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Immunotherapy for prostate cancer: Requirements for a successful regime transfer

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Despite the revolutionary progress in cancer treatment using immune checkpoint inhibitors (ICIs), remarkable responses in prostate cancer treatment have not yet been achieved. The disappointing previous results of ICIs have required further studies towards combined treatment targeting other pathways and restricted the eligibility criteria for patients with high mutation burdens, especially those with mismatch repair deficiency. Cancer immunotherapies activate adaptive immune systems, rather than directly attack tumor cells with their own cytotoxicity. Therefore, refractoriness to ICIs can not only be derived from the intractable nature of tumor cells *per se*, but also from their hostile milieu. Here, we reviewed the prostate cancer immunotherapies exploring clinical trials to date, along with the molecular characteristics of prostate cancer and its microenvironment.

Keywords: Immunotherapy; Prostatic neoplasms; Tumor microenvironment

INTRODUCTION

Prostate cancer is the second-most prevalent cancer and leads the sixth-highest cancer-related mortality rate in men [1]. Localized prostate cancers are curable by regional treatment, such as radical prostatectomy or radiotherapy, and can even be supervised with special caution via active surveillance in well-matched low-risk patients [2]. However, approximately 20% to 30% of patients experience recurrences requiring further therapeutic modalities. Androgen deprivation therapy (ADT) is generally used for treatment of metastatic prostate cancer, achieving durable responses and manageable adverse effects. However, most prostate cancers finally develop resistance to ADT, classified as castration-resistant prostate cancer (CRPC) [3]. CRPC can proliferate and survive under androgen deficiency through various strategies, including the *de novo* synthesis of androgen, modification of androgen receptors (ARs), cross-talk with other molecular pathways to improve the AR pathways, and cellular plasticity involving epithelial to mesenchymal transition (EMT) and cancer stem cells [4,5]. CRPC can be classified as non-metastatic CRPC (nmCRPC) and metastatic CRPC (mCRPC) according to the clinical metastasis. In the struggle to subdue CRPC, first-line treatment involves second-generation antiandrogens and taxane-based chemotherapies [6]. However, these therapeutics increase the overall survival (OS) by a few months, and CRPCs remain incurable.

Tumor immunotherapy has revolutionized cancer treatment by providing durable responses and a broad range of applications in treating many cancers [7-9]. Cancer immunotherapy primarily intends to promote the activation of cytotoxic T cells, which have anti-tumor effects, by recognizing tumor antigens and executing apoptosis through granzyme and perforin [10]. Immunotherapy has accomplished

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remarkable progresses in the field of urology. In metastatic renal cell carcinoma with intermediate and poor risk, as defined by the International Metastatic RCC Database Consortium, combined treatment with nivolumab and ipilimumab, which are inhibitors of programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4), respectively, achieved superior OS and complete response rates to sunitinib, with fewer adverse effects in the phase-3 Checkmate 214 trial [11,12]. Other phase-3 trials, including KEYNOTE-426, JAVELIN Renal 101, and IMmotion161, demonstrated the benefits of anti-PD-1 or PD-L1 agents in combination with axitinib or bevacizumab, with superior OS and progression-free survival to sunitinib [13,14]. In metastatic bladder cancer, phase-2 trials with anti-PD-1 and PD-L1 agents exhibited complete remission in 9% to 10% of patients, and they have since become a first-line treatment for cisplatin-ineligible patients with positive PD-L1 expression [15,16]. Furthermore, for muscle-invasive bladder cancer, neoadjuvant pembrolizumab (anti PD-L1 inhibitor) achieved pT0 in 42% of patients, and its performance was notably better in PD-L1 high patients [17].

In prostate cancer, the first immunotherapeutic agent, sipuleucel-T, was adopted for treating mCRPC with minimal metastatic burden in 2010 [18], which uses autologous peripheral blood mononuclear cells activated *ex vivo* by the recombinant fusion protein PA2024 to activate cytotoxic T cells. Sipuleucel-T improves OS by four months; however, progression-free survival in mCRPCs has not been observed [19]. The insufficient yet promising results achieved for sipuleucel-T suggest an encouraging future for immune checkpoint inhibitors (ICIs). However, benefits in terms of OS have not yet been observed. In this review, we summarize the ongoing clinical trials on immunotherapy for prostate cancer, review the molecular mechanism of resistance to immunotherapy in prostate cancer, and discuss future directions to improve prostate cancer immunotherapy.

CLINICAL TRIALS OF IMMUNE CHECKPOINT INHIBITORS ON PROSTATE CANCER

1. CTLA4 inhibitor

CTLA4 negatively regulates T cell activation by binding to its ligand B7.1 and B7.2 costimulatory molecules [20]. Ipilimumab, an anti-CTLA4 monoclonal antibody, blocks CTLA4 to switch off the inhibitory mechanism and potentiate cytotoxic T cell effects against tumors. Ipilimumab monotherapy failed to achieve significant benefits in prostate cancer treatment (Table 1) [21-31]. In the randomized double-blind phase-3 CA184-095 trial, there was no significant difference in OS between the ipilimumab-and placebo-treated groups (median OS 28.7 and 29.7 months, respectively) in chemotherapy-naïve mCRPC patients with minimal disease burden [21]. The CA184-043 trial was conducted in mCRPC patients with bone metastasis, with ipilimumab or a placebo administered after bone-directed radiotherapy. Ipilimumab achieved favorable OS, but without a significant difference [22].

