Gene therapy of prostate cancer: current and future directions

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Abstract

Prostate cancer (PCA) is the second most common cause of death from malignancy in American men. Developing new approaches for gene therapy for PCA is critical as there is no effective treatment for patients in the advanced stages of this disease. Current PCA gene therapy research strategies include cytoreductive approaches (immunotherapy and cytolytic/pro-apoptotic) and corrective approaches (replacing deleted or mutated genes). The prostate is ideal for gene therapy. It is an accessory organ, offers unique antigens (prostate-specific antigen, prostate-specific membrane antigen, human glandular kallikrein 2 etc.) and is stereotactically accessible for in situ treatments. Viral and non-viral means are being used to transfer the genetic material into tumor cells. The number of clinical trials utilizing gene therapy methods for PCA is increasing. We review the multiple issues involved in developing effective gene therapy strategies for human PCA and early clinical results.

Introduction

New therapeutics like gene therapy are needed urgently for advanced prostate cancer (PCA). PCA remains the most common solid tumor and the second leading cause of cancer-related deaths among men in the USA. An estimated 180,400 new cases and 31,900 PCA-related deaths are expected for 2000 (Cookson 2001). The current standard therapies employed for organ-confined PCA include radiation or surgery, in some circumstances incorporating neoadjuvant or adjuvant hormonal therapy. While these therapies are relatively effective in the short-term, a significant proportion of patients initially presenting with localized disease ultimately relapse. Moreover, each of these therapies may incur unwanted side-effects. Ultimately, the major risk faced by patients with PCA is the development of metastatic disease. For 60 years the mainstay of therapy for metastatic disease has been androgen ablation. Androgen-independent disease typically occurs within the order of 14–20 months (Crawford et al. 1989). A great number of clinical research studies have been reported in the field of chemotherapy for advanced androgen-independent PCA during the last ten years (Culine & Droz 2000). So far, no combination of chemotherapy reported has improved overall survival of patients. Docetaxel combined with estramustine, however, is in randomized trials to assess its impact on survival. An advance in medical oncology of the 1990s is the demonstration of the impact of chemotherapy (mitoxantrone+ prednisone) on quality of life as compared with prednisone alone in advanced hormone-refractory PCA. At the present time, chemotherapy should be considered as a palliative or investigational treatment in patients with symptomatic androgen-independent disease (Morris & Scher 2000).

An improving understanding of the biology of PCA has changed the outlook for patients with this disease. We now have available approaches designed to modulate the immune system, inhibit the metastatic cascade, block angiogenesis, promote apoptosis and alter signal transduction.

Gene therapy has been much heralded since its inception with the first patient treated in 1990, but in oncology has yet to reach phase III trials or show clear cancer patient benefits in large statistically powered phase II trials (Anderson 1998). Nonetheless, in recent years there has been progress in the design and execution of gene transfer clinical studies. Progress in this field provides a rational basis for optimism that gene therapeutic strategies may significantly improve PCA treatment outcome administered either alone or in combination with current therapies. In this report we review the multiple issues involved in developing effective gene therapy strategies for human PCA.

Rationale for PCA gene therapy

The promise of gene therapy lies in its potential for selective potency. To achieve this aim, cancer gene therapy strategies
attempt to exploit the biological uniqueness of each particular tumor. The human prostate is an accessory organ and is not required for potency or urinary continence. Thus unique targets showed by PCA and the normal prostate are candidates for new treatment. The Prostate Expression Database is an online resource designed to access and analyze gene expression information derived from the human prostate (Nelson et al. 2000). It has revealed more than 55,000 expressed sequence tags (ESTs) from 43 cDNA libraries; of these approximately 500 are prostate-unique (Nelson et al. 2000). The presence of this large number of prostate-unique promoters and candidate antigens promises to facilitate the design of prostate-specific gene therapy. Ideally, both the clinical and genetic features of PCA should be taken into account in the design of effective prostate gene therapeutic strategies (Table 1).

Cancer gene therapy may be defined as the transfer of recombinant DNA into human cells to achieve an antitumor effect. Depending upon the strategy, DNA may be introduced either into cells removed from the body – the ex vivo approach – or introduced directly into cells in their normal location – the in vivo approach (Anderson 1998).

### Gene transfer systems

Efficient gene transfer requires the use of a vector. All vectors contain at a minimum the transgene of interest linked to a promoter to drive its expression (Galanis et al. 2001). Increasingly wider ranges of viral and synthetic vectors are available, each with characteristic advantages and limitations (Table 2). However, most vectors used for gene therapy contain promoters that are tissue-non-specific, allowing gene expression to occur in unintended tissues and resulting in the potential for systemic toxicity.

In choosing between vectors, attributes to consider include maximal transgene size permissible, maximal titer of viruses attainable, tendency to provoke inflammatory/immune response, persistence of gene expression, ability to transduce non-dividing cells, effect of vector genome on desired therapy, target cell-specificity, and transduction effi-

<table>
<thead>
<tr>
<th>Table 1 PCA-specific gene therapy strategies</th>
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<tbody>
<tr>
<td><strong>PCA-specific features</strong></td>
<td><strong>Relevance to gene therapy</strong></td>
</tr>
<tr>
<td>Anatomy</td>
<td>Accessibility for localized gene therapy and combined modality therapy in vivo by transurethral/transrectal routes</td>
</tr>
<tr>
<td>Localized tumor accessible for procedures such as brachytherapy</td>
<td>Complete ablation of the normal organ in addition to the tumor is clinically undeterious</td>
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<tr>
<td>Accessory organ</td>
<td>Cytoreductive transgene expression required only for duration necessary for prostate tissue ablation</td>
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<tr>
<td>Natural history</td>
<td>Preventive gene therapy strategies may be feasible</td>
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<tr>
<td>Long pre-clinical latency</td>
<td>Definition of genetic/epigenetic events underlying molecular progression provides rational basis for preventive/corrective strategies</td>
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<tr>
<td>Propensity for metastatic spread to lymphatics/bone</td>
<td>Targeting of systemic gene therapy for advanced disease must effectively target bone and lymphatics</td>
</tr>
<tr>
<td>Current treatment efficacy</td>
<td>Supports use of novel combined modality treatments to improve local control</td>
</tr>
<tr>
<td>Failures even for localized disease</td>
<td>Pressing need for alternative systemic therapies</td>
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<tr>
<td>Ineffective for advanced disease</td>
<td>Effective vectors for in vivo gene delivery must transduce both non-dividing as well as dividing cells</td>
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<tr>
<td>Biology</td>
<td>Tumors are resistant to S-phase-dependent cytotoxic strategies</td>
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<tr>
<td>Low mitotic rate</td>
<td>Define gene therapy targets including tumor suppressors, oncogenes etc., for prevention and treatment of disease at various stages</td>
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<tr>
<td>Clinical/biological transitions:</td>
<td></td>
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<tr>
<td>● Prostatic intraepithelial neoplasial (PIN)→carcinoma</td>
<td></td>
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<tr>
<td>● Androgen-dependent→androgen-independent tumor growth</td>
<td></td>
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<tr>
<td>Prostate-specific genes and antigens</td>
<td>Facilitate prostate specific promoter/antigen targeting and therapeutic monitoring</td>
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<tr>
<td>~500 or more prostate-specific ESTs in addition to current known markers such as PSA/PSMA</td>
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### Table 2  Vectors for gene therapy

<table>
<thead>
<tr>
<th>Vector</th>
<th>Gene therapy applications</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Insert size</th>
<th>Immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA virus RV vectors RRs (oncoviruses)</td>
<td>MMLV-based used in majority of current clinical trials Ex vivo transduction for stable gene expression</td>
<td>Transmitted to progeny of transduced cell Potentially long term transeqne expression</td>
<td>Infect actively dividing cells only Risk of insertional mutagenesis Relatively low titers Low transduction efficiency Inactivated by complement in vivo Difficult to target attachment</td>
<td>6–8 kb</td>
<td>No</td>
</tr>
<tr>
<td>Lentiviruses</td>
<td>Preclinical testing, stable infection of non-dividing cells</td>
<td>Can infect non-dividing cells Long-term transgene expression Not inactivated by human serum</td>
<td>Safety: possible recombination with endogenous HIV/other viruses Risk of insertional mutagenesis</td>
<td>6–8 kb</td>
<td>No</td>
</tr>
<tr>
<td>HFVs</td>
<td>Preclinical testing, stable infection of non-dividing cells</td>
<td>Non-pathogenic Infect non-dividing cells Polycations not required for efficient transduction Not inactivated by human serum</td>
<td>Low titers</td>
<td>5–7 kb</td>
<td>No</td>
</tr>
<tr>
<td>DNA virus vectors Vaccinia virus</td>
<td>Vaccine-based gene therapy</td>
<td>Large insert size Replicates in vivo</td>
<td>Low efficiency Pre-existing immunity from prior vaccinations limits expression duration to ~1 month</td>
<td>25 kb</td>
<td>Yes</td>
</tr>
<tr>
<td>HSV</td>
<td>Neural tropism</td>
<td>Potentially large insert size (amplicons) Episomal no risk insertional mutagenesis Infect non-dividing cells Moderate efficiency</td>
<td>Expression transient Cytopathic Difficult to produce high titers</td>
<td>10–100 kb</td>
<td>Yes</td>
</tr>
<tr>
<td>Ad</td>
<td>In vivo use</td>
<td>Infect non-dividing cells Wide target cell range Concentrate to high titers High transduction efficiency Episomal, no risk of insertional mutagenesis High transgene expression</td>
<td>Transient expression without passage to progeny Ad infection provokes cell-mediated immune response limiting duration of expression Humoral response makes reinfection less feasible with rapid clearance of vector Local tissue inflammation</td>
<td>7.5 kb</td>
<td>Yes</td>
</tr>
<tr>
<td>AAV</td>
<td>In vivo use</td>
<td>Non-pathogenic Infect non-dividing cells Wide target cell range High transduction efficiency Site-specific integration chr 19</td>
<td>Limited insert size Propensity for rearrangement during integration Risk of helper Ad and wild-type AAV contamination Insertional mutagenesis possible if rep gene deleted</td>
<td>2–4.5 kb</td>
<td>No</td>
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Table 2 cont.

