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EXPERT REVIEWS

Prostate cancer vaccines

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In 2010, the US FDA approved the first therapeutic cancer vaccine for the treatment of castration refractory prostate cancer – sipuleucel-T. Prostate cancer is an ideal model for cancer vaccine development based on the ready demonstration of humoral and cellular immunity to a range of cancer antigens as well as often slow progression which means that patients who are otherwise well may have a radiologically evaluable minor progression, after conventional treatment and can undergo vaccine therapy over sufficient periods of time, so as to allow the generation of a robust antitumor response. The association of prostate cancer with one of the few serum cancer biomarkers in general use has also allowed assessment of response and risk stratification of patients. In this review, we will examine key aspects of the evolution of prostate cancer vaccines, which provides an accurate prototype for other cancers, and the challenges we face.

Keywords: immune tolerance • immunotherapy • prostate cancer • tumor microenvironment • vaccines

There have been genuine advances in the diagnosis and treatment of prostate cancer in the last decade. Prostate cancer continues to be an extremely challenging disease to treat, particularly in the advanced stage, and remains the leading cause of cancer-related morbidity or mortality in men in the western world [1,2]. In terms of advanced disease there have been key practice-changing developments such as the establishment of docetaxel chemotherapy for castrate-resistant metastatic disease and more recently treatment of patients post docetaxel with drugs such as abiraterone [3], cabazitaxel [4] and more recently MDV3100 [5]. The US FDA approval of sipuleucel-T was a landmark development as the first vaccine approved for an advanced solid malignancy [6]. In many respects the potential for vaccines to impact on longevity (the 'gold standard' outcome parameter) has already been demonstrated and recent studies have shown immunotherapy is at least comparable with cytotoxic and novel hormonal therapies (Table 1). Prostate cancer is an extremely complex disease and many aspects of the natural history are still largely not understood. Early organ-confined disease is treated by surgery, radiation treatment or in a proportion of men, just monitored (active surveillance). The molecular 'switches' which dictate quiescence versus progression after each of these approaches are a subject of intensive research. Once radiation therapy or surgery fails, patients are treated with androgen deprivation therapy (orchiectomy, a luteinizing hormone releasing hormone agonist or antiandrogens). The relapse is usually indicated by a rising PSA; once PSA starts rising despite hormonal therapy, the patient is designated 'castrate resistant'.

The clinical course of metastatic castration-resistant disease has changed as a result of recent developments and the duration of the course of metastatic disease may now extend to several years [4,7-10]. The optimal timing of treatments in the castrate-resistant disease is still debated and ongoing clinical trials will provide more answers.

The majority of prostate cancer immunotherapy studies have focused on men with castrate-resistant disease. There has been extensive debate as to the optimal timing of any immunotherapy intervention, immune monitoring and radiological assessment of disease status post treatment. These discussions have led to the development of novel end points for clinical trials specifically evaluating immunotherapy.

In many respects, prostate cancer is an ideal model for a cancer vaccine for a number of reasons. The presence of well-defined prostate cancer antigens such as PSA, PAP and PSMA is an advantage and opens up many potential options for immunotherapy approaches. These

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Table 1. Survival outcomes of castrate-resistant prostate cancer: comparison of immunotherapy versus other modalities.

Agent	Type of therapy	Improvement in median overall survival (months)	Hazard ratio	Reduction in death rate (%)	Ref.
Sipuleucel-T	Vaccine	4.1	0.78	22	[6]
PROSTVAC	Vaccine	8.5	0.56	44	[56]
Abiraterone	Hormone	3.9	0.66	34	[3]
Enzalutamide	Hormone	4.8	0.63	37	[8]
Docetaxel	Chemotherapy	2.4	0.76	24	[10]
Cabazitaxel	Chemotherapy	2.4	0.70	30	[4]

antigens are specific for prostatic tissue; however, as self-proteins they are not inherently immunogenic, therefore targeting these antigens becomes a lot more challenging [11]. The precise mechanism of immune response in any cancer is complex and the lack of effective biomarkers to predict response makes this treatment modality more challenging. The aim of the treatment is to activate cellular and humoral immunity, generate memory T cells that destroy cancer cells and consequently extend overall survival (OS). This activation can be achieved in a variety of ways; however, the magnitude of the response as well as targeting the right patient population remains a major challenge.

