Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Novel immunotherapeutic agents for castration-resistant prostate cancer: update from clinical trials

Clin. Invest. (2013) 3(7), 651–663

Immunotherapy has been recognized as a viable therapeutic approach since the regulatory approval of the autologous cell-based vaccine sipuleucel-T in 2010. Emerging preclinical evidence and early-stage clinical studies point to a potential synergy between currently available immunotherapeutic agents and standard anticancer therapies such as radiation and chemotherapy. Several other immunotherapeutic platforms, such as immune checkpoint inhibitors and DNA- and peptide-based vaccines are also in development and clinical testing. Based on the logistics of their production, these platforms are broadly categorized as patient-specific or off-the-shelf. Together, they are beginning to transform the therapeutic landscape in prostate cancer. This article reviews the rationale behind immunotherapeutic approaches in castration-resistant prostate cancer, plus the latest available clinical data.

Keywords: cancer vaccine • combination immunotherapy • immune checkpoint inhibitors • immunogenic modulation • immunotherapy • ipilimumab • metastatic castration-resistant prostate cancer • PSA-TRICOM • radiation therapy • sipuleucel-T

Nishith Singh, Ravi Madan & James L Gulley*

[1]Laboratory of Tumor Immunology & Biology & Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
*Author for correspondence:
Tel.: +1 301 435 2956
Fax: +1 301 480 5094
E-mail: gulleyj@mail.nih.gov

For patients with metastatic castration-resistant prostate cancer (mCRPC), eventual resistance to therapy is inevitable, despite the use of androgen-deprivation therapy (ADT) and antiandrogen therapy. The median time to development of castration resistance, as indicated by an increase in PSA after the initiation of ADT, is approximately 19 months in nonmetastatic prostate cancer (biochemical failure with no radiographic disease) and approximately 13 months in metastatic disease [1]. Efforts to find safe, effective and durable therapeutic modalities for these patients are ongoing.

In 2004, two Phase III clinical trials reported a survival advantage with the chemotherapy agent docetaxel in men with symptomatic mCRPC [2–4]. The evidence of palliation and overall survival (OS) benefit with chemotherapy led to the approval of docetaxel by the US FDA. 6 years later, following the positive published results of a Phase III trial, cabazitaxel, a docetaxel analog, was approved by the FDA as a second-line chemotherapeutic agent in men with symptomatic mCRPC [5]. Treatment options for advanced prostate cancer have expanded with better understanding of the molecular biology of castration resistance. The last few years have seen the emergence of a number of novel second-generation antihormonal therapies, such as androgen receptor antagonists (ARAs), and newer inhibitors of androgen synthesis, such as CYP17A1 enzyme inhibitor (abiraterone), that improve OS in mCRPC [6,7].

Immunotherapy has also shown considerable success as an alternative therapeutic strategy in prostate cancer, which may be especially amenable to immunotherapeutic approaches for several reasons. Prostate cancer cells express a number of targetable antigens such as PSA and PAP. Furthermore, the slow progression of prostate cancer allows time for an immune response to develop [8–10]. The potential of immunotherapy in prostate cancer is highlighted by the success of sipuleucel-T (PROVENGE®, APC8015;

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Dendreon, Seattle, WA, USA), which was approved by the FDA in 2010 for asymptomatic or minimally symptomatic mCRPC [11]. Sipuleucel-T is a patient-specific therapeutic cancer vaccine, and the first therapeutic vaccine to show survival benefit in any malignancy.

In the last few years, multiple novel vaccine platforms have emerged that target different biological pathways (Figure 1; see Table 1 for vaccine platforms in Phase II/III clinical trials in prostate cancer). These immunotherapeutic agents are categorized as either patient-specific or off-the-shelf. Patient-specific immunotherapeutic agents such as sipuleucel-T are produced from immune cells or tumor cells isolated from the patient [12–14]. A major, relevant clinical difference between the two types of vaccine lies in the fact that while patient-specific vaccines have been proven safe and effective, they are resource-intensive. An off-the-shelf approach, on the other hand, avoids the resource-intense manufacture and release of patient-specific vaccines.

This article reviews the clinical evidence on sipuleucel-T and newer experimental immunotherapeutic strategies for CRPC.