<table>
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<tr>
<th>Vector</th>
<th>Gene therapy applications</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Insert size</th>
<th>Immune response</th>
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<tr>
<td>Synthetic vectors</td>
<td></td>
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<tr>
<td>Naked DNA</td>
<td>DNA vaccines</td>
<td>Low cost</td>
<td>Poor efficiency</td>
<td>Unlimited</td>
<td>No</td>
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<td></td>
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<td>Simple preparation</td>
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<td></td>
<td></td>
<td>Non-toxic</td>
<td></td>
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<tr>
<td>Liposomes</td>
<td>In vivo/ex vivo use</td>
<td>Large insert size</td>
<td>Poor efficiency</td>
<td>50 kb</td>
<td>No</td>
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<td></td>
<td></td>
<td>Safe for repeated administration</td>
<td>Prone to degradation/ short expression</td>
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<td></td>
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<td>Non-toxic</td>
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<td></td>
<td></td>
<td>Low cost</td>
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</tr>
<tr>
<td>DNA–protein complex</td>
<td>In vivo</td>
<td>Large insert size</td>
<td>Poor efficiency</td>
<td>50 kb</td>
<td>No</td>
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<td>Low cost</td>
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<td>Suiited for targeting</td>
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Viral vectors

Viral vectors are used in the vast majority of gene therapy trials owing to their relatively higher efficiency at gene transfer compared with the synthetic vectors. Viral vectors may be RNA virus-based, or DNA virus-based. RNA viruses include the retroviruses (RVs) among which are the oncornaviruses such as murine leukemia virus (MuLV) and mouse mammary tumor virus (MMTV), the lentiviruses derived from human immunodeficiency virus (HIV), and the spumaviruses such as human foamy virus (HFV). The DNA viruses include adenovirus (Ad), adeno-associated viruses (AAVs), vaccinia virus, and herpes simplex virus (HSV). To increase their safety, viral vectors may be designed to be replication-deficient, with no further virus particles generated following infection of the target cells. Alternatively the viral vectors may be replication-competent or replication-attenuated, in which case viral replication can occur in permissive cells.

Ad vectors

Ad vectors are well suited to a variety of gene therapy strategies owing to their broad host range, low potential for pathogenicity, and relatively high gene transfer efficiency. Ad consists of a linear double-stranded DNA genome of 30–40 kb. Binding and internalization of Ad to target cells are dependent on the presence of the coxsackie Ad receptor (CAR) and of the αβ3 and αβ5 integrins (Wickham et al. 1993, Roelvink et al. 1998). The interaction of Ad particles with CAR is mediated by the Ad fiber protein knob domain (Roelvink et al. 1998). The fiber protein may be reengineered to bind to a variety of cell-surface molecules (Miller et al. 1998). Fiber protein reengineering together with prostate-specific promoter/enhancers may allow construction of Ad vectors targeted for binding to prostate-specific cell-surface markers such as prostate-specific membrane antigen (PSMA) and at the same time restricted for replication or transgene expression to prostate cells (Lee et al. 1996, Rodriguez et al. 1997, Gotoh et al. 1998). Following attachment, Ad is internalized through coated pits into endosomes, then is dis-assembled and extruded into the cytosol prior to lysosomal fusion, thereby avoiding lysosomal degradation (Silman & Fooks 2000). The DNA-protein core then penetrates the nuclear pores into the nucleus and gene expression commences. The first of two phases of Ad gene expression results in expression of the early genes (E1–E4), which are essential to DNA replication, and subversion of host antiviral defenses. If replication-competent, the Ad proceeds to replicate episomally, culminating in host cell lysis and the release of newly produced viral particles. The lack of proviral integration results in gene expression that is less sustained than with the RVs and gradually lost with subsequent cell divisions. Ad vectors have high safety owing to their mild associated clinical pathogenicity and extrachromosomal life cycle, which eliminates the risk of insertional mutagenesis.

The administration of an Ad vector triggers both cellular and humoral immune responses, which clear remaining virus and also function to eliminate transduced cells, thereby limiting the duration of transgene expression. The anti-Ad immune response rapidly clears subsequent injections of Ad vector, severely limiting the efficacy of readministered vector (Yang et al. 1996). Intratumoral readministration of Ad elicits profound humoral and cellular immune responses to Ad and their transgenes (Nagao et al. 2001). Following endocytosis, virions are released from endosomes and undergo
MHC-1 processing and presentation prior to any de novo Ad protein synthesis.

It has been possible to increase the duration of Ad transgene expression through the use of cytotoxic T-lymphocyte (CTL) blocking agents such as cytotoxic T-lymphocyte antigen-4 Ig (Kay et al. 1995) or immunosuppressive drugs (Dai et al. 1995). In addition to specific anti-Ad/transgene-directed immune responses, non-specific inflammation may also significantly reduce transgene expression (Otake et al. 1998). The ability to suppress immunogenicity towards Ad vectors would be of enormous benefit to in vivo gene therapy and would allow multiple reinfections with the same or different transgenes delivered.

The resulting improvements in gene localization from targeted Ad vectors are likely to reduce immunogenicity and toxicity, increase safety, and enable the systemic administration of these vectors for cancer. Two basic requirements are necessary to create a targeted Ad vector: interaction of Ad with its native receptors must be removed and novel, tissue-specific ligands must be added to the virus. Two general approaches have been used to achieve these basic requirements. In the ‘two-component’ approach, a bispecific molecule is complexed with the Ad. The bispecific component simultaneously blocks native receptor binding and redirects virus binding to a tissue-specific receptor. In the ‘one-component’ approach the Ad is genetically modified to ablate native receptor interactions and a novel ligand is genetically incorporated into one of the Ad coat proteins. Two-component systems offer great flexibility in rapidly validating the feasibility of targeting via a particular receptor. One-component systems offer the best advantages in producing a manufacturable therapeutic agent and in more completely ablating all native Ad receptor interactions. The coming challenges for targeted Ad vectors will be the demonstration that the technology performs in vivo. Ultimately, or in parallel, ‘receptor-targeting’ technology can be combined with improved Ad backbones and with ‘transcriptional-targeting’ approaches to create Ad which deliver genes selectively, safely, and with minimal immune response (Wickham 2000).

In addition to their immunogenicity, another limitation of Ad vectors is their limited transgene capacity of approximately 8 kb. Increased insert capacity and decreased immunogenicity has been observed with modified Ad vectors known as ‘gutless’ Ad vectors, or as encapsidated Ad minichromosomes (EAMs). In EAMs, almost the entire Ad genome has been deleted with the exception of the inverted repeats necessary for replication, and the encapsidation/packaging signals (Kumar-Singh & Farber 1998). EAMs have a theoretical cloning capacity of 36 kb and have facilitated the transfer of multi-gene expression units as large as 28 kb. These vectors offer exciting prospects for the transfer of genes with large regulatory regions intact, or of multiple gene cassettes. An even more radical Ad modification for gene transfer is the ‘Ad dodecahedron’ which is composed of only two of the 11 Ad structural proteins and contains no Ad DNA. The vector consists of the penton base and fiber protein, which are sufficient to mediate efficient attachment, cell entry, and escape from endosomal degradation (Fender et al. 1997). The reduction in virion proteins presented to the immune system is intended to decrease the immunogenicity of this vector, making it even more suitable than EAMs for repeat application.

**AAVs**

The potential applications of the AAV as a gene transfer system vector have expanded dramatically in the last decade. AAVs are a non-enveloped DNA virus belonging to the family Parvoviridae. AAVs have a linear 4.7 kb genome encoding a group of regulatory genes – the rep genes – and a group of structural genes – the cap genes. AAVs must be coinfectected with a helper virus such as Ad for productive lytic infection, otherwise they remain a latent provirus in the host genome. The Ad E1/E4 gene products are essential for AAV transcription and the AAV lytic phase (Richardson & Westphal 1981). Another unique feature of the AAVs is their capacity for site-specific integration. The preferred site of integration for wild-type AAV is chromosome 19q14.3 (Kotin et al. 1990). The capacity for site-specific integration is rep gene-dependent and deletion of rep results in random integration. AAVs are limited to a transgene size of approximately 4.5 kb, smaller than the capacity of the Ad vectors.

Unlike RVs, AAVs may infect non-dividing cells. In addition, the AAVs elicit little or no immune/inflammatory response, which together with their integration into the target cell genome confers the potentially repeated administrations and stable long-term transduction. The AAVs are not associated with any known pathogenicity or oncogenicity. Recent advances in the production of high-titer purified rAAV vector stocks have made the transition to human clinical trials a reality (Monahan & Samulski 2000).

**RV vectors**

RVs of the oncovirus subfamily are single-stranded RNA viruses with a 7–10 kb genome. The RV life cycle begins with attachment to a cell-surface receptor via an envelope (env) surface protein. Depending on their env glycoprotein, RVs may be either amphotropic – able to infect all species, or ecotropic – able to infect only mouse cells. The amphotropic variety of MuLV attaches to cells via the RAM-1 receptor (Kavanaugh et al. 1994), a widely expressed inducible sodium phosphate symporter. Receptor-mediated endocytosis occurs following attachment. Then, the outer env protein coat is shed and the virus genome undergoes reverse transcription by viral reverse transcriptase resulting in the synthesis of a double-stranded DNA intermediate. This viral nucleoprotein complex then enters the cell nucleus and integrates randomly via the viral long terminal repeat sequences...
(LTRs) into the host genome. The stably integrated viral genome is termed a provirus. Because of their stable integration into the target cell genome and transmission to the progeny of the transduced parent cell, RV vectors have the potential for sustained duration of transgene expression, a major advantage particularly for corrective gene therapy strategies (Lin 1998).

