Revisiting Immunotherapy: A Focus on Prostate Cancer

Ha-Ram Cha[1,2], Joo Hyoung Lee[1,3], and Selvarangan Ponnazhagan[1]

ABSTRACT

Therapeutic interventions to harness the immune system against tumor cells have provided mixed results in the past for several solid tumors and hematologic malignancies. However, immunotherapy has advanced considerably over the last decade and is becoming an integral combination for treating patients with advanced solid tumors. In particular, prostate cancer immunotherapy has shown modest efficacy for patients in the past. With several key discoveries on immune mechanisms and advanced molecular diagnostic platforms recently, immunotherapy is re-emerging as a viable option for prostate cancer, especially castration-resistant prostate cancer (CRPC), to stimulate antitumor immunity. Combination of patient-tailored immunotherapy and immune checkpoint blockers with conventional cytotoxic agents and androgen receptor–targeted therapies should move the field forward. With a recent adaptation that the application of immune checkpoint inhibitors has been successful in the treatment of more than a dozen solid tumors, including melanoma, lymphoma, liver, cervical, gastrointestinal, and breast cancers, it is a timely endeavor to harness immunotherapy for prostate cancer. Here, we provide an account on the progression of immunotherapy with new discoveries and precision approaches for tumors, in particular CRPC, from mechanistic standpoint to emerging limitations and future directions.

Introduction

The last decade has seen a tremendous increase in the number of immunotherapy trials for various solid tumors. The advances made in cancer immunotherapy extend beyond understanding the dialog between cancer and the immune system to being used as predictors of cancer prognosis (1, 2). Although surgery, followed by chemotherapy and/or radiotherapy, remains the mainstay of management in many solid tumors, immunotherapy is rapidly being incorporated with other therapies to improve patient survival. Although immunotherapy appears to be promising for many solid tumors, progress made in prostate cancer is relatively moderate. Evidences from studies on genetic, epidemiologic, and pathophysiologic aspects of prostate cancer imply that inflammation plays an important role at different stages of prostate cancer growth and metastasis. From the onset of prostatic inflammation, leading to tumorigenesis and further evolution of the disease characterized by molecular heterogeneity of driver mutations, various signalling pathways play crucial roles in the development of resistance and immunosuppression (3–6). Thus, understanding the pathophysiology of prostate cancer, with particular emphasis on disease responsiveness to different immunomodulatory agents, will shed more light on developing new combination therapy approaches.

Once diagnosed as a localized disease, conventional interventional approach includes radical prostatectomy or radiotherapy, followed by a continuous monitoring of the levels of PSA for biochemical recurrence. Development and progression of prostate cancer is highly associated with chronic inflammation by prostatitis-induced cellular and genomic damage (7). Chronic inflammation in the prostate causes extracellular matrix remodeling and epithelial–mesenchymal transition, which plays a key role in the disease development and progression (7). Prostate cancer is known as a slow-growing inflammatory disease compared with other malignancies, which allows prostate cancer to be an ideal candidate for immunotherapy. Based on initial set of potential prostate cancer antigens including PSA, different immunotherapy approaches have been attempted in patients with prostate cancer (**Fig. 1**). The following details provide an account of immunotherapy, including mechanistic aspects and updates on patient data from ongoing clinical trials with special emphasis on castration-resistant prostate cancer (CRPC).

Passive and Active Immunotherapies

Passive approaches

Cancer immunotherapy can be largely classified into two categories: passive and active immunotherapies. Dating back to the work of Dr. William Coley in the late 1800s, passive immunotherapy adopts a short-term innate immune boost or adoptive immune restoration of T-helper cell (Th)-1 response by providing exogenous proinflammatory cytokines and monoclonal antibodies to patients with cancer. With steady improvements in our understanding of key immune mechanisms that fight invading microbes and prompt cellular transformation or aberrant cells in the host system, recombinant protein technologies started taking over therapeutic approaches applying recombinant cytokines IL2 and IL12 in multiple solid tumor models including lung, metastatic melanoma, and disseminated renal carcinoma (8–12) to activate immune responses. It is noteworthy that in such immune inductions by applying specific cytokines with high purity, early clinical studies have shown modest response in extending patient survival (13). This initial response paved the way for utilizing/testing other proinflammatory cytokines and growth factors activating immune response against the tumor.

An example of passive immunotherapy is a recombinant TNFα (TNFerade) therapy. Although clinical use of TNFerade as a cancer immunotherapy agent was limited only for locally advanced tumors, metastatic melanoma, and soft-tissue sarcoma, due to uncontrolled systemic innate immune response that caused toxicity in patients (14), its application has been discontinued in recent times. In a similar manner, recombinant IFNγ therapy triggered uncontrolled adaptive

[1]Department of Pathology, The University of Alabama at Birmingham, Birmingham, Alabama. [2]McWhorter School of Pharmacy, Samford University, Birmingham, Alabama. [3]PharmAbcine Inc., Yuseong-gu Daejeon, Republic of Korea.

