cell lines104, 105 and alterations of p16 were also reported in prostate cancer.103 Initially p16 mutations in primary human prostate cancer were thought to be rare. Subsequently controversy arose about whether p16 inactivation was critical only in rapidly dividing cancer cells in tissue culture rather than in primary human prostate cancer because homozous deletions or intragenic mutations of p16 were apparently rare.106 However, this controversy was laid to rest by the recent discovery of frequent microdeletions within the p16 gene.

These microdeletions were difficult to confirm by standard molecular techniques because normal cells were present within the tumor specimen.106 Microsatellite analysis using markers close to the p16 gene revealed that a wide range of tumor types, including prostate cancer, had less than 200 kb deletions of each p16 allele.106 Unlike other tumor suppressor genes that are primarily inactivated by point mutation, small homozygous deletions represent a major mechanism of p16 inactivation in cancer.106 In fact, using this technique Cairns102 and Jarrett104 et al identified p16 homozygous deletions in 40% of human primary prostate cancers. Moreover, with progression 46% of prostate cancer metastatic lesions involve the loss of p16 heterozygosity.104 Even more interesting is the fact that when androgen deprivation therapy fails, there is a 71% allelic loss of 9p in the region of p16.21 Using the adenoviral vector GTX-001 containing p16 Steiner et al noted that p16 replacement suppresses cell growth and induces cell senescence in various prostate cancer cell lines.94 A single in vivo intratumoral injection of GTX-001 resulted in a 70% decrease in PPC-1 human prostate xenografts in nude mice and prolonged animal survival.94, 107, 108 In another animal prostate cancer model Gotot et al had similar results using an AdCMVp16 vector.96 Oncogene over expression is another way that cancer cells commonly lose control of the cell cycle.109 An approach to decrease the growth response directly the expression of a reverse or antisense strand of mRNA, which is complementary to the normal or sense strand for that gene. Antisense mRNA binds or anneals to the sense strand and effectively prevents translation of the protein from that mRNA, suppressing its translation into protein. Since prostate cancer commonly involves c-myc over expression, Steiner et al constructed a retroviral LXS N vector containing a prostate specific mouse mammary tumor virus promoter driving anti-sense to the c-myc gene.29 A single intratumoral injection of retroviral mouse mammary tumor virus antisense c-myc markedly suppressed and even eradicated some DU145 prostate cancer xenografts growing in nude mice. They determined that the molecular mechanism was the down regulation of c-myc mRNA and protein expression as well as the induction of apoptosis by the down regulation of bcl-2 protein.49 Using a similar approach Kim et al observed that an adenoviral vector containing an antisense erb-B-2 gene (Ad anti-erb-B-2) inhibited the over expression of growth factor erb-B-2 in prostate cancer cells, resulting in their destruction.28 This tactic was used in vitro to purge selectively metastatic prostate cancer cells from bone marrow.25

In vivo animal model systems have also been used to identify possible alterations in peptide growth factor and growth factor receptor expression during prostate cancer development and progression as potential targets for gene corrective therapy. Interestingly peptide growth factor receptor fibroblast growth factor receptor 2 IIIb is altered with prostate cancer progression.110 Matsubara et al reported that in the Dunning rat adenocarcinoma cell line AT3 fibroblast growth factor receptor 2 IIIb restored the suppression of the prostate
cancer growth rate after transfection with fibroblast growth factor receptor 2 kinase.111 Thus, restoring a single underlying mutation may change the malignant phenotype. Similarly when investigating changes in the insulin-like growth factor family axis, Kaplan et al recently observed that prostate specific insulin-like growth factor-I mRNA expression increased and insulin-like growth factor-2 mRNA expression decreased during prostate cancer progression.112 However, the expression of types 1 and 2 insulin-like growth factor receptor mRNA was not altered during primary prostate cancer progression in intact transgenic adenocarcinoma of mouse prostate mice but dramatically decreased in metastatic lesions and in androgen independent disease. As in reports of clinical disease, serum insulin-like growth factor-1 levels increased early as prostate disease developed. These results imply that the insulin-like growth factor growth factor axis may also serve as a therapeutic target for treating prostate cancer.

