

# In Situ Gene Therapy for Prostate Cancer

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**Abstract:** The incidence of prostate cancer has dramatically increased worldwide in the past decade, with mortality rates also increasing in many countries. Once prostate cancer is diagnosed, it is important to rapidly begin a treatment regimen that is either potentially curative or impedes disease progression. When the disease is confined to the prostate, it can be cured by radical prostatectomy or irradiation therapy. However, there are no curative therapies for locally advanced, recurrent, or metastatic diseases. Clearly, new therapies are needed for these patients. Gene therapy may provide additional therapeutic options with the potential to affect both localized and metastatic disease. Virus-mediated transduction of the herpes simplex virus thymidine kinase (HSV-*tk*) gene transfer, followed by a course of the prodrug ganciclovir (GCV), so-called suicide gene therapy, has been demonstrated by several investigators. The present *in situ* gene therapy clinical trial for human prostate cancer demonstrated safety, clinical efficacy, and biological effects of antitumor activity. HSV-*tk* clinical trials for prostate cancer are also ongoing in Japan, the Netherlands, and Mexico. Currently, numerous preclinical studies have reported immunomodulatory cytokine gene therapy, such as interleukin-2, interleukin-12, B7-1 (CD80), B7-2 (CD86) and granulocyte-macrophage colony-stimulating factor. Several clinical studies have been approved that potentially will show that these immunomodulatory gene therapies may generate an effective local and systemic antitumor activity and that should provide options for patients with prostate cancer. We review the multiple issues involved in current *in situ* gene therapy (gene/immunotherapy), its outcome, and future directions for patients with prostate cancer.

**Keywords:** Gene therapy, prostate cancer, immunomodulatory approaches.

## 1. INTRODUCTION

Prostate cancer is now the most commonly diagnosed cancer in U.S. men. In 2003, 200,900 new cases were detected, and 28,900 patients died [Jemal *et al.*, 2003]. The incidence of prostate cancer is on the rise, and it presently ranks as the eighth most common cause of male cancer death in Japan. There were 10,940 cases in 1994, and the number may increase to as many as 30,285 in 2015 [Kitagawa *et al.*, 1999]. Currently available therapies for prostate cancer are limited—potentially curative localized therapy (radical prostatectomy or irradiation) or palliative androgen ablation therapy for advanced disease. The apparent inadequacy of these treatment options for presumed localized disease, appears to be related, in part, to the presence of occult micrometastasis at the time of diagnosis and treatment. The reported failure rate, within 5 years, as manifested by a rising serum prostate-specific antigen (PSA) level for patients undergoing radical prostatectomy ranges from 20% [Ohori *et al.*, 1995] to 57% [Zietman *et al.*, 1994], which indicates the presence of either local tumor recurrence and/or metastasis. Indeed, although there is a general relationship of tumor volume with metastatic progression, relatively small tumors that are confined to the prostate may also seed metastasis [Sakr *et al.*, 1994]. These clinical observations have been supported by the results of *in vivo* experiments that have indicated that metastasis does not necessarily originate from the most abundant clone of malignant cells at the primary

site [Thompson *et al.*, 1995]. An additional confounding problem with prostate cancer is that the prevalence of histological cancers with low malignancy potential is high (about 40% in men >50 years old [Franks *et al.*, 1954; Stamey *et al.*, 1993]), which suggests that an increasing proportion of cancers that have been detected over the past decade, and that are currently being detected, may in fact be “clinically unimportant” [Stamey *et al.*, 1993]. In fact, data from some studies have shown that 10%–26% of nonpalpable cancers detected by PSA screening are “clinically unimportant” on the basis of pathologic criteria—for example, <0.5 cc, Gleason sum 6, and disease confined to the prostate [Epstein *et al.*, 1994; Ohori *et al.*, 1994; Goto *et al.*, 1996; Noguchi *et al.*, 2001]. However, the detection and treatment of potentially “clinically unimportant cancers” and their treatment with potentially harmful therapy, such as radical prostatectomy and irradiation therapy, may not be a reasonable therapeutic decision in many cases. It would be useful to have safe treatments that can supplement the current treatments for localized prostate cancer. In addition, and perhaps more important, a treatment that provides effective antimetastatic activity will be required to substantially reduce the mortality rate of this disease. Novel approaches, such as *in situ* gene/immunotherapy, may provide this antimetastatic activity. In the present article, we review the multiple issues involved in current *in situ* gene therapy for patients with prostate cancer.

