Gene therapy for prostate cancer

Review Article

Mitchell S Steiner and Jeffrey R Gingrich
Department of Urology, University of Tennessee, Memphis, Tennessee 38163

Correspondence: Mitchell S. Steiner, MD, Department of Urology, University of Tennessee Medical Center, 956 Court Avenue, Memphis, Tennessee 38163. Telephone: 901-448-1492; Fax: 901-448-4743; E-mail: MSteiner@utmem.edu

Abbreviations: GDEPT, gene directed enzyme prodrug treatment; 6 MPDR, 6-methyl-9 (2 deoxy-β-D erythro-pentofuranosyl) purine; Ad, adenovirus; CMV, cytomegalovirus; MMTV, mouse mammary tumor virus; HSV, herpes simplex virus; tk, thymidine kinase; GCV, ganciclovir; DT, dipheria toxin; EBRT, external beam radiation treatment; PSA, prostate-specific antigen; PSE, prostate specific enhancer; ARE, androgen responsive element; PB, probasin; MHC, major histocompatability complex; TIL, tumor infiltrating lymphocytes; CTL, cytotoxic T cells; IL, interleukin; GM-CSF, granulocyte maturation-colony stimulating factor; bFGF, basic fibroblast growth factor

Key Words: Prostate cancer, gene therapy, gene replacement, immunotherapy, oncolytic virus, suicide gene, prostatectomy, prodrug

Received: 7 July, 1999; accepted 15 July 1999

Summary

The advent of recombinant DNA technology has sparked the age of molecular medicine. The ability to deliberately recombine pieces of DNA and then transfer these specific genes into diseased cells has revolutionized medical research. In fact, the ability to modify these genes in the living person is now possible. Several innovative approaches are being developed to circumvent the limitations of current vectors including more effective delivery routes for gene therapy, the incorporation of tissue specific promoters and other enhancers into vectors, and increasing cell death by a phenomenon known as the bystander effect. Gene therapy strategies are rapidly evolving as new gene targets, better vectors and improved gene expression systems become available. Innovative gene therapy strategies currently being employed for the treatment of prostate cancer include: immunotherapy, gene corrective therapy, exploitation of programmed cell death therapy, gene therapy to target critical biological functions of the cell, suicide gene therapy, oncolytic virus gene therapy, and finally combination gene therapy. At this time, 17 gene therapy trials have been approved by the NIH for the treatment of prostate cancer. Overall, current gene therapy to treat advanced localized prostate cancer has been shown to be safe and feasible. There are many challenges that lie ahead for gene therapy. Nonetheless, it is almost certain that gene therapy will be part of the armamentarium against prostate cancer and other human diseases in the next century.

I. Introduction

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer deaths in American men today with an estimated 179,300 new cases of prostate cancer and 37,000 deaths predicted this year (Landis et al, 1999). The risk of prostate cancer rises steeply with age and will continue to increase by 3-4% each year in older men as fewer men are dying from cardiovascular diseases (Walsh, 1994). Despite concerns that increased detection of early prostate cancer by the wide spread use of serum PSA would lead to many more patients being treated unnecessarily for small indolent cancers, no change in the proportion of such cases has been observed by many large medical centers between 1983-1996 (Soh et al, 1997). In fact, the majority of patients that are carefully selected for treatment of clinically localized disease by radical prostatectomy are found pathologically to have advanced localized disease. Badalament et al (1996) evaluated 4 large prostatectomy series totaling 5,661 patients and found that 55% of patients indeed had extracapsular disease at the time of surgery. Locally advanced prostate cancer, defined as the presence of extracapsular extension, increases the likelihood of positive surgical margins at radical prostatectomy and portends a poor prognosis. Re-examination of the role radical prostatectomy as monotherapy for T3 disease suggests that it is really not curative as the 10 and 15 year survival following radical prostatectomy are 12-60% and 20-28%, respectively. The local recurrence rates are as high as 41% by 5 years following radical prostatectomy (Partin et al, 1993). Hence, radical prostatectomy alone is not curative in the majority of patients with significant extracapsular disease.

It is also a well established fact that testosterone,
hormonal deprivation alone does not cure prostate cancer (Schröder, 1995). Neoadjuvant hormone deprivation has been recently used to “down size” or “down stage” locally advanced prostate cancer prior to radical prostatectomy in an attempt to improve the chances of achieving local cancer control. Although earlier studies have shown a reduction in positive surgical margins rate (Wieder and Soloway, 1998), there has been no change in the rate of PSA recurrence either from a retrospective analysis (Wood et al, 1997) or by prospective randomized studies with 2 years follow-up (Goldenberg et al, 1997; Soloway et al, 1997). Thus, neoadjuvant hormone deprivation has not been shown to alter tumor progression or survival rates (Abbas et al, 1996; Cookson and Fair, 1997; Goldenberg et al, 1997; Soloway et al, 1997).

External beam radiation treatment (EBRT) alone also has a high local failure rate in advanced prostate cancer. Zagars et al (1991) have reported a rising serum PSA following EBRT in 17% of patients with a PSA less than 40 ng/ml and as high as 60% in patients whose PSA is > 40 ng/ml. Overall, Holzman et al have shown a 53% local recurrence rate by 8 years after EBRT (Holzman et al, 1991). However, pathologically the rate of local control is even more disappointing. The Stanford series found, that after a mean follow-up of 17 months, following definitive EBRT, greater than 60% of patients had a rising serum PSA indicating cancer progression (Stamey and McNeal, 1992) and over 90% of these patients had a positive biopsy for prostate cancer (Kabalin et al, 1989). Even more alarming, the grade of the recurrent prostate cancer has been shown to be higher than the original cancer (Cumming et al, 1990; Wheeler et al, 1993). The reported disease free survival for T3 disease is 64% at 5 years, 10-35% at 10 years, and 15-18% by 15 years (Bagshaw, 1993; Schellhammer and Lynch, 1997). If a serum PSA criterion is used then the biochemical failure rate exceeds 90% at 10 years for stage T3 prostate cancer (Schellhammer and Lynch, 1997).

Thus, surgery, radiation, or hormone deprivation alone will not be adequate enough to locally control clinical or pathologic stage T3 prostate cancer which will ultimately lead to a higher incidence of morbidity, distant metastasis, and decreased survival (Schellhammer and Lynch, 1997). Clearly, other novel therapies for this devastating and common disease are desperately needed to achieve long term local cancer control. The focus of new therapies should be to intervene at the cellular level as a way to locally directly affect prostate cancer cells in way not possible by current standard therapies. As it is the androgen independent prostate cancer cells that eventually kill the patient (Isaacs, 1995), any strategy that will modify the biologic behavior of these cells may potentially have the most significant clinical impact to achieve local cancer control.

