Gene and Cell Therapy for Prostate Cancer

Bhupesh Parashar1 • Dattatreyyudu Nori1 • Brij Saxena2

1 Stich Radiation Center, Weill Cornell Medical Center, New York Presbyterian Hospital, NY, NY 10021, USA
2 Reproductive Endocrinology, Department of Obstetrics and Gynecology, Weill Cornell Medical Center, New York Presbyterian Hospital, NY, NY 10021, USA

ABSTRACT

Prostate cancer is one of the leading causes of cancer death in men. A number of local curative treatments are available for patients with early localized prostate cancer. These include radiation therapy, radical prostatectomy, cryotherapy or brachytherapy. Locally advanced prostate cancer requires the addition of hormone therapy in addition to radiation therapy or radical prostatectomy. Many such patients go on to develop hormone refractory cancer or distant metastases. In such patients, gene therapy or cell therapy may be useful modalities in addition to or as alternatives to chemotherapy. In this review, we discuss various gene therapy vectors and the new cell based approaches as well as the pre-clinical and clinical data that are available for prostate cancer management.

Keywords: adenovirus, cell therapy, gene therapy, liposomes, sleeping beauty transposons, SV40, vaccinia virus

INTRODUCTION AND BACKGROUND

Prostate cancer is the most commonly diagnosed cancer in men and is the second leading cause of cancer death after lung cancer. Early stage, low risk prostate cancer (PSA ≤10, T1/T2 N0, Gleason score <7) is treated using external beam radiation therapy (EBRT), brachytherapy, radical prostatectomy, or cryotherapy either alone or infrequently as a combined modality approach. Intermediate and high risk prostate cancer (PSA ≥10, Gleason score≥7, T3/T4 ± nodes) is treated generally using a multimodality approach that may include a combination of surgery, radiation, hormones and chemotherapy.

Historically, surgery has been the earliest treatment used in the management of prostate cancer and was used initially to relieve urinary obstruction. There was, however, no systematic technique for removal of the prostate until the pioneering work of Hugh Hampton Young (Young 1905) who in 1904, performed the first radical perineal prostatectomy at the Johns Hopkins Hospital. Thereafter, advances in surgical techniques including retropubic, nerve sparing, laparoscopic and robotic techniques have revolutionized the surgical treatment of prostate cancer.

The history of radiation therapy for prostate cancer dates almost from the time of the discovery of radium by Madame Curie (Curie et al. 1898) at the end of the 19th century. The very first use of the newly discovered “X-rays” was to alleviate the pain of bony pelvic metastases. The first attempts to use radiation to treat localized prostate cancer were limited to introduction of radium sources into the urethra and rectum. At present, external radiation is delivered using advances linear accelerators and brachytherapy treatments are given using radioactive isotopes. External Beam radiation therapy (EBRT) involves delivery of high energy X-ray beams to the prostate as well as areas considered at risk of microscopic disease. The areas at risk may include the periprostatic tissue, seminal vesicles or lymph nodes. Commonly used radiation dose to the prostate is in the range of 7000-8100 cGy. Patients are immobilized on the treatment table and radiation is delivered in multiple fractions over a period of several weeks. Brachytherapy involves the introduction of radioactive pellets (seeds) into the...
prostate. The radioactive seed placement is done under loc- 
al, regional or general anesthesia utilizing ultrasound guid-
ance. Commonly used radioactive seeds such as Iodine-125 
(I-125) and Palladium-103 (Pd-103) emit low energy radia-
tion that is absorbed in the tissue immediately surrounding 
the seeds. Anywhere from 40 to 130 seeds may be placed in 
the prostate. However, the exact seed number is determined 
based on the prostate volume.

Radical Prostatectomy is the surgical removal of pros-
tate gland and possibly seminal vesicles and the surround-
ing nerves and veins. A part of the urethra traveling 
through the gland’s transitional zone is also removed. The 
two ends of the remaining urethra are reattached to form an 
anastomosis. Depending on the type of surgical technique, 
prostatectomy could be open, laparoscopic or robotic. 
Based on the site of surgical incision, prostatectomy 
could be retropubic or perineal. In retropubic approach, incision 
is made in the lower abdomen and lymph node dissection as 
well as nerve sparing operation can be performed, while the 
incision is in perineum in the latter approach.

The goal of cryotherapy is to ablate tissues using ex-
tremely cold temperatures. The major advances in the past 
15 years have included the use of real-time transrectal ultra-
sound (TRUS) monitoring of probe placement and freezing 
(Onik et al. 1988), the simultaneous use of multiple cryo-
probes, and the standard use of urethral warming catheters 
(Saiken et al. 2002). Another significant recent develop-
ment is the introduction of cryotherapy probes based on 
argon gas rather than liquid nitrogen. Argon rapidly cools 
the probe tip to -187°C (-304.6°F) and can be rapidly ex-
changed with helium at 67°C (152.6°F) for an active thaw-
ing phase, producing a faster response to operator input and 
significantly speeding 2-cycle treatment (de la Taille et al. 
2000). Moreover, argon-based probes have a much smaller 
diameter, thus permitting direct, sharp transperineal inser-
tion, avoiding the need for tract dilation and facilitating 
more conformal cryosurgery by allowing placement of 
more probes (Zisman et al. 2001).