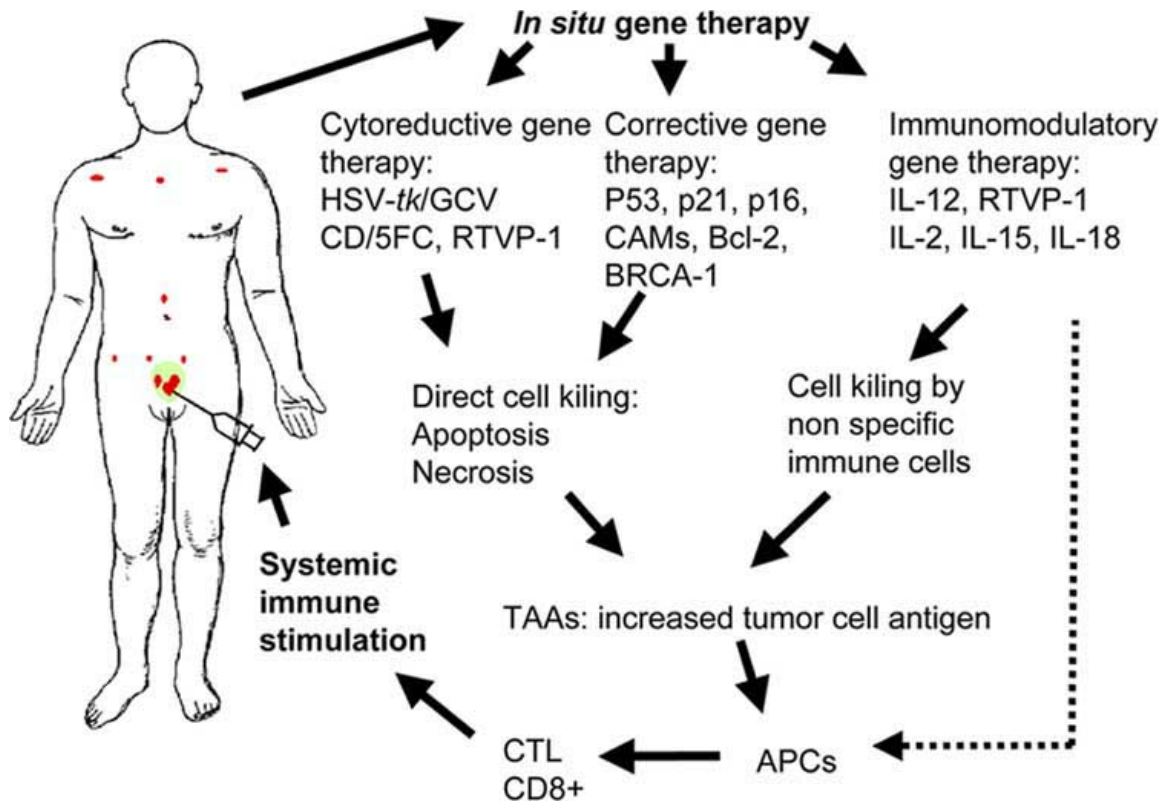
## 2. CURRENT CLINICAL TRIALS OF IN SITU GENE THERAPY FOR PROSTATE CANCER

*In situ* gene therapy, the direct injection of a viral vector into the tumor, is ideal for prostate cancer for a number of

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**Table 1. *In Situ* Gene Therapy Clinical Trials for Patients with Prostate Cancer**