2. PD-1/PD-L1 inhibitor

PD-1 is a surface receptor on T cells that abrogates immune activation by binding to its ligand PD-L1 as a substantial immune checkpoint [32,33]. In the tumor microenvironment (TME), tumor cells exhibit high PD-L1 expression, which binds to the PD-1 on the T cells to be exhausted, leading to immune surveillance evasion [34]. Anti-PD-1/PD-L1 antibodies reinvigorate exhausted T cells to recover the antitumor immune activity [35]. Although several investigations into anti PD-1/PD-L1 inhibitors are ongoing, discouraging results have been reported (Table 1). In a phase-1 trial (CA209-003), the first clinical trial on nivolumab, an anti-PD-1 monoclonal antibody achieved objective responses in non-small cell lung cancer, melanoma, and renal cell cancer, but not in mCRPC [26]. The KEYNOTE-028 trial employing pembrolizumab, an anti-PD-1 inhibitor, was conducted on advanced prostate cancer expressing PD-L1 over 1% of the tumor or stroma, and 17.4% of patients exhibited an overall response without complete response, and 39.1% of patients experienced progressive disease, which hinders the applicability of pembrolizumab in metastatic prostate cancer treatment [27]. The phase-2 KEYNOTE-199 trial on mCRPC involved 258 patients in three cohorts who were previously treated with docetaxel and one or more androgen-deprivation treatments (Table 1). The cohorts were defined as PD-L1-positive (cohort 1), PD-L1-negative (cohort 2) with RECIST-measurable disease, and predominant bone metastasis (cohort 3). PD-L1 positivity had no impact on the objective response or disease control rates (5% vs. 3%, 10% vs. 9%, respectively). However, encouragingly, two patients attained complete response in cohort 1. Full genome sequencing in these cohorts revealed that BRCA1/2 or ATM gene aberrations were somewhat related to higher objective responsive rate (11%) than aberrations in other homologous recombination repair (HRR) genes (0%) or no aberrations in HRR genes (3%) [28].

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Table 1. Clinical trials of immune check point inhibitors on prostate cancer

Study	Study ID/NCT number	Study arm	Phase	Indications	Results
Beer et al. [21]	CA184-095/NCT01057810	Ipilimumab vs. placebo	Phase III	Chemotherapy-naive CRPC	Failed to improve OS, but benefits in PFS and PSA response.
Kwon et al. [22]	CA184-043/NCT00861614	Ipilimumab vs. placebo following radiotherapy	Phase III	Docetaxel-treated CRPC	Failed to improve OS.
Tollefson et al. [23]	MC0253/NCT00170157	ADT+ipilimumab vs. ADT	Phase II	Advanced prostate cancer	Favorable PSA response in combination therapy. Final report is missing.
Zhang et al. [24]	NCI-2014-00318/NCT01804465	Sipuleucel-T with immediate vs. with delayed ipilimumab	Phase II	mCRPC	Durable response in 12% of patients regardless timing.
Graff et al. [25]	CA184-059/NCT01498978	Ipilimumab IV every 3 months for 5 cycles	Phase II	mCRPC	PSA response in 30% of patients, but the study was halted.
Topalian et al. [26]	CA209-003/NCT00730639	Nivolumab 0.1 to 10 mg/kg every 2 weeks up to 12 cycles	Phase I	CRPC	No ORR.
Hansen et al. [27]	KEYNOTE-028/NCT04825990	Pembrolizumab 10 mg/kg every 2 weeks until progression	Phase Ib	Advanced prostate cancer expressing PD-1 ≥1%	ORR of 17.4% without CR.
Antonarakis et al. [28]	KEYNOTE-199/NCT02787005	Pembrolizumab 200 mg every 3 weeks up to 35 cycles	Phase II	Docetaxel & ADT-pretreated mCRPC	Encouraging results in bone-predominant mCRPC and BRCA1/2 or ATM aberrations.
Ross et al. [29]	MK-3475/NCT02489357	Cryotherapy+pembrolizumab	Pilot	Oligo-metastatic prostate cancer	PSA under 0.6 ng/mL in 42% of patients. Progression-free survival of 14 months.
Sweeney et al. [30]	CO39385/NCT03016312	Atezolizumab+enzalutamide vs. enzalutamide	Phase III	mCRPC	Failed to improve OS.
Brown et al. [31]	Pro00080869/NCT03179410	Avelumab IV every 2 weeks until progression	Phase II	Neuroendocrine prostate cancer	Dismal response but CR in one patient.

CRPC, castration-resistant prostate cancer; OS, overall survival; PFS, progression free survival; PSA, prostate-specific antigen; ADT, androgen deprivation therapy; mCRPC, metastatic CRPC; ORR, objective responsive rate; PD-1, programmed death 1; CR, complete response.

MOLECULAR CHARACTERISTICS OF PROSTATE CANCER AND THE TUMOR MICROENVIRONMENT

1. Neoantigen and mutational burden of prostate cancer

Sufficient activation of adaptive immunity can be promoted by immunogenic cell death or neoantigens expressed on tumor cells., which are derived from mutations in tumor cells that provide anti-tumor immunity and aid in avoiding non-selective autoimmune responses in the TME. Neoantigens are generated coincidentally and engaged in the major histocompatibility complex (MHC) to activate cytotoxic T cells. The tumor mutation burden is correlated with the ICI response, as proven by the durable effects of ICI on high mutational loads, including melanoma, NSCLC, SCCHN, and bladder cancer, with a response rate of at least 15% [33]. However, primary prostate cancer exhibits a paucity of neoantigen frequency and tumor mutation burden, which leads to insufficient results in ICIs [36]. Microsatellite instability-high (MSI-H) or mismatch repair deficiency (dMMR) results in an extensive DNA mutation burden contributing to tumor immunogenicity, which motivated the expedited U.S. Food and Drug Administration (FDA) approval of pembrolizumab for metastatic solid tumors accompanying MSI-H or dMMR, regardless of tumor origin, in May 2017. Only 2% to 3% of prostate cancers exhibit MSI-H or dMMR [37].