The treatment of metastatic and advanced prostate can-
cer may include hormones, chemotherapy and radiation ther-
apy. High risk prostate cancer patients have a high risk of 
nodal and systemic recurrence despite surgery and radiation 
treatments. Studies have demonstrated a suppression of sys-
temic disease and an increase in local control (Lawton et al. 
2001; Pilepich et al. 2001; Hanks et al. 2003; Roach et al. 
2003) and survival (Bolla et al. 2002) with addition of hor-
omones and radiation. One advantage of adding androgen 
ablation during radiation is the cytoreduction caused by 
hormones, and radiation has in consequence less tissue to 
eradicate. Chemotherapy is another option to control sys-
temic and local disease. Several early studies showed 
benefit of adding chemotherapy to radiation (Kumar et al. 
2004; Swanson et al. 2006). In the management of meta-
static prostate cancer refractory to hormone treatments, che-
motherapy has been shown to have a modest effect with 
some studies reporting an overall response rate of ~9% (Ya-
goda et al. 1993). A recent randomized trial comparing doc-
etaxel plus prednisone vs. mitoxantrone plus prednisone in 
metastatic prostate cancer showed a 2.4 month median sur-
vival benefit in the docetaxel group (Tannock et al. 2004). 
A PSA response in 45% and pain response in 35% was seen 
in the better group. Another phase II study showed that a 
combination of docetaxel, vinorelbine and zolendronic acid 
in hormone-refractory prostate cancer is associated with 
improve-ment in biochemical, objective and pain response and 
is well tolerated as a first line therapy for such patients 
(di Lorenzo et al. 2007). A recent pre-clinical study evalu-
ated the antiproliferative and cytotoxic effects of a com-
bination of selective inhibitors of EGFR (epidermal growth 
factor receptor) tyrosine-kinase and smoothened hedgehog 
signaling element gefitinib and cyclopamine with docetaxel 
in prostate cancer cell lines (Mimeault et al. 2007). The 
results indicated that the drugs alone or in combination in-
hibited the growth of both androgen dependent and inde-
pendent prostate cancer cell lines and could be used for 
high-risk hormone refractory patients. Table 1 lists the stand-
ard therapies for prostate cancer and the toxicities associ-
ated with them.

In addition to the above mentioned `standard’ therapies 
for prostate cancer, there is laboratory and clinical evidence 
of the efficacy of complementary and alternative therapies 
for treatment and prevention of this disease. These therapies 
include carotenoids, Vitamins A, C, D, E, dietary fat, phyto-
estrogens, selenium and herbs such as PC-SPECS (Wilkin-
son et al. 2003).

The treatment of metastatic prostate cancer is palliative 
that may include hormones, chemotherapy and radiation 
therapy. Despite the recent development of more effective 
chemotherapeutic regimens, the overall prognosis of high 
risk and metastatic prostate cancer is poor. This has led to 
the investigations of novel cell and gene-based approaches 
to treat both localized and disseminated prostate cancer.

MOLECULAR BIOLOGY OF PROSTATE CANCER

Studies have demonstrated multiple alterations in tumor 
suppressor genes in human prostate cancer including alte-
ratings in p53, PTEN1, Msi-1 and Kai-1. In addition, loss of 
hetozygosity (LOH) at polymorphic loci on 8p, 10p, 16q 
and 18q (Effert et al. 1992; Veronese et al. 1996; Jenkins et al. 
1998; Whang et al. 1998; Verma et al. 1999) has also 
been reported. An important regulator of PSA expression is 
androgen receptor (AR). Human AR is a ligand-dependant 
nuclear transcription factor that controls expression of genes 
responsible for growth and development of normal and ma-
lignant prostate tissue. AR activity is repressed by p53. A

Table 1 Toxicity associated with standard therapies for prostate cancer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td>Genitourinary (GU): Cystitis/urethritis, hematuria, urethral stricture, bladder contracture</td>
</tr>
<tr>
<td>Gastrointestinal (GI): Diarrhea, tenesmus, rectal and anal stricture, hematochezia, bowel ulceration, obstruction or perforation</td>
<td></td>
</tr>
<tr>
<td>Sexual: Erectile dysfunction, impotency, leg or genital edema, pain during ejaculation, hematospermia</td>
<td></td>
</tr>
<tr>
<td>Second cancers, fatigue</td>
<td></td>
</tr>
<tr>
<td>Complication associated with anesthesia (brachytherapy)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>Complication associated with anesthesia</td>
</tr>
<tr>
<td>Blood loss, rectal injury, deep vein thrombosis</td>
<td></td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td></td>
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<tr>
<td>Bladder neck and anastomotic strictures</td>
<td></td>
</tr>
<tr>
<td>Impaired bowel function including diarrhea, rectal urgency, fecal incontinence</td>
<td></td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td></td>
</tr>
<tr>
<td>Hormones</td>
<td>Impotence, hot flashes, osteoporosis, anemia, loss of libido, weight gain, muscle wasting, diarrhea, breast and nipple tenderness, liver toxicity, gynecomastia</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Docetaxel; Cytopenias, nausea/vomiting, hair loss, edema, liver functions, skin</td>
</tr>
<tr>
<td>Mitoxantrone; Cytopenias, nausea/vomiting, hair loss, cholestasis, cardiac</td>
<td></td>
</tr>
<tr>
<td>Cryotherapy</td>
<td>Impotence, incontinence, tissue sloughing, pelvic-rectal pain, penile numbness, recto-urethral fistula, urethral stricture, hydronephrosis, small bowel obstruction</td>
</tr>
</tbody>
</table>