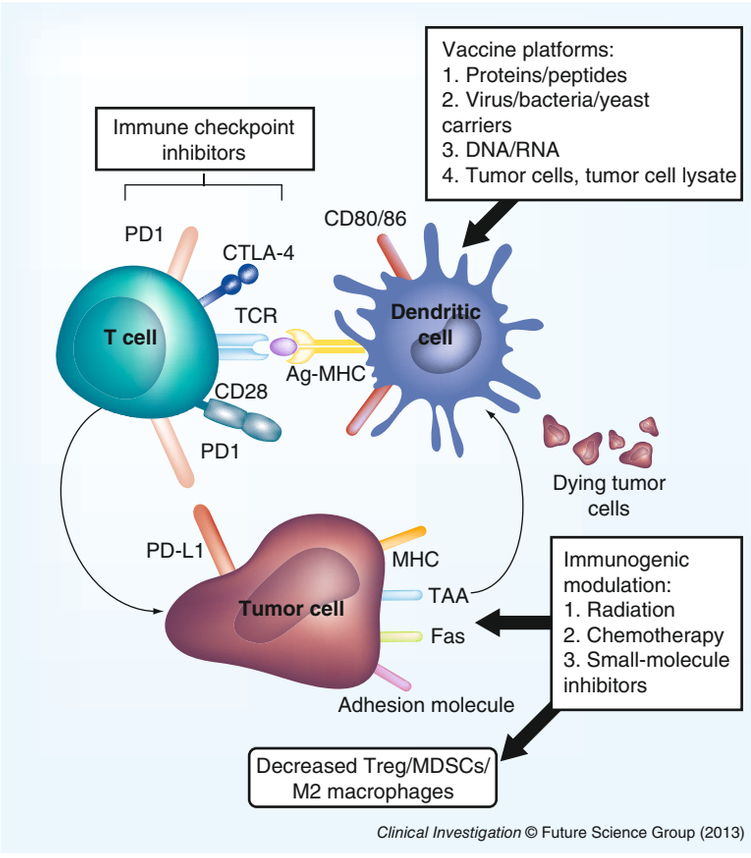

Figure 1. Mechanism-based sites of action of current immunotherapeutic agents in prostate cancer.
MDSCs: Myeloid-derived suppressor cells; TCR: T-cell receptor.

Mechanism of immunotherapy

While both the humoral and cellular (T cells and natural killer [NK] cells) immune system take part in surveillance against cancer, the latter plays the major role. T cells (T helper, $CD8^+$ and regulatory T cells) in particular, along with antigen-presenting cells (APCs; dendritic cells) form the fundamental basis of the adaptive host immune system [15]. Depending on the delivery system used (including host mononuclear cells pulsed with antigen, viral vector and whole tumor cells), current vaccines are designed to elicit an antitumor immune response against one or multiple tumor antigens. APCs can activate T cells by efficiently processing antigens (PSA, for instance) carried by the vaccines and presenting them to T-cell receptors, thereby triggering a cytotoxic antitumor response and antigenic memory in the long term. MHC antigen-processing machinery plays a critical role in the processing of antigens for recognition of tumor cells by cytotoxic T cells. Diversified prime-boost regimens such as PSA-TRICOM, or concurrent vaccination with two distinct vaccine platforms targeting the same antigen, may elicit higher antitumor immunity [16,17].

Whole tumor cell vaccines, such as GVAX, generate an immune response to several antigens both known and unknown. Vaccines such as PSA-TRICOM have been designed with the goal of using PSA as the specified target, although the downstream epitope landscape generated by this vaccine may be broader. As discussed below, several standard chemotherapeutic agents, as well as radiation therapy, are capable of causing immunogenic modulation of the tumor bed and host immune system that renders tumors susceptible to cytotoxic cell killing. This forms the basis of combinatorial regimens, for host compromise is primarily driven by cancer cells that have escaped standard strategies. Conversely, negative co-stimulatory molecules on T cells, such as CTLA-4, PD-1 and PD-L1, on the tumor bed can induce immune tolerance. Immune checkpoint inhibitors such as ipilimumab help abrogate this tolerance and may potentially synergize with vaccines to control tumor growth (Figure 1) [18].

Standard anticancer agents versus immunotherapeutic agents

There are major mechanistic differences between standard antitumor approaches, such as chemotherapy, and immunotherapy using cancer vaccines. The former principally target the tumor and its microenvironment, while the latter targets the immune system to initiate a potentially expandable attack on the tumor through not only a quantitative expansion of tumor-directed T cells, but also by enhancing two

Table 1. Immunotherapeutic platforms in Phase II/III prostate cancer trials.

Name	Constituents	Mode of administration	Ref.
Patient-specific immunotherapeutic agents			
Sipuleucel-T†	Autologous mononuclear cells pulsed with PAP–GM-CSF fusion protein	Three cell product (with minimum of 40 million large cells expressing the co-stimulatory molecule CD54) infusions, 2 weeks apart for a total of three doses	[11]
DeCIDe™ (PSMA)	Therapeutic DC vaccine targeting PSMA designed to manipulate autologous DCs *in situ*	BPX–101, which targets PSMA, is administered intradermally twice weekly for six doses, followed 24 h after each dose by intravenous infusion of DC signal molecule 'dimerizer' AP1903 (0.4 mg/kg)	[100]
Off-the-shelf immunotherapeutic agents			
PSA-TRICOM	Poxviral vector with three co-stimulatory molecules and PSA	rV-PSA-TRICOM on day 1 with GM-CSF followed by boost rF-PSA-TRICOM starting day 15, every 28 days thereafter	[8]
GVAX	Prostate cancer cell lines (LNCaP and PC-3) engineered to express GM-CSF at the vaccine site	500 million cells with the prime dose followed by 300 million cells with each booster dose every 3 weeks for ten cycles, followed by maintenance immunotherapy alone (every 4 weeks)	[101]
DNA-based	Plasmid DNA vaccine encoding PAP	Intradermal, monthly boosters	[22]
PPV	Peptide set selected on the basis of highest levels of peptide-specific IgG	Subcutaneous, 6 weekly	[66]
mRNA-based	DC transfected with mRNA encoding a LAMP hTERT protein	Intradermally, weekly	[102]
Ipilimumab‡	Monoclonal antibody against CTLA-4	3–10 mg/kg intravenous infusions, induction/maintenance regimens, every 3 weeks for four cycles	[18]

