Immunotherapy and Targeted Therapies in Advanced Castration Resistant Prostate Cancer

Joaquim Bellmunt and Irene Moreno

1 Introduction

Over the last decade, the management of PC has become increasingly complex and controversial for both early and advanced disease. Androgen deprivation therapy (ADT) remains a mainstay of treatment in a noncurative setting but progression to castration-resistant PC (CRPC), where the ADT is not anymore useful, eventually occurs. Exploring other therapeutics is key to further improving the quality and quantity of life of our patients.

In the last few years, cancer immunotherapy has changed the natural history and treatment strategies of a number of solid tumors, including melanoma, lung cancer, renal cell carcinoma, and bladder cancer. Immunotherapy is now becoming a mainstay in the management strategy for this type of patients. PC was historically not considered immunogenic in its nature, and first attempts to stimulate an immune response in the prostate cancer were unsuccessful [1, 2]. However, PC generates a variety of tumor-associated antigens, as PSA, prostatic acid phosphatase, and prostatic-specific membrane antigen, which are potentially capable of producing a clinical response through inducing immunogenicity [3]. In fact, PC was the first solid tumor to demonstrate improved survival with a cancer-specific vaccine [4], encouraging researchers to further explore immunotherapy in prostate cancer and other solid tumors.

In this chapter, we will start discussing the basic biology of PC, focusing on issues that relates to immune environment and immune response in PC to then outline some of the immunotherapy approaches that have been approved and the investigational ones that are currently being studied. We will emphasis on the immunologic biomarkers that can help us on the selection of patients. Finally, we will explore some others targeted therapies that are currently available for PC treatment.

2 Rationale

The concept that the immune system acts as a tumor suppressor was introduced in the early twentieth century by Ehrlich [5]. Since then, several studies have provided evidence supporting the role of immunity in cancer development, progression and suppression, conceptually under the term "immune surveillance" [6].

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In order to better understand immunotherapy, we will first briefly discuss the normal response of the human immune system. This system can be classified into subsystems, such as the innate immune system [7] versus the adaptive immune system [8], or humoral immunity versus cell-mediated immunity. Both divisions have been shown to be involved in tumor immune surveillance.

2.1 Innate Immune System

The innate response is usually triggered when foreign organisms or particles are identified by pattern recognition receptors [9] or when damaged or stressed cells send out alarm signals. Thus, innate immune cells are responsible for the initial response [10]. The main components of this type of immune response include macrophages, natural killer cells, and antigen-presenting cells. The macrophages are initially recruited and can be classified as pro-inflammatory M1 (CD68+) cells and anti-inflammatory M2 (CD163+) cells [11]. Inducers or inhibitors of these different types of macrophages are now targets of the new immuntherapeutic agents. In the cancer context the relationship between M1 and M2 cells can become unbalanced [12], resulting in a gain of M2 cells. A recent study reported that in localized PC, the prevalent macrophage phenotype was M1, whereas in PC with extracapsular extension, M2 macrophages were more frequently seen [13]. These findings, together with another observation of reduced infiltration of CD68+ macrophages, associated with higher clinical stage and lymph node positivity, indicate that reduced numbers of macrophages with cytotoxic capabilities parallel more aggressive disease [14].

Natural killer cells (NK) are the responsible of targeting tumor cells without prior sensitization. They recognize altered cells by detecting the loss of human leukocyte antigen (HLA) class I molecules (a change that is associated with injured cells) or by recognition of specific ligands (tumor associated antigens or TAAs) that are expressed by these altered cells [15]. In PC these include the serine protease prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), mucin-1 (MUC-1), prostate stem cell antigen (PSCA), and NY-ESO-1 [16]. Preclinical data show that PC cells induce the expression of inhibitory receptor (ILT2/LILRB1) and down-regulate the expression of activating receptors NKp46 (NCR1), CD16 (FCGR3) and NKG2D (KLRK1) by NK cells, thus preventing their recognition of tumor cells. Notably, blood levels of NKp46 also decrease in PC patients and are inversely correlated with levels of PSA, PC [17].

Antigen-presenting cells (macrophages and dendritic cells)(APC) are the link between the innate and adaptive immunity. The role of these professional antigen-presenting cells is to get ready the naïve T cells for being activated when contacting with foreign antigens.

Cancer employs numerous immune escape strategies such as down regulation of HLA class I antigens and beta-2 microglobulin to escape killing by cytotoxic T cells.

2.2 Adaptive Immune System

This division of the immune system is composed of T and B lymphocytes which develop highly specialized functions via cell surface or secreted effector molecules. The main effector cells in cancer immune response are the CD8+ cytotoxic T-lymphocytes. The TAAs (peptide fragments from the initial tumor cell destruction by innate effectors) can activate this type of lymphocytes, undergoing clonal expansion after that. CD4+ T cells (helper T cells) induce antibody production in B cells and activate macrophages [18]. CD4+ T cells can be divided in: Th1 (involved in intracellular immunity), Th2 (involved in extracellular-humoral immunity), Th17, and regulatory T cells. The last ones are able to suppress effector T cells in order to maintain immune tolerance [19]. CD8+ T cells constitutively express cytotoxic T-lymphocyte-associated protein 4-CTLA-4 (a well-known immunotherapy target [20, 21]).

T cells recognize antigens presented by the MHC on the surface of cancer cells through their T-cell receptor. Activation of T cells requires two signals: first antigens need to be presented on the setting of HLA receptor and second a signal delivered by the B7 stimulatory molecules in APC is required interacting with CD28 receptor on T cells. In order to maintain self-tolerance and prevent hyperactivation, there is a co-inhibitory signal that binds B7 with greater affinity, inactivating T cells-like CTAL-4. The interaction between CTLA-4 and the costimulatory molecules happens primarily in the priming phase of a T-cell response within lymph nodes. Activated T cells can also upregulate programmed cell death protein 1 (PD1), a cell surface receptor that is expressed on T cells and pro-B cells. The PD1 inhibitory receptor is expressed by T cells during long-term antigen exposure and results in negative regulation of T cells. Inflammatory signals in the tissues induce the expression of PD1 ligands, downregulate the activity of T cells binding PD1 in lymphocytes, and thus limit collateral tissue damage in the light effect her face of a T-cell response in peripheral tissues.

Regulatory T cells can be found in large proportions of tumor infiltrating lymphocytes (which has been associated with poor prognosis of certain cancers [22], including prostate cancer). Early studies reported that greater tumor infiltration of CD4+ T-reg cells can predict poorer prognosis [23] in PC, and a high tumor infiltration of forkhead box P3- (foxp3-) expressing cells (T-regs) was also found to correlate with higher baseline PSA levels [24]. This data suggest that therapeutic blockade of these cells may induce beneficial clinical responses.

