

# Gene and Cell Therapy for Prostate Cancer

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## 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

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## 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  $\leq 10$ , 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  $\geq 10$ , Gleason score  $\geq 7$ , T3/T4  $\pm$  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 19<sup>th</sup> 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 advanced 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

**Table 1** Toxicity associated with standard therapies for prostate cancer.

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

prostate. The radioactive seed placement is done under local, regional or general anesthesia utilizing ultrasound guidance. Commonly used radioactive seeds such as Iodine-125 (I-125) and Palladium-103 (Pd-103) emit low energy radiation 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 prostate gland and possibly seminal vesicles and the surrounding 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 extremely cold temperatures. The major advances in the past 15 years have included the use of real-time transrectal ultrasound (TRUS) monitoring of probe placement and freezing (Onik *et al.* 1988), the simultaneous use of multiple cryoprobes, and the standard use of urethral warming catheters (Saiken *et al.* 2002). Another significant recent development is the introduction of cryotherapy probes based on argon gas rather than liquid nitrogen. Argon rapidly cools the probe tip to  $-187^{\circ}\text{C}$  ( $-304.6^{\circ}\text{F}$ ) and can be rapidly exchanged with helium at  $67^{\circ}\text{C}$  ( $152.6^{\circ}\text{F}$ ) for an active thawing 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 insertion, 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 cancer may include hormones, chemotherapy and radiation therapy. 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 systemic 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 hormones and radiation. One advantage of adding androgen ablation during radiation is the cyoreduction caused by hormones, and radiation has in consequence less tissue to eradicate. Chemotherapy is another option to control systemic 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, chemotherapy has been shown to have a modest effect with some studies reporting an overall response rate of ~9% (Yagoda *et al.* 1993). A recent randomized trial comparing docetaxel plus prednisone vs. mitoxantrone plus prednisone in metastatic prostate cancer showed a 2.4 month median survival 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 zoledronic 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 evaluated the antiproliferative and cytotoxic effects of a combination of selective inhibitors of EGFR (epidermal growth factor receptor) tyrosine-kinase and smoothed 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 inhibited the growth of both androgen dependent and independent prostate cancer cell lines and could be used for high-risk hormone refractory patients. **Table 1** lists the standard therapies for prostate cancer and the toxicities associated 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, phytoestrogens, selenium and herbs such as PC-SPECS (Wilkinson *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 alterations in p53, PTEN1, Mxi-1 and Kai-1. In addition, loss of heterozygosity (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 malignant prostate tissue. AR activity is repressed by p53. A

mutation of p53 disrupts the p53 negative activity on the AR (Shenk *et al.* 2001). Some studies have implicated alleles at the CAG and GGC trinucleotide repeats of the AR gene with high-grade, aggressive prostate cancer (Kittles *et al.* 2001). E-cadherin and  $\alpha$ -catenin are components of adherens 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 1995). Several genes have been found to be over-expressed in prostate cancer. These include  $\delta$ -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 prostatic intraepithelial neoplasia and prostate cancer (Kang 2002).

Novel genes that have been identified in the pathogenesis of prostate cancer include tumor suppressor genes LAPSER1 (Sudo *et al.* 2007) and Deleted in Liver Cancer-3 (DLC-3) (Durkin *et al.* 2007), SIM2 (Halvorsen 2007), 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; Trajcevski *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 sui-

cide 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) treatment, 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 cytosine deaminase (CD) and HSVtk to malignant cells. Vector delivery was followed by 1-2 week delivery of prodrugs 5-fluorocytosine (5FC) and GCV. Biological response was seen as evidenced by a decrease in PSA and tumor cell destruction histologically (Freitag *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 cytotoxic 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 normal 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<sup>®</sup> is a cellular vaccine generated from the 2 allogeneic cell lines LNCaP 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). Sipuleucel-T (Provenge<sup>®</sup>) 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  $\alpha_v\beta_3$  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) (Parks *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  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  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 intraprostatic delivery of CV706 (a conditionally replicative adenovirus) could be safely administered to patients even at high doses and it 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 intratumoural 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 cytolytic 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 each end: 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 these genes with the gene or genes to be delivered. Additional promoters, transcriptional stop signals, etc., may also 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 vehicles. *In vitro*, 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 polyplexes