The capacity for random proviral integration into the host genome raises the concern of potential insertional mutagenesis. Wild-type Moloney murine leukemia virus (MMLV) can induce leukemias and lymphomas in mice after long latency. To limit this risk, the minimum total RV dose necessary to achieve a therapeutic endpoint must be utilized. The use of replication-deficient virus also minimizes the amount of virus applied to the target tissue.

For the oncovirus subfamily of RVs which includes MMLV- and MMTV-based RVs, the entry into the cell nucleus undergoes proviral integration is cell-division dependent (Miller et al. 1990). This is a significant limitation on the usefulness of oncovirus family-based vectors for gene therapy in vivo, particularly in PCA with its low mitotic rate. Another feature of RV vectors which may limit their suitability for targeted gene therapy strategies in vivo is the relatively non-specific tropism of the amphotropic viruses to the ubiquitous RAM-1 receptors. The tropism of RV vectors can be modified through modification or replacement of the envelope gene product. Modification of RV targeting through the display of single-chain antibodies to antigens such as carcinoembryonic antigen, transferrin receptor, Her2/neu, and CD34 has been recently reported, suggesting that this approach may improve the suitability of RV vectors for use in vivo (Jiang et al. 1998). Another major hindrance to the use of RV vectors in vivo is the rapid inactivation of RVs by human complement.

In view of the above limitations, RV vectors are typically used for ex vivo gene therapy for which they are well suited, as the issues of non-specific transduction and inactivation by complement are avoided (Cornetta et al. 1990). The most common use of the RV vectors for PCA gene therapy has been for ex vivo cytoreductive immunotherapy.

**Lentivirus and spumavirus vectors**

The lentiviruses are a subfamily of the RVs, which includes HIV, bovine leukemia virus, and human T-cell leukemia virus (HTLV)-1 and HTLV-2. Unlike members of the oncoviridae subfamily such as MMLV and MMTV, the lentiviruses are able to integrate into non-dividing cells, an important advantage for gene therapy in vivo. For the lentivirus HIV, this ability is a result of the viral proteins vpr and viral matrix protein p17 (Bukrinsky et al. 1993, Gallay et al. 1995a). The phosphorylation of a terminal tyrosine residue on p17 permits transport of the HIV pre-integration complex into the nucleus even in the absence of mitosis (Gallay et al. 1995a,b). Another favorable feature of the lentiviral vectors is their ability to direct sustained transgene expression for greater than 6 months’ duration (Miyoishi et al. 1997). The target cell range for wild-type HIV is restricted mainly to CD4+ cells. This limitation has been circumvented experimentally for HIV-based vectors by substituting env sequences from other RVs for those of HIV to confer varying and broader cell specificity (Naldini et al. 1996, Nascone & Mercola 1997). Safety concerns have tempered enthusiasm for the use of HIV-based recombinant vectors. While helper-free virus stocks of HIV-based vectors have been successfully produced, the small risk of pathogenic recombination in vivo supports the development of non-HIV-based/non-human lentiviral vectors for clinical application. Recently, Loimas et al. (2001) found that lentiviral vectors carrying a fusion gene of HSV-thymidine kinase (TK) (HSV-TK) were efficient vehicles for three human PCA cell lines DU-145, LNCaP and PC-3. Despite sufficient gene transfer rates (25–45%) in the ganciclovir (GCV)-sensitivity experiment, only DU-145 cells were efficiently destroyed under clinically relevant GCV concentrations (Loimas et al. 2001).

The spumaviruses are another subfamily of the RVs and include the HFVs. HFV is non-pathogenic and has the capacity for integration into the genome of a wide range of non-dividing cells (Russell & Miller 1996). These are not inactivated by human serum as are the oncoviruses and may represent a safer alternative for clinical gene therapy than HIV.

**Herpes virus vectors**

The herpes viruses are enveloped DNA viruses of the family Herpesviridae. The HSV-1 genome consists of a double-stranded linear DNA of 152 kb. Other members of the herpes virus family include HSV-2, varicella zoster, cytomegalovirus (CMV), Epstein–Barr virus, human herpes virus (HHV)-6 and HHV-7. The herpes viruses replicate in the nucleus episomally. Following replication, viral particles reach the cell surface via the endoplasmic reticulum. HSV-1-based vectors have been used extensively in gene therapy for neural tissue and tumors, given their inherent neural tropism. Advantages of HSV-1-based vectors include their large insert capacity of 35 kb, and their ability to infect dividing as well as non-dividing cells. Disadvantages of the HSV-based vectors include their potential pathogenicity, poor transduction efficiency, and the short duration of gene expression achieved.

**Vaccinia virus vectors**

Vaccinia virus-based vectors have been used extensively for in vivo immunotherapy strategies. These DNA virus vectors have a wide host range and may transfer large inserts of up to 25 kb, facilitating multi-gene transfer (Peplinski et al. 1998). They have been used extensively for delivery of antigen and immune-stimulatory genes for vaccine-based gene therapy (Hodge et al. 1994). These vectors may replicate in...
vivo, further increasing the efficiency of host cell transduction. Their utility for in vivo gene therapy is limited by the strong immune response they elicit, and pre-existing antivaccinia immunity is common as a result of childhood smallpox immunization. The immunological elimination of the vaccinia-transduced cells limits gene expression to about 4 weeks. For the vaccine applications for which vaccinia vectors are typically used, this duration of expression is sufficient for priming an immune response. Booster vaccine administrations may be more effective if given using a heterologous vector, which is not immunologically cross-reactive (Irvine et al. 1997).

**Synthetic gene transfer systems**

Several non-viral methods for gene delivery are under intense investigation. These include chemical methods such as a variety of liposomes and physical methods such as microinjection electroporation and biobalistics (Prince 1998).

**Plasmid DNA vectors**

The most technically simple form of gene therapy is the use of naked DNA. A plasmid containing the gene of interest and appropriate promoter is injected directly into a desired site. Administered in this way, the efficiency of transgene uptake/expression is very low. The transforming DNA does not integrate into the host genome and the duration of expression is short. Of all sites tested so far, the greatest transduction efficiency is observed in muscle (Wolff et al. 1990). In this application, brief low-level expression may be sufficient for eliciting a therapeutic immune response. Animal studies have indicated that the use of syngeneic dendritic cells (DCs) that have been transfected ex vivo with DNA for tumor-specific antigen results in tumor regression and decreased number of metastases. Additional studies have also suggested the possibility of modulating the DCs in vivo either by ‘naked’ DNA immunization or by injecting replication-deficient viral vectors that carry the tumor-specific DNA. Using PSMA as a target molecule, Mincheff et al. (2000) have initiated a clinical trial for immunotherapy of PCA. They have included the extracellular human PSMA DNA as well as the human CD86 DNA into separate expression vectors (PSMA and CD86 plasmids), and into a combined PSMA/CD86 plasmid. In addition, the expression cassette from the PSMA plasmid was inserted into a replication-deficient Ad expression vector. Twenty-six patients with PCA were entered into a phase I/II toxicity–dose escalation study. No immediate or long-term side-effects following immunizations have been recorded. All patients who received initial inoculation with the viral vector followed by PSMA plasmid boosts showed signs of immunization as evidenced by the development of a delayed-type hypersensitivity reaction after the PSMA plasmid injection. In contrast, of the patients who received a PSMA plasmid and CD86 plasmid, only 50% showed signs of successful immunization. However, several responders, as evidenced by a change in the local disease, distant metastases, and prostate-specific antigen (PSA) levels, were reported (Mincheff et al. 2000). A phase II clinical study to evaluate the effectiveness of the therapy is currently underway.

**Liposome vectors**

Liposomes are the second most frequently used gene transfer method in current clinical trials. Liposome vectors consist of a DNA plasmid surrounded by a liposomal coat. The charge characteristics of the liposomal coat play a major role in determining the targeting capacity, intracellular stability and transgene size capacity of the vector. Anionic liposome-based vectors do not display non-specific cell-surface binding and thus may be targeted to specific cell-surface moieties (Lee & Huang 1996). Anionic liposomes are endocytosed by the target cell, targeted to endosomes, and subsequently prone to degradation by lysosomal nucleases severely limiting efficiency of transgene expression. Cationic liposomes bind avidly and non-specifically to the cell surface and are taken up into cells by a non-receptor-mediated process. They are less likely to undergo endosomal degradation than anionic liposomes and can also package much larger transgenes. A commonly used cationic liposomal vector in clinical trials is Lipofectin. Liposomes display preferential uptake in cells of the reticuloendothelial system (RES), complicating attempts at targeting. Modification to decrease this unwanted uptake includes the addition of sialic acid residues to make ‘stealth’ liposomes (Lasic et al. 1991) which may evade RES uptake. It has been suggested that the presence of leaky vessels in solid tumors may favor the selective accumulation of sterically stabilized large vectors such as liposomes (Brown & Giaccia 1998). Liposomal vectors are inexpensive and relatively easy to prepare in large quantities. Their large insert capacity permits the transfer of multi-gene cassettes of unlimited size. Since they lack proteins, they elicit weaker or no immune/inflammatory responses. A disadvantage of liposomal gene transfer is its inefficiency; thousands of liposomes are necessary per cell for successful transduction (Yotsuyanagi & Hazemoto 1998).

**DNA–protein complex vectors and hybrid vectors**

DNA–protein complexes have been developed for gene transfer in which the transgene is complexed with a targeting protein. This allows the possibility of high-specificity gene transfer of large inserts with minimal immune response. DNA–protein complexes, like anionic liposomes are, however, highly susceptible to endosomal targeting and lysosomal nuclease degradation, greatly reducing their transduction efficiency.

