Immunotherapy for Localized Prostate Cancer
The Next Frontier?

Devin Patel, MD[a], Rana McKay, MD[b], J. Kellogg Parsons, MD, MHS[a,*]

KEYWORDS

• Immunotherapy • Prostate cancer • Checkpoint inhibitors • PD-L1 • CDK12 • ProstVac-VF • Sipuleucel-T

KEY POINTS

- Whereas vaccine-based immunotherapy has been promising, other immunotherapy agents, including checkpoint inhibitors, have shown limited efficacy in prostate cancer.
- Ongoing trials of combination therapy and promising biomarkers, including mutations in CDK12, may enhance the efficacy of checkpoint inhibitors for advanced prostate cancer.
- New treatments, including chimeric T lymphocytes and bispecific antibodies, provide future opportunities to enhance the immune response to prostate tumors.

INTRODUCTION

Interactions of the immune system and cancer have been appreciated since the late nineteenth century. Over the past few decades, an increasingly sophisticated understanding of these interactions has driven the development of a novel class of anticancer therapies: immunotherapy. Immunotherapy is the treatment of cancer through suppression or activation of the immune system. Prostate cancer has provided unique opportunities for, and challenges to, immunotherapy drug development.

In this article, we review the mechanisms of the immune response as it relates to cancer biology; outline broad strategies of immunotherapy and key concepts of immunotherapy as it relates to prostate cancer; describe prostate cancer immunotherapy drugs, including pivotal clinical trials and specific indications; and, finally, highlight the emerging role of immunotherapy for localized prostate cancer.

OVERVIEW OF THE IMMUNE RESPONSE AND CANCER

Innate Versus Adaptive Immunity

The immune system relies on an interplay between innate and adaptive immune responses. The innate immune system, which is present at birth and not learned or adapted, includes physical barriers (eg, skin, mucosal barriers), protein barriers (eg, complement components), and cellular barriers. Innate immune cells involved in tumor immunobiology include natural killer cells and macrophages. In addition to creating a nonspecific immune response, innate immune cells are essential for creating the cytokine environment needed for effective antigen presentation to adaptive immune cells. Adaptive immune cells, including cytotoxic $CD8^+$ lymphocytes and helper Th1/Th2 subclasses of $CD4^+$ T lymphocytes, rely on antigen presentation to produce a specific immune response.[1] Adaptive immune cells create a specific response to antigens, including tumor antigens. Most of the effort in using the immune

Conflicts of interest: The authors declare no conflicts of interest.

[a] Department of Urology, University of California San Diego, 9400 Campus Point Drive, MC7987, La Jolla, CA 92093, USA; [b] Division of Hematology-Oncology, Department of Internal Medicine, University of California San Diego, La Jolla, CA 92093, USA
* Corresponding author.
E-mail address: k0parsons@ucsd.edu

Urol Clin N Am 47 (2020) 443–456
https://doi.org/10.1016/j.ucl.2020.07.008

response for cancer immunobiology has focused on harnessing the adaptive immune response.

Basics of Tumor Immunobiology

Tumor immunobiology is best understood in the context of the cancer immunity cycle. This process starts with release of cancer antigens. Released antigens are then captured and presented by antigen-presenting cells (APCs) to adaptive response T lymphocytes. This process involves the creation of an immune synapse. During first contact between antigen-specific T lymphocytes and an antigen, T lymphocytes are primed. Subsequently the primed cells become activated and differentiate either into effector cells or memory cells. This priming and activation step is dependent on interplay among APCs, T lymphocytes, and stimulatory molecules in the immune microenvironment. T-lymphocyte activation is followed by T-lymphocyte trafficking to tumors through the vascular system and subsequently by T-lymphocyte tumor infiltration through the vascular endothelium into the tissue. Antigen-specific T-cell receptors are then able to recognize cancer cells and lead to cancer cell death. Immune-mediated killing in turn promotes tumor antigen release.

Antigen Release and Presentation

The first step in the cancer immunity cycle is antigen release. Immunogenic tumor cell death, in contrast to apoptotic tumor cell death, leads to necrosis and antigen release. Chemotherapy and radiation therapy also increase antigen release, promoting the framework for combined treatment strategies. Importantly, because of genetic alterations, tumors release neoantigens that are distinguishable from normal counterparts.[2] Released antigens, which are short stretches of amino acids, are then presented by two different classes of major histocompatibility complex (MHC) molecules. MHC class 1 is expressed by all nucleated cells, whereas MHC class 2 molecules are constitutively expressed by APCs, such as dendritic cells and macrophages. Antigen presentation is a complex phenomenon essential for T-lymphocyte function.

T-Lymphocyte Activation, Localization, and Response

The interaction between the T-cell receptor complex and the MHC influences the immune response. The T-cell receptor complex consists of a highly variable CD4 or CD8 subunit that binds to MHC. MHC class 1 is recognized by $CD8^+$ T cells, whereas MHC class 2 is recognized by $CD4^+$ T cells. The T-cell receptor also consists of a CD3 molecule, which plays a role in relaying extracellular signaling to intracellular effector molecules. Successful immune activation requires two components. First, the variable CD4 or CD8 molecule must bind to an appropriately matched antigen. Second, this binding must occur in the presence of other costimulatory signals. Without sufficient costimulatory signals, antigen tolerance (or anergy) occurs.[3] The most important costimulatory signal in T cells is the binding of CD28 on lymphocytes to B7-1 (CD80) and B7-2 (CD86) on the APCs. Costimulation is tightly regulated by positively stimulating agonist molecules and negatively regulating immune checkpoint molecules, including cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1 (PD-1), which have become important targets for cancer immunotherapy agents.

Activation of T lymphocytes is followed by clonal expansion, wherein many copies of lymphocytes are created that share affinity with and specificity of the same antigen. A subset of these clonal lymphocytes become memory cells and a subset become effector cells. Effector lymphocytes are then trafficked through the vascular system to areas of tumor. This T-lymphocyte homing is mediated by various chemokine signals, including CX3CL1, CX3CL9, and CX3CL10. T lymphocytes must then exit through the vascular endothelium and infiltrate the tumor. Lastly, these activated and homed T lymphocytes must recognize antigens to in turn promote tumor cell killing.

Tumor Escape

Immunologic tumor escape is the phenomenon by which tumor cells escape immune surveillance.[4] Tumors have the ability to evade each step of the cancer immunity cycle. Tumor cells have the ability to generate immune tolerance that in turn decreases antigen release. Escape of T-lymphocyte activation can occur by several mechanisms. This includes manipulation of the cytokine microenvironment through increased production of anti-inflammatory cytokines, such as interleukin (IL)-10, which directly and indirectly suppress T lymphocytes.[5] Tumor cells can also upregulate the expression of checkpoint molecules to negatively regulate costimulation.[6] T-lymphocyte tumor infiltration is inhibited by increased tumor production of growth factors, such as vascular endothelial growth factor.[7] Tumor cells may also escape recognition through decreased MHC class 1 molecule expression, resulting in reduced antigen presentation.[8,9]

STRATEGIES FOR CANCER IMMUNOTHERAPY

Knowledge of the tumor immune response has led to several strategies to harness these processes and develop rational, immune-based tumor therapies. These strategies include cytokine therapies, checkpoint inhibition drugs, and antitumor vaccines (Fig. 1).