As indicated earlier, prostate cancer evolves through a number of stages and is a relatively slow-growing cancer. This provides windows of opportunity for observation and immunotherapeutic intervention. It has been recognised for some time that in men with prostate cancer, testosterone withdrawal (i.e., medical or surgical castration) leads to rapid tumor apoptosis but also a clonal CD4 and CD8 T-cell infiltration within days [12]. Prostate cancer cells express a wide array of tumor-associated antigens that may potentially be targeted (**Box 1**). PSA is the most commonly used prostate cancer marker, and is useful for monitoring disease status and response to treatment [13]. Finally, *in vivo* preclinical and early clinical trials indicate that passive and active immunotherapy has resulted in antitumor immune responses and, in some cases, tumor regression [14].

The tumor microenvironment

The aim of vaccines in cancer treatment is to induce adaptive anti-cancer immunity. Among the many potential barriers to success is the tumor microenvironment; this is a hostile arena where an evolving tumor deposit is protected against immune rejection. Overcoming, or at least abrogating, these negative factors has to become a prerequisite and incorporated into cancer vaccine design and patient selection (**Box 2**). The physical and immunological factors include the prevention of the diffusion of molecules such as antibodies and effector T cells into the tumor environment, compounded by the high interstitial pressure and hypoxemia associated with large tumor masses [15]. T cells may be of low avidity, anergic and exhausted, characterized by the expression of molecules such as PD1, B7x (B7-H4 or B7 S1) and B7-H3 [16]. Effector T cells may be dysfunctional due to local secretion of inhibitory cytokines and contact inhibition by $CD4^+CD25^+$ Tregs, myeloid-derived suppressor cells, tumor-associated macrophages and regulatory natural killer cells [17]. The large range of soluble immunosuppressive factors includes: IL-10 [18–26], TGF-β [27–29], indoleamine-pyrrole 2, 3 dioxygenase [30] and VEGF [31–33]. The negative effects of the tumor microenvironment may be overcome by removing tumor bulk, *in situ* tumor killing using agents such as oncolytic viruses, expression of immune-enhancing cytokines and a number of pharmacological agents.

Clinical trial design in prostate cancer vaccines

It has been apparent for a number of years that traditional clinical trial design involving chemotherapy and/or radiotherapy is not appropriate and relevant for agents administered for passive or active immunomodulation. Three key issues have been highlighted that may potentially influence outcomes in vaccine studies:

- Selection of patients at specific disease stage;
- Dose/scheduling;
- Evaluation of end points beyond the conventional clinical and radiological parameters.

On the whole, it is accepted that greater vaccine efficacy has been observed in patients with small-volume low-grade disease which behaves in an indolent way [34]. Assessment of efficacy is problematic post vaccination: in patients treated with chemotherapeutic agents, improved time to disease progression is thought to be essential for an improvement in OS. Chemotherapeutic agents affect tumor growth during the period of treatment and possibly for a short time post treatment, with the emergence of resistance in weeks or months. Progression or recurrence of tumor is apparent by restaging radiological scan (usually after every two cycles, using RECIST or equivalent criteria [35]), by symptomatic changes or alterations in serum cancer biomarkers such as PSA. By contrast, cancer vaccines are associated with a very different mechanism of action and the response may take over 6 months to develop [36]. Central to this is the fact that vaccine-induced humoral and/or cellular antitumor responses have indirect effects on tumor cells. These responses take time to develop and may require both a priming vaccine then frequent treatments to boost or sustain this response. Tumor cell destruction in this way may lead to a gradual cross-priming of additional tumor-associated antigens and this broadens the effect by epitope spreading [37]. This longer, more sustained response is probably more useful to the patient, but may take a long time to evolve and may lead to an OS improvement without a prerequisite progression-free survival. Therefore, treating patients early, when the tumor burden and local and systemic immunosuppression is low, would possibly allow a better long-term response to vaccine. It is likely that historically a large number of clinical trials involving

