Immunotherapy for Metastatic Prostate Cancer: Current and Emerging Treatment Options

Dimple Chakravarty, DVM, PhD[a,*], Li Huang, MD, PhD[b,c], Matthew Kahn, MS[b], Ashutosh K. Tewari, MD[a]

KEYWORDS

• Prostate cancer • Immunotherapy • Vaccines • Checkpoint inhibition therapy • Preclinical models

KEY POINTS

- The advent of immunotherapy has revolutionized treatment for patients with cancer.
- Recent studies have reported that, despite low mutation burden, prostate cancer has a high number of DNA damage and repair gene defects that makes prostate cancer immune sensitive.
- Immunotherapies that have been tested in prostate cancer so far have been mainly vaccines and checkpoint inhibitors.
- What holds promise is a combination of genomically targeted therapies (gene and cell therapies), with approaches to alleviate immune response and thereby make the tumor microenvironment immunologically "hot."

PROGRESSION

Cancer is a disease of major concern worldwide and is the second leading cause of mortality.[1] The United States remains one of the countries with the highest incidence rates of prostate cancer.[2] Histologically, 93% of prostate cancer occurs as acinar adenocarcinoma. The remaining 7% of the prostate cancers are variations of ductal adenocarcinoma, basal cell carcinoma, and neuroendocrine tumors. The latter cancer forms are not as common as the acinar adenocarcinoma in the preliminary stages of prostate cancer. It is also difficult to distinguish between acinar adenocarcinoma and intraductal carcinoma because they frequently present together.[3] However, through the progression of drug treatments and different therapeutic regimes for patients with prostate cancer, neuroendocrine tumors can appear in much as 20% in patient populations with castration-resistant prostate cancer (CRPC).[4–7]

Like other tumor types, the occurrence and subsequent development of prostate cancer seem to be driven by genetic aberrations, mutations, and variations. Specifically, the genetic alterations found to be associated with primary prostate cancer include ERG, ETV1, ETV4, FLI1, SPOP, FOXA1, and IDH1.[8] To understand the complexity of prostate cancer is to not only recognize the differences between tumors in patients, but also the heterogeneity between the tumor cells within the patient. In this case, the molecular heterogeneity is grounded in differences from genes to transcriptomic expression. There is not one determinant of tumor development and pathogenesis.[9] TMPRSS2-ERG fusion is seen is approximately 40% to 50% of patients diagnosed with prostate cancer,[10,11] whereas SPOP missense mutations are present in 6% to 15% of cases.[12] TMPRSS2-ERG fusion results from the fusion of these 2 genes on 2 different chromosomes. The result of

[a] Department of Urology and the Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; [b] Department of Urology, Icahn School of Medicine at Mount Sinai, New York, New York, USA; [c] Department of Urology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
* Corresponding author.
E-mail address: dimple.chakravarty@mountsinai.org

Urol Clin N Am 47 (2020) 487–510
https://doi.org/10.1016/j.ucl.2020.07.010

this fusion is the enhancement of androgen dependency and the development of cancer.[11,13] Despite occurring in such a high fraction of the patients with prostate cancer, it does not seem to be a good prognosticator.[14] SPOP mutations are seen to be present in both localized and metastatic prostate cancers.[12] It has been shown to be involved in the ubiquitination of proteins and possibly has a role in maintaining genomic stability.[15] Another important genetic alteration associated with prostate cancer is the genomic deletion of PTEN gene.[8,16] PTEN deletion is seen in 40% to 60% of patients with prostate cancer[8,17] and it functions by inhibiting the PI3K/Akt signaling and inducing cell cycle arrest.[18]

Specific tumor suppressor proteins like Rb1 and Trp53 assist in the maintenance of the cell cycle by halting aberrant growth. Genomic alterations in TP53 involve mostly loss of function mutations or homozygous deletion and it is evidenced in 40% to 60% of prostate cancer cases, predominantly in metastatic prostate cancer.[8,19] Their relation to prostate cancer is important because the inhibition of the genes coding for Rb1 and Trp53 facilitates the cancer's ability to develop androgen deprivation therapy resistance thus paving the way for metastatic development. It was shown that the loss of both Rb1 and Trp53 in mice led to a significant decrease in survival after several weeks compared with single knockout mice groups. Thus, their loss controls linear plasticity in cancer cells in the sense that mutation can eventually lead to insensitivity to drug therapy.[20] Furthermore, SOX2 expression is significantly amplified with double loss of Trp53 and Rb1, indicating that its elevation is correlated with these other genes in controlling cancer.[21]

Primary prostate cancer or early stage prostate cancer is a very localized disease with limited growth. Accompanying this stage are low prostate-specific antigen (PSA) levels and Gleason scores, compared with later metastasis and more severe cases of prostate cancer. If identified at an early stage, several treatment options are available to the patient, such as active surveillance, radiation therapy, and even radical prostatectomy.[22] Active surveillance involves monitoring blood PSA levels or using digital rectal examinations. Radiation therapy is a technique that uses a high beam of radiation to kill dividing cancer cells. Radical prostatectomy is another standard procedure for removing the entire prostate organ and has shown significant efficacy in decreasing cancer development.[22] Patients can be divided into different groups depending on the characteristics of primary prostate cancer, such as low-, intermediate-, and high-risk groups.

Prostate cancer progresses from localized forms in the prostate gland, spreading to surrounding tissues, and subsequently metastasizing to distant sites like the vertebral bone. As the cancer cells grow and mutate, there are changes in the biochemical and pathologic development of the tumor microenvironment that can be controlled by various drug treatments and therapeutic regimes. When a patient has advanced prostate cancer, androgen deprivation therapy is recommended because the cancer cells rely heavily on androgen receptor signaling. These androgen receptors respond to testosterone and dihydrotestosterone for effective signaling and cell growth. Luteinizing hormone-releasing hormone (LHRH) antagonists are useful drugs in this case because they inhibit the production of the luteinizing hormone and prevent the synthesis of testosterone in the testes. These drug therapies are usually administered when the primary prostate cancer is ADT sensitive.

In the castrate-sensitive phase, local therapies include external beam radiation and implant radiation, high-intensity ultrasound therapy, surgeries such as radical prostatectomy and orchiectomy (very uncommon owing to psychological implications), and androgen deprivation therapy in the form of LHRH analogs that interfere with normal hormonal balances by diminishing the production of testosterone and thus dihydrotestosterone. However, cancer can become resistant to the hormonal drug therapies and become insensitive to androgen deprivation therapy. This specific form is known as CRPC. Treatments for castrate-resistant disease include combinations of abiraterone and prednisolone/enzalutamide, as well as docetaxel.

The discussion of drug therapies elsewhere in this article represents the common protocol in the treatment of prostate cancer. However, novel drugs and phase I trial combination therapies are continually being researched. It is important to think about the medical interventions and potential areas for targeted therapy that can obstruct and inhibit cancer survival.

The purpose of external beam radiation therapy is to ablate the prostate tumor using an external source of high energy. Tomita and colleagues[23] looked at the effect of high-intensity radiation on clinical relapse along with neoadjuvant androgen deprivation therapy and found significant improvements for intermediate as well as high-risk patients with prostate cancer. Other studies from Preisser and colleagues[24] discovered that external beam radiation in patients who underwent previous radical prostatectomy showed different patterns in terms of surrounding tissue toxicity. Tissue toxicity is an important factor in deciding whether

or not to use certain treatment options and age differences should be taken into account. What they found was that secondary primary cancers resulting from external beam radiation–induced bladder cancer as the second most common site outranking skin cancer.[24] Furthermore, another study sought to understand changes in PSA levels resulting from the use of implant radiation. D'Amico and colleagues[25] found that implant radiation combined with neoadjuvant androgen deprivation therapy had significantly reduced PSA levels compared with just radical prostatectomy or radiation therapy alone. In terms of the surgical options, there are different forms of prostatectomies. These options include open, laparoscopic, and minimally invasive or robotic-assisted surgeries. Graefen and colleagues[26] elucidated on the fact that research suggests that there are few to no differences in terms of the oncologic outcomes among the surgical practices. Many of the reported differences may stem from prior experience and expertise performing such operations.[26] Some patients opt for minimally invasive interventions compared with radiation or surgery. Ultrasound therapy is one such intervention that has shown interesting clinical progress. The treatment is not as effective as conventional invasive techniques, and Bass and colleagues[27] demonstrated that high-intensity focused ultrasound therapy was not able to assist all intermediate or high-risk groups given that 49% of patients had local recurrence. Although high-intensity focused ultrasound therapy had a high failure rate, it is very clear that the negative side effects were minimal and inconsequential.[27]

Another therapy to discuss is the androgen deprivation therapy. Hormonal manipulation is effective in treating local prostate cancer because the tumor is still castrate sensitive. LHRH agonist affects the hormonal balances in the production of gonadotropin releasing hormone and its effect on the production of testosterone in the testes. There are several LHRH agonists, including leuprorelin, goserelin, and triptorelin. Comparison for the effectiveness of LHRH agonists, goserelin, triptorelin, and leuprolide in treating prostate cancer showed significant differences in the castration levels at less than 10 ng/dL, whereas the efficacy was comparable at 20 ng/dL or higher doses.[28]

When prostate cancer is in its advanced stages and has become resistant to hormonal therapy, this is known as the castrate-resistant phase. Patients usually present with CRPC. Abiraterone and enzalutamide are 2 vital drugs that are used to treat patients presenting with this form of prostate cancer. A study conducted by Hahn and colleagues[29] found that androgen deprivation therapy in combination with abiraterone showed significant improvement in overall survival and promising cancer-free survival after 3 years. Furthermore, Cornford and colleagues[30] highlighted the benefits of enzalutamide on numerous clinical determinants for improved course of prostate cancer.

As second-generation drugs lose effectiveness and potency in the fight against prostate cancer, adjuvant chemotherapies seem to be the last resort. Docetaxel is a common taxane used for metastasis. Although it is used in combination with the aforementioned treatments for improved efficacy of therapeutic intensity and delivery, docetaxel has not been shown to be effective after radical prostatectomy.[31] Cursano and colleagues[32] look at how combinations of radium-223 with docetaxel and cabazitaxel affected patients who had bone metastases. Strikingly different clinical outcomes have emerged from this study, which warrants the need to investigate the effects of these combinations.

Novel agents that are used to treat prostate cancer include the use of proxalutamide, an androgen receptor antagonist. Furthermore, nanoparticles are being developed to enhance delivery of chemotherapeutic agents such as docetaxel. The potency of docetaxel and doxorubicin was increased by enhancing their codelivery using nanocarriers.[33] Furthermore, Hammer and colleagues[34] describe how a novel antibody-based therapy called thorium-227 may be helpful in targeting prostate-specific membrane antigen (PSMA) and alleviating metastatic CRPC.

IMMUNOTHERAPY IN CANCER

Over the past decades, the conventional strategies for cancer treatment include surgery, chemotherapy, and radiotherapy. The concept that the immune system can recognize and control tumor growth was first report in 1891 by William Coley, who demonstrated that bacterial toxins cause antitumor immune responses in some patients, particularly sarcomas,[35] but with limited clinical efficacy. After that, immunotherapy has become an appealing strategy for various types of tumors. Different cancer immunotherapy approaches have proven efficacy,[36] such as cell-based therapies, monoclonal antibodies, cancer vaccinations, and even immune checkpoint blockade therapy.

