Cancer immunotherapy: a paradigm shift for prostate cancer treatment

Dev Karan, Jeffrey M. Holzbeierlein, Peter Van Veldhuizen and J. Brantley Thrasher

Abstract | Prostate cancer remains a significant health problem for men in the Western world. Although treatment modalities are available, these do not confer long-term benefit and are accompanied by deleterious side effects. Immunotherapy represents a valuable alternative to conventional treatments by inducing tumour-specific immune responses that control the growth of cancer cells. Sipuleucel-T is approved by the FDA as an immunotherapeutic agent for the treatment of patients with asymptomatic or minimally symptomatic castration-resistant prostate cancer (CRPC). Although this approval has raised cost-versus-benefit issues, it has provided proof of concept for the therapeutic potential of active immunotherapy approaches for metastatic CRPC. Numerous clinical studies have demonstrated clinical benefit using immunotherapy compared to traditional chemotherapy and several active immunotherapy approaches (at various developmental stages) have demonstrated the potential to change the face of prostate cancer treatment.

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Introduction

Among men, prostate cancer is the most common noncutaneous cancer and the second leading cause of cancer deaths in the Western world.¹ Although this disease predominantly affects older men, a diagnosis of clinical-grade prostate cancer is not uncommon in men in their forties, or even in their thirties.² Ethnicity is an independent risk factor for prostate cancer and African American men are at a 2.5-fold increased risk compared to white men. Thus, a variety of risk factors—including age, race, lifestyle, environment, and genetic predisposition—collectively contribute towards the development of prostate cancer.³ Given the complexity of its aetiology, it is important that the disease is detected in a localized state so that it can be targeted more effectively by curative therapies. Encouragingly, more than 90% of prostate tumours are detected prior to metastasis and patients are thus eligible for standard treatments such as radical prostatectomy, radiation therapy, cryotherapy, and active surveillance (watchful waiting). However, approximately 30% of these men will experience treatment failure and, ultimately, develop metastatic prostate cancer. Treatment for metastatic prostate cancer relies heavily on androgen ablation, which limits the growth of androgen-dependent cancer cells and results in clinical tumour control. Despite excellent initial responses to hormonal castration, prostate cancer cells eventually become castration resistant and the tumour continues to grow.

Novel treatment approaches for patients with progressive castration-resistant prostate cancer (CRPC) have been developed that involve chemotherapy with docetaxel followed by cabazitaxel.⁴ The CYP17 inhibitor

abiraterone has been approved as a second-line therapy following failure of docetaxel-based chemotherapy.⁵ Unfortunately, the overwhelming majority of tumours still progress, leading to an estimated 30,000 deaths every year in the USA.¹ This continues to pose a significant health threat to men in developed countries. In light of the increasing average life expectancy of men in the Western world, there is potential for an epidemic of advanced prostate cancer within the next 50 years, which could constitute a significant economical burden.^{6,7} Thus, novel treatments for advanced prostate cancer are desperately sought.

An increased understanding of basic immunology, combined with the identification of various tumour-associated antigens (TAAs), has fuelled the development of novel immunotherapy approaches for various cancers, including prostate cancer. In this Review, we describe several active immunotherapy approaches that have the potential to transform the way in which patients with prostate cancer are treated. We also highlight future areas of research that might help to improve the efficacy of immunotherapy for prostate cancer.

Cancer immunotherapy

The aim of immunotherapy is to induce immunity to diseases such as cancer using the body's own immune system. This approach is based on the concept of immune surveillance.^{8,9} According to this theory, the immune system constantly patrols the body for signs of abnormal (potentially precancerous) cell growth, and eliminates these abnormal cells before they become harmful to the host. During immune patrolling, various types of immune cells migrate towards the site of infection or injury, triggering an inflammatory response. In

Department of Urology
(D. Karan,
J. M. Holzbeierlein,
J. B. Thrasher),
Department of Internal
Medicine (P. Van
Veldhuizen), University
of Kansas Medical
Center, Kansas City,
3901 Rainbow
Boulevard, Kansas City,
KS 66106, USA.

Correspondence to:
D. Karan
dkaran@kumc.edu

Competing interests

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support of this hypothesis, chronic inflammation has been linked to the development of neoplastic formation in several organs and is widely considered to be a risk factor for cancer.^{10,11}

Inflammation in the prostate is primarily regulated by T cells, B cells, and macrophages.^{12–14} Precancerous cells could harness the functions of infiltrating immune cells (released during the inflammatory response) to support cell growth. The escape of precancerous cells from immune surveillance could arise as a result of multiple factors, including weak immunogenicity, lack of proper communication between immune cells and precancerous cells, reduced (or absent) expression of major histocompatibility complex molecules, immunologic tolerance, increased levels of regulatory (suppressor) T cells, and suppressive activities produced by the neoplastic cells. Prolonged immune surveillance evasion could ultimately lead to the development of cancer.^{15–18}

This neoplastic process is thought to occur in three distinct phases: elimination, equilibrium, and escape.^{19,20} Following clearance attempts by the immune system, abnormal (precancerous) cells that have survived the elimination phase enter a dormant phase (equilibrium), producing new cell variants but remaining clinically undetectable. With time, the new variants of abnormal cells—which are not generally susceptible to attack by immune cells—develop into clinically detectable tumours.