2.3 Androgen Deprivation and Immune System Response

Early results in this field show that neoadjuvant androgen deprivation (before PC surgery) results in a CD4+ T cell infiltration into the gland [25]. Contrarily, the analysis of a postcastration PC tissue reveals a CD8+ T cell infiltration [26]. These findings are also observed in mice models, where it was found that androgen ablation decreases CD4+ T cell tolerance to a PC-associated antigen, showing that clonotypic CD4+ T cells could respond to specific vaccination after androgen deprivation but not in intact, tumor-bearing mice [27]. Moreover, androgen deprivation is related to an increase in the number of cells expressing the co stimulatory molecules B7.1 and B7.2, which are necessary for effective T cell activation [28]. According to these data hormone ablation may have an additive effect with immunotherapy, taking in consideration the timing of treatments (obtaining better results if the immunotherapy is given prior to castration) [29].

2.4 Tumor Immune Scape (Immunoediting)

As described in the beginning of the chapter, functional cancer immunosurveillance process indeed exists that acts as an extrinsic tumor suppressor. However, it has also become clear that the immune system can facilitate tumor progression, at least in part, by sculpting the immunogenic phenotype of tumors as they develop. The recognition that immunity plays a dual role in the complex interactions between tumors and the host prompted a refinement of the cancer immunosurveillance hypothesis into one termed "cancer immunoediting." Tumor cells are normally suppressed by the immune system, however, as part of tumor immunoediting, they sometimes gain properties to escape detection and present themselves as disease [5]. This modern hypothesis, first put forth by Schreiber, describes the three phases (elimination, equilibrium, and escape) where the balance between the tumor and the immune system is discussed. In the first stage, the immune system recognizes and eliminates the high immunogenic tumor cells by effectors such as NK cells or CD8+ T-lymphocytes. This can result in the selection of tumor cells with reduced immunogenicity and thus become resistant to immune effectors, leading the process to the equilibrium phase (where the elimination of tumor cells is balanced by the selection of less

immunogenic variants, known as functional dormancy) [30]. As tumor size increases, tumor-derived soluble factors help to modify the microenvironment causing several mechanisms of immune escape. Some of them are the increasing extracellular matrix that binds tumor antigens (reducing the amount of TAAs) or the attraction of immature DCs which inhibit T cell activation [31]. New immunological therapies try to force the tumor backs towards either the equilibrium phase or, in the best scenario, to the elimination stage (meaning a complete response of the disease).

Sipuleucel T is one potential example that immunoediting plays a role in the immunotherapy of prostate cancer. Despite a benefit seen in terms of overall survival, it has been quite worrisome as patient's tumors very rarely shrink on this treatment with few objective responses described.

If we think about the cancer immunoediting hypothesis, maybe what is happening is not elimination, but maybe the vaccine is just pushing patients back toward an equilibrium phase, where both tumor and an antitumor response are present, but neither one is really winning.

In conclusion, all these data show that PC remains an attractive target for immunotherapy. This type of treatment can also be potentially useful in the biochemical recurrence setting, where the immunosuppressive mechanisms (such as TReg cells, myeloid-derived suppressor cells) and transforming growth factor-β (TGFβ) [32], usually seen associated with an advanced tumor stage-, are expected to be at a minimum at this stage.

Another characteristic of PC which can predict a good response to immunotherapy is that it is a slowly progressing disease, allowing sufficient time for the immunologic response to be build [33]. In terms of a potential risk of adverse events with a prostate cancer-specific immunotherapy, we can take into account that the prostate is a nonessential organ for life, meaning that even if immunotherapy destroys normal prostatic tissue, it would not be life-threatening.

3 Approved Agents

3.1 Immunomodulating Properties of Standard ("Nonimmunotherapy-Based") Agents

It is now believed that many conventional treatments for prostate and others cancers have beneficial immunological effects, making combinatorial trials an attractive strategy. ADT, radiation therapy and chemotherapy (which was broadly viewed as immunosuppressive in the past), might to some extent boost an antitumor response, modulating immune cells and their milieu. For example, ADT may produce changes in the patient immune system and an additive effect with immunotherapy might be expected.

In the setting of chemotherapy and targeted therapies, multiple studies (both in murine and in human models) have shown that various agents (such as the VEGF TKI sunitinib, specific inhibitors of $BRAF^{V600E}$, gemcitabine, 5-fluorouracil or doxorrubicin-cyclophosphamide) can promote a more active anticancer immune environment by enhancing dendritic cell function and decreasing inhibitory T cell populations such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [34–39].

There are also some early studies with taxanes (widely used in advanced PC) that report their capability of modulation the immune system in tumor-bearing mice [40] and in human samples of nonsquamous cell carcinoma [41], breast cancer [42], or melanoma [43]. For example, in a phase II clinical trial [44] published in 2012, the levels of circulating MDSC were assessed in 41 women diagnosed with HER-2 neu-negative breast cancer in stages II-IIIa. They received three chemotherapeutic drugs: doxorubicin-cyclophosphamide followed by docetaxel every 3 weeks followed by NOV-002, a disodium glutathione disulfide. In this study, 15 out of 39 patients achieved a pCR. It was found that patients who achieved pathologic complete response (pCR) had lower levels of circulating MDSC ($Lin^{-}HLA\text{-}DR^{-}CD11b^{+}CD33^{+}$) in the blood compared to patients who did not achieve

pCR. The authors contended that MDSC suppression may increase the efficacy of chemotherapy regimens currently used in the clinic.

There is increasing evidence that radiation therapy may induce or help synergize immunotherapeutic effects on PC [45]. Evidence for an immunological effect of radiotherapy is provided by data showing that the tumoricidal effects of radiation require CD8+ T cells. It seems that the uptake of dying tumor cells by APCs plays an important role [46] where new antibody specificities appear following radiotherapy treatment [47], as well as the induction of a proinflammatory microenvironment by this type of treatment [48]. Radiation may modulate host immunity by increasing CD8+ effector T cells and dendritic cells at the radiation site; increasing antigen availability; inducing immune stimulating cytokines such as Type 1 interferon and chemokines and reducing immunosuppressive cell populations such as MDSCs [49–51]. Some recent work has also shown that HMGB1 (high mobility group box 1) released from dying tumor cells can function as a TLR4 agonist, activating APCs in either the tumor parenchyma or in the lymph nodes [33, 45].

It has been also described in case reports from several cancers [52] that radiation therapy may induce tumor cell death through a rare indirect out-of-field phenomenon described as the abscopal effect [53], in which distant metastatic lesions regress following radiation to an unrelated primary treatment field. The etiology of this scenario is not well known but evidence suggests that is immune mediated [54].