Later in the 1960s, Thomas and Burnet[37] put forward the theory of cancer immune surveillance. According to this theory, the body's immune system would use tumor-associated antigens to eliminate malignant cells.[37] It took about one-half of a

century for this theory to be accepted.[38] In the 1990s, it was demonstrated that the CD8-positive T lymphocytes had the ability to kill tumor cells that presented antigens for melanocyte differentiation.[39] It was also demonstrated that the absence of interferon gamma led to incidence of sarcoma and lung cancer in mice.[40] A subsequent study was done to assess the role of T cells in antitumor immune responses, that ultimately led to the use of IL-2, a T-cell growth factor, in clinics. Briefly, tumor-infiltrating lymphocytes (TILs) from patients were activated with IL-2 in vitro, which was subsequently injected to patient.[41] The US Food and Drug Administration (FDA) later approved IL-2 in 1991 for treating metastatic renal cell carcinoma. However, IL-2 treatments had some caveats. The response rate was relatively low with high toxicity, underlining the need to improve immunotherapeutic strategies.[42,43]

In 1975, monoclonal antibodies were made with the development of the hybridoma technology.[44] Subsequently, treatments using monoclonal antibody started evolving and rituximab was the first FDA-approved drug for treating B-cell lymphomas. This drug is a monoclonal antibody that targets the CD20 antigen, expressed ubiquitously in B cells. This treatment brings about cell death as a result of cytotoxicity, the activation of complements and induction of apoptosis.[45,46] During the same era, chimeric antigen receptor (CAR) T cells were also developed, which had the ability to combine the self-renewal and cytolytic capacity of T cells with the antigen-binding properties of antibodies.[47,48] CAR T cells are chimeric fusion proteins that express an extracellular domain that has the antigen recognition ability, including single chain variable fragments, which are derived from the antibody, and the T-cell activation end domains. In CAR T-cell therapy, the $CD8^+$ T cells of a patient are manipulated ex vivo to elicit an immune response when subsequently reinfused into the patient. The most promising results was seen with CAR T cells targeting CD19 in hematologic malignancies.[49,50] Two FDA-approved CART19 therapies are tisagenlecleucel and axicabtagene ciloleucel.[51–54]

Checkpoints have channelized immune response to pathogens as well as self-antigens. Memory T cells as well as cytokine secretion in addition to an individual's $CD8^+$ cytotoxic T lymphocyte (CTL) cells are required to activate immune response at cellular level. Ligands like CD80 or CD86 bind to CD28 and the CD28 gets replaced by CTL antigen 4 (CTLA)-4, which send inhibitory signals on the T-cell surface. This leads to switching off of the signal or a checkpoint being applied. Cancer cells express increased levels of CTLA-4. The other inhibitory mechanism in cancer cells is between antigen-presenting cells (APCs) and programmed death 1 (PD-1) and PD ligand 1 (PD-L1)/PD-L2 leading to inactivation by programmed death of CTLs. Over recent years, several antibodies that target cellular immune checkpoints (eg, PD-1/PD-L1 and CTLA-4) were developed to promote the activation of T-cell and tumor regression. This therapeutic strategy has demonstrated benefits in tumors having a high mutation burden, enabling tumor-mutated antigens (neoantigens) to enter stage in cancer immunotherapy.[55–61] In patients with melanoma, blockage of both PD-1 and CTLA4 lead to better survival.

However, CAR T cells and checkpoint blockade are not always effective, mainly owing to the immune suppressive environment locally created by the cancer. Further several cytokines with immunosuppressive properties are occasionally turned on by the cancer cells. This facilitates the cancer cells to attract regulatory T cells and myeloid-derived suppressor cells that have the ability to block autoimmunity. Regulatory T cells act by suppressing B-cell Ig production and activation.[62–65] So therapeutic antibodies targeting regulatory T cells and myeloid-derived suppressor cells in combination with checkpoint inhibitors or CAR T cells show promise.

IMMUNOTHERAPY IN GENITOURINARY CANCERS

Genitourinary malignancies represent a heterogeneous group of diseases, such as kidney cancer, bladder cancer, and prostate cancer. Treatment of those diseases involves surgery, radiation, and systemic therapy. Immunotherapies have been used in genitourinary cancers with promising clinical benefits and outcomes.

Kidney cancer lead to more than 175,000 deaths in 2018, and there is a constant increase in its incidence worldwide.[66] In the United States, it is the eighth most common cancer, with an estimated number of 73,750 for new cancer cases and estimated deaths of 14,830 in 2020.[1] Among the different subtypes of kidney cancer, the most prevalent form is the clear cell renal cell carcinoma and represents about 60% to 80% of all the primary kidney cancers.[67]

Studies showed the association between the immune system and kidney cancer.[68,69] For kidney cancer, cytokines have been used as immunotherapies for more than a decade. In 10% to 25% of patients with kidney cancer, the cytokines interferon-alpha and IL-2 improved objective response rates and provided sustained

remissions in a subset of the patients.[70–72] For a long time, IL-12 was the first-line therapy for advanced kidney cancer, but because it has severe side effects, it is no longer a treatment of choice. In addition to cytokines and targeted therapies, several new types of immunotherapy have become important in the treatment of kidney cancer. The most notable immune checkpoint inhibitors are those that block the functions of CTLA-4 and PD-1. CTLA-4 helps to decrease the inflammatory T-cell response by facilitating the activated T cells to be disengaged.[73] The CTLA-4–blocking antibody ipilimumab showed partial response.[74] Treatment with nivolumab, the anti PD-1 antibody, showed treatment response in 27% patients with RCC, making nivolumab a treatment of choice.[75] However, current studies indicate that single agent immunotherapy may not benefit all patients, highlighting the need for combined treatment strategies to improve efficacy.

Atezolizumab is a monoclonal antibody against PD-L1. Bevacizumab is a vascular endothelial growth factor inhibitor. The combination treatment with Atezolizumab and bevacizumab potentiates PD-L1 inhibition.[76,77] In a phase II trial on treatment-naïve patients with metastatic RCC, better antitumor activity was demonstrated with combination therapy with atezolizumab plus bevacizumab compared with atezolizumab alone or sunitinib alone. In PD-L1 patients treated with combination therapy, a higher progression-free survival was reported.[78] Another trial (IMmotion 151) demonstrated that combination therapy had a longer progression-free survival and improved objective response rates in PD-L1–expressing patients.[79] Avelumab (anti–PD-L1) combined with axitinib (vascular endothelial growth factor inhibitor) showed superior progression-free survival (13.8 months vs 7.2 months).[80]

A clinical trial in phase Ib for the use of pembrolizumab (an anti–PD-1 agent) with axitinib (an anti-vascular endothelial growth factor agent) demonstrated promising antitumor activity; 73% patients achieved an objective as well as similar toxicity of each monotherapy.[81] A phase III study comparing axitinib plus pembrolizumab with sunitinib monotherapy (NCT02853331) is ongoing to further evaluate whether or not the combination works better than a vascular endothelial growth factor inhibitor monotherapy. Compared with the group treated with sunitinib, the combination group demonstrated significantly longer overall survival, longer progression-free survival, an improved objective response rate, and a prolonged response.[82]

IMMUNOTHERAPY IN BLADDER CANCER

In the United States, bladder cancer is the fifth most common cancer, with estimated new cancer cases of 81,400 and estimated deaths of 17,980 in 2020.[1] Immunotherapy for bladder cancer has a long history. Both early and advanced stages of bladder cancer has been treated with different immunotherapies, including bacillus of Calmette and Guerin (BCG) intravesical immunotherapy[83,84] and anti–PD-1/PD-L1 immune checkpoint blockade.

BCG has been applied to treat patients with non–muscle invasive bladder cancer since 1976.[85] In addition, BCG was the first immunotherapy developed for non–muscle invasive bladder cancer approved by the FDA. BCG suppresses the tumor cell growth by infiltrating the bladder with inflammatory cells and upregulating cytokines. BCG immunotherapy was superior to various intravesical chemotherapy drugs and was more effective in preventing tumor recurrence.[86–91] However, BCG treatment failed in approximately 40% of patients with non–muscle invasive bladder cancer.[92] This is a main problem that requires alternative strategies.

There are other FDA-approved immunotherapy options for bladder cancer, including PD-1/PD-L1 inhibitors, including atezolizumab,[93] durvalumab,[94] avelumab,[95] nivolumab,[96] and pembrolizumab.[97]

Anti–programmed Death Ligand 1 Immunotherapies

The first anti–PD-L1 antibody to be tested in bladder cancer immunotherapy was atezolizumab, which was approved by the FDA in 2014.[98] Atezolizumab showed good activity in metastatic urothelial bladder cancer, which was associated with positive PD-L1, had significantly higher response rates. A multicenter phase II trial of atezolizumab showed an improved overall objective response rate compared with a historical platinum-based chemotherapy control (15% vs 10%).[93] A postprogression study from Imvigor210 also demonstrated that platinum-treated patients with either locally advanced or with metastatic urothelial carcinoma still benefited from continued atezolizumab treatment.[99]

After the success of atezolizumab, durvalumab, another anti–PD-L1 drug, was tested in advanced bladder cancer.[94] Durvalumab, an engineered human antibody that selectively blocks the binding of PDL-1 to PD-1 and CD80, demonstrated encouraging clinical activity in locally advanced/metastatic bladder patients with cancer.[94]

Avelumab is the third anti–PD-L1 inhibitor approved for locally advanced or metastatic

bladder cancer with disease progression. Avelumab is a fully humanized antibody developed against PDL-1 that assists in using the immune response of the human body against the cancer. Avelumab successfully showed a significant improvement in overall survival.

Anti–programmed Death 1 Immunotherapies

Nivolumab, a PD-1–blocking antibody, demonstrated safety and efficacy in locally advanced or metastatic urothelial carcinoma.[96,100] Nivolumab demonstrated antitumor activity and survival in the global population.[101]

Another highly selective humanized monoclonal antibody, pembrolizumab, blocks the interaction between PD-1 and PD-L1/PDL-2. Compared with chemotherapy, pembrolizumab demonstrated better response in all patient and also in patients having PD-L1–positive score of 10% or higher. Compared with the chemotherapy group, the median overall survival of the pembrolizumab-treated group was significantly longer.[102] Pembrolizumab has been approved by FDA as a second-line therapy after platinum treatment and as a first-line therapy for patients with locally advanced/metastatic urothelial carcinoma that are ineligible for cisplatin treatment.

Testicular Germ Cell Tumors

The majority of patients with metastatic germ cell tumors could be cured with first-line or salvage chemotherapy.[103] However, a group of 15% to 20% patients who failed after those treatment need develop additional therapeutic options.[104] Recent targeted therapies trials did not show promising efficiency.

IMMUNOTHERAPY FOR METASTATIC PROSTATE CANCER

Immunotherapy is a growing area of research for prostate cancer, given the importance and need for alternative treatments. Immunotherapy fundamentally encompass harnessing and exploitation of the patient's individual immune system to fight against the cancer. Augmenting the strength of the immune system by inducing specific interactions to take place between T cells and their compliment antigens on cancer cells may prove to be useful in the targeted destruction of tumors. Furthermore, immune checkpoint pathway CTLA-4/B7 inhibitors like ipilimumab, tremelimumab, and prostvac, as well as PD-1 pathway inhibitors such as nivolumab, pembrolizumab, and pidilizumab are specific types of drugs that may be helpful in boosting an immune response that was initially suppressed by the growth and spread of cancer.

Although several immunotherapies have been FDA approved for metastatic castration-resistant prostate cancer, there is still a major lack of efficient use of immunotherapeutic agents for this disease. This is mainly because prostate cancer for a prolonged period of time has been conceived to be an immune desert. Unlike other solid tumors, prostate has never shown a strong immune infiltrate within the tumor. Further, prostate tumors present a tumor microenvironment that is metabolically hostile, with increased glycolysis. This environment suppresses T-cell function. Further, tumor-infiltrating T cells also have a reduced mitochondrial function.[105] Tumor immune score is computed based on the TIL expression within the tumor. This along with the tumor specific inflammatory gene signature is used to categorize tumors as "hot" or "cold."[106] Unlike other solid tumors like urothelial and lung cancers, prostate tumors have more immunosuppressive factors than immunostimulatory factors leading to an impaired TIL activity. However, studies that looked for other T-cell populations report increased expression of $CD4^+$ and $CD8^+$ forkhead box P3 ($Foxp3^+$) regulatory T cells in tumors.[107,108] Further, a study also reports that increased Foxp3+ TILs were associated with worse survival outcomes.[109] Besides immunosuppressive lymphocytes, protolerogenic tumor-associated macrophage has been reported to be infiltrated in high numbers in prostate cancer tumor microenvironment.[110] M2-associated cytokines and chemokines that are immunosuppressive are also secreted along with transforming growth factor-β2, by the macrophages. So, some studies have elucidated the need for targeting transforming growth factor-β before checkpoint inhibition to get better therapeutic benefit.[111]

Prostate cancer has a low tumor mutation burden, which results in low neoantigen expression compared with other tumor types.[112] As a result, immunotherapy is speculated to be less effective. Despite having a low somatic alteration burden, prostate cancer does present with a high number of DNA damage and repair gene defects.[8,113,114] Mutations in DNA damage and repair genes especially in members of the homologous recombination repair pathway both somatic and germline, makes prostate cancer immune sensitive. So prostate cancer cannot be called a cold tumor or an immune desert.

The various immunotherapies that have been tested on prostate cancer can be broadly classified into vaccines, cell therapies, checkpoint blockade therapies, oncolytic virus therapy, and targeted antibodies.