cancer vaccines have failed due to poor patient selection, including patients with end-stage disease where the tumor burden was extremely high and life expectancy short. It has been common practice to follow disease evaluation schedules (2-monthly scans) used for chemotherapy leading to withdrawal of vaccine at first evaluation after several months rather than allowing a useful response to evolve over 6–12 months [38]. These issues have been discussed extensively and led to the concept of 'immune response criteria' designed to capture delayed response to immunotherapy studies. The immune-related response criteria have been agreed in 2008 and prospectively applied to studies with ipilimumab [39]. An obvious disparity between progression-free survival, RECIST criteria and OS have been highlighted in two studies of prostate cancer (sipuleucel-T and PROSTVAC). In patients with metastatic malignant melanoma treated with ipilimumab, several examples of early disease progression on treatment followed by regression after continuation of the same antibody treatment at the same dose/schedule has been documented; as in the prostate studies a significant advantage in survival was reported without statistically significant difference in time to disease progression [6,38].

Novel end points have been proposed for vaccine studies and are currently under evaluation in a number of studies. The second Prostate Cancer Clinical Trials Working Group recently reassessed the outcome measures for vaccine trials in prostate cancer, and proposed drug evaluation pathways for cytotoxic and noncytotoxic agents be developed separately. The discussions also highlighted the often paradoxical role of PSA as a biomarker in vaccine studies. PSA is widely used to measure efficacy of treatment in castration-resistant prostate cancer (CRPC). PSA level broadly follows disease progression but the kinetics (doubling time, slope) may be more useful. New biomarkers are clearly needed and the emergence of technology to capture and enumerate circulating tumor cells and circulating endothelial cells may herald a new era in the assessment of vaccine efficacy, particularly in the setting of patients who have only a rising PSA but clear scans. Harmonization of immunological readouts has been long awaited, and may address the heterogeneity of immune responses seen in different patients on the same vaccines. As mentioned earlier, immune response-related criteria may more accurately reflect the overall biological effects of vaccination, and effectively provide an assessment of tumor volume as a continuous variable. The criteria encompass kinetics of response, response after initial progression and response in the face of new lesions. Response categories are defined as:

- Complete resolution of lesions: complete response in two consecutive observations not less than 4 weeks apart;
- Partial response: ≥50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart;
- Stable disease: 50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir;
- Progressive disease: at least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart.

Box 1. Tumor-associated antigens with potential for immunotherapy.

Proteins expressed mainly in prostate tissue:
- PSA
- PSMA
- PAP
- PSCA
- TARP
- STEAp1

Proteins overexpressed in prostate and other cancers:
- PTHrP
- hTERT
- Survivin
- EGF receptor family (HER-2/neu, EGF receptor, HER-4)
- N-cadherin
- SSX

The advantage of this system of assessment is that patients are not taken off study for the appearance of small-volume new lesions that do not increase tumor burden by ≥25%. This allows the patients to stay on study long enough to generate a sustained and useful antitumor response [38,39].

Specific approaches in prostate cancer

Cell-based immunotherapy

The rationale behind using whole prostate cancer cells as vaccines were largely based around the potentially huge antigenic repertoire expressed by prostate cancer cells which would not be HLA-restricted. There would be no need to identify individual antigens and early preclinical work indicated high efficacy of vaccines involving irradiated whole tumor cells [40]. However, tumor cells themselves are generally poorly immunogenic so a logical progression of the concept was to engineer cells to express cytokines which would enhance antigen presentation or express proinflammatory cytokines which were shown to be advantageous. Although the autologous vaccines have generally resulted in the best responses in murine models, the use of autologous cells is clearly problematic as many patients have had their prostate glands removed and any metastatic deposits are difficult to access (such as bone). Coupled with the fact that prostate cancer cells from patients are notoriously difficult to grow *in vitro*, using allogeneic whole cells was clearly the only feasible way forward. Using a combination of three nonmodified

Box 2. Factors limiting antitumor response.
- Advanced cancer with multiple metastases
- Defective antigen presentation on tumor cells
- Immunosuppressive cytokines (IL-4, IL-6, IL-10, TGF-β)
- Immunosuppressive molecules (indoleamine 2,3-dioxygenase, arginine, nitric oxide)
- Upregulation of Tregs
- Myeloid-derived suppressor cells
- Upregulation of coinhibitory signaling (PD-1, B7 family) in T cells