Corresponding Author: Selvarangan Ponnazhagan, University of Alabama at Birmingham, 1825 University Blvd., Birmingham, AL 35294. Phone: 205-934-6731; Fax: 205-975-4919; E-mail: sponnazh@uabmc.edu

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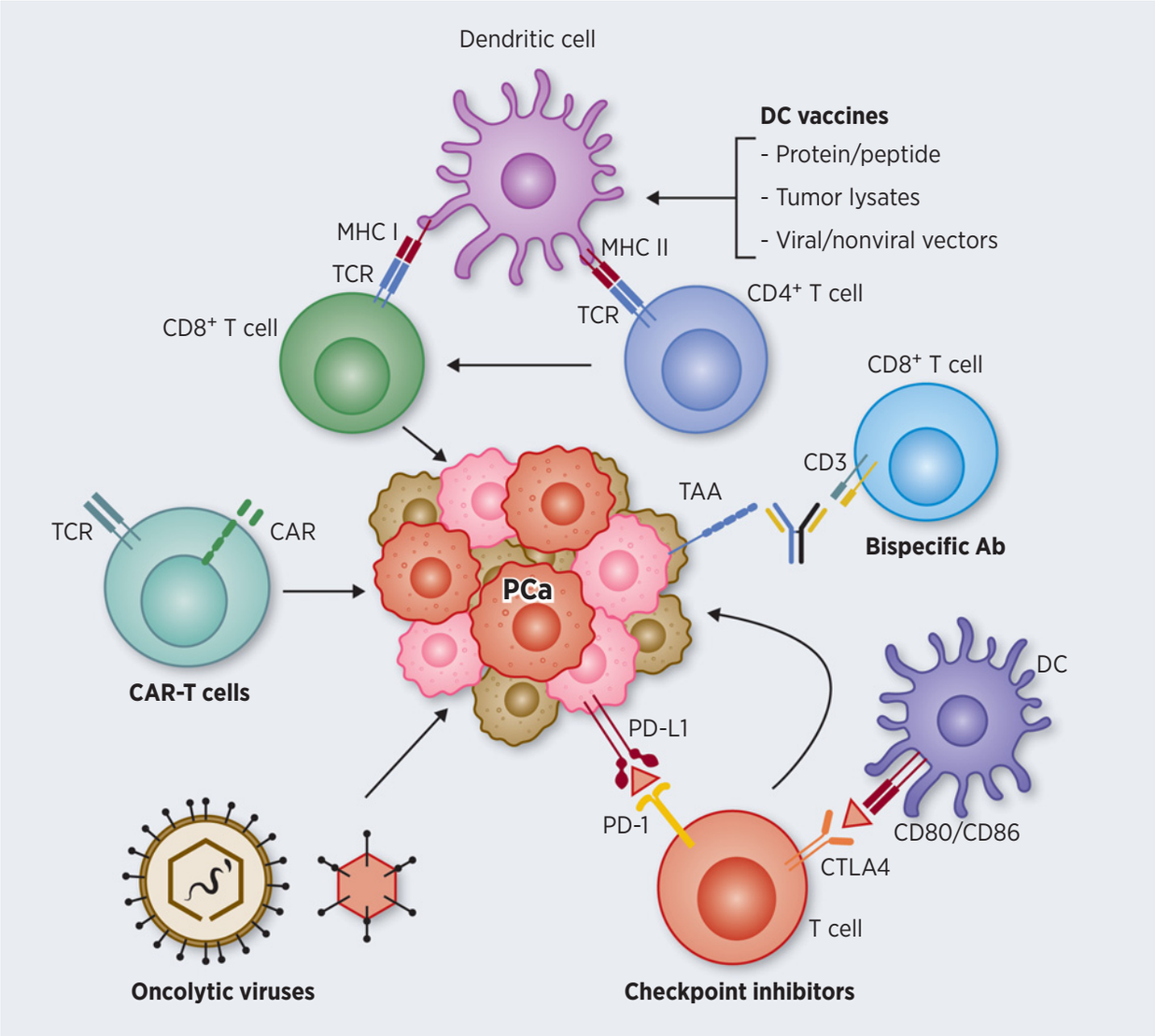

Figure 1.

