



Immunotherapy in Metastatic Castration-Resistant Prostate Cancer: Past and Future Strategies for Optimization

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Abstract

Purpose of Review To date, prostate cancer has been poorly responsive to immunotherapy. In the current review, we summarize and discuss the current literature on the use of vaccine therapy and checkpoint inhibitor immunotherapy in metastatic castration-resistant prostate cancer (mCRPC).

Recent Findings Sipuleucel-T currently remains the only FDA-approved immunotherapeutic agent for prostate cancer. Single-agent phase 3 vaccine trials with GVAX and PROSTVAC have failed to demonstrate survival benefit to date. Clinical trials using combination approaches, including combination PROSTVAC along with a neoantigen vaccine and checkpoint inhibitor immunotherapy, are ongoing. Checkpoint inhibitor monotherapy clinical trials have demonstrated limited efficacy in advanced prostate cancer, and combination approaches and molecular patient selection are currently under investigation.

Summary The optimal use of vaccine therapy and checkpoint inhibitor immunotherapy in metastatic castration-resistant prostate cancer remains to be determined. Ongoing clinical trials will continue to inform future clinical practice.

Keywords Metastatic prostate cancer · Castration resistance · Immunotherapy · Vaccines · Checkpoint inhibitors · Neoantigen vaccine

Introduction

Prostate cancer remains the second most common cause of death among men in the USA, with an additional estimated 15,891 cases of metastatic prostate cancer by 2025 [1, 2]. Despite six FDA-approved therapies for metastatic castration-resistant prostate cancer (mCRPC), the clinical efficacy and utilization of immunotherapy agents are still in its relative infancy in this disease.

Harnessing the immune system to treat cancer has become a cornerstone of modern oncology therapeutics, with

particular rapidity within the last two decades. In 2000, James Allison, Tasuku Honjo, and colleagues demonstrated an immune response in the prostate tumors of transgenic mice treated with a combination anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) therapy and a granulocyte-macrophage colony-stimulating factor (GM-CSF) expressing vaccine [3], and had also seen similarly impressive results in a melanoma model [4]. Based on this, ipilimumab, an anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) monoclonal antibody, eventually became the first checkpoint inhibitor immunotherapy drug to gain FDA approval in 2011 after a survival benefit was demonstrated in patients with metastatic melanoma [5]. Since that time, anti-PD-1 checkpoint inhibitors (pembrolizumab, nivolumab, cemiplimab) and anti-PD-L1 checkpoint inhibitors (atezolizumab, avelumab, durvalumab) have been approved across multiple malignancies [6–10]. Despite these important advances, patients with prostate cancer have not yet benefitted to as great an extent as those with more “immunologically responsive” cancers such as melanoma and urothelial carcinoma [11, 12]. Prostate cancers have historically been largely deemed immunologically “cold” tumors that generally have a lower tumor mutation burden (TMB) than other tumor types [13, 14]. The

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complexities of the prostate cancer immune milieu and tumor microenvironment (TME) are reviewed in other articles in this Special Edition, and our understanding of this biology will be critical for advancing the field of immunotherapy in prostate cancer. Here, we will focus on past vaccine-based and checkpoint inhibitor treatment modalities in prostate cancer, as well as emerging treatment approaches across both modalities.

Prior Approaches: Vaccines, Checkpoint Inhibitor Therapy

Vaccines

1. Sipuleucel-T

Sipuleucel-T is a therapeutic cancer vaccine consisting of autologous antigen-presenting cells (APCs), stimulated with PA2024, a recombinant fusion protein of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Several randomized, placebo-controlled trials had suggested a survival benefit, but no clear effect on progression free survival (PFS) [15, 16]. In 2010, Kantoff and colleagues conducted the phase 3 IMPACT trial of 512 men with metastatic castration-resistant prostate cancer, randomized in a 2:1 fashion to receive sipuleucel-T or placebo [17••]. The study met its primary endpoint of overall survival (OS), with a median survival of 25.8 months in the sipuleucel-T arm compared with 21.7 months in the control arm, a 4.1-month improvement. However, no difference in time to disease progression (PFS) or PSA response was observed, with only 2.6% of patients having a PSA decline of 50% or greater. However, based on the observed survival benefit, sipuleucel-T was FDA approved in 2010. Importantly, this was the first cellular therapeutic vaccine for any cancer type that was approved by the FDA, marking an important milestone in the field of cancer immunotherapy. Retrospective analysis of the IMPACT trial stratified patients by PSA levels, and found a 13-month improvement in OS in the lowest quartile compared with placebo, while the difference was only 2.8 months in the highest quartile, suggesting patients benefit the most at time of lowest tumor burden [18]. More recently, Holl et al. conducted a retrospective analysis of 336 patients with metastatic castration-resistant prostate cancer treated with sipuleucel-T. Interestingly, a subset of 44 patients showed PSA stabilization and long-term disease control, with 79% surviving 36 months with a median time to subsequent therapy of 17.8 months. There was a trend toward a higher percentage of African American (AA) patients falling into this category, compared with Caucasian counterparts [19]. Additional analyses of a prospective registry trial, PROCEED, found that AA patients had significantly longer median OS (39.5 months) compared with matched Caucasians (28.1 months; $p < 0.001$). AA race also emerged as an