II. Gene therapy and prostate cancer

There are several features of prostate cancer that make it a particularly useful model to study gene therapy: 1) Prostate cancer is common and for the majority of patients who are diagnosed with advanced disease there is no cure. 2) The prostate gland produces over 500 unique gene products and each specific prostate antigen, or protein may be exploited for vector targeting or gene vaccine immunization. Moreover, these prostate specific promoters and other enhancers that direct transcription of these prostate unique proteins may also be incorporated into vectors to direct prostate specific expression of therapeutic genes (Simons et al, 1999). 3) The prostate gland does not serve any critical life sustaining functions which obviates the need to distinguish between normal and cancerous prostate tissues for targeted gene therapy. 4) The prostate gland is also easily accessible by transurethral, transperineal, and transrectal approaches for intratumoral administration of gene therapy. 5) The prostate may be easily evaluated by transrectal ultrasound, digital rectal examination, and other standard radiologic imaging (magnetic resonance imaging and computer tomography) following gene therapy. 6) The pattern of prostate cancer spread is also predictable; it spreads to pelvic lymph nodes and then to the axial skeleton. This allows for the development of gene therapy strategies to purge the bone marrow of malignant cells or develop vectors that have tropism for bone (Kim et al, 1997; Ko et al, 1996; Malkowicz and Johnson, 1998). 7) Although controversial, prostate cancer gene therapy may be followed by serum PSA. Even though it should not be an endpoint by which to make decisions, it may serve as a surrogate marker of prostate cancer progression (Cech and Bass, 1986; Partin and Oesterling, 1994).

Prostate cancer also poses some interesting challenges for current gene therapy technology as the doubling time of prostate cancer is usually greater than 150 days with less than 5% of cells actively dividing at any one time (Berges et al, 1993). By having such a low proliferative index, vectors are required that are capable of providing high gene transfer independent of cell division. Another concern is that prostate cancer is a heterogeneous cancer, not only from individual to individual, but also within the same individual. For example, the underlying genetic mutations that dictate the phenotype of primary tumor may not be identical to those that are responsible for metastasis (Isaacs, 1995). Thus, corrective gene therapy approaches to correct leading gene mutations for one individual may not necessarily be useful to treat another individual or a different lesion in the same patient.

III. Prostate targeted delivery of gene therapy

Current vector technology cannot achieve the ultimate
goal of in vivo cancer gene therapy; that is, to administer a vector systemically that will result in the expression of therapeutic gene exclusively in 100% of target cells. Several innovative approaches are being developed to circumvent these limitations of current vectors including more effective delivery routes for gene therapy, the incorporation of tissue specific promoters/enhancers into vectors, and increasing cell death by enhancing the phenomenon known as the bystander effect.

A. Delivery approaches

Theoretically, metastatic disease may only be treated by the systemic delivery of gene vectors. However, for locally advanced prostate cancer (stages T3 and T4), variations in delivery strategy may help target gene therapy vectors to the prostate. Lu et al (1999) compared the effectiveness of gene transfer by an adenoviral vector containing β-galactosidase reporter gene delivered by three routes of administration: intravenous, intraarterial, and intraprostatic injection in the canine model. The intraarterial approach was performed by cannulating the internal iliac artery and threading the catheter to the inferior vesicle and prostatic arteries. The transduction efficiencies of adenovirus β-gal were assessed by X-gal staining of prostate tissue sections, by colorimetric β-gal assay, and by PCR. The intraprostatic administration of the adenoviral vector resulted by far in the highest gene transfer rate with the least adenoviral systemic dissemination (Lu et al, 1999; Steiner et al, 1999). This study provides direct support for the use of intratumoral prostatic injections of gene therapy vectors for prostate cancer gene therapy trials either by the transrectal route or by the transperineal approach under ultrasound guidance (Steiner et al, 1998).

B. Tissue specific promoters

Viral vectors will transfer therapeutic genes to any mammalian cell exposed to that vector. One way for vectors to theoretically target only prostate cells is by incorporating a prostate tissue specific promoter and/or enhancer that will limit the expression of the therapeutic gene to prostate cells. Only prostate cells will have the appropriate complement of transcription factors to activate the prostate specific promoter, and thus, the therapeutic gene will be expressed only in prostate cells. This assumes, however, that this prostate tissue specific promoter/gene vector expression system is tightly controlled and not active or “leaky” resulting in the inadvertent expression of the therapeutic gene in unintended tissues. Unfortunately, prostate promoters currently employed in gene therapy are leaky to some degree, but what is not known is whether this low level of expression in non-prostate tissues is clinically relevant.

Prostate epithelial cell promoters that have been used for gene therapy include prostate specific antigen (PSA) promoter, (Dannull and Belldegrun, 1997; Dannull et al, 1999; Pang et al, 1997; Pang et al, 1995; Steiner et al, 1999) probasin (PB) promoter (Greenberg et al, 1994; Zhang et al, 1998), mouse mammary tumor virus (MMTV) promoter, (Muller et al, 1990; Steiner et al, 1998; Tutrone et al, 1993) and the prostate specific membrane antigen promoter (Israeli et al, 1993). The PSA promoter has been most commonly employed in vector constructs (Dannull and Belldegrun, 1997; Dannull et al, 1999). One theoretical concern is that the PSA promoter requires the presence of both functional androgen receptors and circulating androgens to be active. The majority of patients who have advanced and hormone refractory prostate cancer, however, are also undergoing androgen deprivation therapy which may not optimally activate the PSA promoter. Addressing this potential obstacle, Gotoh et al (1998) have shown that a better promoter is the long PSA promoter (5837 bp) which is more active than the short PSA promoter (631 bp) both in an androgen depleted environment and in androgen insensitive cells in vitro. Another strategy is to be able to preserve the tissue specificity of the promoter like the PB promoter, but be able to activate the promoter with another hormone.

Zhang et al (1998) have developed a retroviral construct containing a glucocorticoid responsive element upstream of the androgen responsive element (ARE). This allows the activation of the PB promoter with dexamethasone. Similarly, Rodriguez et al (1999) demonstrated that the androgen sensitive probasin promoter may also be activated by phenylbutyrate in the absence of androgens. Thus, gene therapy using constructs containing modified prostate specific promoters may be used to treat patients who have advanced prostate cancer and are concomitantly receiving androgen deprivation therapy.

Another experimental observation is that the activity of prostate specific promoters tend to be less than that of other non specific or viral promoters (Steiner et al, 1999). There appears to be an inverse relationship between promoter activity and tissue specificity. In the canine prostate model, PSA, PB, and MMTV promoters are prostate specific, but have a 10 to 100 fold less activity than the Rous sarcoma virus promoter in vivo (Steiner et al, 1999). To help circumvent this problem, Pang et al (1997) have cloned a mutated PSA promoter (PCPSA) from a prostate cancer patient who had a high serum PSA. The PCPSA promoter has 50-fold greater activity than the wild type PSA promoter. Similarly, upstream regulatory sequences of the PSA promoter referred to as prostate specific enhancer (PSE) sequences were cloned from normal and cancerous prostate tissue. In vitro, PSE sequences increased the PSA promoter activity by 72 fold when isolated from normal prostate compared to 100 fold when cloned from prostate cancer tissue (Dannull and Belldegrun, 1997). Recently, Dannull et al (1999) incorporated the PSE (822 bp) and PSA promoter (611 bp) into an E1 deleted adenoviral vector.