†Cost is $31,000 per infusion [209].
‡Cost is $30,000 per injection [210].
DC: Dendritic cell.

important qualitative aspects of T-cell response: avidity and expandability [19]. Only high-avidity T cells can efficiently lyse tumor cells with low concentrations of antigen [20]. Conversely, T-cell expandability, also known as epitope spreading or antigen cascade, involves cross-presentation of tumor antigens released during the lysis of tumor cells [21]. In addition, unlike a cytotoxic agent, a vaccine promulgates a T-cell memory response, leading to different kinetics of clinical response. The initial immune response is slow and narrow, with no perceptible impact on tumor growth rate. However, over time the immune response takes off. Furthermore, through antigen cascade the target antigen landscape also evolves [22,23]. The immune response to the vaccine thus grows broader and more potent, lasting beyond the time of treatment and potentially exerting a more sustained impact on tumor growth rate. These unique biological characteristics may provide an explanation for the substantial improvement of OS seen with the use of immunotherapeutic agents, despite no improvement in median progression-free survival (PFS). This lack of short-term clinical benefit was evident in the Phase III trials of sipuleucel-T in prostate cancer, and ipilimumab in metastatic melanoma and the randomized Phase II trial of PSA-TRICOM vaccine [11,24,25].

Antigen cascade has additional implications for immunotherapy. Since therapeutic vaccines are relatively well tolerated and have the potential for long-term clinical benefit, administration of vaccines at earlier stages of disease will probably provide the most benefit. However, early visible shrinkage of tumors may not be expected from this approach, and there are no clinically validated biomarkers of response on follow up.

Sipuleucel-T

Sipuleucel-T ushered in a new age of therapy for prostate cancer [14]. This patient-specific therapeutic vaccine is designed to stimulate the immune system to target and eliminate prostate cancer cells. The approach involves obtaining peripheral blood mononuclear cells by leukapheresis from a prostate cancer patient. After centrifugation and washing, the remaining cells, including APCs, are incubated with PA2024 at 37°C for 36–44 h. PA2024 is a recombinant protein of PAP fused at its C-terminus to the

N-terminus of GM-CSF [26]. PAP is the target tumor-associated antigen (TAA). After postincubation processing, the autologous cell product, now enriched for $CD54^+$ cells, is transfused back into the patient following standard pretreatment.

An initial Phase I/II trial of sipuleucel-T conducted in 31 patients with nonmetastatic CRPC demonstrated safety [27]. Patients were treated with escalating doses of activated cells at 0, 4 and 8 weeks, with an option to receive another dose at 24 weeks. Those receiving higher doses were more likely to have improved time-to-progression (TTP) and enhanced T-cell proliferation and antibody responses. A second Phase II trial in patients with mCRPC again demonstrated safety and tolerability [28]. One patient experienced a complete response during the study period. Median TTP was 118 days and two patients showed PSA declines of 25–50%. Immune responses were predominantly T-cell proliferation responses to PA2024.

A consistent improvement in OS was demonstrated in two subsequent randomized controlled trials (D9901 and D9902A) in minimally symptomatic mCRPC [29,30]. The placebo in each study was the patient's APCs not pulsed with PA2024. The two trials enrolled a total of 225 men, and had a primary end point of TTP. Sipuleucel-T brought about an insignificant improvement in TTP in the first trial. However, a follow-up analysis of the first trial (D9901; n = 127) demonstrated an OS advantage in favor of sipuleucel-T (25.9 vs 21.4 months for placebo; p = 0.01). At 36 months follow up, OS was 34% for sipuleucel-T versus 11% for placebo (p = 0.005). A trend toward improved OS was demonstrated in the treatment arm of D9902A, without a significant improvement in TTP. Based on these compelling data, and the lack of treatment effect on TTP, a larger randomized Phase III trial, IMPACT, was designed with OS as the primary end point [11].

In the IMPACT trial, 512 asymptomatic or minimally symptomatic mCRPC patients were randomized 2:1 to receive three infusions of sipuleucel-T versus placebo. Placebo was prepared using a third of the APCs obtained from leukapheresis. The trial demonstrated an OS benefit in favor of sipuleucel-T, with a median survival of 25.8 versus 21.7 months for patients in the placebo arm (p = 0.02). While approximately 55% of men in both groups received docetaxel after the study, a sensitivity analysis discovered that subsequent chemotherapy did not alter the difference in outcomes. Notably, the survival benefit was seen despite a crossover of 49.1% of placebo subjects to receive cryopreserved sipuleucel-T. However, as in earlier studies, TTP was not significantly different between the two treatment groups. A subsequent meta-analysis of the three randomized, placebo-controlled trials (n = 737) found OS to be significantly longer with sipuleucel-T compared with placebo (hazard ratio [HR]: 0.73; 95% CI: 0.61–0.88; p = 0.001) [31].