Institution	Principal investigators	Vector	Gene	Phase	Country	NIH no.
Vanderbilt University School of Medicine	Holt & Steiner	RV	Antisense myc RNA	I	U.S.	9509-123
Baylor College of Medicine, Memorial Sloan-Kettering Cancer Center	Scardino&Thompson	Ad serotype 5	HSV- <i>tk</i> cDNA	I	U.S.	9601-144
University of California, Los Angeles	Belldegrum	Cationic liposome complex/DMRIE-DOPE	IL-2 cDNA	I	U.S.	9703-184
Mount Sinai School of Medicine	Hall & Woo	Ad serotype 5	HSV- <i>tk</i> cDNA	I	U.S.	9705-187
University of California, Los Angeles	Belldegrum	Ad serotype 5	p53 cDNA	I	U.S.	9706-192
The University of Texas MD Anderson Cancer Center	Logothetis	Ad serotype 5	p53 cDNA	I-II	U.S.	9710-217
Baylor College of Medicine	Kadmon&Thompson	Ad serotype 5	HSV- <i>tk</i> cDNA	I-II	U.S.	9801-229
Johns Hopkins University	Simons	Ad serotype 5	Promoter and enhancer elements of PSA	I	U.S.	9802-236
University of Virginia Health Science System	Gardner & Chang	Ad serotype 5	HSV- <i>tk</i> cDNA	I	U.S.	9812-276
University of California, Los Angeles	Belldegrum	Cationic liposome complex/DMRIE-DOPE	IL-2 cDNA	II	U.S.	9905-312
Henry Ford Health System	Freytag & Kim	Ad serotype 5	<i>E. coli</i> CD cDNA/HSV- <i>tk</i> DNA	I	U.S.	9906-321
Baylor College of Medicine	Butler & Aguilar-Cordova	Ad serotype 5	HSV- <i>tk</i> cDNA	I-II	U.S.	9906-324
University of Tennessee, Coleman College of Medicine	Gingrich	Ad serotype 5	p16 cDNA	I	U.S.	9909-338
Stanford University Palo Alto Veterans Administration Medical Center	Terris	Ad serotype 5/replication-competent virus	Elements of PSA	I-II	U.S.	9910-344
University of California, Los Angeles	Belldegrum	Cationic liposome complex/DMRIE-DOPE	IL-2 cDNA	I-II	U.S.	9910-352
The University of Texas MD Anderson Cancer Center	Pollack	Ad serotype 5	p53 cDNA	II	U.S.	0010-418
Indiana University Medical Center	Gardner	Ad serotype 5	Osteocalcin promotor	I	U.S.	0010-426
Henry Ford Health System	Freytag & Kim	Ad serotype 5	<i>E. coli</i> CD cDNA/HSV- <i>tk</i> DNA	I	U.S.	0010-428
Baylor College of Medicine	Miles&Thompson	Ad serotype 5	IL-12 c DNA	I	U.S.	0101-449
Johns Hopkins University	DeWeese	Ad serotype 5	Promotor and enhancer elements of the PSA	II	U.S.	0101-450
Henry Ford Health System	Freytag & Kim	Ad serotype 5	<i>E. coli</i> CD cDNA/HSV- <i>tk</i> DNA	I	U.S.	0104-464
Okayama University School of Medicine	Kumon	Ad serotype 5	HSV- <i>tk</i> cDNA	I	Japan	—
Erasmus University	Bangma	Ad serotype 5	HSV- <i>tk</i> cDNA	I	Netherlands	—
Universidad Autonoma de Nuevo Leon	Rojas-Martinez	Ad serotype 5	HSV- <i>tk</i> cDNA	I-II	Mexico	—
Kobe University School of Medicine	Gotoh	Ad serotype 5	Osteocalcin promotor	I-II	Japan	—



**Fig. (1).** *In situ* gene therapy for prostate cancer may result in antimetastatic benefits through the generation of immune cell-mediated cytotoxic activities. TAAs become available for presentation by APCs and subsequent induction of cytotoxic T lymphocyte with systemic anti-tumor cell activities.

reasons. Relative to many other tumor sites, prostate cancer is highly accessible to gene transfer. Transrectal ultrasound is a routinely used imaging modality that has been adapted for performing biopsies as well as other clinical applications. Gene therapy is routinely administered through ultrasound-guided needle injections directly into presumed tumor foci, which are visible as hypoechoic areas, and the spread of the vector is monitored by ultrasound during the injection. Additionally, because the prostate in older men is not an essential organ, gene-therapy effects on normal prostate cells are not likely to be life threatening. The relatively slow growth of prostate cancer makes the combined use of *in situ* gene therapy with surgery or irradiation therapy as a neoadjuvant/adjuvant approach a reasonable therapeutic option. *In situ* gene therapy for prostate cancer may result in antimetastatic benefits through the generation of immune cell-mediated cytotoxic activities that affect not only the primary tumor but also metastatic disease.

**2-1. Herpes Simplex Virus Thymidine Kinase Plus Ganciclovir**

Baylor College of Medicine conducted the first *in situ* herpes simplex virus thymidine kinase (HSV-*tk*) gene therapy phase I clinical trial for human prostate cancer and demonstrated its safety. In that clinical trial, men with a biochemical recurrence of localized prostate cancer after radiation therapy received a single injection of the adenoviral vector. Herman *et al.*, [1999], reported that some toxicity was observed at the highest dose ( $1 \times 10^{11}$ IU), and there were