Cancer Vaccines

Vaccines are composed of an adjuvant that can activate APCs like dendritic cells, as well as a target protein that is associated with the cancer.[115] Using the patient's own dendritic cells that are pulsed with tumor antigens, is an approach that has lot of popularity in the vaccine world. Peptides from tumor antigens are used to pulse dendritic cells and have shown good response in preclinical models.[116] Therapeutic cancer vaccines that facilitate the body's immune system to recognize tumor-associated antigens and generate a T-cell response have shown considerable success in prostate cancer. Prostate cancer is a promising target for vaccine-based therapy owing to the expression of several specific tumor-associated antigens like PSA and PSMA, as well as prostatic acid phosphatase.[117]

Sipuleucel-T

Interest in immunotherapy has gained momentum with the relative success of the FDA-approved treatment of sipuleucel-T. Sipuleucel-T, an autologous vaccine, has been derived from peripheral dendritic cell collection by leukapheresis. This collection of cells is stimulated by PA2024, a fusion protein of an immune-activating cytokine, granulocyte-macrophage colony-stimulated factor linked to the target antigen, prostatic acid phosphatase.[118,119] After 36 to 44 hours, the dendritic cells, which are now primed, are reinfused back into the patient for generating a $CD4^+$ and $CD8^+$ T-cell response that is prostatic acid phosphatase specific.[120,121] This process is repeated 3 times at 2-week intervals over the course of 1 month to complete the full course of therapy.[122]

Sipuleucel-T was the first immunotherapy to be approved by FDA for patients with metastatic CRPC. The IMPACT trial randomized patients in 2:1 fashion and received 3 doses of sipuleucel-T or of placebo. This study demonstrated a improvement in median overall survival by 4.1 months and a 22% decrease in the risk of death.[118] Despite the beneficial effect of sipuleucel-T, several other prostate cancer vaccines that were tested in phase III trials have not been that promising.

GVAX

Another cell-based vaccine, GVAX, has been synthesized using prostate cancer cell lines, LNCaP and PC3, by transducing with a retrovirus that was made replication defective and had also been modified genetically to be able to bear granulocyte-macrophage colony-stimulated factor and bring about the recruitment of APC to the injection site.[123,124] Although an initial phase I/II trial in hormone-naïve patients with prostate cancer with relapse in PSA, showed promising results,[125] 2 subsequent phase III clinical trials (VITAL-1 and VITAL-2) did not show significant benefit and so were terminated early. In the VITAL-1 trial, randomization of patients with asymptomatic metastatic CRPC was done to receive either GVAX or docetaxel-prednisone. Initial analysis showed that less than 30% of patients would meet the primary end point, which was overall survival, and so the trial was terminated early. In contrast, the phase III trial, VITAL-2, on symptomatic taxane naïve metastatic CRPC patients who received GVAX alone or GVAX plus docetaxel/prednisone, also terminated early as a result of increased mortality among patients in the intervention group.

The flaws in the trial design could have possibly led to the negative outcomes. There was no placebo control in the study. Moreover, before the phase II trial VITAL-2, the recommended doses for the combination therapy of GVAX and docetaxel were not determined, which may have also contributed to experimental flaws.[121,126]

PROSTVAC

PROSTVAC is also another vaccine that includes in viral vectors PSA gene and several T-cell costimulatory molecules. It creates a heterologous prime boost by combining recombinant fowlpox and vaccinia virus.[127] This vaccine infects APCs to generate cell surface proteins expressed on the surface of APCs finally leading to tumor cell destruction as a result of interaction of APCs with the T cells.[127] PROSTVAC has been used in several clinical trials. An increase in PSA progression-free survival was seen in 63% of the patients for a period of 6 months. Furthermore, the phase II trial showed a significant reduction of the PSA doubling.[128] Other studies have been established using PROSTVAC. In another phase II study, patients with minimally symptomatic metastatic CRPC were included in the study and were randomized to either receive the vaccine or placebo. Even though the study showed negative results for its primary end point (progression-free survival), overall survival was seen to be significantly increased.[129] PROSTVAC has shown no effect on overall survival in patients with metastatic CRPC.[130]

Polyinosinic-polycytidylic acid and poly-L-lysine

Although the capacity to activate a T-cell response has been tested for several dendritic cell-based vaccines, their effects has been limited. Polyinosinic-polycytidylic acid, and poly-L-lysine, an immunostimulant, is a double-stranded RNA complex that acts like a viral mimic. It is composed

of poly-L-lysine double-stranded RNA and carboxymethylcellulose, polyinosinic-polycytidylic acid. It activates dendritic cells by binding to toll-like receptor 3, MDA5, and other pathogen receptors.[131–133] The synergy between MDA5 and toll-like receptor 3 activation makes Poly IC a superior vaccine. Toll-like receptor 3 contributes to $CD8^+$ T-cell activation and MDA5 is required for the survival of CD8 memory T cells. Poly-ICLC (Hiltonol®) is being tested in several clinical trials as an immune stimulant.

There are 4 clinical trials for Poly ICLC in prostate cancer. Out of the 2 completed studies, 1 trial tested PSMA and TARP peptide vaccine in combination with Poly IC-LC in HLA-$A2^+$ patients with rising PSA (NCT00694551), and the other tested combination therapies of Poly IC LC with MUC1 vaccine in patients with advanced prostate cancer (NCT00374049). One of the studies at our institute is testing IT/IM Poly-IC LC in patients with high-risk, clinically localized prostate cancer (NCT03262103).

Checkpoint Inhibition Therapy

Another type of drug is the immune checkpoint inhibitor that blocks proteins on the immune cells. This drug assists in making the immune system more effective at destroying cancer cells. They function by releasing inhibitory responses that regulate T-cell–mediated immunity. Immune checkpoints are inhibitory mechanism of immune cells used to regulate immune response. Antibodies blocking immune checkpoint receptors and that are approved for clinical use include the CTLA-4 as well as PD-1 and its ligand PD-L1.

The first successful immune checkpoint inhibitor to receive FDA approval was anti–CTLA-4 (ipilimumab).[134] Ipilimumab was also the first immune checkpoint that was studied in prostate cancer. CTLA4 controls T-cell activation and competes with CD28, the costimulatory receptor, for ligand binding, to CD80 and CD86. This leads to translocation and expression of CTLA4 on the cell surface of T cells. CTLA-4 pathway blockade was achieved using a fully human monoclonal antibody called ipilimumab.[135] Another phase III clinical trial in metastatic CRPC patients who had progressed on docetaxel-chemotherapy was randomized to receive either ipilimumab or placebo after bone-directed radiotherapy. Although there was no benefit in the primary end point of overall survival, some benefit was observed in progression-free survival with ipilimumab over placebo. There was a significant reduction in the PSA in patients who were treated with ipilimumab. Additionally, a greater benefit was also seen in subset of patients, such as lower alkaline phosphatase concentrations, higher hemoglobin concentrations, and finally absence of visceral metastases. The median overall survival was significantly higher with ipilimumab compared with placebo.[136,137]

Pembrolizumab (Keytruda) has been shown effective for a low rate hypermutated subtype of prostate cancers.[138] The effects of pembrolizumab were studied on a large group of patients who had metastatic CRPC. They were divided into cohorts 1, 2, and 3 based on if they were PD-L1 positive, PD-L1 negative, or bone predominant, respectively. Treatment with 200 mg of pembrolizumab showed that median overall survival was highest for cohort 3 at 14.1 months and the estimated 12-month survival rates were 41% for cohort 1%, 35% for cohort 2%, and 62% for cohort 3. This study warrants the need to further research the effects of pembrolizumab on bone-predominant disease.[139,140]

PD-1 is also expressed on activated immune cells including B cells, T cells, and natural killer cells. PD-1 has a tyrosine-based inhibitory motif and also an immune-receptor tyrosine-based switch motif that gets phosphorylated when it binds to the B7 ligands PD-L1 or PD-L2. This caused SH2 domain containing tyrosine phosphatase 2 to be recruited and finally leads to inhibition of T-cell proliferation. PD-L1 is expressed on APCs, T cells, vascular endothelial cells, stromal cells, and cancer cells.[141,142] PD-L1 expression is induced owing to production of inflammatory cytokines, interleukins (IL-2, IL-7, and IL-15), when antigens expressed by MHC complex get presented to T cells. T-cell effector functions are inhibited owing to PD-1/PD-L1 interactions through distinct mechanisms compared with CTLA-4.[143,144] Antibody-based blockade of the PD-1 receptor or its ligand increases the antitumor immunity and tumor growth suppression.[144] The PD-1/PD-L1 pathway is activated in several tumor types such as in the lung, kidney, and bladder.[145] It is clear that early phase clinical trials that are investigating nivolumab (PD-1 blockade) and prostate cancer have shown very small success and that further research experiments are needed to investigate these complex biochemical mechanisms Studies of nivolumab monotherapy, showed no measurable responses.[75,134,146,147] These studies demonstrate that the success of immune checkpoint inhibitors in patients with metastatic CRPC has been limited,[59,75,134] expect for some isolated response seen in patients having mutations in either BRCA 1/2, CDK12, or microsatellite instability–high mutations. Ipilimumab has been FDA approved as a drug therapy for metastatic melanoma and has shown potential in treatment

for other types of cancers like renal, lung, and prostate. Currently, combination therapies using ipilimumab would be more useful in the treatment of metastatic CRPC as opposed to treatment alone. Nevertheless, studies have illustrated that the response rates to ipilimumab have ranged in the 10% to 15% range, further supporting the notion that it is not a completely effective therapy.

More in-depth and comprehensive research is needed to assist the immune system recognize prostate tumors and as well as activating the immune cells for targeted destruction of the cancer. Various studies are looking at whether combinations of immunotherapy drugs may be more effective in treating prostate cancer compared with single immunotherapies.

Adoptive Cell Therapy

In this form of treatment, the immune cells of the body are geared toward eliminating cancer. The immune cells are either isolated and expanded or genetically engineered to improve their ability to fight cancer. Cell therapy include TIL therapy, T cell therapy, CAR T-cell therapy, as well as natural killer cell therapy.

In TIL therapy, the T cells from the tumors are expanded in the presence of IL-2 and reinfused into the patients.[148] TIL therapy has been successful in melanoma as well as other solid tumors. Recent studies have also demonstrated the ability of using TIL therapy for patients with prostate cancer.[149]

In both T-cell therapy and CAR T-cell therapy, the T cells from patients are genetically modified ex vivo, expanded, and readministered to the patient. In T-cell therapy, the T cells from the patient are genetically modified to be able to target specific cancer antigens, whereas in CAR T-cell therapies, the patients T cells are coupled with synthetic receptors, CAR. CAR T-cell therapy is advantageous over the other adoptive cell therapies mainly because CARs can bind to the cancer cells even if their antigens are not presented on cell surface. However, 1 disadvantage with CAR T-cell therapy is that the range of potential antigen targets are limited, owing to the intercellular expression of most proteins, which makes them unavailable for CARs.

For effective CAR T-cell therapy in prostate cancer, the most critical step is the identification of tumor-associated antigens that are constitutively expressed by the cancer cells. The proteins that have been used as TAAs for prostate cancer CAR T cell therapy are mainly PSA, prostatic acid phosphatase, prostate stem cell antigen (PSCA), PSMA, and epithelial cell adhesion molecule. Of these, PSMA and PSCA are the CAR T-cell targeted antigens that have been mostly used in metastatic prostate cancer. In the phase III trials of sipuleucel-T for metastatic CRPC patients, the antigen presenting cells were pre-exposed to human granulocyte macrophage colony stimulating factor and prostatic acid phosphatase fused protein.[149] PSCA is another cell surface glycoprotein expressed in prostate cells. CTL response HLA-A2 restricted anti-PSCA peptides has been evaluated in vitro in several studies.[150–152] CTL response has also been studied in TRAMP mouse models vaccinated with PSCA-encoded viral vectors.[153,154] Further in xenograft models prostate cancer inhibition has been evaluated using anti-PSCA antibodies.[155–157] PSMA, a transmembrane glycoprotein, shows higher expression in high grade prostate tumors. Invitro studied have demonstrated CTL response using HLA-2–restricted PSMA peptides.[158–161] In several recent studies, chimeric anti-PSMA immunoglobulin T-cell receptor constructs have been used to promote a T-cell response. In mouse models PSMA-CAR T cells were able to abolish metastatic prostate cancer.[162] PSMA CAR T-cells coupled with CD28 showed significant decrease in tumor volume in mice.[163] In another study anti-PSMA CAR T cells resistant to transforming growth factor-β caused cell lysis of PSMA expressing cells with an increase of interferon-γ, IL-2, and $CD8^+$ cells.[164]

CAR T-cell therapy shows promise mainly in preclinical studies on metastatic prostate cancer. However, more investigation on risk to patients is essential to develop plans for management of toxicity. Further among all the TAAs the one that's most safe an effective is yet to be determined.