allogeneic cells, the Onyvax vaccine (KAEL-GemVax, Seoul, South Korea) resulted in a reduction in PSA velocity (PSAV) and a statistically significant increase in time to disease progression [40]. Out of the 26 patients, 11 showed statistically significant reduction in their PSAV. There was no significant toxicity reported. Median time to disease progression was 58 weeks; this compares favorably to historical reports of approximately 28 weeks. PSAV-responding patients showed an increased Th1 cytokine profile in response to restimulation with a vaccine lysate, while nonresponders showed a mixed Th1 and Th2 response. However, a follow-up randomized Phase IIb study failed to establish any advantage over placebo [Michael A *et al.*, Manuscript in Preparation]. The prostate GVAX program consisted of allogeneic prostate-cancer cell lines LNCap and PC-3 transfected with the *GM-CSF* gene [41]. Attempt to use this vaccine alone or in combination with docetaxel was found to be ineffective, although it did show clear evidence of prostate cancer specific humoral immunity [42]. A Phase II dose-escalation study in men with CRPC indicated a PSA stabilization in 19% of men and increased median survival (35 vs expected 23 months) with the high-dose group [43]. The GVAX program included two studies at Phase III level comparing GVAX to docetaxel plus prednisone in men with asymptomatic CRPC as well as GVAX in combination with docetaxel/prednisone versus docetaxel/prednisone alone (VITAL-1 and VITAL-2, respectively). VITAL-1 failed a futility interim analysis with a <30% chance of seeing benefit and VITAL-2 stopped due to an imbalance of deaths in the vaccine arm. However, follow-up of VITAL-2 study patients have shown no excessive death rates in the vaccine arm [41,42]. The GVAX program continues in other cancers (melanoma, pancreatic cancer and breast cancer).

Peptide vaccines

A large number of studies have evaluated peptide vaccines in prostate cancer. This was a result of identifying tumor-associated antigens and subsequently HLA-restricted epitopes most likely to induce a useful antitumor T-cell response. Although peptides have the advantage of ease of production and storage and are directed against specific tumor-associated antigens, they have largely resulted in weak immunogenicity, particularly when used as single epitopes due to tumor escape from immune recognition for antigen mutation or loss [44]. Furthermore, peptide-based strategies were based on HLA restriction and so excluded largely patients who are not HLA-A2 positive. Finally, there are indications that peptide vaccines result in optimal balance of CD4 and CD8 active T-cell leukocyte activation, which is believed to be essential for effective long-lasting antitumor immunity [44].

A new, interesting approach was introduced in a study with a novel DNA-based and peptide-based vaccine targeting melanoma antigen (PRAME) and PSMA (vaccine known as MKC1106-PP) [45]. This involved a DNA prime, dual-peptide boost immunization regimen, which comprised a recombinant plasmid (pPRA-PSM encoding fragments derived from both antigens) and two peptides (E-PRA and E-PSM derived from PRAME and PSMA, respectively). In a multicenter Phase I study, 26 HLA-A2-positive patients with refractory CRPC were treated with MKC1106-PP administered by intra-lymph node injection in a prime–boost sequence. There were no significant toxicities, and 15 out of 24 evaluable patients showed an immune response (PRAME-specific or PSMA-specific T cells in the blood). No objective response was seen by RECIST but seven patients had stable disease for ≥6 months, or PSA decline (four out of ten with prostate cancer) [45].

DNA vaccines

DNA vaccines are composed of naked DNA plasmids encoding tumor antigens. To date, DNA vaccines have had limited immunogenicity, possibly related to the low level of *in vivo* infection of antigen-presenting cells by these vaccines [46]. The optimal dose and immunization protocol has yet to be defined, and targets to date include PSMA, PSA, PSCA and STEAP. New approaches have included multiple immunizations with simultaneous administration of cytokines such as GM-CSF and IL-2 [47]. The development of new plasmid platforms encoding non-cell antigens and improved delivery systems such as liposomes, electroporation and gene gun have made significant improvements in terms of immunogenicity in cancer models [28-30]. Clinical studies using plasmid DNA with a vaccine adjuvant have increased PSA doubling time but to date this has not resulted in radiological responses nor sustained increased disease-free survival. Several studies have combined DNA vaccines encoding PAP together with GM-CSF with demonstration of PAP-specific T-cell responses and increased PSA doubling time [48]. Recently, a Phase I/II dose-escalation trial of a DNA fusion vaccine was reported which encodes a domain (DOM) from fragment C of tetanus toxin linked to an HLA-A2-binding epitope from PSMA [47-55]. Delivery by intramuscular vaccination without or with electroporation was employed. A total of 32 HLA-A2$^+$ patients were vaccinated and monitored for immune and clinical responses for a follow-up period of 72 weeks. The vaccine induced DOM-specific CD4$^+$ and PSMA(27)-specific CD8$^+$ T cells. PSA doubling time increased significantly from 11.97 months pretreatment to 16.82 months over the 72-week follow-up.

