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This is the Accepted version of the following publication

Hossain, MK, Nahar, K, Donkor, Osaana and Apostolopoulos, Vasso (2018)
Immune-based therapies for metastatic prostate cancer: An update.
Immunotherapy, 10 (4). 283 - 298. ISSN 1750-743X

The publisher's official version can be found at
<https://www.futuremedicine.com/doi/10.2217/imt-2017-0123>
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Immune-based therapies for metastatic prostate cancer: an update

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Abstract

Prostate cancer (PC) is a common malignancy among elderly males and is non-curable once it becomes metastatic. In recent years, a number of antigen delivery systems have emerged as a viable and promising immunotherapeutic agents against PC. The approval of Sipuleucel-T by the US FDA for the treatment of males with asymptomatic or minimally symptomatic castrate resistant PC was a landmark in cancer immunotherapy, making this the first approved immunotherapeutic. A number of vaccines are under clinical investigation, each having its own set of advantages and disadvantages. Here, we discuss the basic technologies underlying these different delivery modes, we discuss the completed and current human clinical trials, as well as the use of vaccines in combination with immune checkpoint inhibitors.

KEYWORDS: prostate cancer • vaccine • preclinical study • clinical trial • vaccine delivery • immune checkpoint inhibitor • immunotherapy

Background

Prostate cancer (PC) is the second most commonly diagnosed malignant tumor in men and a major cause of mortality, with an estimated 385,560 deaths globally expected by 2020 [1]. In 2016, prostate cancer was the 3rd most common cause of cancer deaths in Australia and the 2nd most common cause of cancer deaths amongst males [2]. In a systematic review of over 71,000 PC patients 10–20 % of the cases progressed to castration-resistant prostate cancer (CRPC), for which there is no effective cure [3]. The vast majority (> 80 %) of patients diagnosed with CRPC already have distant metastases and, one third of the remaining subjects are likely to develop metastases within 2 years [4, 5]. This was also shown recently, based on the Danish PC registry where 19,487 males with PC had died in the 18 year period (1995-2013), the majority of those who had died were those who had lymph node and elsewhere metastasis at the time of diagnosis. This was supported by a decreased number of cases of metastatic disease at the time of diagnosis over time which correlated with overall increased median survival [6]. The current standard chemotherapeutic treatment regime for patients with CRPC is based on docetaxel plus prednisone. This approach only

modestly enhances patient survival and, as with most chemotherapeutics, a range of undesirable side effects ensues [7]. Other novel treatment options include radium-223 and androgen receptor targeted therapy for CRPC which has shown to improve survival compared to placebo [8, 9]. However, there is a need for less toxic alternative treatments, such as, active immunotherapy. PC is a viable candidate for the development of an immunotherapeutic or vaccine, as current standard treatments for the clinical management of CRPC are inadequate., PC cells express an array of tissue specific proteins that could act as therapeutic targets, including prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) [3]. In the last decade, a number of vaccines against PC (i.e., virus based, gene based, peptide/protein based and cell based vaccines) have been developed and tested in pre-clinical models and in human clinical trials for safety and therapeutic profile [10-12].

Tumor development and progression results from a cancer-induced immunosuppressive state, in which the patient's immune system is not able to recognize and destroy cancer cells (cancer immunoediting), hence, escaping immune surveillance. Therefore, immune evasion is recognized as a hallmark feature of cancer and, in the last 5 years, immune checkpoints have surfaced as key players. As a result, much interest in anticancer research is aimed at blocking immune checkpoints alone or in combination with immunotherapeutic agents/vaccines to restore and enhance cellular-mediated antitumor immunity and achieve durable tumor regression [13].

Herein, we describe the current knowledge regarding various types of immunotherapeutic/vaccine strategies along with human clinical trial outcomes. In addition, we present the effects and importance of immune checkpoint inhibitors mediating cancer regression.