Major immunotherapy pathways targeting prostate cancer cells. Attempts to activate tumor-specific $CD8^+$ T cells against prostate cancer involved loading dendritic cells (DC) with proteins and peptides of tumor antigens or transducing antigen genes into dendritic cells using viral and nonviral vectors by *ex vivo* or *in vivo* approaches. Such antigen-loaded dendritic cells, prompted by additional signals for maturation and APC function, result in augmenting CTL effector function, both in number and in activity. Optimizing dendritic cell function further enables $CD4^+$ T cells to promote T helper function against growing tumor. Genetic approaches to harness tumor-specific $CD8^+$ T cells directly involve harvesting T cells from prostate cancer, transfecting them with CAR genes directed against the patients' tumor, expanding the modified T cells *ex vivo*, and reinfusing them back into the patients for antitumor activity. Bispecific antibody conjugates help direct tumor-specific $CD8^+$ T cells to tumor target using an adapter molecule involving CD3 and tumor surface antigen-specific antibodies. Whereas the above approaches help in the generation and activation of tumor-specific T cells, immune checkpoint inhibitors on the other hand help in blocking inhibitory pathways that dampen T-cell function. The two major checkpoint molecules on T cells that block effector function are PD-1 and CTLA4 that interact with PD-L1 produced by tumor cells and CD80/86 on APCs, respectively. Recent immunotherapy approaches using monoclonal antibody blockade of their inhibitory interaction are highly promising in the clinic to improve CTL function. Oncolytic viruses, which are engineered to selectively replicate and kill tumor cells, further improve immunotherapy approaches for effective cross-presentation of tumor antigens to the immune system, either as standalone treatment or in combination with other immunotherapy approaches. PCa, prostate cancer.

immune boosting, Th-1 response, which unleashed cytotoxic T cells that resulted in autoimmune-like organ damage (9).

Chimeric antigen receptor (CAR)-T cell therapy is another example of well-established passive immunotherapy approach in which T cells from patients with cancer are genetically modified *ex vivo*, to express a specific *CAR* gene, targeting a tumor-specific antigen, and culture-expanded CAR-T cells infused back into the patient. Recent studies have shown promising results from CAR-T cell therapy in solid tumors, including CAR-T strategy targeting a cancer cell surface antigen, mesothelin, in malignant pleural disease, which has shown a favorable response in an ongoing phase I clinical trial (NCT02414269; ref. 15). In addition, an ongoing phase I clinical trial (NCT03159819) of CAR-T cell therapy targeting claudin 18.2, a protein highly expressed on gastric and pancreatic

adenocarcinomas, has shown antitumor activity in patients with advanced gastric and pancreatic adenocarcinomas (16). Despite these potentials, CAR-T cell therapy has shown a better clinical response in hematologic malignancies than in solid tumors (17–22). For targeting prostate cancer, CAR-T cells were generated against prostate-specific membrane antigen (PSMA) and embedding CD28 as a costimulator (23). The CAR-T cell strategy targeting PSMA has shown improved antitumor effects *in vivo*, compared with IgCD28TCR T cells, suggesting a translational potential for targeting CRPC. In line with other cell-based immunotherapies, CAR-T cell therapy also faces difficulties in treating solid tumors including prostate cancer. One of the major limitations in CAR-T therapy is the immunosuppressive tumor microenvironment (TME). In addition to immunosuppressive cytokines and growth factors, the TME is generally replete with protumorigenic tumor-associated macrophages (TAM), regulatory T cells (Treg), and myeloid-derived suppressor cells (MDSC), as encountered in lung cancer and renal cell carcinoma (24–27). Well-characterized T-cell–inhibitory factors in the TME are programmed cell death ligand-1 (PD-L1), which is expressed on cancer cells and interacts with PD-1 on T cells inducing $CD8^+$ T-cell anergy, and TGFβ, which suppresses effector immune cell function (28, 29). In addition, the emergence of resistant clones with neoantigens further warrants next-generation CAR-T cell technology with multiple single-chain variable fragment targeting multiple tumor-specific antigens.

Another strategy of passive immunotherapy currently being studied is radiolabeled monoclonal antibodies targeting PSMA, which is highly expressed specifically on prostate cancer cells. The PSMA strategy has advantage of effective local delivery of the agent because of its high specificity and internalization into prostate cancer cells upon PSMA binding the agent. A phase II clinical trial testing anti-PSMA labeled with lutetium-177 (177Lu-J591) demonstrated PSA decline after receiving single treatment in 59.6% of 47 patients with metastatic CRPC (mCRPC; ref. 30). Also, a recent phase I/II study with 177Lu-J591 showed promising therapeutic efficacy in patients with mCRPC, when combined with higher cumulative radiotherapy (31). In addition, the most updated outcome of a study with alternative PSMA ligand, PSMA-617 radiolabeled with lutetium-177 (177Lu-PSMA-617), described that 50% or greater decrease in PSA was observed in 32 of 50 patients with mCRPC (32). Besides anti-PSMA monoclonal antibody conjugates being tested as promising immunotherapeutic agent in prostate cancer, they serve as a useful tool for CRPC diagnosis and imaging by identifying metastatic sites (33, 34).