independent predictor of longer OS in multivariate analyses [20]. Taken together, these suggest that patient selection and host factors may be keys for optimizing responses to sipuleucel-T.

A recent randomized phase II trial of sipuleucel-T with or without sensitizing radiation therapy, administered to a single metastatic site up to 30 Gy, in patients with asymptomatic or minimally symptomatic mCRPC, did not enhance humoral and cellular responses to the vaccine therapy [21]. Several clinical trials have also evaluated the use of sipuleucel-T earlier in the course of prostate cancer. In a multicenter phase II trial of neoadjuvant sipuleucel-T administered to men with localized prostate cancer prior to planned radical prostatectomy (RP), 37 of 42 patients received three sipuleucel-T treatments. All 37 of the patients who received RP were found to have peripheral immune responses and immune infiltrates. CD3+, CD4+FOXP3-, and CD8+ T cell infiltrates were evident in the RP tissues of sipuleucel-T-treated patients and were concentrated primarily at the tumor interface. Nearly half of CD3+ T lymphocytes at the tumor interface of treated patients expressed PD-1 [22].

The ProVent trial (NCT03686683) is an ongoing randomized phase III, open-label clinical trial of sipuleucel-T administered to patients on active surveillance who have newly diagnosed prostate cancer, ISUP grade groups 1–3, and an estimated life expectancy of ≥ 10 years. Patients are randomized in a 2:1 fashion to receive sipuleucel-T or to standard of care active surveillance. The primary objective is to assess the efficacy of sipuleucel-T in reducing histopathologic reclassification to a higher Gleason grade in prostate cancer subjects on active surveillance 3 years after randomization. Such efforts are of value in this patient population given the progressive risk of distant metastases and prostate cancer-related mortality in patients with higher grade prostate cancer. Nonetheless, it is noteworthy that despite the documented survival benefit associated with sipuleucel-T, a recent large cohort study of its use in more than 7000 patients with metastatic castration-resistant prostate cancer found that only 1 of 10 patients receive this therapy, highlighting the relatively limited use of this therapy—the only FDA-approved immunotherapy for prostate cancer—in a real-world population [23].

2. GVAX

GVAX is a GM-CSF-secreting tumor cell vaccine generated using LNCaP and PC-3 cell lines. A phase 1/2 study in patients with hormone-naïve prostate cancer and PSA relapse demonstrated a statistically significant decrease in PSA velocity in 16 of 21 patients (76%) at 20 weeks after first therapy [24]. However, two subsequent phase 3 clinical trials were terminated early based on the results of a previously unplanned futility analysis. VITAL-2 (NCT00133224) was a phase 3, randomized open-label study of docetaxel with or

without GVAX in patients with taxane-naïve mCRPC, and VITAL-1 (NCT00089856) was a phase 3 clinical trial which randomized chemotherapy-naïve patients with mCRPC to receive either GVAX or docetaxel and prednisone. In VITAL-2, the combination GVAX and docetaxel arm was associated with an increased mortality rate compared with the chemotherapy monotherapy arm. This vaccine therapy has subsequently not been developed further and represents an important cautionary lesson in the importance of large, randomized phase 3 clinical trials to demonstrate therapeutic efficacy of novel drugs, particularly in the challenging immunotherapy landscape in prostate cancer.

