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Advances in Prostate Cancer Immunotherapies

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Abstract

Prostate cancer is a major cause of mortality in men in the Western world. Although treatment of early stage prostate cancer with radiation therapy or prostatectomy is efficient in most cases, some patients develop a fatal hormone-refractory disease. Treatments in this case are limited to aggressive chemotherapies, which can reduce serum prostate-specific antigen (PSA) levels in some patients. Taxane- and platinum-compound-based chemotherapies produce a survival benefit of only a few months. Therefore, it is crucial to develop novel, well tolerated treatment strategies.

Over the past years, immunotherapy of hormone-refractory prostate cancer has been studied in numerous clinical trials. The fact that the prostate is a non-essential organ makes prostate cancer an excellent target for immunotherapy. Administration of antibodies targeting the human epidermal growth factor receptor-2 or the prostate-specific membrane antigen led to stabilisation of PSA levels in several patients. Vaccination of prostate cancer patients with irradiated allogeneic prostate cell lines has demonstrated that whole cell-based vaccines can significantly attenuate increases in PSA. Two different recombinant viral expression vectors have been applied in prostate cancer treatment: poxvirus and adenovirus vectors. Both vaccines have the advantages of using a natural method to induce immune responses and achieving high levels of transgene expression. Vaccinia viruses in combination with recombinant fowlpox or canarypox virus have been used to express recombinant PSA. Several studies demonstrated that this approach is safe and can lead to stabilisation of PSA values. A very promising approach in prostate cancer immunotherapy is vaccination of patients with dendritic cells. Thereby, peptides, recombinant proteins, tumour lysates or messenger RNA have been used to deliver antigens to autologous dendritic cells. Loading of dendritic cells with up to five different peptides derived from multiple proteins expressed in prostate cancer demonstrated that cytotoxic T-cell responses could be elicited in prostate cancer patients. Sipuleucel-T (APC8015), an immunotherapy product consisting of antigen-presenting cells, loaded *ex vivo* with a recombinant fusion protein consisting of prostatic acid phosphatase linked to granulocyte-

macrophage colony-stimulating factor, demonstrated in a phase III, placebo-controlled trial an improvement in median time to disease progression. The improvement in overall survival was 4.5 months for sipuleucel-T-treated patients compared with the placebo group.

Although there is a minor increase in overall survival of metastatic prostate cancer patients with some approaches, more effective therapeutic strategies need to be developed.

Prostate cancer is the most frequently diagnosed cancer among men, with 234 460 new cases being expected in 2006 in the US alone, and the third leading cause of cancer-related deaths in both the US and Europe.[1] Although localised prostate cancer is often curable, metastatic hormone-independent prostate cancer is usually fatal and 27 350 men were expected to die of prostate cancer in the US in 2006.[1] In recent years, the mortality rate for prostate cancer may have been reduced as a result of early diagnosis using detection techniques such as digital rectal examination and screening for prostate-specific antigen (PSA). Nearly three decades ago, PSA was first examined as a serum marker for the early detection of prostate cancer.[2] The 34 kDa glycoprotein PSA is a serine protease belonging to the glandular kallikrein gene family and is produced by prostatic epithelial cells lining the glandular acini and ducts. When PSA is secreted into prostatic and seminal fluid, it hydrolyses seminal vesicle proteins important in semen liquefaction.[3]

Treatment of early stage (localised) prostate cancer includes radical prostatectomy, external beam radiotherapy, brachytherapy (interstitial radiotherapy) and surveillance (also known as expectant management, watchful waiting or observation). After radical prostatectomy, serum PSA drops rapidly and should be undetectable within 3 or 4 weeks. Within 5 years, 15–40% of resected patients exhibit a rise in PSA level above the limit of detection.[4] About one-third of newly diagnosed patients have advanced or metastatic prostate cancer and are treated with anti-testosterone therapy, consisting of either bilateral orchidectomy or hormonal therapy using estrogens or analogues of hypothalamic luteinising hormone-releasing hormone. Some tumour cells eventually become androgen-independent and progress rapidly. Patients with locally advanced or disseminated metastases usually succumb with hormone-refractory disease. Because there is presently no effective therapy available for such advanced tumours, it is crucial to develop novel therapeutic tools against prostate cancer.

The objective of this article is to review current knowledge and recent developments in the treatment of prostate cancer patients, including antibody-, DNA-, whole cell-, viral- and dendritic cell-based immunotherapy. In order to compare immunotherapeutic strategies with conventional strategies, such as chemotherapy and cytokine treatment, recent advances in these fields are also included in this review.

1. Chemotherapy

Although metastatic prostate cancer initially responds well to androgen deprivation therapy, most cancers eventually develop resistance. Patients with metastatic androgen-independent prostate cancer have a progressive and morbid disease with an estimated median survival of 12 months.[5] Chemotherapy can reduce serum PSA levels in patients with hormone-refractory prostate cancer and relieves pain in some patients, but tolerability is a concern.

1.1 Mitoxantrone

Mitoxantrone is an anthracenedione that has demonstrated activity against a variety of malignancies.[6,7] Kantoff et al.[8] treated 242 patients with hormone-refractory prostate cancer with either mitoxantrone and hydrocortisone or hydrocortisone alone. Treatment was well tolerated but no improvement in survival was observed with either approach, although mitoxantrone plus hydrocortisone reduced pain and improved quality of life in some men with advanced, hormone-refractory prostate cancer. Mitoxantrone and hydrocortisone also generated more frequent responses to treatment and a delay in both time to treatment failure and disease progression compared with hydrocortisone alone.

1.2 Taxanes

Docetaxel, which belongs to the taxane class of chemotherapy drugs, acts by forming stable microtubule bundles and phosphorylating the membrane protein Bcl-2 *in vitro*, leading to its inactivation and to eventual cell death by apoptosis.[9,10] Docetaxel is derived from the needles of the European yew tree (*Taxus baccata*). Administration of this medication has been shown in several phase II studies to reduce serum PSA levels in >50% of patients.[11,12] Furthermore, the combination of docetaxel and either estramustine or calcitriol has led to a reduction in PSA levels in >80% of patients.[13-15] Docetaxel plus estramustine chemotherapy for metastatic, hormone-refractory prostate cancer has been shown to significantly prolong life by approximately 2–3 months, compared with the previous standard therapy mitoxantrone plus prednisone or hydrocortisone treatment.[16] Tannock et al.[17] compared docetaxel plus prednisone and mitoxantrone plus prednisone treatment in patients with advanced prostate cancer. When prednisone plus docetaxel was given every 3 weeks, superior survival and improved rates of response in terms of pain, serum PSA level and quality of life were observed, compared with mitoxantrone plus prednisone treatment. However, Berry et al.[18] found no difference between docetaxel plus estramustine and mitoxantrone plus prednisone treatment in pain palliation in patients with advanced stage prostate cancer.

Cabrespine et al.[19] compared treatment with the taxane paclitaxel and carboplatin versus mitoxantrone in patients with hormone-refractory prostate cancer. Paclitaxel treatment leads to microtubular stabilisation resulting in mitotic arrest at the G2/M transition of the mitotic cell cycle.[20] Paclitaxel also facilitates apoptosis by inducing tubulin polymerisation and Bcl-2 phosphorylation.[10,21] The PSA response to paclitaxel and carboplatin in the study by Cabrespine et al.[19] was significantly greater than the response to mitoxantrone. The median survival was 14.5 months for the paclitaxel and carboplatin arm compared with 11.1 months for the mitoxantrone arm, but the paclitaxel and carboplatin arm had significantly greater rates of sensitive neuropathy.

Oh et al.[22] evaluated mitoxantrone treatment followed by taxane-based treatment or vice versa in 68 patients with hormone-refractory prostate cancer. The response rate to taxane-based chemotherapy was greater than the response rate to mitoxantrone treatment; however, total progression-free survival was similar, irrespective of whether taxane-based chemotherapy was administered first or second.