In addition to PSMA, prostate stem cell antigen (PSCA) has emerged as an ideal immunotherapeutic target because of its overexpression in prostate cancer including metastatic and hormone refractory tumors, but not in normal prostate tissue. A phase I/II clinical trial with PSCA is currently ongoing to evaluate safety and clinical activity of PSCA-specific CAR-T cells (BPX-601), where T cells were engineered to recognize PSCA-expressing prostate cancer cells, in patients with previously treated for PSCA (NCT02744298). Also, a recently initiated clinical trial of anti-PSCA adopted PSCA-targeting CAR-T cell strategy in patients with PSCA-positive mCRPC (NCT03873805). This phase I clinical trial has not yet reported primary outcomes. Along with anti-PSMA antibody, properties of iodine-labeled anti-PSCA, [124I] PSCA-minibody, as a useful drug for PET imaging have been evaluated that resulted in positive outcomes (NCT02092948). Collectively, these clinical outcomes point to the potential of prostate cancer antigens targeting in coadjuvant setting to improve survival.

Active approaches

Active immunotherapy stimulates a patient's own immune response, resulting in the activation of immune cells, natural killer cells, or cytotoxic T cells, or antibody production targeting tumor-specific antigens. This approach is intended to provoke adaptive immune response, particularly, to establish a long-term T-cell memory that actively and specifically targets tumor-specific antigens. Various tumor-specific and tumor-associated antigens have been identified, cancer antigen (CA)-125 in ovarian cancer (35), HER2 in breast cancer, carcinoembryogenic antigen in breast and colon cancers (36), melanoma antigen gene in melanoma, and alpha-fetoprotein in hepatocellular carcinoma, and tested in clinical trials with moderate success (37, 38).

Sipuleucel-T (Provenge) is an example of active immunotherapy targeting prostatic acid phosphatase (PAP), one of PSAs (39). This FDA-approved autologous active cellular therapy is designed to induce T-cell–mediated immune response via *ex vivo* stimulation of patient's immature antigen-presenting cells (APC) in combination with recombinant PAP and costimulatory GMCSF. A completed phase III clinical trial of Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT: NCT00065442) indicated that the Sipuleucel-T improved overall survival (OS) by 4.1 months and a 22% reduction of relative mortality risk in patients diagnosed with mCRPC (40). The IMPACT study further indicated that the patients with lower disease burden demonstrated the greatest benefit (41, 42), suggesting a higher efficacy of the therapy in early stages of prostate cancer. However, only minimal antitumor responses were observed, in spite of OS benefit, which is possibly due to that the concept of Sipuleucel-T is to achieve remission of advanced prostate cancer, not regression, by exerting antigen presentation of APCs. Although phase II trial of sipuleucel-T in combination with pidilizumab (anti–programmed cell death-1; anti–PD-1) and cyclophosphamide (chemotherapy) in patients with mCRPC was initiated in 2012 (NCT01420965), unfortunately it was terminated due to drug supply issues. Another clinical trial to investigate the effect of combination therapy of sipuleucel-T and ipilimumab and anti-cytotoxic T-lymphocyte antigen (CTLA)-4 (NCT01832870) in mCRPC was also terminated without any result reported at phase I (43).

An example of active immunotherapy agent that was clinically tested involved use of viral vectors. A poxvirus-based cancer vaccine, composed of rilimogene galvacirepvec (V-PSA-TRICOM; PROSTVAC-V), a recombinant vaccinia virus, and rilimogene glafolivec (F-PSA-TRICOM; PROSTVAC-F), a recombinant fowlpox virus, with potential immunostimulatory and antineoplastic activities have been tested as immunotherapeutic agents (44). Both viruses encoded modified forms of human PSA and the three costimulatory molecules (triad of costimulatory molecules; TRICOM), B7-1 (CD80), intercellular adhesion molecule-1 (ICAM-1), and lymphocyte function-associated antigen-3 (LFA3). In spite of positive outcomes in patients who received PROSTVAC-VF resulting in 8.5-month prolongation of median OS in phase II trial (45), a large phase III confirmatory trial (PROSPECT: NCT01322490), where 1,200 asymptomatic patients with mCRPC were randomly assigned to PROSTVAC-VF with or without GMCSF, failed to confirm previous results with no significant differences in OS between the treatment arms (46). A currently ongoing clinical trial (NCT03315871) of PROSTVAC is investigating if the combination therapy (PROSTVAC + M7824: monoclonal antibody targeting PD-L1 and TGFβ R II + CV301: recombinant vaccine of Avipoxvirus) exerts an antitumorigenic effect on patients with prostate cancer with biochemical recurrence, defined as the state at which PSA level is increased.

In spite of development and clinical application of active and passive immunotherapies, clinical outcomes have only been modest due to the limitations, including low levels of targeting molecules, side effects, and short half-life of the agents (46–49). Moreover, TME-induced immunosuppression hindered the efficacy of these immunotherapies (50). Such limitations promoted the development of alternative approaches in cancer immunotherapy. Recent clinical trial reports and studies have shown that the application of immune checkpoint inhibitors has been successful in the treatment of various malignancies (51, 52), indicating a promising cancer therapy that overcomes the limitations of conventional therapies.