2. PD-1/PD-L1 expression on prostate cancer

PD-1/PD-L1 expression does not assure objective responses to anti-PD-1/PD-L1 inhibitors, although they are required for treatment effects [38]. Previous studies on PD-1/PD-L1 expression in prostate cancer have reported conflicting results. In several studies, PD-L1 expression was low in primary prostate cancer, irrespective of PTEN loss [26,38]. ADT is believed to activate adaptive immunity; this was the basis of a study reporting that CRPCs treated with enzalutamide expressed high levels of PD-L1, which could be associated with resistance to ADT by employing immune surveillance evasion [39]. However, aggressive ADT in combination with abiraterone, prednisone, and leuprolide does not increase the PD-L1 levels, resulting in poor responses to anti-PD-1/PD-L1 inhibitors [39]. These contradictory data indicate that PD-L1 expression is not necessarily induced by ADT, but depends on an unknown mechanism for each specific ADT agent, which should be addressed with clear evidence [40].

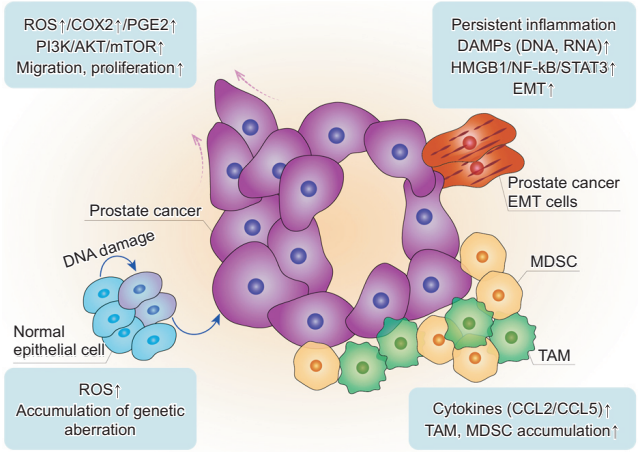

Fig. 1. Inflammatory tumor microenvironment of prostate cancer. Chronic inflammation interferes to establish anti-tumor immunity and promotes tumor progression as well as carcinogenesis derived from genetic aberration. Cytokines produced in inflammatory TME invigorate tumor cells to proliferate and migrate, thus facilitate tumor cell invasion, metastasis, and resistance to chemotherapeutics through EMT. Inflammatory cytokines attract MDSC and TAM to occupy major proportions in TME. TME, tumor microenvironment; EMT, epithelial to mesenchymal transition; MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage.

3. Chronic inflammation of prostate cancer microenvironment

Prostate cancer is accompanied by chronic inflammation related to DNA damage-induced inflammation, which can be derived from carcinogenesis accompanied by exaggerated DNA damage (Fig. 1) [41]. There are notable reports of chronic inflammation contributing to carcinogenesis in other organs, such as the skin, liver, breasts, colon, and lungs, through cytokine and reactive oxygen species-driven genetic aberrations. Accordingly, a prospective study linking PCPT and SELECT cohorts revealed that chronic prostatic inflammation is a significant risk factor for prostate carcinogenesis [42,43]. Moreover, a previous report demonstrated that the severity of chronic inflammation is associated with poorer prognosis in the five-year recurrence-free survival rate (61% vs. 66.7%) and biochemical recurrence (p=0.03) [44]. Concomitant evidence has shown that prolonged inflammatory conditions with increased cytokines, such as PGE2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), affect tumor progression. PGE2 generated via the COX2-related pathway increases prostate cancer cell migration and proliferation by upregulating the PI3K/AKT/mTOR pathway [45]. GM-CSF plays a multifaceted role in TME, providing anti-tumorou activity and pro-tumoral progression, depending on the situation. GM-CSF facilitates massive inflammation, induces immune cell deposition and tumor antigen presentation, and provokes pro-tumoral activity by

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promoting tumor cell proliferation and migration via increased matrix metalloproteinase [46]. It has been assumed that cytokines gathering tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) to the TME are crucial for the invasiveness and distant metastasis of tumors [47]. Chronic inflammation not only aggravates prostate cancer progression by modulating the hostile TME, but also by EMT. EMT conversion from edged inflammation to a constant and firm TME is mediated by transcriptional factors, including NF-kB, STAT3, and HMGB1. Notably, HMGB1 encodes inflammasomes facilitated by damage-associated molecular patterns, such as DNA and RNA, which lead to chronic and persistent inflammation in the TME. Chronic inflammation suppresses anti-tumor immunogenicity by inhibiting immune responses. Inflammatory tumor sites contain various immune cells with innate and adaptive immunity, which largely affect the treatment results of immunotherapies [48]. In the following sections, we discuss each component of the inflammatory TME of prostate cancer, along with molecular characteristics and strategies for breakthrough.

TUMOR-ASSOCIATED MACROPHAGES IN PROSTATE CANCER

Chronic inflammation results in the transcriptional activation of cytokines and chemokines, triggering TAMs. TAMs are abundant in prostate cancer microenvironments consisting of 70% of immune populations with other myeloid lineage cells, which play a critical role in tumor progression by inducing tumor cell proliferation, angiogenesis, immunomodulation, and metastasis (Fig. 2) [49]. CCL2, the monocyte chemoattractant protein1, is predominantly secreted by prostate cancer cells, such as the PC3 and LnCaP cell lines, attracting macrophages to the TME and activating the PI3K/AKT signaling pathway for the proliferation of prostate cancer cells [50].