Generally corrective gene therapy holds the promise that restoring the expression of 1 or more genes may revert the malignant phenotype of the cancer cell to or toward normal. Other corrective gene therapy approaches indicate that gene replacement induces cell death in a proportion of tumor cells. Still others may incite host responses, such as the bystander effect and other immunological responses, which result in additional tumor cell death via complementary or synergistic mechanisms.

Preliminary studies of corrective gene therapy also raise important clinical concerns unique to gene therapy. It has always been a dictum in cancer therapy that each cancer cell must be
A GAL-4 responsive element is placed upstream of the polyglutamine gene. Polyglutamine is a potent apoptotic protein that in this study was produced selectively in PSA producing cells. Similarly Hyer et al observed that adenovirus mediated transduction of the fas ligand, a component of cell death pathways, induced apoptosis in LNCaP, PC3 and DU145 prostate cancer cell lines in vitro.100 Marcelleti et al reported that transducing prostate cancer LNCaP cells with an adenoviral vector containing caspase-7, a potent and critical modulator of apoptosis, also induced programmed cell death.114 Another molecular approach targeted bcl-2, an oncogene with apoptotic pathways, also known as programmed cell death, are also being investigated genetic mutations responsible for the malignant phenotype. The types of suicide gene therapy strategies that gene therapy design may be to target critical cellular processes. Gene therapy for targeting critical cell machinery (fig. 3, C). In as in classic pharmacology, the basis of rational anticancer gene therapy design may be to target critical cellular processes. Lee et al designed liposomal vectors containing a PSA promoter upstream of antisense topoisomerase II or antisense DNA polymerase α.117 Topoisomerase II and DNA polymerase α are critical molecular components of DNA replication. The treatment combination of PSA-antisense topoisomerase II and PSA-antisense DNA polymerase α liposomes had the greatest inhibitory effects on the prostate cancer cell lines LNCaP, DU145 and PC3 in vitro. Similarly Williams et al used a retroviral vector that incorporated the antisense eIF4E gene for treating prostate cancer cells.115 Prostate cancer cells were previously shown to have over expression of eIF4E, which is a rate limiting factor in the translation initiation of growth controlling genes such as cyclin D1, c-fos, c-myc, v-epidermal growth factor and b-fibroblast growth factor. A single intratumoral injection of retroviral antisense eIF4E suppressed prostate cancer xenograft growth for up to 65 days.118 Thus, the rational design of gene therapy vectors for disrupting critical molecular events required for cellular function is an enticing strategy against prostate cancer.

Suicide gene therapy (fig. 3, D). A suicide type of gene therapy strategy may have the most promising clinical potential. In this strategy vectors are used to introduce the therapeutic gene into cancer cells. After the gene product is expressed the cell is destroyed without regard to the underlying genetic mutations responsible for the malignant phenotype. The types of suicide gene therapy strategies that have emerged are gene directed enzyme prodrug treatment and gene directed production of a cellular toxin.

Gene Directed Enzyme Prodrug Treatment: The gene directed enzyme prodrug treatment approach involves prodrug enzyme gene therapy followed by the systemic administration of its specific prodrug. After gene transfer of the prodrug enzyme gene cells producing the prodrug enzyme render the cell capable of converting a nontoxic prodrug into an activated lethal drug. This activated drug not only kills the cell that produced the toxic drug, but also its neighboring cancer cells. This bystander effect may be impressive and kill 100 to 1,000-fold more cells than predicted by the gene transfer rate only. Thus, low gene transfer efficiency may be compensated by a high bystander effect. Local activation of the cancer killing drug maximizes the toxic metabolite concentration at the tumor site and minimizes systemic toxicity as the drug becomes diluted in the total blood stream volume of distribution.