Checkpoint Blockade Therapy to Improve Effector T-Cell Function

Therapy targeting PD-1 and PD-L1

One of the important mechanisms by which cancer cells evade immune surveillance is the activation of immune checkpoint pathways, which suppress antitumor responses by causing T-cell exhaustion or anergy as seen in different types of solid tumors (53–59). Immune checkpoint inhibitors sustain antitumor activities by interfering T-cell coinhibitory signaling pathways, thus enhancing immune-mediated tumoricidal effect (60). Examples of immune checkpoint inhibitors are nivolumab (Opdivo) and pembrolizumab (Keytruda) that block an immune checkpoint protein PD-1, resulting in the restoration of T cells to target cancer cells. These drugs have shown to inhibit the progression of certain types of solid tumors (61–65). Currently, two phase II clinical trials of pembrolizumab targeting PD-1 are ongoing to investigate its effects against progression of the disease (NCT02787005: KEYNOTE-199) in mCRPC after androgen-deprivation therapy (ADT) and in patients with mCRPC treated with enzalutamide (NCT02312557). A recent update on the KEYNOTE-199 trial stated that pembrolizumab responses are durable, and the observed OS benefit is promising (66).

In addition to agents targeting PD-1, anti–PD-L1 immunotherapies are currently being studied using avelumab (Bavencio) and atezolizumab (Tecentriq). Recent updates on ongoing phase I clinical trial of avelumab (NCT01772004) showed that only 3 of 17 patients with mCRPC resulted in prolonged PSA doubling time (67). A phase III clinical trial of atezolizumab (NCT03016312) in combination with enzalutamide for patients with mCRPC, which is designed to measure OS with the time frame of 42 months, has been ongoing since January 2017. It is noteworthy to highlight that currently two distinct PDLs, PD-L1 and PD-L2, have been identified. Given the fact that T cells interact with APCs expressing both PDLs during initial priming phase, it has been suggested that PD-1 blockade, which inhibits interaction with both PD-L1 and PD-L2, is more effective immunotherapeutic strategy in exerting T-cell priming than targeting PD-L1 alone (68).

CTLA4 as a potential immune checkpoint inhibitor

Ipilimumab (Yervoy) is an immune checkpoint inhibitor that blocks CTLA4, expressed on the surface of cytotoxic T cells, preventing T-cell–mediated antitumor immune responses (53). Administration of this monoclonal antibody has already been approved by the FDA as cancer immunotherapy agents (69). Initial clinical trial with ipilimumab monotherapy was discontinued at phase III due to only a marginal improvement of patient OS when compared with the placebo arm (70). As an alternative strategy, ongoing clinical trials for mCRPC adopt combinations of immune checkpoint inhibitors. For instance, a phase II clinical trial, CheckMate 650, was initiated to study a combination of ipilimumab and nivolumab in patients with mCRPC who developed resistance to androgen receptor (AR)–targeted therapies (71, 72). However, recently Cancer Discovery 2019 reported that the combination of the two drugs resulted in only 25% of objective response rate (73). In addition, discontinuation of the therapy in the study population was reported due to the disease progression and increased side effects (72, 73). Another phase III trial, in which patients with mCRPC that had progressed after Taxol chemotherapy (NCT00861614) received radiotherapy targeting bone metastasis followed by ipilimumab treatment, resulted in prolonged median OS (74). Furthermore, the result showed that OS rate at 1 year in patients who received ipilimumab therapy was 46.5%, compared with 40.8% in the placebo group.

Bispecific Antibody Conjugates for Therapeutic Targeting of Prostate Cancer

Bispecific antibodies, conjugated to tumor antigens expressed on prostate cancer cells and CD3 molecule on T cells, have emerged recently as a promising new approach to treat hormone-refractory disease. In this context, various combinations of bispecific conjugates have been tested with encouraging results. A site-specific, bispecific antibody, containing moieties of PSMA and anti-CD3 Fab, has shown excellent potency and activity in *in vitro* and *in vivo* xenograft models (75–77). Translation of this approach using a bispecific conjugate targeting CD3 and Her2 on tumor cells in a phase I clinical study demonstrated encouraging results with no dose-limiting toxicities, and with partial response as well as significant decreases in PSA levels and pain scores in a few patients. Immune evaluations of responders showed increases in IFNγ and Th1 serum cytokines (78), indicating a strong rationale for future application of this approach. Further, this antibody conjugate approach was recently tested in prostate cancer tissue specimens using oncolytic viral platform, fibroblast activation protein-bispecific T-cell engager (FAP-BiTE), combining virolysis with endogenous T-cell activation signals. Interestingly, this approach has also shown efficacy of targeting cancer-associated fibroblasts in addition to prostate cancer cells prompting a multimodal treatment strategy within a single therapeutic agent (79). Based on encouraging data from preclinical studies, a phase I study using PSMA-targeted bispecific T-cell agent pasotuxizumab in mCRPC recently reported antitumor activity in a dose-dependent manner, with 2 patients showing durable response for over 1 year (80).