The ultimate goal of cancer immunotherapy is to identify and destroy tumour cells without exerting detrimental effects on the host. As cancer immunotherapy establishes a tumour-specific immunological memory, one focus of research has been to generate high-potency cytotoxic T lymphocytes (CTLs). Various immunotherapy approaches utilize whole cancer cells, parts of cancer cells (for example, RNA or DNA), or a particular TAA to stimulate the immune response.^{21–25} In particular, progress in this field has been driven by the identification of TAAs such as melanoma antigen family A (MAGEA) 1, MAGEA2, MAGEA3, melanoma antigen recognized by T cells 1 (MART1), glycoprotein 100 (gp100), carcinoembryonic antigen (CEA), human epidermal growth factor receptor 2 (HER2), mucin 1 cell surface associated antigen (MUC1), and prostate-cancer-specific antigens—including PSA, prostate stem cell antigen (PSCA), prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA). Immune-based therapies that target these antigens are under investigation in various clinical trials to assess overall survival benefits.^{26–29}

The number of tumour-antigen-specific CTLs has been shown to correlate with clinical response in various cancer vaccine studies to date.^{30,31} Although there are no defined criteria for measuring the CTL response that is mounted following immunization, increased expression of interferon (IFN)- γ —which is produced by antigenic CD8 T cells—and increased *in vitro* CTL activity are routinely used to define the potential of the developed anti-tumour immunity. Over time, we have learned that the utilization of effector CTLs to provide potent antitumour

Key points

- Prostate cancer is responsive to active immunotherapy, as evidenced by the clinical efficacy of sipuleucel-T and PROSTVAC®-VF vaccination
- Sipuleucel-T is the first FDA-approved immunotherapy option for the treatment of minimally symptomatic or asymptomatic metastatic castration-resistant prostate cancer
- Cancer vaccines containing adenovirus and plasmid DNA are emerging as potential approaches for active immunotherapy; these are at various stages of development and clinical evaluation
- Overall survival remains the gold-standard study end point; alternative end points (such as novel biomarkers) are sought for patients with early-stage disease
- Immunotherapeutic approaches might be most effective for patients with minimal residual disease and for use early in the treatment paradigm
- Future studies should focus on identifying which patients are most likely to benefit from treatment, as well as on the development of combination therapies and novel multitargeted approaches that can increase survival advantage

immunity depends on multiple factors, including antigen selection and delivery method.

Antibody-based targeted immunotherapy

Therapies targeting CTL-associated antigen 4 (CTLA4) have been a particular research focus for the treatment of various solid tumours, including prostate cancer.^{32–35} CTLA4 is a cell-surface molecule on T cells that is engaged in the suppression of T-cell activation. Circulating T cells demonstrate increased expression of CTLA4 in patients with cancer;³⁶ thus, targeted inhibition of CTLA4 might induce the function of effector T cells. Ipilimumab and tremelimumab—two antibodies that target and inhibit CTLA4—were initially tested in patients with melanoma. In phase III clinical trials of patients with advanced melanoma, only ipilimumab improved overall survival compared to standard chemotherapy (no survival benefit was evident with tremelimumab).^{37,38} On this basis, Small *et al.*^{35,39} evaluated the effect of administering ipilimumab to patients with CRPC, both as monotherapy and in combination with granulocyte-macrophage colony-stimulating factor. When given as monotherapy, a single dose of 3.0 mg/kg was safe and tolerable. However, the toxicity of combination therapy was a limiting factor, despite its clinical efficacy.

In another trial, a combination of ipilimumab and the gp100 peptide vaccine Gp100:209-217(210M) was associated with improved overall survival in patients with metastatic melanoma compared to monotherapy with either agent.³⁸ This suggests that combining antibody-based therapy with selected antigen targets might improve the therapeutic potential of immunotherapy for other solid tumours. Two phase I studies have demonstrated the safety of administering ipilimumab combined with either PROSTVAC®-VF or GVAX® prostate cancer vaccines to patients with metastatic CRPC.^{40,41} It is likely that a phase II trial will follow to establish the efficacy of these combinations. Currently, two phase III clinical trials are investigating the efficacy of ipilimumab therapy for metastatic CRPC; either alone⁴² or in combination with radiotherapy.⁴³

Another promising candidate for antibody-based targeted therapy is denosumab—a humanized monoclonal

antibody against the receptor activator of NF κ B ligand (RANKL) that is used for the prevention of skeletal-related events in patients with bone metastases. In a phase III double-blind randomized study, denosumab treatment was associated with a reduction in skeletal fractures compared to zoledronic acid therapy in men with CRPC and associated bone metastases.⁴⁴