indications of efficacy—serum PSA levels in 3 of 18 patients were suppressed by 50%. The trial was extended to an additional 18 patients, most of whom received a dose of  $1 \times 10^{10}$  IU. Additional safety studies confirmed that this dose was safe even when it was administered at multiple sites or repeated up to three times [Shalev *et al.*, 2000]. Further analysis of the patients in this gene therapy protocol indicated that this experimental treatment led to an increased PSA doubling time, a significantly increased mean percentage PSA reduction, and a significantly increased mean time to return to initial PSA levels after initial or repeat vector injections. An immune component in the response to this gene therapy protocol was evidenced by increased levels of activated (HLA DR-positive) CD8<sup>+</sup> T cells in the peripheral blood after treatment, and, of particular interest, an increase in the density of CD8<sup>+</sup> T cells in posttreatment biopsies. This latter observation was correlated with an increased number of apoptotic cells [Miles *et al.*, 2001]. Having demonstrated the safety and potential efficacy of herpes simplex virus thymidine kinase (HSV-*tk*) plus ganciclovir (GCV) gene therapy in men with a biochemical recurrence of their prostate cancer after radiation therapy, the clinical trial was expanded to include a group of men with newly diagnosed prostate cancer with clinical markers that suggested high-grade disease and who elected to undergo a radical prostatectomy. In this neo-adjuvant trial, the *in situ* gene therapy was delivered 4–6 weeks before surgery. The availability of the radical prostatectomy specimen allowed a clear demonstration that *in situ* gene therapy induced local inflammation within prostate cancer foci that was

accompanied by an increased infiltration of CD4 and CD8 T cells [Ayala *et al.*, 2000]. Remarkably, this form of therapy induced necrosis within prostate cancer lesions in preference to adjacent normal prostatic tissues [Ayala *et al.*, 2000]. Further studies confirmed that HSV-*tk* + GCV gene therapy treatment led to HLA DR-positive CD8<sup>+</sup> T cell activation in the peripheral blood of these men as well [Satoh *et al.*, 2002]. An additional phase I-II trial in progress combines two to three doses of HSV-*tk* with intensity-modulated radiation therapy and replaces intravenous GCV with the oral bioequivalent drug valacyclovir. Men in this trial were stratified to three groups, low-stage disease (PSA <10 ng/ml, biopsy Gleason score <7, and clinical stage T1-T2a), high-stage disease (PSA ≥10 ng/ml, biopsy Gleason score ≥7, and clinical stage T2b-T3), or stage D1 (regional lymph-node metastases). The latter two groups also received concurrent hormonal therapy. Mild hematologic and hepatic abnormalities could be attributable to the gene therapy, whereas genitourinary and gastrointestinal side effects were typical radiation-related side effects. There was no added toxicity attributable to the combination therapeutic approach [Teh *et al.*, 2001].

In Japan, a phase I trial is ongoing at the Okayama University School of Medicine and the Kobe University School of Medicine. The protocol design of the Okayama University trial is similar to the first Baylor trial, except for the enrollment criteria (Table 2). Patients with locally advanced hormone-refractory prostate cancer without definitive distant metastasis have been enrolled. The neoadjuvant gene therapy trials are also ongoing in the Netherlands [Nasu *et al.*, 2000] and in Mexico [Rojas-Martinez *et al.*, 2003].

## 2-2. Double-Suicide Gene Therapy

Freytag *et al.*, [1998] developed double-suicide gene therapy, which uses replication-competent Ad-5CD/Tkrep (Ad5-CD/tkrep) adenoviral vector concomitant with 5-fluorocytosine and GCV [Rogulski *et al.*, 2000]. In a phase I study, the patients received an escalating dose ( $10^{10}$ ,  $10^{11}$ , and  $10^{12}$  virus particles) of the replication-competent Ad5-CD/Tkrep adenovirus on day 1, followed by 1 week (days 3-9) of 5-FC (150 mg/kg/day) and GCV (10 mg/kg/day) prodrug therapy [Freytag *et al.*, 2002]. If none of the patients had a dose limiting toxicity (defined as any irreversible grade 3 or 4 toxicity), the study proceeded to the next cohort. A total of 16 patients in four cohorts were treated, and the vast majority (94%) of toxicity levels were mild (82% were grade 1) or moderate (12% were grade 2) in nature. The most frequent toxicities were increased in creatine phosphokinase (81%), decreased blood CO<sub>2</sub> (63%), anemia (44%), and thrombocytopenia (38%). Only one event required medical intervention. Seven (44%) of 16 patients exhibited a reduction in serum PSA ≥25% from pretreatment levels. Three (19%) of 16 patients achieved a partial response, defined as a reduction in serum PSA ≥50% for at least 4 weeks. Ad5-CD/Tkrep viral DNA could be detected in blood as far out as day 76, and no infectious adenovirus was detected in patients' serum and urine samples. The Henry Ford Health System has suggested that this double-suicide gene therapy can be safely applied to humans and shows signs of biological activity.