Cytokine-Based Therapy

Cytokines are glycoproteins produced by immune cells to generate a local and systemic response. Cytokines play a role in initiating, sustaining, and regulating immune responses by stimulating T-cell growth and natural killer cells. Importantly, they create a nonspecific immune response. Cytokine-based therapy was the first type of tumor immunotherapy developed for urologic malignancies. IL-2, the first cytokine found to have therapeutic benefit, was discovered in 1976 by Robert Gallo, MD, and Francis Ruscetti, PhD.[10] IL-2 achieves a durable response in a subset of patients with renal cell carcinoma and leads to improved survival when combined with cytoreductive nephrectomy in patients with metastatic disease.[11] IL-2 has also shown efficacy in advanced melanoma.[12] Interferon alfa-2b, which promotes $CD8^+$ lymphocytes, and bacille Calmette-Guérin, which induces cancer cells to produce cytokines and present tumor antigens to lymphocytes, have shown efficacy in superficial urothelial carcinoma.[13]

Checkpoint Inhibition

Immune checkpoints are physiologic constraints on unrestrained cytotoxic T-effector function. The interaction between PD-1, a transmembrane protein expressed on T cells, and PD-1 ligand (PD-L1), expressed on normal cells and many tumor cells, is an important checkpoint for T lymphocytes.[14] The prevalence of PD-L2, the other known ligand of PD-1, and its relationship to response to anti-PD-1 therapy is unknown. The PD-1 inhibitors that target ligand (ie, anti-PD-L1) as opposed to receptor (anti-PD-1) interfere with ligand binding to PD-L2, but the clinical relevance of these interactions remains uncertain.[15,16]

The binding of PD-1 and PD-L1 acts as a physiologic brake on unrestrained T-lymphocyte function. Binding of PD-1 to tumor cell PD-LI leads to

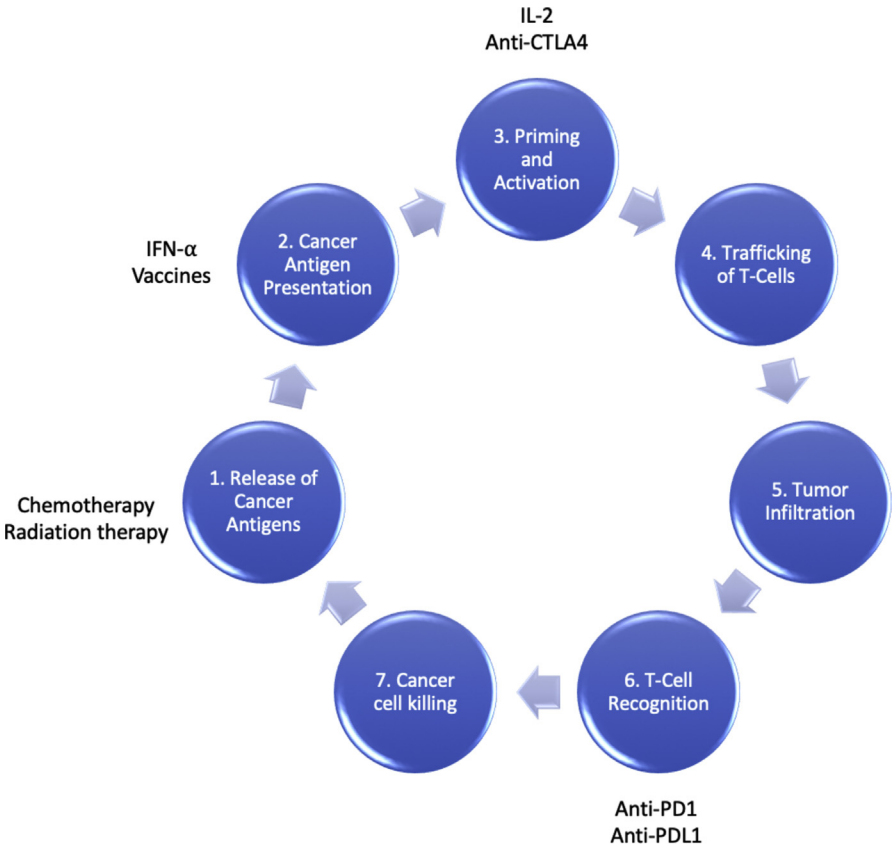

Fig. 1. The cancer-immunity life cycle. IFN, interferon. (*Adapted from* Chen, Daniel S., and Ira Mellman. "Oncology meets immunology: the cancer-immunity cycle." Immunity 39.1 (2013): 1-10.)

inhibition of tumor cell apoptosis and T-lymphocyte tolerance.[17] Checkpoint inhibitors are drugs that target these interactions. PD-1 inhibitors include pembrolizumab and nivolumab. PD-L1 inhibitors include atezolizumab, avelumab, and durvalumab. Currently, pembrolizumab is approved for microsatellite instability–high metastatic prostate cancer. Pembrolizumab and atezolizumab have been approved for use in metastatic urothelial cell carcinoma. Pembrolizumab has also been approved for use in bacille Calmette-Guérin–unresponsive, non–muscle invasive bladder cancer. Combination treatment with nivolumab and ipilimumab, pembrolizumab and axitinib, and avelumab and axitinib have been approved for advanced renal cell carcinoma.

CTLA-4 is a second transmembrane protein expressed on T lymphocytes and acts by competitively binding B7-1 (CD80) and B7-2 (CD86), located on APCs, to form a negative feedback loop on activated lymphocytes. The anti-CTLA-4 antibody ipilimumab was the first immune checkpoint inhibitor to be approved for metastatic cancer.[18]

Vaccines

Vaccines generate an adaptive immune response by relying on a combination of antigen presentation and accompanying immune adjuvants, which act to create the necessary immune microenvironment to stimulate immune cells. Vaccine antigens can be peptides, which are easier to prepare but limited in spectrum, or whole cell, which offers a broader range of antigens but are more labor intensive to generate. Viral-based cancer vaccines, such as ProstVac-VF, have also been developed for prostate cancer. Sipuleucel-T, a dendritic cell vaccine for advanced prostate cancer, is the only currently approved cancer vaccine. Dendritic cell vaccines are unique in that they rely on ex vivo manipulation. Dendritic cells are removed from the patient's body and isolated. The cells are subsequently primed to an antigen and these primed, antigen-presenting dendritic cells are reintroduced to the patient.