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 in 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 *a*-isopropyl 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, DU-145 and PC-3 human prostate cells, with little effect on normally proliferating fibroblast cells. The hydrophobic nature of betulonic acid and consequent lack of solubility in aqueous solvents had remained a limitation in achieving full expression of its anti-cancer activity *in vivo*. Hence, a lysinated derivative of betulonic acid was synthesized to yield a more hydrophilic compound. The higher activity of lysinated-betulonic acid against prostate cancer was further confirmed by using xenograft tumors *in vivo*. Lysinated-betulonic acid, 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 polyplexes 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) (SS-PAEI) synthesized by addition copolymerization of triethylenetetramine 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 perform 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 *Tc1/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 diffusible 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 transfect 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 anti-

gen-derived 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 $\zeta$  chain or the FcR $\gamma$  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 leucopheresis of monocyte precursors and acquire the form of mature DC after culture with cytokines such as GM-CSF 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 (Fuesel *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<sup>+</sup> T-cell activation against prostein, 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-Kassel *et al.* 2006), patients received 4 vaccinations with a median of  $2.7 \times 10^7$  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 13.4 months, DTH-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.

Vector	Gene	Phase	Results	Reference
adenovirus	osteocalcin	Phase I/II	3 patients (pts) tested, 1 partial response, safe.	Hinata <i>et al.</i> 2006
	promoter-driven HSV-tK CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus	Phase I	23 pts tested, 5 patients had decrease in PSA, no partial or complete responses, flu-like symptoms most common.	Small <i>et al.</i> 2006
	adenovirus expressing IL-2	Phase I	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.	Trudel <i>et al.</i> 2003
	replication-competent adenovirus (Ad5-CD/TKrep) to deliver a cytosine deaminase/HSV-tK fusion gene	Phase I	16 pts tested, 44% showed $\geq 25\%$ decrease in PSA, 19% $\geq 50\%$ decrease, 2 pts transgene negative for adenoca at 2 yrs, 94% patients had mild to moderate side effects.	Freytag <i>et al.</i> 2002
	CV706-prostate-specific antigen (PSA)-selective, replication- competent adenovirus	Phase I	20 pts tested, no grade 3 or 4 toxicity, 5 pts with high doses $\geq 50\%$ reduction in PSA.	de Weese <i>et al.</i> 2001
	INGN 201(Ad-p53), an adenoviral vector that encodes a CMV driven wild-type p53 gene	Phase I	30 pts tested, no grade $\frac{3}{4}$ toxicity, 10 of 11 pts with negative p53 before injection became + after injection.	Pisters <i>et al.</i> 2004
Retrovirus	GM-CSF secreting vaccines	Phase I	8 pts tested, T and B cell immune responses to prostate cancer generated, 2 pts had delayed-type hypersensitivity.	Simons <i>et al.</i> 1999
Vaccinia virus	prostate-specific antigen (PSA)	Phase II	64 pts tested, minimal toxicity, 45.3% free of PSA progression at 19 months.	Kaufman <i>et al.</i> 2004
	MUC-1 and IL-2 genes	Phase I	No grade $\frac{3}{4}$ toxicity observed, maximum tolerated dose of $5 \times 10^7$ pfu is safe and well tolerated.	Pantuck <i>et al.</i> 2004
Liposomes	IL-2	Phase I	24 pts tested, no grade $\frac{3}{4}$ reactions, transient PSA responses seen after treatment.	Belldgrun <i>et al.</i> 2001
Dendritic cells	prostate stem cell antigen and PSA	Phase I/II	12 pts tested vaccination 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 4 <sup>th</sup> vaccination.	Thomas-Kaskel <i>et al.</i> 2006
	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)]	Phase I	8 pts tested, local skin reaction only side effect, 1 pt partial response, 3 had stable PSA.	Fuessel <i>et al.</i> 2006
	PA2024 (fusion protein of PAP and GMCSF)	Phase II	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.	Burch <i>et al.</i> 2004