## 2-3. IL-2

IL-2 is one of the most effective antitumor cytokines used in clinical practice. It is known to promote activated T

**Table 2. Comparison of Clinical Trials of HSV-*tk* Gene Therapy with Adenovirus Vector for Patients with Prostate Cancer**

Institution	Baylor	Baylor	Baylor	Baylor	Mt. Sinai	U. Virginia	Erasmus	Okayama	Nuevo Leon	Kobe	Kitasato <sup>a</sup>
Principal investigator	Scardino	Miles	Kadmon	Butler	Hall	Gardner	Bangma	Kumon	Rojas-Martinez	Gotoh	Baba
Injection site	Prostate	Prostate	Prostate	Prostate	Prostate	Bone, L/N	Prostate	Prostate	Prostate	Bone, L/N	Prostate
No. of treatment cycles	1	2-3	1	2-3	1	1	1	1	1	1	2
Enrollment											
Stage	C	C	A-B	B-D <sub>1</sub>	A-B	D	A-B	C	A-B	D	A-B
Age	Any	Any	35-75	Any	Any	Any	35-75	>20	35-75	>20	35-75
Prior therapy	Radiation	Radiation	None	None	None	Hormone	Radiation	Hormone	None	Hormone	None
FU therapy	None	None	Neoadj. RPx	XRT/Hormone	Neoadj. RPx	None	Neoadj. RPx	None	Neoadj. RPx	None	Neoadj. RPx
Vector											
Promotor	RSV	RSV	RSV	RSV	RSV	Osteocalcin	RSV	RSV	RSV	Osteocalcin	RSV
Dose	$10^8$ - $10^{11}$ PFU	$10^{10}$ PFU	$10^{10}$ PFU	$10^{11}$ vp	$10^{10}$ - $10^{12}$ PFU	$10^8$ - $10^{10}$ PFU	$10^{10}$ PFU	$10^9$ - $10^{11}$ PFU	$10^{11}$ vp	$10^9$ - $10^{10}$ PFU	$10^{10}$ PFU

<sup>a</sup>Under review.

cell growth and to augment their cell-killing activity. Preliminary results have been reported of the clinical trial ongoing at the University of California at Los Angeles. In a phase I trial, intratumoral injection of a plasmid coding for IL-2, formulated in a liposomal, cationic lipid mixture vehicle, revealed decreases in serum PSA levels in 16 of (67%) 24 patients on day 1. Fourteen (58%) of the patients showed a persistence in this decrease that lasted until day 8. During the entire course of treatment, there were no significant changes in American Urologic Association symptom scores, hematologic disturbances, electrolyte imbalances, or hepatic functions. IL-2 gene therapy was well tolerated, with no grade 3 or 4 toxicity occurring [Belledegrun *et al.*, 2001].

#### 2-4. IL-12

IL-12 is a heterodimeric protein that is composed of two disulfide-linked subunits of 35 and 40 kD [Scott 1993; Gately *et al.*, 1998]. It is predominantly secreted by APCs, including monocytes, macrophages, B cells, and dendritic cells. IL-12 interacts with specific cell receptors, which in turn can activate gene expression through the Stat4 signal-transduction pathway [Gately *et al.*, 1998]. The effect of IL-12 plays an important role in initiating and orchestrating an immune response direct by central lymphoid effector cells, including NK cells, LAK cells, and both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Nasu *et al.* [1999] reported that enhanced NK-cell cytolytic activity was seen during the first 7 days after an injection with AdmIL-12. An immunohistochemical analysis of tumor specimens revealed enhanced macrophage activity such as nitric oxide synthase (NOS) activation and the support of cytokine production from and possible cytolytic activities of CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells within the local tumor tissues. The observation of multiple immunocyte activities that potentially could develop into a systemic antitumor immune response involving the generation of memory T cells was evident, and the results of an analysis of distant antimetastatic activity in response to local injection of AdmIL-12 further supported this notion. In a series of experiments in which AdmIL-12 was directly compared with AdHSV-*tk* + GCV, results showed somewhat superior activity in regard to local cytotoxicity, overall survival increased so did antimetastatic effects [Nasu *et al.*, 2001]. These preclinical data have led to the generation of a replication-defective human IL-12 transducing adenoviral vector that has recently been tested and found to be suitable for clinical trials. A phase I clinical trial involving an intraprostatic injection of AdIL-12 injection in patients who have failed irradiation therapy or who have metastatic prostate cancer is planned at Baylor College of Medicine. In another clinical trial, Kang *et al.*, [2001] reported that a phase I dose-escalation trial of peritumoral injections of IL-12-transduced autologous fibroblasts was done in patients with disseminated cancer. Nine patients were enrolled in this dose-escalation study, and treatment-related adverse events were limited to mild to moderate pain at the injection site; clinically significant toxicities were not encountered. Transient but clear reductions in tumor sizes were observed at the injection sites in four of nine patients. They suggested that gene therapy by peritumoral injection of IL-12-producing autologous fibroblasts is feasible in patients with advanced cancer.