Oncolytic Virus Therapy

Immunotherapies are currently used to activate the immune system enabling it to target and kill the cancer cells. These new drugs can enhance and facilitate antitumor activity by promoting the function of specific cells, such as the T cells and natural killer cells. However, recent studies have suggested that, even with the use of these immunotherapies, cancer cells can adapt to the environment, evade the immune system, and induce immunosuppression. Therefore, oncolytic viruses have been proposed as a novel method to counteract this tumor-associated immunosuppression and evasion. Oncolytic viruses have even been shown to potentiate the effectiveness of several immunotherapeutic approaches that involve immune checkpoint inhibition. Therefore, it is important to consider the potential applications and

practical usefulness of oncolytic viruses in combination with other drugs and immunotherapies in targeting metastatic prostate cancer.[165]

Viruses typically work by infecting cells through the injection and incorporation of their DNA or RNA elements into the host's genetic machinery. Therefore, viruses can be used to target specific cancer cells that may be susceptible and vulnerable to such molecular modification. Cancer cells may not have protective qualities that allow for antiviral defense mechanisms. As such, the development of specific oncolytic viruses that target cancer cells, as opposed to healthy cells, can potentially induce antitumor responses. Furthermore, oncolytic viruses can potentially induce a lytic reaction in the cancer cells in which the multiplication and growth of progeny in the host cells eventually induces a burst effect. In conjunction with immunotherapeutic stimulation of the immune cells, this effect may help in synergistically eliminating tumor growth.[166]

Viral infection occurs because the virus recognizes its target cell based on cellular surface receptors. One oncolytic virus that was engineered from the herpes simplex virus is called T-VEC. T-VEC is able to recognize surface receptors such as the herpesvirus entry mediator, nectin-1, and nectin-2.[167] Engineering these oncolytic viruses can allow researchers to manipulate them in very specific ways. For example, current research involves the modification of their receptors for entry into cancer cells as well as restriction and deletion of certain viral protein expression via oncogenic promoter function.[168] In the context of prostate cancer, oncolytic viruses have been engineered to involve a PSA or a PSA promoter. Effectively, this leads to E1A expression and viral proliferation specific to prostate cancer cells.[169]

One study investigated the role of using virotherapy in inhibiting prostate cancer growth and metastasis. An oncolytic adenovirus, ZD55, was developed to target SATB1. ZD55-SATB1 inhibited both viability and invasion in prostate cancer cell lines.[170] Another study aimed to understand how oncolytic rhabdovirus VSV-GP functioned as a therapeutic modality in prostate cancer cells. VSV-GP infected 6 out of 7 prostate cancer cell lines and the mouse models achieved long-term remission. Several of the cancer cell lines developed resistance to interferon type I, suggesting a reduced antiviral responses.[171]

Targeted Antibodies

Another immunotherapy that has been shown to be effective in eliminating cancer cells involves targeted antibodies. Antibodies are proteins naturally produced from mature B cells. Antibodies can precisely target, bind to cell surfaces, and disrupt pathogenic cancer cell activity. After the antibodies bind to the cell surface of the cancer cells, immune cells can recognize them and induce different immune mechanisms.[172] Several different classifications of targeted antibodies include monoclonal antibodies, bispecific antibodies, and antibody–drug conjugation.

One study evaluated the efficacy of a specifically engineered antibody–drug conjugate called MEDI3726. This molecule contains an antibody called J591 that specifically binds to PSMA presented on cancer cell surfaces. Cancer cells typically have increased surface expression of PSMA making this a very attractive biotarget for tumor-mediated destruction. Also, MED13726 has another element called the pyrrolobenzodiazepine dimer tesirine that is, conjugated to the anti-PSMA antibody. As the antibody binds to PSMA express on cancer surface membranes, the entire MEDI3726 is engulfed into a vesicle and incorporated into the tumor cell. Pyrrolobenzodiazepine subsequently dissociated from the drug and binds to DNA and leads to cell death. MEDI3726 has shown potential as it led to specific cytotoxicity and antitumor activity in prostate cancer cell lines.[173]

Several studies have illustrated the efficacy of using monoclonal antibodies to target cancer cells. For example, it was found that N-cadherin expression is increased in metastatic tumors of individuals with CRPC. Administering monoclonal antibodies that targeted the ectodomain of N-cadherin effectively reduced proliferation and growth of prostate cancer cells in CRPC xenografts.[174] Another molecule upregulated in prostate cancer is TRIM24. A study revealed that silencing TRIM24 through a human monoclonal PSMA antibody-mediated TRIM24 small interfering RNA, drastically suppressed proliferation and invasion of the PSMA-positive CRPC cells.[175]

Furthermore, bispecific antibodies are used to recognize both the receptor on the surface of T cells as well as the specific tumor antigens. Thus, this antibody bridges the 2 cells together, which activates the T cells. TSAxCD28 bispecifics for targeting prostate cancer malignancy shows promising tolerance in immunocompetent mouse models and minor toxicity.[176] Also, 3E10-AR441 bispecific antibodies were developed and blocked genomic signaling of androgen receptor in LNCaP cells and inhibited prostate cancer cell growth under androgen-simulated conditions.[177] In xenograft models, AMG 160, a bispecific antibody that targets PSMA expressing prostate cancer cells and CD3 of T cells, caused significant decrease in tumor

growth.[178] Fig. 1 gives a representation of current and emerging therapies for metastatic prostate cancer. Further, in Table 1, we summarize some of the recent clinical trials for immunotherapies in metastatic prostate cancer.

PRECLINICAL MODELS FOR IMMUNOTHERAPY RESEARCH FOR METASTATIC PROSTATE CANCER

With a revolution in development of therapies that harness the immune response of tumors, the need to develop preclinical models that replicate the disease and can be used to test novel immunotherapies becomes extremely important. However, developing a preclinical model that can appropriately recapitulate the disease and help test the efficacy of immunotherapy is extremely challenging.

Cell Line Models

Several cell lines have been used to study to model immunotherapy for prostate cancer. The RM1, RM-9 murine prostate cancer cells were derived from ras and myc transformed mouse prostate reconstitution C57BL/6 mice model.[179,180] Syngeneic prostate cancer model from gp100-transfected murine RM1 cells has complete immunity and can be used to evaluate $CD8^+$ lymphocyte-mediated antitumor immunity.[181] The combination of nitroxoline and PD-1 blockade in RM9-Luc-PSA mouse model can significantly enhance antitumor immunity by increasing $CD44^+CD62L^+CD8^+$ memory T cells and reducing myeloid-derived suppressor cells.[182]

Transgenic adenocarcinoma mouse prostate (TRAMP)-C1, TRAMP-C2, and TRAMP-C3 were derived from a TRAMP model. Both TRAMP-C1 and TRAMP-C2 are tumorigenic, whereas TRAMP-C3 easily grows in vitro but does not form tumors.[183] Dexamethasone plus octreotide enhances the antitumor efficiency of docetaxel in a TRAMP-C1 prostate cancer model.[184] TRAMP-C1 cells showed increased expression of CXCL16 after radiation therapy, indicating a radiotherapy combination with immunotherapy.[185]

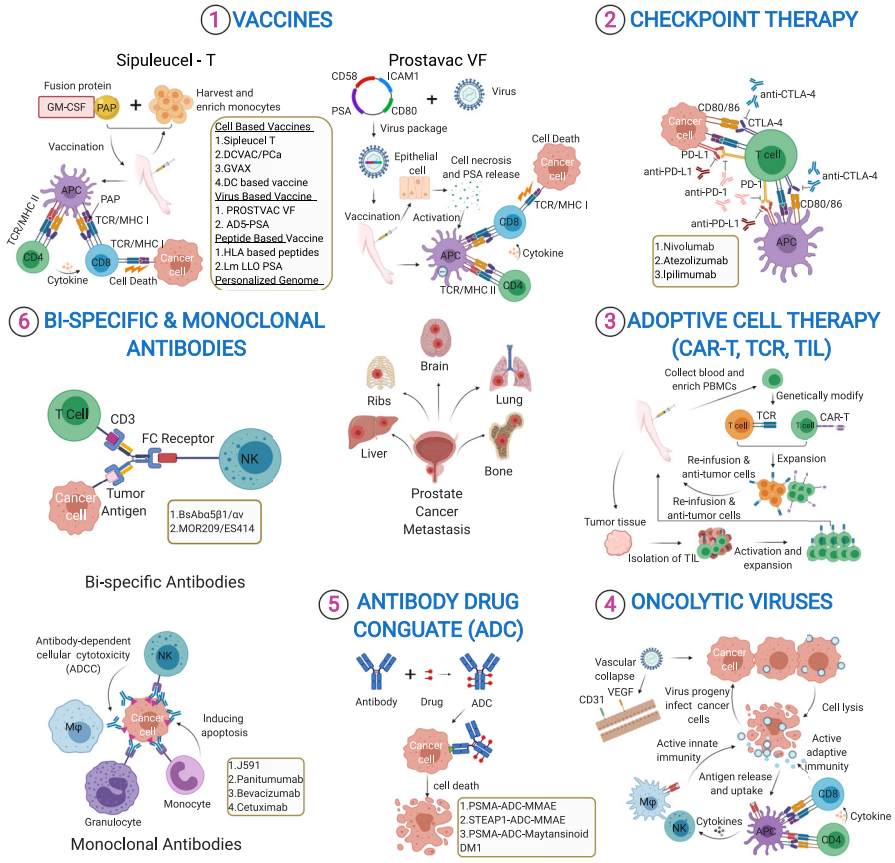

Fig. 1. Current and emerging therapies for metastatic prostate cancer. Clockwise from top left, vaccines, checkpoint therapy, adoptive cell therapy, oncolytic viruses, antibody–drug conjugate and bispecific and monoclonal antibodies.

Table 1
Current clinical trials testing novel immunotherapies for metastatic prostate cancer

Trial Number	Drug	Phase	Tumor Type	Mechanism of Action
NCT03834506[179]	Pembrolizumab + docetaxel	Phase III	Metastatic CRPC	Pembrolizumab targets the cellular pathway of proteins found on immune cells and cancer cells, known as PD-1/PD-L1.
NCT03834519[180]	Pembrolizumab + olaparib vs abiraterone acetate or enzalutamide	Phase III	Metastatic CRPC	Pembrolizumab targets the cellular pathway of proteins found on immune cells and cancer cells, known as PD-1/PD-L1.
NCT04262154[181]	Abiraterone, atezolizumab, lupron, and radiation therapy	Phase II	Metastatic hormone-sensitive prostate cancer	Atezolizumab is a monoclonal antibody of IgG1 isotype against the protein PD-L1.
NCT04104893[182]	Pembrolizumab	Phase II	Metastatic castrate resistant prostate cancer	Pembrolizumab targets the cellular pathway of proteins found on immune cells and cancer cells, known as PD-1/PD-L1.
NCT03693612[183]	GSK3359609 and tremelimumab	Phase II	Castrate-resistant prostate adenocarcinoma	GSK3359609 is an anti-Inducible T cell co-stimulator receptor agonist antibody. Tremelimumab is a fully human monoclonal antibody against CTLA-4.
NCT03575819[184]	FOR46	Phase I	Metastatic CRPC	FOR 46, an antibody–drug conjugate targeting CD46 protein.
NCT03577028[185]	HPN424	Phase I	Metastatic CRPC	HPN424 targets PSMA.
NCT02985957[186]	Nivolumab + ipilimumab or cabazitaxel	Phase II	Metastatic CRPC	Nivolumab blocks cancer cells' protective proteins against T cells.
NCT03972657[187]	REGN5678 + cemiplimab	Phase I/II	Metastatic CRPC	Cemiplimab targets PD-1 so it acts as a checkpoint inhibitor.
NCT04227275[188]	CART-PSMA-TGFβRDN	Phase I	Metastatic CRPC	Co-expression of TGFβRdn on PSMA-redirected CAR T cells increase T-cell proliferation and greater tumor eradication.
NCT03725761[189]	Sacituzumab govitecan	Phase II	Metastatic CRPC	Sacituzumab govitecan targets the Trop-2 receptor that helps the cancer to grow, divide, and spread.
NCT03554317[190]	Testosterone cypionate and nivolumab	Phase II	Metastatic CRPC	Nivolumab blocks cancer cells' protective proteins against T cells.
NCT03338790[191]	Nivolumab in combination with rucaparib, docetaxel, or enzalutamide	Phase II	Metastatic CRPC	Nivolumab blocks cancer cells' protective proteins against T cells.