## REFERENCES

- Baroudy BM, Venkatesan S, Moss B (1982) Incomplete base-paired flipflop terminal loops link the two DNA strands of the vaccinia virus genome into one uninterrupted polynucleotide chain. *Cell* **28**, 315-324
- Bastide C, Maroc N, Bladou F, Hassoun J, Maitland N, Mannoni P, Bagnis C (2003) Expression of a model gene in prostate cancer cells lentivirally transduced *in vitro* and *in vivo*. *Prostate Cancer and Prostatic Disease* **6**, 228-234
- Baum C, Schambach A, Bohne J, Galla M (2006) Retrovirus vectors: toward the plentivirus? *Molecular Therapy* **13**, 1050-1063
- Belldgrun A, Tso CL, Zisman A, Naitoh J, Said J, Pantuck AJ, Hinkel A, de Kernion J, Figlin R (2001) Interleukin 2 gene therapy for prostate cancer: phase I clinical trial and basic biology. *Human Gene Therapy* **12**, 883-892
- Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW (1997) Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**, 1320-1323
- Bolla M, Collette L, Blank L, Warde P, Dubois JB, Mirimanoff RO, Storme G, Bernier J, Kuten A, Sternberg C, Mattelaer J, Lopez Torecilla J, Pfeffer JR, Lino Cutajar C, Zurlo A, Pierart M (2002) Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): a phase III randomised trial. *Lancet* **360**, 103-106
- Brand TC, Tolcher AW (2006) Management of high risk metastatic prostate cancer: the case for novel therapies. *Journal of Urology* **176**, S76-80
- Brunetti-Pierri N, Palmer DJ, Beaudet AL, Carey KD, Finegold M, Ng P (2004) Acute toxicity after high-dose systemic injection of helper-dependent adenoviral vectors into nonhuman primates. *Human Gene Therapy* **15**, 35-46
- Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, Richardson RL, Valone FH, Vuk-Pavlovic S (2004) Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a Phase 2 trial. *Prostate* **60**, 197-204
- Burger MJ, Tebay MA, Keith PA, Samarantunga HM, Clements J, Lavin MF, Gardiner RA (2002) Expression analysis of delta-catenin and prostate-specific membrane antigen: their potential as diagnostic markers for prostate cancer. *International Journal of Cancer* **100**, 228-237
- Cemazar M, Wilson I, Dachs GU, Tozer GM, Sersa G (2004) Direct visualization of electroporation-assisted *in vivo* gene delivery to tumors using intravital microscopy - spatial and time dependent distribution. *BMC Cancer* **4**, 81
- Cheng L, Ziegelhoffer PR, Yang NS (1993) *In vivo* promoter activity and transgene expression in mammalian somatic tissues evaluated by using particle bombardment. *Proceedings of the National Academy of Sciences USA* **90**, 4455-4459
- Cordelier P, Bienvenu C, Lulka H, Marrache F, Bouisson M, Openheim A, Strayer DS, Vaysse N, Pradayrol L, Buscail L (2007) Replication-deficient rSV40 mediate pancreatic gene transfer and long-term inhibition of tumor growth. *Cancer Gene Therapy* **14**, 19-29
- Cozzi PJ, Burke PB, Bhargava A, Heston WD, Huryk B, Scardino PT, Fong Y (2002) Oncolytic viral gene therapy for prostate cancer using two attenuated, replication-competent, genetically engineered herpes simplex viruses. *Prostate* **53**, 95-100
- Curie P, Curie MS, Benoit G (1898) Sur une nouvelle substance fortement radio-active, contenue dans la pitchblende. *Comptes Rendus* **12**, 1215
- de la Taille A, Benson MC, Bagiella E, Burchardt M, Shabsigh A, Olsson CA, Katz AE (2000) Cryoablation for clinically localized prostate cancer using an argon-based system: complication rates and biochemical recurrence. *BJU International* **85**, 281-286
- de Weese TL, van der Poel H, Li S, Mikhak B, Drew R, Goemann M, Hamper U, de Jong R, Detorie N, Rodriguez R, Haulk T, de Marzo AM, Piantadosi S, Yu DC, Chen Y, Henderson DR, Carducci MA, Nelson WG, Simons JW (2001) A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Research* **61**, 7464-7472