IMMUNOTHERAPY CONSIDERATIONS FOR PROSTATE CANCER

Although the basics of tumor immunobiology apply to all solid tumors, there are several important considerations specific to implementing immunotherapy for prostate cancer. The development of immunotherapy strategies for prostate cancer has differed slightly when compared with other solid tumors. For instance, therapeutic cancer vaccines have shown greater clinical activity in prostate cancer than in other tumor types. This is most notably because prostate cancer cells express several tumor-associated antigens. These cancer-specific antigens can be used to develop vaccines, which function by enhancing the immune recognition of these antigens to generate a targeted T-lymphocyte-mediated immune response. Several unique tumor-associated antigens are produced by prostate cells. Examples include prostatic acid phosphatase, which may regulate prostate cancer cell growth; prostate-specific antigen (PSA), which serves as a sensitive biomarker for low-volume disease and recurrence; and prostate-specific membrane antigen, a transmembrane protein that is induced to higher expression levels with androgen deprivation therapy (ADT).[19,20]

Despite the promise of vaccines in prostate cancer, results of treatment with other forms of immunotherapy, such as with checkpoint inhibitors, have been less robust compared with other malignancies.[21,22] Relative to other tumor types, prostate cancer cells have a low tumor mutational burden and a low expression of PD-L1.[23,24] Similarly, prostate cancer is a "cold" tumor with minimal T-cell infiltrates.[25] Furthermore, prostate cancer characteristically has absent or downregulated MHC class 1 expression, in primary and metastatic tumors, posing a challenge for immunotherapy agents, such as checkpoint inhibitors, which rely on MHC class 1–mediated antigen presentation.[26,27] These factors have been challenges for implementing newer forms of immunotherapy for prostate cancer.

In addition to differing responses to types of immunotherapy, there are important treatment-related and disease-related considerations that are specific to prostate cancer. For example, hormonal therapy, a cornerstone of prostate cancer treatment, has numerous implications related to immunotherapy. ADT has been shown to modulate the immune system by restoring thymic function and promoting T-cell proliferation.[28] In addition to these systemic effects, ADT leads to increased prostate immune infiltrates, increased cancer-targeted antigens, and decreased T-cell antigen tolerance. Together, these results suggest that hormonal therapy should augment the effect of immunotherapy.

In contrast, the predilection for prostate cancer to metastasize to bone proves to be a challenge in implementing immunotherapy. The bone microenvironment is oxygen poor and rich in lymphocyte regulatory cells and myeloid-derived suppressor cells, which act to dampen the immune response.[29] Furthermore, prostate cancer cells promote osteoblast- and osteoclast-mediated

growth factor production. Many of these growth factors, such as transforming growth factor-β, suppress the immune response (Table 1).[30]

Several clinical trials have investigated the impact of immunotherapy on metastatic prostate cancer. In addition to advanced disease, there is growing rationale for and use of immunotherapy in localized disease. Importantly, unlike cytotoxic therapy, immunotherapy may not cause dramatic changes in tumor burden over a short period of time and relies on immunologic memory. Accordingly, starting immunotherapy earlier in the disease course may lead to much greater improvements in outcomes than starting later.[31] Vaccine-based therapies that have been studied include ProstVac-VF and Sipuleucel-T. Various checkpoint inhibitors, including ipilimumab, a humanized anti-CTLA-4 monoclonal antibody, and pembrolizumab, a PD-1 inhibitor, have also been studied (Table 2).

ProstVac-VF

ProstVac-VF is a viral-based recombinant vaccine that uses viral vectors containing transgenes for PSA and multiple proprietary T-cell costimulatory molecules (TRICOM) that act to bolster the local immune response.

Mechanisms

Primary vaccination is done using a vaccinia virus vector and subsequent booster doses are given using a fowlpox virus vector. The vaccinia virus produces a strong immune response with a single dose. However, if given in repeated doses, it is neutralized by the host immune response.[32] As such, subsequent doses are given using fowlpox, which although able to penetrate APCs, does not lead to production of high volumes of neutralizing antibodies by the host.[33]

ProstVac-VF in Advanced Prostate Cancer

Several clinical trials have evaluated the safety and efficacy of ProstVac-VF monotherapy. Initial phase I trials established the safety of vaccinia-based vaccines and subsequently of combined regimens of vaccinia virus priming with fowlpox virus boost.[34,35] In addition to establishing safety, these trials showed a tissue immune response and a PSA response. Several phase II trials of ProstVac-VF monotherapy for advanced prostate cancer again confirmed a PSA response and suggested an improvement in survival.[36,37]

Although a large phase III trial of 1200 chemotherapy-naive men with asymptomatic metastatic castration-resistant prostate cancer (mCRPC) showed no improvement in overall or progression-free survival between men randomized to ProstVac-VF or to placebo,[38] there is robust biologic and early clinical evidence to suggest that combining vaccine-based immunotherapy with traditional advanced prostate cancer therapies, including chemotherapy, androgen-targeted therapy, and bone radionucleotides, may improve outcomes. Docetaxel leads to tumor cell cytolysis, which in turn leads to an increase in tumor-associated antigens. Furthermore, preclinical data have suggested that docetaxel and other chemotherapeutic agents may have an immunostimulatory effect, by increasing cytokine production and increasing MHC class I expression.[39] A phase II study of docetaxel plus vaccine treatment showed that patients who received vaccine had an increase in antigen-specific T cells and a longer progression-free survival on docetaxel.[40] Larger studies of combination immunotherapy and chemotherapy are ongoing. In addition to chemotherapy, there is biologic probability that vaccine-based therapies may have increased efficacy with androgen-targeted therapies. Testosterone has an antiproliferative effect on T cells and ADT is known to enhance T-cell infiltration of the prostate.[41,42]

Studies combining vaccine-based treatments with newer androgen-targeted therapies, such as abiraterone or enzalutamide, are lacking. However, early results from a randomized phase II study of flutamide with or without vaccine in non-mCRPC have shown longer progression-free survival in the arm treated with vaccine compared with those treated with antiandrogen alone.[43] Along with androgens, other studies have looked at the impact of combining ProstVac-VF with bone-targeting therapies in men with bone mCRPC. Radiation treatment of tumors, even at low doses, is thought to increase tumor antigen generation and presentation. Sm-153 consists of radioactive samarium and a tetraphosphate chelator and acts by targeting low levels of radiation to metastatic lesions in bone. A phase II study of men with nonvisceral mCRPC showed longer progression-free survival (3.7 months vs 1.7 months; $P = .041$) and PSA response in men treated with Sm-153 combined with vaccine therapy compared with those treated with Sm-153 alone.[44]

ProstVac-VF in Localized Prostate Cancer

Phase I clinical trials have shown that of ProstVac-VF generates an inflammatory response in localized prostate cancer.[45] Studies of ProstVac-VF in early stage prostate cancer are ongoing. A