235
Intratumoral injection of this vector into a variety of different human prostate xenografts growing subcutaneously in SCID mice resulted in PSA promoter activity that was not as robust as seen in vitro. In fact, the promoter activity was markedly less than the CMV viral promoter in the same system.

Another tactic uses gene therapy to treat the supporting bone stromal cells in an effort to erode prostate epithelial cells metastatic to bone. Using the osteocalcin promoter which is active in bone stroma including osteoblasts (Ko et al, 1996), prostate cancer gene therapy may be directed to the bony metastatic sites. Ko et al (1996) have shown that osteosarcoma tumors are inhibited following intratumoral injection with adenoviral vector composed of the osteocalcin promoter controlling thymidine kinase (tk) followed by ganciclovir (GCV). Moreover, the osteocalcin promoter is active in spontaneous canine prostate cancer bony metastasis suggesting that the osteocalcin may be useful for targeting bone metastasis in humans (Ou et al, 1999). Combinations of prostate specific antigen enhancers and other prostate specific epithelial and stromal promoters are currently under intense investigation. Whether or not any of these promoter combinations will be ultimately effective in the systemic treatment of prostate cancer with the required level of promoter activity remains to be shown.

C. Bystander effect

A unique observation that became apparent only after the application of gene therapy in clinical in vivo models is the bystander effect. The bystander effect occurs when more cells are destroyed or biologically altered following gene therapy than would have been predicted by transduction rates alone. This is fortuitous as no one vector system is currently available that can transfer therapeutic genes into 100% of target cells; thus, the ability to affect more cells than those that have been transfected makes gene therapy more clinically applicable. There are several hypotheses to explain the mechanism of the bystander effect: 1) There is intratumoral cell to cell transfer of the therapeutic gene, gene product, or gene activated toxic prodrug from cell to cell by cellular vesicles and endocytosis or by diffusion through gap junctions and cell channels. 2) The therapeutic gene or vector antigens may induce an intense immune response which contributes to cell kill. 3) Tissue ischemia resulting from endovascular injury secondary to toxic gene product or non-specific immunologic response (Simons and Marshall, 1998). Although the exact mechanism is not known, the bystander effect is a real clinical phenomenon that may help compensate in part for the inefficient in vivo gene transfer limitations of the currently available vectors.

IV. Gene therapy strategies to combat prostate cancer

Gene therapy strategies are also rapidly evolving with new gene targets, better vectors and improved gene expression systems. Initially, ex vivo gene therapy, the transfer of genes into cells growing outside the body in tissue culture, was the primary approach. Primarily because of the new viral-based gene therapy technologic advances, in vivo gene therapy, the ability to transfer genes into cells that are still part of a living organism, has also become possible. The innovative gene therapy strategies that are being currently employed for the treatment of prostate cancer include: immunotherapy, corrective gene therapy, exploitation of programmed cell death gene therapy, gene therapy to target critical biological functions of the cell, suicide gene therapy, oncolytic virus gene therapy, and combination gene therapy.

A. Immunotherapy

Class I major histocompatibility complex (MHC) proteins are critical for appropriate antitumor immunologic responsiveness. Alterations or loss of Class I MHC is one common way that prostate cancer cells may evade the host’s immune system (Blades et al, 1995; Sanda et al, 1995). Several approaches have been used to stimulate or augment the body’s own antitumor immune response to essentially circumvent the loss of the critical Class I MHC proteins. Four general immunologic approaches have evolved for the immunotherapy of prostate cancer: 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 to treat effector immune cells such as dendritic cells, tumor infiltrating lymphocytes (TIL), or cytotoxic T cells (CTL) by ex vivo gene transfer techniques, and lastly, cytokine immunotherapy, which is not truly gene therapy as the patient is treated systemically with purified cytokines such as Interleukin 4 (IL-4), IL-2, granulocyte maturation-colony stimulating factor (GM-CSF), or B7 (Dannull and Belldegrun, 1997).

1. Gene vaccine therapy

Autologous tumor or fibroblast cells harvested by patient biopsy or surgical specimen, or alternatively nonautologous cells are modified by ex vivo gene therapy with genes that encode for immunity enhancing proteins (Fisher et al, 1989). The genetically altered cells are irradiated to destroy their capacity to replicate prior to subcutaneous patient re-inoculation. CTL, not only recognize tumor specific antigens present on the surface of these inoculated, irradiated cells, but also they are induced by local secretion of the transferred stimulatory cytokine.
The activated CTL cells expand, target, and destroy tumor cells that share these same antigens on their cell surface throughout the body (Sikora and Pandha, 1997). Gene therapy using cytokine genes, IL-2 and GM-CSF, theoretically will stimulate antitumor responses independent of Class I MHC levels, alternatively utilizing class II MHC expression and natural killer cell mediated tumor lysis (Fearon et al, 1990; Golumbek et al, 1991). In contrast, TNF-α and IFN-γ depend on Class I MHC proteins which are altered in prostate cancer to mediate their immune effects (Catalona et al, 1991; Lange et al, 1989).

Preclinical studies have clearly shown that gene vaccines are not efficacious in the presence of a large tumor burden, but theoretically may be more useful against micrometastasis following debulking of the primary tumor (Simons et al, 1998a, 1999a,b). Other concerns about autologous prostate cancer gene vaccines include the fact that prostate cancer tissue may not be resectable, the tumor tissue may not be easily cultured, poor cytokine gene transfer efficiencies, and the inability to produce large scale yields of genetically modified tumor cells (Jaffee et al, 1993). Gene vaccines that have shown efficacy against prostate cancer in animal models include gene transfer of cytokine genes GM-CSF (Sanda et al, 1994), IL-2 (Kawakita et al, 1997; Vieweg et al, 1994), IFN-gamma, TNF-, and B7 (Kawakita et al, 1997). Cytokine producing cancer gene vaccines have been evaluated for IL-2, GM-CSF, and IFN-γ in MAT LyLu cells grown in Copenhagen rats (Moody et al, 1994; Sandra et al, 1995; Schmidt et al, 1995; Vieweg et al, 1994; Yoshimura et al, 1996). The retroviral delivery of IL-2 or GM-CSF inhibited prostate cancer tumors and increased animal survival with up to 30% of animals cured when treated with GM-CSF gene vaccines (Gansbacher et al, 1990; Vieweg and Gilboa, 1995; Vieweg et al, 1994; Yoshimura et al, 1996). Similar results have been reported for ex vivo IL-2 liposomal gene vaccine therapy (Vieweg et al, 1995). In contrast, Kawakita et al (1997) used canary pox virus for utilizing genes IL-2, IFN-γ, TNF-α, or B7 and found that only TNF-α or IL-2 delayed RM1 tumors in C57BL/6 mice (Kawakita et al, 1997).