- di Lorenzo G, Autorino R, Perdoni S, de Laurentiis M, D'Armiento M, Cancelli G, Mirone V, Imbimbo C, Longo N, Altieri V, Tortora G, Figg WD, de Placido S (2007) Docetaxel, vinorelbine, and zoledronic acid as first-line treatment in patients with hormone refractory prostate cancer: A phase II study. *European Urology*, in press
- Durkin ME, Ullmannova V, Guan M, Popescu NC (2007) Deleted in liver cancer 3 (DLC-3), a novel Rho GTPase-activating protein, is downregulated in cancer and inhibits tumor cell growth. *Oncogene*, in press
- Effert PJ, Neubauer A, Walther PJ, Liu ET (1992) Alterations of the P53 gene are associated with the progression of a human prostate carcinoma. *Journal of Urology* 147, 789-793
- Foley R, Lawler M, Hollywood D (2004) Gene-based therapy in prostate cancer. *Lancet Oncology* 5, 469-479
- Freytag SO, Khil M, Stricker H, Peabody J, Menon M, de Peralta-Venturina M, Nafziger D, Pegg J, Paielli D, Brown S, Barton K, Lu M, Aguilard-Cordova E, Kim JH (2002) Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. *Cancer Research* 62, 4968-4976
- Fuessel S, Meye A, Schmitz M, Zastrow S, Linne C, Richter K, Lobel B, Hakenberg OW, Hoelig K, Rieber EP, Wirth MP (2006) Vaccination of hormone-refractory prostate cancer patients with peptide cocktail-loaded dendritic cells: results of a phase I clinical trial. *Prostate* 66, 811-821
- Gabizon A, Goren D, Cohen R, Barenholz Y (1998) Development of liposomal anthracyclines: from basics to clinical applications *Journal of Controlled Release* 53, 275-279
- Gade TP, Hassen W, Santos E, Gunset G, Saudemont A, Gong MC, Brentjens R, Zhong XS, Stephan M, Stefanski J, Lyddane C, Osborne JR, Buchanan IM, Hall SJ, Heston WD, Riviere I, Larson SM, Koutcher JA, Sadelain M (2005) Targeted elimination of prostate cancer by genetically directed human T lymphocytes. *Cancer Research* 65, 9080-9088
- Gu Z, Thomas G, Yamashiro J, Shintaku IP, Dorey F, Raitano A, Witte ON, Said JW, Loda M, Reiter RE (2000) Prostate stem cell antigen (PSCA) expression increases with high gleason score, advanced stage and bone metastasis in prostate cancer. *Oncogene* 19, 1288-1296
- Halvorsen OJ, Rostad K, Oyan AM, Puntervoll H, Bo TH, Stordrange L, Olsen S, Haukaas SA, Hood L, Jonassen I, Kalland KH, Akslen LA (2007) Increased expression of SIM2-s protein is a novel marker of aggressive prostate cancer. *Clinical Cancer Research* 13, 892-897
- Hanks GE, Pajak TF, Porter A, Grignon D, Brereton H, Venkatesan V, Horwitz EM, Lawton C, Rosenthal SA, Sandler HM, Shipley WU; Radiation Therapy Oncology Group (2003) Phase III trial of long-term adjuvant androgen deprivation after neoadjuvant hormonal cytoreduction and radiotherapy in locally advanced carcinoma of the prostate: the Radiation Therapy Oncology Group Protocol 92-02. *Journal of Clinical Oncology* 21, 3972-3978
- Hinata N, Shirakawa T, Terao S, Goda K, Tanaka K, Yamada Y, Hara I, Kamidono S, Fujisawa M, Gotoh A (2006) Progress report on phase I/II clinical trial of Ad-OC-TK with VAL therapy for metastatic or locally recurrent prostate cancer: Initial experience at Kobe University. *International Journal of Urology* 13, 834-837
- Ikegami S, Yamakami K, Ono T, Sato M, Suzuki S, Yoshimura I, Asano T, Hayakawa M, Tadakuma T (2006) Targeting gene therapy for prostate cancer cells by liposomes complexed with anti-prostate-specific membrane antigen monoclonal antibody. *Human Gene Therapy* 17, 997-1005