Vaccine approach: developments in preclinical studies

Prior to potential vaccines entering human clinical trials, it is important to determine its immune efficacy using *in vitro* studies and *in vivo* animal models. Many prospective vaccines will not progress beyond this stage due to unacceptable adverse reactions in animal models or a lack of immunogenicity. However, careful pre-clinical analysis is required, despite over the decades of development of cellular therapies, mouse models have produced often

contradictory and conflicting results. Hence, comprehensive and convincing pre-clinical data (to include appropriate animal models, use of humanized mouse models, appropriate immune cell analysis etc.) is a major pre-requisite prior to moving into human trials. Pharmacological and toxicology studies of new vaccines must also be assessed prior to clinical development. The goals of preclinical safety evaluation include single-dose toxicity; repeated-dose toxicity; primary pharmacodynamics (immunogenicity); secondary pharmacodynamics (safety); pharmacokinetics and local tolerance. For the *in vivo* phase of preclinical testing, selection of the relevant animal species, age of test animals, their physiological state, vaccine delivery (including dose, route of administration and treatment regimen) and stability of the test material under the conditions of use are necessary regulatory requirements prior to human clinical studies.

Viral vector-based approach

Viral vectors are attractive for use in cancer immunotherapy as they mimic natural infection and lead to the induction of robust immune responses. The advantage of viral vectors is their immunogenicity, and the off-the-shelf nature of the ensuing construct, which does not need to be individualized for each patient. The major disadvantage of a viral vaccine construct is the complex nature of its backbone, which means that the majority of the immune response is targeted against the virus itself, rather than the target antigen. To circumvent these limitations, significant innovations have been implemented [14], such as, immunogenicity of the standard vaccinia virus vector is dramatically improved by the addition of 3 immunomodulatory proteins (LFA-1, ICAM and B7-1) [15]. Preclinical studies showed that this combination was strikingly synergistic in terms of generating robust immune responses, and the technology was adapted for clinical development. Modification could utilize heterologous prime boost regimens that prime the immune system against a target antigen and subsequently boosting antigen-specific immune responses with a distinct antigen, provided another viral backbone was employed.

One of the earliest vaccines for prostate cancer was GVAX[®], which at the time used the Dunning R3327 rat model of human PC. The high grade and metastatic subline MAT-LyLu transduced with a retroviral vector carrying the gene for granulocyte macrophage-colony stimulating factor (GM-CSF), was used to immunize tumor-bearing rats, and showed

that one third of the treated rats were tumor free. This approach was translated into human clinical trials, first using GM-CSF-secreting, irradiated autologous patient prostate tumor cells [16] and later followed by allogeneic tumor cells, also engineered to secrete GM-CSF [17].

Furthermore, the uses of vaccinia viral vectors and adenovirus (Ad) vectors have shown promise in preclinical studies [18-21]. Vaccinia viral vectors have been used both as replication competent vaccine strains [22] and a modified, limited replication variant [19], whilst all of the Ad based vaccines are based on the replication deficient strain [21]. Preclinical studies conducted with vaccinia virus vector carrying the gene encoding for human PSA induces strong CD8⁺ T cells in mice [23, 24], and shown to have a safety profile in monkeys [25]. On the other hand, Ad vectors have been shown to be more immunogenic compared to vaccinia virus vectors. However, a major challenge to Ad-based vaccines is the presence of pre-existing neutralizing antibodies resulting from prior environmental exposure, which can neutralize adenoviruses prior to activation of immune responses. In animal models, the use of replication-deficient Ad serotype 5 vector (Ad5), transduced with the full-length human PSA (Ad5-PSA) [26-28] stimulates strong anti-PSA antibody and CD8⁺ T cell immune responses which lyse RM11/PSA⁺ tumor cells in mice [29]. On the other hand, in established tumor studies, the effectiveness of such vaccine was only noted in 20 % of mice which was enhanced to 80 % when combined with cytokine genes (IL-2, IL-12, TNF- α) [29]. Interestingly, incorporation of Ad5-PSA in a gelfoam matrix further enhanced antibody and CD8⁺ T cell responses [28]. Ad5-PSA priming in gelfoam matrix also removed the inhibitory effects of adenoviral immunity on CD8⁺ T cell activation in mice naive to PSA but immune to Ad. The ability to immunize with Ad vaccines in the presence of anti-Ad antibodies has important implications for their use in clinical trials as most males would have been exposed to Ad5 viruses and possess significant levels of anti-Ad antibodies. Moreover, the combination of Ad5-PSA vaccine with immunostimulatory TLR9 agonist, CpG (injected simultaneously, or Ad5-PSA followed by CpG days later), enhances ensuing immune responses [26, 27, 30] and protects mice in both prophylactic and therapeutic settings [31]. Likewise, Ad5 vector transduced with a truncated gene for prostate specific membrane antigen (Ad5-PSMA) and pulsed with mouse dendritic cells (DCs) induces strong CD8⁺ T cell responses and protect against PSMA⁺ tumors in mice [21]. Thus, the advantage

of Ad5 platform is its relative immunogenic potential in preclinical settings, and its translation into human clinical trials.

