Clinical development of immunotherapy for prostate cancer
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Abstract: Prostate cancer is the most common cancer in men, and the second leading cause of cancer-related death in Western countries. Prostate cancer-related death occurs in patients with metastatic castration-resistant prostate cancer. Although several new drugs for castration-resistant prostate cancer have been approved, each of these has prolonged survival by just a few months. Consequently, new therapies are sorely needed. Recently, it has been recognized that immunotherapy is an effective treatment for prostate cancer patients. Several strategies, such as cancer vaccines and immune checkpoint inhibitors, have been investigated in clinical studies for prostate cancer patients. In the present review, the results of the most recent clinical studies investigating immunotherapy in prostate cancer patients are reported, and the future clinical development of immunotherapy for prostate cancer is discussed.

Key words: cancer vaccine, chimeric antigen receptor T cells, immunotherapy, prostate cancer, tumor microenvironment.

Introduction
Although prostate cancer patients with metastatic disease treated with ADT alone usually respond well, ultimately, most patients fail ADT. Progressive status despite ADT is recognized as CRPC, and most prostate cancer-related deaths occur in patients with mCRPC. Recently, four new drugs, abiraterone acetate, enzalutamide, cabazitaxel and radium-223, for CRPC patients have been approved by the FDA. These new drugs showed OS benefits in phase 3 studies.⁴–⁵ Despite the approval of these new drugs for CRPC patients, each of these has prolonged survival by just a few months. Treatments that can provide durable disease control and long-term survival benefits are still required. In the past few decades, immunotherapy has become an important part of treating some types of cancer. Recent trials showed that immunotherapy can increase survival for patients with CRPC, metastatic melanoma and other malignancies using sipuleucel-T, CTLA-4 and PD-1 blockade, respectively.⁶–⁷ Newer types of immune treatments are now being studied, and will impact how we treat cancer in the future.

In the present review, the results of the most recent clinical studies investigating immunotherapy in prostate cancer patients are summarized and the future clinical development of immunotherapy for prostate cancer is discussed.

Mechanism of immunotherapy for cancer
Antigen-specific immune cells mediated adoptive immunity for systemic antitumor immunity. There are several cell types that recognize and destroy tumor cells, such as macrophages, APCs, CD8⁺ CTL cells and natural killer cells. Presentation of tumor antigens to T cells by APCs including monocytes and DCs is required to stimulate adaptive immunity. Proliferation and generation of antitumor effects are stimulated by antigen-specific T cells through cytokines, and direct killing by CTL. However, tumor cells are developing pathways to suppress immune responses and escape from the immune system, and clinical progression.⁸ Most investigated mechanisms of escape from the immune system include the modulation of immune-inhibitory (checkpoint) pathways to suppress T-cell activity, and the disruption of antigen processing and presentation.⁹ Tumors can also recruit and promote...
the developing immunosuppressive cells, such as Tregs and MDSCs. In addition, the release of immunosuppressive factors, such as transforming growth factor-β, interleukin-6 and interleukin-10, might be directly or indirectly mediated by tumors. These factors contribute to the development of the immunosuppressive microenvironment of the tumor.11,12 Figure 1 shows the targets of immunotherapy in the tumor microenvironment.

The cancer immunotherapies currently under investigation can be classified into four strategies for cancer treatment according to their target. The first strategy is designed to augment the frequency of T cells in a patient, specific for one or more tumor-associated antigens. The second strategy is T-cell checkpoint blockade, such as CTLA-4, PD-1 or PD-L1. The third strategy is the use of T cells engineered to express a CAR. The last strategy of immunotherapy is to disrupt or

Fig. 1 Targets of immunotherapy. (a) Antigen peptides are presented by APCs, such as monocytes and DCs, to T cells, and antigen-specific T cells are stimulated to proliferate and generate antitumor effects through cytokines, and direct killing by CTL. (b) Immune checkpoint inhibitors, including CTLA4, PD-1 and PD-L1 antibodies, block negative signals to T-cell cytotoxicity. (c) Engineered or modified CAR-T cells permit recognition of a cell-surface antigen without MHC restriction, and have antitumor activity. (d) Tregs and MDSCs suppress the activation of T cells in the tumor microenvironment.
otherwise modify the immunosuppressive tumor microenvironment.