Dendritic-cell-based vaccination

A new generation of cancer immunotherapies has been developed based on our increased understanding of dendritic cells, which play a central role in the development of effector T cells. Active immunization methods that induce TAA-specific CTLs using *ex vivo* dendritic cells loaded with antigen, cell-based, plasmid DNA, or viral vaccines have undergone clinical evaluation for the treatment of various cancers, including prostate cancer. The question regarding whether dendritic cells are a homogeneous cell population with multiple functions or a heterogeneous group of cells that exhibit differences in morphology, function, and localization, remains unanswered. Despite increasing evidence to show that dendritic cells display varying degrees of plasticity and cannot be rigidly categorized according to a particular phenotype,⁴⁵ these cells are most commonly classified as either plasmacytoid or myeloid, based on phenotypic markers.⁴⁶

Dendritic cells are professional antigen-presenting cells (APCs) that process and present TAAs to T cells, stimulating the production of a costimulatory signal and triggering the development of effector (tumour-reactive) T cells. Various studies have examined the direct activation and antigen loading of dendritic cells in *ex vivo* systems and demonstrated an induction of antigen-specific immune response.⁴⁷ In one study, autologous dendritic cells from patients with CRPC were loaded with antigenic peptides derived from four different PSAs (PSCA, PAP, PSMA, and PSA). These antigen-loaded dendritic cells were injected intradermally in sets of six immunizations (three injections into each thigh) every other week for 3 months, followed by monthly booster injections where appropriate (total treatment duration of up to 20 weeks). This vaccination regimen was well tolerated and induced significant CTL responses against all PSAs. Furthermore, it was associated with a prolongation in PSA doubling time.⁴⁸

Similarly, a phase I clinical trial studied the effects of administering dendritic cells transfected with PSA mRNA to patients with metastatic CRPC.⁴⁹ For this treatment approach, autologous dendritic cells were generated from peripheral blood mononuclear cells (PBMCs).⁵⁰ After 7 days in culture, the dendritic cells were co-incubated with a vector containing PSA mRNA (pGEM4/PSA/A64) to enable transient transfection. These transfected cells were washed and incubated overnight to allow time for the expression and presentation of the PSA antigen. Patients with metastatic CRPC were vaccinated with PSA-mRNA-transfected dendritic cells at biweekly intervals to assess the safety and toxicity of this approach. The study revealed a small, but statistically significant, decrease in PSA velocity in treated patients.

Sipuleucel-T

Sipuleucel-T is the only immunotherapy approved by the FDA for the treatment of asymptomatic or minimally symptomatic metastatic CRPC. It is an autologous cellular immunotherapy that has been designed to stimulate a patient's own immune response against prostate cancer. It acts in a similar way to a vaccine and is thus referred to as a dendritic-cell-based vaccine immunotherapy. Treatment begins with leukapheresis to isolate a patient's PBMCs, which are a mixture of APCs, T cells, B cells, and other immune cells. These PBMCs are then activated *ex vivo* by the recombinant human PAP/GM-CSF fusion protein (PA2024) and reinfused back into the patient to target and treat the prostate cancer.⁵¹ Each dose of sipuleucel-T contains a minimum of 40 million cells expressing the costimulatory molecule CD54. The number and cellular composition of the final dose depends on the composition of the cells harvested during leukapheresis.⁵² Cells expressing CD54 are considered to be the 'active ingredient' of the treatment as CD54 is predominantly localized to APCs, which are responsible for antigen uptake.⁵³ Although the precise mechanism of action is unknown, it is thought that PAP/GM-CSF is internalized by the APCs during *ex vivo* culture and PAP-specific peptides—such as major histocompatibility complex (MHC) class I and class II peptides—are expressed on the surface of the APCs.

In phase III studies, treatment with sipuleucel-T resulted in a median survival advantage of 4.1 months, with a 3-year survival rate of 31.7% (compared with 23.0% for patients receiving placebo).⁵¹ However, no difference was observed between the two study arms in terms of progression-free survival (PFS), time to clinical progression, or time to PSA progression. The reason for the dissociation between overall survival and measurable anti-tumour effect is unclear. It is, therefore, difficult to determine which patients are likely to benefit from treatment and all patients are treated empirically at present.

The exclusivity of FDA approval for sipuleucel-T immunotherapy for prostate cancer has raised issues regarding cost versus benefit. Although traditional chemotherapy is expensive, treatment cost can be regulated by the discontinuation of therapy when this proves to be ineffective. Sipuleucel-T, on the other hand, is administered in full at the start of treatment, prior to any assessment of benefit. Moreover, evidence is lacking to support a direct correlation between the proposed mechanism of action of sipuleucel-T (via induction of antigenic immune and tumour responses) and the observed clinical benefit of this drug.