2. Direct in vivo intratumoral injection of gene therapy vectors containing cytokine genes

This second immunotherapy approach treats the tumor directly by intratumoral injection of vectors containing cytokine genes. Utilizing animal models of prostate cancer, Naitoh et al (1998) have shown that liposome and adenoviral vectors containing the IL-2 gene produced IL-2 following intratumoral injection resulting in the activation of specific T cell antitumor responses. Similarly, Sanford et al (1999) have employed adenoviral vector containing IL-12 (Ad IL-12) to intratumorally inject primary prostate cancer tumors in mice which significantly reduced the number of lung metastasis. The molecular mechanism may include stimulation of T cell and NK cells, induction of IFN-γ, and upregulation of fas expression (Hyer et al, 1999; Sanford et al, 1999). Phase I clinical trials utilizing IL-2 gene transfer vectors for intratumoral injection of prostate cancer are nearing completion (Naitoh and Belldegrun, 1998).

3. Adoptive immunotherapy

Effector immune cells such as dendritic cells, TIL, or CTL cells are genetically modified by ex vivo gene transfer techniques. This form of therapy for prostate cancer is still in its infancy. The difficulty with this approach in prostate cancer has been the ability to selectively obtain specific effector cell types from the patient, ex vivo amplification of the effector cells, and ex vivo gene transfer of specific biological modifiers such as cytokine genes.

In summary, immunotherapy may ultimately be effective against micrometastatic disease, but patients with greater metastatic or primary tumor volumes will require some other non-immunologic therapeutic intervention. Another concern is the theoretical possibility that by autologous or nonautologous cells producing low levels of tumor associated antigens they may paradoxically induce immune tolerance which would suppress the host’s immunity against prostate cancer. Finally, tumor antigens in general are also very weak inducers of the immune system. Thus, it is only through well-designed clinical trials employing gene immunotherapy that these critical questions may be answered.
B. Corrective gene therapy

Corrective gene therapy seeks to replace inherited or acquired defective genes which are important for normal growth regulation of the cell cycle. The molecular components of the cell cycle targeted include proto-oncogenes, tumor suppressor genes, and growth factors and their receptors. Since prostate cancer is estimated to be a consequence of an average of 5 genetically accumulated mutations, it is hard to conceptualize that the correction of any single gene alteration would have any major biological consequence on the cancer cell’s phenotype. This is confounded by the fact that current vector technology does not achieve the stable integration of therapeutic genes into 100% of prostate cancer cells comprising the tumor. Unexpectedly, the replacement or correction of one gene alteration has been shown to indeed alter the malignant phenotype and in some cases even completely eradicate prostate tumors in preclinical studies. In fact, this phenomenon has been repeatedly shown for different genes and vectors (Bookstein et al, 1990; Isaacs et al, 1991; Kleinerman et al, 1995; Steiner et al, 1998, 1999). These observations suggest that some genetic mutations are more critical to cell control than others and that the bystander effect may be playing an important role as well (Gotoh et al, 1997; Hall et al, 1997, 1998; Steiner et al, 1998b, c).

Most corrective gene therapy strategies have employed either retroviral or adeno viral vectors administered by intratumoral injection. Prostate cancer preclinical studies have been reported for the replacement of an assortment of tumor suppressor genes including AdCMVp53 (Asgari et al, 1998; Eastham et al, 1995; Gotoh et al, 1997; Ko et al, 1996), retroviral LXSN BRCA-1 (Steiner et al, 1998), AdCMVp21 (Eastham et al, 1995; Gotoh et al, 1997), and AdCMV CAM1 (Hsieh et al, 1995). Another critical cell cycle component, cell cycle dependent kinase inhibitor p16, is commonly altered in prostate cancer (Cairns et al, 1994; Cairns et al, 1995). In prostate cancer, p16 inactivation is a common event observed in the majority of human prostate cancer cell lines (Itoh et al, 1997; Jarrard et al, 1997), and alterations of p16 have also been reported in patients who have prostate cancer (Cairns et al, 1995). Controversy arose as to whether p16 inactivation was critical only in rapidly dividing cancer cells in tissue culture rather than in primary human prostate cancer because homozygous deletions or intragenic mutations of p16 were apparently infrequent (Liggett and Sidransky, 1998). This controversy, however, was laid to rest by the recent discovery of microdeletions within the p16 gene. These microdeletions of the p16 gene were difficult to confirm by standard molecular techniques because of the presence of normal cells within the tumor specimen (Liggett and Sidransky, 1998). Microsatellite analysis employing markers close to the p16 gene revealed that a wide range of tumor types including prostate cancer had small (< 200 kb) deletions of both p16 alleles (Liggett and Sidransky, 1998). Unlike other tumor suppressor genes that are commonly inactivated by point mutation, small homozygous deletions represented a major mechanism of p16 inactivation in cancer (Liggett and Sidransky, 1998). In fact, using this technique Cairns et al found that p16 homozygous deletions occurred in 40% of human primary prostate cancers (Cairns et al, 1995; Jarrard et al, 1997). Moreover, with progression 46% of prostate cancer metastatic lesions demonstrate loss of heterozygosity (Jarrard et al, 1997). Even more interesting, patients who have failed androgen deprivation have a 71% loss of 9p allelic loss (Isaacs, 1995). Using an adenoviral RSV vector containing p16, Steiner et al (1999) have shown that p16 replacement suppresses cell growth and induces cell senescence in a variety of prostate cancer cell lines. Moreover, in vivo, a single intratumoral injection of Adp16 resulted in 70% reduction of PPC-1 human prostate xenografts in nude mice and prolonged animal survival (Lu et al, 1999; Lu et al, 1998; Steiner et al, 1999). Using a different prostate cancer animal model, Gotot et al (1997) have shown similar results employing an AdCMVp16 vector. Interestingly, peptide growth factor receptor FGFR 2 IIIb becomes altered with prostate cancer progression (Feng et al, 1997). Matsubara et al (1998) have shown that following transfection with FGFR2 kinase, AT3 had restoration of KGF response resulting in suppression of AT3 prostate cancer growth. Thus, restoration of a single underlying growth factor pathway may favorably alter the malignant phenotype.