The most widely used gene directed enzyme prodrug treatment system against prostate cancer is the Herpes simplex virus (HSV)-thymidine kinase and ganciclovir system.119,120 The nucleoside analogue ganciclovir is converted by HSV-thymidine kinase, an enzyme not normally expressed in mammalian cells, into a phosphorylated compound that is then incorporated into DNA during DNA replication. This incorporation causes DNA chain termination and the selective killing of dividing cells. Eastham et al used an adenoviral vector containing h. simplex virus-thymidine kinase to sensitize human and murine prostate cancer cells to the toxic effects of ganciclovir in vitro and in vivo models.119 Ad HSV-thymidine kinase gene therapy followed by ganciclovir suppressed prostate cancer growth and prolonged survival in mice with prostate tumors.119 Hall et al also reported that Ad HSV-thymidine kinase injection of orthotopic mouse prostate reconstitution model cell line tumors followed by ganciclovir administration suppressed tumor growth and decreased the rate of spontaneous lung metastasis.121,122 An immune basis for these effects was demonstrated in the cytolytic activity challenged mice with an injection of prostate cancer cells into the tail vein followed by excising subcutaneous prostate cancer tumors. In the animals with previously treated primary tumors lung metastasis decreased 40%. This effect appeared to be partially mediated by natural killer cells.123

Other gene directed enzyme prodrug treatment strategies investigated in prostate cancer include the cytosine deaminase-flucytosine system, in which cytosine deaminase converts flucytosine to the chemotherapeutic agent 5-fluorouracil.119,120 Kim et al transferred the cytosine deaminase or HSV-thymidine kinase gene into the murine bone marrow stroma cell line D1.121 Co-cultures of D1 cells and human prostate cancer cell lines followed by the appropriate prodrug destroyed prostate cancer cells when as little as 20% of the D1 cells in the co-culture produced the prodrug enzyme. Blackburn et al used an adenoviral vector incorporating the heat shock protein (HSP) 70 promoter and the cytosine deaminase or HSV-thymidine kinase gene for treating PC3 cells.120 In this system hyperthermia to 41°C activated the HSP-70 promoter, which then directed prodrug enzyme expression. Thus, systemic administration of the prodrug and local heat enabled selective expression of the prodrug enzyme in the intended tissue.120 Martiniello-Wilks et al used another system with an E1a deleted adenovirus containing the Escherichia coli DeoD gene purine nucleoside phosphorylase as a prodrug enzyme under the control of the PSA promoter.121 The prodrug used was 6-methyl-9 (2 deoxy-β-D erythro-pentofuranosyl) purine (6 MDP), which converts 6 MDP into a toxic nonphosphorylated purine capable of killing quiescent and proliferating cells when incorporated in mRNA or DNA during synthesis.121 The purine nucleoside phosphorylase-6 MDPR system was efficacious against the human prostate cancer cell line PC3.121 Other gene directed enzyme prodrug treatment systems using assorted prostate specific promoters and vector types are currently under intense investigation.

Gene Directed Production of Cell Toxin: With this strategy, which is similar to gene directed enzyme prodrug treatment,
the transferred gene kills the cell independent of the underlying cancer mutations. Unlike gene directed enzyme prodrug treatment this approach does not require a prodrug. Rodriguez et al screened numerous direct biological toxins known to kill mammalian cells by cell cycle independent mechanisms to determine which would be best against human prostate cancer.123 Diphtheria toxin was the most toxic substance used. This toxin kills rapidly independent of p53 or androgen sensitivity status, and kills dividing and nondividing cells alike.123 However, this approach has limitations. This toxic gene must be incorporated into vectors that contain highly prostate specific promoters that are under tight regulatory control since diphtheria toxin is so biologically toxic that even a small amount of leaky promoter activity in nonprostatic tissue may be lethal. Also, mass production of adenoviral vector containing the diptheria toxin gene is difficult because of the toxic effects of the diptheria toxin gene product on the packaging cell line, resulting in low production titers.251