To better understand the functional activity of sipuleucel-T, a phase II study is evaluating the magnitude of immune response to different concentrations of PA2024 antigen in patients with metastatic CRPC.⁵⁴ Another phase II study, set up to measure antigen-specific immune responses over time, is currently monitoring men who have failed primary therapy and are given sipuleucel-T before or after androgen deprivation therapy.⁵⁵ In the wake of encouraging preliminary results regarding novel combination therapy with antiandrogens, a trial

Table 1 Results of selected immunotherapy trials for patients with prostate cancer				
Study	Phase	Study design	Study end points	Study outcomes
Sipuleucel-T				
Kantoff et al. ⁵¹	III	Patients with asymptomatic metastatic CRPC randomized to receive either sipuleucel-T or placebo (ratio of 2:1)	OS; TTP	Increase in mean OS (4.1 months) in treated patients compared to controls; no difference in TTP reported between groups
NCT00715078 ⁵⁴	II	Dose-dependent antigen-based (PA2024) evaluation of sipuleucel-T therapy for patients with metastatic CRPC	Immune response; safety; OS	Data collection ongoing
NCT01431391 ⁵⁵	II	Sipuleucel-T given as a neoadjuvant treatment for localized prostate cancer	Immune response	Data collection ongoing
NCT01487863 ⁵⁶	II	Patients with metastatic CRPC randomized to receive sipuleucel-T plus abiraterone acetate and prednisone (administered either sequentially or concomitantly)	Immune response (including PAP-specific T-cell response); safety	Data collection ongoing
PROSTVAC®-VF				
Kantoff et al. ⁶⁹	II	Patients with minimally symptomatic metastatic CRPC randomized (double-blind) to receive either PROSTVAC®-VF or placebo	OS; PFS	Increase in OS (8.5 months) in treated group compared to controls; no reported improvement in PFS with treatment
NCT01322490 ⁷³	III	Patients with minimally symptomatic metastatic CRPC randomized to receive either PROSTVAC®-VF, PROSTVAC®-VF plus GM-CSF, or placebo	OS; PFS	Data collection ongoing
NCT00450619 ⁷²	II	Patients with metastatic CRPC randomized to receive 153Sm-EDTMP with or without PROSTVAC®-VF	TTP; immune efficacy	Data collection ongoing
NCT00450463 ⁷⁴	II	Patients with nonmetastatic CRPC randomized to receive flutamide with or without PROSTVAC®-VF	TTP; immune efficacy	Data collection ongoing
GVAX®				
Small et al. ¹¹¹	III	Patients with metastatic CRPC randomized to receive GVAX® immunotherapy plus docetaxel (with or without prednisone)	Efficacy; OS	Prematurely terminated as a result of an increase in the incidence of fatalities
Ipilimumab				
NCT00861614 ⁴³	III	Patients with CRPC that have received prior treatment with docetaxel randomized to receive either ipilimumab or placebo following radiotherapy	Efficacy; OS	Data collection ongoing
NCT01057810 ⁴²	III	Patients with minimally symptomatic or asymptomatic metastatic chemotherapy-naïve CRPC randomized to receive either ipilimumab or placebo	Efficacy; OS	Data collection ongoing
Ad/PSA				
NCT00583752 ⁹⁷	II	Patients with recurrent prostate cancer after local therapy randomized to receive either vaccine only or vaccine plus ADT	PSA doubling time (primary); PSA levels (secondary); immune response (secondary)	Data collection ongoing
NCT00583024 ⁹⁸	II	Nonrandomized study of the effect of administering vaccine to patients with metastatic CRPC	PSA doubling time (primary); PSA levels (secondary)	Data collection ongoing
pTVG-HP				
NCT01341652 ²⁹	II	Patients with nonmetastatic prostate cancer randomized (double-blind) to receive GM-CSF with or without pTVG-HP	Metastasis-free survival; TTP; PSA doubling time	Data collection ongoing
Abbreviations: Ad/PSA, replication-deficient PSA-expressing adenovirus vaccine; CRPC, castration-resistant prostate cancer; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVAX®, GM-CSF modified tumour cell vaccine; OS, overall survival; PAP, prostatic acid phosphatase; PFS, progression-free survival; pTVG-HP, plasmid DNA containing PAP antigen; TTP, time to progression.				

has been instigated to assess the safety and efficacy of administering sipuleucel-T—given either simultaneously or sequentially with antihormonal agents such as abiraterone acetate plus prednisone—to patients with metastatic CRPC (Table 1).⁵⁶

Microbial vectors for cancer immunotherapy

The ability of microbial vectors to carry foreign antigens has been harnessed by various vaccine approaches to targeted immunotherapy. Bacterial strains have been engineered to express cancer-specific antigens and

cytokines, as well as prodrug-converting cytokines. A variety of microbial vectors—including BCG, *Listeria monocytogenes* (LM), *Salmonella*, *Escherichia coli*, and *Shigella*—have demonstrated efficacy as vaccine vectors in preclinical animal models of infectious disease and cancer.⁵⁷ These microbial vectors are useful because of their strong ability to infect APCs and induce potent antitumour responses to targeted antigens. However, utilization of microbial agents as delivery vehicles is limited because the properties of these vaccine vectors have not yet been fully characterized.