1.3 Epothilones

Epothilones A and B are new classes of cytotoxic agents that, similarly to paclitaxel, cause microtubular stabilisation and mitotic arrest. Treatment of chemotherapy-naive patients with castrate-metastatic prostate cancer with epothilone B analogue (BMS-247550 or ixabepilone) and estramustine phosphate resulted in a >50% decline in PSA in 11 of 12 evaluable patients in one study[23] and in 31 of 45 patients in a similar study.[24] Hussain et al.[25] also evaluated the effect of epothilone B analogue

alone in chemotherapy-naive metastatic hormone-refractory prostate cancer patients. Major toxicities were neutropenia and neuropathy. Fourteen of 48 patients had confirmed PSA responses after treatment. Seventy-two percent of PSA responders had declines >80%, and two patients achieved an undetectable PSA value. To evaluate the clinical cross-resistance of epothilone B analogue and taxanes in hormone-refractory prostate carcinoma, 49 patients who received epothilone B analogue with or without estramustine subsequently received second-line taxane therapy.[26] Second-line taxane chemotherapy after epothilone B analogue resulted in a substantial frequency of PSA declines, although patients with epothilone B analogue-refractory disease were less likely to respond to second-line taxane chemotherapy.

1.4 Platinum Compounds

Platinum compounds (cisplatin, carboplatin) used in chemotherapy have shown activity against several human tumours. Satraplatin (JM-216, BMS-182751) is a novel platinum (IV) complex which has also demonstrated *in vitro* cytotoxicity. In a phase III trial, 50 patients with hormone-refractory prostate carcinoma were randomised to treatment with satraplatin for 5 days plus prednisone or to prednisone alone.[27] Toxicity was minimal in both arms and the median overall survival was 14.9 months in the satraplatin plus prednisone arm compared with 11.9 months when prednisone was administered alone. A >50% decrease in PSA was seen in 9 of 27 patients in the satraplatin plus prednisone arm versus 2 of 23 in the prednisone only arm, supporting anti-tumour activity in the combination arm. Although satraplatin has moderate activity in hormone-refractory prostate carcinoma when given on a daily basis for 5 days, it is associated with significant treatment-related toxicities such as thrombocytopenia, neutropenia, anaemia, nausea, vomiting and diarrhoea.[28]

1.5 Triamcinolone

Most patients with recurrent prostate cancer disease initially respond to androgen deprivation therapy with tumour regression. However, this treatment eventually fails and the tumour becomes androgen-independent. The androgen receptor is implicated in disease progression. Several mutations have been identified in the ligand binding site of the androgen receptor in metastatic prostate cancer. It has been demonstrated that the common mutation T877A allows binding of nonandrogenic corticosteroid hormones (such as deoxycorticosterone, corticosterone, cortisol and cortisone) to the androgen receptor, thereby stimulating tumour cells.[29,30]

Srinivas et al.[31] administered oral triamcinolone twice daily to 24 patients with androgen-independent prostate cancer. The corticosteroid triamcinolone does not bind to androgen receptors harbouring the T877A mutation, but clearly exerts potent corticosteroid effects through the corticosteroid receptor. Thus, it was hypothesised that triamcinolone might suppress endogenous corticosteroids and inhibit stimulation of androgen-independent tumour growth. In this study, 29% of patients had a <50% decrease in serum PSA level and another 21% achieved stable disease. The median time to progression was 7.5 months. The frequency of PSA response correlated with cortisol suppression, with 8 of 12 non-responders not having cortisol suppression.

1.6 Summary

Taken together, determined efforts to generate and test new chemotherapeutics for the treatment of prostate cancer have been rewarded with small but significant successes but which do not lengthen life by more than a mean of 2–3 months.

2. Cytokines

Cytokines are secreted low-molecular weight proteins that regulate the intensity and duration of the immune response by exerting a variety of effects on lymphocytes and other immune cells. These proteins can be administered alone or in combination with vaccines. Thereby, cytokines can be given systemically or injected directly into the tumour. Several clinical trials have evaluated interleukin (IL)-2 or granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant for poxvirus, adenovirus or gene-therapy treatments.

2.1 Interleukin-2

IL-2 is a 15 kDa glycoprotein produced by activated lymphocytes that plays a major role in immune regulation and is the most effective anti-tumour cytokine used in clinical trials.[32] Systemic administration of IL-2 can be associated with severe, potentially lethal adverse effects while the cytokine concentration around the tumour remains low. In order to increase local IL-2 concentration, Belldegrun et al.[33] conducted a phase I clinical trial involving 24 patients with locally advanced prostate cancer. A functional DNA-lipid complex encoding the IL-2 gene was administered intraprostatically into the hypoechogenic tumour lesion under transrectal ultrasound guidance. IL-2 therapy was well tolerated and evidence of systemic immune activation was observed. Transient decreases in serum PSA were seen in 16 of 24 patients on day 1, and the decrease persisted in 14 patients to day 8. A different approach to increasing the cytokine concentration directly around the tumour was chosen by Ko et al.,[34] who used the EMD 273066 huKS-IL2 fusion protein. This protein is composed of two molecules of IL-2 genetically fused to a humanised monoclonal antibody directed against human adenocarcinoma-associated antigen (KSA). KSA (also known as epithelial cell adhesion molecule [EpCAM]) is highly expressed on many epithelial cancers, including prostate cancer. In their phase I dose escalation study in patients with advanced prostate cancer, Ko et al.[34] demonstrated that this fusion protein is well tolerated at doses above a level demonstrating systemic biological activity. Further clinical studies of EMD 273066 administered alone and in combination with chemotherapeutic agents demonstrating its benefit against prostate cancer are planned.

2.2 Granulocyte-Macrophage Colony-Stimulating Factor

GM-CSF regulates the proliferation and differentiation of myeloid precursor cells and is capable of inducing tumour necrosis factor (TNF) and IL-1 expression (which leads to indirect T-cell activation) as well as activation of macrophage and dendritic cell anti-tumour activity. In an efficacy evaluation, patients with hormone-refractory prostate cancer were treated with subcutaneously administered GM-CSF.[35] All but 1 of 12 patients experienced a decline in PSA (median decline 32%), but a PSA decline >50% was seen in only one patient. In a similar study, Rini et al.[36] demonstrated that GM-CSF has a biological effect in patients with serological progression of prostate cancer, as measured by declines in PSA and modulation of PSA kinetics. In a follow-up phase II clinical trial, 7 of 29 evaluable patients remained free of disease progression at a median of 5.1 years after the start of GM-CSF treatment.[37] An increase in the number of circulating monocytes and dendritic cells was observed after 14 days of GM-CSF treatment. However, Schwaab et al.[38] demonstrated that GM-CSF therapy is safe but unable to induce PSA-specific T-cell immunity, and concluded that little therapeutic benefit can be attributed to this single agent.

In a phase II trial, patients with androgen-independent metastatic prostate cancer were treated with GM-CSF in combination with thalidomide.[39] *In vitro* data have suggested that thalidomide has an-

tiangiogenic activity,[40] and two clinical trials have investigated the effect of thalidomide in prostate cancer patients.[41,42] In these trials, a >50% decline in PSA level was seen in <20% of patients. In the phase II trial of GM-CSF in combination with thalidomide,[39] the treatment was well tolerated and 5 of 22 patients with androgen-independent metastatic prostate cancer had a >50% decline in PSA level.

2.3 Fetal Liver Tyrosine Kinase 3 Ligand

Fetal liver tyrosine kinase 3 (Flt3) ligand is a growth and differentiation factor for dendritic cells that produces high concentrations of circulating dendritic cells in Flt3 ligand-treated patients. In a phase II clinical study, 32 patients with hormone-refractory prostate cancer were randomly assigned to receive Flt3 ligand or placebo.[43] Dendritic cells increased markedly in Flt3 ligand-treated patients and a significant slowing in velocity of PSA was observed while patients were on-study, suggesting a potential clinical application in the immunotherapy of prostate cancer.