**Oncolytic virus gene therapy** (fig. 3, E). Because of safety reasons, almost all current vectors are engineered to be replication incompetent. This term means that after the virus with the gene is transported into a cell, the virus cannot express its viral genes that commandeer the cell to produce more virus and enter the lytic cycle, which would release more virus particles to infect additional cells. Consequently viral vector effectiveness directly correlates with transduction efficiency and the ability to be administered in repeat doses. Recently 2 types of replication competent viral vectors were developed. A conditionally competent adenoviral vector was mutated, such that the virus does not express the viral protein E1b.124 The wild-type adenovirus uses the E1b protein to block p53 from performing its normal function, which is to prevent the replication of cells with damaged DNA. Theoretically mutant E1b virus may infect, replicate and lyse p53 deficient cells but it does not affect normal cells with functional p53.124 Thus, these mutant viruses are oncolytic to cancer cells harboring p53 mutations. However, in prostate cancer there is generally a lower rate of p53 mutations than in other cancer types. Only 10% to 20% of prostate cancers have mutated p53 and most such mutations tend to develop in higher grades and stages of prostate cancer.119, 125, 126

Other replication competent oncolytic viruses were designed based on an attenuated cytotoxic adenovirus type 5 vector incorporating a prostate specific enhancer and promoter coupled to the E1a gene.127, 128 The E1a viral product enables the virus to reproduce and enter the lytic cycle. The PSA promoter theoretically limits E1a production to PSA producing cells.127 The level of E1a production was higher in PSA producing cells, such as LNCaP, than in those producing little or no PSA.251 In vivo CN706 viral vector caused the regression of LNCaP tumors and decreased PSA production after a single intratumoral injection.25, 26, 27 Although intratumoral injection of CN706 in patients with prostate cancer is the basis of a clinical phase I trial, CN706 raises several clinical concerns. Due to a lack of adequate large animal models to our knowledge there is no experimental support that systemically injecting CN706 lyases prostate cells exclusively. In some systems wide variation in the level of E1a expression has little effect on inducing viral replication, implying that the level of E1a does not correlate with viral replication and subsequent cell lysis. This implication is especially worrisome since the PSA promoter has been shown to be leaky because other types of cells produce PSA in addition to prostate cells. For example, cells that line the urethra produce abundant PSA. Theoretically CN706 only ceases replication and lysis when all PSA producing cells are eradicated. Furthermore, there is some question of the usefulness of any PSA promoter vector for treating cells that do not produce PSA and in patients on androgen deprivation therapy. Nevertheless, studies of the CN706 or any other vector containing the PSA promoter that involve intratumoral injection would be critical for increasing our understanding of this field until newer, tissue specific vector technology becomes a reality.

**Combined gene therapy approaches and other treatment modalities.** As discussed, it is clear that neither surgery, radiation therapy, hormonal therapy nor chemotherapy is currently adequate alone or in combination for treating advanced prostate carcinoma. Gene therapy as monotherapy against prostate cancer currently remains in its infancy. Although preventive strategies are being entertained, the ultimate clinical use of gene therapy for improving cancer treatment would most likely be in combination with surgery, radiation or chemotherapy in men with pathologically advanced disease or those at high risk for locally advanced disease. Moreover, clinical trials using this multimodal approach are more justified until the science of gene therapy becomes better understood. The most commonly used strategies involve gene therapy combined with DNA damaging agents, although effective regimens combining gene therapy with antimitabolites were also recently reported.129 Improved prostate cancer tumor suppression and apoptotic induction were described for Ad5CMVp53 combined with pacitaxel130 and Ad5CMVp53,131 Adp16 or Ad.Egr tumor necrosis factor-α132 combined with ionizing radiation. Taxol appears to up-regulate Coxsackie adenovirus receptors on the cell surface, enhancing transgene delivery and, therefore, better gene expression.133 Other combined approaches to prostate cancer include gene directed enzyme prodrug treatment Ad cytosine deaminase and flucytosine with radiation,134 and gene directed enzyme prodrug treatment. Ad HSV-thymidine kinase with ganciclovir and AdIL-12 cytokine therapy were studied in prostate cancer.135 These types of multimodal therapy for improving conventional prostate cancer treatment represent the most promising immediate clinical applications for prostate gene therapy.