Listeria monocytogenes

Although the clinical development of bacteria-based vectors has generally lagged behind that of other currently used vaccine approaches, several unique characteristics of *Listeria monocytogenes* have focused attention on the use of this vector for active immunotherapy, including its ability to induce both arms of the immune response (innate and adaptive).⁵⁸ As *L. monocytogenes* can access both the phagosomal and cytosolic compartments of APCs, *L. monocytogenes*-delivered antigens can be presented in the context of MHC class I and class II molecules, resulting in stronger immune responses.^{59,60} A number of live attenuated strains of *L. monocytogenes* have been developed to express several human antigens, including HER2, human papilloma virus (HPV) E7 antigen, MAGE, and PSA.^{61–64} Preclinical studies of PSA-expressing *L. monocytogenes* have shown that this strain is immunogenic and could have therapeutic potential for treating prostate cancer. Interestingly, the PSA-expressing *L. monocytogenes* strain was more effective at inducing tumour regression than vaccinia virus vectors carrying human PSA.^{65,66} Although this strain is in the early stages of development, trials of patients with metastatic cervical cancer who have been treated with *L. monocytogenes* expressing the HPV E7 antigen and patients with lung metastases treated with human mesothelin (CRS-207) have indicated the safety and tolerability of *L. monocytogenes*-based vaccination.^{67,68} Although additional clinical trials are needed to confirm these initial promising results, it seems that *L. monocytogenes*-based vaccination could induce immune activation with a potential clinical benefit against prostate cancer.

PROSTVAC®-VF

Another novel form of immunotherapy for prostate cancer that utilizes a microbial vector is PROSTVAC®-VF. This vaccine approach uses a 'prime-boost strategy', involving vaccinia and fowlpox viral vectors that express a PSA-targeted molecule and a triad of costimulatory molecules (B7.1, ICAM-1, and LFA-3)—termed the vaccinia-PSA-TRICOM and fowlpox-PSA-TRICOM vaccines.^{69,70} Using this approach, the patient's immune system is first primed with vaccinia-PSA-TRICOM. Subsequent vaccination with fowlpox-PSA-TRICOM boosts the CTL immune response against PSA-expressing tumour cells. The efficacy of PROSTVAC®-VF has been analyzed in a randomized multicenter, double-blind phase II study of patients with metastatic CRPC ($n = 125$). Although no improvement in PFS—the primary end point of the study—was observed in the treated cohort, patients receiving PROSTVAC®-VF therapy experienced an 8.5 month improvement in overall survival relative to controls.⁶⁹

In a separate randomized phase II study of 64 patients treated with PROSTVAC®-VF, 45.3% of patients remained free of PSA progression at 19 months (primary end point of the study) and 78.1% of patients demonstrated a clinical benefit in PFS compared to controls.⁷⁰ Analysis of PSA-specific immune response revealed an

increased number of PSA-specific T cells relative to the baseline (PBMCs collected prior to immunization) in 46% of treated patients. However, no differences were observed between the treated and untreated cohorts in relation to anti-PSA antibody response.⁷⁰ In a similar, albeit smaller, study of 32 patients, vaccination with PROSTVAC®-VF was linked to an increase in levels of PSA-specific T cells in association with improved survival.⁷¹ Encouraged by the outcomes of these trials, as well as demonstrations of PSA-specific immune responses, PROSTVAC®-VF is currently being tested in a phase III study, as well as two phase II combination therapy studies (Table 1).^{72–74}

Adenoviruses

Increasing attention is being directed towards the use of adenoviruses as expression vectors and recombinant virus vaccines. For gene-therapy-based and vaccine-based approaches, adenovirus serotype 5 (Ad5) has been the most extensively studied vector.^{75–77} The Ad5-vector has been genetically modified for vaccine therapy to allow insertion of foreign genes. Current vectors have deletions in the E1a and E1b regions, as well as a partial deletion of the E3 region. Ad5-vector-based vaccines for prostate cancer are produced by inserting the gene for full-length human PSA into a replication-deficient Ad5-vector to create Ad5-PSA, which induces a strong anti-PSA CD8⁺ T-cell response.^{78,79} Moreover, Ad5-PSA has been shown to induce stronger anti-PSA immune responses than other viral PSA vaccines, including vaccinia virus vaccines (both replication competent and replication deficient) and a canarypox vaccine.^{79,80} The Ad5-vector is able to transduce DNA between a wide range of dividing and nondividing cells types, making this gene delivery system a valuable tool for vaccine therapy.^{81,82} Furthermore, it is genetically and biochemically well characterized and can be produced at high titres under good manufacturing practice conditions.^{83,84}