Sipuleucel-T was very well tolerated, especially when compared with established chemotherapies for prostate cancer. Infusion-related chills, fever, headache, flu-like symptoms, myalgia, hypertension, hyperhydrosis and groin pain were more frequent in the sipuleucel-T group than in the placebo group. Most of these toxicities resolved in 1–2 days. Grade 3 adverse events (AEs) within 1 day of infusion were seen in 6.8% of sipuleucel-T-treated patients, with AE-related drug interruptions seen in <1% of patients. The FDA's approval of sipuleucel-T in mCRPC was based on these results, and the National Cancer Center Network Prostate Panel has added sipuleucel-T as a category 1 treatment recommendation for mCRPC. Despite cost concerns, the Centers of Medicare and Medicaid Services have stipulated sipuleucel-T as a necessary and reasonable treatment modality.

A prespecified immune analysis of 151 patients from both treatment arms of the IMPACT trial revealed interesting correlative data: 66.2% in the sipuleucel-T arm and 2.9% in the placebo arm had antibody titers to PA2024 > 1:400 [11], and these patients had improved survival compared with patients with titers <1:400 (p < 0.001). Antibodies to PAP were found in 28.5% of evaluable patients in the sipuleucel-T arm, compared with 1.4% of evaluable patients in the placebo arm. T-cell proliferation responses to both PA2024 and PAP were higher in the sipuleucel-T group compared with the placebo group. A follow-up immunological analysis of data from three Phase III trials of sipuleucel-T found evidence of antigen-specific immune responses in 78.8% of monitored subjects, and these responses correlated with OS (p = 0.003) [32].

An exploratory analysis of IMPACT divided the enrolled subjects (n = 512) into baseline quartiles (≤22.1, >22.1–50.1, >50.1–134.1 and >134.1 ng/ml) [33]. Although not prespecified and not powered for significance, consistent survival benefit with sipuleucel-T over placebo was seen across the quartiles. A trend towards higher survival benefit (41.3 months OS with sipuleucel-T vs 28.3 months with placebo; HR: 0.51; 95 % CI: 0.31–0.85) was seen in the lowest quartile, suggesting that the vaccine may have the greatest efficacy in lower-burden disease (in the highest quartile, the OS difference between sipuleucel-T and placebo was 2.8 months in favor of the former; HR: 0.84; 95% CI: 0.55–1.29).

In a separate retrospective analysis, 26 patients (16 in the long OS group and ten in the short OS group) who had bone scans on IMPACT and who received

sipuleucel-T were stratified as those with low bone metastatic burden (<7 baseline lesions, an increase of ≤2 lesions at week 10, and an increase of ≤5 lesions at week 18) and high metastatic burden [34]. Investigators could correctly predict long OS in 76% of patients and short OS in 67% of patients (81% sensitivity, 60% specificity, 73% crude agreement [p = 0.029 vs uninformed 50% rate of correct prediction]). Although the study lacked a placebo comparator, investigators concluded that lower tumor burden and slow disease progression may increase the likelihood of prolonged OS, which is consistent with emerging data showing that therapeutic vaccines may have the most benefit in lower-burden disease [35].

Randomized trials of sipuleucel-T have raised a number of concerns, including the effect on outcome of advanced age and immune-depletion brought about by leukapheresis [36]. However, a subgroup analysis of the IMPACT trial did not confirm an association between advanced age and vaccine efficacy. In addition, leukapheresis removes only 0.1–1.4% of the total body pool of lymphocytes [37], with a decline in peripheral circulating lymphocyte count of approximately 10% after two to nine procedures without a significant impact on patients' immune status [38]. Furthermore, no difference in infection rates between the arms was noted in the Phase III trial, suggesting a preserved immune status for patients in the placebo arm [39]. Lymphocytic proliferation that probably follows any leukapheresis-associated transient leukopenia would be expected to drive an antitumor response [40].

PSA-TRICOM

A collaborative effort between Bavarian-Nordic Immunotherapeutics (CA, USA) and the National Cancer Institute led to the development of an off-the-shelf poxviral-based cancer vaccine consisting of a recombinant vaccinia prime and multiple boosts of recombinant fowlpox. As with cellular-based vaccines such as sipuleucel-T, the goal of vector-based strategies is to induce a dynamic tumor response propagated by the adaptive immune system [41,42]. In clinical trials for prostate cancer, the poxviral vector is encoded with transgenes for PSA, which is overexpressed in the vast majority of prostate cancer patients, as well as the co-stimulatory molecules B7.1 (CD80), ICAM-1 (CD54) and LFA-3 (CD58), to enhance immune responses [43]. This vaccine is designated PSA-TRICOM (PROSTVAC®). Preclinical studies demonstrated that the three co-stimulatory molecule transgenes act synergistically to greatly enhance the number and avidity of T cells [44–47].

A heterologous prime and boost strategy with poxviral vaccines was tested in an earlier Phase II trial [48]. Vaccinia and fowlpox complement each other, as the former initiates an immune response while the latter boosts it without stimulating neutralizing antibodies. A vaccinia priming dose followed by fowlpox boosts was found to be the optimal dosing schedule. In the Phase II trial, 78.1% of patients demonstrated clinical PFS. A Phase I trial of PSA-TRICOM combined with GM-CSF administered monthly on the same 'prime-and-boost' schedule was proven to be safe [49], with no toxicity > grade 2. The most common grade 2 toxicity was injection-site reaction (~50%); less common were grade 2 systemic bone pain, pyogenic granuloma and hyperhydrosis (6.7%).