3. PROSTVAC

PROSTVAC-VF is an immunotherapeutic vaccine that incorporates the genes for prostate-specific antigen (PSA) and multiple T cell co-stimulatory molecules (TRICOM) in viral vectors to generate a T cell response in prostate cancer patients. It utilizes both recombinant vaccinia virus (“V”) and recombinant fowlpox virus (“F”) components, thus taking advantage of a heterologous prime-boost strategy [25]. Several clinical trials have been conducted using PROSTVAC, including a phase 2 randomized, double-blind placebo-controlled study of PROSTVAC in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with mCRPC, which, similar to sipuleucel-T, showed no improvement in their primary endpoint of PFS, but did show a significant OS benefit, compared with placebo (26.2 versus 16.3 months, respectively; stratified log-rank $p = 0.0019$) [26, 27]. Based on this, a large phase 3 placebo-controlled clinical trial of PROSTVAC combined with GM-CSF was subsequently conducted to confirm the findings of the phase 2 trial [28]. A total of 432 patients received vaccine-based therapy, and 433 patients were assigned to the placebo arm. The primary endpoint of the study was overall survival (OS), and at the third interim analysis, the trial was stopped early due to futility. No OS benefit was observed, and patients on the vaccine arm also had no improvement in other clinical outcome parameters of interest, including radiographic progression, pain progression, or chemotherapy initiation. The authors hypothesize that the earlier phase 2 trial may have generated a false positive signal due to being underpowered for OS evaluation, or that survival may have been equilibrated due the interim approval of multiple systemic chemotherapy and androgen receptor pathway therapies associated with survival benefit.

Checkpoint Inhibitor Immunotherapy

CTLA-4 inhibitor monotherapy has been evaluated in patients with advanced prostate cancer in two large, randomized, phase III clinical trials. Kwon et al. conducted a trial of 799 patients

with metastatic castration-resistant prostate cancer with bone metastases who had progressed on docetaxel chemotherapy [12]. Patients received directed radiation to 8 Gy to an osseous metastasis and were randomized 1:1 to then receive either ipilimumab 10 mg/kg IV or placebo every 3 weeks. The primary endpoint was overall survival (OS), and there was no statistically significant difference between the groups; the median OS was 11.2 months in the ipilimumab arm and 10.0 months in the placebo arm (HR 0.85, 0.72–1.00, $p = 0.053$). However, a post hoc assessment demonstrated that patients with more favorable prognostic features, including an alkaline phosphatase level < 1.5 the upper limit of normal, hemoglobin concentration of ≥ 11 g/dL, and absence of visceral metastases, had a prolonged survival of 22.7 months with ipilimumab compared with 15.8 months with placebo (HR = 0.62, 95% CI 0.45–0.86, $p = 0.0038$). A subsequent double-blind phase III trial by Beer et al. randomized 598 patients with chemotherapy-naïve metastatic castration-resistant prostate cancer in a 2:1 fashion to ipilimumab 10 mg/kg versus placebo [11]. As in the preceding study, the primary endpoint of overall survival was again not met, but there was a longer progression free survival (PFS) among ipilimumab-treated patients compared with the placebo arm (5.6 months versus 3.8 months, HR = 0.67, 95.87% CI 0.55–0.81). The ipilimumab group also demonstrated a higher PSA response rate (23% versus 8%), suggesting a population of patients more likely to respond to immunotherapy, but with overall disappointing response rates.

KEYNOTE-028 (NCT02054806) was a phase Ib trial of pembrolizumab, an anti-PD-1 checkpoint inhibitor, in patients with locally advanced or metastatic solid malignancies. This study included 23 patients with castration-resistant metastatic prostate cancer, a RECIST 1.1. measurable lesion, and PD-L1 expression in $\geq 1\%$ tumor or stromal cells. Patients received pembrolizumab 10 mg/kg every 2 weeks until disease progression or unacceptable toxicity. The objective response rate (ORR) was 17.4%, with 4 of 23 patients achieving a partial response [29].

The preceding modest, and largely negative, efficacy data on vaccine-based therapies and single-agent checkpoint immunotherapy in mCRPC underscore the need for novel and combination approaches in this disease space.

Future Approaches: Combination Vaccine and Checkpoint Inhibitor Trials, Molecularly Driven Approaches

Vaccine Combination Therapy

1. Vaccine and cytokine combination therapy

Given the relatively limited efficacy of vaccine monotherapy in prostate cancer to date, one emerging therapeutic

approach is combining vaccine and cytokine therapy. IL-7 is a homeostatic growth factor for T cells and is capable of inducing proliferation, maintaining T cell responsiveness, and preventing and reversing T cell anergy [30]. A phase 2 randomized, controlled clinical trial is currently underway in which patients with asymptomatic or minimally symptomatic mCRPC were randomized to receive CYT107, a recombinant glycosylated human interleukin-7, after standard sipuleucel-T administration (NCT01881867). The primary objective of this study is to determine whether CYT107 administration increases the vaccine-induced antigen-specific T cell immune response to the sipuleucel-T fusion protein vaccine construct PAP-GM-CSF (PA2024). Patients received CYT107 therapy within 3–7 days of completion of sipuleucel-T therapy, compared with control (no CYT107). This trial has completed accrual and preliminary results presented show CYT107 can induce significant expansion of T cells compared with controls (Pachynski et al. SITC Annual Meeting 2018).