Adenovirus-based cancer vaccines have been used to induce active immunization in murine models for various types of cancer, as well as in some clinical studies.^{85–88} Ad5-vectors that express prostate cancer antigens such as PSA, PSMA, PSCA, or six-transmembrane epithelial antigen of the prostate (STEAP) have all demonstrated therapeutic effects in preclinical models.⁸⁹ A study with 10-year follow-up safety data has supported the continued use and development of adenoviruses as gene delivery vehicles.^{90,91} The Vaccine Research Center at the US National Institutes of Health has developed several candidates for cancer vaccines based on the Ad5-vector and has performed biodistribution and toxicological safety studies that support the investigational use of Ad5-based vaccine candidates in humans.⁹² In addition, wild-type adenovirus infections are extremely common in the general population, providing a well-documented safety record. Indeed, it could be argued that adenovirus-mediated gene delivery is associated with lower morbidity than most currently used treatments for prostate cancer including surgery, hormonal therapy, and chemotherapy.

Overcoming anti-adenovirus immunity

Despite potential advantages, the use of the Ad5-vector as a delivery vehicle is hampered by the prevalence of pre-existing anti-adenovirus immunity in human populations.^{93,94} To improve the immunogenicity of the Ad5-vector, the adenovirus can be administered with GELFOAM® to enhance CTL activity in the presence of anti-Ad5 immunity.⁹⁵ This finding is important as most humans have pre-existing levels of anti-adenovirus antibodies, resulting from prior natural exposure to the virus.

In support of Ad5-vector-based vaccination, a phase I trial showed the development of an anti-PSA T-cell response in 68% of patients with prostate cancer treated with the Ad5-PSA vaccine injected with GELFOAM®.⁹⁶ This study, which included 32 patients with advanced CRPC, demonstrated the safety of Ad5-vector vaccination for prostate cancer. Following on from these results, a phase II continuation of this study is currently evaluating the efficacy of Ad5-PSA treatment for patients with a rising PSA after local therapy, and for patients with CRPC.^{97,98} Interim results for 44 patients—presented at the American Association for Cancer Research annual meeting in 2012—showed that three injections of Ad5-PSA vaccine in GELFOAM® (administered at intervals of 30 days) induced anti-PSA T-cell responses above the baseline (pre-immunization) level in 100% of patients with a rising PSA after local therapy and 67% of patients with CRPC. The majority (64%) of patients demonstrated an increase in PSA doubling time.⁹⁹ These observations suggest that the use of GELFOAM® with Ad5-based vaccines induces an immune T-cell response against the self-antigen and circumvents pre-existing anti-Ad5 immunity.

In vivo dendritic cell targeting using Ad5-vectors

It is widely accepted that dendritic cells have a pivotal role in the development of effector T cells; thus, the development of immune-based therapies has focused on the activation of dendritic cells. Despite advantages of autologous dendritic-cell-based vaccination, the status of dendritic cell maturation during the preparation of a dendritic-cell-based vaccine is a critical issue that presents many challenges.^{100,101} Vaccine development is constrained by clinical practicalities as the vaccine must be patient-specific, and vaccine preparation techniques are cumbersome, laborious, and costly. Furthermore, *in vivo* dendritic cell migration is a complex and tightly regulated process that involves multiple cellular components, including cytokines, chemokines, and adhesion molecules, and *ex vivo* cultured and modified (antigen-loaded) dendritic cells have demonstrated poor migration *in vivo*, adversely affecting the efficacy of the vaccine.¹⁰²

Various studies have shown that the Ad5-vector is capable of modulating dendritic cell maturation by increasing expression of MHC antigens and costimulatory molecules, making this delivery system a valuable tool for studying disease pathology, vaccine therapy mechanisms, and potential clinical use.^{81,82} It is likely that adenoviruses, which contain adjuvant-like properties, provide the costimulatory signals necessary to generate a strong antigen-specific effector T-cell response.

Interaction of CD40 with its ligand (CD40L) constitutes one of the basic costimulatory signals that activate dendritic cells to generate CTLs with enhanced antitumour activity. Multiple studies have utilized adenoviruses carrying CD40L to activate CD40 on dendritic cells, generating high-potency tumour-reactive CD8⁺ T cells.^{103,104} Therefore, Ad5-vector-based *in vivo* targeting of tumour-specific antigens, combined with dendritic cell activation, might represent an important immunotherapy approach.