The major limitation of viral vectors is maximum insert size. The major limitation of the synthetic vectors is their...
poor gene transfer efficiency, a result of poor uptake of vector DNA into cell/nucleus and the lysosomal degradation that occurs following internalization. As a means to circumvent some of the limitations of both synthetic and viral vectors, hybrid vectors combining viral and synthetic approaches have been devised. DNA segments can be complexed to Ad virions to which polylysine or DEAE-dextran has been conjugated (Wagner et al. 1992, Curiel 1994, Forsayeth & Garcia 1994). These conjugates improve transfer efficiency by orders of magnitude in comparison with naked DNA. Similar improvements have been noted for an Ad–liposome complex which resulted in a 1000-fold increase in gene transfer efficiency relative to naked plasmid (Raja-Walia et al. 1995). Transgenes of up to 48 kb have been successfully transferred using this technique (Cotten et al. 1992). The Ad to which the DNA is complexed may be replication-defective or UV-inactivated. The presence of the virion proteins in the DNA–virus complex are sufficient to improve vector attachment and uptake, and more importantly, allow escape of the vector DNA from endosomes prior to fusion with the lysosomes, where they may be degraded (Cristiano et al. 1993, 1998).

Table 3 Corrective gene therapy strategies for PCA

<table>
<thead>
<tr>
<th>Corrective gene therapy targets</th>
<th>Normal function</th>
<th>Defect in PCA</th>
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<tbody>
<tr>
<td><strong>Tumor suppressors</strong></td>
<td></td>
<td></td>
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<tr>
<td>p53</td>
<td>Regulation of cell cycle G1/S checkpoint, apoptosis, response to stress/DNA damage</td>
<td>Mutated in 20–75% of PCAs</td>
</tr>
<tr>
<td>Rb</td>
<td>Cell cycle regulation and differentiation</td>
<td>Allelic loss in 27–40% of PCAs</td>
</tr>
<tr>
<td>pTEN</td>
<td>Regulation of PI3K cell survival pathway</td>
<td>Decreased expression in 50% of PCAs</td>
</tr>
<tr>
<td>p16 (CDKN2)</td>
<td>Cyclin-dependent kinase inhibitor involved in cell cycle regulation</td>
<td>Markedly reduced expression in 43% untreated primary PCAs; no alteration in BPH Down regulation by androgen</td>
</tr>
<tr>
<td><strong>KAI-1</strong></td>
<td>Cell–cell and cell–matrix interactions</td>
<td>Decreased expression in metastatic PCAs</td>
</tr>
<tr>
<td><strong>Oncogenes</strong></td>
<td></td>
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<tr>
<td>myc</td>
<td>Transcription factor regulating proliferation/differentiation</td>
<td>Amplified in 20% of advanced PCAs</td>
</tr>
<tr>
<td>bcl-2</td>
<td>Inhibition of apoptosis</td>
<td>Uniformly elevated in androgen-independent PCAs</td>
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<tr>
<td><strong>Ras</strong></td>
<td>GTP-binding proteins involved in growth regulation</td>
<td>Mutated in 25% PCA in US men; in Japanese cases PCA ~25% mutated</td>
</tr>
<tr>
<td>c-met</td>
<td>Receptor tyrosine kinase for hepatocyte growth factor</td>
<td>Elevated in 100% bone metastases</td>
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<tr>
<td><strong>Growth factors</strong></td>
<td></td>
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<tr>
<td>IGFs/IGFBPs</td>
<td>Growth factors/growth factor regulatory proteins</td>
<td>Increased IGF-1 levels associated with increased risk of PCA</td>
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<tr>
<td><strong>TGF-β</strong></td>
<td>Growth factor – growth-inhibitory in normal prostate</td>
<td>Tumor progression may involve dysregulation of TGF-β growth inhibition</td>
</tr>
<tr>
<td><strong>Cell-adhesion molecules</strong></td>
<td></td>
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<tr>
<td>E-cadherin</td>
<td>Signal transduction/intercellular adhesion</td>
<td>Deficient or absent expression in ~50% of PCAs</td>
</tr>
<tr>
<td>α-catenin</td>
<td>Signal transduction/intercellular adhesion</td>
<td>Deficient expression in 42% of PCAs</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Signal transduction/intercellular adhesion</td>
<td>Mutated in 5% of PCAs</td>
</tr>
<tr>
<td>CD44</td>
<td>Intercellular adhesion/cell–matrix interactions</td>
<td>Decreased expression with increasing stage</td>
</tr>
<tr>
<td><strong>Detoxification</strong></td>
<td></td>
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<tr>
<td>GSTP1</td>
<td>Detoxification of electrophilic carcinogens</td>
<td>Absent expression secondary to promoter hypermethylation in PIN and 98% of clinical PCAs</td>
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</table>

Corrective PCA gene therapy

Corrective gene therapy involves the functional complementation of an abnormal or absent gene. Corrective strategies may be designed to prevent tumorigenesis in phenotypically normal cells. Alternatively, corrective gene therapy may be administered to induce phenotypic reversion or regression of transformed cells to a non-neoplastic or less transformed phenotype. Numerous potential candidate genes for corrective gene therapy approaches are suggested by the wide variety of genetic and epigenetic changes known to occur accompanying prostate tumorigenesis (Table 3) including allelic loss, alterations in DNA methylation patterns, activating mutations of oncogenes, and inactivating mutations of tumor suppressors and cell-adhesion molecules (Isaacs et al. 1995, Konishi et al. 1997, Roylance et al. 1997).

The most commonly observed allelic loss in PCA involves chromosomes 8, 10, 11 and 16 (Isaacs et al. 1994, 1995), suggesting the presence of tumor-suppressor genes on these chromosomes. The potential for functional tumor suppression by genes encoded in these deleted chromosomal regions has been demonstrated in microcell-mediated chro-
mosome transfer studies. Transfer of portions of chromosomes 8, 10, 11, 17 and 19 into Dunning R-3327 rat PCA cells results in decreased formation of metastases and decreased tumorigenicity (Isaacs et al. 1995, Gao et al. 1999). Analysis of allelic loss involving chromosome 11 led to the cloning of the KAI1 metastasis-suppressor gene (Dong et al. 1995), a potential candidate for use in PCA gene therapy strategies aimed at prevention of metastases. Further analysis of allelic loss during tumor progression may identify new candidates suitable for corrective gene therapy specific to various stages of tumorigenesis.

Tumor-suppressor genes such as p53, retinoblastoma (Rb), p16, and pTEN show evidence of mutation or aberrant expression in some PCAs (Bookstein et al. 1993, Isaacs et al. 1994, 1995, Pesche et al. 1998, Suzuki et al. 1998). The tumor-suppressor gene p53 encodes a transcription factor involved in regulation of the cell cycle and apoptosis. Mutations in p53 are widespread in human cancer and are observed in between 20 and 75% of PCAs, more commonly in advanced metastatic tumors (Heidenberg et al. 1995). The ability of overexpressed p53 to inhibit the growth of primary cultures derived from radical prostatectomy specimens has been demonstrated, surprisingly even when p53 status is normal (Asgari et al. 1997).

Mutations of the tumor-suppressor pTEN have been frequently observed in PCA (Pesche et al. 1998, Suzuki et al. 1998, Whang et al. 1998), pTEN appears to be involved in a regulation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, a pathway involved in cell survival. Candidates for effectors downstream in this signaling pathway include GSK-3/β-catenin (Bullions & Levine 1998), and members of the bcl-2 family. Another tumor-suppressor, p16 (CDKN2) shows markedly reduced expression in 43% of untreated primary PCAs but is unaltered in benign prostate hyperplasia (BPH) (Chi et al. 1997). p16 is a cyclin-dependent kinase inhibitor involved in cell cycle regulation. Down-regulation of p16 by androgen, promoter methylation, or other inactivating mutations may increase progression through the cell cycle (Jarrard et al. 1997, Lu et al. 1997).

Methylation changes affecting gene expression have been widely observed in prostate and other tumors (Lee et al. 1994, Nelson et al. 1997). A frequent target for inactivating methylation changes is the glutathione S-transferase P1 gene, a gene involved in detoxification of electrophilic carcinogens (Lee et al. 1994). The absence of this mutation in normal prostate or BPH, and its presence in prostatic intraepithelial neoplasia (PIN) and greater than 98% of clinical prostate tumors suggests that it is an early step in prostate tumorigenesis, and thus may be an appropriate target for PCA preventive gene therapy.

Oncogenes that may be activated in PCA include c-Myc, Ras, bcl-2 and c-met (Table 3) (Isaacs et al. 1995, Konishi et al. 1997, Roylanle et al. 1997). Gene therapy strategies to correct oncogene activation include the use of dominant negative gene products, antisense oligodeoxynucleotides, hammerhead ribozymes, and single-chain antibodies. The overexpression of the oncogene c-Myc is frequently observed in advanced prostate tumors (Fleming et al. 1986, Buttyan et al. 1987). Disruption of c-Myc overexpression using antisense c-Myc transduced by a replication-deficient MMTV-derived RV (Steiner et al. 1998) resulted in a 94.5% reduction in tumor size of DU145 PCA cell xenografts. Another oncogene, which could be targeted in a corrective gene therapy approach, is bcl-2, which is frequently overexpressed in androgen-independent PCAs (McDonnell et al. 1992, Furuya et al. 1996, Beham et al. 1998). A hammerhead ribozyme designed to disrupt bcl-2 expression in LNCaP PCA cells showed pro-apoptotic activity and resulted in increased cell sensitivity to secondary pro-apoptotic agents including phorbol ester (Dorai et al. 1997).

Cell-adhesion molecules such as the cadherins and catenins show frequent alterations accompanying tumorigenesis and may constitute appropriate targets for corrective gene strategies. The cadherins are a family of transmembrane cell-adhesion proteins involved in calcium-dependent cell–cell adhesion, and in transduction of extracellular growth regulatory signals. Deficient or absent E-cadherin expression due to allelic loss, or promoter hypermethylation, is observed in approximately half of PCAs. The level of E-cadherin expression is prognostic for survival, metastases, tumor stage, grade, and risk of post-prostatectomy recurrence (Umbas et al. 1994, Richmond et al. 1997). E-cadherin-mediated cell adhesion also requires the function of the cytoplasmic protein α-catenin which couples E-cadherin to the cytoskeleton. Deficient α-catenin expression has been observed in approximately 42% of PCAs and is prognostic for patient survival (Richmond et al. 1997). The constitutive expression of α-catenin in PC-3 PCA cells through microcell-mediated transfer of chromosome 5 results in suppression of tumorigenicity in an E-cadherin-dependent fashion (Ewing et al. 1995). β-catenin, another catenin involved in E-cadherin-mediated cell–cell adhesion as well as in signal transduction through the Wnt/wingless gene signaling pathway, has recently been shown to be mutated focally in some prostate tumors, suggesting that it may play a role in tumor progression (Voeller et al. 1998).