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mutation of p53 disrupts the p53 negative activity on the AR (Shenk et al. 2001). Some studies have implicated allelic losses at the CAG and GCC trinucleotide repeats of the AR gene with high-grade, aggressive prostate cancer (Kittles et al. 2001). E-cadherin and α-catenin are components of adhesion junctions which mediate calcium dependent cell-cell adhesion in a homotypic manner. Reduced levels of E-cadherin have been reported in advanced prostate cancer (Nelson et al. 1981). Several genes have been found to be over-expressed in prostate cancer. These include δ-catenin, an adhesion junction-associated protein that promotes cell scattering and prostate-specific membrane antigen (PSMA/FOLH1) (Burger et al. 2002). Prostate stem cell antigen (PSCA) a 123 amino-acid glycoprotein is strongly expressed in the prostate and has been shown to be over-expressed by more than 80% of prostate cancers and is correlated with an aggressive phenotype and a high Gleason score (Gu et al. 2000). Annexin1 is a calcium-dependent binding protein down-regulation of which is a common finding in high-grade prostate intraepithelial neoplasia and prostate cancer (Kang 2002).

Novel genes that have been identified in the pathogenesis of prostate cancer include tumor suppressor genes LAPSER1 (Suido et al. 2007) and Deleted in Liver Cancer-3 type III TGF-beta receptor (TbetaRIII or betaglycan) (Turley et al. 2007) and Prosaposin (Koochekpour et al. 2007).

AN OVERVIEW OF GENE AND CELL THERAPY VECTORS

Gene therapy involves the transfer of a transgene to the target using a viral or a non-viral vector. The main objectives of gene therapy are (a) augmentation of a missing gene, (b) expression of pharmacologic gene products e.g. suicide genes in cancer gene therapy, (c) interference of an unwanted gene expression and (d) expression of genes that are not normally expressed in the tissues to produce the desired therapeutic effect. Commonly used viral vectors include adenoviruses, adeno-associated viruses, retroviruses (e.g. lentivirus), herpes-simplex virus (HSV), vaccinia virus (VV) and SV40 virus (simian virus) (Kay et al. 2001). Many of these viral vectors display a high efficiency in gene transfer and expression. However, their major limitations such as toxicity, immunogenicity, limited DNA carrying capacity, production and packaging problems, recombination and high cost, hamper their successful clinical applications (Tenenbaum et al. 2003; Brunetti-Pierri et al. 2004; Trajevski et al. 2005).

In contrast, non-viral vectors are relatively less immunogenic than viral vectors and are easier to produce on a larger scale, but display a less-efficient gene transfer to the tissues and have a limited period of gene expression. Non-viral vectors can be (a) chemical (e.g. liposomes and F-proteins) and (b) physical (e.g. electroporation, gene guns, nucleofection) (Foley et al. 2004).

Cellular vectors involve the use of dendritic cells (DCs), macrophages and T-cells from peripheral circulation to migrate into tumors. For example, in ex-vivo gene transfer, cells are isolated from a patient, transfected with the required gene, and are injected back into the same patient.

CANCER GENE THERAPY

Numerous in-vitro and preclinical animal model studies have shown remarkable efficacy of cancer gene therapy to induce cancer cell lysis and death, and decrease blood supply to the cancer cells in various types of cancer (Tseng 2002; Prieto et al. 2004). Cancer gene therapy can be broadly divided into gene transfer, oncolytic virotherapy and immunotherapy.

Gene transfer involves the transfer of a foreign gene into cancer cells or the surrounding tissues. Genes that have been selected for this kind of treatment include suicide genes such as herpes simplex virus type 1-thymidine kinase gene (HSVtk) (Sadeghi et al. 2005) and anti-angiogenesis genes (e.g. sFLT1 and statin-AE) (Ohlfest et al. 2005). The most common viral vector that has been used for gene transfer is the replication incompetent adenovirus though the type of vector depends on the effect for which it is being utilized. In suicide gene therapy, the presence of the herpes simplex virus type 1 (HSVtk) gene in transduced cells allows to eliminate by ganciclovir (GCV) any cell that may have undergone transformation (Sadeghi et al. 2005). A recently reported phase I trial utilized intraprostatic injections of lytic replication competent adenoviruses that delivered cytotoxic deaminase (CD) and HSVtk to malignant cells. Vector delivery was followed by 1-2 week delivery of produgs 5-fluorocytosine (5FC) and GCV. Biological response was seen as an decrease in PSA and tumor cell destruction histologically (Freytag et al. 2002). Solid tumors such as prostate, lung (nineteen patients with nonmetastatic non-small cell lung cancer who were not eligible for chemoradiation or surgery were treated with radiation therapy to 60 Gy over 6 weeks in conjunction with three intratumoral injections of Adenovirus-p53 (INGN 201)-tumor regression was seen at primary injected tumor) and pancreatic tumors (adenovirus vector encoding a soluble form of Flk1 (Flk1-Fc), a receptor for vascular endothelial growth factor, in 3 murine models of pancreatic adenocarcinoma-tumor regression seen after injection of the viral vector) and brain cancers (xenograft-bearing mice showed tumor regression on delivery of soluble-vascular endothelial growth factor receptor and angiostatin-endostatin fusion gene using sleeping beauty (SB) transposon coadministered with SB-transposase encoding DNA) have been treated successfully in various models using a variety of gene transfer methods (Tseng 2002; Swisher et al. 2003; Ohlfest et al. 2005; Satoh et al. 2005).