#### 2-5. p53

The p53 gene is one of the most studied tumor suppressor genes. Located on chromosome 17, it is mutated in many high-grade or metastatic cancer variants. It has been found to be mutated in >50% of advanced prostate cancer and in up to 70% of prostate cancer metastasis [Thompson *et al.*, 1996]. The p53 gene is involved in many aspects of cell proliferation. In the G1-S interface of the cell cycle, it induces cell cycle arrest through the p21 gene product, which allows DNA damage to repair before replication. Defective DNA repair can result in an induction of apoptosis by p53-mediated activities [Levine *et al.*, 1997]. Thus, a defective p53 gene is a major contributor to the development and progression of malignancy. Preclinical studies testing the adenoviral-mediated expression of the wild-type p53 gene in patients with prostate cancer are in progress at several institutions, and results from the University of Texas M.D. Anderson Cancer Center have suggested that this vector is safe [Merritt *et al.*, 2001].

#### 2-6. p16

Tumor suppressor gene p16 (also known as MTS1, INK4A, and CDKN2) is a cyclin-dependent kinase inhibitor and an important negative cell cycle regulator. It prevents phosphorylation, and therefore inactivation of the Rb(retinoblastoma) gene [Serrano *et al.*, 1993]. Inactivation of p16 has been reported in most prostate cancer cell lines and 46% of metastatic prostate lesions exhibit loss of heterozygosity [Jarrard *et al.*, 1997; Steiner *et al.*, 2000]. This suggests that most patients with advanced prostate cancer may be suitable candidates for p16 gene therapy. Preclinical studies using an adenoviral vector delivering p16 demonstrated wild-type p16 inhibited prostate-cancer proliferation *in vitro* and markedly suppressed tumors *in vivo* [Steiner *et al.*, 2000; Allay *et al.*, 2000] and a Phase I clinical trial is currently underway at University of Tennessee.

#### 2-7. Osteocalcin Promotor-HSV-*tk*

Osteocalcin (OC), a major non-collagenous bone-matrix protein, is expressed prevalently in prostate cancer epithelial cells, adjacent fibromuscular stromal cells, and osteoblasts in locally recurrent prostate cancer and prostate cancer bone metastasis [Matsubara *et al.*, 2001]. A phase I dose-escalation clinical trial of the intralesional administration of Ad-OC-HSV-*tk* followed by oral valacyclovir was conducted at the University of Virginia (Charlottesville) in 11 men with localized recurrent and metastatic hormone-refractory prostate cancer [Kubo *et al.*, 2003]. In that trial, the therapeutic adenoviruses were injected directly into prostate cancer lymph-node and bone metastasis. All patients tolerated this therapy with no serious adverse events, and local cell death was observed in treated lesions in seven patients (63.6%), as assessed by Tdt-mediated dUTP digoxigenin nick-end labeling assay. One patient showed stabilization of the treated lesion for 317 days with no alternative therapy administered. They concluded that this form of gene therapy requires further development for the treatment of androgen-independent prostate cancer metastasis, although histopathologic and immunohistochemical evidence of apoptosis was observed in the specimens treated.

## 2-8. PSA Selective Oncolytic Adenovirus

CV706 is a prostate-specific antigen (PSA)-selective, replication-competent oncolytic adenovirus that has been shown to selectively kill human prostate cancer xenografts in preclinical models [Rodriguez *et al.*, 1997; Chen *et al.*, 2001]. Phase I dose-ranging study for the treatment of patients with locally recurrent prostate cancer after radiation therapy was conducted [DeWeese *et al.*, 2001], and twenty patients in five groups were treated with between  $1 \times 10^{11}$  and  $1 \times 10^{15}$  viral particles delivered by a transrectal ultrasound-guided transperineal technique. In this study, CV706 was found to be safe and was not associated with irreversible grade 3 or any grade 4 toxicity. No grade >1 alterations in liver function tests associated with CV706 administration were observed. Post-treatment prostatic biopsies and detection of a delayed "peak" of circulating copies of virus provided evidence of intraprostatic replication of CV706. The study defined the timing of CV706 shedding into blood and urine as well as the appearance of circulating Ad5 neutralizing antibodies. This study documents the serum PSA response of treated patients and reveals a dose response showing that all five patients who achieved a > or =50% reduction in PSA were treated with the highest two doses of CV706.