Such combination trials are important steps in improving the efficacy of this already FDA approved therapeutic vaccine; sipuleucel-T has the potential to serve as an important backbone in prostate cancer immunotherapy studies.

2. Vaccine and checkpoint inhibitor combination therapy

Another vaccine-based strategy is centered around the use of attenuated bacterial strains such as *Listeria monocytogenes* (*Lm*) as an antigen delivery vector. *Listeria monocytogenes* (*Lm*)-listeriolysin O (LLO) therapies can induce antigen-specific T cell responses, and have shown significant anti-tumor efficacy in prostate cancer preclinical models [31, 32]. ADXS31-142 is a live attenuated *Lm*-LLO which targets PSA and secretes an antigen-adjuvant fusion protein [33]. Preclinical murine models have demonstrated that *Lm*-LLO in conjunction with an anti-PD-1 antibody inhibits the PD-1/PD-L1 interaction, resulting in inhibition of tumor growth and prolonged survival in treated animals [34]. This approach is currently being evaluated in the phase 1/2 clinical trial KEYNOTE-046, in which patients with mCRPC are either treated with ADXS310142 alone or in combination with pembrolizumab (NCT02325557). The primary endpoint of the phase 1 portion of the trial is safety as measured in the frequency of adverse events; the secondary outcome measure is progression-free survival per RECIST 1.1. Preliminary results were recently presented, showing that 2 (14%) patients receiving monotherapy and 16 (43%) patients receiving combination therapy had a decreased PSA post-baseline, with 8 (22%) of the combination patients having PSA reductions of $\geq 50\%$ from baseline. These data support the added benefit of checkpoint inhibition in the setting of a vaccine strategy.

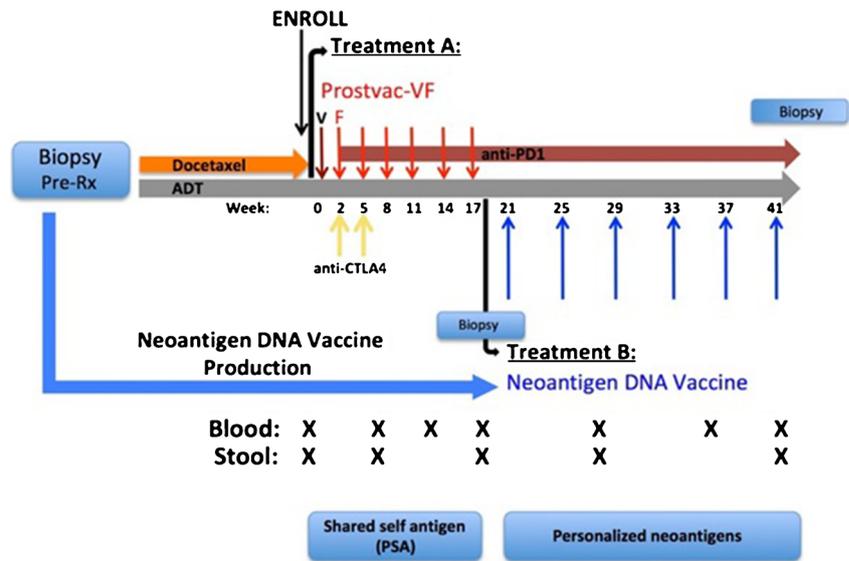
Other vaccine combination approaches employ DNA vaccines in conjunction with checkpoint inhibitor immunotherapy. One vaccine-based approach is being tested in an ongoing

phase I clinical trial (NCT02616185) utilizing escalating doses of a vaccine-based immunotherapy regimen (VBIR, Pfizer). Planned accrual is for 133 patients who have either a prior history of prostate cancer and hormone-sensitive biochemical relapse, those with disease progression during post-surgical castration or ongoing androgen deprivation therapy (pre-secondary hormone CRPC), or who have documented disease progression after secondary hormone therapy such as abiraterone acetate or enzalutamide (post-secondary hormone CRPC). Patients will receive three different biologic agents administered at various dosing intervals: an adenoviral vaccine (PF-06755992) on day 1 of each cycle, a DNA plasmid vaccine (PF-06755990) on days 27, 57, and 85 via a TDS-IM electroporation device, and an anti-PD1 monoclonal antibody (PF-06801591) every 28 days. Concurrently, patients will receive oral sunitinib, with the maximum tolerated dose (MTD) identified during the course of the study, as well as tremelimumab, an anti-CTLA-4 monoclonal antibody, every 28 days. The primary outcome measure is incidence and grade of treatment-emergent adverse events, including dose-limiting toxicities (DLTs). Multiple secondary outcome measures are planned, including immune response to selected prostate cancer tumor antigens and antibody response to PSMA antigen. Study completion is anticipated in late 2021.