Oncolytic gene therapy vectors are viruses that have been genetically engineered to infect and destroy cancer cells through the propagation of the virus, expression of cytokotoxic proteins and cell lysis (Mullen et al. 2003). The key issue to use this strategy is the “conditional” oncolytic virus, that is, the virus is altered to specifically target a desired cell type or attenuated in a way that the desired target cells are much highly sensitive to its oncolytic cell lysis than non-targeted cells. A number of different viruses have been used for this purpose, including VV, adenovirus, HSV type I, Reovirus and Newcastle disease virus (Mullen et al. 2002). G207, a multi-mutated HSV, that was genetically modified and has a deletion of both ICP34.5 genes and an insertion inactivation of the ICP6 gene, permits replication within cancer cells but limits replication in non-targeted cells (Mineta et al. 1995). In a nude mice model of xenograft human prostate tumors, intratumoral injection of G207 caused a reduction of tumor size and a complete eradication of >22% of tumors and intravenous injection of G207 suppressed distant subcutaneous tumor growth (Walker et al. 1999).

Immunotherapy involves boosting the immune system to destroy cancer cells. Cancer cells are harvested from the patient or from established cancer cell lines and then are grown in vitro. These cells are then engineered to be more recognizable to the immune system by the addition of one or more genes, which are often cytokine genes that produce pro-inflammatory immune stimulating molecules, or highly antigenic protein genes. These altered cells are grown in vitro and killed, and the cellular contents are incorporated into a vaccine (Kowalczyk et al. 2003). GVAX™ is a cellular vaccine generated from the 2 allogeneic cell lines LN Cap and PC-3, which have been genetically modified to secrete GM-CSF and then irradiated to prevent further cell division. Two phase II trials have shown clinical response to GM-CSF tumor cell vaccine in metastatic and hormone refractory prostate cancer patients (Brand et al. 2006). Si-puleucel-T (Provenge®) is another immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells that have been cultured in vitro with a recom-
binant fusion protein composed of prostatic acid phosphatase (PAP) and GM-CSF. This has also shown clinical response in phase II (P-16) and phase III (D-9901 and D-9902A) clinical trials for hormone refractory prostate cancer patients (Brand et al. 2006).

VIRAL VECTORS

Adenovirus

Adenovirus is a double stranded DNA virus and has a DNA genome of ~38 kb. Serotypes 5 and 2 are most commonly used as vectors. The viral capsid is non-enveloped and consists of the following proteins: Hexon that binds to the Coxsackie and Adenovirus Receptor (CAR) on the target cell surface and Penton that binds to the α,β,γ integrins on the target cell surface. The adenovirus genome has four early transcription units (E1-4) and a late unit that codes for structural proteins. In the first generation of adenoviral vectors, E1 region was removed to insert the therapeutic gene and inhibit viral replication. However, despite the removal of this region, there was still a low level of transcription of viral genes that led to host cellular immune response against the virus resulting in limited gene expression. Later generations of adenoviral vectors have deletions of E2a, E2b and E4 that further reduce immunogenicity and help to generate replication-competent viruses (Relph et al. 2005). A third generation of viruses has been generated (utilizing helper viruses-viruses that provide necessary proteins in trans) in which virtually all viral genes have been deleted except for the packaging signal (gutless vectors) (Park et al. 1996).

There is a requirement of targeted adenoviral vectors in clinical applications in order to allow systemic administration, reduce normal tissue toxicity and reduce immune response. There are two main approaches for targeted expression: transductional targeting and transcriptional targeting. The identification of the route by which cells take up adenoviral vectors is called transductional targeting. The adenovirus anchors on to the cell surface by means of CAR and interaction of capsid penton proteins and integrins α,β, and α,β allows internalization of the vector (Wickham et al. 1993; Bergelson et al. 1997). CAR expression is higher in metastatic prostate cancer cells compared to primary tumors, and therefore makes adenoviruses suitable vectors for gene transfer and metastatic prostate cancer (Rauen et al. 2002). A phase I clinical trial showed that intraprostastic delivery of CV706 (a conditionally replicative adenovirus) could be safely administered to patients even at high doses and resulted in a clinical response shown by a decrease in PSA (de Weese 2001). In transcriptional targeting, targeting of gene expression to specific cell types can be achieved through the use of a tumor or tissue-specific promoter. For example, replication-competent adenovirus, CV706, has been developed with a selective toxicity for PSA-positive prostate cancer cells, using minimal enhancer/promoter constructs derived from the 5’ flank of the PSA gene, to drive the E1A gene (Rodriguez et al. 1997). A single intratumoral injection of the virus destroyed large LNCaP tumors and abolished PSA production in mice.