Table 1
Immunotherapies for prostate cancer

Drug Classes	Specific Agents	Mechanism	Applicability to Localized Prostate Cancer
Vaccine-Based Treatment			
Virus-based vaccine	PROSTVAC-VF[34]	Vaccinia and fowlpox virus genetically engineered to contain human PSA	Possible Ongoing phase II trial (NCT02326805) in patients with clinically localized prostate cancer on active surveillance. Primary outcome is tumor immune response.[46]
Dendritic cell vaccine	Sipuleucel-T[51]	Autologous dendritic cell vaccine to enhance T-cell response to prostatic acid phosphatase	Possible A current trial (NCT03686683) is examining the impact on active surveillance patients. Primary outcome is reduction in reclassification to a higher Gleason grade.[88]
Checkpoint Inhibitors			
PD-1 inhibitor	Pembrolizumab, nivolumab[60,61]	Monoclonal antibody against inhibitor molecule PD-1 expressed on T-cells	Unknown Pembrolizumab plus prostatic cryotherapy evaluated in men with low-volume hormone-sensitive metastatic prostate cancer. Results showed that 42% (5/12) of patients had a PSAs of <0.6 ng/mL at 1 y.[67]
PD-L1 inhibitor	Atezolizumab, avelumab, durvalumab[89]	Monoclonal antibody against inhibitor molecule PD-1 ligand expressed on tissue cells	Unknown
CTLA-4 inhibitor	Ipilimumab[56]	Monoclonal antibody against inhibitor molecule cytotoxic T-lymphocyte-associated protein 4	Unknown A phase I trial of the CTLA-4 inhibitor ipilimumab plus ADT in men with PSA only recurrent prostate cancer after local treatment showed improved PSA kinetics with the addition of immunotherapy.[64]

randomized phase II trial of ProstVac-VF in patients with clinically localized prostate cancer on active surveillance has completed accrual and follow-up.[46] Patients with low- or intermediate-risk prostate cancer (stage $\leq$T2a, grade group $\leq$2 [Gleason $\leq$3 + 4 = 7], $\leq$50% of the biopsy cores containing cancer, and PSA <20 ng/mL) were randomized (2:1) to 5 months of treatment with either vaccine or placebo. Prostate biopsy was performed following treatment. The primary outcome is tumor immune response as defined by tissue and serum biomarkers. Results from this study are expected in 2021 (ClinicalTrials.gov NCT02326805).

There has also been interest in combining ProstVac-VF with radiation therapy for localized cancer. Radiation treatment can induce tumors to upregulate expression of MHC molecules and tumor-associated antigens, thereby making these cells more susceptible to a T-lymphocyte response.[47] Initial phase II studies of vaccine plus radiation therapy treatment showed an increase in PSA-specific T cells among patients treated with vaccine compared with control

Table 2
Selected clinical trials of immunotherapy for prostate cancer

Trial	Phase	Arms	N	Patient Population	Primary End Point	Median OS (mo); ORR[a]	Hazard Ratio (CI) vs Placebo
Kantoff et al,[53] 2010	3	Sipeulecel-T vs placebo	512	mCRPC	OS	25.8 vs 21.7	0.78 (0.61–0.98)
Kwon et al,[56] 2014	3	Radiotherapy with ipilimumab vs placebo	799	mCRPC progressed on docetaxel	OS	11.2 vs 10.0	0.85 (0.72–1.00)
Beer et al,[57] 2017	3	Ipilimumab vs placebo	602	Asymptomatic mCRPC without prior therapy	OS	28.7 vs 29.7	1.11 (0.88–1.39)
Hansen et al,[60] 2018, KEYNOTE-028	1b	Pembrolizumab	23	Metastatic prostate cancer failing prior therapy with PD-L1 expression in $\geq$1%	ORR	17.4%	n/a[a]
Antonarakis et al,[59] 2020, KEYNOTE-199	2	Pembrolizumab	258	mCRPC treated with docetaxel and >1 targeted endocrine therapy with PD-L1-positive (cohort 1) or PD-L1-negative (cohort 2) disease	ORR	5% (cohort 1) and 3% (cohort 2)	n/a[a]
Sharma et al,[58] 2019 CheckMate 650	2	Nivolumab + ipilimumab	78	Asymptomatic mCRPC who progressed after second-generation hormone therapy without prior chemotherapy (cohort 1) vs patients who progressed after taxane therapy (cohort 2)	ORR	26% (cohort 1) and 10% (cohort 2)	n/a[a]

Abbreviations: CI, confidence interval; mCRPC, metastatic castration-resistant prostate cancer; ORR, objective response rate; OS, overall survival.
[a] Patients enrolled in KEYNOTE-028, KEYNOTE-199, and CheckMate 650 were nonrandomized.

subjects.[48] However, studies with longer term follow-up have shown that the addition of vaccine does not seem to have a significant difference with regard to PSA control and that long-term immune response may be limited.[49]

SIPULEUCEL-T

Sipuleucel-T is an autologous dendritic cell vaccine that enhances the immune response to prostatic acid phosphatase antigen. It is the only currently approved vaccine-based therapy for advanced cancer. In the setting of CRPC, Sipuleucel-T has had a favorable safety profile and prolonged survival compared with placebo.

Mechanisms

To prepare the vaccine, peripheral blood mononuclear cells are isolated by leukapheresis.[50] These cells are exposed ex vivo to a prostatic acid phosphatase antigen fused to human granulocyte-macrophage colony–stimulating factor. Once cells are activated to the antigen, they are infused back into the patient. A total of three treatments are performed over a 6-week period.[51]

Sipuleucel-T in Advanced Prostate Cancer

Evidence for the efficacy of Sipuleucel-T in CRPC has come from three large randomized trials. Patients eligible for inclusion in these trials had radiologic evidence of asymptomatic or minimally symptomatic mCRPC and good performance status, defined as an Eastern Cooperative Oncology Group score of less than 1. A total of 225 men were evaluated in a pooled analysis of two separate trials. Results showed that when compared with placebo, treatment with Sipuleucel-T was associated with an improved, albeit statistically nonsignificant, progression-free survival (11.1 vs 9.7 months; $P = .11$). Overall survival, a secondary end point, was significantly longer in the Sipuleucel-T group compared with placebo (median, 23.2 vs 18.9 months; $P = .01$).[50,52] The phase III IMPACT trial evaluated overall survival as the primary end point.[53] A total of 512 patients were randomized in a 2:1 ratio to receive either Sipuleucel-T (341 patients) or placebo (171 patients). Among men receiving Sipuleucel-T, there was a relative reduction of 22% in the risk of death as compared with the placebo group (hazard ratio [HR], 0.78; $P = .03$). Median survival was improved by 4.1 months in the treatment group (25.8 months in the Sipuleucel-T group vs 21.7 months in the placebo group). Patients receiving vaccine had more frequent T-cell proliferation responses to prostatic acid phosphatase. Among patients receiving Sipuleucel-T, results from this study also showed that patients with an antibody response to vaccine antigens had a significantly longer survival.[53] In all three trials, Sipuleucel-T was generally well tolerated, with the most common adverse events being chills (53%), fatigue (41%), fever (31%), nausea (21%), and headache (7%).