Oncogene overexpression is another way that cancer cells commonly lose control of the cell cycle (Steiner et al, 1995). One therapeutic approach utilizes expression of an antisense mRNA to the oncogene. The antisense mRNA anneals to the sense strand and effectively prevents the translation of the protein from that mRNA, thereby suppressing the protein level. Since prostate cancer commonly has overexpression of c-myc, Steiner et al (1998a) constructed a retroviral LXSN vector containing a prostate specific MMTV promoter driving the antisense c-myc gene. A single intratumoral injection of retroviral MMTV antisense c-myc was able to markedly suppress and even eradicate some of the DU145 prostate cancer xenografts growing in nude mice. The molecular mechanism was down regulation of c-myc expression and protein and the induction of apoptosis and downregulation of bcl-2 protein (Steiner et al, 1998a). Using a similar approach, Kim et al (1997) have shown that an adenoviral vector containing the antisense erb-B-2 gene (Ad anti-erb-B-2) inhibited the overexpression of growth factor erb-B-2 in prostate cancer cells resulting in their destruction. This tactic was used to selectively purge bone marrow cells of metastatic prostate cancer cells in vivo (Kim et al, 1997).
malignant phenotype of the cancer cell may be restored towards a more normal cell. Other corrective gene therapy approaches like AdCMVp53 have shown that gene replacement induces cell death, while others like retroviral antisense c-myc may incite host responses such as the bystander effect and other immunologic host responses (Gotoh et al, 1997; Hall et al, 1997, 1998; Steiner et al, 1998). Preliminary studies that employ corrective gene therapy also raise important clinical concerns unique to gene therapy. It has always been the dictum in cancer therapy that every cancer cell must be eradicated to effect a long-term cure. Corrective gene therapy, whose goal is to correct or repair alterations of the cell cycle and its components, challenges this concept. It is quite possible that the clinical endpoint of a corrective gene therapy strategy would simply be that the cell behaves more normally and no longer threatens the life of the patient. To this end, leaving a restored to more normal cancer cell in the patient may be an acceptable clinical endpoint. As the human genome project progresses and new prostate cancer genes are identified, corrective gene therapy will play a pivotal role in the treatment of prostate cancer.

C. Exploitation of programmed cell death gene therapy

Gene therapy strategies are being developed to activate apoptotic pathways toward the ultimate goal of forcing the cancer cell to irreversibly commit to programmed cell death. Segawa et al (1998) have used an elaborate PSA promoter based system (GAL-4-VP16) to activate GAL-4 responsive elements. One GAL-4 responsive element is placed upstream of the polyglutamine gene. Polyglutamine is a potent apoptotic protein which in this case is selectively expressed in PSA producing cells. Similarly, Hyer et al (1999) have shown 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. Marcelli et al (1999) have reported that transduction of prostate cancer cell line LNCaP with adenoviral vector containing caspase-7, a potent and critical modulator of apoptosis, also induced programmed cell death. Another molecular approach has targeted the bcl-2, an oncogene that has anti-apoptotic activity, with an adenoviral vector containing a hammerhead ribozyme directed against bcl-2 (Dorai et al, 1997, 1999). A bcl-2 ribozyme is an RNA molecule which specifically catalyzes or disrupts bcl-2 mRNA making the cell more susceptible to apoptosis. Interestingly, adenoviral hammerhead bcl-2 ribozyme treatment induced apoptosis in androgen sensitive, but not androgen insensitive prostate cancer cells. Although no preclinical in vivo studies have yet been reported, exploitation of programmed cell death gene therapy approaches are attractive and may potentially be quite effective.

D. Gene therapy to target critical biological functions of the cell

Like classical pharmacology, gene therapy may be used to target critical cellular processes as the basis of rational anticancer gene therapy design. Lee et al (1996) have designed liposomal vectors that contain a PSA promoter upstream of either antisense topoisomerase II or antisense DNA polymerase α. Both topoisomerase II and DNA polymerase α are critical molecular components of DNA replication. The treatment combination of both liposome PSA-antisense topoisomerase II and liposome PSA-antisense DNA polymerase α had the greatest inhibitory effects on prostate cancer cell lines (LNCaP, DU145, and PC3) in vitro. Similarly, a retroviral vector that incorporated antisense eIF4E gene was used to treat prostate cancer cells (Williams et al, 1998). Prostate cancer cells have been previously shown to have overexpression of eIF4E which is a rate limiting factor in the translation initiation of growth controlling genes like cyclin D1, c-fos, c-myc, VEGF, and bFGF. A single intratumoral injection of retroviral antisense eIF4E suppressed prostate cancer xenograft growth for up to 65 days (Williams et al, 1998). Thus, the rational design of gene therapy vectors to disrupt critical molecular events required for cellular function is an enticing strategy against prostate cancer.

E. Suicide gene therapy

Suicide gene therapy may have the most promising clinical application. Vectors introduce the therapeutic gene into the cancer cells, and once the gene product is expressed, the cell is destroyed without regard to the underlying genetic mutations responsible for the malignant phenotype. Two types of suicide gene therapy strategies have emerged: gene directed enzyme prodrug treatment (GDEPT) and gene directed production of a cellular toxin.

1. Gene directed enzyme prodrug treatment (GDEPT)

GDEPT approach utilizes a system that couples prodrug enzyme gene therapy followed by systemic administration of its specific prodrug. Following gene transfer of the prodrug enzyme gene, the cancer cell produces that prodrug enzyme, and as a consequence, is capable of converting a nontoxic prodrug into an activated, lethal metabolite. This activated drug not only kills the cell that produced the toxic drug, but also its neighboring cancer cells. This bystander effect can be quite impressive with the ability to kill 100 to 1000 times more cells than would be predicted by gene transfer rates alone. Thus, a low gene transfer efficiency may be compensated by the high bystander effect. By having the cancer cell itself manufacture the activated cancer killing drug which acts locally minimizes systemic toxicity as the
The most widely used GDEPT system against prostate cancer is the Herpes simplex virus thymidine kinase (HSV-tk) and ganciclovir (GCV) system (Eastham et al, 1996; Hall et al, 1997). The nucleoside analogue GCV is converted by HSV-tk into a phosphorylated compound that is then incorporated into DNA during DNA replication. This causes DNA chain termination and selective killing of dividing cells. Eastham et al (1996) have used an adenoviral vector containing HSV-tk (AdHSV-tk) to sensitize both human and murine prostate cancer cells to the toxic effects of GCV both in vitro and in vivo models. AdHSV-tk gene therapy followed by GCV in both suppressed prostate cancer growth and prolonged survival rates in mice bearing prostate tumors (Eastham et al, 1996). Hall et al (Hall et al, 1997; Hall et al, 1998) have also shown that AdHSV-tk followed by GCV in the mouse prostate reconstitution orthotopic model suppressed tumor growth and decreased the rate of spontaneous prostate metastases to the lung. An immune basis for these effects was demonstrated by challenging mice with an injection of prostate cancer cells into the tail vein followed by excision of primary prostate cancer tumors. The animals that had treated primary tumors had a 40% reduction in lung metastases. This bystander effect appeared to be mediated in part by NK cells (Hall et al, 1998).