Identification of the optimal dose, fractionation regimens, and timing are an important issue to be planned in future clinical trials.

A study of TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice demonstrated optimal mitigation of tolerance with a tumor vaccine at 3–5 weeks following radiotherapy, when tumor burden is at its lowest [56].

Following these observations, there is a remarkable potential for synergistic combinations of radiation therapy with such immune-based agents. Several preclinical studies support this notion in terms of the antitumor response. This concept has been evaluated clinically in a randomized trial of men undergoing primary radiotherapy for PC [57], that will be described in Sect. 4.2.

3.2 Sipuleucel-T (Provenge)

Sipuleucel-T is an autologous cellular immunotherapy, approved in 2010 by the US Food and Drug Administration (FDA) for the treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant PC [58]. It has been shown to increase overall survival [59] and generate antigen-specific immune responses that correlate with increased overall survival [60]. Similar to traditional vaccines, cellular immunotherapy tries to engage the immune system by activating effector T cells and dampening immunosuppressive factors, facilitating the infiltration of lymphocytes into the tumor microenvironment. The concept of this type of treatment approach was originated in lymphoma, where antigen-loaded, autologous APCs showed clinical promise [61].

Sipuleucel-T is a personalized product that is individually manufactured for each patient with PC. First, leukopheresis is carried out, and monocytes are enriched in the leukopheresis product through density–gradient centrifugation. Autologous cells are cultured in vitro with a proprietary protein cassette (PA2024) that couples the vaccine target (prostatic acid phosphatase, PAP; chosen based on preclinical studies in a murine model [62]) to the granulocyte–macrophage colony-stimulating factor (GM-CSF), before intravenous administration. The infusion contains at least 50 million autologous activated CD54+ dendritic cells, and a variable number of T cells, B cells, natural killer cells, and others [63]. Treatment is repeated three times over 4–6 weeks [33, 64]. Once infused, it is thought that these autologous monocytes present the PAP antigen to host T cells (PAP-specific CD4+ and CD8+ T cells), resulting in the T-cell activation and proliferation [65] (Fig. 1).

An analysis of culture during the manufacture process showed an increase in APC activation

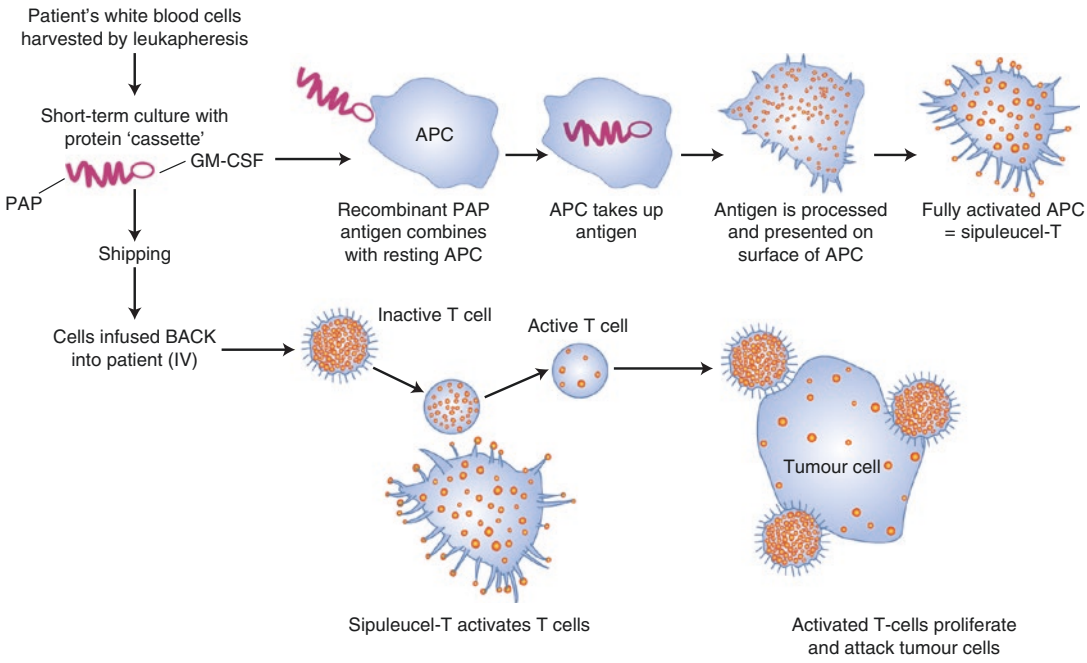

Fig. 1 The predicted mechanism of action and stages of Sipuleucel-T treatment for patients with castration-resistant prostate cancer (CRPC)

cytokines (macrophage inflammatory protein (MIP)-1a and -1b; interleukin (IL)-1a, IL-23), T cell activation markers (IL-2, IL-3, IL-4, IL-5, IL-10, and IL-17) and APC/T cell activation-associated cytokines (IL-12, tumor necrosis factor-TNF) [60]. The GM-CSF component of the fusion protein is an immune modulatory cytokine that stimulates the development and maturation of APCs, including type 1 dendritic cells (DC1), the subset responsible for initiation of cytotoxic immune responses [65, 66].

Sipuleucel-T is the first antigen-specific immunotherapy approved for cancer treatment. Three Phase III studies have been completed.

The first sipuleucel-T phase-3 trials (D9901 and D9902A) used the traditional measure of response, time to disease progression (TTP) as the primary endpoint. The improvement in the primary end point TTP did not achieve statistical significance [67]. There was, however, a significant benefit in the prespecified endpoint of 3-year survival with sipuleucel-T versus placebo in D9901 (median survival benefit 4.5 months; $p=0.01$; hazard ratio [HR] 0.586; 95 % confidence interval [CI] 0.39–0.88), suggesting that sipuleucel-T may provide a survival advantage to asymptomatic HRPC patients. The subsequent IMPACT (IMmunotherapy Prostate AdenoCarcinoma Treatment) trial met its primary end-point of significantly improved overall survival (OS) with sipuleucel-T versus placebo (median survival benefit 4.1 months: 25.8 months versus 21.7 months; $p=0.03$; HR 0.78; 95 % CI 0.61–0.98) [4]. This trial, where 512 patients with asymptomatic or minimally symptomatic metastatic castration-resistant PC were studied, served as the basis for the licensing approval of sipuleucel-T. Overall, in an integrated analysis of survival across the three trials (D9901, D9902A, and IMPACT; $n=737$), sipuleucel-T provided a survival benefit compared with placebo ($p<0.001$; HR 0.735 [95 % CI 0.613–0.882]) [68]. The greatest magnitude of benefit was observed among patients with better baseline prognostic factors, particularly among patients with lower baseline PSA values [69].