Growth factors are another potential target for corrective gene therapy. The insulin-like growth factors (IGFs) are potent mitogens for prostate epithelial cells. The ability of IGF-I to activate the androgen receptor (AR) even in the absence of hormone suggests a potential role in PCA progression (Culig et al. 1995a,b). The IGF-binding proteins (IGFBPs), produced in the prostate and elsewhere, are a family of seven related proteins which can modulate epithelial cell growth, either via binding and modulation of IGF-I and -II activity or through IGF-independent mechanisms (Oh 1997). The IGFBPs are induced by growth inhibitory molecules such as transforming growth factor (TGF)-β.

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Figure 1 Ex vivo cytokine-transduced tumor vaccine gene therapy. Tumor cells transduced ex vivo with cytokine such as GM-CSF are irradiated then reintroduced into the patient. Transduced GM-CSF improves tumor cell antigen presentation and fully activates DCs, the most potent APCs in the immune system. DCs present prostate tumor vaccine-associated antigens they have processed to both CD4 (helper) and CD8 (cytolytic) T cells in the draining lymph node of the vaccination sites, promoting systemic tumoricidal immunity.


It is estimated that approximately 9% of PCAs occurring prior to the age of 85 are due to genetic predisposition (Gronberg et al. 1997, Walsh & Partin 1997). The basis for hereditary PCA has been mapped to several loci – the first susceptibility locus identified, HPC-1, resides on chromosome 1q24–25 (Smith et al. 1996). A second susceptibility locus, the recently identified HPC-X, resides on the X chromosome at Xq27–28 (Xu et al. 1998). Identification of the involved gene(s) at these loci should provide targets for preventive/corrective gene therapy, particularly for high genetic susceptibility risk probands.

A potential limitation of corrective gene therapy is the requirement that not even a single neoplastic clonogen must develop. To achieve this goal would require 100% efficiency of gene transfer, as well as sustained transgene expression, or tolerance for repeated vector application. Even if efficiency could be improved to the extent required, alternative mechanisms of progression may circumvent whatever the corrective strategy employed.

Cytoreductive PCA gene therapy

Immunotherapy strategies

PCA offers potentially unique antigens which may be exploited for the genetic induction of autoimmune antitumor immune responses. Immunotherapy strategies for cancer gene therapy utilize gene transfer to facilitate a dormant host immune response directed against the tumor. Evasion of autologous host cellular immunity is a common feature of tumor cell neoantigens. Tumor cells are poor antigen-presenting cells (APCs). In PCA in particular, defects in MHC class I expression are observed in a striking 85% of primary and 100% of metastatic tumors (Blades et al. 1995), suggesting that evasion of MHC class I tumor-associated antigen is important in prostate tumor development. ‘Cancer vaccine’ strategies are based on optimization of the context in which tumor antigens or tissue-specific antigens are presented to the host immune system. When appropriately primed, the activated host immune system can then act against tumor cells systemically.

One means to utilize gene therapy to optimize tumor-antigen presentation is through the targeted expression of cytokines in tumor cells. Targeted paracrine expression eliminates the toxicities associated with systemic cytokine administration. The transduced cytokines result in a combination of improved tumor cell vaccine antigen presentation, and activation of APCs, both essential for effective priming of the cellular immune response (Fig. 1). Granulocyte-macrophage colony stimulating factor (GM-CSF) has emerged as a cytokine with significant efficacy in the induction of an antitumor immune response (Dranoff et al. 1993). GM-CSF is the most potent cytokine signal tested for activation of antigen processing and presentation by macrophages and DCs (Cella et
GM-CSF may be transduced into autologous or allogeneic tumor cells \textit{ex vivo} using either an RV or other vectors. The transduced tumor cells are then irradiated both to minimize malignant potential and to improve immunogenicity (Simons & Mikhak 1998). The cells are then reintroduced by vaccination into the patient and tumor response monitored. Tumor cell vaccine-expressed GM-CSF may activate quiescent DCs which then capture prostate tumor cell vaccine antigens. DCs are the most potent APCs in the immune system and are central to the success of these genetically engineered tumor vaccine strategies. Activated DCs can present prostate tumor vaccine-associated antigens they have processed to both CD4 (helper) and CD8 (cytolytic) T cells in the draining lymph node of the vaccination sites (Fig. 1) (Banchereau & Steinman 1998, Simons & Mikhak 1998), activating a systemic tumoricidal immune response.

By presenting tumor antigen/MHC I in the context of supraphysiological local levels of transduced cytokines such as GM-CSF or interleukin (IL)-2, the CTL-based clinical antitumor immune response has been enhanced (Simons & Mikhak 1998). A potent antitumor CTL response has also been attained through co-presentation of tumor antigen/MHC II with a transduced costimulatory signal such as B7 (Baskar \textit{et al.} 1993). The duration of the transduced cytokine expression must be of sufficient duration to permit cell harvesting \textit{ex vivo} and for antigen processing/presentation \textit{in vivo}. For autologous vaccine, RV and Ad vectors have been favored for \textit{ex vivo} tumor vaccine trials. GM-CSF gene transfer has also been accomplished using cationic immunoliposome AAV vectors (Koppenhagen \textit{et al.} 1998).

\textit{Ex vivo} cancer vaccine strategies have also directly employed APCs such as DCs to facilitate effective tumor-antigen presentation. The rationale for these strategies is that the APC, properly primed, will express the most appropriate combination of factors for eliciting an effective immune response. DC-based vaccines have been generated by RV vector-mediated transduction of antigen (Specht \textit{et al.} 1997), pulsing with tumor-antigen RNA (Boczkowski \textit{et al.} 1996), and pulsing with tumor- or tissue-specific peptide (Murphy \textit{et al.} 1999).

As complete ablation of PSA-expressing tissue is an ideal therapeutic endpoint, \textit{in vivo} cytoductive immunotherapy approaches have centered on prostate-specific recombinant viral vaccines. The vector-induced inflammatory/imune response functions as an adjuvant to the transduced antigen, resulting in local release of cytokines and influx of APCs to the vaccine site. The direct transduction and expression of tumor-associated antigens in DCs can enhance the efficacy of viral vaccines (Bronte \textit{et al.} 1997). Cotransduction of cytokines together with the tumor-associated antigen may further enhance viral vaccine efficacy (Bronte \textit{et al.} 1995).

Another vaccine strategy is the DNA or polynucleotide vaccine. In this approach, an expression cassette containing the transgene against which an immune response is desired is injected directly into host cells, typically i.m. (Benton & Kennedy 1998). The expression of the transfected gene \textit{in vivo} may then engender humoral and cellular immune responses. DNA vaccines are technically easier to prepare than peptide- or viral-based vaccines and pose no risk of pathogenicity as they are non-replicating. A modification of the DNA vaccine technique which may improve its efficacy is the fusion of the desired transgene with the sequence for a pathogen-derived gene to engender a stronger immune response. Fragment C of tetanus toxin has been used in a fusion with single-chain Fv sequences from B-cell lymphoma, and resulted in striking enhancement of both humoral and cellular immunity (Falo & Storkus 1998, King \textit{et al.} 1998). Recently, the preclinical testing of a PSA-based DNA vaccine in mice was reported (Kim \textit{et al.} 1998). Injection i.m. of the PSA expression cassette resulted in a strong CTL/humoral response against PSA-positive tumor cells. Evaluation of the effects of vaccines incorporating various regions of the PSA-coding sequence identified four dominant coding sequences for Th epitopes of PSA. Future vaccine approaches can be predicted to target other prostate-unique antigens such as PSMA, mucin (MUC-2), human glandular Kallikrein 2 (hK2), and other novel prostate-unique antigens whose identification will be facilitated through the use of genomics and proteomics techniques (Pang \textit{et al.} 1997, Nelson \textit{et al.} 1998).

A major limitation of cyto reduc tive immunotherapy is the limited tumor burden which the immune system can eliminate in experimental models. Thus, applicability is probably limited to clinical settings of low bulk or micrometastatic disease, or in combination with other debulking therapies. Additionally, the harvesting and culture of autologous or allogeneic tumor or immune cells for \textit{ex vivo} gene therapy is expensive and technically challenging. The observed safety of cytokine gene-transduced vaccines is in no small measure responsible for ongoing enthusiasm for clinical testing as an outpatient adjuvant therapy.

\textbf{Polyvalent vs single antigen PCA vaccines}

Polyvalent cancer vaccines targeting the entire antigenic spectrum on tumor cells may represent a superior therapeutic strategy for cancer patients than vaccines solely directed against single antigens (Heiser \textit{et al.} 2001). Heiser \textit{et al.} (2001) showed that autologous DCs transfected with RNA amplified from microdissected tumor cells are capable of stimulating CTL against a broad set of unidentified and critical PSAs. In this strategy of gene therapy, the tumor’s candidate antigens were transfected as a cDNA library. Although the polyclonal CTL responses generated with amplified tumor RNA-transfected DCs encompassed as a subcomponent a response against PSA as well as against telomerase reverse
transcriptase, the tumor-specific CTLs were consistently more effective than PSA or telomerase reverse transcriptase CTLs in lysing tumor targets, suggesting the superiority of the polyclonal response. Although tumor RNA-transfected DCs stimulated CTLs which recognized not only tumor but also self-antigens expressed by benign prostate tissue, these cross-reactive CTLs were exclusively specific for the PSA, indicating an immunodominant role of PSA in the PCA-specific immune response. These data suggest that tumor RNA-transfected DCs may represent a broadly applicable, potentially clinically effective vaccine strategy for PCA patients, which is not limited by tumor tissue availability for antigen preparation and may minimize the risk of clonal tumor escape.