Two Phase II studies of PROSTVAC-VF/TRICOM on a monthly dosing schedule have been completed in mCRPC. A multicenter Phase II trial randomized patients (n = 125) 2:1 in favor of vaccine versus empty vector (wild-type poxvirus). Patients enrolled had a Gleason score of ≤7 and no evidence of visceral metastasis [24]. As with sipuleucel-T, TTP (the primary end point) was not found to be significantly different between the vaccine and placebo arms, but median OS was 25.1 months in the vaccine arm compared with 16.6 months in the control arm (HR = 0.56; p = 0.0061). In a single-arm Phase II study of PSA-TRICOM at the National Cancer Institute, 32 patients with mCRPC were enrolled regardless of Gleason score. The median OS was 26.6 months, and immunologic analysis revealed that 13 of 29 evaluable patients had a greater than twofold increase in PSA-specific T cells [50]. An ongoing randomized, double-blind, Phase III trial will evaluate the efficacy of PSA-TRICOM with or without GM-CSF as local immune adjuvant in patients with asymptomatic or minimally symptomatic mCRPC [201]. With an estimated enrollment of 1200 patients and OS as the primary end point, this three-arm study will compare PSA-TRICOM with and without GM-CSF versus placebo.

Immune checkpoint inhibitors

Ipilimumab (Yervoy®; Bristol-Myers Squibb, NY, USA) is a fully human IgG1κ monoclonal antibody (mAb) that targets CTLA-4. CTLA-4 is a CD28 homolog membrane protein on T cells that regulates the early activation of naive and memory T cells following engagement of T-cell receptors with APCs [201]. Ipilimumab was the first in a class of therapies targeting T-cell activation and regulation to be licensed in the broad category of agents known as immune checkpoint inhibitors. Ipilimumab has been shown to extend survival in metastatic melanoma, and has been tested in multiple studies as monotherapy and in combination with other agents in the treatment of prostate cancer [25].

A pilot study (n = 14) of intravenous ipilimumab as monotherapy in mCRPC showed evidence of safety [51].

Two of 14 patients had a decline in PSA of >50% that lasted 135 and 60 days, respectively; eight of 14 patients had a <50% decline in PSA. A unique mechanism-based set of toxicities known as immune-related AEs has been noted with the use of anti-CTLA-4 inhibitors. The most common immune-related AEs, involving the skin, mucous membrane, liver and pituitary, are thought to result from an unchecked autoimmune expansion of self-reactive T cells, which often dictates interruption of therapy and administration of immunosuppressive agents [18]. In this study, one patient developed grade 2 pruritus and grade 3 rash attributable to ipilimumab, both of which responded to steroids.

Anti-CTLA-4 mAbs have been combined with several different anticancer modalities in clinical trials in mCRPC. In a Phase I/II study of ipilimumab and radiation in mCRPC (with or without prior chemotherapy), eight of 50 PSA-evaluable patients had a PSA decline of ≥50% [52]. The ability of radiation to induce phenotypic modulation of tumor cells [53,54], leading to antigen cascade and antitumor immune response, was the rationale for the combination of ipilimumab and radiation [55]. Another Phase II randomized trial of 3 mg/kg ipilimumab with or without concurrent docetaxel in mCRPC showed a confirmed PSA decline of ≥50% in three of 43 patients [56]. A Phase I trial that tested the combination of GM-CSF with escalating doses (0.5–10 mg/kg) of ipilimumab demonstrated acceptable tolerability and PSA declines of ≥50% in three of six patients treated with 3 mg/kg [57].

By increasing T-cell avidity, CTLA-4 blockade can potentially improve the efficacy of antitumor vaccines [58]. This combination approach was tested in a Phase I study that combined GVAX (a whole tumor cell vaccine engineered to express GM-CSF) with escalating doses of ipilimumab (0.3–5.0 mg/kg) in patients with mCRPC [59]. Seven patients (25%) had a PSA decline of ≥50%; two patients in the escalation phase showed a clear regression of bone metastases. Stable bone metastases (lasting 3–27 months) were noted in 15 patients. In a similar approach, PSA-TRICOM was tested in combination with ipilimumab (1.0–10 mg/kg) in 30 patients with mCRPC [60]. No dose-limiting toxicity was identified, 14 of 24 patients (58%) had confirmed PSA declines, and six (25%) had a PSA decline of >50% (two were >90%). Three of 12 patients with measurable disease had unconfirmed partial responses on computed tomography. Notably, median OS was 29.2 months (95% CI: 9.6–48.8) for patients receiving ipilimumab in combination with GVAX, and 34.4 months (95% CI: 29.6–41) for patients receiving ipilimumab in combination with PSA-TRICOM [59,60]. Randomized, controlled, Phase III studies are needed to confirm these findings.

Two ongoing Phase III studies with OS as the primary end point will further characterize the role of CTLA-4 blockade in prostate cancer. A Phase III randomized, placebo-controlled trial aims to enroll 800 patients with mCRPC who have been previously exposed to docetaxel [202]. Following radiation therapy, intravenous ipilimumab (10 mg/kg) will be given every 3 weeks for up to four doses in the induction phase (up to 24 weeks) and every 12 weeks in the maintenance phase (48+ weeks), or until treatment-halting criteria are met. A second randomized, placebo-controlled trial of ipilimumab will evaluate survival in patients with asymptomatic or minimally symptomatic chemonaive mCRPC [203]. Projected enrollment for the trial is 600 patients.