DNA-based approach

DNA vaccines have emerged as promising approach to antigen delivery based on injection of DNA into the host with the aim of transducing cells at the injection site, resulting in the subsequent production of immunogenic protein and stimulation of antitumor immune responses. The advantage of DNA-based vaccines is the ease of manufacture, yield and purity, highly reproducible and cost-effective for large scale up [25, 27]. It has been well documented that DNA vaccination is a highly potent strategy for inducing both prophylactic and therapeutic responses [23].

One of the earliest uses of DNA technology for the development of tumor vaccines was reported by Irvine in 1995 [32] and further pursued by Disis et al. in 2003 [33]. Preclinical studies have used the prostate tumor-associated antigens PAP, PSA, PSMA and prostate stem cell antigen (PSCA) [18, 34, 35]. The experimental plans and antigens used in DNA vaccine approaches are varied, with some co-administering of GM-CSF [36] whilst others make use of the prime–boost approach [18]. Most studies reported use of DNA that encoded for the native protein, whereas Vittes et al. immunized with DNA that encoded for different PSMA peptides [35]. Interestingly, the latter study demonstrated that 2/3 of the peptides used were able to induce T cell responses and were able to lyse tumor target cells that expressed the native protein [35]. Hence, DNA-based vaccines offer a viable alternative for the development of PC vaccines.

Cell-based approach

Dendritic cells (DC) are the most potent antigen presenting cells in initiating adaptive immune responses and present an attractive platform for cancer vaccines [37, 38]. The source of tumor antigen in such approach is important, and may involve synthetic peptides, autologous tumor cell lysates or lysates from cultured cancer cells. Using autologous tumor cell lysates is particularly attractive, as ensuing immune responses are directed against the antigen repertoire derived from a patient's own individual tumor. However, autologous cells vary greatly in number, viability and recovery, and an adequate surgical sample is required

for vaccine to be formulated [39]. The advantages of DC-based vaccine includes the presentation of a broad spectrum of antigens, as well as the potential augmentation of the immune response conferred by the non-self major histocompatibility complex (MHC) molecules present in allogeneic tumor cells [40]. Likewise, the *ex vivo* maturation step also has benefits as it has been shown in cancer patients that DC are deficient in numbers and function. Disadvantages include the complexity of manufacture, especially the shipping of leukopheresis product to a central processing facility, *ex vivo* culture for 5-7 days, maturation of DCs, pulsing with antigen, and shipping the final product back to the treatment center for infusion., The costs involved in such approach are also extremely high [41]

DCs pulsed with a number of proteins have been used in vaccine studies and are the prototypical cellular vaccines. One of the early DC vaccine was that developed by Heiser et al., where DCs were pulsed with PSA RNA and anti-PSA and T-cell responses were induced [42]. Furthermore, anti-prostate tumor cytotoxic T cells (CTL) were induced *in vitro* using DC pulsed with RNA extracted from human PC cells [43]. The PC vaccine that has received the most publicity and attention is Sipuleucel-T or Provenge®, a cell-based approach. In early preclinical studies using rats, immunization with antigen-presenting cells (APCs) pulsed with a fusion protein of rat PAP and rat GM-CSF [44] did not show anti-tumor effects although normal rat prostate tissues developed lymphocytic infiltrates. Subsequent *in vitro* studies using the Dunning R3327 tumor cells provided support that lymphocytes from APC/fusion protein immunized rats could have an antitumor effect [44].