The use of adenoviruses as delivery vehicles potentially infects dendritic cells *in vivo* to induce an antigen-specific effector T-cell response. In mouse models, subcutaneous injection of bone-marrow-derived dendritic cells (BMDCs) treated with Ad5-vector carrying a model antigen induced immune and antitumour responses.^{105,106} Bone marrow cells were harvested by flushing the murine femurs and tibias to generate dendritic cells. Following overnight treatment with Ad5-OVA (Ad5-vector carrying ovalbumin as a model antigen), BMDCs were washed with phosphate buffer saline and injected subcutaneously into treatment-naïve mice. A week after injection, immune response (level of IFN- γ -secreting CD8⁺ T cells) was measured by *in vitro* assay for BMDC-immunized mice. Mice challenged with OVA-expressing EG7 tumour cells demonstrated significant inhibition of tumour growth.¹⁰⁶ These observations further suggest that the Ad5-vector can be used as a vaccine system to deliver cancer-specific antigens by infecting *ex vivo* dendritic cells, which then process and present antigens to induce a protective immune response *in vivo* upon reinsertion.

Multigene-targeted immunotherapy

The generation of a tumour-specific CD8⁺ T-cell response is generally considered to be the most potent immunotherapy approach. It is well documented that adenoviruses can be used as vehicles for antigen delivery, as well as vaccine carriers, to strongly induce cytotoxic T-cell responses, which are actively involved in the destruction of tumour cells that express cognate antigens. However, vaccines for prostate cancer have only, as yet, employed a single antigen, despite the fact that patients have often been heavily pretreated and frequently harbour advanced tumour burdens with a high propensity for changing their expressed antigens. Prostate cancer is a heterogeneous multifocal disease that undergoes significant genetic changes during its transition from a hormone-responsive to a hormone-refractory malignancy. Thus, the use of a single-antigen-based immunotherapy might not provide an efficient immune response against the prostate tumour cells directing the immunoediting process.

To expand the range of antitumour responses, some investigators have loaded dendritic cells with multiple peptides *ex vivo* to generate antitumour immunity.^{48,107} However, multi-peptide-loaded dendritic cells lack the appropriate costimulatory molecules required for activating effector T cells, which limits the antitumour response.¹⁰⁸ Using a preclinical mouse model, a recent study demonstrated the therapeutic benefit of administering dendritic cells loaded with a virus-expressed

Figure 1 | Schematic presentation of the mechanism of action of an adenovirus vector encoding the PSA-PSCA fusion protein. Antitumour immunity develops via simultaneous targeting of multiple tumour-associated antigens.

cDNA library specific to prostate cancer.¹⁰⁹ Clinical studies are now needed to confirm the safety and efficacy of this approach in humans.

GVAX®

GVAX® is a GM-CSF modified tumour cell vaccine produced by engineering the prostate tumour cell lines PC3 and LNCaP to secrete GM-CSF.²⁸ This vaccine, which is one of the most extensively studied vaccines for treating prostate cancer, was designed to induce a broad range of prostate tumour-specific immune responses. Despite promising early results,¹¹⁰ Vital-2—a GVAX® phase III trial—was terminated because of safety concerns.¹¹¹ The reason for this is not entirely clear. As GVAX® vaccination utilizes irradiated prostate tumour cells (cellular vaccine), the immune responses associated with this approach could be undesirable and might outweigh the clinical benefit. It is highly likely that uncontrolled levels of GM-CSF in both GVAX® and sipuleucel-T could cause counter-regulatory immune responses, as higher levels of GM-CSF are also associated with an increased number of suppressor T cells.^{112,113} Administering GVAX® vaccination concurrently with ipilimumab therapy has demonstrated safety in a phase I trial of patients with metastatic CRPC. However, development of autoimmune response remains a concern.⁴¹

Ad5-PSA/PSCA bivalent vaccine

As the heterogeneity of prostate cancer might limit the therapeutic benefit of single-antigen-based targeted therapies, one alternative approach is to amplify the potential of antitumour immunity using a combination of PSA and PSCA antigens, which are both highly specific to the prostate. PSCA is stably expressed in advanced prostate cancer and has been proposed as a prospective therapeutic target for CRPC.^{114–117} It is likely that initiating a strong line of attack on a heterogeneous tumour mass by inducing an amplified immunity against multiple target antigens will significantly reduce the ability of tumour cells to escape immune surveillance.

An Ad5-based bivalent vaccine encoding a fusion of human PSA and PSCA cDNA (Ad5-PSA/PSCA) has been proposed as a potential therapy for prostate cancer.¹⁰⁶ The Ad5-PSA/PSCA vaccine infects dendritic cells and the cytomegalovirus promoter of the adenovirus

drives the synthesis of a PSA-PSCA fusion protein. This protein undergoes antigen processing, leading to the presentation of PSA and PSCA gene-specific peptides on the surface of dendritic cells and the induction of an antigen-specific T-cell response (Figure 1). The Ad5-PSA/PSCA vaccine has been shown to simultaneously induce PSA-specific and PSCA-specific CD8⁺ T-cell responses (determined by IFN- γ cytokine secretion) in mice. Furthermore, this approach induced a strong antitumour immunity that inhibited the growth of established tumours coexpressing cognate antigens; 80% of mice were tumour free following treatment.¹⁰⁶ These observations also suggest that multigene-targeted TAA-specific CTLs can be generated simultaneously (with an additive antitumour effect) to improve therapeutic efficacy of an immunotherapy approach. This preclinical study provides a strong rationale for further developing and investigating the Ad5-PSA/PSCA bivalent vaccine to determine safety, dose tolerance, and clinical proof of concept in patients with prostate cancer.