3. Antibody-Based Immunotherapy

Tumour-specific antibodies may contribute directly to tumour destruction by antibody-mediated complement-dependent cellular cytotoxicity. Alternatively, antibodies to tumour-specific antigens may be coupled to cytotoxic agents or radioisotopes. Several factors contribute to inefficient antibody treatment of tumour patients:[44] (i) low affinity and cross-reactivity of the antibody; (ii) expression of the target not only in tumours but also in normal tissue; (iii) inefficient penetration of the antibody into solid tumours; and (iv) technical problems with the production of radioisotope and toxin conjugates.

The fact that the prostate is a non-essential organ makes prostate cancer an excellent target for antibody-based therapy. Furthermore, tissue-specific antigens can be targeted without identification of cancer-specific antigens. Several promising targets for antibody treatment of prostate cancer have been identified:

- human epidermal growth factor receptor-2 (HER-2/neu);
- prostate-specific membrane antigen;
- prostate stem cell antigen;
- vascular endothelial growth factor (VEGF);
- polymorphic epithelial mucin;
- mindin/RG-1;
- six-transmembrane epithelial antigen of prostate.

3.1 Human Epidermal Growth Factor Receptor-2 (HER-2/neu)

The HER-2/neu oncoprotein is a transmembrane tyrosine kinase receptor of the epidermal growth factor receptor family. Its oncogenic character is probably based on its action on cellular cascades involved in the proliferation and differentiation of epithelial cells. Different studies have reported that HER-2 gene amplification and protein overexpression are observed in <60% of patients with hormone-refractory prostate carcinoma. However, a phase II clinical trial using an antibody recognising HER-2/neu receptor (trastuzumab) demonstrated poor efficacy in treating hormone-refractory prostate carcinoma.[45] Lara et al.[46] conducted a phase II trial using trastuzumab plus docetaxel in HER-2-positive patients with prostate cancer. One hundred patients with hormone-refractory prostate carcinoma were screened for HER-2 receptor. The trial was closed because of non-feasibility due to a low HER-2-positivity rate (<20%). The authors estimated that 1000 patients needed to be screened to complete accrual for a 40-patient efficacy trial. No patient responded to trastuzumab alone. Schwaab et al.[47] used a bispecific antibody (MDXH210) to target Fcγ class I receptor (FcγRI) and HER-2/neu in patients whose prostate cancer overexpressed HER-2/neu. The bispecific antibody targets phagocytic cells expressing FcγRI (monocytes, macrophages, dendritic

cells, interferon-γ-activated neutrophils) to tumours overexpressing HER-2/neu. Patients received an intravenous infusion of the bispecific antibody three times per week for 2 weeks. The antibody was well tolerated and circulating plasma HER-2/neu levels decreased by 80% at days 12 and 29. Five of six patients had stable PSA levels over the ≥40 day course.

3.2 Prostate-Specific Membrane Antigen

J591 (MLN591) is a monoclonal IgG1 antibody recognising the external domain of the type II transmembrane glycoprotein (100 kDa) prostate-specific membrane antigen, which is expressed primarily in prostate epithelium. Prostate-specific membrane antigen expression increases across the range of benign prostate epithelium to prostate cancer, with the highest intensity occurring in highest-grade cancers, but is minimally expressed in non-prostate tissues.[48] The antibody to prostate-specific membrane antigen can be labelled with a variety of radioisotopes, such as yttrium-90 (^{90}Y), indium-111 (^{111}In), and lutetium-177 (^{177}Lu), which form stable complexes that are rapidly internalised after binding to prostate-specific membrane antigen at the cell surface. In order to remove T-helper (T_h) epitopes, J591 was deimmunised by humanisation of murine antibody variable domains.

Different studies have demonstrated the safety of radioconjugated J591.[49,50] Bander et al.[49] demonstrated that 4 of 35 patients had a decrease in PSA following treatment with ^{177}Lu-J591 and 16 of 35 had stabilisation of PSA. In a clinical trial, Morris et al.[51] treated 14 patients with progressive metastatic prostate cancer with ^{111}In-labelled J591. J591 was well tolerated in repetitive dose-escalating administrations, but only one patient showed a >50% reduction in PSA level. The investigators proposed that future studies should target patients with a lesser burden of disease than those in this study, in order to maximise anti-tumour effects.

Recently, a recombinant prostate-specific membrane antigen-specific single chain immunotoxin (A5-PE40) has been described as being selectively toxic to prostate cancer cells.[52] The PE40 domain is a truncated version of *Pseudomonas* exotoxin A, which is not cytotoxic so long as it remains in the extracellular space. Once linked to a single chain antibody fragment directed against a cell surface antigen (in this case prostate-specific membrane antigen) capable of internalising the exotoxin, it becomes a potent immunotoxin. Because of its high and specific toxicity, this recombinant immunotoxin is a promising candidate for treatment of prostate cancer.

4. DNA-Based Immunotherapy

Various groups have demonstrated tumour protection using DNA immunisation in different models. Vaccination with DNA has several advantages: (i) gene sequences can be manipulated to deliver several epitopes stimulating specific cellular and humoral immune responses; (ii) DNA immunisation is safe in humans; (iii) DNA is very stable and can be produced in large scale; and (iv) DNA vaccines containing unmethylated sequential cytosine-guanine (CpG) motifs skew the immune system to a T_h1 cell immune response.

In a mouse model, *in vivo* electroporation has emerged as a potent method for DNA vaccine delivery[53] compared with other methods. Different animal models have demonstrated that DNA immunisation can elicit host immune responses to PSA. Kim et al.[54] immunised mice with a DNA vaccine encoding the human PSA gene. The vaccine induced a strong and persistent antibody response against PSA, a significant PSA-specific T_h cell proliferation and a cytotoxic T lymphocyte that recognised tumour cell targets expressing PSA. The safety and the immunogenicity of the DNA vaccine were confirmed in rhesus macaques.[55] Marshall et al.[56] demonstrated that mice immunised with DNA

encoding PSA protected the mice from subsequent tumour challenge. In a follow-up study, the same investigators demonstrated that co-administration of a plasmid encoding IL-18 enhances T_h1 immunity and tumour protection by a DNA vaccine.[57] Similar results were observed when DNA encoding PSA was co-administered with plasmids coding for GM-CSF and/or IL-2.[58] In order to evaluate the safety, feasibility and biological efficacy of a plasmid-encoding PSA, a phase I trial of this agent combined with GM-CSF and IL-2 was conducted in patients with hormone-refractory prostate cancer.[59] The vaccine was safe and a PSA-specific cellular immune response together with an increase in anti-PSA IgG were observed in 2 of 3 patients after vaccination with 900μg DNA in five cycles. In a follow-up study, patients were monitored for their ability to mount PSA-specific cellular response after receiving the plasmid-encoding PSA.[60] Interferon-γ enzyme-linked immunosorbent spot (ELISPOT) assays demonstrated PSA-specific T cells in some patients. Analysis of other cytokines showed IL-4 and IL-6 but not IL-10 producing cells after vaccination in any of the patients.

Plasmid DNA encoding prostatic acid phosphatase was used to immunise rats.[61] The vaccine was found to be effective in eliciting prostatic acid phosphatase-specific CD4 and CD8 T cells as well as prostatic acid phosphatase-specific IgG that were detected in a dose-dependent manner. These studies demonstrated that immunisation with DNA encoding prostatic acid phosphatase is safe and support further clinical evaluation in prostate cancer patients.[62]

In a phase I/II clinical trial, Mincheff et al.[63] vaccinated prostate cancer patients with DNA encoding the extracellular domain of human prostate-specific membrane antigen in combination with CD86-encoding plasmids and soluble GM-CSF. The vaccine was compared with an adenovirus encoding the same prostate-specific membrane antigen sequence. All patients who received initial inoculation with the viral vector followed by prostate-specific membrane antigen-plasmid boosts showed signs of immunisation, whereas only 50% of patients who received prostate-specific membrane antigen/CD86 plasmids demonstrated successful immunisation. All patients receiving prostate-specific membrane antigen/CD86 plasmids plus soluble GM-CSF became immunised. Several responders, as evidenced by a change in local disease, distant metastases and PSA levels, could be identified. Positive reactions were detected in 86% of vaccinated prostate cancer patients.[64]

5. Whole Cell-Based Vaccines

Vaccination with attenuated viral or bacterial vaccines often results in immunogenicity, memory cell production and subsequent protection against exposure to the live pathogen. The same immunisation concept could in theory be adapted to tumour immunology. Patient-derived tumour cells should be the best source of antigen, but in practice it is extremely difficult to establish and maintain human prostate cancer cells *in vitro*. In order to prevent growth of the applied tumour cells in the body, these cells must be irradiated prior to vaccination.