In pre-clinical studies, McNeel and colleagues showed that vaccination using a DNA vector platform resulted in increased PD-1 expression on antigen-specific CD8+ T cells which limited anti-tumor efficacy. They were able to successfully reverse this using concomitant PD-1 blockade resulting in significantly improved anti-tumor responses [35, 36]. A subsequent pilot clinical trial evaluated sequential or concurrent administration of a DNA-based vaccine and anti-PD-1 checkpoint inhibitor immunotherapy with pembrolizumab in patients ($n = 26$) with mCRPC [37]. Overall, the concurrent treatment was tolerated well with no unanticipated adverse events. Interestingly, 8/13 (62%) of patients treated concurrently, while only 1/12 (8%, $p = 0.01$) of patients treated sequentially, experienced PSA declines from baseline. Both groups had increases in PAP-specific interferon gamma or granzyme B-secreting T cells, but PSA declines were associated with the development of PAP-specific Th1-biased T cell immunity and CD8+ T cell infiltration in metastatic tumor biopsy specimens in those patients who received concurrent treatment. This suggests the efficacy of a combinatorial approach utilizing concurrent checkpoint blockade may be successful in increasing immune infiltration in an otherwise immunologically “cold” tumor.

To this end, an ongoing clinical trial at our institution is evaluating a personalized neoantigen DNA vaccine in combination with ipilimumab/nivolumab and PROSTVAC in metastatic prostate cancer (NCT03532217) (see schema, Fig. 1). Of note, given the previously discussed negative phase III trial of single agent PROSTVAC in patients with mCRPC, this

Fig. 1 A pilot trial of neoantigen DNA vaccine in combination with nivolumab/ipilimumab and Prostavac in metastatic hormone-sensitive prostate cancer (NCT03532217). Patients who successfully complete an initial course of docetaxel chemotherapy for mHSPC will then receive the combination of Prostavac-VF plus checkpoint inhibitors (“Treatment A”), as indicated. A personalized neoantigen DNA vaccine is produced during that time, and subsequently administered with continued anti-PD-1 checkpoint blockade (“Treatment B”)



study seeks to evaluate the immune responses, safety/tolerability, and efficacy of a vaccine-based combination immunotherapeutic approach in patients with metastatic hormone-sensitive prostate cancer (mHSPC)—thus moving combination immunotherapy earlier in the disease state. Patients with untreated high-volume metastatic disease receive planned standard of care docetaxel chemotherapy dosed every 3 weeks for a planned 6 cycles, along with continuous standard androgen deprivation therapy (ADT). After completion of chemotherapy, patients will receive nivolumab/ipilimumab plus PROSTVAC followed by a personalized neoantigen DNA vaccine. Within 60 days after the last dose of docetaxel, patients will start a priming dose of PROSTVAC-V as a single agent, and subsequent doses of PROSTVAC-F in combination with ipilimumab (1 mg/kg every 3 weeks for 2 doses), and nivolumab (3 mg/kg every 3 weeks for 6 doses) over a course of approximately 17 weeks. Patients will then receive a personalized neoantigen DNA vaccine based off of their metastatic biopsy for a total of 6 treatments every 28 days along with nivolumab 480 mg IV. The neoantigen vaccine is delivered via electroporation, which has been shown to enhance vaccine delivery and responses [38]. The primary efficacy endpoint of the trial is failure-free survival (FFS) and milestone survival of the combination therapy.

Ongoing Checkpoint Inhibitor Immunotherapy Trials

Several checkpoint inhibitor clinical trials are aiming to sub-stratify patients by parameters such as disease burden, prior therapy, and other potential biomarkers of response such as PD-L1 status and tumor mutation burden (TMB), with completed and ongoing trials summarized in Table 1. KEYNOTE-199 (NCT02787005) is a phase II clinical trial of