Cytolytic/pro-apoptotic strategies

Enzyme/prodrug gene therapy

Enzyme/prodrug gene therapy, also referred to as ‘suicide gene therapy’, relies on the conversion of an inactive prodrug into a toxic drug using an enzyme vectored only to the target tumor cells. In this way, active drug is limited spatially to the transduced cells and adjacent surrounding cells, facilitating higher tumor drug concentrations without increased normal tissue toxicity. As opposed to the corrective gene therapy approach noted above, it is essential that the vector be targeted with great specificity to tumor cells to avoid increased normal tissue injury. Prodrug-activating enzymes which have been employed in this approach include cytosine deaminase (CD) which catalyzes the conversion of the non-toxic 5-fluorocytosine (5-FC) to the cytotoxic 5-fluorouracil (5-FU), and HSV-TK which together with cellular enzymes facilitates the conversion of GCV into the toxic GCV triphosphate. Using this approach it has been shown that even when only 2% of a tumor contains CD-transduced cells, significant tumor regression is observed, suggesting the presence of a significant bystander effect (Kim et al. 1998). The cytotoxic efficacy of suicide gene therapy in vivo may benefit from a systemic antitumor response precipitated by tumor lysis and inflammation, which may be mediated by natural killer (NK) cells (Hall et al. 1998). The capacity for prostate-specific expression of suicide genes has been demonstrated using PSA, PSMA, and hKLLK2 promoters (Harris et al. 1994).

Cytotoxins

Another cytoreductive strategy involves the targeted expression of a cytotoxin such as the diphtheria toxin A chain, or pseudomonas exotoxin A. In a screen of cytotoxins using a wide range of PCA cell lines, Rodriguez et al. (1998) demonstrated a cell cycle- and p53-independent cytotoxic activity of the diphtheria toxin A chain in PCA cell lines through both apoptotic and non-apoptotic pathways. Another cytoreductive agent that has shown activity in PCA cells in vitro is expanded polyglutamine. This is a pro-apoptotic molecule implicated in eight inherited neurodegenerative disorders (Ikeda et al. 1996). Its pro-apoptotic potential can be modulated by varying the expression level or the length of the expanded polyglutamine. This molecule has demonstrated pro-apoptotic activity in PCA cell lines when expressed from a vector under control of a PSA promoter (Segawa et al. 1998). A critical concern with the use of potent cytotoxins for cytoreductive gene therapy is the target cell specificity of the vectoring strategy. From a practical viewpoint, even low levels of expression of a cytotoxin may be toxic to the packaging cell line if a recombinant viral strategy is employed. With tumor/tissue-specific promoter or antigen targeting, unwanted expression of the cytotoxin in non-target cells either through non-specific antigen targeting or through a ‘leaky’ tissue-specific promoter could result in significant adverse normal tissue effects.

Oncolytic viruses

Viral vectors may themselves be designed to target and kill tumor cells without insertion of a foreign transgene. Proof of this principle was demonstrated therapeutically as early as the 1950s with tumor responses noted following injections of wild-type Ad into patients with cervical cancer. The Ad life cycle includes a lytic phase, which can result in host cell death independently of entry into the cell cycle – an important advantage for PCA therapy given the low mitotic rate. Ad has evolved a potent repertoire of gene products, which may exert profound effects on the growth regulation of the host cell, in order to facilitate viral replication. A replication-competent Ad designed to preferentially replicate in p53 mutant cells, the ONX-015 vector, is currently in clinical trials (Kim et al. 1998). The design of this vector was based on the findings that a consequence of Ad infection was the induction of p53, whose pro-apoptotic activity would be detrimental to the viral life cycle and was therefore blocked directly by the virally encoded E1B 55 kDa protein (Debbas & White 1993). In addition, E1B 19 kDa protein expression resulted in the production of a bcl-2 type molecule with anti-apoptotic effects downstream of the p53 pathway (Chiou et al. 1994). It was reasoned therefore that an E1B-deficient Ad would replicate preferentially in p53-deficient tumors (Bischoff et al. 1996). Recent findings suggest, however, that control of viral replication is more complex than the initial model suggests, as the E1B-deficient virus replicates in p53 wild-type tumor cells as well (Rothmann et al. 1998). To evaluate the selectivity of ONX-015 replication and cytopathic effects for the first time in humans, a phase II clinical testing of intratumoral and peritumoral ONX-015 injection in 37 patients with recurrent head and neck carcinoma was carried out (Nemunaitis et al. 2000). Post-treatment biopsies docu-
mented selective ONYX-015 presence and/or replication in the tumor tissue of 7 of 11 patients biopsied. Tissue destruction was also highly selective; significant tumor regression (>50%) occurred in 21% of evaluable patients, whereas no toxicity to injected normal peritumoral tissues was demonstrated. p53 mutant tumors were significantly more likely to undergo ONYX-015-induced necrosis (7 of 12) than were p53 wild-type tumors (0 of 7; P = 0.017). High neutralizing antibody titers did not prevent infection and/or replication within tumors. ONYX-015 is the first genetically engineered replication-competent virus to demonstrate selective intratumoral replication and necrosis in patients. This agent demonstrates the promise of replication-selective viruses as a novel therapeutic platform against cancer (Nemunaitis et al. 2000).

Among the Ad gene products which may function to induce cell death are E1A (Rao et al. 1992), the E3–11.6k protein also known as the Ad death protein, which is required for efficient cell lysis during productive viral infection (Tollefson et al. 1996b), and the E4orf4 protein, which may induce p53-independent apoptosis (Marcellus et al. 1998). The E1A 243-residue protein is able to induce a p53-dependent apoptosis in the absence of other viral proteins using a caspase effector cascade which involves activation of procaspase-8, followed by mitochondrial redistribution of cytochrome c and activation of procaspase-3 (Fearnhead et al. 1998, Nguyen et al. 1998). An Ad vector with the E1A gene placed under the control of a PSA minimal promoter enhancer, the CN706 vector (Fig. 2) showed potent PSA-selective cytototoxic activity in preclinical testing and is currently being utilized in a phase I clinical trial (Rodriguez et al. 1997) (see below; Principal Investigator J W Simons, Johns Hopkins University).

Searching for additional approaches that would induce therapeutic apoptosis of PCA cell lines, Li et al. (2001) recently used a binary Ad system to overexpress the pro-apoptotic molecule Bax. Bax was dramatically overexpressed and caused apoptosis of every cell line infected by engaging the mitochondrial pathway, including proteolytic cleavage and catalytic activation of the caspases, cleavage of caspase substrates, release of cytochrome c from the mitochondria, and DNA fragmentation. Furthermore, three injections of the Bax-overexpression system into PC-3 cell tumors in nude mice in vivo caused a 25% regression in tumor size corresponding to a 90% reduction relative to continued tumor growth in animals that received injections with the control binary system expressing Lac-Z. These experiments show that Ad-mediated Bax overexpression is capable of inducing therapeutic programmed cell death in vitro and in vivo by activating the mitochondrial pathway of apoptosis. On the basis of these studies, it can be concluded that manipulation of Bax expression is an attractive new gene therapy approach for the treatment of PCA (Li et al. 2001).

Prostate-specific targeting

Regardless of the vector or gene therapy strategy used, restriction of cytoreductive effects to target tissue is an essential factor in determining overall therapeutic index. Strategies to restrict cytoreductive effects of gene therapy may utilize tissue- or tumor-specific antigens or promoters. The presence of several well-characterized prostate-specific markers such as PSA and PSMA, and 500 or more prostate-unique genes, provides a biological foundation for prostate-localized gene therapy treatment. The prostate-restricted expression of PSA has stimulated a variety of PSA-dependent gene therapy strategies. Antigen-based strategies include the PSA vaccines in clinical trial discussed below. Several groups have placed therapeutic genes under the control of various PSA gene cis-regulatory regions. Varying segments of the 5′-flanking region of PSA have been evaluated for their suitability for prostate-specific expression of a desired gene. A minimal composite PSA promoter/enhancer element (PSE) was used to drive expression of Ad E1A in the attenuated replication-competent vector CN706 (Fig. 2) (Schuur et al. 1996, Rodriguez et al. 1997). Within the PSA enhancer region employed in the PSE, a functional androgen response element (ARE) at position 4136 (Fig. 2) results in an up to 100-fold increase in expression in the presence of testosterone or the steroid analog R1881. The regulation of E1A expression by the PSE element effectively limits cytolysis viral replication to PSA-expressing cells, with a resulting therapeutic ratio of between 20:1 and 3000:1 depending upon the cell line tested.

One issue with PSA-promoter/ enhancer-based targeting is whether sufficient levels of transgene expression are obtained, particularly in the absence of androgen. For clinical PCA gene therapy, efficient transgene expression in the absence of androgen would be preferred. In a PSA promoter-based strategy in preclinical testing, the HSV-1 tk gene was placed under control of a long 5.8 kb PSA promoter in an Ad construct. This vector showed activity in both the presence and absence of androgen (Gotoh et al. 1998). Another approach to amplify PSA-specific expression independently of androgen is through a two-step transcriptional activation system in which the PSA promoter drives the expression of a potent transcriptional transactivator which in turn regulates expression of the desired transgene (Segawa et al. 1998).

PSMA is a potential target in PCA patients because it is very highly expressed and because it has been reported to be upregulated by androgen deprivation. O’Keefe et al. (2000) recently described analysis of the PSMA enhancer for the most active region(s) using the enhancer in combination with the E. coli CD gene for suicide-driven gene therapy that converts the non-toxic prodruk 5-FC into the cytotoxic drug 5-FU in PCA cells. Deletion constructs of the full-length PSMA enhancer were subcloned into a luciferase reporter vector containing either the PSMA or SV-40 promoter. The
most active portion of the enhancer was then determined via luciferase activity in the C4–2 cell line. The luciferase gene was then replaced with the *E. coli CD* gene in the subclone that showed the most luciferase activity. The specificity of this technique was examined *in vitro*, using the PCA cell line LNCaP, its androgen-independent derivative C4-2, and a number of non-prostatic cell lines. Deletion constructs revealed that at least two distinct regions seem to contribute to expression of the gene in PCA cells, and therefore the best construct for prostate-specific expression was determined to be 1648 bp long. Transfection with the 1648 nucleotide PSMA enhancer and the PSMA promoter to drive the CD gene enhanced toxicity in a dose-dependent manner more than 50-fold, while cells that did not express the PSMA gene were not significantly sensitized by transfection.