The Effect of ADT in Immune Modulation

Androgen deprivation by surgical castration or antiandrogens is a mainstay therapy to target AR signaling in treating prostate cancer. Short-term increase in the number of naïve T cells and Th-1 cells, and decrease in the number of Tregs after the initiation of ADT have been reported (81, 82). In addition, increased number of tumor-infiltrating T cells, transiently Th-1 biased, has been observed in animal models, supporting antitumor immune response of ADT (83). Although studies have shown immunostimulatory benefits of ADT in prostate cancer treatment, patients who have undergone standard ADT eventually result in relapse. This may be due to short-term Th-1 response caused by ADT, which eventually fails to establish immunostimulatory response, resulting in tumor-infiltrated immune cells polarizing toward immunosuppressive cells. Therefore, combination therapy of

ADT with immunotherapies blocking such protumorigenic events will be beneficial in treating prostate cancer.

There are emerging evidences that, in fact, ADT exerts immunosuppressive responses. A recent study has reported that T-cell–suppressive activity of AR antagonists, including flutamide and enzalutamide, results in decreased IFNγ production by T cells and/or APCs *in vivo* (84). Furthermore, the study revealed that immunosuppression induced by AR antagonists occurs during initial T-cell priming phase rather than at later stages of T-cell stimulation (84), suggesting that the accurate timing of ADT when treating prostate cancer in combination with other immunotherapies is crucial to avoid unintended immunosuppressive effect of AR antagonists. Indeed, a follow-up study of a combination therapy with Prostvac and nilutamide showed that patients who received Prostvac followed by nilutamide resulted in significantly increased survival rate compared with population that received nilutamide followed by Prostvac (85), suggesting vaccine followed by antiandrogen sequence may be a preferred approach to increase the efficacy of combination therapy.

Existing Limitations of Current Immunotherapy in General and Prostate Cancer in Particular

Although immunotherapies (e.g., immune checkpoint inhibitors) have shown encouraging clinical responses in certain types of cancer including melanoma, their application to other cancers needs further optimization. Unpredictable efficacy and toxicity of the therapy often become hindrances of successful immunotherapy in many cancers. Various patient responses to the same immunotherapy in patients with different types and stages of cancers have been observed (86). In addition, the patient response depends on multiple factors including intratumor heterogeneity and previous treatment history, which suggests the need of personalized and combination therapy as important future direction for successful immunotherapy.

Prostate cancer grows slowly compared with other types of malignancies, which allows it to be an ideal candidate where immunotherapy can be effective. However, various clinical trials by active immunotherapy, passive immunotherapy, adoptive T-cell therapy, and immune checkpoint inhibitors in combination with chemotherapy thus far have only shown modest clinical outcomes in mCRPC when compared with other genitourinary cancers. There are proposed hypotheses why immunotherapy trials were not successful in particular for prostatic malignancies. Particularly, TME in prostate lesions is known for establishing a niche unsuitable for tumor-infiltrating immune cells with antitumor activities, leading to limited efficacy of immunotherapy (87). In fact, a study has revealed a significantly smaller number of tumor-infiltrating $CD8^+$ T cells in primary prostate tumors in patients who underwent abiraterone treatment, an antiandrogen agent inhibiting biosynthesis of androgen, when compared with other types of malignancies (46). Generally, blocking the interaction between PD-1 and PD-L1 is expected to restore antitumor responses induced by tumor-infiltrating $CD8^+$ T cells. However, there are many other immunosuppressive characteristics associated with prostate TME, which possibly renders immunotherapeutic strategies using immune checkpoint inhibitors ineffective. For example, increased level of plasma TGFβ that directly suppresses $CD8^+$ T cells was observed in bladder cancer (88). Also, increased number of immunosuppressive cells including TAM, Tregs, and MDSC affect the antitumor response of $CD8^+$ T cells (89–92). Another explanation of different responses to immunotherapies, especially immune checkpoint inhibitors, can be supported by different types of tumors with various levels of tumor mutation burdens. Types of cancer with higher response rate to anti–PD-1/PD-L1 immunotherapy are melanoma and non–small cell lung carcinoma, which are known to have higher tumor mutation burden (93). These tumors are prone to be recognized by T cells because they express more number of neoantigens. On the other hand, tumors with low tumor mutation burden and less somatic mutations, such as prostate cancer, will less likely to respond to these immune checkpoint inhibitors (93), which explains why immunotherapies in prostate cancer have been relatively unsuccessful than in high mutation burden tumors (93). Another clinical speculation suggests that low level of PD-L1 expression is associated with prostate cancer progression. Studies have demonstrated, surprisingly, a downregulation of PD-L1 expression in primary prostate cancer (46–48, 94), which may explain why early clinical trials of anti–PD-1 monotherapy in mCRPC were not successful. For example, PD-L1 expression was rarely observed in specimens of patients with prostate cancer, whereas the level of PD-L1 expression increased in response to proinflammatory signals, IFNγ *in vitro* (48). In addition, gene analysis study revealed that PD-L1 expression was low, whereas the level of PD-L2 (another ligand for PD-1) expression remained significantly high in prostate cancer (95). Given that the number of effector T cells is low in the immune-privileged prostate lesions, downregulation of PD-L1 expression can be explained by relatively low levels of proinflammatory cytokines, secreted from $CD8^+$ T cells. However, other reports have suggested an increase in PD-L1 expression in CRPC (96). A study has shown that prostate cancer that has progressed after receiving enzalutamide resulted in upregulated PD-L1 expression in prostate cancer and circulating dendritic cells in patients and preclinical model (97). Increased number of circulating PD-1^+ T cells has also been observed in preclinical model (NCT02312557; ref. 97). Interestingly, it is noteworthy that prostate cancer under abiraterone acetate therapy in combination with prednisone showed a downregulation of PD-L1 expression in the tumors (46). The variations in PD-L1 expression in prostate tumors partly suggest that the levels of immune checkpoint molecule expression vary in different stages of prostate cancer progression, in response to ADT (96). Because clinical trials have been performed with patients only in advanced stages of cancer, PD-L1 expression levels may also vary depending on the types of previous therapies received before the progression of the disease.