Retroviruses

Retroviruses are RNA viruses that replicate through a DNA intermediate facilitated by a RNA-directed DNA polymerase. Members of the retroviridae family have three common features, a receptor-mediated uptake of a membrane-coated viral particle into target cells and reverse transcription of a plus-stranded RNA genome into a double-stranded DNA that is integrated into cellular chromosomes, as well as cytoplasmic assembly of particles with incorporation of the full-length retroviral mRNA as the mobile form of genetic information (Miller et al. 1992; Baum et al. 2006). The genes required in the process are gag (encoding viral matrix, capsid, and nucleocapsid proteins), pol (encoding a protease, reverse transcriptase, and integrase), and env (encoding a bipartite membrane-anchored surface protein mediating target cell recognition and particle uptake). Replication-competent retrovirus vectors contain genes in addition to the gag-pol-env genome (Tai 2005). The replication-deficient retroviral vectors are generated by coexpressing the basic retroviral trans-acting genes from transcripts that are not intended to be incorporated into retroviral particles. The transgene is encoded within a transcript that contains all cis-regulatory sequences required for its retroviral packaging (Baum et al. 2006). The following three genera of retroviruses have been studied extensively: (1) simple gamma-retroviruses with vectors derived from murine leukemia virus (MLV), (2) complex lentiviruses with the vectors derived from the human immunodeficiency virus type 1 (HIV) and (3) spumaviruses with vectors derived from ‘human’ foamy virus (HFV). The reverse-transcribed DNA poxviruses are integrated into the host genome and DNA is then transcribed using the host machinery. Although this integration is beneficial for long term viral gene expression, it has potential adverse effects including insertional mutagenesis and the activation of proto-oncogenes. Except for Lentiviruses, retroviruses require actively dividing cells to complete their lifecycle. The limitation of retroviruses other than their potential mutagenic potential is the limited size of their genome (<10 kbp of DNA) and difficulty in achieving high viral titers for in-vivo gene transfer. Lentivirus vectors have been known to infect prostate cancer cells and induce cell death efficiently in vitro and in vivo (Bastide et al. 2003).

Herpes-Simplex Virus (HSV)

HSV-1 is a large double-stranded DNA viruses (152 Kb) whose capsule is surrounded by a thick protein layer and a lipid bilayer. HSV-1 is attractive for cancer therapy because of, (a) its ability to infect a broad range of cell types and species, (b) it is cytoplytic by nature, (c) the 152 kb genome that can be replaced with multiple therapeutic transgenes, (d) many anti-herpetic drugs are available as a safeguard against unfavorable replication of the virus and (e) the virus remains as an episome within the infected cell, even during latency decreasing the risk of insertional mutagenesis as is seen in retroviruses (Varghese et al. 2002). Following infection, the viral DNA circularizes in the nucleus and viral transcription starts with five immediate early genes that encode factors responsible for the transcription of the remaining viral genes and for evasion of the host immune system. Early gene expression initiates the onset of viral DNA synthesis and many early viral proteins are responsible for DNA metabolism while the late genes encode virion structural proteins (Roizman et al. 1996). Oncolytic HSV is highly effective against experimental prostate cancer both in vitro and in vivo. A conditionally replicating HSV-1 G207 has been shown to efficiently destroy DU-145 and PC-3 prostate cancer cell lines within 7 days of infection. In addition, subcutaneous inoculation in athymic mice bearing prostate cancer xenografts showed a significant reduction in tumor growth (Oyama et al. 2000; Cozzi et al. 2002).

Vaccinia Virus (VV)

VV is a member of the genus Orthopoxvirus of the family Poxviridae. The VV genome is a linear, double-stranded DNA molecule with hairpin loop at its ends. The two strands are joined at the ends, essentially resulting in a single stranded circular DNA molecule (Baroudy et al. 1982). Several features of VV make it an excellent choice as a gene delivery vehicle in vivo. These include the ability to have a wide host range, ability to infect almost all human cell types efficiently, the ability to accommodate at least 25 kb of foreign DNA sequence. This quantity could be further expanded by deleting viral DNA that is not required for replication in cultured cells. In addition, VV replication oc-
curs exclusively in the cytoplasm, eliminating the possibility of chromosomal integration thereby significantly reducing the chances of insertional mutagenesis. VV vectors can infect both dividing and non-dividing cells. These viruses have been used to generate a protective immune response to pathogens as well as humoral response against certain types of cancer. For example, insertion of the PSA gene can be used to induce an immune response against the expressed protein. A phase II clinical trial utilizing vaccinia-PSA vaccine in prostate cancer patients showed significant freedom from clinical progression as well as minimal toxicity (Kaufman et al. 2004).

**SV40 Virus**

Recombinant SV40 (rSV40) viruses can potentially be used for gene therapy of prostate cancers. Vectors are made from wild type (wt) SV40 by modifying the viral genome to delete some or all SV40 genes, and replacing them with the gene or genes to be delivered. Additional promoters, transcriptional stop signals, etc., may be added (Strayer et al. 2005). The maximal total DNA that can be packaged in a rSV40 vector using this approach ~5.7 kb. rSV40 vectors are generally highly efficient gene delivery vectors. In vitro, DU-145 cells at virus:cell ratios > 10, they will deliver their genes to virtually every target cell available (Strayer et al. 2002). In vivo, high transduction efficiencies require repeated administrations (e.g., to the liver), but effectiveness of gene delivery to such solid organs as the liver can exceed 90% of cells (Sauter et al. 2000). rSV40s appear to be equally effective in transducing cells that are actively cycling as in cells that are not. rSV40-derived vectors can be and have been administered, and multiply, without generating detectable neutralizing immune responses. This observation, which has been documented by several different investigators, represents a unique feature of rSV40 vectors that endows them with several important properties. Like oncoretroviruses, SV40 integrates at random into cellular DNA. In so doing, it may activate or inactivate cellular genes. Disruption of a critical gene may cause a cell to become dysfunctional or die. BxPC3 and Capan-1 pancreatic cancer cells were efficiently transduced using SV40 vectors generated by combining an hTR tumor-specific promoter with sst2 somatostatin receptor tumor-suppressor gene. In vitro cell proliferation was strongly impaired following administration of SV (hTR-sst2). In vivo, intratumoral gene transfer of sst2 using rSV40 vectors resulted in a marked inhibition of Capan-1 tumor progression, and proliferation (Cordelier et al. 2007).