(*continued on next page*)

Table 1
(*continued*)

Trial Number	Drug	Phase	Tumor Type	Mechanism of Action
NCT02601014[192]	Nivolumab and ipilimumab	Phase II	Metastatic CRPC	Ipilimumab turns off cancer mediated T-cell inhibition.
NCT03217747[193]	Avelumab, utomilumab, anti-OX40 antibody PF-04518600, and radiation therapy	Phase I/II	Metastatic CRPC	Utomilumab targets (CD137) to stimulate a more intense immune system attack on cancers.

Abbreviation: TGF, transforming growth factor.

Radiotherapy induced tumor growth delay in TRAMP-C1 prostate cancer model, but increased macrophages and dendritic cells and also causes upregulation of PD-1/PD-L1, $CD8^+$ T cells.[186] TRAMP-C1P3 cells have been derived from TRAMP-C1 and showed tumorigenic and metastatic to lymph nodes. It was demonstrated that Fms-like tyrosine kinase-3 ligand induced inflammatory cell infiltrate, including dendritic cells, macrophages, granulocytes and $CD4^+$ and $CD8^+$ T cells, significantly inhibited the growth of preexisting orthotopic TRAMP-C1P3 tumors and also the development of metastatic disease.[187]

IL-15 combination with CTLA-4 and PD-L1 blockade increased $CD8^+$ T cells, increased antitumor activity, suppressed tumor growth and prolonged the animal survival in a TRAMP-C2 prostate tumor model.[188] Imiquimod was showed to enhance antitumor activation of $CD8^+$ T cells, and imiquimod inhibits TRAMP-C2 cells in vivo and in vitro.[189,190] Enhanced IL-15Ralpha expression increased the $CD8^+$ T cells in TRAMP-C2 tumors, which resulted in inhibition of tumor growth.[191]

Syngeneic mouse models

Syngeneic mouse models, such as C57BL/6, BALB/C and FVB mice, are widely used preclinical models to study anticancer therapeutics. These models are immunocompetent and useful for testing immunotherapeutic agents. Carcinogens have been used to induce tumor formation in various strains of mice allowing researchers to understand and measure subsequent immune response and antitumor activity. This carcinogen-induced model presents a relatively useful level of genomic instability that warrants investigation of antitumor response.[192] Furthermore, genetic manipulation of the syngeneic model allows for the analysis of the effects of various biomarkers involving sensitivity or resistance responses.[193] It is very clear that owing to the ease of using these models and also because of high reproducibility, they are most commonly used preclinical models to test immunotherapies. However, these models lack the microenvironment and also the genomic heterogeneity seen in human cancer. Owing to the limited number of mice strains that researchers work with, there is a lack of interpatient genomic heterogeneity. Also, normal progression that defines the cancer growth in these models does not adequately represent the intrapatient genetic heterogeneity seen in normal individuals with metastatic prostate cancer. This posits a challenge for modeling the effects of cancer immunotherapy.[194] The other caveat is that in these syngeneic mice the implanted tumors are poorly differentiated and do not recapitulate the tumor evolution seen in human beings. Thus, the typical plasticity of the tumor evolution as well as the adaptability of the immune editing response by typical human beings is overtly absent in the syngeneic mouse models.[195] Finally, a crucial component of cancer development encompasses the surroundings microenvironment that can both facilitate and inhibit to tumor growth. Various tissue elements such as the microvasculature and stem cell progenitor populations naturally respond and adapt to tumor growth. However, these molecular components are largely absent in the subcutaneous implantation sites located in syngeneic models.[196]

Genetically engineered mouse models

A better understanding of the genetic subclasses of cancer have led to invention of genetically engineered mouse models having the specific genetic alterations incorporated and tissue specific tumor development. Tissue-specific promoters are predominantly used for driving either the expression of an oncogene or expression of recombinase

enzymes that drive the deletion of tumor suppressors. Viral oncogenes such as SV40 large T antigen,[197] or Kras and MYC[198] and so on, and tumor suppressors like PTEN and TP53 in prostate caner,[199] APC in colon cancer,[200] among others, are modeled. These models help in developing autochthonous cancer development and also the precancerous lesions such as intraepithelial neoplasia in the prostate.[201] Most important, targeting tissue specific promoters that alter the expression of normal tumor suppressor activity as well as oncogenic function engenders a long window for tumor development. Thus, there is ample time for immunotherapeutic intervention of gradual and adaptive immune responses.[202] Mimicking the complexity of oncogenesis and tumor burden seen in human patients is a goal with genetically engineered mouse models. It is important to consider the effects that increased mutational rates have on the immune system. Genetically altering tissue-specific genes leads to this increase mutational response, thus promoting the formation and existence of neoantigens. Neoantigens are recognized by cytotoxic T cells for targeted tumor destruction, which can provide helpful clues as to how to develop immunotherapeutic vaccines.[202]

Studies in mouse models could potentially aid in clinical trial design and expected outcome. One of the mouse models that has been widely studied to determine immunologic response in the TRAMP model (transgenic adenocarcinoma of the mouse prostate model).

Patient-derived xenograft

Human xenograft models are one of the oldest models for evaluating cytotoxic therapies against cancer. These models have priory advantage in the evaluation of antitumor efficacy. The hosts include athymic nude or severe combined immunodeficiency (SCID) animals.[203]

Athymic nude mice have neutrophils and dendritic cells, B cells, and natural killer cells, many aspects of the immune response, although they lack normal thymic development and are deficient in T-cell function. SCID mice lack a DNA-dependent protein kinase, which is essential for T-cell and B-cell development. Therefore, athymic nude mice were good for engraftment of human cancer cell lines, whereas the NOD/SCID mice are sufficient for engraftment of primary human tumors.[204,205] NOD/SCID mice demonstrated PD-1 targeted immunotherapy inhibited both cell line–derived xenograft and patient-derived xenograft tumor growth, indicating an important preclinical immunotherapy model for research.[206]

Humanized tumor models

Humanized tumor models depart from the previous models in the sense that they are the most relevant and representative of the cancer growth in humans. Various humanized mice models are available including humanized $CD34^+$ mouse models, humanized PBMC mouse models, and knock-in humanized mouse models.[207] These models are vital in the study of immune responses because they use patient derived xenografts, which contained human tumor tissue. The human tumor tissue is implanted into these mice models that exhibit an intact humanized immune system. They resemble human tissue in the sense that they accurately create the complexity associated with genetic heterogeneity as well as the tissue microenvironment.[208] In the humanized $CD34^+$ mouse model, the mice are initially irradiated to destroy the host immune system. Subsequently, they are injected or reconstituted with umbilical cord human $CD34^+$ cells. The development of the human immune system is monitored over 12 to 15 weeks and then the effects of the immune response to engrafted human patient-derived xenograft tumor is analyzed.[209] Recent studies have shown the efficacy of the $CD34^+$ humanized mice and positive antitumor responses in the regression of human tumor xenograft.[206] Another model, the humanized PBMC mouse models, is usually performed owing to its very short-term and robust reaction. This mouse model can be used in immunocompromised mice, to reconstitute the human immune system using peripheral blood mononuclear cells. Subcutaneous or orthotopic implantation of patient-derived xenograft or cell line derived xenograft can then be used to assess antitumor response. One of the problems with this model is that it involves a quick engraftment, which limits the time for observation of T-cell immune modulation, as well as overall antitumor activity.[210]

FUTURE DIRECTIONS: COMBINATION THERAPY OF VACCINES AND CHECKPOINT INHIBITORS, WITH MOLECULARLY DRIVEN APPROACHES

The introduction of immunotherapy to cancer treatment has brought a revolutionary change in the treatment for patients with cancer. As results of studies involving single agent immunotherapies for prostate cancer are coming out, there is a clear need for combinatorial approaches to evoke immune responses in patients with prostate cancer. Currently, there are numerous clinical trials of combined immunotherapy that are ongoing for prostate cancer. As we start to see the results

from these studies as well as new therapeutics, we will learn novel methods to improve the benefits from immunotherapies for prostate cancer.

Future research directions of prostate cancer immunotherapy are mainly focus on identifying molecular mechanism of immune resistance and developing combination therapies. To develop beneficial combination immunotherapies that show promising clinical outcomes, it is important to investigate their efficacy using complex mouse models. The humanized tumor models can prove to be useful because they are used in the study of immune responses that use patient derived xenografts, which contain human tumor tissue. Mimicking the complexity of the human tumor tissue, genetic heterogeneity, and microenvironment through these mouse models, is a vital step in improved treatment outcomes. Personalized therapies combining genomically targeted therapies (gene and cell therapies), with approaches to alleviate immune response is the future direction for metastatic diseases.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Avery Davis, Department of Urology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, for help with the figure of this manuscript. The authors wish to acknowledge funding support from The Arthur M. Blank Family Foundation to AT. The Figure, either in part or whole, was exported under paid subscription and created with Biorender.com.

DISCLOSURE

No competing financial and/or non-financial interests.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7–30. https://doi.org/10.3322/caac.21590.
2. Culp MB, Soerjomataram I, Efstathiou JA, et al. Recent global patterns in prostate cancer incidence and mortality rates. Eur Urol 2020;77:38–52. https://doi.org/10.1016/j.eururo.2019.08.005.
3. Baig FA, Hamid A, Mirza T, et al. Ductal and acinar adenocarcinoma of prostate: morphological and immunohistochemical characterization. Oman Med J 2015;30:162–6. https://doi.org/10.5001/omj.2015.36.
4. Miyoshi Y, Uemura H, Kitami K, et al. Neuroendocrine differentiated small cell carcinoma presenting as recurrent prostate cancer after androgen deprivation therapy. BJU Int 2001;88:982–3. https://doi.org/10.1046/j.1464-4096.2001.00936.x.
5. Shah RB, Mehra R, Chinnaiyan AM, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209–16. https://doi.org/10.1158/0008-5472.CAN-04-2442.
6. Tanaka M, Suzuki Y, Takaoka K, et al. Progression of prostate cancer to neuroendocrine cell tumor. Int J Urol 2001;8:431–6. https://doi.org/10.1046/j.1442-2042.2001.00347.x [discussion: 437].
7. Turbat-Herrera EA, Herrera GA, Gore I, et al. Neuroendocrine differentiation in prostatic carcinomas. A retrospective autopsy study. Arch Pathol Lab Med 1988;112:1100–5.
8. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25. https://doi.org/10.1016/j.cell.2015.10.025.
9. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22. https://doi.org/10.1016/j.ccr.2010.05.026.
10. Tomlins SA, Bjartell A, Chinnaiyan AM, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol 2009;56:275–86. https://doi.org/10.1016/j.eururo.2009.04.036.
11. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8. https://doi.org/10.1126/science.1117679.
12. Barbieri CE, Baca SC, Lawrence MS, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9. https://doi.org/10.1038/ng.2279.
13. Gasi Tandefelt D, Boormans J, Hermans K, et al. ETS fusion genes in prostate cancer. Endocr Relat Cancer 2014;21:R143–52. https://doi.org/10.1530/ERC-13-0390.
14. Nam RK, Sugar L, Yang W, et al. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007;97:1690–5. https://doi.org/10.1038/sj.bjc.6604054.
15. Geng C, He B, Xu L, et al. Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. Proc Natl Acad Sci U S A 2013;110:6997–7002. https://doi.org/10.1073/pnas.1304502110.
16. Yoshimoto M, Cunha IW, Coudry RA, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. Br J Cancer 2007;97:678–85. https://doi.org/10.1038/sj.bjc.6603924.

17. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;162:454. https://doi.org/10.1016/j.cell.2015.06.053.
18. Ramaswamy S, Nakamura N, Vazquez F, et al. Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 1999;96:2110–5. https://doi.org/10.1073/pnas.96.5.2110.
19. Shoag J, Barbieri CE. Clinical variability and molecular heterogeneity in prostate cancer. Asian J Androl 2016;18:543–8. https://doi.org/10.4103/1008-682X.178852.
20. Mu P, Zhang Z, Benelli M, et al. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. Science 2017;355:84–8. https://doi.org/10.1126/science.aah4307.
21. Ku SY, Rosario S, Wang Y, et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. Science 2017;355:78–83. https://doi.org/10.1126/science.aah4199.
22. Simmons MN, Berglund RK, Jones JS. A practical guide to prostate cancer diagnosis and management. Cleve Clin J Med 2011;78:321–31. https://doi.org/10.3949/ccjm.78a.10104.
23. Tomita N, Soga N, Ogura Y, et al. High-dose radiotherapy with helical tomotherapy and long-term androgen deprivation therapy for prostate cancer: 5-year outcomes. J Cancer Res Clin Oncol 2016;142:1609–19. https://doi.org/10.1007/s00432-016-2173-9.
24. Preisser F, Mazzone E, Knipper S, et al. Effect of external beam radiotherapy on second primary cancer risk after radical prostatectomy. Can Urol Assoc J 2019. https://doi.org/10.5489/cuaj.6087.
25. D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA 1998;280:969–74. https://doi.org/10.1001/jama.280.11.969.
26. Graefen M, Beyer B, Schlomm T. Outcome of radical prostatectomy: is it the approach or the surgical expertise? Eur Urol 2014;66:457–8. https://doi.org/10.1016/j.eururo.2013.12.010.
27. Bass R, Fleshner N, Finelli A, et al. Oncologic and functional outcomes of partial gland ablation with high intensity focused ultrasound for localized prostate cancer. J Urol 2019;201:113–9. https://doi.org/10.1016/j.juro.2018.07.040.
28. Shim M, Bang WJ, Oh CY, et al. Effectiveness of three different luteinizing hormone-releasing hormone agonists in the chemical castration of patients with prostate cancer: Goserelin versus triptorelin versus leuprolide. Investig Clin Urol 2019;60:244–50. https://doi.org/10.4111/icu.2019.60.4.244.
29. Hahn AW, Higano CS, Taplin ME, et al. Metastatic castration-sensitive prostate cancer: optimizing patient selection and treatment. Am Soc Clin Oncol Educ Book 2018;38:363–71. https://doi.org/10.1200/EDBK_200967.
30. Cornford P, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. Eur Urol 2017;71:630–42. https://doi.org/10.1016/j.eururo.2016.08.002.
31. Lin DW, Shih MC, Aronson W, et al. Veterans Affairs Cooperative Studies Program Study #553: chemotherapy after prostatectomy for high-risk prostate carcinoma: a phase III randomized study. Eur Urol 2020;77:563–72. https://doi.org/10.1016/j.eururo.2019.12.020.
32. Cursano MC, Iuliani M, Casadei C, et al. Combination radium-223 therapies in patients with bone metastases from castration-resistant prostate cancer: a review. Crit Rev Oncol Hematol 2020;146:102864. https://doi.org/10.1016/j.critrevonc.2020.102864.
33. Li K, Zhan W, Chen Y, et al. Docetaxel and doxorubicin codelivery by nanocarriers for synergistic treatment of prostate cancer. Front Pharmacol 2019;10:1436. https://doi.org/10.3389/fphar.2019.01436.
34. Hammer S, Hagemann UB, Zitzmann-Kolbe S, et al. Preclinical efficacy of a PSMA-targeted thorium-227 conjugate (PSMA-TTC), a targeted alpha therapy for prostate cancer. Clin Cancer Res 2019. https://doi.org/10.1158/1078-0432.CCR-19-2268.
35. DeWeerdt S. Bacteriology: a caring culture. Nature 2013;504:S4–5. https://doi.org/10.1038/504S4a.
36. Klener P Jr, Otahal P, Lateckova L, et al. Immunotherapy approaches in cancer treatment. Curr Pharm Biotechnol 2015;16:771–81. https://doi.org/10.2174/1389201016666150619114554.
37. Burnet FM. Immunological aspects of malignant disease. Lancet 1967;1:1171–4. https://doi.org/10.1016/s0140-6736(67)92837-1.
38. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001;2:293–9. https://doi.org/10.1038/86297.
39. Romero P, Valmori D, Pittet MJ, et al. Antigenicity and immunogenicity of Melan-A/MART-1 derived peptides as targets for tumor reactive CTL in human melanoma. Immunol Rev 2002;188:81–96. https://doi.org/10.1034/j.1600-065x.2002.18808.x.
40. Kaplan DH, Shankaran V, Dighe AS, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice.

Proc Natl Acad Sci U S A 1998;95:7556–61. https://doi.org/10.1073/pnas.95.13.7556.
41. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17:4550–7. https://doi.org/10.1158/1078-0432.CCR-11-0116.
42. Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 1985;313:1485–92. https://doi.org/10.1056/NEJM198512053132327.
43. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999;17:2105–16. https://doi.org/10.1200/JCO.1999.17.7.2105.
44. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7. https://doi.org/10.1038/256495a0.
45. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997;90:2188–95.
46. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994;83:435–45.
47. Eshhar Z, Waks T, Gross G, et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci U S A 1993;90:720–4. https://doi.org/10.1073/pnas.90.2.720.
48. Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood 2010;116:4099–102. https://doi.org/10.1182/blood-2010-04-281931.
49. Brentjens RJ, Riviere I, Park JH, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 2011;118:4817–28. https://doi.org/10.1182/blood-2011-04-348540.
50. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013;368:1509–18. https://doi.org/10.1056/NEJMoa1215134.
51. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507–17. https://doi.org/10.1056/NEJMoa1407222.
52. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med 2017;377:2545–54. https://doi.org/10.1056/NEJMoa1708566.
53. Pehlivan KC, Duncan BB, Lee DW. CAR-T cell therapy for acute lymphoblastic leukemia: transforming the treatment of relapsed and refractory disease. Curr Hematol Malig Rep 2018;13:396–406. https://doi.org/10.1007/s11899-018-0470-x.
54. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 Anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. Mol Ther 2017;25:285–95. https://doi.org/10.1016/j.ymthe.2016.10.020.
55. McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9. https://doi.org/10.1126/science.aaf1490.
56. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015;350:207–11. https://doi.org/10.1126/science.aad0095.
57. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189–99. https://doi.org/10.1056/NEJMoa1406498.
58. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013;31:e439–42. https://doi.org/10.1200/JCO.2012.47.7521.
59. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–13. https://doi.org/10.1126/science.aan6733.
60. Domingo E, Freeman-Mills L, Rayner E, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 2016;1:207–16. https://doi.org/10.1016/S2468-1253(16)30014-0.
61. Eggink FA, Van Gool IC, Leary A, et al. Immunological profiling of molecularly classified high-risk endometrial cancers identifies POLE-mutant and microsatellite unstable carcinomas as candidates for checkpoint inhibition. Oncoimmunology 2017;6:e1264565. https://doi.org/10.1080/2162402X.2016.1264565.
62. Lim HW, Hillsamer P, Banham AH, et al. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. J Immunol 2005;175:4180–3. https://doi.org/10.4049/jimmunol.175.7.4180.
63. Iikuni N, Lourenco EV, Hahn BH, et al. Cutting edge: regulatory T cells directly suppress B cells in systemic lupus erythematosus. J Immunol

2009;183:1518–22. https://doi.org/10.4049/jimmunol.0901163.
64. Xu A, Liu Y, Chen W, et al. TGF-beta-induced regulatory T cells directly suppress B cell responses through a noncytotoxic mechanism. J Immunol 2016;196:3631–41. https://doi.org/10.4049/jimmunol.1501740.
65. Zhao DM, Thornton AM, DiPaolo RJ, et al. Activated CD4+CD25+ T cells selectively kill B lymphocytes. Blood 2006;107:3925–32. https://doi.org/10.1182/blood-2005-11-4502.
66. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424. https://doi.org/10.3322/caac.21492.
67. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs-part A: renal, penile, and testicular tumours. Eur Urol 2016;70:93–105. https://doi.org/10.1016/j.eururo.2016.02.029.
68. Noessner E, Brech D, Mendler AN, et al. Intratumoral alterations of dendritic-cell differentiation and CD8(+) T-cell anergy are immune escape mechanisms of clear cell renal cell carcinoma. Oncoimmunology 2012;1:1451–3. https://doi.org/10.4161/onci.21356.
69. Itsumi M, Tatsugami K. Immunotherapy for renal cell carcinoma. Clin Dev Immunol 2010;2010:284581. https://doi.org/10.1155/2010/284581.
70. Minasian LM, Motzer RJ, Gluck L, et al. Interferon alfa-2a in advanced renal cell carcinoma: treatment results and survival in 159 patients with long-term follow-up. J Clin Oncol 1993;11:1368–75. https://doi.org/10.1200/JCO.1993.11.7.1368.
71. Fyfe G, Fisher RI, Rosenberg SA, et al. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995;13:688–96. https://doi.org/10.1200/JCO.1995.13.3.688.
72. McDermott DF, Regan MM, Clark JI, et al. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J Clin Oncol 2005;23:133–41. https://doi.org/10.1200/JCO.2005.03.206.
73. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015;27:450–61. https://doi.org/10.1016/j.ccell.2015.03.001.
74. Yang JC, Hughes M, Kammula U, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother 2007;30:825–30. https://doi.org/10.1097/CJI.0b013e318156e47e.
75. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54. https://doi.org/10.1056/NEJMoa1200690.
76. Wallin JJ, Bendell JC, Funke R, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun 2016;7:12624. https://doi.org/10.1038/ncomms12624.
77. Elamin YY, Rafee S, Toomey S, et al. Immune effects of bevacizumab: killing two birds with one stone. Cancer Microenviron 2015;8:15–21. https://doi.org/10.1007/s12307-014-0160-8.
78. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med 2018;24:749–57. https://doi.org/10.1038/s41591-018-0053-3.
79. Rini BI, Powles T, Atkins MB, 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. https://doi.org/10.1016/S0140-6736(19)30723-8.
80. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380:1103–15. https://doi.org/10.1056/NEJMoa1816047.
81. Atkins MB, Plimack ER, Puzanov I, et al. Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomised, open-label, dose-finding, and dose-expansion phase 1b trial. Lancet Oncol 2018;19:405–15. https://doi.org/10.1016/S1470-2045(18)30081-0.
82. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380:1116–27. https://doi.org/10.1056/NEJMoa1816714.
83. Shelley MD, Kynaston H, Court J, et al. A systematic review of intravesical bacillus Calmette-Guerin plus transurethral resection vs transurethral resection alone in Ta and T1 bladder cancer. BJU Int 2001;88:209–16. https://doi.org/10.1046/j.1464-410x.2001.02306.x.
84. Bohle A, Jocham D, Bock PR. Intravesical bacillus Calmette-Guerin versus mitomycin C for superficial bladder cancer: a formal meta-analysis of comparative studies on recurrence and toxicity. J Urol 2003;169:90–5. https://doi.org/10.1097/01.ju.0000039680.90768.b3.
85. Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 1976;116:180–3. https://doi.org/10.1016/s0022-5347(17)58737-6.