During the early phase of tumorigenesis, inflammatory TAMs defeat tumors; however, they are subdued by T_H2 T cells to support tumorigenesis. The macrophages with contradictory properties are classified as M1 (anti-tumoral, inflammatory) or M2 (pro-tumoral, immunosuppressive) polarized macrophages [51]. M1 macrophages are associated with favorable recurrence-free survival in prostate cancer (hazard ratio=3.26) [52]. The TAMs employed by prostate cancers hamper anti-tumor-immunity [53]. TAMs reciprocally communicate with cancer stem-like cells through the CCL5-β-catenin/STAT3 signaling axis, which differentiate into clusters composed of highly heterogenic subpopulations with self-renewal properties associated with drug resistance, and evolve into CRPCs [54-56]. However, ADT promotes reciprocal interaction between TAMs and prostate cancer cells, which then release a CSF1 to invite TAMs and provoke the emergence of prostate cancer macrophage-targeted treatment [46]. The prognosis of PTEN-deficient prostate cancer is worse, with more aggressive features and resistance to ADT and radiotherapy, which is partly associated with enhanced M2 TAM infiltration [57].

Furthermore, TAMs contribute to the sequential metastasis process of intravasation, circulation through vessels, extravasation, and adaptation in a hostile environment. Additionally, TAM clusters construct a pre-metastatic niche to mediate cancer cells to readily settle in hostile distant microenvironments [58,59]. Bone metastasis is a common and devastating feature of advanced prostate cancer, which is promoted by the release of inflammatory cytokines and chemokines, such as IL-6 and CXCL5, from M2 macrophages [49,60]. Accordingly, macrophage-targeting strategies that inhibit TAM recruitment or M2 polarization are undergoing clinical trials. However, concordant therapy against TAM recruitment by CCL2-CCR2 signaling, anti-CCL2 monoclonal antibodies, and carlumab (CNTO888) failed to provide efficacy in CRPCs as a monotherapy in phase-2 clinical trial [61].

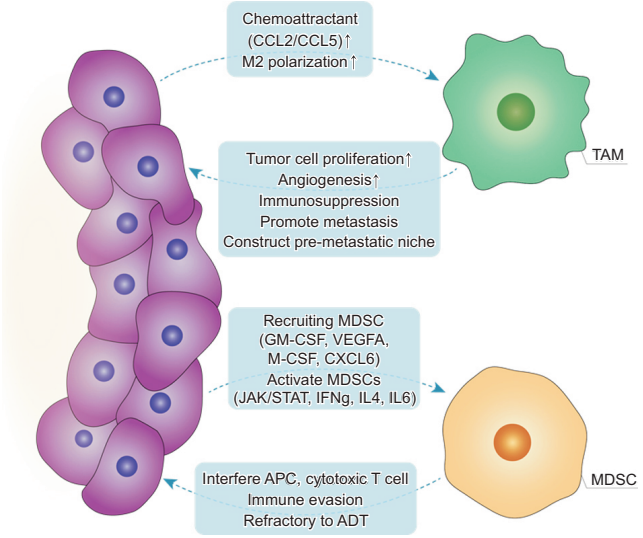

Fig. 2. TAM and MDSC shape the immunosuppressive milieu in prostate cancer. Tumor cell released cytokines attract TAM and MDSC to be rich in tumor microenvironment. TAM and MDSC reciprocally communicate with tumor cells to construct tumor-favorable immunosuppressive milieu. TAM, tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; APC, antigen presenting cell; ADT, androgen deprivation therapy.

MYELOID-DERIVED SUPPRESSOR CELLS RESTRICT THE ANTI-TUMOR EFFECT OF IMMUNE CHECKPOINT INHIBITORS

Prostate cancer employs MDSCs to suppress cytotoxic T cells and lead them to exhaustion, which are the main constituent of the TME in prostate cancers, with their higher expressions approximately 40 times compared with those in the normal prostate. MDSCs suppress immune activation against tumor cells by disrupting the activation of antigen-presenting and cytotoxic T cells, which alleviates the anti-tumor effect of ICIs [62]. MDSCs predominantly drive immune evasion by suppressing $CD8^+$ T cell infiltration and impeding anti-tumor effects. Although the mechanism by which MDSCs accumulate in prostate cancer is unclear, the YAP1-CXCL5-CXCR2 signaling axis has been shown to promote MDSC accumulation in a murine prostate cancer model [63]. MDSCs may be derived from myeloid progenitors traveling from the bone marrow to tumors after expansion by stem cell factors, GM-CSF, VEGFA, and M-CSF from tumor cells. Tumor-secreted chemokines, such as JAK/STAT signaling, IFNγ, IL4, and IL6, are responsible for the immunosuppressive activity of MDSCs. In a clinical trial, CXCL6, the homolog of CXCL5 in humans, was associated with high Gleason scores and poor prognosis, which was partly due to MDSC recruitment. Furthermore, the MDSC component in TME correlates with the prostate-specific antigen (PSA) level and PTEN deficiency with worse progression, contributing to refractoriness to anti-androgen therapy via the secretion of IL-23 to enhance AR signaling and facilitate the survival and proliferation of CRPCs [64,65]. There are various treatment strategies targeting MDSCs with representative methods, including MDSC depletion, MDSC recruitment, MDSC activity suppression, and encouraging MDSC differentiation [66]. Additionally, cancer-associated fibroblasts (CAFs) mediate MDSC infiltration by suppressing CSF-1 [67]. Targeting the MDSCs in CRPC enhanced the anti-tumor effects of ICIs in preclinical experiments [68]. Accordingly, strategic targeting of MDSCs in combination with ICIs should be considered to reinvigorate innate and adaptive immune responses [68].