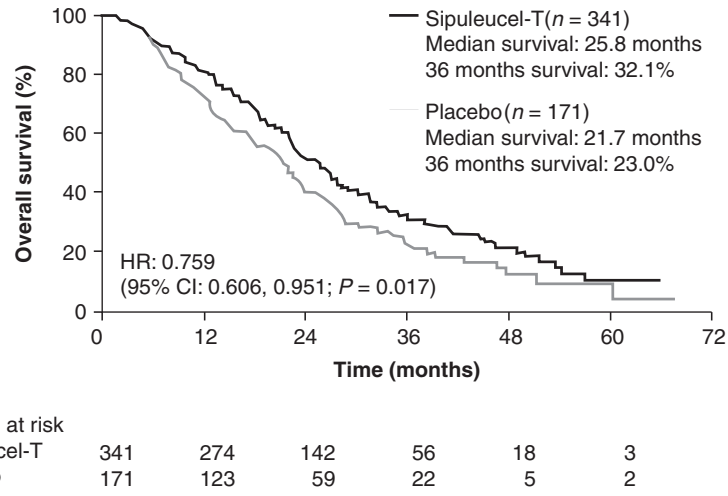

Fig. 2 Kaplan-Meier estimates of overall survival from the phase III (IMPACT) trial of sipuleucel-T in patients with metastatic CRPC

Although the median survival time was greater for sipuleucel-T-treated patients over placebo in all the trials, no difference in progression-free survival was observed between the two groups. Possible explanations relate to how progression is defined (in which a responding scenario can be interpreted as progression) or the idea that the treatment gradually slows down progression, being reflected in prolongation of overall survival, but short-term improvements are not apparent [14].

In the study, sipuleucel-T was generally well tolerated. Adverse events were reported more commonly by patients in the treatment group than in the placebo. These included chills, fever, myalgia, headache, influenza-like illness, hyperhidrosis, hypertension, and groin pain, most of which occurred within 1 day after infusion and resolved in a few days. Grade 3/4 adverse events were uncommon, being reported in 23 of 338 patients (6.8%) in the sipuleucel-T group and 3 of 168 patients (1.8%) in the placebo group (Fig. 2).

Sipuleucel-T has also been studied in the neoadjuvant setting with the single-arm phase 2 NeoACT (NEOadjuvant Active Cellular immunotherapy) trial. It was undergone in 42 patients with localized and treatment-naive PC prior to radical prostatectomy to characterize the immune infiltrate in this type or tumor before and after treatment with sipuleucel-T, and not to look at patient-specific outcomes [70]. The NeoACT trial was the first to demonstrate that sipuleucel-T induced a local immune effect, with an increased T and B cell infiltration (such as CD3+ cells, CD4+ cells, CD8+ cells, CD4+/FOXP3+-T helper, and CD20+ cells) at tumor interface after treatment with Sipuleucel-T. In addition, an examination of peripheral blood mononuclear cells revealed a significant change in antigen-specific T-cell circulation at 12 weeks postradical prostatectomy relative to baseline. This fact was also shown in a subsequent study where it was examined whether sipuleucel-T altered adaptive T cell responses by expanding preexisting T cells or by recruiting new T cells to prostate tissue [71]. Next-generation sequencing of the T cell receptor (TCR) genes from blood or prostate tissue was used to quantitate and track T cell clonotypes in these treated subjects with PC. A significantly greater diversity of circulating TCR sequences in subjects with PC compared with healthy donors was seen, supporting the hypothesis that sipuleucel-T treatment facilitates the recruitment of T cells into the prostate.

Despite all the controversy, sipuleucel-T is the first anticancer therapeutic vaccine that has demonstrated an overall survival improvement in solid cancer patients. It is also interesting the way that this approach can be adaptable to other tumor types by changing the nature of the immunogen–the antigen coupled to GM-CSF in the fusion protein.

Key clinical trials based on the four selected immunotherapies for prostate cancer.

Drug	Trial design	Number of patients	Phase	Key finding	Reference
Sipuleucel-T	Randomized, double-blind, placebo-controlled trial for asymptomatic metastatic CRPC	127	III	Improved OS by sipulcucel-T compared to placebo (25.9 versus 21.4 months)	Pasero et al. [17]
	Randomized, double-blind, placebo-controlled trial for asymptomatic metastatic CRPC	98	III	Improved OS by sipuleucel-T compared to placebo (19 versus 15.7 months)	Zhu and Paul [18]
	Randomized, double-blind, placebo-controlled trial for asymptomatic metastatic CRPC	512	III	Improved OS by sipuleucel-T compared to placebo (25.8 versus 21.7 months)	Wing and Sakaguchi [19]
Ipilimumab	Randomized, double-blind, placebo-controlled trial for metastatic CRPC after docetaxel	799	III	No difference in OS between the two groups, but trend of improved PFS rate by ipilimumab at 6 months (30.7 % versus 18.1 %)	Wei et al. [20]
Prostvac-VF	Randomized placebo-controlled trial of Prostvac-VF for metastatic CRPC	125	II	Improved OS by Prostvac-VF compared to control vector placebo (25.1 versus 16.6 months)	Hodi et al. [21]
	Nonrandomized trial for chemotherapy-naive CRPC	32	II	Improved OS by Prostvac-VF compared to historical controls (Halabi nomogram): (26.6 versus 17.4 months)	Nishikawa and Sakaguchi [22]
GVAX	Randomized trial of GVAX with docetaxel versus docetaxel with prednisone in taxane-naïve patients with symptomatic CRPC	408	III	Trial terminated early due to excess deaths in GVAX plus docetaxel group compared to control (docetaxel plus prednisone) (67 versus 47), and shorter median OS (12.2 versus 14.1 months).	Dalgleish et al. [23]
	Randomized trial of GVAX with docetaxel versus docetaxel with prednisone in taxane-naïve patients with asymptomatic CRPC	626	III	Trial terminated early based on futility analysis showing <30 % chance of meeting primary endpoint (improved OS)	Dunn et al. [24]

Tse et al. [14]

4 Investigational Agents

Multiple immune approaches beyond sipuleucel-T are under development, including monoclonal antibodies against immune checkpoints as well as antigen-directed therapies. Moreover, combinations of these immunotherapies and conventional therapies are also under investigation. In addition, finding the ideal setting and timing for these therapies is also a priority. It is at early

stages of the disease when the immune system of patients may be more intact. That might be the best setting where to apply this approach.