Unlike some of the high-responsive tumors for immunotherapies, such as melanoma and non–small cell lung carcinoma, characterized by increased tumor-infiltrating lymphocytes, prostate cancer is considered as a "cold tumor" not only from the perspective of limited number of tumor-associated antigens and neoantigens available for immune targeting, but also from the existence of a complex TME, resisting T-cell infiltration, even in the combat of blockade with immune checkpoint inhibitors. Hence, adjusting strategies to overcome histologic barriers, including tissue hypoxia and dense stromal network, would complement immunotherapy approaches for effective cytotoxic T lymphocyte infiltration. A recent preclinical study demonstrated that reducing hypoxia using a hypoxia-activated prodrug, TH-302, significantly reduced hypoxia in prostate cancer TME and improved efficacy of immune checkpoint inhibitors (98).

Precision Immunotherapy for Prostate Cancer Therapy

Tumor is composed of subpopulations of cancer cells with distinct phenotypic and genotypic profiles, defined as tumor

heterogeneity. Tumor heterogeneity allows subpopulations of cells to present different behaviors and response rates to cancer immunotherapies. Different types of mutated proteins exist in a tumor, including KRAS and TP53 (99). The number of mutations in certain tumors can be used as a tool to predict their response to immunotherapies, anti–PD-1/PD-L1, and anti–CTLA4 monoclonal antibodies (99). For example, tumors with high mutational burden show high response rates to immune checkpoint inhibitors, anti–PD-1 immunotherapy (99). A study has revealed that prostate cancer bears 35 mutated peptides, whereas lung adenocarcinoma and melanoma resulted in 197 and 276 mutated proteins, respectively, describing relatively low tumor mutation burden in prostate cancer through the cancer genome atlas profiles (99). Recent studies have identified the existence of a large heterogeneity in mutation types in different foci within the same patients with prostate cancer (100). Further, existence of clonal evolution of genetically distinct mutations in multifoci prostate cancer, even in younger patients (101), suggests the importance of identifying patient-specific molecular signatures to design rational immunotherapy strategies. The complex nature of cancer with genomic heterogeneity and immunosuppressive TME highlights a need for personalized genomic therapy, which possibly will benefit clinical outcomes in CRPC. For a successful cure for prostate cancer, a combinatorial approach with individualized medicine, for instance, cancer genomics targeting newly identified gene components in the TME as part of therapeutic regimen, is essential. Cancer genomics compares the genomes of tumors with noncancerous cells to identify the specific mutations, especially in heterogeneous tumors. A recent next-generation genome sequencing analysis has identified that the patients with prostate cancer who have undergone a course of ipilimumab therapy have increased expression of v-domain Ig suppressor of T-cell activation (VISTA), a newly discovered immune checkpoint on macrophages (71, 102), suggesting a new potential immunotherapy target in prostate TME. Although VISTA-mediated signaling pathways are yet to be determined, VISTA expression on immunosuppressive subpopulation (e.g., MDSC, TAM, and Treg) is known to induce T-cell anergy/exhaustion through its interaction with potential binding counterparts (e.g., VSIG3 and PSGL-1), expressed on T-cell surface (102, 103). More recently, it has been reported that VISTA–VISTA transinteraction also directly induces naïve T-cell quiescence (104, 105), demonstrating a complex mechanism of VISTA biology in suppressing effector T-cell function in TME. These new findings pave way to further investigate additional modulators on VISTA expression in T cells. Given the fact that AR activation is reported to regulate adaptive immunity, including effector T cells (106), it is conceivable whether AR target genes include VISTA, along with others like PD-L1. Further studies in this angle may underscore the effectiveness of AR-directed therapies in combination with current immunotherapy regimens.