**NON-VIRAL VECTORS**

Non-viral vectors generally are less immunogenic than viral vectors and are easier to produce on a large scale. However, they have limited transfection efficiencies and have limited application in anti-cancer gene therapies.

**Liposomes and polycations**

Liposomes are in vitro-generated, self-enclosed vesicles consisting of a spherical lipid bilayer and a hydrophilic inner compartment. The versatility of liposomes permits drug or antigen for delivery to be integrated into the lipid membrane, encapsulated by the vesicle, or both. With a stable and inert liposomal formulation, encapsulated material is protected from rapid extracellular degradation. In terms of improving drug delivery, encapsulation may decrease the required dose and increase the efficacy of the entrapped drug at the target organ or tissue (Gabizon et al. 1998). Modification of the liposome surface with antibodies or ligands recognized by specific cell types can enhance tissue targeting and modulation of antigen processing as well. Liposomes are easy to produce in large numbers and are non-immunogenic. The route of administration can affect the physical properties and the transfection ability of the lipoplex. Prostate specific membrane antigen (PSMA) is a membrane antigen of the prostate epithelium and prostate cancer cells, and the expression of PSMA in prostate cancer cells is characterized by its upregulation under androgen ablation conditions (Wright et al. 1996). Anti-PSMA-liposome complex containing a suicide gene, thymidine kinase, demonstrated a selective growth-inhibitory effect on LNCaP cells in vitro. Significant enrichment of plasmid DNA was observed at the target prostate. LNCaP xenografts with anti-PSMA-liposome complex in comparison with normal IgG-liposome complex in xenograft model of LNCaP cells in nude mice. Anti-PSMA-liposome complex exerted a significant inhibitory effect on the growth of LNCaP xenograft, in contrast to normal IgG-liposome complex (Ikegami et al. 2006).

Liposomes can also be used to transfer chemicals and drugs into tumor cells. One such chemical against prostate cancer that has recently been studied is betulonic acid (Saxena et al. 2006). Betulinol is extracted from the outer bark of the white birch tree. The structure of betulinol is based on a 30-carbon skeleton comprising of four, six-member rings and one 5-member E-ring containing an iso-propyl group. Betulonic acid, a derivative of betulinol, showed high cytotoxicity in vitro in an MTT assay and significant inhibition of colony and tumor growth in an AIG assay on LNCaP human prostate cancer xenografts with anti-betulonic acid liposome complex in comparison with normal IgG-liposome complex (Ikegami et al. 2006).

Betulinol at a dose of 30 mg/kg body weight, showed up to 92% growth inhibition of the LNCaP xenograft tumors transplanted in athymic male mice (Saxena et al. 2006). RNA interference (RNAi) has the ability to silence gene expression in a sequence-specific manner and shown enormous potential as a powerful therapeutic strategy for treating various gene-related diseases (McManus et al. 2002; Lieberman et al. 2003). RNAi is induced by 21–23bp short interfering RNA, which elicits RNA-mediated endonucleolytic cleavage of a target mRNA by incorporating into RNA-induced silencing complex (RISC). However, due to its inherent instability and poor permeability across biological membranes, the successful application of small interference RNA (siRNA) in mammalian cells largely depends on the development of safe and efficient carriers for its delivery (Oishi et al. 2005). Several polycations have been introduced as non-viral gene carriers with a capability of forming stable complexes by electrostatic interaction with siRNA. A reducible poly (amido ethylenimine) (SPEI) synthesized by addition copolymerization of triethylene tetramine and cystamine bis-acrylamide (poly (TETA/CBA)) was used as a carrier for siRNA. Poly (TETA/CBA) could efficiently condense siRNA to form stable complexes under physiological conditions and performed complete release of siRNA in a reductive environment. When formulated with VEGF-directed siRNA, poly (TETA/CBA) demonstrated significantly higher suppression of VEGF than linear-polyethylenimine (PEI) (L-PEI, 25 kDa) in human prostate cancer cells (PC-3).

**Sleeping Beauty transposons**

Transposons are mobile genetic elements that can mediate transgene integration. Sleeping Beauty (SB) transposon is referred to as a synthetic transposon, as it was resurrected experimentally. SB is member of the Tcl/mariner superfamily of transposons and is a two-part system consisting of a transposase enzyme and a transposon DNA substrate. SB transposase binds to specific DNA sequences in the inverted repeat/direct repeat (IR/DR) termini of the transposon substrate as a homodimer, and mediates integration of the
transposon into a TA dinucleotide (Ivics et al. 1997). This technology has the potential to transfer and integrate anti-tumor genes (e.g. against prostate cancer) thereby resulting in continuous bombardment of tumor with anti-tumor proteins.