## 2-9. Oncolytic Herpes Simplex Virus

G207 [Mineta *et al.*, 1995; Yazaki *et al.*, 1995] is a multimutated herpes simplex virus 1 (HSV) vector that replicates within cancer cells, causing cellular death. However, replication is limited in normal cells, including those of the nervous system. *In vitro*, G207 at a low multiplicity of infection (MOI of 0.01) is oncolytic for multiple human prostate cancer cells. In athymic mice, a single intraneoplastic inoculation of G207 completely eradicates >22% of established subcutaneous human prostate cancer tumors irrespective of hormonal responsiveness. Two intraneoplastic inoculations of G207 completely eradicated two of three recurrent previously irradiated tumors and two intravenous administration of G207 induced tumor regression in distant subcutaneous tumors and completely eradicated one-fourth of the tumors [Walker *et al.*, 1999]. Phase I clinical trial of three vectors, G207, 1716, and NV1020, are either ongoing or completed, with no adverse events attributed to the virus. These and other HSV-1 vectors are effective against a myriad of solid tumors in mice, including glioma, melanoma, breast, prostate, colon, ovarian, and pancreatic cancer. Enhancement of activity was observed when HSV-1 vectors were used in combination with traditional therapies such as radiotherapy and chemotherapy, providing an attractive strategy to pursue in the clinic [Varghese *et al.*, 2002].

## 3. FUTURE DIRECTIONS

### 3-1. Immunomodulatory Gene Therapy

#### 3-1-1. RTVP-1

In prostate cancer progression, p53 mutations do occur at significant levels in metastatic disease, and the pattern of p53 mutations indicates a role in metastasis suppression

[Thompson *et al.*, 1996]. To identify prostate cancer-related genes under the transcriptional regulation of p53, a mouse prostate cancer cell line deficient in p53 was transduced with an adenoviral vector that expresses wild-type p53, and differential-display polymerase chain reaction was used to compare them with the same cells transduced with a control vector. Thompson *et al.* isolated genes not previously associated with p53 regulation, which was a mouse sequence homologous to the human gene, RTVP-1 (related to testes-specific, vepsid and pathogenesis proteins) [Ren *et al.*, 2002]. This gene induces p53 and significantly down-regulated in metastatic mouse and human prostate cancer. In prostate cancer cells, the adenoviral vector-mediated overexpression of the mouse RTVP-1 gene (mRTVP-1) induced apoptosis and growth suppression *in vitro* [Ren *et al.*, 2002]. *In situ* gene therapy with mRTVP-1 can have therapeutic effects that include the suppression of tumor growth and lung metastasis. These therapeutic responses may be associated with RTVP-1-mediated activities other than apoptosis, given that increased numbers of specific tumor-infiltrating immune cells were also observed in these studies [Satoh *et al.*, 2003]. Thus, RTVP-1 could become extremely useful as a therapeutic gene for prostate cancer, and this gene therapy may have an advantage of immunostimulatory functions over AdHSV-*tk* or AdIL-12.

#### 3-1-2. B7

B7 is known to comprise at least two molecules, B7-1 and B7-2 (CD80 and CD86, respectively), which are expressed on APC such as activated B cells, dendritic cells, and activated macrophages. The costimulatory B7-CD28 interaction enhances and sustains the T-cell activation signals transmitted by the peptide-MHC-TCR interaction. B7-1 is poorly expressed on most tumor cells, thus, genetically engineered tumor cells that express B7-1 have been studied as *in situ* gene therapy or whole-cell vaccines in several preclinical models [Chen *et al.*, 1994]. Hull *et al.* [2000] directly compared the effectiveness of an adenovirus that expresses both IL-12 and B7-1 (AdmIL-12/B7) with one that expresses IL-12 alone (AdmIL-12) using the poorly immunogenic RM-9 orthotopic murine model of prostate cancer. A significant reduction in orthotopic tumor size and increased survival was demonstrated in mice treated with a single orthotopic injection of AdmIL-12/B7 compared with AdmIL-12 or controls. Interestingly, orthotopic treatment of tumors with both vectors led to an infiltration of both CD4+ and CD8+ immunoreactive cells, with AdmIL-12/B7 treatment having a more prolonged infiltration of CD8+ cells. AdmIL-12/B7 was also more effective than AdmIL-12 or controls at suppression of pre-established metastasis.

#### 3-1-3. IL-15

IL-15 is a member of the four-helix bundle cytokine family that shares many *in vitro* biological activities with IL-2 [Cosman *et al.*, 1995]. It is unique in its influence on the development of NK-cells and CD+8 memory T-cells. *In vivo* studies in nude mice found that IL-15-transfected PC-3 tumors contained necrotic areas with high apoptotic index. These observations suggest that NK cell-mediated, anti-tumor effects of IL-15 could provide a potential rationale for gene therapy of prostate cancer [Suzuki *et al.*, 2001].