One approach that has been applied to whole-cell immunisation is to transfect tumour cells with the gene encoding GM-CSF. These genetically engineered tumour cells, when re-infused back into the patient, will secrete GM-CSF, enhancing differentiation and activation of host antigen-presenting cells. As dendritic cells accumulate around the tumour cells, the GM-CSF secreted by the tumour cells will enhance the presentation of tumour antigens to T_h and cytotoxic T cells by dendritic cells. In a phase I study, eight patients were treated with autologous, GM-CSF-secreting, irradiated tumour cells prepared from *ex vivo* retroviral transduction of surgically harvested cells.[65] Vaccine site biopsies manifested infiltrates of dendritic cells and macrophages and

seven of eight patients exhibited activation of new T- and B-cell responses against PSA antigens, as determined by delayed-type hypersensitivity reactions against non-transduced autologous tumour cells. Seven of eight vaccinated men had new antibodies recognising three polypeptides in protein extracts derived from prostate cells; these new antibodies were not recognised in extracts from prostate stromal cells.

A major difficulty for future clinical development of this autologous treatment approach is the low yield of autologous prostate cancer vaccine cells recovered using cell culture approaches to expand prostate cancer cell numbers. Therefore, this approach appears clinically impractical for the conduct of large phase II studies required to assess efficacy. To circumvent this problem, cell lines established in culture from various individuals differing in MHC tissue type may provide a source of whole-cell tumour vaccines. The efficacy of this allogeneic approach bas been demonstrated in animal models for prostate cancer.[66] Based on this finding, 60 patients with hormone-refractory prostate cancer were immunised with three cell lines in conjunction with the immunostimulant *Mycobacterium vaccae* (SRL-172).[67] The vaccine was safe and well tolerated, although no significant decline in PSA could be observed, which is not surprising considering the stage of disease in patients in this study. In contrast, the immunological response was encouraging, because several patients had an increase in cytokine production, increases in specific antibodies and evidence of T-cell proliferation in response to the vaccination.

Recently, 21 patients with PSA relapse following radical prostatectomy were immunised intradermally with GM-CSF-transduced, irradiated LNCaP or PC-3 cells every week for 8 weeks.[68] Use of cell lines circumvented the limitation of the small size of the resected autologous prostate tumours.[65] At 20 weeks after the first treatment, 16 patients showed a statistically significant decrease in PSA elevation compared with the pre-vaccination state. Patients developed new oligoclonal antibodies reactive against at least five identified antigens present in LNCaP or PC-3 cells. The dose and schedule employed in this trial were at the low end of a potential dose-response relationship. Higher cell doses and a more prolonged schedule of boost injections are warranted.

Michael et al.[69] vaccinated 26 patients with asymptomatic hormone-resistant prostate cancer who showed increasing PSA levels but little or no evaluable disease. Patients were intradermally vaccinated with three irradiated allogeneic prostate cell lines (OnyCap23, LNCaP, P4E6). The vaccine was administered monthly and the first two doses were supplemented with bacillus Calmette-Guérin as adjuvant. The vaccine was well tolerated and 11 patients showed statistically significant, prolonged decreases in their PSA velocity. Median time to disease progression was 58 weeks, compared with historical control values of around 28 weeks.

6. Recombinant Viral Vaccines

Use of viral vaccines offers the advantage of a natural way of inducing immune responses and achieving high levels of transgene expression. Proteins expressed by recombinant viruses are more immunogenic than proteins in adjuvant.[70] However, the safety aspects of this approach have to be considered carefully. Two different recombinant viral expression vectors have been used in prostate cancer treatment: poxvirus and adenovirus vectors.

6.1 Poxvirus Vectors

Vaccinia and fowlpox are double-stranded DNA viruses belonging to the family of poxviruses and have been extensively studied in different models. One advantage of the poxviral vectors is the large size of the genomes, which allows the expression of up to seven different genes in one vector.[71] In an

early study, Hodge et al.[72] used a recombinant vaccinia virus expressing human PSA to immunise rhesus monkeys. Human and rhesus PSA share 94% homology between amino acid sequences. Immunised monkeys showed a short-lived PSA-specific IgM antibody response and PSA-specific T-cell responses that were maintained for up to 270 days. Several phase I clinical trials of vaccinia-PSA demonstrated that the vaccine is well tolerated in men.[73,74] A number of approaches have been developed to further enhance the immune response to vaccinia-PSA. For example, Eder et al.[75] administered recombinant vaccinia virus (rV)-PSA to 33 men with rising PSA levels. PSA levels in 14 of 33 patients remained stable for at least 6 months after primary immunisation. Additionally, ten patients were treated with the cytokine GM-CSF, which has been reported to enhance T-cell responses.[76,77] PSA-3 peptide-specific T-cell response was augmented at least 2-fold in five of seven patients possessing the correct MHC restriction element (HLA-A2). In four of these five patients with an increased PSA-specific immune response, stabilisation of serum PSA levels for at least 6–11 months was observed.

Repeated administration of vaccinia-based vaccines results in the rapid appearance of strong neutralising antibodies against the vaccinia virus itself, preventing the ability of the recombinant protein to induce T-cell responses after boosting.[75,78,79] To overcome this hurdle, new strategies have been developed. Use of recombinant avian pox viruses (avipox) such as canarypox (ALVAC) or fowlpox are potential candidates for immunisation protocols in that they can infect mammalian cells and express the inserted transgene, but do not replicate in mammalian cells. Lack of replication in host cells results in weak neutralising antibody responses, permitting subsequent boosting after initial exposure to these viruses. Avipoxviruses offer the advantage of non-pathogenicity and expression of antigens for longer periods than vaccinia viruses. Kaufmann et al.[80] evaluated the feasibility and tolerability of a prime/boost vaccine strategy using recombinant vaccinia virus (rV-PSA) and fowlpox virus (rF-PSA) expressing human PSA. Sixty-four eligible patients were randomly assigned to receive four vaccinations with rF-PSA, three rF-PSA vaccines followed by one rV-PSA vaccine, or one rV-PSA vaccine followed by three rF-PSA vaccines. The therapies were well tolerated and a significant portion of men remained free of PSA progression after 19 months. Furthermore, 46% of the patients treated in this trial exhibited an increase in PSA-specific T-cell responses. There was a trend toward greater efficacy in the treatment group that received a priming dose of rV-PSA.