**PHYSICAL METHODS**

**Electroporation** is a technique of exposing cells to a strong electrostatic field that temporarily alters the structure of cell membrane, facilitating entry of naked DNA. The optimal electrical parameters have to be defined for each tissue type. Electrodes are placed in the tissue of interest followed by application of an external electric field leading to an increase in transmembrane conductivity and diffusive permeability. This method of nonviral gene delivery is gaining popularity and has been used to mediate gene transfer into tumors (Cemazar et al. 2004). Because gene expression and protein production from plasmids are dependent on viable cells, it is critical that the majority of cells being transfected are not killed. Therefore, the optimal electroporation conditions are a subtle balance between enhancing permeability without causing extensive cell death and tissue trauma. In a mouse model, evaluation of the CD8+ T lymphocyte response to a prostate cancer DNA vaccine encoding prostate-specific antigen (PSA) after intradermal electroporation was performed. A significantly increased gene expression (100- to 1000-fold) and higher levels of PSA-specific T cells, compared to DNA delivery without electroporation, was demonstrated (Roos et al. 2006). Nucleofection is a new electroporation-based technique that uses a combination of electrical parameters and cell-type specific solutions to achieve the delivery of plasmid DNA into cell nucleus trigger rapid expression of transgenes (Zaragosi et al. 2007). The advantage of this technique is the ability to transflect non-proliferating and primary cells such as DCs and monocytes. It has been successfully applied to hematological and immunological cells and also to embryonic and adult stem cells and may find an application in gene or plasmid transfer in prostate cancer cells.

**Gene Gun Transfection or Particle Mediated Transfection**, entails coating very small gold particles with plasmid DNA and using pressured gas or an electrical current to bombard a tissue with the gold/DNA particles at high velocity (Cheng et al. 1993). The transfection efficiency is generally low and varies from 1% to 20% depending on the type and depth of the tissue. However, gene gun inoculation of DNA vaccine pSLC-3P-Fc induced a strong anti-tumor response in a mouse tumor model, which significantly inhibited tumor growth and prolonged the survival time of the tumor-bearing mice (Qin et al. 2006).

**CELLULAR THERAPY**

This approach utilizes cells that are known to migrate into tumor cells readily. These include T-cells, macrophages and DCs. Cells are isolated from a given patient, transfected *ex-vivo* and injected back into the same patient. Therefore, these cells are recognized as ‘self’ by a patient and are non-immunogenic. Some forms of bacteria are also being utilized to infiltrate and grow preferentially in tumors.

**T cells**

Cancer vaccines are capable of expanding tumor reactive T-lymphocytes through immunization. These vaccines rely on the activation and expansion of host CD8 cytotoxic T lymphocytes (CTLs) and CD4 helper T cells that bind to specific tumor antigens in context with human leukocyte antigen (HLA) class I or II molecules. T-cell activation and expansion is determined, to a large extent, by the interaction between the T cell and antigen-presenting cells (APCs), such as dendritic cells (DCs), during which antigenderived peptides complexed with the APC’s HLA molecules are presented to the T cell’s antigen receptor (TCR) (Steinman et al. 2002). T-cells with specificity for tumor-associated antigens can kill tumors directly by releasing proteolytic enzymes, such as perforins and granzymes. Other cells of the innate immune system (i.e., macrophages or neutrophils) may be attracted to the tumor site, thereby further enhancing the inflammatory anti-tumor response (Kubler et al. 2006). Vaccine-mediated T-cell stimulation can lead to the establishment of immunologic memory in vaccinated cancer patients, thus making anti-tumor responses more durable and sustained (Su et al. 2005). A novel strategy is to use genetically engineered T cells to accelerate the generation of tumor-specific T cells. Chimeric antigen receptors (CARs) are essential constituents of this new armamentarium. Unlike the physiologic T-cell receptor, CARs encompass immunoglobulin variable regions or receptor ligands as antigen-recognition elements, thus permitting T cells to recognize cell surface tumor antigens in the absence of HLA expression. T-cell activation is mediated by the cytoplasmic domain of the CAR, which is typically derived from the CD3- chain or the FcRγ chain. In vivo function of Pz1, a CAR-targeting human PSMA (prostate specific membrane antigen) was evaluated in three tumor models, orthotopic, s.c., and pulmonary and it was shown that PSMA-targeted T cells effectively eliminate prostate cancer (Gade et al. 2005).

**Dendritic cells**

Dendritic cells (DC) are the only cells in the body that stimulate naive T cells, and can activate B-cells to trigger antibody formation. DC can be isolated by leucophoresis of monocyte precursors and acquire the form of mature DC after culture with cytokines such as GMCSF and IL-4 (Sallusto et al. 1994). DCs are the most efficient cell type for processing exogenous antigen to major histocompatibility complex (MHC) class I and II pathways. In a phase I clinical trial using vaccination with DCs loaded with a cocktail consisting of HLA-A0201-restricted peptides derived from five different prostate cancer-associated antigens, prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), survivin, prostein, transient receptor potential p8 (trp-p8), 8 hormone-refractory prostate cancer patients received a total of four vaccinations every other week (Fuessel et al. 2006). Apart from local skin reactions, no side effects were noted. One patient displayed a partial response (PR; PSA decrease >50%) and three other patients showed stable PSA values or decelerated PSA increases. In ELISPOT analyses, three of four PSA responders also showed antigen-specific CD8+ T-cell activation against protein, survivin, and PSMA.