Further discovery of new genes to target heterogeneous TME through genomics/deep sequencing will play a significant role in the successful personalized treatment. Furthermore, a combination therapy of Kristen rat sarcoma viral oncogene (KRAS) inhibitor and existing immunotherapies (e.g., anti–PD-1/PD-L1) to target KRAS mutation-induced neoantigens in mutant KRAS tumors will be another multimodal therapeutic regimen. Hence, combination of multimodal immunotherapy that is personalized based on cancer genomics would lead to more effective interventions.

Immunotherapy Targeting Cancer Stem Cells

Cancer stem cells are defined as subpopulations of heterogeneous cancer cells with self-renewing capability for continuous tumorigenesis. Different types of cancer stem cells are distinguished depending on types of cell surface proteins they express. Common cell surface markers to identify cancer stem cells in solid tumors include CD133, CD44, CD24, and epithelial cell adhesion molecule (EpCaM). Subpopulations of prostate cancer cells with stem cell–like properties are known to coexpress cell surface markers, CD44, $\alpha 2\beta 1$ integrin, CD133, CD49f, and CD176 (107). High expression of aldehyde dehydrogenase (ALDH) was observed more in stem-like cells in metastatic prostate cancer compared with tumors without metastasis (108). The expression of ALDH was positively associated with expression of other prostate cancer stem cell markers including EpCaM, CD44, and integrin (108). Cancer stem cell–targeting immunotherapy has recently been attempted in preclinical models of prostate cancer with CAR T cells engineered against EpCaM-expressing cancer stem cell population, and results indicate promising outcome with murine prostate cancer model (109). Conventional prostate cancer therapies targeting differentiated or differentiating cancer cells with non–stem cell-like characteristics can cause tumor relapse by allowing tumorigenesis of cancer stem cells, whereas combinational therapy of traditional prostate cancer therapy and cancer stem cell–specific immunotherapy will provide a better clinical outcome by targeting different cell populations in heterogeneous tumor.

Future Directions

Collective analysis of existing limitations and renewed promise in immunotherapy clinical trials lends more optimism to further refine different aspects of this treatment paradigm for improved clinical outcomes in prostate cancer. Some of the key directions in the field of cancer immunotherapy, in general, are geared toward identifying molecular immune mechanisms in nonresponders and developing combination therapies targeting with engineered biomolecules in the TME (110, 111), improving the potential of systemic oncolytic virotherapy (112), adaptations to improve tumors, refractory to T cell infiltration (113), and improving preclinical animal models that recapitulate the human immune mechanisms (114). Prostate cancer progression in particular adopts immune evasion, involving multilayered cellular alterations, where cancer cells interact with and regulate immune and various stromal components, eventually polarizing them to form an immunosuppressive TME. Such complex events mediated by various molecular signaling pathways, including immune checkpoint expression patterns, may also differ depending on the microenvironment of metastatic sites or organs. Thus, immunotherapy targeting prostate tumors in the early stage before acquiring phenotypic heterogeneity during disease progression may be critical for therapeutic success.

Protumorigenic immunoediting events begin at the very first step of immune infiltration to primary tumors of prostate, where prostate cancer cells hijack danger signals to recruit innate immune cells. Indeed, high levels of antimicrobial peptides (but far less than the levels found in pathogenic infection) are expressed by prostate cancer epithelia with a pattern of gradual increase with tumor growth (115, 116). A study of cabiralizumab (cabira: FPA-008), a monoclonal antibody targeting TAM expressing macrophage colony-stimulating factor receptor, is currently in a phase II clinical trial

(NCT02471716). Even within the context of immune checkpoint targeting strategy, a phase II clinical trial of cabiralizumab in combination with nivolumab is also under investigation in advanced pancreatic cancer (117).

Regarding immunotherapy combination with AR-directed therapy, it is also interesting to note that a specific AR-targeting agent enzalutamide and ipilimumab combination is so far the only ongoing phase III clinical trial in patients with mCRPC. As noted, androgen–AR axis is still a mainstay of targeting CRPC (118, 119); however, AR-directed therapies appear to induce cross-resistance when combined with immune checkpoint inhibitors. For instance, prostate cancer under abiraterone acetate therapy, which inhibits biosynthesis of androgens, in combination with prednisone and leuprolide showed a downregulation of PD-L1 expression in the tumors (46), rendering checkpoint immunotherapy ineffective. However, it is also noteworthy that PD-L1 expression is highly upregulated in enzalutamide-resistant clones and circulating dendritic cells with no classical AR activation, suggesting that AR in hormone-naïve setting may downregulate PD-L1 expression in prostate cancer (97). These variations in PD-L1 expression in prostate tumors partly suggest that the expression levels of immune checkpoint molecule differ based on clinical grade of the disease (96). These results also suggest, in part, that PD-1/PD-L1 expression is differentially regulated depending on ligand availability and AR activation status (including AR gene mutations) in CRPC. Therefore, it may be of great interest to analyze AR regulation of immune checkpoint expression in CRPC, where nuclear AR levels may be a potential indicator of checkpoint immunotherapy success toward tumors progressing over AR-targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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