Another approach taken to further enhance the efficacy of poxvirus vaccines was administration of recombinant viruses expressing costimulatory molecules in combination with poxviruses expressing PSA. Efficient costimulation of T cells is essential for activation, especially when weak antigens are involved. T-cell receptor engagement is required for T-cell activation and ensures antigen specificity and MHC restriction of the response. Nevertheless, additional signals delivered by costimulatory molecules sustain and integrate T-cell receptor signalling, resulting in optimal cell proliferation and differentiation. Delivery of the first signal (T-cell receptor engagement) in the absence of a second signal(s) (costimulation) leads to apoptosis or anergy. Anergic T cells do not produce IL-2 or proliferate upon restimulation. Numerous costimulatory molecules have been identified as playing roles in the initiation of immune responses by T and B lymphocytes. Signals provided through CD28-B7.1/2 (costimulatory molecules B7.1 or B7.2) interactions are essential for initial naive T-cell activation and lead to increased IL-2 production and IL-2Rα (CD25) expression. The receptors intercellular adhesion molecule-1 (ICAM-1) and leukocyte func-

tion-associated antigen-3 (LFA-3) on antigen-presenting cells are additional costimulatory molecules. DiPaola et al.[81] conducted a phase I study to evaluate the safety and immunogenicity of vaccinia and fowlpox vaccine incorporating the PSA gene and a TRIad of COstimulatory Molecules (TRICOM™).[1] The vaccine designated TRICOM™ contains a triad of T-cell costimulatory molecules (B7.1, ICAM-1 and LFA-3) in poxviral vectors. Ten patients with androgen-independent prostate cancer were treated with a recombinant vaccinia virus expressing PSA (PROSTVAC®-V) followed by a booster with recombinant fowlpox virus with gene sequences for PSA (PROSTVAC®-F) and TRICOM™. The vaccine was well tolerated and generated an immune response to vaccinia, although no anti-PSA antibodies were induced. During the 8-week study period, four patients had stable disease with <25% increase in PSA. The PSA-specific cytotoxic T-lymphocyte response was not addressed in this study.

In a randomised, phase II clinical trial, Gulley et al.[82] combined the rV-PSA vaccine with radiotherapy in patients with clinically localised prostate cancer. Patients received a priming vaccine with rV-PSA plus rV-B7.1 (vaccinia virus expressing the costimulatory molecule B7.1) followed by monthly booster vaccines with rF-PSA. The vaccines were administered with local GM-CSF and low-dose systemic IL-2. Standard external beam radiation therapy was given between the fourth and sixth vaccinations. The vaccinations were well tolerated and 13 of 17 patients receiving all eight vaccinations had an increase in PSA-specific T cells of at least 3-fold compared with the radiotherapy-only arm. Whether the enhanced PSA-specific immune response can translate into an improved clinical outcome was not addressed in this study.

In another randomised, phase II clinical trial, Arlen et al.[83] evaluated the concurrent administration of docetaxel and poxvirus-PSA in men with metastatic androgen-independent prostate cancer. Patients were immunised with rV-PSA admixed with rV-B7.1 and received sequential booster vaccinations with rF-PSA. Patients also received GM-CSF with each vaccination. In addition, 14 patients received weekly doses of docetaxel. No deleterious effect on the ability to mount immune responses was observed with use of monthly vaccines in combination with weekly docetaxel. PSA-specific T-cell precursor levels increased 3.33-fold irrespective of whether patients received vaccine and docetaxel or vaccine alone. Furthermore, it seemed that vaccine either in combination with or before docetaxel therapy may have had a positive effect on patients' PSA levels when compared with a historical control.

6.2 Adenoviral Vectors

Adenovirus (ADV; Ad)-based gene therapy possesses great potential for prostate cancer control. The ADV genome is composed of a linear double-stranded DNA molecule which does not integrate into the host cell genome. ADV gene transcription can be divided into an early and a late phase which occur, respectively, before and after virus DNA replication. Two main approaches for ADV-based gene therapy have been developed: (i) the viral deletion approach (replication-defective ADV); and (ii) use of tissue and/or tumour-specific promoters (replication-competent ADV) to drive critical early viral gene expression.

Replication-defective ADVs have the E1 and E3 genes removed, allowing for introduction of up to 7kb of foreign DNA. In 'suicide gene therapy', a genetically modified ADV encoding the herpes simplex virus thymidine kinase (HSV-TK) gene delivers this gene into host cells. Administration of ganciclovir, aciclovir or valaciclovir, which are poor substrates for mammalian thymidine kinase, leads to

1 The use of trade names is for product identification purposes only and does not imply endorsement.

phosphorylation of these drugs by HSV-TK. The phosphorylated drugs are nucleotide analogues, which are highly toxic for mammalian cells because they are incorporated into DNA during cell division and cause termination of DNA replication and cell death.

Replication-competent ADVs have the ability to replicate within cells, lyse those cells and infect neighbouring cells. In order to make replication-competent ADVs tumour- or tissue-specific, the E1A gene (which controls the viral gene expression cascade) can be placed under transcriptional control of tumour- or tissue-specific promoters. Several ADV systems have been investigated in localised prostate cancer clinical trials. For example, CV706 (also named CN706 or CG7060) is a replication-competent, E3-deleted, cytolytic Ad5-based virus that utilises PSA promoter-regulated replication and which has been shown to selectively kill human prostate cancer xenografts in preclinical models.[84] This restricted replication was achieved by insertion of a minimal promoter-enhancer construct of the human PSA gene 5´ of E1A, 3´ of the E1A promoter, resulting in PSA-regulated expression of E1A. CN706 destroys human PSA^+ cells 400 times more efficiently than PSA^- cells.[84] In a phase I study, deWeese et al.[85] treated patients with different doses of CV706 delivered intraprostatically. The vaccine was well tolerated and five of five patients treated with the highest two doses of CV706 achieved a >50% reduction in PSA value. These results suggest that CV706 treatment has potential for disease stabilisation.

CV787 (also named CG7870) expresses E1A under control of the rat probasin promoter and E1B under control of the PSA promoter-enhancer. In contrast to CV706, this virus expresses E3 (which encodes several proteins that have the capacity to modulate the immune response of the host to adenovirus-infected cells) in order to decrease the immune response to the adenoviral vector itself.[86] CV787 destroys PSA^+ prostate cancer cells 10 000 times more efficiently than PSA^- cells.[87] *In vitro* and *in vivo* (animal model) experiments have demonstrated that CV787-mediated replication-dependent cytotoxicity is synergistic with the chemotherapeutic agents paclitaxel and docetaxel.[88] In a dose escalation phase I study, CV787 was administered as a single intravenous infusion to 23 patients with hormone-refractory metastatic prostate cancer.[89] Patients receiving the highest dose had detectable CV787 genome copies in the peripheral blood throughout the 29 days of the study. All patients developed antibodies to CV787 and dose-related increases in IL-6 and IL-10 were detected in the blood. No patient had a ≥50% decline in PSA value, but five patients had a decrease in serum PSA of 25–49% following a single treatment.

Numerous *in vitro* and animal studies have shown that HSV-TK gene delivery is effective against prostate cancer cell lines and prostate tumours in animal models.[90-93] Herman et al.[94] conducted a phase I dose escalation clinical trial of a replication-deficient ADV containing the HSV-TK gene injected directly into the prostate, followed by intravenous administration of the prodrug ganciclovir. All cultures of blood and urine specimens were negative for growth of ADV, and minimal toxicity (grade 1–2) was encountered in 4 of 18 patients. Three patients, one each at the three highest dose levels, achieved an objective response, defined as a decrease in serum PSA levels by ≥50% sustained for 6 weeks to 1 year. This study was the first to demonstrate the safety of ADV/HSV-TK plus ganciclovir gene therapy in human prostate cancer. Several clinical trials of HSV-TK have subsequently demonstrated that the therapy is well tolerated in men.[95-98]

Satoh et al.[99] evaluated the systemic T-cell response after intraprostatic injection of the adenoviral vector HSV-TK followed by systemic administration of ganciclovir or valaciclovir in combi-

nation with radiotherapy. There was an increase in activated CD8+ T cells in the peripheral blood after vector injection. Addition of radiotherapy to *in situ* gene therapy seemed to further increase total CD8+ T cells and activated CD4+ T cells. Fujita et al.[100] further combined adenoviral *in situ* gene therapy with radiotherapy and hormonal therapy. Sustained long-term systemic T-cell responses were noted after combined radio-gene-hormonal therapy. Ayala et al.[101] focused on the tissue effects observed in cancer foci and surrounding noncancerous prostate as well as evidence for local and systemic immune response in patients receiving intraprostatic viral injections of ADV/HSV-TK followed by 2 weeks of ganciclovir and prostatectomy 2–4 weeks later. Local (CD8+ cells and macrophages) and systemic immune responses (CD8+, activated CD8+ and IL-12) were increased in patients treated with HSV-TK. Increased apoptosis and decreased microvessel density were also noted in these patients. Hence, these results suggest a tumour-specific effect mediated by systemic and local immune responses, an antiangiogenic effect and modulation of apoptosis.