In another phase I/II trial performed to demonstrate feasibility, safety and induction of antigen-specific immunity by vaccination with DCs presenting prostate stem cell antigen (PSCA) and PSA peptides in patients with hormone- and chemotherapy-refractory prostate cancer (Thomas-Kaszel et al. 2006), patients received 4 vaccinations with a median of 2.7 x 10^7 peptide-loaded mature DC subcutaneous in biweekly intervals. Twelve patients completed vaccination without relevant toxicities. Six patients had stable disease after 4 vaccinations. One patient had a complete disappearance of lymphadenopathy despite rising PSA. Four patients with stable disease and 1 progressor developed positive delayed-type hypersensitivity (DTH) after the 4th vaccination. With a median survival of all patients of 3.5 months, PSA positivity was associated with significantly superior survival (p = 0.003). HLA tetramer analysis detected high frequencies of peptide-specific T cells after 2 vaccinations in 1 patient who was also the sole responder to concomitant hepatitis B vaccination as an indicator of immune competence and survived 27 months after start of vaccination. Table 2 is a list recent clinical trials using viral, non-viral and cell based therapies for prostate cancer.
Table 2 Recent Clinical trials using viral, non-viral and cell-based therapies for prostate cancer.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Gene</th>
<th>Phase</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenovirus</td>
<td>osteocalcin</td>
<td>Phase I/II</td>
<td>3 patients (pts) tested, 1 partial response, safe.</td>
<td>Hinata et al. 2006</td>
</tr>
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<td></td>
<td>CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus</td>
<td>Phase I</td>
<td>23 pts tested, 5 patients had decrease in PSA, no partial or complete responses, flu-like symptoms most common.</td>
<td>Small et al. 2006</td>
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<td></td>
<td>adenovirus expressing IL-2</td>
<td>Phase I</td>
<td>12 pts tested, PSA fell in 5 of 5 evaluable pts, no dose-limiting toxicity, side effects were perineal discomfort, flu-like symptoms, hematuria, urinary hesitancy.</td>
<td>Trudel et al. 2003</td>
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<td></td>
<td>replication-competent adenovirus (Ad5-CDTKrep) to deliver a cytosine deaminase/HSV-1K fusion gene</td>
<td>Phase I</td>
<td>16 pts tested, 44% showed ≥25% decrease in PSA, 19% ≥50% decrease, 2 pts transgene negative for adenoca at 2 yrs, 94% patients had mild to moderate side effects.</td>
<td>Freytag et al. 2002</td>
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<td></td>
<td>CV706-prostate-specific antigen (PSA)-selective, replication-competent adenovirus</td>
<td>Phase I</td>
<td>20 pts tested, no grade 3 or 4 toxicity, 5 pts with high doses ≥50% reduction in PSA.</td>
<td>de Weese et al. 2001</td>
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<tr>
<td></td>
<td>INGN 201(Ad-p53), an adenoviral vector that encodes a CMV driven wild-type p53 gene</td>
<td>Phase I</td>
<td>30 pts tested, no grade ≥3 toxicity, 10 of 11 pts with negative p53 before injection became + after injection.</td>
<td>Pisters et al. 2004</td>
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<tr>
<td>Retrovirus</td>
<td>GM-CSF secreting vaccines</td>
<td>Phase I</td>
<td>8 pts tested, T and B cell immune responses to prostate cancer generated, 2 pts had delayed-type hypersensitivity.</td>
<td>Simons et al. 1999</td>
</tr>
<tr>
<td></td>
<td>prostate-specific antigen (PSA)</td>
<td>Phase II</td>
<td>64 pts tested, minimal toxicity, 45.3% free of PSA progression at 19 months.</td>
<td>Kaufman et al. 2004</td>
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<tr>
<td></td>
<td>MUC-1 and IL-2 genes</td>
<td>Phase I</td>
<td>No grade ≥3 toxicity observed, maximum tolerated dose of 5 x 10⁷ PFU is safe and well tolerated.</td>
<td>Pantuck et al. 2004</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>Phase I</td>
<td>24 pts tested, no grade ≥3 reactions, transient PSA responses seen after treatment.</td>
<td>Beldregrun et al. 2001</td>
</tr>
<tr>
<td>Liposomes</td>
<td>prostate stem cell antigen and PSA</td>
<td>Phase I</td>
<td>12 pts vaccinated without relevant toxicities. 6 patients had stable disease after 4 vaccinations. 1 patient had a complete disappearance of lymphadenopathy despite rising PSA. 4 pts with SD and 1 progressor developed a positive DTH after the 4th vaccination.</td>
<td>Thomas-Kaskel et al. 2006</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>HLA-A*0201-restricted peptides derived from five different prostate cancer-associated antigens (prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), survivin, prostein, transient receptor potential p8 (trp-p8)]</td>
<td>Phase I</td>
<td>8 pts tested, local skin reaction only side effect, 1 pt partial response, 3 pts stable PSA.</td>
<td>Fuessel et al. 2006</td>
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<td></td>
<td>PA2024 (fusion protein of PAP and GMCSF)</td>
<td>Phase II</td>
<td>21 pts enrolled, most toxicities were grade 1/2, 4 pts gr 3/4 effects, 2 pts showed 25-30% decrease in PSA, one patient had pelvic, retroperitoneal lymphadenopathy resolved.</td>
<td>Burch et al. 2004</td>
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