pembrolizumab monotherapy in patients with docetaxel-refractory mCRPC. Patients were stratified into cohorts according to PD-L1 status and presence or absence of RECIST-measurable disease. Cohort 1—enrolled patients with measurable disease and PD-L1 positive tumors; cohort 2—enrolled patients with measurable disease and PD-L1 negative tumors, and cohort 3—enrolled patients with non-measurable, bone-predominant disease. Initial data for cohorts 1–3 was reported in 2018, with an overall disease control rate (CR + PR + SD) across cohorts of 11% [39]. CheckMate-650 is evaluating combination anti-CTLA-4 and anti-PD-1 immunotherapy in patients with mCRPC and progression on second generation hormone therapy. Patients receive ipilimumab 3 mg/kg IV and nivolumab 1 mg/kg IV every 3 weeks for 4 doses followed by nivolumab 480 mg IV every 4 weeks [40]. A pre-planned interim analysis after 78 patients had a minimum of 6 months of follow-up demonstrated an ORR of 26% in chemotherapy-naïve patients and 10% in patients who had received prior chemotherapy. ORR rates were higher in patients with PD-L1 ≥ 1%, DNA damage repair (DDR), homologous recombination deficiency (HRD), or a high tumor mutation burden (TMB), defined as greater than 74.5 mutations. Importantly, high TMB (above the median) was associated with significantly improved rPFS vs low TMB (below the median) (p < 0.0001) [40], suggesting even within prostate cancer, mutational burden status likely impacts responses to checkpoint inhibitors. The PERSEUS1 trial (NCT03506997) is a phase II, single-arm study of pembrolizumab in patients with mCRPC with disease progression on at least one prior line of therapy in the metastatic castration-resistant setting, either chemotherapy or hormonal therapy. Patients in this trial must have a high mutational load (> 11 mutations per targeted panel), and/or a DNA repair defect that can increase

Table 1 Completed and Ongoing Trials in mCRPC

Study and drug	Phase	Patient population	Primary endpoint	Trial outcome	Year reported	Status
Kantoff et al. sipuleucel-T (NCT00065442)	3	mCRPC	Overall survival	4.1 month improvement in survival vs. placebo	2010	Completed
Twardowski et al. sipuleucel-T ± radiation therapy (NCT01807065)	2	mCRPC	Safety and immune activation	Safe; no enhancement of responses	2019	Completed
Fong et al. neoadjuvant sipuleucel-T (NCT00715104)	2	Localized	Change in Infiltrating CD3+ T cells	3-fold increase in infiltrating T cells	2014	Completed
Sipuleucel-T ProVent (NCT03686683)	3	Localized	Reduce histopathologic classification to higher Gleason grade	Pending	TBD	Ongoing
Simons et al. GVAX (NIH protocol 9708-205)	1/2	Biochemical recurrence	Safety and PSA response	Safe; 16 of 21 (76%) with declining PSA velocity	2006	Completed
GVAX ± docetaxel VITAL-2 (NCT00133224)	3	mCRPC (taxane- naïve)	Overall survival	N/A	2008	Early Termination
GVAX vs. docetaxel VITAL-1 (NCT00089856)	3	mCRPC (chemo- naïve)	Overall survival	N/A	2008	Early termination
Kantoff et al. PROSTVAC (NCT00078585)	2	mCRPC	Progression-free survival (PFS)	Primary endpoint not met	2017	Completed
Gulley et al. PROSTVAC (NCT01322490)	3	mCRPC	Overall survival	Primary endpoint not met	2019	Completed
Kwon et al. ipilimumab vs. placebo (NCT00861614)	3	mCRPC	Overall survival	Primary endpoint not met	2014	Completed
Beer et al. ipilimumab vs. placebo (NCT01057810)	3	mCRPC	Overall survival	Primary endpoint not met	2016	Completed
KEYNOTE-028 (NCT02054806)	1b	mCRPC	ORR	ORR 17.4% (4 of 23 pts)	2018	Completed
Study and drug	Phase	Patient population	Primary endpoint	Year initiated	Status	
CYT107 after sipuleucel-T (NCT01881867)	2	mCRPC	Quantification of T cell responses in PAP-GM-CSF	2013	Ongoing	
Pembrolizumab KEYNOTE-046 (NCT02325557)	1/2	mCRPC	Safety and progression-free survival (PFS)	2014	Ongoing	
Neoantigen DNA vaccine + PROSTVAC + ipilimumab and nivolumab (NCT03532217)	2	mHSPC	Failure-free survival (FFS) and milestone survival at 3 years	2018	Ongoing	
Pembrolizumab KEYNOTE-199 (NCT02787005)	2	mCRPC	ORR by RECIST 1.1	2016	Ongoing	
Ipilimumab and nivolumab CheckMate-650 (NCT02985957)	2	mCRPC	ORR	2016	Ongoing	
Pembrolizumab PERSEUS 1 (NCT03506997)	2	mCRPC	ORR, PSA ≥ 50% response, or CTC conversion from > 5 to < 5	2018	Ongoing	
Pembrolizumab patients progressing on enzalutamide (NCT02312557)	2	mCRPC	PSA ≥ 50% response	2014	Ongoing	
Pembrolizumab DNA repair deficient and proficient cohorts (NCT03248570)	2	mCRPC	ORR in DNA damage repair proficient and deficient groups	2017	Ongoing	
KEYNOTE-365 enzalutamide ± pembrolizumab (NCT02861573)	1b/2	mCRPC	Safety, PSA ≥ 50% response, ORR by RECIST 1.1	2016	Ongoing	
IMbassador250 atezolizumab (NCT03016312)	3	mCRPC	Overall survival	2017	Ongoing	
KEYNOTE-641 pembrolizumab (NCT03834493)	3	mCRPC	Overall survival	2019	Ongoing	
CDK12 loss ipilimumab and nivolumab (NCT03570619)	2	mCRPC	ORR by PSA ≥ 50% response	2018	Ongoing	