Other immunotherapeutic agents in clinical development

GVAX (Cell Genesys, CA, USA), a whole tumor cell vaccine consisting of a GM-CSF-transduced androgen-sensitive prostate cancer cell line (LNCaP) and a CRPC cell line (PC3), has been studied extensively in prostate cancer. A randomized Phase III trial (VITAL-1) enrolled 626 patients with mCRPC and administered GVAX versus docetaxel every 3 weeks [203]. The trial was prematurely terminated when a futility analysis determined that GVAX had a <30% chance of meeting the predefined superiority end point of OS. At the most recent analysis, patients randomized to vaccine had an almost identical OS compared with those randomized to chemotherapy (HR = 1.01). A second Phase III trial (VITAL-2) enrolled 408 docetaxel-naive patients with symptomatic mCRPC and randomized them to GVAX plus docetaxel or docetaxel and prednisone. The results revealed an excess of deaths (67 vs 47) and shorter median OS (12.2 vs 14.1 months; p = 0.0076) for the GVAX-docetaxel combination [61]. The failure to show a clinical benefit has been potentially attributed to patient selection, although the role of GM-CSF and the lack of prednisone (known to have clinical activity in prostate cancer) in the vaccine-treated patients are alternative explanations [62]. While the failure of GVAX in two Phase III trials in prostate cancer has received much attention, GVAX-based vaccines continue to be tested in other cancers such as breast, pancreatic, colorectal and, most recently, leukemias [63]. Based on preclinical evidence of efficacy and early clinical efficacy data in the combinatorial setting [64,65], there is hope that this platform will continue to be explored in prostate cancer.

Peptide-based vaccines are derived from host peptides selected on the basis of higher antigen-specific humoral response. They are faster and cheaper to produce and are less likely to induce self-antigens capable of generating an autoimmune response compared with cell-based

therapies [65]. Personalized peptide vaccines (PPVs) derived from PSA, PAP, PSMA, multidrug-resistant proteins and other epithelial tumor antigens have been clinically tested in prostate cancer. In a randomized trial, PPV plus low-dose estramustine phosphate (EMP) was compared with standard-dose EMP in 57 HLA-A2$^-$ or -A24$^+$ patients with mCRPC [66]. The combination was well tolerated. Even with crossover, the HR for OS was 0.3 (95% CI: 0.1–0.91) in favor of the PPV plus low-dose EMP group (log-rank, p = 0.0328).

Adenoviral vectors encoding PSA have also been tested. In a Phase I clinical trial in 32 patients with mCRPC, a single subcutaneous dose of an adenovirus/PSA vaccine was found to be safe, with 34% of patients showing anti-PSA antibodies and 68% showing anti-PSA T-cell responses [67]. PSA doubling time (PSADT) increased in 48% of patients. A Phase II nonrandomized trial is evaluating adenovirus/PSA vaccine in mCRPC [204]. An expected 88 patients will receive three subcutaneous doses of vaccine, with PSADT as the primary end point.

DNA-based vaccines, essentially plasmids encoding PAP or PSA, have been shown to be safe, easy to manufacture and amenable to incorporation with other immunomodulatory agents. DNA vaccines have been evaluated in Phase I and II clinical trials in castration-sensitive prostate cancer. In a Phase I trial in eight patients with stage D0 prostate cancer, a PSA-specific cellular immune response and humoral response were detected in two of three patients in the highest-dose cohort [68]. In a Phase I/II trial, 22 patients with mCRPC received an intradermal plasmid DNA vaccine expressing PAP, with GM-CSF [69]. The vaccine was well tolerated and had an excellent toxicity profile. Three patients (14%) had ELISPOT responses in the form of PAP-specific IFN-γ-secreting CD8$^+$ T cells. Nine patients (41%) developed PAP-specific CD4$^+$ and/or CD8$^+$ T cell-proliferative responses, but no antibody responses against PAP. Further analysis revealed a suggestion of antitumor activity. Median PSADT increased from 6.5 months at baseline to 8.5 months during treatment (p = 0.033) and 9.3 months (p = 0.054) in the year post-treatment; HLA-A2$^+$ patients derived the greatest benefit [22]. Based on these early results, an ongoing randomized Phase II trial at the University of Wisconsin (WI, USA) is accruing a projected 34 patients with nonmetastatic CRPC to test a DNA vaccine (pTVG-HP; 100 µg) encoding PAP in combination with recombinant human GM-CSF (200 µg) [205]. The study drugs will be administered intradermally biweekly for six total doses, then at the same doses every 3 months until radiographic disease progression. Safety and immune responses will be the primary end points.

PSMA is a widely expressed epithelial cell membrane-restricted antigen that is progressively expressed in mCRPC [70]. Unlike PSA and PAP, PSMA is not secreted, which makes it a good target for mAb therapy [70,71]. Among a number of antibodies developed to the extracellular domain of PSMA, J591, a deimmunized mAb, is the most well studied in the field of immunotherapy for prostate cancer [72]. This approach is similar to 'passive immunotherapy' regimens, such as trastuzumab in breast cancer and rituximab in hematological malignancies, as opposed to the 'active immunotherapy' of therapeutic cancer vaccines.