- Ivics Z, Hackett PB, Plasterk RH, Izsvak Z (1997) Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. *Cell* 91, 501-510
- Jenkins R, Takahashi S, DeLacey K, Bergstrahl E, Lieber M (1998) Prognostic significance of allelic imbalance of chromosome arms 7q, 8p, 16q, and 18q in stage T3N0M0 prostate cancer. *Genes Chromosomes Cancer* 21, 131-143
- Jeong JH, Christensen LV, Yockman JW, Zhong Z, Engbersen JF, Kim WJ, Feijen J, Kim SW (2007) Reducible poly(amido ethylenimine) directed to enhance RNA interference. *Biomaterials* 28, 1912-1917
- Kang JS, Calvo BF, Maygarden SJ, Caskey LS, Mohler JL, Ornstein DK (2002) Dysregulation of annexin I protein expression in high-grade prostatic intraepithelial neoplasia and prostate cancer. *Clinical Cancer Research* 8, 117-123
- Kaufman HL, Wang W, Manola J, di Paola RS, Ko YJ, Sweeney C, Whiteside TL, Schlom J, Wilding G, Weiner LM (2004) Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. *Journal of Clinical Oncology* 22, 2122-2132
- Kay MA, Glorioso JC, Naldini L (2001) Viral vectors for gene therapy: The art of turning infectious agents into vehicles of therapeutics. *Nature Medicine* 7, 33-40
- Kittles RA, Young D, Weinrich S, Hudson J, Argyropoulos G, Ukoli F, Adams-Campbell L, Dunston GM (2001) Extent of linkage disequilibrium between the androgen receptor gene CAG and GGC repeats in human populations: implications for prostate cancer risk. *Human Genetics* 109, 253-261
- Koochekpour S, Lee TJ, Wang R, Sun Y, Delorme N, Hiraiwa M, Grabowski GA, Culig Z, Minokadeh A (2007) Prosaposin is a novel androgen-regulated gene in prostate cancer cell line LNCaP. *Journal of Cellular Biochemistry* 101, 631-641
- Kowalczyk DW, Wysocki PJ, Mackiewicz A (2003) Cancer immunotherapy using cells modified with cytokine genes. *Acta Biochimica Polonica* 50, 613-624
- Kubler H, Vieweg J (2006) Vaccines in renal cell carcinoma. *Seminars in Oncology* 33, 614-624
- Kumar P, Perrotti M, Weiss R, Todd M, Goodin S, Cummings K, di Paola RS (2004) Phase I trial of weekly docetaxel with concurrent three-dimensional conformal radiation therapy in the treatment of unfavorable localized adenocarcinoma of the prostate. *Journal of Clinical Oncology* 22, 1909-1915
- Lawton CA, Winter K, Murray K, Machtay M, Mesic JB, Hanks GE, Coughlin CT, Pilepich MV (2001) Updated results of the phase III Radiation Therapy Oncology Group (RTOG) trial 85-31 evaluating the potential benefit of androgen suppression following standard radiation therapy for unfavorable prognosis carcinoma of the prostate. *International Journal of Radiation Oncology Biology and Physics* 49, 937-946
- Lieberman J, Song E, Lee SK, Shankar P (2003) Interfering with disease: opportunities and roadblocks to harnessing RNA interference. *Trends in Molecular Medicine* 9, 397-403
- McManus MT, Sharp PA (2002) Gene silencing in mammals by small interfering RNAs. *Nature Reviews. Genetics* 3, 737-747
- Miller AD (1992) Retroviral vectors. *Current Topics in Microbiology and Immunology* 158, 1-24
- Mimeault M, Johansson SL, Vankatraman G, Moore E, Henichart JP, Depreux P, Lin MF, Batra SK (2007) Combined targeting of epidermal growth factor receptor and hedgehog signaling by gefitinib and cyclopamine cooperatively improves the cytotoxic effects of docetaxel on metastatic prostate cancer cells. *Molecular Cancer Therapy* 6, 967-978
- Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL (1995) Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nature Medicine* 1, 938-943
- Mullen JT, Tanabe KK (2003) Viral oncolysis for malignant liver tumors. *Annals of Surgical Oncology* 10, 596-605
- Mullen JT, Tanabe KK (2002) Viral oncolysis. *Oncologist* 7, 106-119
- Mulligan RC (1993) The basic science of gene therapy. *Science* 260, 926-932
- Nelson NJ (1995) Combining prostate markers may yield useful screening tool. *Journal of National Cancer Institute* 87, 1281-1283
- Oishi M, Nagasaki Y, Itaka K, Nishiyama N, Kataoka K (2005) Lactosylated poly(ethylene glycol)-siRNA conjugate through acidlabile beta-thiopropionate linkage to construct pH-sensitive polyion complex micelles achieving enhanced gene silencing in hepatoma cells. *Journal of the American Chemical Society* 127, 1624-1625
- Onik G, Cobb C, Cohen J, Zabkar J, Porterfield B (1988) US characteristics of frozen prostate. *Radiology* 168, 629-631
- Ohlfest JR, Demorest ZL, Motooka Y, Vengco I, Oh S, Chen E, Scappaticci FA, Saplis RJ, Ekker SC, Low WC, Freese AB, Largaespada DA (2005) Combinatorial antiangiogenic gene therapy by nonviral gene transfer using the sleeping beauty transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma. *Molecular Therapy* 12, 778-788
- Oyama M, Ohigashi T, Hoshi M, Murai M, Uyemura K, Yazaki T (2000) Oncolytic viral therapy for human prostate cancer by conditionally replicating herpes simplex virus 1 vector G207. *Japanese Journal of Cancer Research* 91, 1339-1344
- Pantuck AJ, van Ophoven A, Gitlitz BJ, Tso CL, Acres B, Squiban P, Ross ME, Beldegrun AS, Figlin RA (2004) Phase I trial of antigen-specific gene therapy using a recombinant vaccinia virus encoding MUC-1 and IL-2 in MUC-1-positive patients with advanced prostate cancer. *Journal of Immunotherapy* 27, 240-253
- Parks RJ, Chen L, Anton M, Sankar U, Rudnicki MA, Graham FL (1996) A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proceedings of the National Academy of Sciences USA* 93, 13565-13570
- Pilepich MV, Winter K, John MJ, Mesic JB, Sause W, Rubin P, Lawton C, Machtay M, Grignon D (2001) Phase III radiation therapy oncology group (RTOG) trial 86-10 of androgen deprivation adjuvant to definitive radiotherapy in locally advanced carcinoma of the prostate. *International Journal of Radiation Oncology Biology and Physics* 50, 1243-1252
- Pisters LL, Pettaway CA, Troncso P, McDonnell TJ, Stephens LC, Wood CG, Do KA, Brisbay SM, Wang X, Hossan EA, Evans RB, Soto C, Jacobson MG, Parker K, Merritt JA, Steiner MS, Logothetis CJ (2004) Evidence that transfer of functional p53 protein results in increased apoptosis in prostate cancer. *Clinical Cancer Research* 10, 2587-2593
- Prieto J, Qian C, Hernandez-Alcoceba R, Gonzalez-Asequinolaza G, Mazzolini G, Sangro B, Kramer MG (2004) Gene therapy of liver diseases. *Expert Opinion on Biological Therapy* 4, 1073-1091
- Qin H, Zhou C, Wang D, Ma W, Liang X, Lin C, Zhang Y, Zhang S (2006) Enhancement of antitumor immunity by a novel chemotactic antigen DNA vaccine encoding chemokines and multi-epitopes of prostate-tumour-associated antigens. *Immunology* 117, 419-430
- Rauen KA, Sudilovsky D, Le JL, Chew KL, Hann B, Weinberg V, Schmitt LD, McCormick F (2002) Expression of the coxsackie adenovirus receptor in normal prostate and in primary and metastatic prostate carcinoma: Potent-