**Prostate cancer gene therapy clinical trials**

**Challenges of gene therapy clinical trials.** In many ways identifying a promising gene therapy target and generating preclinical supporting data in the laboratory are the easy initial steps on a long pathway to clinical trials. As with any new drug, before performing a clinical trial basic in vivo pharmacology and toxicology studies are mandatory. However, gene therapy trials are currently complicated by the obligation to establish not only the pharmacology and toxicology of the therapeutic gene, but also the toxicity of the gene vector, for example adenovirus, retrovirus, liposomes and so forth. Successful efforts to improve the production of increasingly higher viral titers have resulted in the ability to deliver efficiently trillions of viral particles directly to a site. The local, regional and systemic host distribution of and response to these vectors must be established and monitored. When a replication deficient viral vector is used to limit potential transmission and expression toxicity, assays must be developed and performed to detect replication competent viruses in addition to screening for contaminating viruses. Optimizing vector delivery by intravenous, intra-arterial or direct injection may require additional investigation. Furthermore, viral shedding from a patient to the surrounding environment, personnel and family must be considered. As standard gene delivery vectors and techniques are developed and experience with them accumulates, these additional requirements may decrease with time.

The toxicity of gene expression would largely be determined by the particular therapeutic strategy of a trial. For example, gene replacement therapy that attempts to restore physiological levels of gene expression may have limited toxic side effects since most normal cells already express the target gene. In other cases to limit toxicity expression of the therapeutic gene may be restricted to a specific tissue or cell type by tissue specific gene regulatory elements such as PSA.
major challenge in the field of gene therapy is establishing adequate vector delivery systems with acceptable toxicity, a research (see table).136

Some naturally readable text follows...
GENE THERAPY FOR PROSTATE CANCER 1131
direct functional assays using technologies such as positron emission tomography.

Current gene therapy protocols for the prostate. At this time 18 gene therapy trials for prostate cancer have been approved by the NIH (see table).136 To date the approved trials have had a phase I or I-II design. The preliminary results of these trials have only recently been forthcoming as meeting abstracts and peer reviewed publications. The initial approved gene therapy trial in prostate cancer of Simons et al involved patients with metastatic prostate cancer in the lymph nodes at radical prostatectomy (see table).67, 137 As in previous studies of metastatic renal cell carcinoma treatment,138 the ex vivo transduction of autologous, irradiated prostate tumor cells with retroviral MFG-granulocyte macrophage colony stimulating factor was done to generate vaccines that were administered subcutaneously every 2 weeks until available cells were exhausted. Vaccination site biopsy revealed infiltrating macrophages, dendritic cells, eosinophils and T cells. No dose limiting toxicity was observed. At an average followup of 20 weeks ultra-sensitive PSA criteria revealed progression in 7 of 8 patients. This study demonstrated the feasibility of autologous granulocyte macrophage colony stimulating factor transduced prostate cancer vaccines, limited only by the in vitro expansion of vaccine cells.

To circumvent this limitation a followup trial by Simons et al was approved and is currently in progress that is powered to estimate the efficacy of using the ex vivo, granulocyte macrophage colony stimulating factor transduced, allogeneic prostate cancer cell lines PC3139 and LNCaP140 as a vaccine (see table). Patients are vaccinated weekly for 8 weeks with irradiated, granulocyte macrophage colony stimulating factor secreting PC3 and LNCaP prostate cancer cells. Of the 21 patients treated to date 1 has a partial PSA response of greater than 7 months in duration, 6 patients had a greater than 25% decrease in tumor size, as measured by endorectal coil MRI, 3 patients had disease stabilization at 2 months and 7 patients had PSA velocity or slope decreased in 71% of cases. No dose limiting toxicity was identified. Although the dose and schedule are still being optimized to determine potential therapeutic efficacy, numerous new post-vaccination IgG1 antibodies were identified, indicating that immune tolerance to prostate cancer associated antigens may be broken. Therefore, this approach appears to be a clinically feasible means of treating prostate cancer.