Sipuleucel-T in Localized Prostate Cancer

The use of Sipuleucel-T in localized prostate cancer has garnered some interest. Tumor immune recruitment was analyzed in a study of 42 patients given a standard dose of Sipuleucel-T before radical prostatectomy. Results from this study showed a systemic and local tumor response to vaccine treatment. Patients given vaccine had higher peripheral levels of interferon-γ and increased T-cell proliferation. Immunohistochemistry results of tumor specimens showed an increase in cytotoxic and nonregulatory helper T cells.[54]

A current trial (NCT03686683) is examining the impact of Sipuleucel-T administered to active surveillance patients for newly diagnosed prostate cancer: the open label trial ProVent. This study is designed to accrue 450 participants with International Society of Urologic Pathology grade group 1 or 2 prostate cancer diagnosed via either systematic or MRI-targeted biopsy enrolled in active surveillance. The primary outcome of interest is the efficacy of Sipuleucel-T in reducing histopathologic reclassification to a higher Gleason grade within 36 months in prostate cancer subjects on active surveillance. This trial completed accrual ahead of schedule and is currently in follow-up.

CHECKPOINT INHIBITOR IMMUNOTHERAPY

Immune checkpoints act as negative feedbacks on T lymphocytes. CTLA-4, a molecule found on T lymphocytes, is an important inhibitory costimulatory signal that suppresses the T-cell response to antigen presentation when binding to B7 on APCs. PD-1 is a second inhibitory transmembrane protein expressed on T cells that acts by binding PD-L1, found on normal tissue cells. Checkpoint inhibitors act by blocking these signals to in turn stimulate the immune response.

Checkpoint Inhibitors in Advanced Prostate Cancer

Ipilimumab, a humanized anti-CTLA-4 monoclonal antibody, binds to the CTLA-4 receptor on T cells and augments the immune response by blocking the interaction of CTLA-4. Phase I/II trials have

shown that ipilimumab is well tolerated alone and when combined with bone-targeted radiotherapy and may produce a PSA response.[55] However, two large phase III studies of ipilimumab have failed to show any improvement in overall survival over placebo. In both trials ipilimumab was given every 3 weeks for four cycles in men with CRPC. Among 799 patients with bone mCRPC who had received prior treatment with docetaxel, the combination of bone-directed radiotherapy plus immunotherapy showed no benefit in the primary outcome of overall survival when compared with placebo.[56] Median overall survival was 11.2 months (95% confidence interval [CI], 9.5–12.7) in men treated with ipilimumab and 10.0 months (95% CI, 8.3–11.0) with placebo (HR, 0.85; 95% CI, 0.72–1.00). Secondary analyses showed that median progression-free survival was improved with immunotherapy treatment compared with placebo (4.0 vs 3.1 months; HR, 0.70; 95% CI, 0.61–0.82). Also, a larger portion of patients treated with ipilimumab (13.1%; 95% CI, 9.5–17.5) had a greater than 50% PSA response when compared with placebo (5.2%; 95% CI, 3.0–8.4). A second trial, of 600 men with no prior nonhormonal treatment of asymptomatic or minimally symptomatic nonvisceral mCRPC, again compared therapy with ipilimumab versus placebo.[57] Results were similar, and no difference was seen in the primary outcome of overall survival. Median overall survival was 28.7 months (95% CI, 24.5–32.5 months) in the ipilimumab arm versus 29.7 months (95% CI, 26.1–34.2 months) in the placebo arm (HR, 1.11; 95.87% CI, 0.88–1.39). However, median progression-free survival was longer in the ipilimumab arm (5.6 vs 3.8 months; HR, 0.67; 95.87% CI, 0.55–0.81). PSA response also seemed to be higher in the treatment arm (23%; 95% CI, 19%–27%) than with placebo (8%; 95% CI, 5%–13%). Together these studies showed that immunotherapy for advanced prostate cancer has an acceptable and well-tolerated toxicity profile. Moreover, these trials have shown that despite inducing some measurable antitumor activity, via progression-free survival and PSA response, treatment with ipilimumab does not extend overall survival in unselected populations of patients with mCRPC. With the limited clinical benefit of checkpoint inhibitor monotherapy, ongoing trials are attempting to evaluate the efficacy of combination immunotherapy. The CheckMate 650 trial is aiming to evaluate the efficacy of nivolumab plus ipilimumab in men with mCRPC in two cohorts, those who have progressed after second-generation hormone therapy and have not received chemotherapy and those who have progressed after taxane-based chemotherapy.[58] Interim results have shown objective response rates of 26% in chemotherapy-naive patients and 10% in those failing prior taxane therapy. Furthermore, objective response rates in both cohorts were higher among patients with PD-L1 expression greater than 1%, DNA damage repair or homologous recombination mutations, or higher tumor mutational burden.

The PD-1 inhibitor pembrolizumab has been studied in CRPC. KEYNOTE-199, a phase II trial of men with mCRPC who failed prior chemotherapy, enrolled 258 men.[59] Patients were stratified into three different cohorts based on PD-L1 overexpression and location of metastatic disease. A total of 133 men had PD-L1-positive disease, 66 had PD-L1-negative disease, and 59 had bone-predominant disease. Among men with PD-L1 overexpression, there was a 5% objective response and a complete response in two patients. Among patients with bone-predominant disease, the disease control rate was 22%. Results from the smaller KEYNOTE-028 also suggested that pembrolizumab can result in durable responses for individuals with CRPC and PD-L1 overexpression.[60] Data from other cancers have suggested that in addition to PD-L1 expression, tumors with DNA mismatch repair mechanism (dMMR) mutations may derive benefit from treatment with pembrolizumab.[61] Tumors with dMMR mutations seem to have higher rates of mutations and a resultant higher rate of tumor-associated antigens. A hallmark of dMMR is the presence of high levels of microsatellite instability.[61] Based on data from other tumors, pembrolizumab is currently approved for treatment of a variety of advanced solid tumors, including prostate cancers, that have dMMR mutations or microsatellite instability, specifically in men who have progressed following prior treatment and exhausted alternative treatment options. However, results from other studies have suggested that dMMR mutations and microsatellite instability are rare in advanced prostate cancer, occurring as infrequently as 1% to 2%, limiting the widespread use of PD-L1 inhibitors in advanced prostate cancer.[62,63]

Checkpoint Inhibitors in Oligometastatic Prostate Cancer

As a treatment that relies on generating an immunogenic response with treatment memory, there is biologic rationale for starting checkpoint inhibitor therapy earlier in the disease course. A phase I trial of the CTLA-4 inhibitor ipilimumab plus ADT in men with PSA only recurrent prostate cancer after local treatment showed improved PSA kinetics with the addition of immunotherapy.[64] Some

research has posited that adding local therapy to systemic immunotherapy may be more effective than immunotherapy given alone. Localized cell death leads to increased immune presentation that may augment a systemic response, a phenomenon known as the abscopal effect.[65] Preclinical models have suggested that, among forms of local treatment, cryotherapy may produce the most robust immune response.[66] With this evidence in mind, a recent trial evaluated the potential benefit of combining pembrolizumab plus prostatic cryotherapy among men with low volume (≤5 metastases) hormone-sensitive metastatic prostate cancer.[67] Treatment was well tolerated with minimal complications. Results from this study showed that 42% (5/12) of patients had PSAs of less than 0.6 ng/mL at 1 year.