Tissue-specific transcriptional regulatory elements can increase the safety of gene therapy vectors. Unlike PSA/hK3, whose expression displays an inverse correlation with PCA grade and stage, hK2 is upregulated in higher grade and stage disease. Therefore, Xie *et al.* (2001) developed a prostate-specific hK2-based promoter for targeted gene therapy. They identified the minimum ‘full-strength’ hK2 enhancer and built transcriptional regulatory elements composed of multiple tandem copies of this 1.2 kb enhancer, fused to the hK2 minimal promoter. Relative to the weak induction of the minimal hK2 promoter by androgen analog (R1881) in AR-positive LNCaP cells, transcriptional activity was increased by 25-, 44-, 81- and 114-fold when one to four enhancers were spliced to the hK2 promoter respectively. In contrast, the PSEs were inactive in the AR-negative PCA line PC-3 and in a panel of non-prostate lines, including 293, U87, MCF-7, HuH-7 and HeLa cells. These results suggest that the hK2 multi-enhancer/promoter should be a powerful reagent for targeted gene therapy of PCA.

The search for additional prostate-specific genes for use in PCA gene therapy is still going on. Recently, Steiner *et al.* (2000) reported on a novel gene *pHyde* that was cloned from Dunning rat PCA cell lines. A replication-deficient recombinant Ad containing pHyde cDNA gene under the control of a truncated RSV promoter (AdRSVpHyde) was generated. AdRSVpHyde inhibited
Observations in our own laboratory reveal evidence for synergy between the oncolytic CN706 Ad (see above and Fig. 2) and radiation in the LNCaP and LAPC-4 PCA cell lines. An obvious combined modality strategy for PCA is the combined implantation of brachytherapy seed sources with a gene therapy vector. The capacity of Ad proteins such as E1A and E4orf4 to engage cellular pro-apoptotic machinery suggests a mechanistic basis for the observed therapeutic synergy (Marcellus et al. 1998, Nguyen et al. 1998). Supporting this idea is the observation that E1A expression enhances apoptotic cell killing by a variety of chemotherapeutic agents in ovarian cell lines (Brader et al. 1997). Further investigations into the interactions between radiation, cellular and oncolytic virus gene products may clarify the mechanisms by which radiation might potentiate oncolytic gene therapy.

Gene therapy may also be used for radiosensitization strategies in which the activation of a prodrug, such as GCV (Nishihara et al. 1997) or 5-Fc (Hanna et al. 1997, Gabel et al. 1998), can function to sensitize cells to the effects of radiation. While chemotherapy has thus far been ineffective for PCA, novel combinations of gene therapy and chemotherapy may display synergistic improvements in efficacy. The combination of paclitaxel and gene therapy utilizing an Ad expressing p53 under the control of the CMV promoter revealed synergy for the combined modality treatment for a wide variety of tumor cell lines in vitro and in xenografts including the DU-145 PCA cell line (Nielsen et al. 1998).

Combinations of three modalities have also been demonstrated – radiotherapy, viral-cytopathic (E1B-deleted Ad) and radiosensitizing double-suicide gene therapy – with marked enhancement in efficacy in vitro with DU-145 cells (Freytag et al. 1998).

HSV-tk gene therapy may be effective in combination with radiation therapy due to complementary mechanisms and distinct toxicity profiles. In a study (Chhikara et al. 2001) mouse prostate tumors transplanted s.c. were treated by either gene therapy involving intratumoral injection of Ad-tk followed by systemic GCV or local radiation therapy or the combination of gene and radiation therapy. Both single-therapy modalities showed a 38% decrease in tumor growth compared with controls. The combined treatment resulted in a decrease of 61%. The combination led to an additional 50% reduction in lung colonization. Primary tumors that received the combination therapy had a marked increase in CD4 T-cell infiltration. This is the first report showing a dramatic systemic effect following the local combination treatment of radiation and Ad-tk. A clinical study using this strategy has been initiated and patient accrual is ongoing (Chhikara et al. 2001).

**Translation of concepts to human gene therapy clinical trials**

At present there are 40 approved clinical gene therapy trials for PCA in progress in the USA (ORDA 010/2001).
(HUMAN GENE THERAPY PROTOCOLS. http://www4.od.nih.gov/oba/rdna.htm Updated 10–01–2001 edn (National Institutes of Health (NIH) Office of Recombinant DNA Activities 2001). Of these, 22 are for cytoreductive immunotherapy, 14 are for cytoreductive cytolytic/pro-apoptotic strategies, and four are corrective strategies (Table 4).

The preliminary results of only part of these trials have only recently been published. The results of the phase I clinical trial of irradiated GM-CSF-secreting autologous prostate tumor vaccine therapy in eight patients (Principal Investigator J W Simons, Johns Hopkins University) indicated that such treatment was safe and induced both B-cell and T-cell immune responses against PCA cell-associated antigens (Simons et al. 1999). GM-CSF gene-transduced PCA vaccines increased antibody titers against prostate tumor cell line-associated antigens. Increasing titers of antibodies to PCA antigens were detected among three of the men treated with irradiated GM-CSF-secreting autologous PCA cell vaccines. This is the first report of induction of new antibody responses to PCA antigens in patients using cytokine gene-modified tumor vaccines, peptide-pulsed DCs, or any other strategy of immunotherapy.

Phase II studies have commenced with GM-CSF gene-transduced allogeneic vaccines. Preliminary results of the initial trial approved to use direct transrectal prostatic gene therapy injection were reported by Steiner & Gingrich (2000). A total of 21 men with PCA in whom standard therapy failed underwent ultrasound-guided injection of the RV LXSN containing BRCA-1 under the control of the viral promoter LTR. No viral symptoms or evidence of viremia developed. Serum PSA in these cases of metastatic disease remained unchanged. The study indicated the safety of PCA gene replacement therapy by direct injection.

Herman et al. (1999) reported results of a phase I clinical trial of a replication-deficient Ad containing the HSV-tk injected directly into the prostate, followed by i.v. administration of the prodrug GCV. Only 1 of 18 patients at the highest dose level developed spontaneously reversible grade 4 thrombocytopenia and grade 3 hepatotoxicity. Most of the patients at the highest dose levels achieved objective response (fall in serum PSA levels by 50% or more).

Of the clinical trials ongoing at the University of California at Los Angeles preliminary results have been reported. In a phase II trial, intratumoral injection of a plasmid coding for IL-2, formulated in a liposomal, cationic lipid mixture vehicle, revealed decreases in serum PSA levels at 2 weeks post-injection in 80% of the patients treated (Pantuck et al. 2000). IL-2 gene therapy was well tolerated, with no grade 3 or 4 toxicity occurring. In another immunotherapy phase I trial in MUC-1-positive patients using vaccinia virus MUC-1-IL2 revealed an immunological response associated with a clinical PSA decline. In addition, an upregulation of cytokine expression, augmented T-cell activation signals and augmented MUC-1-targeted cytotoxicity were noted (Pantuck et al. 2000).

Integration of the following concepts: (i) PSA-based targeting, (ii) virus-directed cytolytic therapy, (iii) stereotactically-guided vector administration for improved target tissue distribution, and (iv) use of replication-competent virus for improved tissue penetration, formed the basis for a recently initiated clinical trial for patients with a post-radiation therapy local recurrence of PCA (Principal Investigator J W Simons, Johns Hopkins University).

A major limitation in the use of replication-deficient gene therapy in solid tumors in vivo is the diffusion-limited tissue penetration into the target tissue. The ability of viral replication to increase tissue penetration has been observed in vitro and in vivo (Advani et al. 1998, Han et al. 1998). Replication-competent Ad spread in vivo may be facilitated not only through transcellular movement and vector amplification, but also through greater tissue exposure resulting from virus-dependent cell lysis. Thus, a replication-competent Ad can be expected to achieve greater tissue penetration relative to a replication-deficient Ad or other non-lytic vector. CN706 (Fig. 2) is attenuated for replication competence only in PSA-expressing cells, in which it may be expected to amplify titer and achieve greater tissue penetration. In addition, the local immune response against the Ad-infected cells may aid in killing of the target tumor cells.