Other GDEPT systems including the prodrug enzyme cytosine deaminase-flucytosine strategy in which cytosine deaminase converts flucytosine to the chemotherapeutic agent 5 fluorouracil have been investigated in prostate cancer (Blackburn et al, 1998; Kim et al, 1999). Kim et al (1999) transferred either the cytosine deaminase gene or the HSV-tk gene into stromal cells of the bone marrow derived murine cell line D1. Co-cultures of D1 cells and human prostate cancer cell lines followed by the appropriate prodrug resulted in prostate cancer cell death with as low as 20% of D1 cells producing the prodrug enzyme in the co-culture. Blackburn et al (1998) used an adenoviral vector incorporating a heat shock protein (HSP 70) promoter and either cytosine deaminase or HSV-tk gene to treat PC3 cells. In this system, hyperthermia to 41°C activated the HSP-70 promoter resulting in prodrug enzyme expression. Thus, systemic administration of the prodrug and local heat allowed selective expression of the prodrug enzyme in intended tissues (Blackburn et al, 1998). Another system used an E1a deleted adenovirus containing prodrug enzyme E. coli DeoD gene product purine neocleoside phosphorylase (PNP) under the control of the PSA promoter (Martiniello-Wilks et al, 1998). The prodrug is 6-methyl-9 (2 deoxy-β-D erythro-pentofuranosyl) purine (6 MPDR) is converted into a toxic nonphosphorylated purine capable of killing both quiescent and proliferating cells when incorporated in mRNA or DNA during synthesis (Martiniello-Wilks et al, 1998). The PNP-6 MPDR system had efficacy against human prostate cancer cell line PC3 (Martiniello-Wilks et al, 1998). Other GDEPT systems utilizing assorted prostate specific promoters and vector types are currently under intense investigation.

2. Gene directed production of cell toxin

This strategy is similar to GDEPT where the transferred gene kills the cell independent of the underlying cancer gene mutations, but unlike GDEPT, this approach does not require a prodrug. Rodriguez et al (1998) screened numerous direct biological toxins known to kill mammalian cells by cell cycle independent mechanisms to determine which would be the best one against human prostate cancer. Diptheria toxin (DT) was found to be the most toxic. DT kills rapidly, independent of p53 or androgen sensitivity status and it kills both dividing and nondividing cells alike (Rodriguez et al, 1998). This approach, however, has several limitations. First, this toxic gene must be incorporated into vectors that contain promoters that are highly prostate specific and under tight regulatory control; DT is such a toxic biological toxin that even small amounts of “leaky” promoter activity in non-prostatic tissues may be very lethal. In addition, mass production of adenoviral vector- DT gene is very difficult because of the toxic effects of the DT gene on the packaging cell line resulting in low production titers (Simons et al, 1999).

F. Oncolytic virus gene therapy

Because of safety reasons, practically all current vectors are engineered to be replication incompetent meaning that the virus cannot express those viral genes that commandeer cells to enter the lytic cycle producing more virus. Consequently, the effectiveness of the viral vector is directly correlated to its transduction efficiency and its ability to be given in repeated doses. Recently, two types of replication competent viral vectors have been developed. One conditionally competent adenoviral vector has been mutated such that the virus cannot express viral protein E1b (Bischoff et al, 1996). The wild type adenovirus uses the E1b protein to stop p53 from preventing the replication of cells that have damaged DNA. Theoretically, the mutant E1b- virus can infect, replicate, and lyse p53 deficient cells, but does not affect normal cells that have functional p53 (Bischoff et al, 1996). Thus, these mutant viruses are oncolytic to cancer cells that harbor p53 mutations. In prostate cancer, however, the p53 mutation rate is lower than perhaps other types of cancer. Only 10-20% of prostate cancers having nonfunctional p53 and most p53 mutations are only found in tumors that have a higher grade and stage (Brooks et al, 1996; Dahiya et al, 1996; Eastham et al, 1996).

Another oncolytic virus is CN706 which is a replication
competent, attenuated cytotoxic adenovirus type 5 vector with a prostate specific enhancer and promoter coupled to the E1A gene (Rodriguez et al, 1997). The E1A viral product allows the virus to reproduce and to enter the lytic cycle. The PSA promoter theoretically limits E1A production to PSA producing cells (Rodriguez et al, 1997). The level of E1A production has been shown to be several logs higher in PSA producing cells like LNCaP than in cells that produce little or no PSA (Simons et al, 1999). In vivo, CN706 viral vector produced tumor regression of LNCaP tumors and decreased PSA production following a single intratumoral injection (Rodriguez et al, 1997; Simons et al, 1999). Although a clinical Phase I trial of intratumoral injection of CN706 in patients who have prostate cancer is in progress, there are several clinical concerns that are raised about CN706. First, there is no experimental support that systemically distributed CN706 will exclusively lyse prostate cells. In some systems, wide variation in the level of E1A expression has demonstrated little effect on viral replication suggesting that even low level expression may be sufficient to support viral replication and subsequent cell lysis. This is especially worrisome since the PSA promoter has been shown to be leaky as other types of cells in addition to prostate cells produce PSA. For example, cells that line the urethra produce abundant PSA. Theoretically, CN706 will only cease replication and lysis when all PSA producing cells are eradicated. Furthermore, there is questionable utility of any PSA promoter vector for the systemic treatment of PSA negative prostate cancer cells or in patients undergoing androgen deprivation therapy. Nonetheless, studies employing the CN706 or any other vector containing the PSA promoter by using intratumoral injections will be critical in increasing our understanding of this field until newer tissue specific vector technology becomes a reality.

G. Combination of gene therapy approaches and other treatment modalities

Use of gene therapy as monotherapy against prostate cancer is currently in its infancy. The use of gene therapy in combination with surgery, radiation, or chemotherapy to improve cancer treatment will most likely be the way that gene therapy will be used clinically. Moreover, clinical trials using this multimodal approach are more justified until the science of gene therapy improves. The most commonly employed strategy is the use of gene therapy in combination with DNA damaging agents. Improvements in prostate cancer tumor suppression and induction of apoptosis have been reported for the combinations of Ad5CMVp53 and paclitaxel (Nielsen et al, 1998), Ad5CMVp53 (Wilson, et al, 1999), Adp16, Ad.Egr TNF-α (Chung, et al, 1998), and ionizing radiation. Other combination approaches that have been employed in prostate cancer include GDEPT Ad cytosine deaminase and flucytosine and radiation (Yin et al, 1998), or GDEPT AdHSV-Jk/GCV and AdLIL2 cytokine therapy (Hassen et al, 1999). Improved prostate cancer treatment by including gene therapy as part of a multimodal treatment regimen is where gene therapy may have the most immediate clinical application.