Checkpoint Inhibitors in Localized Prostate Cancer

Treatment with checkpoint inhibitors before surgery for prostate cancer has been investigated. A total of 20 patients with localized, high-risk prostate cancer were treated with ADT and two doses of ipilimumab before radical prostatectomy.[68] Tumor specimens were analyzed to better understand prostate tumor immune response. The authors discovered potential compensatory immune inhibitory pathways that may arise in the setting of immune checkpoint inhibition. After treatment with checkpoint inhibitor therapy, tumors had significantly higher levels of PD-1 and PD-L1 expression and increased expression of VISTA, a second inhibitor molecule known to suppress T-lymphocyte response.[69] The results from this study have helped elucidate possible mechanisms of prostate cancer's relative resistance to immune monotherapy.

FUTURE DIRECTIONS

Combination Therapies

Despite the disappointing results of checkpoint inhibitor monotherapy in advanced prostate cancer, there is increasing evidence that combining checkpoint inhibitors with other forms of systemic therapy may enhance their efficacy. Tyrosine kinase inhibitors may perform synergistically with checkpoint inhibitors by allowing for increased tumor perfusion and lymphocyte infiltration.[70] A recent phase 1b study (COSMIC-021) investigated the objective response rate of patients with mCRPC treated with the tyrosine kinase inhibitor cabozantinib combined with the PD-L1 inhibitor atezolizumab. Results showed an objective response rate of 32%, with 4.5% having a complete response and 27% having a partial response.[71] Other trials using a similar approach include an ongoing phase I study investigating tremelimumab (anti-CTLA-4) plus durvalumab (anti-PD-L1) in patients with chemotherapy-naive mCRPC (NCT03204812) and the IMPACT study (NCT03570619) of nivolumab plus ipilimumab in populations with mutations in CDK12. CDK12 mutations, which are present in approximately 5% of mCRPC tumors, confer a distinct phenotype of prostate cancer that is thought to be more immunogenic.[72]

Another approach would be to combine different treatment modalities to potentiate immunotherapies for localized disease. Pairing an established prostate ablation therapy, such as cryoablation, with a checkpoint inhibitor or cancer vaccine holds conceptual promise. Cryoablation lyses tumor cells and provokes a systemic immune response. Treating a prostate tumor with cryoablation would potentially prime it for subsequent immunotherapy by turning a "cold" prostate cancer "hot" and thus rendering it more susceptible to a cancer vaccine or checkpoint inhibitor.[73,74]

T-Cell Engaging Therapies

A promising technology in development is the use genetic engineering for immunotherapy. These treatments include chimeric lymphocytes and bispecific antibodies. Chimeric antigen receptor (CAR) T lymphocytes are genetically engineered cells designed to produce an artificial T-lymphocyte receptor for use in immunotherapy. An ongoing phase I trial is investigating the safety of CAR T lymphocytes directed at prostate-specific membrane antigen. Initial cohorts have completed therapy with no reports of dose-limiting toxicity.[75] A phase I study of CAR-T (NCT04249947) began actively recruiting in January 2020. Bispecific antibodies are genetically engineered antibody proteins that are designed to bind to two different types of antigens. By binding tumor antigens in one arm and T-lymphocyte antigens in the second arm, tumor cells are more effectively cross-linked to effector immune cells.[76] Bispecific antibodies that target the tumor antigen prostate-specific membrane antigen and the T-lymphocyte antigen CD3 have recently been developed for prostate cancer, with early results indicating promising tolerability.[77] A phase I trial (NCT03577028) investigating the efficacy of the bispecific antibody HPN424 is currently accruing patients with CRPC.

Predictors of Immune Response

With immunotherapy becoming more widely used in advanced cancer treatment, there has been increased effort in understanding potential

predictors of response to treatment. Several predictors of immune response have been studied, including PD-L1 expression via immunohistochemistry, tumor mutational burden, gene expression profiling, and multiplex immunohistochemistry/immunofluorescence. These assays have been used to assess pretreatment tumor tissue to predict response to checkpoint inhibitor treatment.[78] Results among men with CRPC suggest that PD-L1 overexpression may predict response to agents that target this pathway. However, many prostate tumors lack PD-L1 expression.[24] Results suggest that men with intraductal tumors, high-grade (grade group 5) tumors, and tumors that are resistant to enzalutamide may have greater levels of PD-L1 expression.[79–81] Other authors have suggested that PD-L2 may be an alternative marker in prostate tumors to predict immunotherapy response.[22] Tumor mutational burden is a well-established marker of response to PD-1 inhibition.[82] However, relative to other solid tumors, prostate cancers tend to have a lower mutational burden. The advent of large-scale and rapid-throughput gene-expression profiling of tumors has led to development of several gene signatures that have shown to predict response to immunotherapy.[83]

Specifically, biallelic inactivation of CDK12 is a promising marker for immunogenic prostate cancer. CDK12 is a cyclin-dependent kinase that controls genetic stability by regulating DNA repair genes. CDK12 mutation is associated with increased genomic instability and leads to increased gene fusion events and neoantigen creation. Tumors with CDK12 mutations also have increased lymphocyte infiltration.[84] Several gene profiles have been developed in metastatic melanoma and non–small cell lung cancer.[85] Many of these genes are immune related and involve chemokine pathways. However, these have yet to be developed or validated for prostate cancer. The immune characteristic of the tumor microenvironment provides an additional way to predict treatment response. With this aim in mind, multiplex immunohistochemistry/immunofluorescence is a novel method of staining immune cells and tumors cells. This technique provides objective, quantitative data describing the immune subset and location within the tumor microenvironment. These data are used to better classify tumors as being T-lymphocyte inflamed versus immune excluded.[86]

In addition to tumor specific predictors of response, increasing evidence suggests that response to immunotherapy involves a complex interplay between somatic inheritance and tumor-related mutations. MHC molecules are highly genetically variable. Recent results have shown that inherited MHC class I genotype plays a role in restricting the ability of T lymphocytes to present certain tumor antigens.[87] As such, future efforts to predict response to immunotherapy may rely on combining patient-specific and tumor-specific data to optimize candidates for this type of therapy.

FUNDING

Dr. Mckay: NCI P30CA023100-34 Prostate Cancer Foundation Young Investigator Award.

SUMMARY

Compared with other solid tumors, prostate cancer poses challenges for immunotherapy. Whereas the vaccine-based treatment Sipuleucel-T has been introduced in the clinic, other immunotherapy agents, including checkpoint inhibitors, have shown limited efficacy in prostate cancer. Ongoing trials of combination therapy may enhance the efficacy of checkpoint inhibitors for advanced prostate cancer. Biomarkers for immunotherapy response, including mutations in CDK12, also show promise. New treatments, including chimeric T lymphocytes and bispecific antibodies, provide future opportunities to enhance the immune response to prostate tumors.