Freytag et al.[102] developed a novel approach that utilises a lytic, replication-competent ADV (Ad5-cytosine deaminase (CD)/TK*rep*) to deliver a CD/HSV-1-TK fusion gene to tumours. The Ad5-CD/TK*rep* virus itself generates a potent anti-tumour effect by replicating in and destroying cancer cells. The therapeutic effect of the Ad5-CD/TK*rep* virus can be significantly enhanced by invoking two suicide gene systems (CD/flucytosine [5-fluorocytosine] and HSV-1-TK/ganciclovir), which render malignant cells sensitive to specific pharmacological agents and sensitise them to radiation. CD converts the prodrug flucytosine to fluorouracil (5-fluorouracil), which on further conversion results in inhibition of thymidylate synthase and depletion of thymidine 5'monophosphate pools. This leads to increased DNA strand breaks and cell cycle redistribution, sensitising cells to the lethal effects of radiation. In the study by Freytag et al.,[102] an escalating dose of the Ad5-CD/TK*rep* virus was injected intraprostatically into 16 patients with locally recurrent prostate cancer. Two days later, patients were given flucytosine and ganciclovir prodrug therapy for 1 or 2 weeks. Seven of 16 (44%) patients exhibited a ≥25% decrease in serum PSA and 3 of 16 (19%) patients exhibited a ≥50% decrease in serum PSA. In a further study, these investigators combined this approach with radiation therapy.[103] As expected for patients receiving definitive radiation therapy, all patients experienced significant declines in PSA. The mean PSA half-life in patients administered >1 week of prodrug therapy was significantly shorter than in patients receiving prodrugs for only 1 week (0.6 vs 2.0 months, respectively; $p < 0.02$) and markedly shorter than that reported previously for patients treated with conventional-dose 3-dimensional conformal radiation therapy alone (2.4 months).

Systemic delivery of recombinant IL-2 induces clinical responses in various malignancies. By providing exogenous cytokines, it may be possible to overcome or prevent anergy of the immune effector cells. In a phase I trial, adenovirus expressing IL-2 (AdCAIL-2) was directly injected into the prostate 4 weeks prior to prostatectomy.[104] Histopathology demonstrated an inflammatory response consisting predominantly of CD3+CD8+ T lymphocytes with areas of tumour necrosis. PSA levels declined in five of five evaluable patients treated at the lowest dose. At higher doses, PSA values initially increased after injection, and then decreased to baseline prior to surgery.

7. Dendritic Cell-Based Immunotherapy

Dendritic cells are the most potent antigen-presenting cells. Interaction of dendritic cells with microbial or viral products (double-stranded RNA, CpG, lipopolysaccharide), proinflammatory cytokines (TNFα, IL-1β) or after ligation of surface

CD40 leads to activation of dendritic cells, resulting in upregulation of costimulatory and MHC molecules as well as changes in homing receptor expression. After capturing antigen in the tissues by phagocytosis or by endocytosis, dendritic cells migrate to the draining lymph node where they present the antigen to T lymphocytes. Dendritic cells have the unique ability to prime naive T cells and elicit potent antigen-specific responses. However, vaccination of patients with autologous dendritic cells carries the disadvantage of the tremendous technical effort required to isolate dendritic cells or precursor from peripheral blood and mature them *in vitro*. Nevertheless, dendritic cell vaccine therapies have been used against >20 different types of tumours.[105] Thereby, peptides, recombinant proteins, tumour lysates, messenger RNA (mRNA) and DNA have been used to deliver antigens to dendritic cells. Numerous clinical trials have demonstrated that vaccination of prostate cancer patients with dendritic cells is safe and can elicit a prostate cancer-specific immune response (table I).

7.1 Dendritic Cells Loaded with Peptides

Murphy et al.[106] demonstrated the safe administration of autologous dendritic cells in combination with HLA-A0201-restricted peptides (named PSM-P1 and PSM-P2) derived from prostate-specific membrane antigen. Dendritic cells used in this study were generated from adherent peripheral blood mononuclear cells in the presence of GM-CSF and IL-4 for 4–6 days *in vitro*. Patients were vaccinated 4–5 times with autologous dendritic cells. Detection of cellular response and decrease in PSA level in some patients who received dendritic cells pulsed with PSM-P2 indicate the potential of this method in prostate cancer therapy. A long-term observation of these patients showed a response persisting for >200 days in some patients.[109] Several follow-up studies have demonstrated safety and a response in patients vaccinated with autologous dendritic cells loaded with PSM-P1 or PSM-P2 peptides.[107,108,110-112] GM-CSF applied as adjuvant did not significantly enhance the measured immune response after administration of autologous dendritic cells loaded with PSM-P1 or PSM-P2 peptides.[113] In all of their studies, Murphy and colleagues[106-113] used *in vitro* cultured dendritic cells without a maturation step. Therefore, these dendritic cells should have an immature phenotype, and it has been reported that immature dendritic cells tolerise (i.e. induce tolerance in)[126,127] rather than activate naive T cells. Nevertheless, it might be possible that dendritic cells mature further *in vivo* after vaccination.

Perambakam et al.[114] immunised patients with autologous dendritic cells pulsed with PSA146-154 peptide (binding to HLA-A2) [intravenous administration] or PSA146-154 peptide plus GM-CSF (intradermal injection). In this study, 28 HLA-A2$^+$ patients with locally advanced or metastatic prostate cancer were randomly assigned to one of these two methods. Strong delayed-type hypersensitivity skin reactions to the PSA peptide became detectable in 50% (14 of 28) of patients over time. In this study, half of the patients were vaccinated with immature monocyte-derived dendritic cells, which were cultured with GM-CSF and IL-4 for 7 days followed by pulsing with PSA peptide and the influenza matrix protein Flu-M1 peptide overnight. This procedure induced PSA-peptide-specific delayed-type hypersensitivity responses in 5 of 14 patients, and specific interferon-γ responses were observed in two of the positive patients tested. The results of this study indicate that vaccination with soluble peptide or dendritic cell-bound peptide elicits strong specific T-cell immunity to the PSA peptide in one-half of patients with locally advanced or hormone-sensitive metastatic prostate cancer.

In an attempt to induce a broader cytotoxic T-cell response against prostate cancer cells, two independent groups loaded autologous dendritic cells with different peptides derived from different pro-

teins.[115,116] Although tumour cells may evade immune recognition by altering antigen processing, multiple-epitope vaccination may overcome this potential limitation by maintaining immunological pressure against different tumour-associated antigens. During immunotherapy, antigen loss may even be promoted, as has previously been shown with the emergence of melanoma antigen recognised by T cells (MART)-1 negative melanoma metastasis following adoptive transfer of MART-1-specific T cells.[128] Fuessel et al.[115] immunised HLA-A*0201+ patients with dendritic cells loaded with a cocktail consisting of five different peptides derived from PSA, prostate-specific membrane antigen, survivin, prostein and transient receptor potential p8 (trp-p8).[115] Four vaccinations every 2 weeks were administered to eight patients. No adverse effects other than local skin reactions were noted. One patient displayed a partial response and three other patients showed stable PSA values or decelerated PSA increases. ELISPOT analyses demonstrated that three of four PSA responders also showed antigen-specific CD8+ T-cell activation against prostein, survivin and prostate-specific membrane antigen. Waeckerle-Men et al.[116] vaccinated six HLA-A*0201+ patients with advanced hormone-refractory prostate cancer with autologous dendritic cells pulsed with four different peptides derived from prostate stem cell antigen ($PSCA_{14-22}$), prostatic acid phosphatase ($PAP_{299-307}$), prostate-specific membrane antigen ($PSMA_{4-12}$) and PSA ($PSA_{154-163}$). Dendritic cells were intradermally applied six times at 2-weekly intervals. Three patients with enhanced immune responses were further vaccinated with monthly booster injections. Vaccination elicited significant cytotoxic T-cell responses against all PSAs tested and all long-term treated patients demonstrated an increase in PSA doubling time, which correlated with the onset of interferon-γ production by cytotoxic T lymphocytes from the peripheral blood after the sixth vaccination. These data support the notion that only after repeated dendritic cell-based vaccinations over several months can an effect on progression of PSA values be expected. The investigators reported that even a sufficiently stimulated immune system will not 'win the uphill struggle' against an established rapidly growing tumour and therefore proposed that treating patients with earlier stage disease may not only result in successful therapy but also in clear clinical benefits for patient. Both studies with dendritic cell-based multi-epitope immunotherapy used dendritic cells generated from monocytes isolated from peripheral blood mononuclear cells of prostate cancer. Immature dendritic cells were matured in the presence of IL-1β, TNFα, IL-6 and prostaglandin E_2 (PGE_2). It has been reported that maturation-induced upregulation of chemokine receptor 7 (CCR7) surface expression is not sufficient for monocyte-derived dendritic cells to migrate toward their ligands CCL19 and CCL21.[129-131] Monocyte-derived dendritic cell migration toward CCL19 and CCL21 was readily observed upon maturation in the presence of the proinflammatory mediator PGE_2, although PGE_2 did not change the expression level of CCR7 on mature dendritic cells. Thus, PGE_2 is an important element in the preparation of monocyte-derived dendritic cells as cellular vaccines in tumour immunotherapy.