mCRPC metastatic castration-resistant prostate cancer, mHSPC metastatic hormone sensitive prostate cancer, ORR objective response rate

mutational load such as dMMR/MSI-H. The primary endpoint is objective response rate (ORR), PSA decline of $\geq 50\%$, and circulating tumor cell (CTC) count conversion from > 5 to < 5 .

There are multiple ongoing clinical trials assessing checkpoint inhibitor immunotherapy in conjunction with anti-androgen therapy, in particular in the setting of prior resistance to androgen-receptor pathway inhibitors. Previously published data has suggested an increased likelihood of response to checkpoint inhibitor immunotherapy in patients with prior enzalutamide resistance [41, 42]. A phase 2 clinical trial (NCT02312557) is currently underway in patients with mCRPC who previously progressed on enzalutamide therapy. Fifty-eight patients will be enrolled and will receive ongoing enzalutamide along with pembrolizumab. The primary endpoint of this study is PSA $\geq 50\%$ response rate. An ongoing multi-center phase II clinical trial (NCT03248570) is enrolling men with mCRPC who received either abiraterone acetate and/or enzalutamide and will receive pembrolizumab but will be stratified to two treatment arms: those that are DNA damage repair proficient or deficient, respectively. The primary endpoint for this trial is the objective response rate in the DNA damage repair proficient and deficient groups.

Cohort C of KEYNOTE-365 (NCT02861573) is evaluating pembrolizumab plus enzalutamide in abiraterone-pretreated patients with metastatic castrate-resistant prostate cancer (mCRPC) [43]. There are several ongoing phase 3 clinical trials evaluation combinations of androgen receptor pathway inhibitors and immunotherapy that should add important data to the current therapeutic landscape in advanced prostate cancer. IMbassador250 (NCT03016312) is a phase 3 clinical trial of enzalutamide with or without atezolizumab in mCRPC patients who have progressed on an androgen synthesis inhibitor and are unable to receive taxane chemotherapy. KEYNOTE-641 (NCT03834493) is a phase 3, randomized, double blind clinical trial of pembrolizumab and enzalutamide versus placebo and enzalutamide in patients with mCRPC.

Despite the overall relatively low response rates in some of the early published data from these clinical trials, it is nonetheless encouraging that subpopulations of patients have demonstrated a response to checkpoint inhibitor immunotherapy, suggesting that appropriate patient selection may be a key factor in future trial design. Moreover, appropriate biomarker selection to screen to potential responsiveness to checkpoint inhibitor therapy is still lacking in prostate cancer. In one series of immunohistochemical analysis of PD-L1 in primary prostate cancers, 52.2 to 61.7% demonstrated moderate to high PD-L1 expression, a finding that is notably discordant from published clinical trial data to date [44]. Thus, additional strategies to identify robust predictive biomarkers of response to immunotherapy are needed in prostate cancer.

Molecular Selection to Optimize Response to Checkpoint Inhibitor Immunotherapy

While one avenue to improving the likelihood of response to checkpoint inhibitor immunotherapy in patients with prostate cancer is developing combination treatments with vaccines or implementing combination immunotherapy treatments, another important consideration is patient selection. Previous clinical trials have demonstrated relatively low response rates in an unselected mCRPC population, but within these cohorts there nonetheless appear to be patient subsets with a higher response rate to immunotherapy. With the advent of more widespread genomic analysis of advanced prostate cancer, tailoring therapies to genomically selected prostate cancer sub-populations are now feasible [45•, 46].

Pembrolizumab was FDA approved in 2017 as second-line therapy in a tumor-agnostic fashion for patients with advanced malignancies who harbor DNA mismatch repair (MMR) deficiencies or are microsatellite instability-high (MSI-H). dMMR/MSI-H aberrations have been identified in about 2–3% of patients with advanced prostate cancer [45•, 47, 48]. Antonarakis et al. reported four dMMR mCRPC patients who received anti-PD-1 checkpoint inhibitor therapy with nivolumab or pembrolizumab in the fourth-line setting or beyond, and two patients attained a $> 50\%$ reduction in PSA; three patients had an objective radiographic response in soft tissue metastases [47]. In a retrospective series by Abida et al., 11 patients with dMMR/MSI-H prostate tumors received anti-PD-1 or anti-PD-L1 immunotherapy, and six of these patients achieved a $> 50\%$ PSA decline [49]. Despite the low incidence of mismatch repair deficiencies in prostate cancer, the promising response rates in these studies highlight the importance of rigorous and ubiquitous molecular evaluation of patients with advanced prostate cancer.