RNA vaccines

Despite the relative lability of RNA, there are a number of advantages of mRNA over DNA in the vaccine context. There is no risk of insertion or mutagenesis, no need to define and incorporate an efficient promoter and, unlike DNA, the nuclear membrane is not a major obstacle for mRNA as it exerts its function in cytoplasm and avoids vector-induced immunogenicity [53]. The RNA approach has been evaluated both through the injection of naked mRNA, the injection of mRNA encapsulated in liposomes, gene gun delivery and *in vitro* transfection of dendritic cells (DCs) followed by subsequent delivery to the patient [53]. mRNA can be produced in large amounts at a higher degree of purity and with a lack of induction of antibodies. The same mRNA molecule can theoretically provide an antigen source for adaptive immunity and simultaneously bind to pattern-recognition receptors, thus stimulating innate immunity. Despite these advantages very few studies have actually been completed [54,55]. DCs transfected with mRNA from allogeneic prostate cancer cell lines (DU145, LNCaP and PC-3) have been used in a clinical trial of patients with prostate

cancer [56]. A total of 12 out of 20 patients treated developed a specific immune response to tumor-mRNA-transfected DCs and 13 patients showed a decrease in log slope PSA. CV9103 is an mRNA-based vaccine encoding for specific prostate antigens (PSA, PSCA, PSMA and STAP1)[57]. An initial Phase I/II study reported a high degree of safety and tolerability and a high level of cellular immunogenicity. Antigen-specific T cells were detected in 79% of patients independent of their HLA background. The majority (19–58%) had induction T-cell response against multiple antigens and regardless of their cellular localization, encouraging stabilization of PSA levels after an initial rise was seen, with one patient developing a greater than 85% drop in his PSA level. Attempts to evolve strategies transfecting mRNA onto autologous DCs have been hampered by cost and complexity, and one study reported immune responses and PSA-stabilizing effects [54,55].

Viral vaccines

There is an accumulating body of evidence supporting the use of viral vectors as cancer vaccines. This advantage centers around high levels of gene expression associated with viral vectors and the strong inflammatory response directed against the viral protein [44]. Viral vaccines are straightforward to engineer and able to carry large amounts of genetic material including multiple antigens. Much of the advanced work has centered on pox viral vectors, and vaccinia in particular [58]. The Pox virus family is composed of double stranded DNA viruses which do not integrate with the host genome and replicate within the cytoplasm of infected cells. Despite a vigorous host immune response, poxvirus vectors induce an immune response by direct infection of antigen-presenting cells such as Langerhans cells in the skin. The use of live oncolytic viruses has improved the efficacy of this approach. Most recently, a heterologous prime–boost approach has been used encompassing oncolytic virus expressing antigen as the priming agent and then a nonreplicating different virus as a boosting agent [59]. The most advanced vaccine using this approach is the PROSTVAC strategy, which comprises two recombinant viral vectors (vaccinia and fowlpox) each encoding transgenes for PSA and TRICOM. TRICOM consists of costimulating molecules including intercellular adhesion molecule-1, B71 and leukocyte function-associated antigen 3 [60]. In a double-blind randomized Phase II study in asymptomatic patients, 82 patients received PROSTVAC and 40 received controlled vectors. There was no difference in the progression-free survival and although initially the trial was reported as negative, after 3-year follow-up of the study patients, the patients with PROSTVAC were found to have significantly improved OS, 25.1 versus 16.6 months ($p = 0.0061$), and a better 3-year survival, 30 versus 17% (and a 44% reduction in death rate) [6]. Based on these results multiple clinical trials in different stages of prostate cancer were designed, including a large Phase III registration study.