4.1 PROSTVAC-VF Tricom

The use of viral vectors is a promising area in treating cancer. Using this approach, with proven efficacy in infectious disease, might have several advantages as they can mimic natural infection and lead to the induction of potent immune responses against the tumor antigens they encode. An increased number of tumor antigens are available for intersection into these vectors. The poxvirus-based vaccines are the most established and well studied. One example of these vaccines is PROSTVAC®-VF, which employs a recombinant poxvirus-based vector encoded with PSA and TRICOM (three immune co stimulatory molecules: B7.1, ICAM-1, and LFA-3). Vaccination is often enhanced by the subcutaneous co administration of GM-CSF, which acts to further boost immune function [72]. The rationale behind this treatment is that the virus will directly infect the APCs (resulting in expression of the costimulatory molecules), or somatic cells (epithelial and/or fibroblasts) at the site of injection, leading to cell death and subsequent uptake of cellular debris containing PSA by the APCs [14]. APCs will lead to the promotion of a T cell-mediated immune response that destroys PSA-expressing cancer cells. The vaccine virus-based vector is followed by fowl pox virus-based vector boots, helping to overcome the host antivector antibody responses to the original vector and maintaining the level of immunity (Fig. 3).

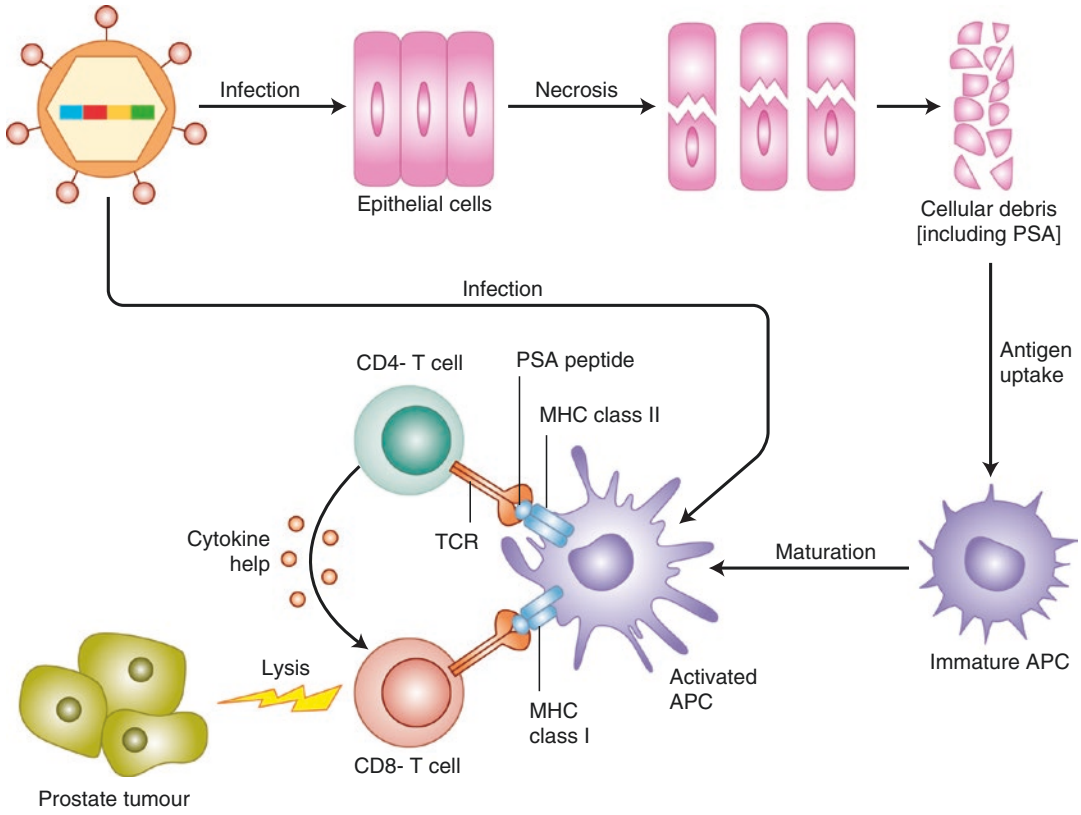

Fig. 3 The ProstVac VF 'vaccine' consists of a DNA plasmid encoding the target antigen (PSA) and a series of co-stimulatory molecules. Then viral vectors are injected intradermally, where they probably infect the patient's epithelial cells. This in turn leads to epithelial cell death, following which the cellular debris (including the target antigen PSA) is taken up by host antigen´presenting cells (APCs) and presented to host CD4+ and CD8+ T cells. The incorporation of CD80 into viral vector facilitates the activation of T cells, through the provision of a co-stimulatory signal for T cell activation

The therapy has been studied in two phases II trials. The first one enrolled 32 patients and evaluated PSA-specific T-cell responses as the primary endpoint, finding a trend towards increased overall survival and a decreased in regulatory T-cell (Treg) suppression in patients with longer survival [18]. These data suggest that PSA-specific T-cell responses and Treg functionality can be used as prognostic markers of efficacy in future trials. The largest phase II randomized 125 patients with minimally symptomatic, metastatic castration resistant PC to treatment or control vectors. The primary end point of progression-free survival was similar between 82 patients treated with PROSTVAC®-VF and 40 patients who received placebo. However, with 3 years of follow-up, patients receiving the vaccine had an 8.5 month improvement in median OS [73]. The therapy was well-tolerated. Most adverse effects were injection site reactions, with only a few patients experiencing associated systemic symptoms such as fatigue, nausea, or fever.

Based on this information, a phase III trial was designed, with and without GM-CSF, in asymptomatic or minimally symptomatic, chemotherapy-naïve, men with metastatic castration resistant PC with or without GM-CSF (NCT01322490). This three-arm trial has overall survival as primary endpoint, and the accrual is already completed ($n=1200$) with results maturing (Fig. 4).

Another type of vaccine that has been studied is the whole-cell-based vaccine or GVAX (BioSante). It is an allogenic cell-based PC vaccine that is composed of both homono-sensitive (LNCaP) and naive (PC3) PC cell lines and that have been genetically modified to constitutively secrete GM-CSF and irradiated to prevent cell replication [74]. The whole tumor cell is used as the antigen, facilitating both humoral and cellular immune responses, with GM-CSF enhancing this process by functioning as chemo attractant for dendritic cells [75]. The use of allogeneic tumor cells as the main component also has advantages in being faster and less expensive to manufacture as compared to autologous cells. Initial phase I/II studies confirmed clinical activity [74]. One phase II trial involving 55 men with chemotherapy-naive metastatic CRPC showed a trend of increased survival time by GVAX in a dose-dependent fashion. Another phase II clinical trial comprised of 80 men with the same clinical characteristics, treatment with high dose was associated with longer median survival time (35 months) as compared with those given medium dose (20 months) and low dose therapy (23.1 months). The proportion of patients that generated an antibody response to either cell line had a median survival of 34 months ($n=30$), compared to 16 months for those who did not ($n=6$), suggesting that immune reaction is associated with better clinical outcomes.