### 3-1-4. IL-18

IL-18, originally identified as interferon-gamma inducing factor (IGIF), is related to the IL-1 family in terms of its structure, processing, receptor, signal transduction pathway and pro-inflammatory properties. IL-18 is also functionally related to IL-12, as it induces the production of Th1 cytokines and participates in cell-mediated immune cytotoxicity [Lebel-Binay *et al.*, 2000]. *In vivo* studies in mice found that combination of IL-12 and IL-18 of electro-gene therapy (EGT) inhibited tumor growth significantly better than IL-12 EGT, which was consistent with significantly higher intratumoral levels of IFN-gamma. Enhanced infiltration of tumor tissues by CD8+ T cells was confirmed with both IL-12 and IL-12 + IL-18 EGT [Tamura *et al.*, 2003].

## 3-2. Corrective Gene Therapy

### 3-2-1. p21

The p21 gene product is a downstream mediator in the p53 induced growth suppression system. It is cyclin-dependent kinase inhibitor that binds to damaged DNA in proliferating cells and inhibits the progression from the G1 to S phase of replication. Its abnormal expression in prostate cancer is associated with a poor prognosis [Aaltomaa *et al.*, 1999]. Eastham *et al.*, reported the effect of transducing prostate cancer cells with adenoviral vectors carrying a p21 transgene *in vitro* and *in vivo* [Eastham *et al.*, 1995]. In direct comparison of the effectiveness of p21 and p53 gene therapy, in prostate cancer models slower tumor cell growth rate and prolongation of animal survival was obtained with adenoviral mediated p21 gene therapy.

### 3-2-2. CAMs

The cell adhesion molecule (CAMs) family of gene products function as cellular anchors and mediate cell-to-cell signaling [Syrigos *et al.*, 1999]. CAMs are often mutated in prostate cancer and such abnormalities are associated with disease progression [Rodriguez *et al.*, 1997]. The androgen regulated C-CAM1 function as a tumor suppressor in prostate cancer [Lin *et al.*, 1999] and is reported to decrease prostate cancer tumor size in nude mice xenograft models when delivered by an adenoviral vector [Lin *et al.*, 1999].

### 3-2-3. Bcl-2

The Bcl-2 oncogene was first identified as a site involved in a t(14;18) chromosomal translocation in follicular lymphomas [Tsujiimoto *et al.*, 1985]. The Bcl-2 gene is the prototype of novel class of oncogenes that contribute to neoplastic progression by enhancing tumor cell survival through inhibition of apoptotic death. Over expression of Bcl-2 is frequently observed in prostate cancer and is implicated in the progression from androgen-dependent to androgen-independent cancer [McDonnell *et al.*, 1992]. Studies have shown that Bcl-2 inactivates of programmed cell death, such as Bax, through heterodimerization and that it is the ratio of Bcl-2, or functionally similar gene products, to apoptosis inducers that determines whether a cell will undergo programmed death [Salomons *et al.*, 1997]. The antitumor activity of radiation and many chemotherapeutic agents appears to results from apoptosis that is dependent on

the expression of Bcl-2 in a variety of preclinical animal models. Thus, Bcl-2 appears to be a particularly attractive target for genetic downregulation in a wide range of tumor types.

### 3-2-4. BRCA1

The breast cancer susceptibility gene BRCA1 on chromosome 17q21 encodes an 1863 amino acid protein that is important for normal embryonic development. Germline mutations of this gene are linked to a significantly increased lifetime risk for breast and/or ovarian cancer, and recent studies suggest that the same may be true for prostate cancer [Rosen *et al.*, 2001]. Phase I-II clinical gene therapy trials using BRCA1 gene are being conducted in ovarian cancer at Vanderbilt University [Tait *et al.*, 1999] and proposed for prostate cancer.

## 4. CONCLUSION

Previously, a number of clinical gene therapy trials were using a single-gene or vaccine approach, in an attempt to generate a therapeutic impact on prostate cancer. Unfortunately, many of these trials were done during the terminal stages of the disease, when there are no other treatment options. However, in the patients with terminal-stage disease, compromised immune system due to an extensive large tumor burden may limit the effectiveness of gene immunotherapy. The biggest change likely to occur in prostate cancer therapy over the next 5 years is the adoption of novel therapies in combination with conventional therapies. The relatively slow growth of prostate cancer makes the combined use of *in situ* gene therapy with surgery or irradiation therapy as a neoadjuvant/adjuvant approach a reasonable option. *In situ* gene therapy for prostate cancer may result in antimetastatic benefits through the generation of immune cell-mediated cytotoxic activities that affect not only the primary tumor but also metastatic lesions. *In situ* gene therapy may work as an "active vaccine".

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