A modified vaccinia virus incorporating the 5T4 antigen has been extensively evaluated as a cancer vaccine known as TROVAX [40]. 5T4 is a cell surface glycoprotein expressed by many cancers [61]. Unlike PROSTVAC, the TROVAX approach consists of homologous boost injections after initial priming and does not include any costimulatory molecules. Preclincial data suggested TROVAX functions through CD4 cell induction and that CD8 cells are not required. A recent study showed PSA reduction in five out of 27 men treated in conjunction with GM-CSF [62].

Viral cDNA libraries as cancer vaccines

One of the key limiting factors to cancer vaccine efficacy is overcoming immune tolerance induced by the evolving cancer. Attempts to overcome this have included *in situ* tumor kill using oncolytic viruses to generate 'danger signals' and upregulation of heat-shock proteins. When immune escape occurred with suboptimal vaccination, the tumor cells were readily treated by another hit of second-line, virus-based immunotherapy. Use of the cDNA library allows presentation of a broad panel of (undefined) tumor-associated antigens, which subsequently reduces emergence of treatment-resistant clones and paves the way to rational, combined-modality approaches in the clinic. In a similar way, multiple intravenous injections of a cDNA library, derived from human melanoma cell lines and expressed using vesicular stomatitis virus, cured mice with established melanoma tumors. Successful tumor eradication was associated with the ability of mouse lymphoid cells to mount a tumor-specific $CD4^+$ IL-17 recall response *in vitro* [63]. The advantages of viral vectors include systemic delivery without the need for tumor targeting as well as the ability to produce the vaccines to a clinical grade. Virus-expressed cDNA libraries represent a novel and promising modality of cancer immunotherapy that can potentially address many of the key issues that have undermined the efficacy of cancer vaccines to date.

Targeting checkpoint blockade molecules CTLA-4 & PD-1

CTLA-4 is a key negative regulator of T-cell responses, inhibiting recognition of cell antigens by T cells, and has the ability to down regulate the antitumor immune response. Ipilimumab and tremelimumab are fully humanized monoclonal antibodies against CTLA-4 [64–67] Ipilimumab has been approved by the FDA after showing improved OS in metastatic melanoma [68]. CTLA-4 is associated with marked toxicities potentially including phenomena such as autoimmunity, enterocolitis, hypophysitis and dermatitis. A large number of clinical Phase I and II trials have been conducted in prostate cancer with ipilimumab with objective clinical and PSA responses. Based on these early studies further randomized clinical trials are currently in progress [69,70].

Interaction between PD-1 and its ligand PD-L1 (also known as B7-H1) leads to the inhibition of T-cell function. Blockade of this pathway is associated with significant antitumor immune response in murine models [71]. B7-H1 has been upregulated in a variety of human cancers and is associated with worse outcomes [72]. In a recent Phase I study, anti-PD-1 showed antitumor efficacy in a range of cancers [71], with a correlation between PD-L1 expression on the patient's tumor and objective response. As PD-L1 is expressed on prostate cancer cells, this strategy is attractive for CRPC patients [73].

Vaccines in combination with other treatment modalities

In keeping with cancer therapy for advanced disease, in general it is most likely that combination rather than single-agent

treatments will most likely lead to long-term disease control. The choice of the second agent depends on a number of factors for synergistic antitumor effects, and may be achieved through a broad range of modalities (**Box 3**). Broadly, the efficacy of cancer vaccines is greatly enhanced when combined either with another agent to increase immunogenicity or with agents that reduce immune suppression [74,75]. A large number of potential immunostimulants have been evaluated often as single agents and then potentially 'retired' by the pharmaceutical industry due to lack of individual efficacy. These include GM-CSF [76], interferon [77], IL-2 [78], IL-15 [78], IL-7 [79,80] and FLT3 ligand [80]. GM-CSF has been used successfully in combination with a prostate cancer vaccine (GVAX) described earlier, where whole tumor cells used as vaccines were stably transfected with *GM-CSF*. *GM-CSF* is now being incorporated into a number of oncolytic viruses to enhance immunogenicity of virus-induced local tumor kill [81]. A number of TLR agonists have also been used in combination with vaccines as immunological adjuvants [82]. Cytokines such as interferon enhance immune response by increasing expression of tumor-associated antigens and MHC molecules on tumor cells [83]. Other cytokines such as IL-2 may have an opposite effect with stimulation of Tregs, thereby increasing immunosuppression, and may also be responsible for T-cell exhaustion [84].