86. Lamm DL, Thor DE, Harris SC, et al. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol 1980;124:38–40. https://doi.org/10.1016/s0022-5347(17)55282-9.
87. Lamm DL, Thor DE, Stogdill VD, et al. Bladder cancer immunotherapy. J Urol 1982;128:931–5. https://doi.org/10.1016/s0022-5347(17)53283-8.
88. Brosman SA. Experience with bacillus Calmette-Guerin in patients with superficial bladder carcinoma. J Urol 1982;128:27–30. https://doi.org/10.1016/s0022-5347(17)52736-6.
89. Schellhammer PF, Ladaga LE, Fillion MB. Bacillus Calmette-Guerin for superficial transitional cell carcinoma of the bladder. J Urol 1986;135:261–4. https://doi.org/10.1016/s0022-5347(17)45603-5.
90. Martinez-Pineiro JA, Jimenez Leon J, Martinez-Pineiro L, et al. Bacillus Calmette-Guerin versus doxorubicin versus thiotepa: a randomized prospective study in 202 patients with superficial bladder cancer. J Urol 1990;143:502–6. https://doi.org/10.1016/s0022-5347(17)40002-4.
91. Lamm DL, Blumenstein BA, Crawford ED, et al. A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette-Guerin for transitional-cell carcinoma of the bladder. N Engl J Med 1991;325:1205–9. https://doi.org/10.1056/NEJM199110243251703.
92. Witjes JA. Management of BCG failures in superficial bladder cancer: a review. Eur Urol 2006;49:790–7. https://doi.org/10.1016/j.eururo.2006.01.017.
93. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909–20. https://doi.org/10.1016/S0140-6736(16)00561-4.
94. Powles T, O'Donnell PH, Massard C, et al. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. JAMA Oncol 2017;3:e172411. https://doi.org/10.1001/jamaoncol.2017.2411.
95. Apolo AB, Infante JR, Balmanoukian A, et al. Avelumab, an anti-programmed death-ligand 1 antibody, in patients with refractory metastatic urothelial carcinoma: results from a multicenter, phase Ib study. J Clin Oncol 2017;35:2117–24. https://doi.org/10.1200/JCO.2016.71.6795.
96. Sharma P, Retz M, Siefker-Radtke A, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2017;18:312–22. https://doi.org/10.1016/S1470-2045(17)30065-7.
97. Bellmunt J, Bajorin DF. Pembrolizumab for advanced urothelial carcinoma. N Engl J Med 2017;376:2304. https://doi.org/10.1056/NEJMc1704612.
98. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515:558–62. https://doi.org/10.1038/nature13904.
99. Necchi A, Joseph RW, Loriot Y, et al. Atezolizumab in platinum-treated locally advanced or metastatic urothelial carcinoma: post-progression outcomes from the phase II IMvigor210 study. Ann Oncol 2017;28:3044–50. https://doi.org/10.1093/annonc/mdx518.
100. Sharma P, Callahan MK, Bono P, et al. Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. Lancet Oncol 2016;17:1590–8. https://doi.org/10.1016/S1470-2045(16)30496-X.
101. Ohyama C, Kojima T, Kondo T, et al. Nivolumab in patients with unresectable locally advanced or metastatic urothelial carcinoma: CheckMate 275 2-year global and Japanese patient population analyses. Int J Clin Oncol 2019;24:1089–98. https://doi.org/10.1007/s10147-019-01450-w.
102. Bellmunt J, de Wit R, Vaughn DJ, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017;376:1015–26. https://doi.org/10.1056/NEJMoa1613683.
103. Hanna NH, Einhorn LH. Testicular cancer–discoveries and updates. N Engl J Med 2014;371:2005–16. https://doi.org/10.1056/NEJMra1407550.
104. Galvez-Carvajal L, Sanchez-Munoz A, Ribelles N, et al. Targeted treatment approaches in refractory germ cell tumors. Crit Rev Oncol Hematol 2019;143:130–8. https://doi.org/10.1016/j.critrevonc.2019.09.005.
105. Menk AV, Scharping NE, Rivadeneira DB, et al. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med 2018;215:1091–100. https://doi.org/10.1084/jem.20171068.
106. Danaher P, Warren S, Lu R, et al. Pan-cancer adaptive immune resistance as defined by the tumor inflammation signature (TIS): results from the Cancer Genome Atlas (TCGA). J Immunother Cancer 2018;6:63. https://doi.org/10.1186/s40425-018-0367-1.
107. Kiniwa Y, Miyahara Y, Wang HY, et al. CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer. Clin Cancer Res 2007;13:6947–58. https://doi.org/10.1158/1078-0432.CCR-07-0842.
108. Kaur HB, Guedes LB, Lu J, et al. Association of tumor-infiltrating T-cell density with molecular subtype, racial ancestry and clinical outcomes in prostate cancer. Mod Pathol 2018;31:1539–52. https://doi.org/10.1038/s41379-018-0083-x.

109. Nardone V, Botta C, Caraglia M, et al. Tumor infiltrating T lymphocytes expressing FoxP3, CCR7 or PD-1 predict the outcome of prostate cancer patients subjected to salvage radiotherapy after biochemical relapse. Cancer Biol Ther 2016;17: 1213–20. https://doi.org/10.1080/15384047.2016.1235666.
110. Lundholm M, Hagglof C, Wikberg ML, et al. Secreted factors from colorectal and prostate cancer cells skew the immune response in opposite directions. Sci Rep 2015;5:15651. https://doi.org/10.1038/srep15651.
111. Mariathasan S, Turley SJ, Nickles D, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 2018; 554:544–8. https://doi.org/10.1038/nature25501.
112. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499: 214–8. https://doi.org/10.1038/nature12213.
113. Freedland SJ, Aronson WJ. Commentary on "Integrative clinical genomics of advanced prostate cancer". Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, Beltran H, Abida W, Bradley RK, Vinson J, Cao X, Vats P, Kunju LP, Hussain M, Feng FY, Tomlins SA, Cooney KA, Smith DC, Brennan C, Siddiqui J, Mehra R, Chen Y, Rathkopf DE, Morris MJ, Solomon SB, Durack JC, Reuter VE, Gopalan A, Gao J, Loda M, Lis RT, Bowden M, Balk SP, Gaviola G, Sougnez C, Gupta M, Yu EY, Mostaghel EA, Cheng HH, Mulcahy H, True LD, Plymate SR, Dvinge H, Ferraldeschi R, Flohr P, Miranda S, Zafeiriou Z, Tunariu N, Mateo J, Perez-Lopez R, Demichelis F, Robinson BD, Schiffman M, Nanus DM, Tagawa ST, Sigaras A, Eng KW, Elemento O, Sboner A, Heath EI, Scher HI, Pienta KJ, Kantoff P, de Bono JS, Rubin MA, Nelson PS, Garraway LA, Sawyers CL, Chinnaiyan AM.Cell. 21 May 2015;161(5):1215-1228. Urol Oncol 2017; 35:535. https://doi.org/10.1016/j.urolonc.2017.05.010.
114. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. N Engl J Med 2016;375:443–53. https://doi.org/10.1056/NEJMoa1603144.
115. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol 2014;11:24–37. https://doi.org/10.1038/nrclinonc.2013.208.
116. Mac Keon S, Ruiz MS, Gazzaniga S, et al. Dendritic cell-based vaccination in cancer: therapeutic implications emerging from murine models. Front Immunol 2015;6:243. https://doi.org/10.3389/fimmu.2015.00243.
117. Westdorp H, Skold AE, Snijer BA, et al. Immunotherapy for prostate cancer: lessons from responses to tumor-associated antigens. Front Immunol 2014;5:191. https://doi.org/10.3389/fimmu.2014.00191.
118. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411–22. https://doi.org/10.1056/NEJMoa1001294.
119. Ren R, Koti M, Hamilton T, et al. A primer on tumour immunology and prostate cancer immunotherapy. Can Urol Assoc J 2016;10:60–5. https://doi.org/10.5489/cuaj.3418.
120. Gilboa E. DC-based cancer vaccines. J Clin Invest 2007;117:1195–203. https://doi.org/10.1172/JCI31205.
121. Drake CG. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol 2010;10: 580–93. https://doi.org/10.1038/nri2817.
122. Harzstark AL, Small EJ. Immunotherapy for prostate cancer using antigen-loaded antigen-presenting cells: APC8015 (Provenge). Expert Opin Biol Ther 2007;7:1275–80. https://doi.org/10.1517/14712598.7.8.1275.
123. Simons JW, Sacks N. Granulocyte-macrophage colony-stimulating factor-transduced allogeneic cancer cellular immunotherapy: the GVAX vaccine for prostate cancer. Urol Oncol 2006;24:419–24. https://doi.org/10.1016/j.urolonc.2005.08.021.
124. Small EJ, Sacks N, Nemunaitis J, et al. Granulocyte macrophage colony-stimulating factor–secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin Cancer Res 2007;13: 3883–91. https://doi.org/10.1158/1078-0432.CCR-06-2937.
125. Higano CS, Corman JM, Smith DC, et al. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 2008; 113:975–84. https://doi.org/10.1002/cncr.23669.
126. Fernandez-Garcia EM, Vera-Badillo FE, Perez-Valderrama B, et al. Immunotherapy in prostate cancer: review of the current evidence. Clin Transl Oncol 2015;17:339–57. https://doi.org/10.1007/s12094-014-1259-6.
127. Madan RA, Arlen PM, Mohebtash M, et al. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs 2009;18:1001–11. https://doi.org/10.1517/13543780902997928.
128. DiPaola RS, Chen YH, Bubley GJ, et al. A national multicenter phase 2 study of prostate-specific antigen (PSA) pox virus vaccine with sequential androgen ablation therapy in patients with PSA progression: ECOG 9802. Eur Urol 2015;68: 365–71. https://doi.org/10.1016/j.eururo.2014.12.010.

129. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 2010;28:1099–105. https://doi.org/10.1200/JCO.2009.25.0597.
130. Gulley JL, Borre M, Vogelzang NJ, et al. Phase III Trial of PROSTVAC in asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. J Clin Oncol 2019;37:1051–61. https://doi.org/10.1200/JCO.18.02031.
131. Martins KA, Bavari S, Salazar AM. Vaccine adjuvant uses of poly-IC and derivatives. Expert Rev Vaccin 2015;14:447–59. https://doi.org/10.1586/14760584.2015.966085.
132. McCartney S, Vermi W, Gilfillan S, et al. Distinct and complementary functions of MDA5 and TLR3 in poly(I:C)-mediated activation of mouse NK cells. J Exp Med 2009;206:2967–76. https://doi.org/10.1084/jem.20091181.
133. Zhang Z, Kim T, Bao M, et al. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. Immunity 2011;34:866–78. https://doi.org/10.1016/j.immuni.2011.03.027.
134. Lipson EJ, Drake CG. Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. Clin Cancer Res 2011;17:6958–62. https://doi.org/10.1158/1078-0432.CCR-11-1595.
135. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol 2002;3:611–8. https://doi.org/10.1038/ni0702-611.
136. Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013;24:1813–21. https://doi.org/10.1093/annonc/mdt107.
137. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15:700–12. https://doi.org/10.1016/S1470-2045(14)70189-5.
138. Pritchard CC, Morrissey C, Kumar A, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014;5:4988. https://doi.org/10.1038/ncomms5988.
139. Antonarakis ES, Piulats JM, Gross-Goupil M, et al. Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: multicohort, open-label phase II KEYNOTE-199 study. J Clin Oncol 2020;38:395–405. https://doi.org/10.1200/JCO.19.01638.
140. Pembrolizumab monotherapy is active in metastatic prostate cancer. Cancer Discov 2020;10:15. https://doi.org/10.1158/2159-8290.CD-RW2019-181.
141. Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med 2016;375:1767–78. https://doi.org/10.1056/NEJMra1514296.
142. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009;114:1537–44. https://doi.org/10.1182/blood-2008-12-195792.
143. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005;25:9543–53. https://doi.org/10.1128/MCB.25.21.9543-9553.2005.
144. Goswami S, Aparicio A, Subudhi SK. Immune checkpoint therapies in prostate cancer. Cancer J 2016;22:117–20. https://doi.org/10.1097/PPO.0000000000000176.
145. McDermott DF, Atkins MB. PD-1 as a potential target in cancer therapy. Cancer Med 2013;2:662–73. https://doi.org/10.1002/cam4.106.
146. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 2014;20:5064–74. https://doi.org/10.1158/1078-0432.CCR-13-3271.
147. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010;28:3167–75. https://doi.org/10.1200/JCO.2009.26.7609.
148. Smith KA, Gilbride KJ, Favata MF. Lymphocyte activating factor promotes T-cell growth factor production by cloned murine lymphoma cells. Nature 1980;287:853–5. https://doi.org/10.1038/287853a0.
149. Yunger S, Bar El A, Zeltzer LA, et al. Tumor-infiltrating lymphocytes from human prostate tumors reveal anti-tumor reactivity and potential for adoptive cell therapy. Oncoimmunology 2019;8:e1672494. https://doi.org/10.1080/2162402X.2019.1672494.
150. Dannull J, Diener PA, Prikler L, et al. Prostate stem cell antigen is a promising candidate for immunotherapy of advanced prostate cancer. Cancer Res 2000;60:5522–8.
151. Kiessling A, Schmitz M, Stevanovic S, et al. Prostate stem cell antigen: identification of immunogenic peptides and assessment of reactive CD8+ T cells in prostate cancer patients. Int J Cancer 2002;102:390–7. https://doi.org/10.1002/ijc.10713.