These results lead to two phase III clinical trials (VITAL-2 and VITAL-1). VITAL-2 was a multicenter, randomized, controlled phase 3 clinical trial designed to evaluate the safety and efficacy of GVAX immunotherapy for prostate cancer used in combination with docetaxel chemotherapy compared to the use of docetaxel chemotherapy and prednisone in hormone-refractory prostate cancer (HRPC) patients with metastatic

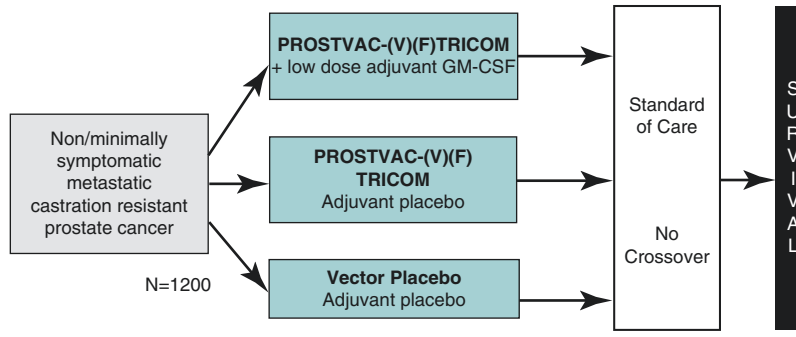

Fig. 4 Phase III ProstVac VF +/- GM-CSF trial study design

disease who were symptomatic with cancer-related pain. The primary endpoint of the trial was an improvement in survival. The trial ended after the Independent Data Monitoring Committee (IDMC) observed an imbalance in deaths between the two treatment arms of the study. VITAL-2 enrolled 408 patients. The IDMC based its recommendation on 114 deaths of which 67 occurred in the GVAX plus docetaxel combination treatment arm and 47 deaths occurred in the docetaxel control arm [76]. VITAL-1, the other Phase 3 clinical trial of GVAX immunotherapy for prostate cancer, was designed to compare GVAX cancer immunotherapy as a monotherapy to docetaxel chemotherapy plus prednisone in earlier stage HRPC patients with metastatic disease who were asymptomatic with respect to cancer-related pain. The primary endpoint of the trial was an improvement in survival. The trial was fully enrolled in 2007 with 626. The study was terminated trial based on the results of a previously unplanned futility analysis conducted by the study's IDMC which indicated that the trial had less than a 30% chance of meeting its predefined primary endpoint of an improvement in survival.

Despite these disappointing results, GVAX is currently being trialed in combination with other immunotherapies, for example, Ipilimumab [77] or mitoxantrone [78] for PC.

4.2 Immune Checkpoint Blockade

As previously mentioned, immune responses are kept in balance by immune checkpoints that oppose co-stimulatory pathways. Alteration of these pathways in tumor cells can provoke sending negative signal into the binding T cells, thus leading to its exhaustion (Fig. 5).

4.1 Anti-CTLA-4 Therapies

Several phase II trials have investigated the role of ipilimumab in PC. A phase I/II study evaluated ipilimumab at up to 10 mg/kg dose with or without radiotherapy in patients with metastatic CRPC who received no more than one prior chemotherapy. PSA decline and radiographic responses were observed in all dose cohorts [79]. A subsequent phase II study randomized 43 chemotherapy naive CRPC patients to ipilimumab at 3 mg/kg versus ipilimumab and docetaxel [80]. These trials lead to plan two phase III trials, which have been completed accrual. The first study evaluated the impact of ipilimumab and radiation (in an effort to prime an initial antitumor immune response) versus radiation alone in the postdocetaxel setting looking for an overall survival (OS) advantage. The study's primary endpoint of OS did not reach statistical significance with median OS at 11.2 months with ipilimumab and 10 months with the placebo (HR = 0.85; 95% CI = 0.72–1.00; $p = 0.053$). Median progression-free survival favored ipilimumab over placebo (HR = 0.70; 95% CI = 0.61–0.82) as did prostate-specific antigen (PSA) response rates. A post hoc analysis was done showing that patients with favorable prognosis (three baseline factors defined by: alkaline phosphatase level, hemoglobin level and no visceral metastases) may derive clinical benefit from ipilimumab [81]. The results of the second study that evaluates ipilimumab versus placebo in metastatic CRPC patients who have not received chemotherapy are still pending (NCT01057810) (Fig. 6).

Tremelimumab, another monoclonal antibody, has been studied in a phase I trial in PSA-recurrent setting. It does show dose-limiting toxicities (diarrhea and skin rash) and PSA doubling time prolongation was observed in 3/11 patients [82].

4.2 Anti-PD1 Therapies

PD1 has been less well studied in PC, although it was found that the CD8+ T cells that infiltrate the prostate gland in men with cancer seem to express PD1 [83].

An earlier phase I study of nivolumab in multiple cancer types enrolled 17 patients with castration-resistant PC, but no objective responses were seen in these patients [84]. Also, there is a phase II trial in the metastatic PC setting, currently ongoing, studying the efficacy of pembrolizumab after androgen-deprivation therapy