**Cancer vaccines**

The four main types of vaccine-based immunotherapies investigated in prostate cancer can be classified as autologous, cell-based, viral-based or peptide-based vaccines. The important clinical trials of immunotherapy for prostate cancer are summarized in Table 1.

Prostate cancer is a disease for which cancer vaccines have shown survival benefits, with sipuleucel-T being the first cancer vaccine to receive FDA approval for the treatment of any malignancy. This autologous dendritic-cell vaccine consists of PAP and GM-CSF. Three phase 3 clinical trials of sipuleucel-T for prostate cancer reported promising findings for this autologous vaccine. In the first two studies, there was no difference in time to tumor progression, but there was a significant increase in OS (25.9 months vs 21.4 months and 19.0 months vs 15.7 months). A third phase 3 clinical trial, known as the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial, showed a 4.1-month improvement in median OS. This provided the first solid evidence that cancer vaccines could provide a real benefit in clinical outcome for patients with prostate cancer.

One early clinical investigation of cancer vaccines for prostate cancer was with GVAX. This vaccine is a cell-based vaccine derived from LNCaP and PC3 cell lines genetically modified to secrete GM-CSF. Although the results of phase 1/2 trials for patients with metastatic prostate cancer were a safety profile, PSA decrease and stabilization, and a median OS time of 35.0 months with high-dose treatment, two phase 3 trials (VITAL-1 and VITAL-2) were closed prematurely due to the lack of superior clinical efficacy compared with chemotherapy in VITAL-1 and an increase in patient mortality observed in VITAL-2. Despite these failure of phase 3 studies, GVAX continues to be explored in combination regimens and for other tumor types.

Other investigators have explored different vaccine strategies and target antigens. One anticipated vaccine for prostate cancer is PROSTVAC (PSA-TRICOM), which is a PSA-targeted poxvirus-based vaccine consisting of a heterologous prime-boost (vaccinia or fowlpox virus vector) and three costimulatory molecules (TRICOM; B7.1, ICAM-1 and LFA-3) serving to increase the PSA-specific immune response. Tumor-specific CTL responses and prolonged OS in CRPC patients are suggested in a multicenter phase 2 clinical trial of PROSTAVAC. Subsequently, a phase 3 randomized, placebo-controlled trial of PROSTAVAC (NCT01322490) is currently enrolling 1200 patients with mCRPC. Patients are randomized to receive PROSTAVAC with GM-CSF, PROSTAVAC with placebo GM-CSF or double placebo, with OS as the primary end-point.

Another new concept of vaccine for prostate cancer is PPV. In this “personalized” cancer vaccine strategy, appropriate peptide antigens for vaccination are screened based on pre-existing host immunity, and up to four peptides are selected from a list of vaccine candidates in each patient. Previous phase 1 studies of PPV for CRPC showed that PPV was well tolerated, and cellular or humoral immune responses, and decreases in the PSA levels in some patients have been reported. In a randomized phase 2 study, the combination treatment of PPV with low-dose EMP for CRPC patients showed a longer survival than that of standard dose EMP. Another combination treatment of PPV with low-dose dexamethasone for patients with chemotherapy-naive CRPC also showed longer PSA progression-free survival and median survival time. In addition, another phase 2 study suggested that the OS of docetaxel-resistant CRPC patients treated with PPV (n = 20) was longer than that of historical controls (n = 17) (17.8 months vs 10.5 months, P = 0.166). Based on these results, a phase 3, randomized, placebo-controlled trial of PPV (UMIN000011308) for docetaxel-resistant CRPC patients with OS as the primary end-point was carried out in Japan. This clinical trial has completed enrolling 333 patients, and is currently under investigation.