Progress in human clinical trials

Clearly, preclinical PC vaccine immunotherapy studies have produced strong foundation for the extension of these therapies into human clinical trials and subsequent treatment of men with PC. DC, DNA and viral vectors are currently in Phase I-III human clinical trials. The identification of prostate tumor-associated antigens and the ability to isolate their genes has propelled antigen-specific vaccine immunotherapy into a new era of vaccine development.

Sipuleucel-T (Provenge®)

The first autologous cellular immunotherapy approved by the Food and Drug Administration (FDA) in 2010 and by the European Medicines Agency (EMA) for the treatment of metastatic castrate resistant prostate cancer (mCRPC) was sipuleucel-T (Provenge®). To date it remains the only FDA-approved immunotherapeutic approach for PC, although it should be noted that the FDA initially declined its use and a new phase III trial was designed and registered. This approval was based on a blinded, randomized controlled trial where those that received sipuleucel-T, had a 22 % reduced risk of death compared to the placebo group [14]. The reduction represented a 4.1-month improvement in median survival (25.8 months vs. 21.7 months) and the 3-year survival rate was 31.7 % in the sipuleucel-T group compared 23.0 % in the placebo group [45].; although only marginal improvements in median survival were noted. A recent phase II study (NCT01487863, results obtained March 2017) evaluated the impact of concurrent versus sequential administration of abiraterone acetate plus prednisone on the ability to manufacture sipuleucel-T (by assessing sipuleucel-T product parameters), and to assess the safety and efficacy of sipuleucel-T administration in patients with mCRPC. The cumulative CD54 upregulation ratio between the cohorts was similar in both the concurrent and sequential groups [46]. CD54 is an intercellular adhesion molecule 1 which is expressed by endothelial and leukocyte cells which upon stimulation secrete IL-1 and tumor necrosis factor. Upregulation of CD54 in PC implies overall survival. Currently the NCT00970203 trial is recruiting participants for intradermal immunization of DC-loaded with allogeneic PC cell lines in combination with androgen ablation in patients with PC. This is a phase II, randomized and open label with cross over intervention study and will assess the feasibility, safety and efficacy of the vaccine. The estimated completion date of the study is December 2018 [47].

Tumor-mRNA transfected DC

A phase I trial using autologous ex vivo monocyte derived DCs transfected with mRNA from allogeneic PC cells (LNCaP, DU145 and PC-3) were injected (2×10^7 cells) either intranodally or intradermally into patients with mCRPC and were reported to be well tolerated [48]. In addition, a decrease in PSA progression in 13/19 patients was noted which correlated to increased T cell proliferative responses; clinical outcome was significantly related to immune responses [49]. This work has been currently extended in the form of an active Phase I/II

trial using autologous tumor cells as an antigen source (NCT01197625) for curative resected PC patients, and the primary outcome measure is expected by September 2019 with a completion date of September 2025.

GVAX®

GVAX is composed of both castrate-sensitive and castrate resistant allogeneic PC cell lines (LNCaP and PC3, respectively) transduced with GM-CSF [14]. Two phase III human clinical trials were conducted which led to their premature discontinuation due to severe adverse reactions. In the first study, (VITAL-1) GVAX prostate was compared to standard chemotherapy (docetaxel) in males with mCPRC. In the second trial (VITAL-2), the combination of GVAX prostate and docetaxel was compared to docetaxel alone; this time a more advanced population, males with symptomatic mCRPC, were enrolled; preliminary results suggested an imbalance in deaths in the combined treated group [50]. Nevertheless, these concerns prompted an unplanned, and slightly underpowered efficacy analysis of the VITAL-1 trial [51], which indicated ineffective and led to its premature termination. In addition, the combination of GVAX and ipilimumab was studied in an open-labeled, single-center, phase I clinical trial [52]. Ipilimumab (Yevroy™) is an-anti CTLA-4 monoclonal antibody which downregulates the immune system and has been shown in two randomized phase III trials to improve the overall survival of patients with metastatic melanoma [52, 53]. In a phase I study of 28 patients with mCRPC and no previous history of chemotherapy, patients received 13 intradermal injections of GVAX (5×10^8 cells) and escalating doses of ipilimumab. At high ipilimumab doses side effects such as, inflammation of pituitary gland and/or sarcoid alveolitis developed whereas, at low doses it was generally safe and well tolerated. In addition, the combination of GVAX and ipilimumab improved overall survival compared to sipuleucel-T or PostVac VF [53, 54]. However, it is not clear whether this combined treatment constitutes a significant improvement over GVAX alone in terms of overall survival.