VI. Prostate cancer gene therapy clinical trials

At this time, 17 gene therapy trials for the treatment of prostate cancer have been approved by the NIH (Table 1). To date, the approved trials have all been either Phase I or Phase I/II in design. Preliminary results of these trials are only recently forthcoming as meeting abstracts and peer reviewed publications. The first approved gene therapy trial for prostate cancer by Simons et al (1998, 1999) (Table 1) was designed for patients who were found to have metastatic prostate cancer in the lymph nodes at the time of radical prostatectomy. Similar to previous studies for the treatment of metastatic renal cell carcinoma (Simons et al, 1997), ex vivo transduction of autologous, irradiated prostate tumor cells with retroviral MFG-GM-CSF was performed to generate vaccines which were administered subcutaneously every 2 weeks until available cells were exhausted. Vaccination site biopsies revealed infiltrating macrophages, dendritic cells, eosinophils, and T cells. No dose limiting toxicities were observed. Seven out of eight patients had progressed by ultrasensitive PSA criteria by an average follow-up of 20 weeks. This study demonstrated the feasibility of autologous GM-CSF transduced prostate cancer vaccines was limited only by the in vitro expansion of vaccine cells. To circumvent this limitation, a follow-up trial by the same investigators powered to estimate efficacy utilizing ex vivo GM-CSF transduced allogeneic prostate cancer cell lines [PC-3 (Kaighn et al, 1979) and LNCaP (Horoszewicz et al, 1983)] as vaccines has been approved and is currently in progress (Table 1). Patients are vaccinated weekly for eight weeks with irradiated, GM-CSF secreting PC-3 and LNCaP prostate cancer cells. One of 21 patients treated to date has had a partial PSA response of >7 months duration, 14/21 have stable disease, and 6/21 progressed (Simons et al, 1999). At three months after treatment, PSA velocity or PSA slope have decreased in 71% of patients. No dose limiting toxicities have been identified. Although the dose and schedule are still undergoing optimization to demonstrate potential therapeutic efficacy, numerous new post-vaccination IgG1 antibodies have been identified demonstrating that immune tolerance to prostate cancer associated antigens may be broken and therefore appears to be clinically feasible for prostate cancer treatment.

Preliminary results of the first trial approved utilizing direct transrectal prostatic gene therapy injection has recently been reported by Steiner et al (1998). This is the first study designed using a gene replacement strategy.
Table 1. Approved prostate cancer gene therapy trials

<table>
<thead>
<tr>
<th>NIH</th>
<th>Phase</th>
<th>PI</th>
<th>Institution</th>
<th>Sponsor</th>
<th>Patient Population</th>
<th>Vector</th>
<th>Gene</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I/II</td>
<td>Simons</td>
<td>Johns Hopkins</td>
<td></td>
<td>Metastatic</td>
<td>Retrovirus</td>
<td>MFG-GM-CSF</td>
<td>Ex vivo, autologous PCA vaccine</td>
</tr>
<tr>
<td>2</td>
<td>I/II</td>
<td>Gansbacher</td>
<td>Memorial Sloan-Kettering</td>
<td></td>
<td>Prostate cancer</td>
<td>Retrovirus</td>
<td>IL-2 + gamma IFN</td>
<td>Ex vivo, allogeneic PCA vaccine</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>Steiner</td>
<td>Univ. of Tennessee/Vanderbilt</td>
<td></td>
<td>Advanced</td>
<td>Retrovirus</td>
<td>Anti-sense myc</td>
<td>In vivo, intraprostatic injection</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>Chen</td>
<td>Bethesda, Naval</td>
<td></td>
<td>Prostate Cancer</td>
<td>Vaccinia virus</td>
<td>PSA cDNA</td>
<td>In vivo, intradermal injection</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>Paulson</td>
<td>Duke Univ.</td>
<td></td>
<td>Locally Advanced, Metastatic</td>
<td>AAV/Liposome</td>
<td>IL-2</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>Scardino</td>
<td>Baylor College of Medicine</td>
<td></td>
<td>Radio-Recurrent</td>
<td>Adenovirus</td>
<td>RSV-HSV-tk/ganciclovir</td>
<td>In vivo, intraprostatic injection</td>
</tr>
<tr>
<td>7</td>
<td>I</td>
<td>Kufe &amp; Eder</td>
<td>Dana-Farber</td>
<td></td>
<td>Prostate Cancer</td>
<td>Vaccinia virus</td>
<td>PSA cDNA</td>
<td>In vivo, intradermal injection</td>
</tr>
<tr>
<td>8</td>
<td>I/II</td>
<td>Sanda</td>
<td>Univ. of Michigan</td>
<td>Thorton Biologics Corp.</td>
<td>PSA Recurrence after RRP</td>
<td>Vaccinia virus</td>
<td>PSA cDNA</td>
<td>In vivo, intradermal injection autologous</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>Beldegrun</td>
<td>UCLA</td>
<td>Vical, Inc.</td>
<td>Locally Advanced</td>
<td>Liposome</td>
<td>IL-2</td>
<td>In vivo, Autologous, intratumoral</td>
</tr>
<tr>
<td>10</td>
<td>I</td>
<td>Hall</td>
<td>Memorial Sloan-Kettering</td>
<td>T1c, T2b &amp; c</td>
<td></td>
<td></td>
<td>Adenovirus</td>
<td>RSV-HSV-tk/ganciclovir</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>Beldegrun</td>
<td>UCLA</td>
<td>Schering-Plough Corp.</td>
<td>Locally Advanced, Recurrent</td>
<td>Adenovirus p53 wild type cDNA</td>
<td>In vivo, intraprostatic injection/RPP</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I/II</td>
<td>Simons</td>
<td>Johns Hopkins</td>
<td></td>
<td>Prostate Cancer</td>
<td>Retrovirus</td>
<td>GM-CSF</td>
<td>Ex vivo, allogeneic PCA vaccine</td>
</tr>
<tr>
<td>13</td>
<td>I/II</td>
<td>Logothetis/ Steiner</td>
<td>MD Anderson/Univ. of Tennessee</td>
<td>Introgen, Inc.</td>
<td>No Prior Therapy, high risk</td>
<td>Adenovirus p53 wild type cDNA</td>
<td>In vivo, intraprostatic injection/RPP</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>I/II</td>
<td>Kadmon</td>
<td>Baylor College of Medicine</td>
<td></td>
<td>No Prior Therapy, high risk</td>
<td>Adenovirus</td>
<td>RSV-HSV-tk/ganciclovir</td>
<td>In vivo, intraprostatic injection/RPP</td>
</tr>
<tr>
<td>15</td>
<td>I</td>
<td>Simons</td>
<td>Johns Hopkins</td>
<td>Calydon</td>
<td>Radio-Recurrent</td>
<td>Adenovirus</td>
<td>PSA-E1a (replication competent)</td>
<td>In vivo, intraprostatic injection</td>
</tr>
<tr>
<td>16</td>
<td>I/II</td>
<td>Figlin</td>
<td>UCLA</td>
<td>Transgene SA</td>
<td>MUC-1 Positive</td>
<td>Vaccinia virus</td>
<td>MUC-1/IL2</td>
<td>Immunotherapy</td>
</tr>
<tr>
<td>17</td>
<td>I</td>
<td>Gardner/ Chung</td>
<td>Univ. of Virginia</td>
<td></td>
<td>Metastatic</td>
<td>Adenovirus</td>
<td>Osteocalcin HSV-tk/ valacyclovir</td>
<td>In vivo, intratumoral injection</td>
</tr>
<tr>
<td>18</td>
<td>Pending</td>
<td>Gingrich</td>
<td>Univ. of Tennessee</td>
<td>Geno-therapeutics</td>
<td>Locally Advanced</td>
<td>Adenovirus</td>
<td>p16</td>
<td>In vivo, intraprostatic injection/RPP</td>
</tr>
</tbody>
</table>

Primary Source: http://www.nih.gov/od/orda
Prostate cancer patients (n=21) who had failed standard therapy underwent ultrasound guided injection of retrovirus LXSN containing BRCA1 under the control of the viral promoter (LTR). No patients developed viral symptoms or evidence of viremia. Viral DNA was detected in prostate tissue by PCR at least 2 years after injection (unpublished results). Although average prostate volume was decreased one month after injection (30.9 ± 6.4cc vs. 36.5 ± 6.5cc), serum PSA in those patients who had metastatic disease remained unchanged. This study demonstrated the safety of direct injection of prostate cancer gene replacement therapy.