152. Matsueda S, Kobayashi K, Nonaka Y, et al. Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2(+) patients with hormone-refractory prostate cancer. Cancer Immunol Immunother 2004;53:479–89. https://doi.org/10.1007/s00262-003-0464-x.
153. Garcia-Hernandez Mde L, Gray A, Hubby B, et al. Prostate stem cell antigen vaccination induces a long-term protective immune response against prostate cancer in the absence of autoimmunity. Cancer Res 2008;68:861–9. https://doi.org/10.1158/0008-5472.CAN-07-0445.
154. Krupa M, Canamero M, Gomez CE, et al. Immunization with recombinant DNA and modified vaccinia virus Ankara (MVA) vectors delivering PSCA and STEAP1 antigens inhibits prostate cancer progression. Vaccine 2011;29:1504–13. https://doi.org/10.1016/j.vaccine.2010.12.016.
155. Ross S, Spencer SD, Holcomb I, et al. Prostate stem cell antigen as therapy target: tissue expression and in vivo efficacy of an immunoconjugate. Cancer Res 2002;62:2546–53.
156. Saffran DC, Raitano AB, Hubert RS, et al. Anti-PSCA mAbs inhibit tumor growth and metastasis formation and prolong the survival of mice bearing human prostate cancer xenografts. Proc Natl Acad Sci U S A 2001;98:2658–63. https://doi.org/10.1073/pnas.051624698.
157. Olafsen T, Gu Z, Sherman MA, et al. Targeting, imaging, and therapy using a humanized antiprostate stem cell antigen (PSCA) antibody. J Immunother 2007;30:396–405. https://doi.org/10.1097/CJI.0b013e318031b53b.
158. Lu J, Celis E. Recognition of prostate tumor cells by cytotoxic T lymphocytes specific for prostate-specific membrane antigen. Cancer Res 2002;62:5807–12.
159. Harada M, Matsueda S, Yao A, et al. Prostate-related antigen-derived new peptides having the capacity of inducing prostate cancer-reactive CTLs in HLA-A2+ prostate cancer patients. Oncol Rep 2004;12:601–7.
160. Schroers R, Shen L, Rollins L, et al. Human telomerase reverse transcriptase-specific T-helper responses induced by promiscuous major histocompatibility complex class II-restricted epitopes. Clin Cancer Res 2003;9:4743–55.
161. Kobayashi H, Omiya R, Sodey B, et al. Identification of naturally processed helper T-cell epitopes from prostate-specific membrane antigen using peptide-based in vitro stimulation. Clin Cancer Res 2003;9:5386–93.
162. Zuccolotto G, Fracasso G, Merlo A, et al. PSMA-specific CAR-engineered T cells eradicate disseminated prostate cancer in preclinical models. PLoS One 2014;9:e109427. https://doi.org/10.1371/journal.pone.0109427.
163. Ma Q, Gomes EM, Lo AS, et al. Advanced generation anti-prostate specific membrane antigen designer T cells for prostate cancer immunotherapy. Prostate 2014;74:286–96. https://doi.org/10.1002/pros.22749.
164. Zhang Q, Helfand BT, Carneiro BA, et al. Efficacy against human prostate cancer by prostate-specific membrane antigen-specific, transforming growth factor-beta insensitive genetically targeted CD8(+) T-cells derived from patients with metastatic castrate-resistant disease. Eur Urol 2018;73:648–52. https://doi.org/10.1016/j.eururo.2017.12.008.
165. Lee P, Gujar S. Potentiating prostate cancer immunotherapy with oncolytic viruses. Nat Rev Urol 2018;15:235–50. https://doi.org/10.1038/nrurol.2018.10.
166. Liu GB, Zhao L, Zhang L, et al. Virus, oncolytic virus and human prostate cancer. Curr Cancer Drug Targets 2017;17:522–33. https://doi.org/10.2174/1568009616666161216095308.
167. Kohlhapp FJ, Zloza A, Kaufman HL. Talimogene laherparepvec (T-VEC) as cancer immunotherapy. Drugs Today (Barc) 2015;51:549–58. https://doi.org/10.1358/dot.2015.51.9.2383044.
168. Jhawar SR, Thandoni A, Bommareddy PK, et al. Oncolytic viruses-natural and genetically engineered cancer immunotherapies. Front Oncol 2017;7:202. https://doi.org/10.3389/fonc.2017.00202.
169. DeWeese TL, van der Poel H, Li S, et al. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. Cancer Res 2001;61:7464–72.
170. Mao LJ, Zhang J, Liu N, et al. Oncolytic virus carrying shRNA targeting SATB1 inhibits prostate cancer growth and metastasis. Tumour Biol 2015;36:9073–81. https://doi.org/10.1007/s13277-015-3658-x.
171. Urbiola C, Santer FR, Petersson M, et al. Oncolytic activity of the rhabdovirus VSV-GP against prostate cancer. Int J Cancer 2018;143:1786–96. https://doi.org/10.1002/ijc.31556.
172. Wustemann T, Haberkorn U, Babich J, et al. Targeting prostate cancer: prostate-specific membrane antigen based diagnosis and therapy. Med Res Rev 2019;39:40–69. https://doi.org/10.1002/med.21508.
173. Cho S, Zammarchi F, Williams DG, et al. Antitumor activity of MEDI3726 (ADCT-401), a pyrrolobenzodiazepine antibody-drug conjugate targeting PSMA, in preclinical models of prostate cancer. Mol Cancer Ther 2018;17:2176–86. https://doi.org/10.1158/1535-7163.MCT-17-0982.
174. Tanaka H, Kono E, Tran CP, et al. Monoclonal antibody targeting of N-cadherin inhibits prostate

cancer growth, metastasis and castration resistance. Nat Med 2010;16:1414–20. https://doi.org/10.1038/nm.2236.
175. Shi SJ, Wang LJ, Han DH, et al. Therapeutic effects of human monoclonal PSMA antibody-mediated TRIM24 siRNA delivery in PSMA-positive castration-resistant prostate cancer. Theranostics 2019; 9:1247–63. https://doi.org/10.7150/thno.29884.
176. Waite JC, Wang B, Haber L, et al. Tumor-targeted CD28 bispecific antibodies enhance the antitumor efficacy of PD-1 immunotherapy. Sci Transl Med 2020;12. https://doi.org/10.1126/scitranslmed.aba2325.
177. Goicochea NL, Garnovskaya M, Blanton MG, et al. Development of cell-penetrating bispecific antibodies targeting the N-terminal domain of androgen receptor for prostate cancer therapy. Protein Eng Des Sel 2017;30:785–93. https://doi.org/10.1093/protein/gzx058.
178. Bailis J, Deegen P, Thomas O, et al. Preclinical evaluation of AMG 160, a next-generation bispecific T cell engager (BiTE) targeting the prostate-specific membrane antigen PSMA for metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 2019;37:301. https://doi.org/10.1200/JCO.2019.37.7_suppl.301.
179. Thompson TC, Southgate J, Kitchener G, et al. Multistage carcinogenesis induced by ras and myc oncogenes in a reconstituted organ. Cell 1989;56:917–30. https://doi.org/10.1016/0092-8674(89)90625-9.
180. Baley PA, Yoshida K, Qian W, et al. Progression to androgen insensitivity in a novel in vitro mouse model for prostate cancer. J Steroid Biochem Mol Biol 1995;52:403–13. https://doi.org/10.1016/0960-0760(95)00001-g.
181. Yeon A, Wang Y, Su S, et al. Syngeneic murine model for prostate cancer using RM1 cells transfected with gp100. Prostate 2020;80:424–31. https://doi.org/10.1002/pros.23957.
182. Xu N, Huang L, Li X, et al. The novel combination of nitroxoline and PD-1 blockade, exerts a potent antitumor effect in a mouse model of prostate cancer. Int J Biol Sci 2019;15:919–28. https://doi.org/10.7150/ijbs.32259.
183. Foster BA, Gingrich JR, Kwon ED, et al. Characterization of prostatic epithelial cell lines derived from transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Cancer Res 1997;57:3325–30.
184. Dalezis P, Geromichalos GD, Trafalis DT, et al. Dexamethasone plus octreotide regimen increases anticancer effects of docetaxel on TRAMP-C1 prostate cancer model. In Vivo 2012;26:75–86.
185. Matsumura S, Demaria S. Up-regulation of the pro-inflammatory chemokine CXCL16 is a common response of tumor cells to ionizing radiation. Radiat Res 2010;173:418–25. https://doi.org/10.1667/RR1860.1.
186. Philippou Y, Sjoberg HT, Murphy E, et al. Impacts of combining anti-PD-L1 immunotherapy and radiotherapy on the tumour immune microenvironment in a murine prostate cancer model. Br J Cancer 2020. https://doi.org/10.1038/s41416-020-0956-x.
187. Ciavarra RP, Holterman DA, Brown RR, et al. Prostate tumor microenvironment alters immune cells and prevents long-term survival in an orthotopic mouse model following flt3-ligand/CD40-ligand immunotherapy. J Immunother 2004;27:13–26. https://doi.org/10.1097/00002371-200401000-00002.
188. Yu P, Steel JC, Zhang M, et al. Simultaneous inhibition of two regulatory T-cell subsets enhanced Interleukin-15 efficacy in a prostate tumor model. Proc Natl Acad Sci U S A 2012;109:6187–92. https://doi.org/10.1073/pnas.1203479109.
189. Rechtsteiner G, Warger T, Osterloh P, et al. Cutting edge: priming of CTL by transcutaneous peptide immunization with imiquimod. J Immunol 2005; 174:2476–80. https://doi.org/10.4049/jimmunol.174.5.2476.
190. Han JH, Lee J, Jeon SJ, et al. In vitro and in vivo growth inhibition of prostate cancer by the small molecule imiquimod. Int J Oncol 2013;42: 2087–93. https://doi.org/10.3892/ijo.2013.1898.
191. Zhang M, Ju W, Yao Z, et al. Augmented IL-15Ralpha expression by CD40 activation is critical in synergistic CD8 T cell-mediated antitumor activity of anti-CD40 antibody with IL-15 in TRAMP-C2 tumors in mice. J Immunol 2012;188:6156–64. https://doi.org/10.4049/jimmunol.1102604.
192. Fantini D, Glaser AP, Rimar KJ, et al. A carcinogen-induced mouse model recapitulates the molecular alterations of human muscle invasive bladder cancer. Oncogene 2018;37:1911–25. https://doi.org/10.1038/s41388-017-0099-6.
193. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature 2009;461: 1071–8. https://doi.org/10.1038/nature08467.
194. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol 2010;5:51–75. https://doi.org/10.1146/annurev-pathol-121808-102113.
195. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331: 1565–70. https://doi.org/10.1126/science.1203486.
196. Bonnotte B, Gough M, Phan V, et al. Intradermal injection, as opposed to subcutaneous injection, enhances immunogenicity and suppresses tumorigenicity of tumor cells. Cancer Res 2003; 63:2145–9.
197. Greenberg NM, DeMayo F, Finegold MJ, et al. Prostate cancer in a transgenic mouse. Proc Natl

Acad Sci U S A 1995;92:3439–43. https://doi.org/10.1073/pnas.92.8.3439.
198. Sinn E, Muller W, Pattengale P, et al. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell 1987;49:465–75. https://doi.org/10.1016/0092-8674(87)90449-1.
199. Chen Z, Trotman LC, Shaffer D, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005;436:725–30. https://doi.org/10.1038/nature03918.
200. Shibata H, Toyama K, Shioya H, et al. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science 1997;278:120–3. https://doi.org/10.1126/science.278.5335.120.
201. Kaplan-Lefko PJ, Chen TM, Ittmann MM, et al. Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. Prostate 2003;55:219–37. https://doi.org/10.1002/pros.10215.
202. Liu J, Blake SJ, Yong MC, et al. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov 2016;6:1382–99. https://doi.org/10.1158/2159-8290.CD-16-0577.
203. Siegler EL, Wang P. Preclinical models in chimeric antigen receptor-engineered T-cell therapy. Hum Gene Ther 2018;29:534–46. https://doi.org/10.1089/hum.2017.243.
204. Puchalapalli M, Zeng X, Mu L, et al. NSG mice provide a better spontaneous model of breast cancer metastasis than Athymic (Nude) Mice. PLoS One 2016;11:e0163521. https://doi.org/10.1371/journal.pone.0163521.
205. Walsh NC, Kenney LL, Jangalwe S, et al. Humanized mouse models of clinical disease. Annu Rev Pathol 2017;12:187–215. https://doi.org/10.1146/annurev-pathol-052016-100332.
206. Wang M, Yao LC, Cheng M, et al. Humanized mice in studying efficacy and mechanisms of PD-1-targeted cancer immunotherapy. FASEB J 2018;32:1537–49. https://doi.org/10.1096/fj.201700740R.
207. Brehm MA, Cuthbert A, Yang C, et al. Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation. Clin Immunol 2010;135:84–98. https://doi.org/10.1016/j.clim.2009.12.008.
208. DeRose YS, Wang G, Lin YC, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med 2011;17:1514–20. https://doi.org/10.1038/nm.2454.
209. Gonzalez L, Strbo N, Podack ER. Humanized mice: novel model for studying mechanisms of human immune-based therapies. Immunol Res 2013;57:326–34. https://doi.org/10.1007/s12026-013-8471-2.
210. King MA, Covassin L, Brehm MA, et al. Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host major histocompatibility complex. Clin Exp Immunol 2009;157:104–18. https://doi.org/10.1111/j.1365-2249.2009.03933.x.