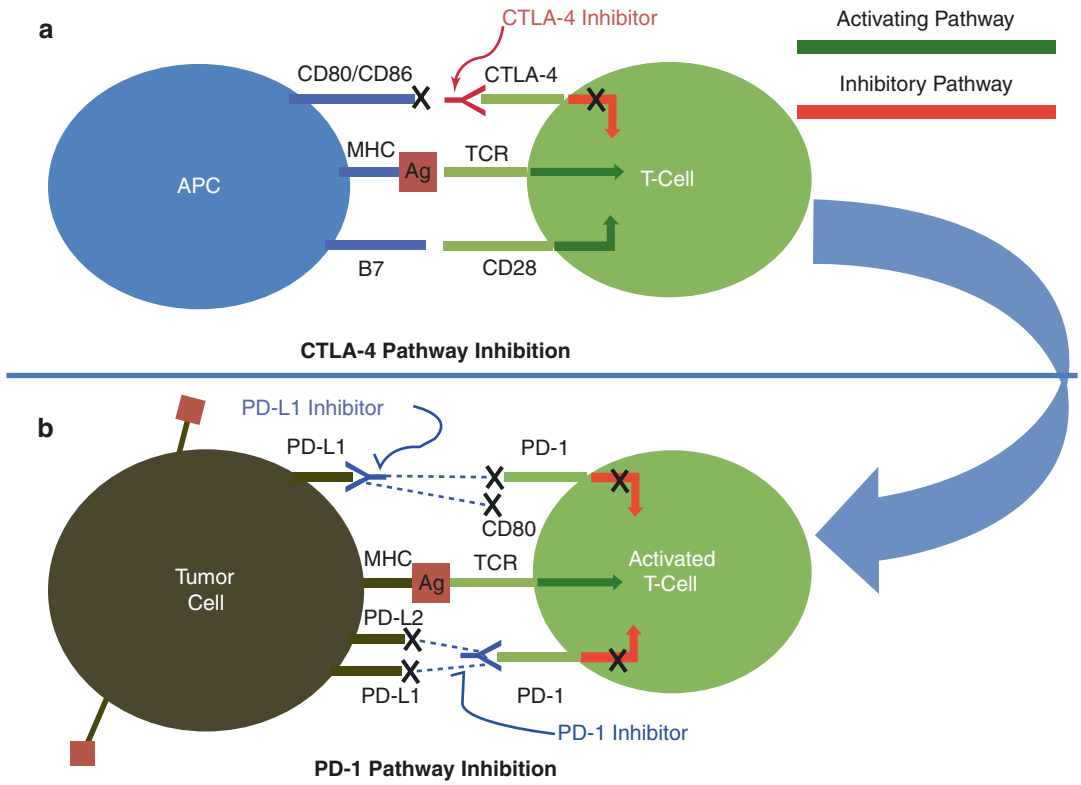

Fig. 5 Schematic of immune checkpoint interactions on T cells and effect of monoclonal antibody inhibition

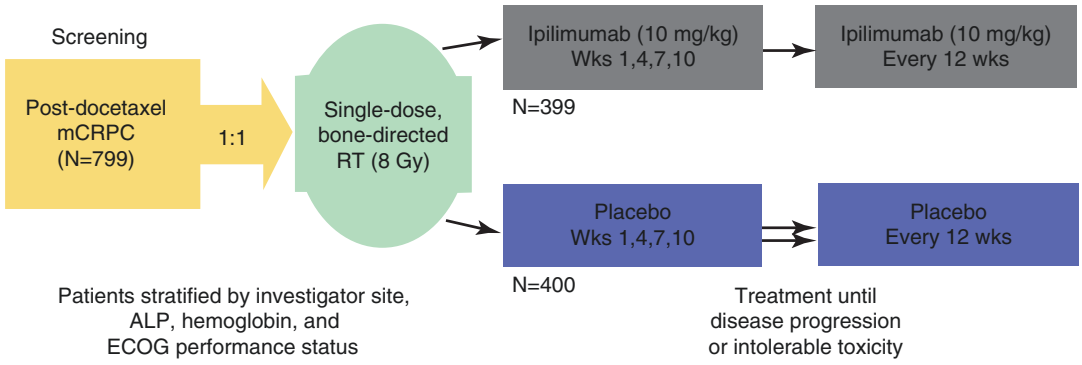

Fig. 6 Phase III trial which evaluates radiation +/- ipilimumab in the post-docetaxel setting. Study design

(NCT02312557). Besides, there are some studies that are focused on the combination of immunotherapy (pembrolizumab) with other types of treatment, such as radium-223, in castration-resistant PC with bone metastases (Investigator initiated trail at Dana Farber)

Pidilizumab, another PD-1 antibody, is being evaluated in a phase II trial for the treatment of androgen-independent PC in combination with sipuleucel-T and cyclophosphamide (NCT01420965).

A promising field of PC treatment is the combination of radiation and immunotherapy. This concept has been evaluated in the previously mentioned phase III clinical trials that combines ipilimumab and radiotherapy and also, in a small randomized trial of men undergoing primary radiotherapy [57];

13 out of 17 patients in the radiotherapy and immunotherapy combination treatment group had a greater than threefold increase in the number of PSA-specific T cells, whereas no increase in the number of PSA-specific T cells was noted in the group that received radiotherapy alone.

New emerging immune checkpoint targets have been identified and include LAG-3, TIM-3, VISTA, and co-stimulatory molecules OX40, ICOS, and 4-1BB [85]. In addition, new next generation sequencing techniques sequencing could help to identify a spectrum of mutation frequencies that can respond to immunotherapy. Some select patients with advanced heavily pretreated PC might harbor microsatellite instability making them more suitable for PD1/PDL1 blockade (P.Nelson, *ASCO* 2016).

4.3 Biomarkers in PC

Due to the emerging development of new therapies, including immune agents, predictive and surrogate biomarkers will be needed. Such biomarkers could identify responders in the earlier phases of treatments, in which the full effects are often not apparent before weeks to months after initiation. Because OS is a more reliable endpoint than PFS with immunotherapy, such biomarkers could provide intermediate surrogate endpoints for trials (while final end points would otherwise take years to complete). Multiple categories of immune biomarkers have already been investigated in PC, in the following table there is a selection of these [86].

Biomarker	Description/examples	Applications	Sampling specimen	Routine clinical availability	Prognostic	Predictive	Pharmacodynamic	Surrogate
Inflammatory biomarkers								
Individual cytokines	IL-6, IL-8, TGF-β1	Diagnostic and prognostic utility in various stages of disease Prediction of responses with chemotherapy, vaccines and Sipuleucel-T	Serum	No	Yes	Yes	FSN	FSN
C-reactive protein (CRP)	Acute-phase protein involved in inflammation, necrosis, and carcinogenesis	Prognostic utility in various stages of disease	Serum	Yes	Yes	FSN	FSN	FSN
Toll-like receptors (TLRs)	Family of transmembrane proteins that can recognize highly conserved molecules in invading pathogens	Postdiagnostic prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Neutrophil-to-lymphocyte ratio	Ratio of peripheral neutrophil to lymphocite count	Postdiagnostic prognostic utility Possible predictive value in enzalutamide-treated-patients	Serum	Yes	Yes	Yes	FSN	FSN
Cellular response to PC								
Increase in Th1 T cell response	Subtype of T-helper cell response	Possible favorable prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Increase in Th2 T cell response	Subtype of T-helper cell response	Possible negative prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Cellular response to immunotherapeutic agents								
Increase in various T cell responses	Cytotoxic and T-helper lymphocytes	Possible prognostic and pharmacodynamic utility in patients treated with vaccines	Serum	No	Yes	FSN	Yes	FSN
Decrease in Treg response	Regulatory T cells	Role to be defined in patients treated with ipilimumab, Sipuleucel-T, and other vaccines	Serum	No	FSN	FSN	FSN	FSN
Increase in eosinophil response	Peripheral eosinophil count	Possible prognostic and predictive utility in Sipuleucel-treated patients	Serum	No	Yes	FSN	FSN	FSN