**T-cell checkpoint inhibitors**

Mechanisms of T-cell checkpoint inhibitors include interference with molecules on T cells that regulate their expansion and function, known as immune checkpoints. CTLA-4, was identified as the first of these T-cell molecules showing high inhibition of cytolytic antitumor T-cell responses, and an antibody of CTLA-4 (ipilimumab) was approved for the treatment of patients with metastatic melanoma by the FDA in 2011. Subsequent investigation has identified many other checkpoint molecules, such as PD-1 and PD-L1. Antibodies blocking PD-1 have recently received FDA approval for the treatment of melanoma, non-small cell lung cancer and renal cell cancer. This treatment is expected to be a more active immunotherapeutic strategy than other immunotherapies. Ipilimumab has been evaluated in several phase 1, 2 and 3 clinical trials. A phase 1/2 clinical trial of ipilimumab for patients with mCRPC showed safety, tolerability and a synergistic activity with radiotherapy. However, a randomized, double-blind phase 3 clinical trial comparing ipilimumab with placebo after radiotherapy in 799 mCRPC patients in whom docetaxel chemotherapy failed did not confirm an OS benefit. Furthermore, a phase 1 clinical trial of PD-1 blockade with nivolumab in solid malignancies showed no objective responses in 17 men with mCRPC. Results to date examining either CTLA-4 or PD-1 blockade alone have suggested little role for these treatments as monotherapy for patients with mCRPC. However, effectiveness of T-cell checkpoint inhibitors with other combination is still investigated.