Preliminary results of the initial trial approved to use direct transrectal prostatic gene therapy injection were recently reported by Steiner et al.144 To our knowledge this study was the first to evaluate gene replacement strategy. A total of 21 men received a single 1.1 cc injection of replication deficient adenovirus containing the HSV-thymidine kinase gene under the RSV promoter, followed by 14 days of intravenous ganciclovir. The dose of Ad HSV-thymidine kinase was escalated from 1 x 10^6 to 1 x 10^11 IU. After treatment 3 and 1 patients had a greater than 50% decrease in PSA and negative biopsy, respectively. Local and systemic toxicity was mild except for the final patient, in whom severe thrombocytopenia developed with abnormal liver function test results. Another study to investigate multiple sites of injection before radical prostatectomy is currently in progress. These followup studies may provide important histological confirmation of the tumor response to intraprostatic cytotoxic gene therapy. In addition, assessing the long-term patient outcomes of beneficial local or possible systemic immunological bystander effects would be interesting.

An alternative approach to intraprostatic gene replacement or cytotoxic therapy that may indirectly enhance a host immunological response against tumor cells is the administration of gene therapy to stimulate intentionally an immune response. Using a liposomal vector Patel et al treated 12 patients before radical prostatectomy and 9 with recurrent prostate cancer after radiation or cryotherapy with 2 injections of intraprostatic IL-2.145 A total of 40 injections of 300 to 1,500 μg. IL-2 were administered. In the men treated before radical prostatectomy average PSA decreased by 53.3 ng/ml preoperatively and in 7 of 9 cases undetectable PSA was maintained 24 to 56 weeks postoperatively. Average PSA decreased 3.6 and 1.3 ng/ml after 1 or 2 injections, respectively, in patients with recurrent disease. Although the significance of these PSA responses and differentiation of the effects of gene therapy from surgery only are not yet clear, this liposomal delivery strategy appears to be safe. In addition, it may be effective locally and promote a systemic immune response.

Three trials were approved and initiated using Prostvac, a recombinant vaccinia virus expressing PSA, as a tumor associated antigen immunotherapy strategy (see table). Chen et al treated 30 men with hormone refractory prostate cancer who were withdrawn from antiandrogen therapy with a vaccinia inoculation followed by Prostvac immunization.144 Patients received 2.65 x 10^6 or 2.65 x 10^6 plaque forming units by dermal scarification, or subcutaneous administration of 2.65 x 10^6 or 2.65 x 10^6 plaque forming units once monthly for 3 months, followed by repeat staging. Local erythema developed at the vaccination site in all cases and all toxicity was grade 0 to 1. Disease was stable in 4 of the 14 patients who completed the treatment course, including 2 with subsequent progression at 5 and 6 months, while disease continued to progress in 10.

In a population with less advanced disease Eder et al treated 24 men with increasing PSA after radical prostatectomy and/or radiation therapy with 2.65 x 10^{0–8} plaque forming units as 3 consecutive monthly doses without significant toxicity.145 Patients were withdrawn from the protocol when there was clinical progression or 3 monthly increases in PSA greater than 50% of baseline. Of the 23 men 12 maintained stable disease status for 10 months or greater. In another study Sanda et al administered Prostvac once in 6 men with androgen modulated prostate cancer recurrence after radical prostatectomy.146 In addition to toxicity, they evaluated time to serum PSA increase after the interruption of androgen deprivation therapy and per-

† Therion Biologics, Cambridge, Massachusetts.
formed Western blot analysis to determine anti-PSA antibody production. Again no dose limiting toxicity was observed. Undetectable serum PSA was maintained in 1 case more than 8 months after antiandrogen therapy was withdrawn and immunization done. This study emphasized the variable interval to the return of serum testosterone levels after antiandrogen therapy is withdrawn, as observed by Oefelein.147 An IgG antibody against PSA developed after immunization in 1 case. Notably 2 of 6 patients had anti-PSA antibodies before immunization. The significance of this finding and the implications of vaccination against tumor associated antigens is unclear at this time. However, it was demonstrated that an immune response may be solicited by this gene therapy strategy. Additional trials of variations of these types of gene therapy strategies were approved for locally advanced and metastatic prostate cancer (see table). The NIH Office of Recombinant DNA Activities regularly updates its comprehensive list of gene therapy trials.136