REFERENCES

1. Hennecke J, Wiley DC. T cell receptor-MHC interactions up close. Cell 2001;104(1):1–4.
2. Boon T, Cerottini JC, Van den Eynde B, et al. Tumor antigens recognized by T lymphocytes. Annu Rev Immunol 1994;12:337–65.
3. Wherry EJ. T cell exhaustion. Nat Immunol 2011;12(6):492–9.
4. Gajewski TF, Woo S-R, Zha Y, et al. Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. Curr Opin Immunol 2013;25(2):268–76.
5. Vinay DS, Ryan EP, Pawelec G, et al. Immune evasion in cancer: mechanistic basis and therapeutic strategies. Semin Cancer Biol 2015;35(Suppl):S185–98.
6. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515(7528):568–71.
7. Franciszkiewicz K, Le Floc'h A, Boutet M, et al. CD103 or LFA-1 engagement at the immune synapse between cytotoxic T cells and tumor cells promotes maturation and regulates T-cell effector functions. Cancer Res 2013;73(2):617–28.

8. Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 2016;375(9):819–29.
9. Rooney MS, Shukla SA, Wu CJ, et al. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 2015;160(1–2):48–61.
10. Rosenberg SA, Mulé JJ, Spiess PJ, et al. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. J Exp Med 1985;161(5):1169–88.
11. Belldegrun AS, Klatte T, Shuch B, et al. Cancer-specific survival outcomes among patients treated during the cytokine era of kidney cancer (1989-2005): a benchmark for emerging targeted cancer therapies. Cancer 2008;113(9):2457–63.
12. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA 1994;271(12):907–13.
13. Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer: a current perspective. Nat Rev Urol 2014;11(3):153–62.
14. Spranger S, Spaapen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 2013;5(200):200ra116.
15. Yearley JH, Gibson C, Yu N, et al. PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. Clin Cancer Res 2017;23(12):3158–67.
16. Yang H, Zhou X, Sun L, et al. Correlation between PD-L2 expression and clinical outcome in solid cancer patients: a meta-analysis. Front Oncol 2019;9:47.
17. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 2009;206(13):3015–29.
18. Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 2015;33(17):1889–94.
19. Kong HY, Byun J. Emerging roles of human prostatic acid phosphatase. Biomol Ther (Seoul) 2013;21(1):10–20.
20. Wright GL, Grob BM, Haley C, et al. Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. Urology 1996;48(2):326–34.
21. Madan RA, Gulley JL. Finding an immunologic beachhead in the prostate cancer microenvironment. J Natl Cancer Inst 2019;111(3):219–20.
22. Zhao SG, Lehrer J, Chang SL, et al. The immune landscape of prostate cancer and nomination of PD-L2 as a potential therapeutic target. J Natl Cancer Inst 2019;111(3):301–10.
23. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163(4):1011–25.
24. Martin AM, Nirschl TR, Nirschl CJ, et al. Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance. Prostate Cancer Prostatic Dis 2015;18(4):325–32.
25. Vitkin N, Nersesian S, Siemens DR, et al. The tumor immune contexture of prostate cancer. Front Immunol 2019;10:603.
26. Blades RA, Keating PJ, McWilliam LJ, et al. Loss of HLA class I expression in prostate cancer: implications for immunotherapy. Urology 1995;46(5):681–6 [discussion: 686–7].
27. Sanda MG, Restifo NP, Walsh JC, et al. Molecular characterization of defective antigen processing in human prostate cancer. J Natl Cancer Inst 1995;87(4):280–5.
28. Brelińska R. Thymic epithelial cells in age-dependent involution. Microsc Res Tech 2003;62(6):488–500.
29. Ahern E, Harjunpää H, Barkauskas D, et al. Co-administration of RANKL and CTLA4 antibodies enhances lymphocyte-mediated antitumor immunity in mice. Clin Cancer Res 2017;23(19):5789–801.
30. Thomas DA, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 2005;8(5):369–80.
31. Gulley JL, Madan RA, Schlom J. Impact of tumour volume on the potential efficacy of therapeutic vaccines. Curr Oncol 2011;18(3):e150–7.
32. Gulley J, Chen AP, Dahut W, et al. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. Prostate 2002;53(2):109–17.
33. Hodge JW, McLaughlin JP, Kantor JA, et al. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. Vaccine 1997;15(6–7):759–68.
34. Eder JP, Kantoff PW, Roper K, et al. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin Cancer Res 2000;6(5):1632–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10815880.
35. Arlen PM, Skarupa L, Pazdur M, et al. Clinical safety of a viral vector based prostate cancer vaccine strategy. J Urol 2007;178(4 Pt 1):1515–20.
36. Kaufman HL, Wang W, Manola J, et al. Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. J Clin Oncol 2004;22(11):2122–32.
37. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted

immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 2010;28(7):1099–105.
38. Gulley JL, Borre M, Vogelzang NJ, et al. Phase III trial of PROSTVAC in asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. J Clin Oncol 2019;37(13):1051–61.
39. Chan OTM, Yang LX. The immunological effects of taxanes. Cancer Immunol Immunother 2000;49(4–5):181–5.
40. Arlen PM, Gulley JL, Parker C, et al. A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. Clin Cancer Res 2006;12(4):1260–9.
41. Sutherland JS, Goldberg GL, Hammett MV, et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol 2005;175(4):2741–53.
42. Drake CG, Doody ADH, Mihalyo MA, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. Cancer Cell 2005;7(3):239–49.
43. Bilusic A-M, Gulley A-JL, Heery A-C, et al. A randomized phase II study of flutamide with or without PSA-TRICOM in nonmetastatic castration-resistant prostate cancer (CRPC). J Clin Oncol 2011;29:163.
44. Heery CR, Madan RA, Stein MN, et al. Samarium-153-EDTMP (Quadramet) with or without vaccine in metastatic castration-resistant prostate cancer: a randomized phase 2 trial. Oncotarget 2016;7(42):69014–23.
45. Merino MJ, Pinto PA, Moreno V, et al. Morphological changes induced by intraprostatic PSA-based vaccine in prostate cancer biopsies (phase I clinical trial). Hum Pathol 2018;78:72–8.
46. Parsons JK, Pinto PA, Pavlovich CP, et al. A randomized, double-blind, phase II Trial of PSA-TRICOM (PROSTVAC) in patients with localized prostate cancer: the immunotherapy to prevent progression on active surveillance study. Eur Urol Focus 2018;4(5):636–8.
47. Sheard MA, Vojtesek B, Janakova L, et al. Up-regulation of Fas (CD95) in human p53wild-type cancer cells treated with ionizing radiation. Int J Cancer 1997;73(5):757–62.
48. Gulley JL, Arlen PM, Bastian A, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res 2005;11(9):3353–62.
49. Kamrava M, Kesarwala AH, Madan RA, et al. Long-term follow-up of prostate cancer patients treated with vaccine and definitive radiation therapy. Prostate Cancer Prostatic Dis 2012;15(3):289–95.
50. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 2009;115(16):3670–9.
51. Tanimoto T, Hori A, Kami M. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363(20):1966 [author reply: 1967–8].
52. Small EJ, Schellhammer PF, Higano CS, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 2006;24(19):3089–94.
53. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363(5):411–22.
54. Fong L, Carroll P, Weinberg V, et al. Activated lymphocyte recruitment into the tumor microenvironment following preoperative sipuleucel-T for localized prostate cancer. J Natl Cancer Inst 2014;106(11). https://doi.org/10.1093/jnci/dju268.
55. Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013;24(7):1813–21.
56. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15(7):700–12.
57. Beer TM, Kwon ED, Drake CG, et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer. J Clin Oncol 2017;35(1):40–7.
58. Sharma P, Pachynski RK, Narayan V, et al. Initial results from a phase II study of nivolumab (NIVO) plus ipilimumab (IPI) for the treatment of metastatic castration-resistant prostate cancer (mCRPC; CheckMate 650). J Clin Oncol 2019;37(7_suppl):142.
59. Antonarakis ES, Piulats JM, Gross-Goupil M, et al. Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: multicohort, open-label phase II KEYNOTE-199 Study. J Clin Oncol 2020;JCO1901638. https://doi.org/10.1200/JCO.19.01638.
60. Hansen AR, Massard C, Ott PA, et al. Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. Ann Oncol 2018;29(8):1807–13.
61. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372(26):2509–20.