Initial clinical gene therapy results utilizing a similar gene replacement strategy of replication-defective adenovirus containing wild-type p53 (AdCMVp53) driven by the CMV promoter injected into patients prior to radical prostatectomy have recently been reported (Logothetis et al, 1999). To date, 17 patients with locally advanced prostate cancer have received at least one course of AdCMVp53 which consists of 3 administrations 14 days apart. Transperineal injections of 3 ml in 4-6 divided doses are delivered under transrectal ultrasound guidance. In this phase I/II study, the number of viral particles (vp) delivered was escalated from 3x10^10 vp per treatment per patient to 3x10^12 vp. Three patients completed a second course of therapy for > 25% reduction in tumor size as measured by endorectal coil MRI after the first course of treatment. There were no grade 3 or 4 toxicities in 14 evaluable patients. This study confirms the relative feasibility and safety of intraprostatic gene therapy injection. Further results regarding efficacy due to the apparent radiologic responses, correlations with gene expression, pathologic findings at prostatectomy, and surgical outcomes are pending at this time.

The first trial utilizing intraprostatic injection of a locally cytotoxic or “suicide” type of gene therapy has recently been completed by Scardino et al (Herman et al, 1999; Scardino et al, 1998). For the treatment of locally recurrent prostate cancer after definitive radiation therapy, eighteen patients received a single 1.1 cc injection of replication-deficient adenovirus containing the HSV-tk gene (AdHSV-tk) under the RSV promoter followed by 14 days of intravenous ganciclovir. The dose of AdHSV-tk was escalated from 1 x 10^8 to 1 x 10^11 IU. Three patients had > 50% reduction in PSA and 1 had a negative biopsy after treatment. Local and systemic toxicity was mild except for the final patient who developed severe thrombocytopenia and abnormal liver function tests. A second study to investigate multiple sites of injection prior to radical prostatectomy is currently in progress. These follow-up studies will provide important histologic confirmation of tumor response to the intraprostatic cytotoxic gene therapy. In addition, assessment of long term patient outcomes due to beneficial local or possible systemic immunologic “bystander” effects will be interesting.

An alternative approach to intraprostatic gene replacement or cytotoxic therapy which may indirectly enhance a host immunologic response against the tumor cells is to administer gene therapy to intentionally stimulate an immune response. Utilizing a liposomal vector, Belldegrun et al have treated 12 patients prior to radical prostatectomy and 9 patients with recurrent prostate cancer after radiation or cryotherapy with two injections of intraprostatic IL-2 (Patel et al, 1999). A total of 40 injections of 300-1500 μg of IL-2 were administered. For patients treated prior to radical prostatectomy, the average PSA declined by 5.3 ng/ml prior to surgery and 75% maintain undetectable PSA levels at 24-56 weeks after surgery. Average PSA declines in patients with recurrent disease were 3.6 ng/ml and 1.3 ng/ml after one or two injections, respectively. Although the significance of these PSA responses is not yet clear, this strategy utilizing liposomal delivery appears to be safe and may be locally and systemically therapeutic.

Three trials (Table 1) have been approved and initiated using recombinant vaccinia virus expressing PSA (PROSTVAC) as a tumor associated antigen (TAA) immunotherapy strategy. Chen et al treated 30 patients with hormone refractory prostate cancer who had undergone withdrawal of anti-androgen therapy with a vaccinia inoculation followed by PROSTAVAC immunization (Chen et al, 1998). Patients received 2.65 x 10^5 or 2.65 x 10^6 plaque forming units (PFU) once a month for 3 months followed by restaging. All patients developed local erythema at the vaccination site with all toxicities grade 0-1. Of 14 patients who completed the treatment course, 4 had stable disease (2 subsequently progressed at 5 and 6 months) and 10 had continued disease progression. In a patient population with less advanced disease, Eder et al treated 24 men with rising PSA after radical prostatectomy, radiation therapy, or both with 2.65 x 10^8 or 2.65 x 10^9 PFU once a month for 3 months followed by restaging. All patients developed local erythema at the injection site with all toxicities grade 0-1. Of 5 patients who completed the treatment course, 4 had stable disease (2 subsequently progressed at 5 and 6 months) and 10 had continued disease progression. In a patient population with less advanced disease, Eder et al treated 24 men with rising PSA after radical prostatectomy, radiation therapy, or both with 2.65 x 10^8-8 PFU as three consecutive monthly doses without significant toxicity (Eder et al, 1998). Patients were removed from the protocol for clinical progression or if PSA > 50% of baseline. Twelve out of 23 men maintained a stable disease status for ≥10 months. In a third study, Sanda et al administered PROSTVAC once to six patients with androgen-modulated recurrence of prostate cancer after radical prostatectomy (Sanda et al, 1999). In addition to evaluation for toxicity, time until rise in serum PSA after interruption of androgen deprivation therapy and Western blot analysis for anti-PSA antibody production were determined. Again, no dose limiting toxicity was observed. One patient maintained an undetectable serum PSA for over 8 months after withdrawal of anti-androgen therapy and immunization. This study emphasizes the variability in time to return of serum testosterone levels after withdrawal of anti-androgen therapy as observed by others (Oefelein,


Jarrard DF, Bova GS, Ewing CM, Pin SS, Nguyen SH, Baylin SB, 245


Scardino PT, Adler HL, Herman JR, Woo SLC, Aguilar-Cordova E, Timme TL, and Thompson TC (1998) In Herpes simplex virus-thymidine kinase (HSV-tk) mediated gene therapy is safe and active against locally recurrent prostate cancer after irradiation therapy (Los Angeles:Lippincott Williams & Wilkins), vol. 17 (suppl), pp. 308a.


Steinier MS, Satterwhite DJ, and Moses HL (1995) Molecular insights into altered cell cycle regulation and genitourinary malignancy. Urol Oncol 1, 3-17.


Steiner and Gingrich: Gene Therapy for Prostate Cancer

CA) pp. 132.