The concept of dose uniformity for cytotoxic PCA therapy has been well developed in radiation therapy. Local underdos- ing is thought to increase the risk of treatment failure – a concept supported by the improved local control observed with conformational radiation dose-escalation trials (Hanks et al. 1998, Zelefsky et al. 1998). It follows that similar principles may apply to the tissue distribution and dosing of viral cytolytics such as CN706. By analogy to radiation dosimetry for prostate brachytherapy, the tissue surrounding a seed implant is exposed to varying concentrations of virus. The extent to which a replication-competent virus spreads depends on initial viral titer, infectivity, lytic ability, and host immune response. Empirical determinations of viral spread from tumor models allows an estimation of the effective radius of virus activity. This can then be used iteratively in a computer 3D model to designate viral injection patterns for optimal tissue distribution (Fig. 3). Existing brachytherapy treatment planning systems may be adapted for 3D viral vector spread modeling. Validation of predicted viral spread through biopsy analysis of Ad hexon protein immunohistochemistry will permit increasing accuracy in viral spread predictions. Spatial evaluation of oncolysis relative to viral spread will help define potentially new parameters involved in oncolytic efficacy and augment comparisons of alternative vectors. Clinical direct injection trials utilizing this precision in vector delivery benefit from the increased uniformity of gene delivery between patients.
Table 4 PCA gene therapy: current clinical trials summary

<table>
<thead>
<tr>
<th>NIH No.</th>
<th>Phase</th>
<th>Principal investigator</th>
<th>Institution</th>
<th>Vector</th>
<th>Gene</th>
<th>Modality</th>
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<td>9408-082</td>
<td>I–II</td>
<td>Simons</td>
<td>Johns Hopkins University</td>
<td>RV</td>
<td>GM-CSF</td>
<td>Ex vivo autologous PCA vaccine</td>
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<tr>
<td>9509-123</td>
<td>I</td>
<td>Holt &amp; Steiner</td>
<td>Vanderbilt University School of Medicine</td>
<td>RV</td>
<td>Antisense myc RNA</td>
<td>In vivo intraprostatic injection</td>
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<td>I</td>
<td>Chen</td>
<td>National Naval Medical Center</td>
<td>Vaccinia virus</td>
<td>PSA cDNA</td>
<td>In vivo i.d. injection</td>
</tr>
<tr>
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<td>I</td>
<td>Lyerly &amp; Paulson</td>
<td>Duke University Medical Center</td>
<td>Cationic liposome complex</td>
<td>IL-2 cDNA</td>
<td>Ex vivo autologous PCA vaccine</td>
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<tr>
<td>9601-144</td>
<td>I</td>
<td>Scardino</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
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<td>HSV-TK cDNA</td>
<td>In vivo intraprostatic injection</td>
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<td>9609-160</td>
<td>I</td>
<td>Eder &amp; Kufe Sanda</td>
<td>Dana-Farber Cancer Institute</td>
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<td>PSA cDNA</td>
<td>In vivo i.d. injection</td>
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<tr>
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<td>I–II</td>
<td>Chou &amp; Chang</td>
<td>The University of California, San Francisco</td>
<td>Vaccinia virus</td>
<td>PSA cDNA</td>
<td>In vivo i.d. injection, autologous</td>
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<td>I</td>
<td>Belldegrun</td>
<td>University of California, Los Angeles</td>
<td>Cationic liposome complex/DMRIE-DOPE</td>
<td>IL-2 cDNA</td>
<td>Intratumoral direct injection</td>
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<td>9705-187</td>
<td>I</td>
<td>Hall &amp; Woo</td>
<td>Mount Sinai School of Medicine</td>
<td>Ad serotype 5</td>
<td>HSV-TK cDNA</td>
<td>Intratumoral direct injection</td>
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<td>University of California, Los Angeles</td>
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<td>p53 cDNA</td>
<td>Intratumoral direct injection</td>
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<td>9708-205</td>
<td>I–II</td>
<td>Simons</td>
<td>Johns Hopkins University</td>
<td>RV</td>
<td>GM-CSF</td>
<td>Intra muscular direct injection</td>
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<td>p33 cDNA</td>
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<td>Baylor College of Medicine</td>
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<td>HSV-TK cDNA</td>
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<td>9802-236</td>
<td>I</td>
<td>Simons</td>
<td>Johns Hopkins University</td>
<td>Ad serotype 5/replication-competent virus</td>
<td>Promoter and enhancer elements of the PSA</td>
<td>Intratumoral direct injection</td>
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<td>I–II</td>
<td>Figlin</td>
<td>University of California, Los Angeles</td>
<td>Vaccinia virus</td>
<td>MUC-1/IL-2</td>
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<td>University of Virginia Health Science System</td>
<td>Ad serotype 5</td>
<td>HSV-TK cDNA</td>
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<td>II</td>
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<td>Vaccinia virus/fowlpox virus</td>
<td>PSA</td>
<td>i.m. injection</td>
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<td>GM-CSF</td>
<td>s.c. injection</td>
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<td>II</td>
<td>Kaufman &amp; DiPaola</td>
<td>Eastern Cooperative Oncology Group</td>
<td>Vaccinia virus/fowlpox virus</td>
<td>PSA</td>
<td>i.m. or i.d. injection</td>
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<td>9904-306</td>
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<td>Vieweg</td>
<td>Duke University Medical Center</td>
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<td>PSA</td>
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<td>IL-2 cDNA</td>
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<td>GM-CSF</td>
<td>s.c. injection</td>
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<td>Freytag &amp; Kim</td>
<td>Henry Ford Health System</td>
<td>Ad</td>
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<td>Gingrich</td>
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<td>p16 cDNA</td>
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<td>9910-344</td>
<td>I–II</td>
<td>Terris</td>
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<td>Ad serotype 5/replication-competent virus</td>
<td>Promoter and enhancer elements of the PSA</td>
<td>Intratumoral injection</td>
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DMRIE, 1,2-Dimyrystylopropyl-3-dimethyl-hydroxyethyl ammonium bromide; DOPE, dioleoyl-phosphatidyl-ethanolamine.
Twenty men with locally recurrent PCA following radiation therapy were treated with CV706 between September 1998 and May 2000 (Principal Investigator J W Simons, Johns Hopkins University). CV706 was found to be safe and was not associated with irreversible grade 3 or any grade 4 toxicity. Post-treatment prostate biopsy and detection of a delayed ‘peak’ of circulating virus provided evidence of intraprostatic replication of CV706. This study revealed that all patients treated with the highest doses of CV706 achieved a 50% or more reduction in serum PSA levels (DeWeese et al. 2001).

### Future directions

The challenges facing the implementation of successful gene therapeutic strategies will be better understood as the early clinical trials for PCA gene therapy begin to return more results. Vector development with increased transgene size capacity, optimized immunogenic properties, and improved transduction efficiency and targeting will facilitate the next generation of gene therapy strategies. The burgeoning field of genomics provides an exciting new resource for the design of prostate-specific gene therapy strategies. The design of oncolytic viruses should be aided by new insights into the mechanism of killing by lytic viruses. Already, the potential of Ad genes such as E4orf4, E3–11k and E1A to function in virus-induced cell death has been observed (Tollefson et al. 1996, Nevels et al. 1997, Marcellus et al. 1998, Nemunaitis et al. 2000, Li et al. 2001). Harnessing these viral gene products and others for use as oncolytic drugs offers exciting prospects for a whole new class of cytoreductive gene therapy strategies.

As the diversity of molecular lesions underlying prostate tumorigenesis is better characterized, new targets for corrective and cytoreductive approaches will emerge. Effective anticancer gene therapy may ultimately require individualized molecular profiles. Manipulation of the cellular...
Apoptotic machinery for cytoreductive PCA gene therapy may permit development of more generalized antineoplastic treatment, which bypasses upstream signal cascade lesions. Components of the apoptotic machinery such as the death receptors, caspases, and the bcl-XL/Bcl2 family may be utilized in strategies which result in efficient oncolysis even in the presence of diverse upstream lesions. Cell death pathways involving the death receptors such as CD95, DR3, 4 and 5, may provide potent therapeutic targets. A recent preclinical study shows that Ad- or RV-transduced fas ligand (FasL) or FADD can efficiently induce apoptosis in human glioma cells (Shinoura et al. 1998).

Dong et al. (1995) showed that intratumoral delivery of FasL using an Ad vector could force PCA cells into apoptosis. They placed the tetracycline transactivator gene under the control of a prostate-specific ARR2PB promoter, and a pro-apoptotic FasL-GFP gene under the control of the tetracycline responsive element. The latter expression cassette was inserted into Ad5. High levels of expression were exclusively observed in LNCaP cells but not in other cell lines of other origins (Hyer et al. 2000). This may hold promise in the future.

Solid tumors meet their demands for nascent blood vessels and increased glycolysis, to combat hypoxia, by activating multiple genes involved in angiogenesis and glucose metabolism. Hypoxia inducible factor-1 (HIF-1) is a constitutively expressed basic helix-loop-helix transcription factor, formed by the assembly of HIF-1alpha and HIF-1beta (Arnt), that is stabilized in response to hypoxia, and rapidly degraded under normoxic conditions. It activates the transcription of genes important for maintaining oxygen homeostasis (Dachs & Tozer 2000, Semenza et al. 2000). Sun et al. (2001) recently demonstrated that engineered downregulation of HIF-1alpha by intratumoral gene transfer of an antisense HIF-1alpha plasmid leads to the down-regulation of vascular endothelial growth factor (VEGF), and decreased tumor microvessel density. Antisense HIF-1alpha monotherapy resulted in the complete and permanent rejection of small EL-4 tumors. It induced NK-dependent rejection

**Figure 3** Injection of CN706 oncolytic Ad into the prostate under stereotactic guidance. (A) Intraoperative ultrasound prostate imaging with superimposed brachytherapy template. (B) Image (A) imported into brachytherapy treatment planning system (Radionics). Yellow and green circles indicate injection needle tracks, filled circles correspond to actual injection sites in the plane of the imaged section. Urethra and prostate outlines contoured in green and red respectively. (C) 3-D reconstruction of prostate volume and injection positions. Urethra imaged in red. (D) Predicted virus spread from injection sites in yellow.
of tumors, but failed to stimulate systemic T-cell-mediated antitumor immunity, and synergized with B7-1-mediated immunotherapy (Sun et al. 2001). This approach holds promise to form the foundation for the transition between the traditional anticancer therapies of the past four decades and the molecular antineoplastic pharmacology of the future.

Other approaches are to develop new gene therapy vectors whose expression is selectively activated by hypoxia. As VEGF is upregulated by hypoxia, such regulatory mechanisms would enable us to achieve hypoxia-inducible expression of therapeutic genes. Constructs with multiple copies of hypoxia-responsive elements (HREs) derived from the 5′ untranslated region of the human VEGF showed excellent transcriptional activation at low oxygen tension relevant to tumor hypoxia (Shibata et al. 2000). It was found that the combination of 5-HRE and a CMV minimal promoter exhibited hypoxia responsiveness (over 500-fold) to a level similar to the intact CMV promoter. Thus it can be proposed that this vector would be useful for tumor-selective gene therapy in the future. Of course many other replication-deficient Ad could be designed to contain multiple copies of HREs and the HSV-tk, which would be injected directly into the prostate and followed by i.v. administration of the prodrug GCV. In this case only hypoxic tumor cells, which are expressing high levels of HIF-1, will be killed.

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