Prostate cancer patients whose tumors harbor DNA repair defects appear to have a consistently higher response rate to checkpoint inhibitor immunotherapy compared with unselected prostate cancer patients. In a phase II trial, patients with mCRPC and disease progression on abiraterone and/or enzalutamide were treated with the combination of the anti-PD-1 antibody durvalumab and the PARP inhibitor olaparib [50]. Nine of 15 patients (53%) had a $\geq 50\%$ PSA decline; of note, four responders had germline alterations in DNA damage repair (DDR) genes, including *BRCA2*, and two additional responders had somatic *BRCA2* alterations. In another phase II clinical trial, 15 patients with mCRPC and AR-V7 positive circulating tumor cells (CTCs) were treated with combination therapy with ipilimumab and nivolumab [51]. Six of the 15 patients (40%) were found to have deleterious mutations in DNA repair genes, both somatic or germline. There was a trend toward improved PSA response rates and ORR rates in the patients with DDR mutations.

Metastatic prostate cancers harboring loss of *CDK12* have been identified at a frequency of about 3–7% [45•, 46, 52•]. In mCRPC, *CDK12* loss results in extensive focal tandem duplications throughout the genome, resulting in a high neoantigen burden [52•]. Pilot clinical data of patients with *CDK12* loss who were treated with anti-PD-1 checkpoint inhibitor immunotherapy demonstrated robust PSA decline and radiographic responses in 2 of 4 patients [52•]. A multi-center, phase II clinical trial of patients with metastatic cancers, including mCRPC, harboring *CDK12* alterations is currently underway (NCT03570619). Patients are treated with combination checkpoint inhibitor immunotherapy with ipilimumab 1 mg/kg IV and nivolumab 3 mg/kg IV every 3 weeks for up to 4 doses, followed by nivolumab 480 mg IV every 4 weeks. The primary objective is the overall response rate in patients with mCRPC; response is defined as 50% decline in PSA as per PCWG3 criteria. Given the multiple antecedent clinical trials with checkpoint inhibitor immunotherapy in unselected mCRPC patients, this study will provide valuable insight into genomically selecting prostate cancer patients for immunotherapy trials.

Conclusions

Despite multiple promising advances—and a current FDA-approved treatment—in the development of prostate cancer immunotherapies, substantial progress in improving the efficacy in prostate cancer patients is still lacking. Nonetheless, it is promising that as further innovative clinical trials are developed, including advances in CAR-T cell therapy in prostate cancer through PSMA-based designs (NCT03089203), more effective immunotherapeutics for prostate cancer continue to become feasible. There are multiple factors contributing to the discrepancy in the abundance of ongoing clinical trials and paucity of resultant practice-changing results. It is evident that our understanding of the heterogeneity of prostate tumors, and the complexities of the prostate cancer tumor immune microenvironment (TIME), are still incomplete [53, 54]. The relative importance and contribution of tumor-infiltrating leukocytes (whether anti- or pro-tumor), interferon gamma and other cytokine responses, and tumor volume all remain incompletely characterized in prostate cancer. While optimal approaches and combinations are still being developed and tested, it is now clear from past clinical trials with a variety of immunotherapies that a “single agent” approach will likely be insufficient to see real efficacy gains in mCRPC. For example, while single agent checkpoint inhibitors have garnered robust responses and FDA approvals across several tumor types, response rates of ~10% are seen in mCRPC. Whether due to differences in mutational burden and neoantigen expression or the immune milieu within the tumor microenvironment, prostate cancer appears to require additional immunomodulation. Combinatorial approaches, many of which have been

discussed here, will be the future of prostate cancer immunotherapy—though, rational combinations must be pursued based on solid prostate tumor immunobiology. Whether off-the-shelf or personalized vaccines, combinations with cytokines or checkpoint inhibitors, or incorporating additional modalities such as radiotherapy, successful treatment regimens will have to take a multi-pronged approach. While the growing number of immunotherapy clinical trials in prostate cancer is encouraging, the relative paucity of robust response data and the limited drug approvals for prostate cancer over the last decade serve as a stark reminder of how much more work there is to be done in this field.

Compliance with Ethical Standards

Conflict of Interest Melissa A. Reimers and Kathryn Slane each declares no potential conflicts of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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