CANCER-ASSOCIATED FIBROBLAST IN PROSTATE CANCER

CAFs are substantial stromal cells that regulate the TME and originate from various sources, including resident fibroblasts and circulating bone marrow-derived cells. CAFs are constantly activated, promoting chronic inflammation,

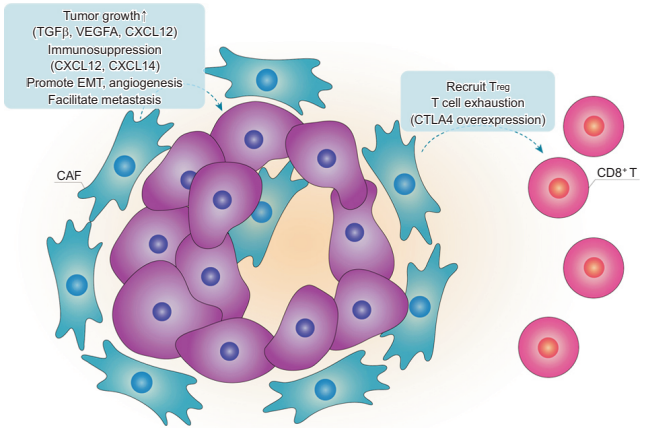

Fig. 3. CAFs hamper $CD8^+$ T cells. CAF abundantly reside in prostate cancer to mediate carcinogenesis and tumor progression. CAFs are constantly activated and interact with immune cells including MDSC, TAM, and T cells. CAFs suppress execution of $CD8^+$ T cell through recruiting regulatory T cells and inducing T cell exhaustions. CAF, cancer-associated fibroblast; MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; EMT, epithelial to mesenchymal transition.

which leads to carcinogenesis and progression (Fig. 3). Cooperation of tumor cells and CAFs mediates tumor growth via the secretion of TGFβ, VEGFA, and CXCL12, and CAF-derived CXCL12 and CXCL14 subsequently lead to immunosuppression by inverting M2 macrophage polarization concomitantly underpinned by Th2 T cells and MDSCs [69]. Specifically, CAF suppresses $CD8^+$ T cells to evade immune surveillance by promoting CTLA-4 overexpression-induced exhaustion through various pathways. Notably, prostate cancers have abundant CAFs, leading to modest responses to ICIs [70]. CAF induces metabolic acidosis by secreting lactic acid, which induces FoxP3-positive T_{reg} cells that subside $CD4^+$ T cells into Th2 cells, enhancing anti-tumor responses [71]. CXCL12 produced by CAFs facilitates the EMT and angiogenesis, promoting tumor migration and distant metastasis [72]. CAFs not only debilitate ICIs, but also increase chemo-resistance by alleviating reactive oxygen species, drug accumulation [73], and TGFβ-mediated GREM2 inhibition [74].

PROSTATE CANCER INFILTRATING $CD8^+$ T CELLS

The tumor immune status can be categorized into four groups depending on the abundance or activity of tumor-infiltrating T cells: hot, altered-excluded, altered-immunosuppressed, and cold [75]. Hot and altered-excluded tumors exhibit relatively abundant T cell infiltration; however, the T cells are segregated to peripheral areas in altered-excluded

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tumors. The paucity of T cells refers to altered immunosuppression, and the classification of cold tumors depends on the degree of altered immunosuppression. Chronic inflammation frequently occurs in prostate cancer; however, this does not necessarily suggest that prostate cancer is a 'hot' tumor. Chronic inflammatory regions in prostate cancer are mainly confined to benign areas adjacent to tumors, and only a few immune cells can be observed in the tumor area, causing prostate cancer to manifest as a 'cold' tumor [76]. The poor results of immune-checkpoint inhibitors in prostate cancer are partly due to the scarce $CD8^+$ T cells. The steps involved in cytotoxic T cell generation against tumor cells should be considered. Tumor-associated antigens (TAAs) released from dying tumor cells are captured and presented on MHC molecules by antigen-presenting cells to prime and activate cytotoxic T cells in the lymphoid organs. Activated T cells travel through blood streams to settle and infiltrate tumors presenting TAAs [10]. Sipuleucel-T is the first dendritic cell (DC) cancer vaccine approved by the FDA for mCRPCs. Autologous DCs derived from patients are supported by a prostate cancer antigen, prostatic acid phosphatase, with encouraging support of the GM-CSF [77]. Recombinant DCs promote cytotoxic T cells to cultivate "cold" TME. Consistent with the trials overcoming cytotoxic T cell anergy, a phase-1b clinical trial has been launched (NCT03024216) to compare combined sipuleucel-T atezolizumab (anti-PD-L1) therapy in time sequences. Although there has been no complete response, manageable adverse effects with partial responses were noted [78].

PERSPECTIVE ON CAR-T THERAPY FOR PROSTATE CANCER

The long journey of chimeric antigen receptor T cells (CAR-T cells), 30 years from conception to the FDA approval of CD19 targeting CAR-T, tisagenlecleucel, for the curative therapy of B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma, has motivated the upcoming CAR-T cell therapies targeting solid tumors [79]. DNA vaccines or CAR-T cell treatments are expected to enhance the anti-tumor effects of immunotherapy and are promising [71]. CAR-T cells are MHC-independent due to the antigen-binding domain recognizing the target cell antigen, provoking a downstream intracellular domain in conjunction with a costimulatory molecule. Prostate cancer is an appealing candidate for CAR-T therapy due to its targetable antigens, such as prostate-specific membrane antigen (PSMA), which is amenable to the tumor antigen-binding domain [80]. Gene therapy inducing antigenic proteins, such as PSA or PSMA, has been adopted to enhance tumor-infiltrating $CD8^+$ T cells, in conjunction with IL-12 coding genes to attract and stimulate T cell activity [81]. PSMA is a membrane protein overexpressed as high as 100 to 1,000 times on prostate cancer cells compared with normal prostate cells, and increases in metastatic cancer cells or CRPCs [82]. Furthermore, the prostate is not a vital organ that should be preserved against cancer cells. Considering the lack of tumor-infiltrating T cells to halt the effect of ICIs on prostate cancer, the combination of ICI and CAR-T is encouraging.