CONCLUSIONS

This decade marks the beginning of history when human gene therapy has become a biomedical commodity. Since the initial gene therapy trial in 1990, there have been more than 125 phase I, 25 phase II and 1 phase III human clinical trials in the United States. Worldwide more than 363 clinical gene therapy trials have been approved with more than 4,000 patients enrolled. Of the various diseases treated malignancy is first at 68% of cases, followed by AIDS at 18% and cystic fibrosis at 8%. Widespread clinical acceptance of this new field and progress in identifying new potential target genes aided by the Human Genome Project have further fueled the rapid expansion of this new technology.

Gene therapy for advanced localized prostate cancer is safe and feasible. However, it is clear that gene therapy for prostate cancer remains in its infancy since to our knowledge no clinical data yet demonstrate even short-term gene therapy activity with a significant clinical response. The challenges that lie ahead for the widespread use of this technology in this century include finding the appropriate target or therapeutic genes for gene therapy, determining the safety of gene therapy in humans, identifying strong tissue specific promoters and other ways to target prostate cancer exclusively, systemically delivering gene therapy to distant prostate cancer cell targets, and ultimately developing appropriate short-term and mid-term end points to assess the efficacy of this new treatment option for human prostate therapy.

APPENDIX: FEATURES AND LIMITATIONS OF VECTORS THAT MAY BE USED FOR PROSTATE CANCER GENE THERAPY

<table>
<thead>
<tr>
<th>Vector Type</th>
<th>Features</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonviral: Cationic liposome-DNA complex</td>
<td>Synthetic DNA complex, No size limitations of gene insert, Low immunogenicity</td>
<td>No integration, Low efficiency gene transfer, Systemic administration unlikely, Toxicity (?)</td>
</tr>
<tr>
<td>Protein/DNA complex</td>
<td>Potential for cell-specific targeting, No size limitations of gene insert</td>
<td>No integration, Immunogenicity, Low efficiency gene transfer, Toxicity (?)</td>
</tr>
<tr>
<td>Colloidal gold DNA complex</td>
<td>No size limitation of DNA</td>
<td>Low efficiency gene transfer, Toxicity (?)</td>
</tr>
<tr>
<td>Gene gun</td>
<td></td>
<td>May require surgical procedure, Systemic administration unlikely, Ex vivo use only (?)</td>
</tr>
<tr>
<td>Viral: Retrovirus</td>
<td>Commonly employed in clinical trials, Transduces many cell types, Integrates into cell's genome, Safety</td>
<td>Transduces only dividing cells, Low efficiency gene transfer, Systemic administration unlikely, Low viral titer production, Does not integrate, Systemic administration unlikely, Immunogenicity, Transient gene expression</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Commonly employed in clinical trials, Transduces many cell types, Transduces both dividing and nondividing cells, High viral titer production, High gene transfer efficiency, Transient gene expression</td>
<td>Low efficiency gene transfer, Systemic administration unlikely, Immunogenicity, Transient gene expression</td>
</tr>
<tr>
<td>Adeno-associated virus (Parvovirus)</td>
<td>Integrates into cell's genome, Transduces many cell types, Transduces dividing and nondividing cells, High viral titer production, High gene transfer efficiency</td>
<td>Immunogenicity, Difficult to mass produce for clinical trials, Systemic administration uncertain</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Immunogenicity, May carry large gene</td>
<td>Low efficiency gene transfer, Transient gene expression, Toxicity (?)</td>
</tr>
<tr>
<td>HSV</td>
<td>Neuronal tropism, Latency expression</td>
<td>Toxicity (?)</td>
</tr>
<tr>
<td>Avianpox virus</td>
<td>Transduces both dividing and nondividing cells</td>
<td>Low efficiency gene transfer, Toxicity (?)</td>
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</table>
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