Combination therapy has aimed at abrogating or deleting immunosuppressive T cells, which inhibit cytotoxic T cells from mounting an antitumor response. Tregs have the phenotype $CD4^+CD25^+FOXP3^+$ and have been implicated in cancer-associated immune suppression for many years [84]. Attempts to delete or abrogate Tregs during vaccine therapy have resulted in improved outcomes in murine models and recently in a study in renal cancer [85]. Methods of targeting Tregs include low-dose cyclophosphamide (bolus or metronomic schedule), denileukin (IL-2 fused to a diphtheria toxin) [86], monoclonal antibodies and small molecule inhibitors of TGF-β [87].

Box 3. Treatment modalities that may enhance vaccine efficacy.

Chemotherapy
- Immunogenic tumor cell death, tumor cell apoptosis
- Expression of proinflammatory cytokines
- Antiangiogenic

Radiotherapy
- Tumor-cell apoptosis
- Immunogenic cell death
- Immunogenic tumor death

Oncolytic virus therapy
- Tumor-cell apoptosis and/or necrosis
- Antiangiogenic

Hormonal therapy
- Clonal T-cell response

Monoclonal antibodies
- Enhanced antibody-dependent cell-mediated cytotoxicity

Prostate cancer vaccines are also being combined with immune checkpoint inhibitors, which are molecules capable of reversing immune suppression. The approval of ipilimumab by the FDA for metastatic melanoma has paved the way for a number of new studies also including prostate cancer. A recent clinical trial involves evaluation of PROSTVAC vaccine in combination with ipilimumab and this has already shown improved survival in patients with metastatic prostate cancer compared with previous trials with PSA-TRICOM as monotherapy [60].

Although initially thought to be contradictory, there is now evidence that a combination of vaccine with traditional anticancer treatment modalities may result in enhanced vaccine efficacy. Several chemotherapeutic agents may enhance antitumor T-cell responses by a number of different mechanisms. These include anthracyclines, gemcitabine and oxaliplatin [88]. Radiotherapy including radiolabeled monoclonal antibodies and bone-targeted radiotherapy agents may also have similar effects [89]. The taxanes as a group have been associated with immune modulation for a number of years. Agents such as docetaxel have been shown to increase expression of tumor-associated antigens, peptide MHC complexes, adhesion molecules and death receptors, it also suppresses myeloid-derived suppressor cells [90]. Low-dose paclitaxel has been shown to enhance DC function [91] and a combination of docetaxel was evaluated in combination with GVAX and more recently PROSTVAC vaccine [60]. In men with prostate cancer, hormonal therapy leads to early vascular collapse, tumor cell apoptosis and the infiltration by a clonal population of T cells; therefore among patients who are or previously hormone naive, antigen deprivation seems to be an ideal opportunity for introducing a cancer vaccine. Randomized trials that are using PROSTVAC vaccine in combination with antiandrogen hormone therapy are ongoing.

Vaccine using antigen-presenting cells

DCs are the most potent antigen-presenting cells known. Their evaluation as cancer vaccines has been intensive over the last 15 years and numerous Phase II studies have evaluated the use of DCs pulsed with peptide or protein infected with viruses or RNA to treat a range of solid malignancies [63]. The sipuleucel-T vaccine was approved in 2010 by the FDA for the therapy of asymptomatic metastatic castrate-resistant prostate cancer. The vaccine consists of antigen-presenting cells (and not a pure population of DCs) extracted from peripheral blood mononuclear cells pulsed with PAP fused to GM-CSF [6]. The vaccine protocol included a leukapheresis step to purify peripheral blood mononuclear cells from patients and vaccine manufactured in a central facility where PAP fusion protein was pulsed onto the cells. The vaccine was subsequently infused back to patients three times at biweekly intervals. In the landmark Phase III trial (IMPACT) 512 men with asymptomatic chemotherapy-naive metastatic CRPC were randomized in a 2:1 ratio to sipuleucel-T or placebo [6]. The primary and secondary end points of IMPACT were OS and progression-free survival, respectively. The results were positive with a significantly improved median OS in the sipuleucel-T group compared with placebo and a relative reduction of 22%