**CAR-T cells**

T cells with engineered or modified CAR have recently emerged as promising tools for treating tumors. Recent studies have shown marked antitumor activity using CAR-T cells that permit recognition of a cell-surface protein using an antibody recognition domain fused to the TCR signaling domain. CAR-T cells targeting CD19 have led to complete responses in some B-cell malignancies. Although the
<table>
<thead>
<tr>
<th>Category</th>
<th>Agent (trial ID/ref)</th>
<th>Phase</th>
<th>n</th>
<th>Setting</th>
<th>Target</th>
<th>Platform</th>
<th>Comparison treatment</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer vaccine</td>
<td>Sipuleucel-T&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3</td>
<td>512</td>
<td>Asymptomatic mCRPC</td>
<td>PAP/GM-CSF fusion</td>
<td>Cultured autologous PBMCs (autologous cells)</td>
<td>Placebo (autologous cells)</td>
<td>14.6 weeks vs 14.4 weeks (HR 0.95, P = 0.63)</td>
<td>25.8 months vs 21.7 months (HR 0.775, P = 0.032)</td>
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<tr>
<td>Cancer vaccine</td>
<td>GVAX&lt;sup&gt;1,8&lt;/sup&gt;</td>
<td>3</td>
<td>626</td>
<td>Asymptomatic mCRPC</td>
<td>GM-CSF transduced cell lines</td>
<td>Docetaxel plus prednisone</td>
<td>NA</td>
<td>20.7 months vs 21.7 months (HR 1.03, P = 0.78)</td>
<td>12.2 months vs 14.1 months (HR 1.70, P = 0.008)</td>
</tr>
<tr>
<td>Cancer vaccine</td>
<td>GVAX plus docetaxel&lt;sup&gt;1,8&lt;/sup&gt;</td>
<td>3</td>
<td>408</td>
<td>Symptomatic mCRPC</td>
<td>GM-CSF transduced cell lines</td>
<td>Docetaxel</td>
<td>NA</td>
<td>25.8 months vs 21.7 months (HR 1.03, P = 0.78)</td>
<td>12.2 months vs 14.1 months (HR 1.70, P = 0.008)</td>
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<td>Cancer vaccine</td>
<td>PROSTAVAC/Vf&lt;sup&gt;2,11&lt;/sup&gt;</td>
<td>2</td>
<td>125</td>
<td>Minimally symptomatic mCRPC</td>
<td>PSA</td>
<td>Vaccine-fowl pox</td>
<td>Control vector</td>
<td>3.8 months vs 3.7 months (HR 0.884, P = 0.6)</td>
<td>25.1 months vs 16.6 months (HR 0.56, P = 0.006)</td>
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<tr>
<td>Cancer vaccine</td>
<td>PROSTAVAC + GM-CSF (NCT01322493)</td>
<td>3</td>
<td>1200</td>
<td>Asymptomatic or minimally symptomatic mCRPC</td>
<td>PSA</td>
<td>Vaccine-fowl pox</td>
<td>Placebo GM-CSF</td>
<td>Ongoing</td>
<td>Ongoing</td>
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<tr>
<td>Cancer vaccine</td>
<td>PPV plus low-dose EMP&lt;sup&gt;26&lt;/sup&gt;</td>
<td>2</td>
<td>57</td>
<td>CRPC</td>
<td>Several TAAs</td>
<td>Multiple peptides</td>
<td>EMP</td>
<td>8.5 months vs 2.8 months (HR 0.58, P = 0.001)</td>
<td>Undefined vs 16.1 months (HR 0.3, P = 0.033)</td>
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<tr>
<td>Cancer vaccine</td>
<td>PPV plus dexamethasone&lt;sup&gt;27&lt;/sup&gt;</td>
<td>2</td>
<td>72</td>
<td>Chemotherapy naive CRPC</td>
<td>Several TAAs</td>
<td>Multiple peptides</td>
<td>Dexamethasone</td>
<td>22.0 months vs 7.0 months (HR 0.59, P &lt; 0.001)</td>
<td>24.9 months vs 18.9 months (HR 0.59, P = 0.001)</td>
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<tr>
<td>Cancer vaccine</td>
<td>PPV&lt;sup&gt;28&lt;/sup&gt;</td>
<td>2</td>
<td>20</td>
<td>Docetaxel resistant mCRPC</td>
<td>Several TAAs</td>
<td>Multiple peptides</td>
<td>Historical control</td>
<td>NA</td>
<td>17.8 months vs 10.5 months (P = 0.166)</td>
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<tr>
<td>Cancer vaccine</td>
<td>PPV (UMIN000011308)</td>
<td>3</td>
<td>333</td>
<td>Docetaxel resistant mCRPC</td>
<td>Several TAAs</td>
<td>Multiple peptides</td>
<td>Placebo</td>
<td>NA</td>
<td>11.2 months vs 10.0 months (P = 0.053)</td>
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<td>Check point inhibitor</td>
<td>Ipilimumab&lt;sup&gt;24&lt;/sup&gt;</td>
<td>3</td>
<td>799</td>
<td>Docetaxel resistant mCRPC</td>
<td>Several TAAs</td>
<td>CTLA-4</td>
<td>Antibody</td>
<td>NA</td>
<td>11.2 months vs 10.0 months (P = 0.053)</td>
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<td>CAR-T cell</td>
<td>CAR-T cell&lt;sup&gt;40&lt;/sup&gt;</td>
<td>1</td>
<td>5</td>
<td>mCRPC</td>
<td>Prostate-specific membrane antigen</td>
<td>CAR-T cell</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tumor microenvironment disruptor</td>
<td>Snitinib&lt;sup&gt;5,7&lt;/sup&gt;</td>
<td>3</td>
<td>873</td>
<td>Docetaxel resistant mCRPC</td>
<td>Angiogenesis and MDSC</td>
<td>Tyrosine kinase</td>
<td>Placebo</td>
<td>5.6 months vs 4.1 months (HR 0.725, P &lt; 0.001)</td>
<td>13.1 months vs 11.8 months (HR 0.9, P = 0.17)</td>
</tr>
<tr>
<td>Tumor microenvironment disruptor</td>
<td>Tasquinmod&lt;sup&gt;32&lt;/sup&gt;</td>
<td>3</td>
<td>1245</td>
<td>Chemotherapy-naive mCRPC</td>
<td>Angiogenesis and MDSC</td>
<td>Oral immunotherapy</td>
<td>Placebo</td>
<td>7 months vs 4.4 months (HR 0.64, P &lt; 0.001)</td>
<td>21.3 months vs 23 months (HR 1.1, P = 0.25)</td>
</tr>
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availability of tissue-specific antigens has limited the development of this approach for prostate cancer, some investigators are exploring targeting prostate-specific membrane antigen and prostate stem cell antigen using the CAR-T cell approach.40–42 CAR-modified T cells directed toward prostate tissue-specific antigens might be a better treatment choice.