62. Middha S, Zhang L, Nafa K, et al. Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. JCO Precis Oncol 2017;2017. https://doi.org/10.1200/PO.17.00084.
63. Abida W, Cheng ML, Armenia J, et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. JAMA Oncol 2019;5(4):471–8.
64. Autio KA, Eastham JA, Danila DC, et al. A phase II study combining ipilimumab and degarelix with or without radical prostatectomy (RP) in men with newly diagnosed metastatic noncastration prostate cancer (mNCPC) or biochemically recurrent (BR) NCPC. J Clin Oncol 2017;35(6_suppl):203.
65. Abdo J, Cornell DL, Mittal SK, et al. Immunotherapy plus cryotherapy: potential augmented abscopal effect for advanced cancers. Front Oncol 2018;8:85.
66. Benzon B, Glavaris SA, Simons BW, et al. Combining immune check-point blockade and cryoablation in an immunocompetent hormone sensitive murine model of prostate cancer. Prostate Cancer Prostatic Dis 2018;21(1):126–36.
67. Ross AE, Hurley PJ, Tran PT, et al. A pilot trial of pembrolizumab plus prostatic cryotherapy for men with newly diagnosed oligometastatic hormone-sensitive prostate cancer. Prostate Cancer Prostatic Dis 2019. https://doi.org/10.1038/s41391-019-0176-8.
68. Gao J, Ward JF, Pettaway CA, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med 2017;23(5):551–5.
69. Lines JL, Sempere LF, Broughton T, et al. VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. Cancer Immunol Res 2014;2(6):510–7.
70. Kwilas AR, Ardiani A, Donahue RN, et al. Dual effects of a targeted small-molecule inhibitor (cabozantinib) on immune-mediated killing of tumor cells and immune tumor microenvironment permissiveness when combined with a cancer vaccine. J Transl Med 2014;12:294.
71. Agarwal N, Loriot Y, McGregor BA, et al. . Cabozantinib (C) in combination with atezolizumab (A) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC): results of Cohort 6 of the COSMIC-021 Study. J Clin Oncol 2020;38(6_supp):139.
72. Robinson D, Van Allen EM, Wu Y-M, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;162(2):454.
73. Yakkala C, Chiang CL-L, Kandalaft L, et al. Cryoablation and immunotherapy: an enthralling synergy to confront the tumors. Front Immunol 2019;10:2283.
74. Schlom J, Gulley JL. Vaccines as an integral component of cancer immunotherapy. JAMA 2018;320(21):2195–6.
75. Narayan V, Gladney W, Plesa G, et al. A phase I clinical trial of PSMA-directed/TGFβ-insensitive CAR-T cells in metastatic castration-resistant prostate cancer. J Clin Oncol 2019;37(7_suppl):TPS347.
76. Sedykh SE, Prinz V, Buneva VN, et al. Bispecific antibodies: design, therapy, perspectives. Drug Des Devel Ther 2018;12:195–208.
77. Clarke S, Dang K, Li Y, et al. A novel CD3xPSMA bispecific antibody for efficient T cell mediated killing of prostate tumor cells with minimal cytokine release. J Clin Oncol 2019;37(7_suppl):324.
78. Lu S, Stein JE, Rimm DL, et al. Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: a systematic review and meta-analysis. JAMA Oncol 2019. https://doi.org/10.1001/jamaoncol.2019.1549.
79. Bishop JL, Sio A, Angeles A, et al. PD-L1 is highly expressed in enzalutamide resistant prostate cancer. Oncotarget 2015;6(1):234–42.
80. Antonarakis ES, Shaukat F, Isaacsson Velho P, et al. Clinical features and therapeutic outcomes in men with advanced prostate cancer and DNA mismatch repair gene mutations. Eur Urol 2019;75(3):378–82.
81. Schweizer MT, Antonarakis ES, Bismar TA, et al. Genomic characterization of prostatic ductal adenocarcinoma identifies a high prevalence of DNA repair gene mutations. JCO Precis Oncol 2019;3. https://doi.org/10.1200/PO.18.00327.
82. Mutation burden predicts anti-PD-1 response. Cancer Discov 2018;8(3):258.
83. Jamieson NB, Maker AV. Gene-expression profiling to predict responsiveness to immunotherapy. Cancer Gene Ther 2017;24(3):134–40.
84. Wu Y-M, Cieślik M, Lonigro RJ, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. Cell 2018;173(7):1770–82.e14.
85. Ulloa-Montoya F, Louahed J, Dizier B, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol 2013;31(19):2388–95.
86. Hofman P, Badoual C, Henderson F, et al. Multiplexed immunohistochemistry for molecular and immune profiling in lung cancer-just about ready for prime-time? Cancers (Basel) 2019;11(3). https://doi.org/10.3390/cancers11030283.
87. Marty R, Kaabinejadian S, Rossell D, et al. MHC-I genotype restricts the oncogenic mutational landscape. Cell 2017;171(6):1272–83.e15.
88. Ross A, Armstrong AJ, Pieczonka CM, et al. A comparison of sipuleucel-T (sip-T) product parameters from two phase III studies: PROVENT in active surveillance prostate cancer and IMPACT in metastatic castrate-resistant prostate cancer (mCRPC). ASCO.:321.
89. Hodi FS, Ballinger M, Lyons B, et al. Immune-modified response evaluation criteria in solid tumors (imRECIST): refining guidelines to assess the clinical benefit of cancer immunotherapy. J Clin Oncol 2018;36(9):850–8.