CONCLUSIONS

Immunotherapy has revolutionized the paradigm of cancer treatment and provided the opportunities to cure metastatic diseases. However, prostate cancer has been excluded from the trend due to the disappointing results of clinical trials. The molecular characteristics of prostate cancer have been determined to elucidate the factors hindering the positive effects of ICIs. Considering the cancer immunotherapy mechanism, every step faces hardships, including antigen retrieval, antigen presentation and T cell priming, immune cell homing, reinvigorating T cells, recognizing cancer cells, and executing cytotoxic activity. In this review, we examine prostate cancer and its microenvironment considering the molecular characteristics and clinical relevance of cancer immunotherapy. To achieve optimal results from immunotherapy, precise modulations are required to harness improved anti-tumor immunity, not only in prostate cancer cells, but also in hostile microenvironments.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Seung-hwan Jeong and Cheol Kwak. Data acquisition: Seung-hwan Jeong. Statistical analysis: Seung-hwan Jeong. Data analysis and interpretation: Seung-hwan Jeong and Cheol Kwak. Drafting of

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REFERENCES

1. Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent global patterns in prostate cancer incidence and mortality rates. Eur Urol 2020;77:38-52.
2. Barocas DA, Alvarez J, Resnick MJ, Koyama T, Hoffman KE, Tyson MD, et al. Association between radiation therapy, surgery, or observation for localized prostate cancer and patient-reported outcomes after 3 years. JAMA 2017;317:1126-40.
3. Klotz L, O'Callaghan C, Ding K, Toren P, Dearnaley D, Higano CS, et al. Nadir testosterone within first year of androgen-deprivation therapy (ADT) predicts for time to castration-resistant progression: a secondary analysis of the PR-7 trial of intermittent versus continuous ADT. J Clin Oncol 2015;33:1151-6.
4. Zhang Z, Zhou C, Li X, Barnes SD, Deng S, Hoover E, et al. Loss of CHD1 promotes heterogeneous mechanisms of resistance to AR-targeted therapy via chromatin dysregulation. Cancer Cell 2020;37:584-98.e11.
5. Ceder Y, Bjartell A, Culig Z, Rubin MA, Tomlins S, Visakorpi T. The molecular evolution of castration-resistant prostate cancer. Eur Urol Focus 2016;2:506-13.
6. Thomas TS, Pachynski RK. Treatment of advanced prostate cancer. Mo Med 2018;115:156-61.
7. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2017;377:1345-56.
8. Paz-Ares L, Luft A, Vicente D, Tafreshi A, GümüşM, Mazières J, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N Engl J Med 2018;379:2040-51.
9. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108-21.
10. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013;39:1-10.
11. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med 2018;378:1277-90.
12. Motzer RJ, Rini BI, McDermott DF, Arén Frontera O, Hammers HJ, Carducci MA, et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. Lancet Oncol 2019;20:1370-85.
13. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380:1116-27.
14. Rini BI, Powles T, Atkins MB, Escudier B, McDermott DF, Suarez C, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. Lancet 2019;393:2404-15.
15. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet 2017;389:67-76.
16. O'Donnell PH, Plimack ER, Bellmunt J, Berger R, Montgomery RB, Heath K, et al. Pembrolizumab (Pembro; MK-3475) for advanced urothelial cancer: results of a phase IB study. J Clin Oncol 2015;33(7_suppl):296.
17. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, LucianòR, et al. Pembrolizumab as neoadjuvant therapy before radical cystectomy in patients with muscle-invasive urothelial bladder carcinoma (PURE-01): an open-label, single-arm, phase II study. J Clin Oncol 2018;36:3353-60.
18. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
19. Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. Clin Cancer Res 2011;17:3520-6.
20. Motz GT, Coukos G. Deciphering and reversing tumor immune suppression. Immunity 2013;39:61-73.
21. Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, 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;35:40-7.
22. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, 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;15:700-12.
23. Tollefson M, Karnes RJ, Thompson RH, Granberg C, Hillman D, Breau R, et al. 668 A randomized phase II study of ipilimumab with androgen ablation compared with androgen ablation alone in patients with advanced prostate cancer. J Urol