In the first study of J591, 17 heterogeneous patients with prostate cancer were given weekly intravenous infusions of the antibody with low-dose IL-2. There was a trend for NK cell expansion in patients without progression [73]. An immunoconjugate of the same internalized antibody designed to deliver the maytansinoid anti-microtubule agent DM-1 was tested in a Phase I trial of 23 patients with mCRPC and shown to be safe. Two (22%) of nine patients treated at 264 or 343 mg/m^2 had a >50% decrease in PSA versus baseline and one patient treated at 264 mg/m^2 showed measurable tumor regression [74].

Combination immunotherapy

A number of standard anticancer strategies have been shown to affect the host immune system and tumor microenvironment in ways that synergize with immunotherapeutic approaches. For instance, sublethal doses of radiation can induce phenotypic changes in tumor cells, generate novel proteins and upregulate many cell-surface proteins involved in T-cell target recognition, adhesion and lysis [74–80]. Proteins affected by radiation include calreticulin, adhesion molecules, MHC class 1 and 2 and Fas. TAAs affected include CEA, MUC-1, CA125, HER2-neu, p53, PSA, PSMA and PAP. There is significant preclinical evidence that these phenotypic changes render tumor cells more susceptible to vaccine-mediated T-cell killing and improve trafficking of TAA-specific effector T cells to the tumor [76–80]. Early clinical trials of vaccine combined with radiation in localized prostate cancer have provided clinical proof-of-concept **(Tables 2 & 3)**.

A randomized Phase II trial evaluated the ability of a poxviral vaccine encoding PSA and the T-cell co-stimulatory molecule B7.1 to induce a PSA-specific T-cell response when combined with radiation therapy in patients with localized prostate cancer [49,50]. In total, 13 of 17 patients who received the complete vaccine schedule showed a ≥ threefold increase in PSA-specific T cells (p < 0.0005) versus no detectable increase in the radiotherapy-only arm. Antigen cascade was noted in the form of *de novo* generation of T cells to well-described prostate-associated nontarget antigens and *de novo*

Table 2. Reported randomized clinical trials of combination immunotherapy in metastatic castration-resistant prostate cancer.

Immunotherapy	Conventional or other immunotherapy	Patients (n)	Phase	Ref.
Dendritic cell: autologous PBMCs activated with a PAP–GM-CSF fusion protein	Docetaxel	82	III (*post hoc* data)	[98]
Viral vector: rV-PSA/rVB7.1 prime/rF-PSA boost	Docetaxel	28	III	[89]
PSA-TRICOM	Sm-153-EDTMP	34	II	[99]
Viral vector: rV-PSA/rVB7.1 prime/rF-PSA boost	Nilutamide	42	II	[94]
Ipilimumab	Docetaxel	43	II	[51,206]
PPV	Estramustine	57	II	[66]
GVAX®	Docetaxel	408	III	[61]

PBMC: Peripheral blood mononuclear cell; PPV: Personalized peptide vaccines; rF: Recombinant fowlpox; rV: Recombinant vaccinia; Sm-EDTMP: Samarium-153-ethylene diamine tetramethylene phosphonate.

humoral responses [81]. The vaccine was well tolerated. Another randomized Phase II trial tested samarium-153, a radionuclide that targets osteoblastic bone lesions, with or without PSA-TRICOM. Of 39 evaluable patients, those in the combination arm had PFS of 3.7 compared with 1.7 months in the samarium-153-only arm (HR = 0.48; p = 0.034). Toxicity profiles were similar in both arms [82].

Preclinical data indicate that certain chemotherapeutic agents can increase the susceptibility of tumor cells to vaccine-mediated T-cell killing through a process of immunogenic modulation. In a preclinical model, sublethal exposure of tumor cells to cisplatin and vinorelbine enhanced the susceptibility of human lung carcinoma cells to CTL-mediated lysis. Gene expression profiling in preclinical studies had shown chemotherapy-induced modulation of tumor phenotype, the cytokine/chemokine milieu, and the proapoptotic:antiapoptotic gene ratio [83]. In preclinical models, where docetaxel has had limited antitumor efficacy, appropriate dose scheduling with vaccine demonstrated optimal enhancement of vaccine-induced antitumor responses [84]. A number of mechanisms have been postulated to explain these immunomodulatory effects. Docetaxel modulates populations of $CD4^+$, $CD8^+$, $CD19^+$, NK and T-regulatory cells in preclinical models [85]. Docetaxel can also upregulate one or more surface molecules (Fas, ICAM-1, MUC-1, CEA and MHC class I) in both sensitive and resistant human carcinoma cell lines, which is associated enhanced killing by HLA-A2-restricted $CD8^+$ CTLs [83–87].

The combination of docetaxel and cancer vaccine was tested in an open-label, randomized, multicenter, crossover Phase II trial in metastatic breast cancer [88]. Docetaxel was administered alone or in combination with PANVAC, a poxviral-based vaccine with transgenes for MUC-1 and CEA, plus TRICOM. At a median follow up of 5.1 months, PFS was 6.6 months in the combination arm versus 3.8 months in the docetaxel-alone arm (HR = 0.67; 95% CI: 0.34–1.31; p = 0.12). Toxicities in both arms were comparable.