in the risk of death in the sipuleucel-T group (hazard ratio: 0.78; p = 0.03). It is expected that the response rate was minimal and the time to objective disease progression was similar in the two groups. Therefore, OS was improved without any measurable antitumor effects and this may reflect the time delay taken for a generation of useful antitumor immunity. Antibody responses against the antigen were observed in 66% of patients in the sipuleucel-T group versus 3% in placebo. Interestingly, although both T-cell and antibody responses to vaccine were observed, only antibody responses were associated with extension of survival [6,11]. The vaccine was well tolerated, with mild to moderate toxicities including fever, fatigue, nausea, chills and headache. Impressively, the survival benefit of sipuleucel-T was consistent across a range of adverse prognostic factors such as PSA level, LDH level and alkaline phosphatase as well as presence of bone metastasis, Gleason's score, performance status and presence of pain. IMPACT reflected the same outcomes as two smaller randomized studies. A number of further studies ongoing include a neoadjuvant Phase II study of sipuleucel-T prior to radical prostatectomy and a randomized Phase II study of patients with nonmetastatic cancer with biochemical recurrence (ClinicalTrials.gov identifier: NCT00715104 [101]). It is not clear whether the optimal scheduling is to administer vaccine before or after androgen ablation [2].

The main drawbacks of sipuleucel-T are the high cost, the requirement for leukapheresis and the limited number of vaccines that can be used. In addition, data extracted from FDA documents revealed that more than 65% (median) of the cells harvested from patients were lost at the manufacturing, indicating the complexity of the process and limitations regarding widespread use [92]. Further analysis of the IMPACT study also suggested that the effect of vaccination varied in the younger and older age group (with 65 years of age used as a cutoff), with older patients deriving a lot less benefit from sipuleucel-T [92]. Ongoing studies will hopefully shed more light on the unanswered questions.

Conclusion

Prostate cancer is an ideal clinical model for the application of cancer vaccines. The first FDA approval of a cancer vaccine was for this disease, and the endorsement of sipuleucel-T has been a significant moment in cancer immunotherapy overall. As well as the identification of new tumor antigens and vaccine delivery platforms, overall efficacy will increase by addressing the powerful limiting factors within the tumor microenvironment. Combined with careful patient selection and the use of new immune response criteria we would expect an incremental improvement in efficacy. The extension of vaccines to the adjuvant and minimal residual or minimally progressive disease scenarios are ongoing, as well as combination with different treatment modalities.

Expert commentary

The treatment of prostate cancer is changing rapidly. More patients are diagnosed early and cured, and those who develop metastatic disease are able to enjoy a longer life. Further developments are needed and many of those will focus on treatment modalities and the correct schedule of various options such as surgery, radiotherapy, chemotherapy, hormonal treatment and immunotherapy. The approval of sipuleucel-T as treatment for prostate cancer gave immunotherapy a defined role and helped to understand the dynamics of response to vaccine in cancer patients.

Five-year view

The next 5 years will hopefully see progress in new immunotherapy approaches in the field of vaccines as well as antibody treatments. The trials will focus on timing of immunotherapy in patients with early disease or those who develop castrate-resistant cancer but without any metastatic disease. The process has to be simplified and vaccines such as whole cell vaccines or gene therapy treatments will hopefully show efficacy equivalent to adoptive cell transfer methods, making the immunotherapy treatments easily accessible and available for more patients. Antibodies targeting checkpoint blockade are likely to become part of the treatment pathway.

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Key issues

- New developments in prostate cancer treatment have had an impact on the survival and quality of life in cancer patients.
- The US FDA approval of sipuleucel-T was a landmark development as the first vaccine approved for an advanced solid malignancy.
- New end points to assess the efficacy of immunotherapy treatments are essential and need to be widely adopted (immune-related response criteria).
- Cell-based immunotherapy approaches are very advanced but none of the treatments have yet been approved and new well-designed trials that take into account immune-related response criteria are essential.
- Currently the only approved vaccine is based on using the host's antigen-presenting cells pulsed with PAP fused to GM-CSF. The production is complex, limited by high cost and not widely available to cancer patients.
- Antibodies targeting checkpoint blockade such as anti-CTLA4 and anti-PD1 antibodies are likely to become part of standard treatment in the near future.

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