https://doi.org/10.4111/icu.20210369

2010;183(4S):e261.
24. Zhang L, Sinha M, Subudhi SK, Chen B, Marquez J, Liu E, et al. The impact of prior radiation therapy on outcome in a phase 2 trial combining sipuleucel-T (SipT) and ipilimumab (Ipi) in patients (pts) with metastatic castration resistant prostate cancer (mCRPC). J Clin Oncol 2021;39(15_suppl):5045.
25. Graff JN, Stein MN, Surana R, Al Rabadi L, Liu E, Fong L, et al. Phase II study of ipilimumab in men with metastatic prostate cancer with an incomplete response to androgen deprivation therapy. Front Oncol 2020;10:1381.
26. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.
27. Hansen AR, Massard C, Ott PA, Haas NB, Lopez JS, Ejadi S, et al. Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. Ann Oncol 2018;29:1807-13.
28. Antonarakis ES, Piulats JM, Gross-Goupil M, Goh J, Ojamaa K, Hoimes CJ, et al. Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: multicohort, open-label phase II KEYNOTE-199 study. J Clin Oncol 2020;38:395-405.
29. Ross AE, Hurley PJ, Tran PT, Rowe SP, Benzon B, Neal TO, et al. A pilot trial of pembrolizumab plus prostatic cryotherapy for men with newly diagnosed oligometastatic hormone-sensitive prostate cancer. Prostate Cancer Prostatic Dis 2020;23:184-93.
30. Sweeney CJ, Gillessen S, Rathkopf D, Matsubara N, Drake C, Fizazi K, et al. Abstract CT014: IMbassador250: a phase III trial comparing atezolizumab with enzalutamide vs enzalutamide alone in patients with metastatic castration-resistant prostate cancer (mCRPC). Cancer Res 2020;80(16 Suppl):CT014.
31. Brown LC, Halabi S, Humeniuk MS, Wu Y, Oyekunle T, Huang J, et al. Efficacy of the PD-L1 inhibitor avelumab in neuroendocrine or aggressive variant prostate cancer: results from a phase II, single-arm study. J Clin Oncol 2021;39(6_suppl):89.
32. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. Nat Rev Immunol 2020;20:75-6.
33. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 2016;16:275-87.
34. Kumagai S, Togashi Y, Kamada T, Sugiyama E, Nishinakamura H, Takeuchi Y, et al. The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. Nat Immunol 2020;21:1346-58.
35. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010;207:2187-94.
36. Subudhi SK, Vence L, Zhao H, Blando J, Yadav SS, Xiong Q, et al. Neoantigen responses, immune correlates, and favorable outcomes after ipilimumab treatment of patients with prostate cancer. Sci Transl Med 2020;12:eaaz3577.
37. Abida W, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. JAMA Oncol 2019;5:471-8.
38. Martin AM, Nirschl TR, Nirschl CJ, Francica BJ, Kochel CM, van Bokhoven A, et al. Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance. Prostate Cancer Prostatic Dis 2015;18:325-32.
39. Bishop JL, Sio A, Angeles A, Roberts ME, Azad AA, Chi KN, et al. PD-L1 is highly expressed in Enzalutamide resistant prostate cancer. Oncotarget 2015;6:234-42.
40. Calagua C, Russo J, Sun Y, Schaefer R, Lis R, Zhang Z, et al. Expression of PD-L1 in hormone-naïve and treated prostate cancer patients receiving neoadjuvant abiraterone acetate plus prednisone and leuprolide. Clin Cancer Res 2017;23:6812-22.
41. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019;25:1822-32.
42. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. Immunity 2019;51:27-41.
43. Platz EA, Kulac I, Barber JR, Drake CG, Joshu CE, Nelson WG, et al. A prospective study of chronic inflammation in benign prostate tissue and risk of prostate cancer: linked PCPT and SELECT cohorts. Cancer Epidemiol Biomarkers Prev 2017;26:1549-57.
44. Irani J, Goujon JM, Ragni E, Peyrat L, Hubert J, Saint F, et al. High-grade inflammation in prostate cancer as a prognostic factor for biochemical recurrence after radical prostatectomy. Pathologist Multi Center Study Group. Urology 1999;54:467-72.
45. Wen S, Tian J, Niu Y, Li L, Yeh S, Chang C. ASC-J9(®), and not Casodex or Enzalutamide, suppresses prostate cancer stem/progenitor cell invasion via altering the EZH2-STAT3 signals. Cancer Lett 2016;376:377-86.
46. Escamilla J, Schokrpur S, Liu C, Priceman SJ, Moughon D, Jiang Z, et al. CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy. Cancer Res 2015;75:950-62.
47. Nakamura K, Smyth MJ. Targeting cancer-related inflammation in the era of immunotherapy. Immunol Cell Biol 2017;95:325-32.
48. Shalapour S, Karin M. Immunity, inflammation, and cancer: an eternal fight between good and evil. J Clin Invest 2015;125:3347-55.
49. Roca H, Jones JD, Purica MC, Weidner S, Koh AJ, Kuo R, et