7.2 Dendritic Cells Loaded with Proteins

Preclinical studies in rats demonstrated that dendritic cells loaded with prostatic acid phosphatase linked to GM-CSF elicited strong cellular immune responses. Sipuleucel-T (APC8015) is an immunotherapy product consisting of autologous antigen-presenting cells (CD54+ dendritic cells, but also CD3+, CD14+, CD19+ and CD56+ cells) loaded *ex vivo* with a recombinant fusion protein consisting of prostatic acid phosphatase linked to GM-CSF.[117] When Small et al.[117] treated patients with hormone-refractory prostate cancer with sipuleucel-T, den-

Table I. Comparison of selected clinical trials for prostate cancer-specific dendritic cell (DC) vaccines

Study	Method to deliver antigenic material	Antigenic source	DC preparation	Results
Murphy et al.,[106-108] Tjoa et al.,[109-111] Salgaller et al.,[112] Simmons et al.[113]	Peptide-loaded DCs	HLA-A0201-restricted peptides derived from PSA (named PSM-P1 or PSM-P2)	DCs generated from adherent PBMCs in the presence of GM-CSF and IL-4 for 4–6 days *in vitro*	Immunisation of prostate cancer patients with DCs was safe Detection of cellular response and decrease in PSA level observed in some patients GM-CSF applied as adjuvant did not significantly enhance immune response
Perambakam et al.[114]	Peptide-loaded DCs	HLA-A2 restricted peptides derived from PSA	Monocyte-derived DCs cultured with GM-CSF and IL-4 for 7 days *in vitro*	PSA peptide-specific response in 5 of 14 patients
Fuessel et al.[115]	Peptide-loaded DCs	Five different peptides derived from PSA, PSMA, survivin, prostein, and trp-p8	DC generated from monocytes isolated from PBMCs in the presence of GM-CSF and IL-4. Matured in the presence of IL-1β, TNFα, IL-6 and PGE_2	Three of four PSA responders showed antigen-specific $CD8^+$ T-cell activation
Waeckerle-Men et al.[116]	Peptide-loaded DC	Four different peptides derived from PSCA, PSA, PSMA and PAP	DC generated from monocytes isolated from PBMC in the presence of GM-CSF and IL-4. Matured in the presence of IL-1β, TNFα, IL-6, and PGE_2	Vaccination elicited significant CTL responses against all prostate cancer-specific antigens Long-term treated patients demonstrated an increase in PSA doubling time
Small et al.,[117,118] Burch et al.,[119] Rini et al.[120]	Protein-loaded DCs	PAP linked to GM-CSF	Autologous antigen-presenting cells ($CD54^+$ DCs, but also $CD3^+$, $CD14^+$, $CD19^+$ and $CD56^+$ cells)	38% of patients developed immune responses to PAP 4.5-month improvement in overall survival

Continued next page

Table I. Contd

Study	Method to deliver antigenic material	Antigenic source	DC preparation	Results
Barrou et al.[121]	Protein-loaded DCs	PSA	DCs differentiated from monocytes with GM-CSF and IL-13 for 7 days	Maximum PSA decreased from 6% to 39% PSA-specific T cells were detected in some patients
Fong et al.[122]	Protein-loaded DCs	PAP	DCs enriched from PBMCs (average DC purity 30%)	All patients developed an antigen-specific immune response regardless of the route of infection (intravenously, intradermally, intralymphatic)
Pandha et al.[123]	DCs loaded with lysates	Lysate from prostate cancer cell lines (DU-145, LN-CaP and JM-RCC)	DCs generated from PBMCs in the presence of GM-CSF and IL-4 for up to 7 days *in vitro*	Reduction in the level of PSA in one patient (out of 11) Reduction in PSA velocity in one patient Reduction in PSA doubling time in six patients
Su et al.[124]	mRNA transfected DCs	hTERT	DCs generated from monocytes isolated from PBMCs in the presence of GM-CSF and IL-4. Matured in the presence of IL-1β, TNFα, IL-6 and PGE_2	Vaccination was associated with a reduction in PSA velocity Vaccination was associated with molecular clearance of circulating micrometastases
Mu et al.[125]	mRNA	mRNA from prostate cancer cell lines (DU-145, LN-CaP and PC-3)	DCs generated from monocytes isolated from PBMCs in the presence of GM-CSF and IL-4. Matured in the presence of IL-1β, TNFα, IL-6 and PGE_2	Specific T-cell responses in 12 of 19 evaluated patients 13 patients developed a decrease in log-slope PSA

CTL = cytotoxic T lymphocyte; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **hTERT** = human telomerase reverse transcriptase; **IL** = interleukin; **mRNA** = messenger RNA; **PAP** = prostatic acid phosphatase; **PBMC** = peripheral blood mononuclear cell; **PGE_2** = prostaglandin E_2; **PSA** = prostate-specific antigen; **PSCA** = prostate stem cell antigen; **PSMA** = prostate-specific membrane antigen; **TNFα** = tumour necrosis factor-α; **trp-p8** = transient receptor potential p8.

dritic cell precursors matured during culture, as evidenced by upregulation of costimulatory molecules. Sipuleucel-T seems to be safe and well tolerated. There was no evidence of development of an autoimmune disease caused by cross-reactivity between the prostatic acid phosphatase antigen and the normal tissue component. All patients developed immune responses to the recombinant fusion protein used to prepare sipuleucel-T, and 38% developed immune responses to prostatic acid phosphatase. In 10 of 26 patients, T cells specific for prostatic acid phosphatase were discovered. Burch et al.[119] treated 13 patients with progressive hormone-refractory metastatic prostate cancer with two infusions of sipuleucel-T administered 1 month apart followed by three subcutaneous monthly doses of prostatic acid phosphatase-GM-CSF fusion protein alone. PSA levels were reduced by more than one-half in the course of treatment in three patients. T cells drawn from patients after infusions of sipuleucel-T, but not before, could be stimulated *in vitro* by GM-CSF and prostatic acid phosphatase, demonstrating broken immune tolerance against these two proteins. Recently, a placebo-controlled phase III trial with sipuleucel-T was conducted in patients with metastatic asymptomatic hormone-refractory prostate cancer.[118] A total of 127 patients were randomly assigned in a 2 : 1 ratio to receive three infusions of sipuleucel-T or placebo every 2 weeks. Sipuleucel-T was well tolerated, but this phase III trial did not demonstrate an improvement in median time to disease progression. The median survival was 25.9 months for sipuleucel-T-treated patients and 21.4 months for placebo-treated patients. This 4.5-month improvement in overall survival was statistically significant ($p = 0.01$). After 3 years, survival was 34% for those treated with the vaccine compared with 11% for those taking the placebo.