**Tumor microenvironment disruptors**

Tumor-associated immunosuppression contributes significantly to tumor progression and resistance to immunotherapies.33 It has been recognized that Tregs suppress the significantly to tumor progression and resistance to immunotherapy.43 MDSCs are associated with immunosuppression with several mechanisms, such as defective dendritic cell function and activation of Tregs.50 Circulating MDSCs are also increased in some malignancies, and are correlated with advanced clinical stages.51

In animal studies, sunitinib, one anti-angiogenic agent with tyrosine kinase inhibitor activity, results in increased tumor-infiltrating lymphocytes infiltration, DC maturation and decreased infiltration of MDSCs into the tumor.52–54 Subsequently, several phase 2 trials were carried out to examine sunitinib as monotherapy for patients with mCRPC.55,56 These trials showed signs of efficacy, PSA declines and objective responses. Although a phase 3 trial of sunitinib in patients with mCRPC showed that sunitinib increased PFS, sunitinib did not impact OS compared with the placebo (13.1 months for sunitinib and 11.8 months for placebo, P = 0.17).57 Another agent that targets the tumor microenvironment in prostate cancer is tasquinimod. Tasquinimod is an oral immunotherapeutic agent with reported effects on the tumor microenvironment by counteracting tumor growth with decreasing MDSCs and angiogenesis.58–60 In a randomized, placebo-controlled phase 2 study of men with mCRPC, tasquinimod significantly improved PFS (7.6 months for tasquinimod and 3.3 months for placebo, P < 0.01).61 However, an international, double-blind, placebo-controlled, phase 3 trial of chemotherapy-naïve men with mCRPC showed no significant OS benefit (21.3 months for tasquinimod and 24 months for placebo, P = 0.25).62 Despite these results, there remains interest in the use of tumor microenvironment disruptors in combination with other immunotherapies.

**Conclusion**

Since sipuleucel-T became the first cancer vaccine to receive approval for patients with CRPC, immunotherapy has emerged as a viable and attractive strategy for the treatment of prostate cancer. Several strategies to enhance the immune response against prostate cancer cells have been investigated. Recently, immune checkpoint inhibitors, such as CTLA-4 and PD-1 or PD-L1 antibodies, have generated excitement because they showed significant and durable responses in several malignancies including metastatic melanoma, renal cell, non-small cell lung and bladder cancer. Early clinical trials of CTLA-4 antibodies for patients with mCRPC showed PSA modulation and objective responses in some cases. However, two phase 3 studies of anti-CTLA-4 antibody in mCRPC did not show improvement in OS. The results suggested that CTLA-4 blockade had little effect as monotherapy in patients with mCRPC. Although there are several promising immunotherapeutic drugs under study, ongoing phase 3 trials for PROSTAVAC in the USA and PPV in Japan might soon broaden the scope of immunotherapies available to patients with CRPC. Current approaches are also investigating optimal combinations and sequencing of immunotherapies with other treatments. In addition, further investigation is required to predict patients with prostate cancer who would most benefit from immunotherapy.

**Conflict of interest**

MN has served as an advisory board consultant for Green Peptide Co. Ltd. KI has served as a consultant and received research funding from Taiho Pharmaceutical Company. NK and TI declare no competing interests.

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