- tial relevance to gene therapy. *Cancer Research* **62**, 3812-3818
- Rolph KL, Harrington KJ, Pandha H** (2005) Adenoviral strategies for the gene therapy of cancer. *Seminars in Oncology* **32**, 573-82
- Roach M 3<sup>rd</sup>, de Silvio M, Lawton C, Uhl V, Machtay M, Seider MJ, Rotman M, Jones C, Asbell SO, Valicenti RK, Han S, Thomas CR Jr., Shipley WS; Radiation Therapy Oncology Group 9413** (2003) Phase III trial comparing whole-pelvic versus prostate-only radiotherapy and neoadjuvant versus adjuvant combined androgen suppression: Radiation Therapy Oncology Group 9413. *Journal of Clinical Oncology* **21**, 1904-1911
- Rodriguez R, Schuur ER, Lim HY, Henderson GA, Simons JW, Henderson DR** (1997) Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Research* **57**, 2559-2563
- Roizman B, Sears AE** (1996) Herpes simplex viruses and their replication. In: Fields BN, Knipe DM, Howley PM (Eds) *Fields Virology*, Lippincott-Raven Publishers, Philadelphia, pp 2231-2295
- Roos AK, Moreno S, Leder C, Pavlenko M, King A, Pisa P** (2006) Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation. *Molecular Therapy* **13**, 320-327
- Sadeghi H, Hitt MM** (2005) Transcriptionally targeted adenovirus vectors. *Current Gene Therapy* **5**, 411-427
- Saliken JC, Donnelly BJ, Rewcastle JC** (2002) The evolution and state of modern technology for prostate cryosurgery. *Urology* **60**, 26-33
- Sallusto F, Lanzavecchia A** (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *Journal of Experimental Medicine* **179**, 1109-1118
- Sauter BV, Parashar B, Chowdhury NR, Kadakol A, Ilan Y, Singh H, Milano J, Strayer DS, Chowdhury JR** (2000) Gene transfer to the liver using a replication-deficient recombinant SV40 vector results in long-term amelioration of jaundice in Gunn rats. *Gastroenterology* **119**, 1348-1357
- Satoh T, Irie A, Egawa S, Baba S** (2005) *In situ* gene therapy for prostate cancer. *Current Gene Therapy* **5**, 111-119
- Saxena BB, Zhu L, Hao M, Kisilis E, Katdare M, Oktem O, Bomshteyn A, Rathnam P** (2006) Boc-lysinated-betulinic acid: a potent, anti-prostate cancer agent. *Bioorganic and Medicinal Chemistry* **14**, 6349-6358
- Shenk JL, Fisher CJ, Chen SY, Zhou XF, Tillman K, Shemshedini L** (2001) p53 represses androgen-induced transactivation of prostate-specific antigen by disrupting hAR amino- to carboxyl-terminal interaction. *Journal of Biological Chemistry* **276**, 38472-38479
- Simons JW, Mikhak B, Chang JF, de Marzo AM, Carducci MA, Lim M, Weber CE, Baccala AA, Goemann MA, Clift SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S, Marshall FF, Nelson WF** (1999) Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer. *Cancer Research* **59**, 5160-5168
- Small EJ, Carducci MA, Burke JM, Rodriguez R, Fong L, van Ummersen L, Yu DC, Aimi J, Ando D, Working P, Kirn D, Wilding G** (2006) A phase I trial of intravenous CG7870, replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer. *Molecular Therapy* **14**, 107-117
- Steinman RM, Pope M** (2002) Exploiting dendritic cells to improve vaccine efficacy. *Journal of Clinical Investigation* **109**, 1519-1526
- Strayer DS, Branco F, Zern MA, Yam P, Calarota SA, Nichols CN, Zaia JA, Rossi J, Li H, Parashar B, Ghosh S, Chowdhury JR** (2002) Durability of transgene expression and vector integration: Recombinant SV40-derived gene therapy vectors. *Molecular Therapy* **6**, 227-237
- Strayer DS, Cordelier P, Kondo R, Liu B, Matskevich AA, McKee HJ, Nichols CN, Mitchell CB, Geverd DA, White MK, Strayer MS** (2005) What they are, how they work and why they do what they do? The story of SV40-derived gene therapy vectors and what they have to offer. *Current Gene Therapy* **5**, 151-65
- Su Z, Dannull J, Yang BK, Dahm P, Coleman D, Yancey D, Sichi S, Niedzwiecki D, Boczkowski D, Gilboa E, Vieweg J** (2005) Telomerase mRNA-transfected dendritic cells stimulate antigen-specific CD8+ and CD4+ T cell responses in patients with metastatic prostate cancer. *Journal of Immunology* **174**, 3798-3807
- Sudo H, Maru Y** (2007) LAPSER1 is a putative cytoskeletal tumor suppressor that shows the same centrosome and midbody subcellular localization pattern as p80 katanin. *FASEB Journal*, in press
- Swanson GP, Faulkner J, Smalley SR, Noble MJ, Stephens RL, O'Rourke TJ, Weiss GR, Quick DP, Thompson IM Jr., Crawford ED** (2004) Locally advanced prostate cancer treated with concomitant radiation and 5-fluorouracil: Southwest Oncology Group Study 9024. *Journal of Urology* **176**, 548-553