In animal models, inadequate dendritic cell differentiation caused by tumour-derived factors, including VEGF, may contribute to defective dendritic cell function in tumour-bearing hosts. To investigate the effect of sipuleucel-T in combination with bevacizumab, a recombinant antibody against VEGF, 22 patients with prostate cancer were treated with this combination therapy.[120] All patients demonstrated induction of an immune response against prostatic acid phosphatase-GM-CSF fusion protein. One patient achieved a >50% reduction in PSA and nine patients exhibited some decrease in PSA from baseline, ranging from 5% to 72%, with the PSA of three patients decreasing by ≥25%. The PSA-modulating and immune effects observed in this combination study warrant further investigation.

Fong et al.[122] immunised 21 patients with metastatic prostate cancer with 2-monthly injections of dendritic cells enriched from peripheral blood mononuclear cells. Following enrichment, the dendritic cells developed an activated phenotype with upregulation of CD80, CD86 and CD83 expression, but CD62 ligand and CCR5 were lost during culture. The investigators loaded dendritic cells *in vitro* with prostatic acid phosphatase and administered the cells via different routes (intravenously, intradermally and intralymphatically) to patients. All patients developed antigen-specific T-cell immune responses following immunisation, regardless of the route of injection but the quality of this response and the induction of antigen-specific antibodies may have been affected by the route of administration.

Barrou et al.[121] immunised prostatectomised prostate cancer patients with autologous dendritic cells pulsed with recombinant human PSA. Dendritic cells were differentiated from monocytes with GM-CSF and IL-13 and were $CD11c^+$, $CD40^+$, $CD80^+$, $CD86^+$ and $HLA\text{-}DR^+$, but $CD83^-$. Patients received nine administrations of PSA-loaded dendritic cells by combined intravenous, subcutaneous and intradermal routes over 21 weeks. PSA-specific T cells were detected *ex vivo* by ELISPOT for interferon-γ in seven patients before vaccination and

in 11 patients post-vaccination. Maximum PSA decrease ranged from 6% to 39%.

7.3 Dendritic Cells Pulsed with Cell Lysates

In order to induce a broader spectrum of T cells, Pandha et al.[123] immunised prostate cancer patients with allogeneic tumour lysate-pulsed dendritic cells. Delayed-type hypersensitivity skin testing and biopsy revealed a cellular infiltrate to intradermal rechallenge with tumour lysate in almost all patients. In addition, there was increased expression of T_h1 cytokine interferon-γ in a few patients. Vaccination resulted in a reduction in the level of PSA in one of nine evaluable patients and an increased PSA doubling time in six patients.

7.4 Dendritic Cells Transfected with Messenger RNA

Use of mRNA to deliver antigens to dendritic cells has several advantages: (i) antigens encoded by mRNA can contain multiple peptide epitopes for different MHC haplotypes; (ii) compared with proteins, mRNA can easily be synthesised; and (iii) in contrast to DNA, mRNA carries little risk of integration into the host genome.

A phase I trial with 13 metastatic prostate cancer patients was performed to evaluate the safety, feasibility and efficacy of immunisation with autologous dendritic cells transfected with mRNA encoding PSA.[132] Induction of PSA-specific T-cell responses was consistently detected in all patients and a significant decrease in log-slope PSA was observed in six of seven evaluable patients. Because human telomerase reverse transcriptase (hTERT) is overexpressed in >85% of human solid tumours (including prostate cancer) but silent in normal tissues, this represents an attractive target for cancer immunotherapy.[133] It has previously been shown that immunisation of mice with hTERT mRNA-transfected dendritic cells stimulates tumour-specific cytotoxic T-lymphocyte responses *in vitro*.[134] A similar approach was chosen by Su et al.,[124] who vaccinated 20 patients with metastatic prostate cancer with hTERT mRNA-transfected dendritic cells. In order to direct hTERT antigen processing into the MHC class II pathway, a lysosomal targeting signal of lysosome-associated membrane protein-1 (LAMP)-hTERT fusion protein can be used to transfect dendritic cells. Dendritic cells transfected with LAMP-hTERT mRNA exhibited enhanced $CD4^+$ T-cell responses while allowing for concomitant induction of hTERT-specific $CD8^+$ T cells. In this study by Su et al.,[124] *ex vivo*-matured and electroporated dendritic cells were clinically applied for the first time in human subjects with metastatic prostate cancer. Despite these modifications, the numbers of vaccine-induced T cells detected in the peripheral blood after three vaccination cycles did not appear to be strikingly different from those observed in a previous clinical trial, in which immature monocyte-derived dendritic cells were passively transfected with PSA mRNA.[132] In the study by Su et al.,[124] 20 patients were vaccinated with hTERT mRNA or its chimeric form. In 19 of these patients, expansion of hTERT-specific $CD8^+$ T cells was measured in the peripheral blood. Patients immunised with the chimeric LAMP-hTERT vaccine developed significantly higher frequencies of hTERT-specific $CD4^+$ T cells as well as enhanced cytotoxic T lymphocyte-mediated killing of $hTERT^+$ target cells.

To obtain a broader antigenicity, Mu et al.[125] transfected dendritic cells with mRNA from allogeneic prostate cancer cell lines (DU145, LNCaP and PC-3). Twenty patients received at least four intranodal or intradermal weekly injections. The vaccine was safe and a total of 12 patients developed a specific immune response to tumour mRNA-transfected dendritic cells. Thirteen patients exhibited a decrease in log-slope PSA.

8. Conclusions

The development of new treatment options for metastatic prostate cancer patients is far from having achieved a breakthrough in tumour vaccination. Several strategies, such as cytokine treatment, antibody therapy, DNA vaccination, viral vectors and dendritic cell immunotherapy, have been explored in the battle against prostate cancer; however, current therapeutic approaches for metastatic prostate cancer remain limited to a palliative role. Only three approaches have demonstrated a minor increase in overall survival (although this parameter has not been investigated in all studies): taxane- and platinum-compound-based chemotherapy[16,17,19,27] and vaccination of patients with sipuleucel-T.[118] All treatments provided a modest survival benefit of a few months, but chemotherapy was also associated with significant adverse effects. Furthermore, one has to ponder whether the complex and cost-intensive procedure required to generate sipuleucel-T justifies the only modest survival benefit seen with this agent.

New molecules could be targeted with chemotherapeutic agents. However, it is highly unlikely that any immunotherapy could elicit an immune response capable of eliminating a growing metastatic tumour. Therefore, early diagnosis may improve the treatment of prostate cancer. At the onset of the disease, when the tumour mass is low, dendritic cell-based immunotherapies may elicit a tumour-specific immune response which can eliminate the tumour or decrease tumour growth. T-cell responses against peptides derived from proteins expressed at later stages of prostate cancer could also be induced at the beginning of the disease and reduce tumour spread. Because of peripheral tolerance, tumour-specific T cells might be tolerised by the tumour and therefore rendered difficult to activate by dendritic cells, whereas early stage T cells might still become activated. However, to induce a profound T-cell response, new tumour-specific epitopes must be identified for prostate cancer. So far, the classical prostate-specific proteins such as prostate-specific membrane antigen, PSA, prostatic acid phosphatase and prostate stem cell antigen have been used to identify cytotoxic T-lymphocyte epitopes by 'reverse immunology'. However, these epitopes are far from optimal because they are self-antigens, they possess homologies to other broadly expressed genes and they exist partially as soluble proteins in the blood. Non-secreted proteins such as six transmembrane epithelial antigen of the prostate, prostate androgen-regulated transcript 1 or trp-p8 might be better candidates for epitopes for immunotherapy. Furthermore, prostate cancer-specific tumour antigens such as mutated or overexpressed proteins have to be identified.

A combination of different approaches to treat prostate cancer such as radiation or chemotherapy combined with immunotherapy might generate a synergistic effect. At present, however, there remains an urgent need for the development of novel therapeutic strategies and improvements in current treatment modalities for prostate cancer.

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