al. Apoptosis-induced CXCL5 accelerates inflammation and growth of prostate tumor metastases in bone. J Clin Invest 2018;128:248-66.
50. Loberg RD, Day LL, Harwood J, Ying C, St. John LN, Giles R, et al. CCL2 is a potent regulator of prostate cancer cell migration and proliferation. Neoplasia 2006;8:578-86.
51. Bertani FR, Mozetic P, Fioramonti M, Iuliani M, Ribelli G, Pantano F, et al. Classification of M1/M2-polarized human macrophages by label-free hyperspectral reflectance confocal microscopy and multivariate analysis. Sci Rep 2017;7:8965.
52. Cao J, Liu J, Xu R, Zhu X, Zhao X, Qian BZ. Prognostic role of tumour-associated macrophages and macrophage scavenger receptor 1 in prostate cancer: a systematic review and meta-analysis. Oncotarget 2017;8:83261-9.
53. Thienger P, Rubin MA. Prostate cancer hijacks the microenvironment. Nat Cell Biol 2021;23:3-5.
54. Huang R, Wang S, Wang N, Zheng Y, Zhou J, Yang B, et al. CCL5 derived from tumor-associated macrophages promotes prostate cancer stem cells and metastasis via activating β-catenin/STAT3 signaling. Cell Death Dis 2020;11:234.
55. Zhang K, Guo Y, Wang X, Zhao H, Ji Z, Cheng C, et al. WNT/β-catenin directs self-renewal symmetric cell division of hTERT^{high} prostate cancer stem cells. Cancer Res 2017;77:2534-47.
56. Huang H, Wang C, Liu F, Li HZ, Peng G, Gao X, et al. Reciprocal network between cancer stem-like cells and macrophages facilitates the progression and androgen deprivation therapy resistance of prostate cancer. Clin Cancer Res 2018;24:4612-26.
57. Armstrong CW, Maxwell PJ, Ong CW, Redmond KM, McCann C, Neisen J, et al. PTEN deficiency promotes macrophage infiltration and hypersensitivity of prostate cancer to IAP antagonist/radiation combination therapy. Oncotarget 2016;7:7885-98.
58. Lin Y, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. J Hematol Oncol 2019;12:76.
59. Lo CH, Lynch CC. Multifaceted roles for macrophages in prostate cancer skeletal metastasis. Front Endocrinol (Lausanne) 2018;9:247.
60. Culig Z, Puhr M. Interleukin-6: a multifunctional targetable cytokine in human prostate cancer. Mol Cell Endocrinol 2012;360:52-8.
61. Pienta KJ, Machiels JP, Schrijvers D, Alekseev B, Shkolnik M, Crabb SJ, et al. Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer. Invest New Drugs 2013;31:760-8.
62. Zhao D, Cai L, Lu X, Liang X, Li J, Chen P, et al. Chromatin regulator CHD1 remodels the immunosuppressive tumor microenvironment in PTEN-deficient prostate cancer. Cancer Discov 2020;10:1374-87.
63. Wang G, Lu X, Dey P, Deng P, Wu CC, Jiang S, et al. Targeting YAP-dependent MDSC infiltration impairs tumor progression. Cancer Discov 2016;6:80-95.
64. Calcinotto A, Spataro C, Zagato E, Di Mitri D, Gil V, Crespo M, et al. IL-23 secreted by myeloid cells drives castration-resistant prostate cancer. Nature 2018;559:363-9.
65. Idorn M, Køllgaard T, Kongsted P, Sengeløv L, thor Straten P. Correlation between frequencies of blood monocytic myeloid-derived suppressor cells, regulatory T cells and negative prognostic markers in patients with castration-resistant metastatic prostate cancer. Cancer Immunol Immunother 2014;63:1177-87.
66. De Cicco P, Ercolano G, Ianaro A. The new era of cancer immunotherapy: targeting myeloid-derived suppressor cells to overcome immune evasion. Front Immunol 2020;11:1680.
67. Kumar V, Donthireddy L, Marvel D, Condamine T, Wang F, Lavilla-Alonso S, et al. Cancer-associated fibroblasts neutralize the anti-tumor effect of CSF1 receptor blockade by inducing PMN-MDSC infiltration of tumors. Cancer Cell 2017;32:654-68.e5.
68. Lu X, Horner JW, Paul E, Shang X, Troncoso P, Deng P, et al. Effective combinatorial immunotherapy for castration-resistant prostate cancer. Nature 2017;543:728-32.
69. Kakarla S, Song XT, Gottschalk S. Cancer-associated fibroblasts as targets for immunotherapy. Immunotherapy 2012;4:1129-38.
70. Galbo PM Jr, Zang X, Zheng D. Molecular features of cancer-associated fibroblast subtypes and their implication on cancer pathogenesis, prognosis, and immunotherapy resistance. Clin Cancer Res 2021;27:2636-47.
71. Liu T, Han C, Wang S, Fang P, Ma Z, Xu L, et al. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. J Hematol Oncol 2019;12:86.
72. Ishii G, Ochiai A, Neri S. Phenotypic and functional heterogeneity of cancer-associated fibroblast within the tumor microenvironment. Adv Drug Deliv Rev 2016;99(Pt B):186-96.
73. Cheteh EH, Augsten M, Rundqvist H, Bianchi J, Sarne V, Egevad L, et al. Human cancer-associated fibroblasts enhance glutathione levels and antagonize drug-induced prostate cancer cell death. Cell Death Dis 2017;8:e2848.
74. Shan G, Gu J, Zhou D, Li L, Cheng W, Wang Y, et al. Cancer-associated fibroblast-secreted exosomal miR-423-5p promotes chemotherapy resistance in prostate cancer by targeting GREM2 through the TGF-β signaling pathway. Exp Mol Med 2020;52:1809-22.
75. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 2019;18:197-218.

https://doi.org/10.4111/icu.20210369

76. Sfanos KS, Yegnasubramanian S, Nelson WG, De Marzo AM. The inflammatory microenvironment and microbiome in prostate cancer development. Nat Rev Urol 2018;15:11-24.
77. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol 2020;20:651-68.
78. Rosser CJ, Hirasawa Y, Acoba JD, Tamura DJ, Pal SK, Huang J, et al. Phase Ib study assessing different sequencing regimens of atezolizumab (anti-PD-L1) and sipuleucel-T (SipT)in patients who have asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer. J Clin Oncol 2020;38(15_suppl):e17564.
79. Braendstrup P, Levine BL, Ruella M. The long road to the first FDA-approved gene therapy: chimeric antigen receptor T cells targeting CD19. Cytotherapy 2020;22:57-69.
80. Schepisi G, Cursano MC, Casadei C, Menna C, Altavilla A, Lolli C, et al. CAR-T cell therapy: a potential new strategy against prostate cancer. J Immunother Cancer 2019;7:258.
81. Shore ND, Morrow MP, McMullan T, Kraynyak KA, Sylvester A, Bhatt K, et al. CD8+ T cells impact rising PSA in biochemically relapsed cancer patients using immunotherapy targeting tumor-associated antigens. Mol Ther 2020;28:1238-50.
82. Patil Y, Shmeeda H, Amitay Y, Ohana P, Kumar S, Gabizon A. Targeting of folate-conjugated liposomes with co-entrapped drugs to prostate cancer cells via prostate-specific membrane antigen (PSMA). Nanomedicine 2018;14:1407-16.