A similar combinatorial approach has been clinically tested in prostate cancer. In a randomized Phase II trial in mCRPC, 28 patients were given either vaccine plus weekly docetaxel (a different dose and schedule than in the GVAX studies) or vaccine alone [89]. Median TTP for the 11 patients who crossed over to docetaxel from vaccine alone was 6.1 months compared with 3.7 months for historical controls. TTP in the combination arm (3.2 months) was similar to historical controls. T-cell responses to PSA and evidence of antigen cascade were seen in both arms, suggesting that chemotherapy (with co-administered peri-infusional steroid) does not impede vaccine-induced responses in a clinical setting.

ADT, the primary treatment modality in advanced prostate cancer, has been shown to have immune-enhancing effects as well. ADT is associated with increased peripheral traffic of effector cells to prostate,

Table 3. Ongoing and planned randomized clinical trials of combination immunotherapy in castration-resistant prostate cancer.

Immunotherapy	Conventional therapy and/or other immunotherapy	Phase	Ref.
Ipilimumab	Radiotherapy	III	[202]
Ipilimumab	GM-CSF	II	[207]
Sipuleucel-T	ADT	III	[208]
Sipuleucel-T	Abiraterone acetate	II	[209]
Sipuleucel-T	Anti-PD-1 and cyclophosphamide	II	[210]

ADT: Androgen-deprivation therapy.

decreased immune tolerance of self-antigens that are overexpressed in many cancers, alteration of $CD4^+$ and $CD8^+$ cell subpopulations, inhibition of T-regulatory cells and increased naive T cell emigrants from the thymus [90–93]. A clinical trial of combined androgen blockade and vaccines in patients with nonmetastatic CRPC illustrates the potential advantages of combining the two strategies [94]. In this trial, which allowed crossover, combined androgen blockade was achieved with nilutamide, an ARA, and ADT. The median time to treatment failure (defined by rising PSA or development of a metastatic lesion) with the combined therapy was 13.9 months in the vaccine arm when nilutamide was added at PSA progression. In contrast, patients who started on nilutamide and added vaccine at PSA progression had a median time to treatment failure of 5.2 months. Pending confirmation in a rigorous clinical setting, these findings favor giving vaccine in early-stage disease followed by nilutamide, compared with nilutamide followed by vaccine. A follow-up survival analysis revealed a 75% 5-year survival rate for patients treated first with vaccine then with added nilutamide, compared with a 43% 5-year survival rate for patients who received nilutamide first and had vaccine added at a later time [95]. In another randomized Phase II trial of flutamide with or without PSA-TRICOM, preliminary evidence suggests improvement in time to treatment failure for the combination arm compared with flutamide alone [96]. Future clinical trials with newer ARAs such as enzalutamide may establish the validity of this combinatorial approach.

Conclusion & future perspective

A growing amount of evidence points to the feasibility, safety and early efficacy of immunotherapeutic agents such as vaccines and immune checkpoint inhibitors in CRPC. Future efforts will be crucial to identifying novel vaccine platforms, patient populations who may receive the most benefit, optimal trial designs and combination approaches with the best clinical outcomes. Off-the-shelf vaccines have obvious logistical advantages over patient-specific vaccines; however, large-scale data analyses may find these approaches to be clinically equivalent in terms of efficacy and tolerability [201]. Patients with minimal disease burden (i.e., nonmetastatic disease on conventional imaging) will probably derive maximum benefit from immunotherapeutic agents because of their safety, biological characteristics and durable effects [97,98]. New pairings and sequencing of disparate agents in combinatorial strategies require further assessment. Combinations of immunotherapy with ADT, chemotherapy, radiotherapy and small-molecule inhibitors may be favored based on preclinical data and clinical proof-of-concept. Options for combinatorial approaches will expand with the development of new vaccine platforms such as PPV, mRNA-, and DNA-based vaccines, as well as newer androgen inhibitors such as abiraterone and enzalutamide and newer checkpoint inhibitors such as anti-PD-1 and anti-PD-L1, all of which are being tested. Bone-seeking radionuclides are also being tested in combination with vaccines [99]. Widespread adoption of immunotherapy for cancer will ultimately depend on other factors as well, including the discovery of relevant biomarkers, better immune assessment methods and greater experience with novel agents.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Characteristics of immunotherapeutic agents

- Cancer vaccines have minimal toxicity and are far better tolerated than conventional anticancer approaches.
- Immunotherapy may have its greatest potential for clinical benefit in patients with low tumor burden/early-stage disease.
- While current vaccine strategies involve *ex vivo* processing (sipuleucel-T), less resource-intensive *in vivo* approaches (off-the-shelf) are also in late stages of clinical development (e.g., PSA-TRICOM).
- Unique tumor response kinetics with immunotherapeutic agents can potentially provide durable clinical benefit.

Combination immunotherapy

- The effects of standard antitumor strategies can potentiate vaccine-mediated T-cell killing of tumor cells, and form the rationale for combination immunotherapy.
- Vaccines have the potential to enhance clinical outcomes when rationally combined with other immune-enhancing or immune-inert cytotoxic regimens.
- Radiation and certain cytotoxic chemotherapies may cause phenotypic changes in tumor cells and induce immunogenic modulation.
- Androgen-deprivation therapy in prostate cancer is associated with host antitumor immune enhancement.

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