Combining immunotherapy with other treatment

Combining immunotherapy with an adjuvant chemotherapeutic drug, radiation therapy, or an immunosuppressant could potentially reduce tumour burden and improve overall survival. Use of an adjuvant therapy, such as the Toll-like receptor 9 (TLR9) agonist CpG, with Ad5-based immunotherapy (for example, Ad5-PSA/PSCA) might enhance the potential of the antitumour response by inducing both innate and adaptive arms of the immune system. Immunotherapy approaches involving sipuleucel-T and PROSTVAC®-VF in combination with chemotherapy drugs are also being evaluated.^{118,119}

Given that inflammation could support the development of a tumour, simultaneous targeting of inflammation in combination with immunotherapy might improve the therapeutic efficacy of a vaccine. Prednisone is an anti-inflammatory agent used for prostate cancer treatment in combination with other chemotherapy drugs. However, this drug is known to weaken the immune system, so the risks of combination therapy might outweigh the beneficial effects. An ongoing study is analyzing the effect of administering prednisone in combination with sipuleucel-T on the development of immune response.⁵⁶

Following immunotherapy with radiation therapy could be advantageous because the release of cell products from tumour cells killed during radiation therapy will further activate the immune system, which has already been triggered by immunotherapy. Similarly, depletion of immune suppressor cells will reduce the suppressive activity of a faulty immune system and provide more space for the expansion of effector T cells, induced following immunotherapy.

Overall, immunotherapy is a promising approach that could significantly reduce the mortality rate associated with CRPC when used in combination with other treatment modalities. However, such combinations need to be tested step-by-step in preclinical and clinical settings. Study design is critical when evaluating the clinical benefit of such combinations as the use of chemotherapy drugs could have a negative impact on the development of immune responses.

Conclusions

Immunotherapy has substantial potential as a nontoxic method of specifically and systematically destroying tumour cells. Multiple approaches for immunotherapy are emerging, including the use of plasmid DNA, microbial vectors, and direct DC manipulations. The success of current cancer immunotherapies—including adoptive cell transfer and cytokine therapy for melanoma, cytokine therapy for renal cell carcinoma, trastuzumab for breast cancer, sipuleucel-T for prostate cancer, and various antibody-based cancer therapies—supports the continuation of research in this field.^{120,121} Among these approaches, sipuleucel-T and PROSTVAC®-VF have been the most successful for treating patients with prostate cancer, with a proven record of safety and clinical benefit.

Analyzing the efficacy of immunotherapy (or therapeutic combinations that include immunotherapy) remains a challenge. Currently, therapeutic responses are monitored based on changes in PSA or PSA-related parameters (including PSA velocity or doubling time), along with other clinical surrogates. Although well established as an indicator of disease progression, PSA-related changes are not always reliable for predicting the efficacy of therapeutic treatments. Currently, no validated immunologic biomarkers have been identified that can

reliably predict clinical response following immunotherapies. Immunologic parameters for measuring the clinical success of immunotherapy are limited to antigen-specific CD8⁺ T-cell levels or IFN- γ secretion.^{122,123} Analysis of PSA-specific or PAP-specific immune responses following PROSTVAC®-VF or sipuleucel-T vaccines has established a limited correlation between these variables and reported clinical benefits. It has been suggested that continuous immune modulation by the initially induced antitumour response that follows vaccination leads to improved overall survival.¹²⁴ This power struggle between the immune system and the tumour might keep the tumour burden in a state of equilibrium. An objective decrease in the tumour burden would then only occur if the immune system overpowered the tumour cells. Immunotherapeutic approaches might, therefore, be most effective against minimal residual disease and when used at an early stage of the treatment paradigm.

As immunotherapy works by inducing an immune system response against cancer cells, a longer follow-up duration could be required to observe clinical benefit and, therefore, overall survival remains the standard study end point. This lack of predictive biomarkers limits our ability to effectively manage the treatment of metastatic CRPC, especially when differentiating between patients who are responding to therapy and those who are not. Thus, the identification of immunological parameters or novel biomarkers that could be used to determine the efficacy of immunotherapy would have significant clinical utility and drive further advances in this field.

Review criteria

The literature for this Review was primarily searched through PubMed using the terms “prostate cancer immunotherapy”, “adenovirus based immunotherapy”, “sipuleucel-T” and “Prostvac-VF”. Relevant articles from the last 10 years were identified. Cross references were checked and relevant articles retrieved. In cases when publications were not available through PubMed, LISTA (EBSCO) and Web of Science databases were also checked. Information about ongoing clinical trials of active immunotherapy for prostate cancer was obtained from <http://clinicaltrials.gov>.

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