Humoral response to PC								
Tumor-associated antigens (TAAs) other than PSA	p90, p62	Possible diagnostic and prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Auto-antibody signatures	Combination of various serum auto-antibodies	Possible diagnostic and prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Humoral response to immunotherapeutic agents								
Antigen spreading	Vaccine-associated response to ubiquitously expressed self-antigens	Possible pharmacodynamic, prognostic and predictive Serum utilities in patients treated with vaccines including Sipuleucel-T	Serum	No	FSN	FSN	FSN	FSN
Immune checkpoints								
PD-1/PD-L1 (B7- H1)	PD-1: Immunoglobulin superfamily member PD-L1: Ligand of PD-1, member of the B7 super-family of costimulatory molecules	Predictive role in patients treated with anti-PD-L1 and tissue anti-PD-1 monoclonal antibodies Possible predictive role in enzalutamide-resistant patients Possible prognostic role in ipilimumab- and Sipuleucel-T-treated patients	Tissue	Yes	Yes	Yes	FSN	FSN
CD276 (B7-H3)	Member of the B7 super-family of costimulatory molecules	Possible postdiagnostic, prognostic and predictive tissue roles New immunotherapy target	Tissue	No	Yes	Yes	FSN	FSN
CD73	Ectonucleotidase catabolizing the hydrolysis of extracellular adenosine monophosphate (AMP) to adenosine	Possible postdiagnostic, prognostic and predictive tissue roles New immunotherapy target	Tissue	No	Yes	Yes	FSN	FSN
Immunologic biomarkers of tumor microenvironment								
Tumor-associated macrophages (TAMs)		Possible adverse prognostic role tissue	Tissue	No	Yes	FSN	FSN	FSN
Cytotoxic CD8 tumor-infiltrating lymphocytes (TILs)		Possible adverse prognostic role tissue	Tissue	No	Yes	FSN	FSN	FSN
Treg tumor- infiltrating lymphocytes (TILs)		Possible adverse prognostic role tissue	Tissue	No	FSN	FSN	FSN	FSN
Mast cells		Role remains to be defined tissue	Tissue	No	FSN	FSN	FSN	FSN

Immunotherapy with check point inhibitors is now a newly rediscovered therapeutic strategy in PC that was initially dismissed. Like in colorectal cancer, in selected patients these agents might be of benefit.

5 Targeted Therapies

In the field of PC, bevies of novel therapeutics with distinct mechanisms of action have been recently tested. Unfortunately although preliminary data were promising, in unselected patients, no one of these new agents has been able to provide clinically meaningful benefit. Here, we briefly report some new therapies that can potentially be useful in PC treatment if the adequate target patient population is identified. An example has been the potential benefit seen with PARP inhibitors is select patient harboring DNA repair genomic alterations (see Sect. 5.8)

5.1 Angiogenesis Inhibitors

Angiogenesis mechanisms play an important role in cancer. It is also well known that a high microvascular density in prostate gland is a poor prognostic factor in PC [87]. There are several angiogenesis-related agents which have been studied, such as thalidomide [88], bevazicumab [89], lenalidomide [90], sorafenib (NCT00619996) [91], most of them with no success in phase III trials.

5.2 Next-Generation Androgen Synthesis Inhibitors and Androgen Receptor Signaling Inhibitors

PC progression usually occurs despite continued castration in patients receiving standard androgen deprivation therapy. There are several mechanisms that have been implicated in castration resistance; such as, overexpression of AR, androgen synthesis by PC cells; alterations in expression of coactivators and corepressors of AR signaling; and constitutively active, ligand-independent AR splice variants [92]. There are currently several androgen synthesis inhibitors in development beyond the ones already approved abiraterone and enzalutamide [93]. AR antagonist in development like ARN-509, competitively inhibits AR signaling in the setting of AR overexpression, with potentially improved efficacy compared with enzalutamide in xenograft models. Phase III studies with ARN-509 are ongoing (NCT01946204).

5.3 HSP90 Inhibitors (Olanespib)

The transcriptional activity of steroid receptors, including AR, is dependent on interactions with the HSP90 chaperone machinery, this is way some early studies are checking the utility of HSP90 inhibitors, specially on PC with androgen receptor variant 7 [94].

5.4 mTOR (Mammalian Target of Rapamycin) Inhibitors (Everolimus, RAD 001)

MTOR inhibition appears to reverse dysregulation of Akt system, thus avoiding the effect of PTEN mutation, which is a common characteristic in 50 % of advanced PC [95].

5.5 EGFR (Epidermal Growth Factor Receptor)-Tyrosine-kinase Inhibitors (Gefitinib, Erlotinib, Pertuzumab)

There are several studies published with poor results, although in vitro test results were promising [96, 97].

5.6 mRNA-Based Therapies

Such as oblimersen, a Bcl-2 antisense oligonucleotide, with negative results in a phase II trial performed in patients with castration-resistant PC [98].

5.7 Histone Deacetylase Inhibitors (HDACs)

HDACs are part of a transcriptional corepressor complex that influences various tumor suppressor genes, included in PC scenario [99]. There are some examples of HDACs that have been studied in this disease with controversial results, such as vorinostat (with a phase II trial where it showed significant toxicities that limited efficacy assessment in the patient population) [100].

5.8 PARP (poly(ADP-ribose) Polymerase) Inhibitors

Previous PC genomic sequencing efforts have identified genetic aberrations, including mutations in DNA repair genes. The researchers hypothesized that olaparib, which targets those tumor cells that are particularly vulnerable to DNA repair defects, may work in this subset of PC patients. A phase II trial [TOPARP, NCT01682772], which is currently recruiting patients will try to determine if this approach can be useful in PC.

Conclusions

PC is a target for immunotherapy approaches. It has a unique natural history characterized by a relatively indolent course, which allows immunotherapies, time to achieve an effect via stimulation of the immune machinery. It was the first type of solid cancer where an immunotherapy drug was the standard of care (sipuleucel-T) upon improving survival. Since that achievement, there are multiple novel therapeutics under investigation (off the shelf vaccines such as PROSTVAC-VF, GVAX; checkpoint inhibitors; or novel homegrown vaccines). However, still some questions remain according to the immune approach: timing and combination with other modalities of treatment need to be explored. Establishing the optimal combination and sequencing of treatment will prove crucial. Also, it is important to keep on finding predictive immune biomarkers as the response is often gradual, and the usual monitored clinical markers are not always affected immediately after treatment initiation. Identifying the best method to measure and quantify such immune responses remains a challenge because of the difficulty in obtaining an adequate quantity of samples and the limitations of current functional assays.

New technologies or platforms, such as T cells receptor (TCR) clonotyping, chimeric antigen receptor T-cell therapies (CARTs), computational analysis approach, or home grown vaccines, are welcomed in this fight against PC.

Achieving long-term remission in most treated patients is an ambitious goal for the scientific community and requires the integration of several modalities in a rational combination therapeutic approach.

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