- Swisher SG, Roth JA, Komaki R, Gu J, Lee JJ, Hicks M, Ro JY, Hong WK, Merritt JA, Ahrar K, Atkinson NE, Correa AM, Dolormente M, Dreiling L, El-Naggar AK, Fossella F, Francisco R, Glisson B, Grammer S, Herbst R, Huaranga A, Kemp B, Khuri FR, Kurie JM, Liao Z, McDonnell TJ, Morice R, Morello F, Munden R, Papadimitrakopoulou V, Pisters KM, Putnam JB Jr., Sarabia AJ, Shelton T, Stevens C, Shin DM, Smythe WR, Vaporciyan AA, Walsh GL, Yin M** (2003) Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy. *Clinical Cancer Research* **9**, 93-101
- Tai CK, Wang WJ, Chen TC, Kasahara N** (2005) Single-shot, multicycle suicide gene therapy by replication-competent retrovirus vectors achieves long-term survival benefit in experimental glioma. *Molecular Therapy* **12**, 842-851
- Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA; TAX 327 Investigators** (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *New England Journal of Medicine* **351**, 1502-1512
- Tenenbaum L, Lehtonen E, Monahan PE** (2003) Evaluation of risks related to the use of adeno-associated virus-based vectors. *Current Gene Therapy* **3**, 546-565
- Thomas-Kaskel AK, Zeiser R, Jochim R, Robbel C, Schultze-Seemann W, Waller CF, Veelken H** (2006) Vaccination of advanced prostate cancer patients with PSCA and PSA peptide-loaded dendritic cells induces DTH responses that correlate with superior overall survival. *International Journal of Cancer* **119**, 2428-2434
- Trajcevski S, Solly SK, Frisen C, Trenado A, Cosset FL, Klatzmann D** (2005) Characterization of a semi-replicative gene delivery system allowing propagation of complementary defective retroviral vectors. *Journal of Gene Medicine* **7**, 276-287
- Trudel S, Trachtenberg J, Toi A, Sweet J, Li ZH, Jewett M, Tshilias J, Zhuang LH, Hitt M, Wan Y, Gaudie J, Graham FL, Dancy J, Stewart AK** (2003) A phase I trial of adenovector-mediated delivery of interleukin-2 (AdIL-2) in high-risk localized prostate cancer. *Cancer Gene Therapy* **10**, 755-763
- Tseng JF, Farnebo FA, Kisker O, Becker CM, Kuo CJ, Folkman J, Mulligan RC** (2002) Adenovirus-mediated delivery of a soluble form of the VEGF receptor Flk1 delays the growth of murine and human pancreatic adenocarcinoma in mice. *Surgery* **132**, 857-865
- Turley RS, Finger EC, Hempel N, How T, Fields TA, Blobe GC** (2007) The type III transforming growth factor-beta receptor as a novel tumor suppressor gene in prostate cancer. *Cancer Research* **67**, 1090-1098
- Varghese S, Rabkin SD** (2002) Oncolytic herpes simplex virus vectors for cancer virotherapy. *Cancer Gene Therapy* **9**, 967-978
- Verma RS, Manikal M, Conte RA, Godec CJ** (1999) Chromosomal basis of adenocarcinoma of the prostate. *Cancer Investigations* **17**, 441-447
- Veronesi ML, Bullrich F, Negrini M, Croce CM** (1996) The t(6;16)(p21;q22) chromosome translocation in the LNCaP prostate carcinoma cell line results in a tpc/hpr fusion gene. *Cancer Research* **56**, 728-732
- Walker JR, McGeagh KG, Sundaresan P, Jorgensen TJ, Rabkin SD, Martuza RL** (1999) Local and systemic therapy of human prostate adenocarcinoma with the conditionally replicating herpes simplex virus vector G207. *Human Gene Therapy* **10**, 2237-2243
- Whang YE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL** (1998) Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proceedings of the National Academy of Sciences USA* **95**, 5246-5250
- Wickham TJ, Mathias P, Cheres DA, Nemerow GR** (1993) Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* **73**, 309-319
- Wilkinson S, Chodak GW** (2003) Critical review of complementary therapies for prostate cancer. *Journal of Clinical Oncology* **21**, 2199-2210
- Wright GL, Grob BM, Haley C, Grossman K, Newhall K, Petrylak D, Troyer J, Konchuba A, Schellhammer PF, Moriarty R** (1996) Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. *Urology* **48**, 326-334
- Yagoda A, Petrylak D** (1993) Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer. *Cancer* **71**, 1098-1109
- Young HH** (1905) Four cases of radical prostatectomy. *Johns Hopkins Bulletin* **16**, 315
- Yu D, Scott C, Jia WW, De Benedetti A, Williams BJ, Fazli L, Wen Y, Gleave M, Nelson C, Rennie PS** (2006) Targeting and killing of prostate cancer cells using lentiviral constructs containing a sequence recognized by translation factor eIF4E and a prostate-specific promoter. *Cancer Gene Therapy* **13**, 32-43
- Zaragosi LE, Billon N, Ailhaud G, Dani C** (2007) Nucleofection is a valuable transfection method for transient and stable transgene expression in adipose tissue-derived stem cells. *Stem Cells* **25**, 790-797
- Zheng JY, Chen D, Chan J, Yu D, Ko E, Pang S** (2003) Regression of prostate cancer xenografts by a lentiviral vector specifically expressing diphtheria toxin A. *Cancer Gene Therapy* **10**, 764-770
- Zisman A, Pantuck AJ, Cohen JK, Beldegrun AS** (2001) Prostate cryoablation using direct transperineal placement of ultrathin probes through a 17-gauge brachytherapy template-technique and preliminary results. *Urology* **58**, 988-993