PROSTVAC

In terms of early development, a trial utilizing vaccinia-PSA prime/boost regimen, showed that a vaccinia-PSA prime, followed by a series of fowlpox-PSA boosts was more

immunogenic than other regimens [55]. A carefully considered series of combination trials followed involving chemotherapy, radiation therapy and androgen ablation. Importantly, a randomized placebo controlled phase II human clinical trial of PROSTVAC was injected in asymptomatic or minimally symptomatic patients with mCRPC [56]. The primary endpoint was time to progression, and upon progression patients were treated at their physicians' discretion – no crossover was permitted. Even though the primary endpoint was not met, long term follow-up showed a statistically significant improvement in overall survival of the ProstVac group compared to control (25.1 months versus 16.6 months).

Furthermore, in a single arm phase II trial of PSA-TRICOM [PROSTVAC expressing PSA together with 3 immune-stimulating molecules (ICAM-1, B7.1 and LFA-3 by poxviral vectors and boosted using fowlpox vectors)], in 32 patients with mCRPC showed an improved survival. The improved survival correlated to a 2-fold increase in PSA specific IFN-gamma secreting T cells [57]. A randomized phase II trial (NCT01145508) using docetaxel with or without PSA-TRICOM vaccine in patients with mCRCP was conducted [58]. to assess the overall survival; however, the study outcome remains inconclusive as an insufficient number of participants were enrolled in the study. A phase III randomized double blind study is currently in progress in order to determine the efficacy of PROSTVAC alone or in combination with GM-CSF in prolonging the overall survival in males with few or no symptoms from mCRPC; the trial is estimated to be completed by June, 2018 [59]. In general, PROSTVAC is well tolerated with common side effects being, reactions at the site of injection, fatigue, nausea and mild fever; PROSTVAC is being developed by Bavarian Nordic (Kvistgaard, Denmark).

TroVax®

TroVax® is being developed by Oxford BioMedica and is based on a vaccine platform utilizing the tumor associated glycoprotein (5T4), expressed on several cancer types, including colorectal, renal and PC [60]. 5T4 is delivered using the poxvirus vector (modified vaccinia virus Ankara (MVA) vector) for delivery. TroVax® does not include a heterologous prime boost, and does not include a series of co-stimulatory molecules like PROSTVAC. A phase III trial in patients with renal cell carcinoma did not improve survival compared to placebo, but subgroup analyses suggested improved survival in patients with a favorable prognosis [61].

The primary advantage of this platform is the novel target antigen, which is broadly applicable to multiple tumor types. The major disadvantages are the univalent nature of the vaccine construct, as well as a homologous prime-boost regimen. Moreover, in an open-label Phase II trial in 27 males with progressive disease with TroVax[®] alone or a combination of TroVax[®] and GM-CSF, was generally well tolerated. Five patients who received TroVax[®] and GM-CSF showed a decline in PSA levels, with none in the TroVax alone group [62]. A randomized open-label phase II trial in castration-resistant PC patients, TroVax was administered in combination with docetaxel compared to docetaxel alone. TroVax was well tolerated in all 25 patients and of the 10 evaluable patients, 6 generated 5T4 specific antibody responses. Patients in the combined treatment group demonstrated a greater median progression-free survival of 9.67 months compared to 5.10 months for docetaxel alone [63].

Ad5-PSA

A phase I trial of Ad5-PSA with or without gelfoam matrix in patients with PC, was shown to be safe (the primary endpoint of the trial), and anti-PSA T cell responses were induced in the majority of patients, however an increase rate rise in PSA (PSA doubling time) was noted in 50 % of patients [64]. A Phase II clinical trial of two separate protocols for patients with recurrent or hormone refractory PC were assessed for toxicity, immune responses, and changes in PSA levels [65]. In Protocol 1, men with recurrent PC following definitive initial treatment for their disease, either received Ad5-PSA alone on days 0, 30, 60, or received Ad5-PSA 14 days after the initiation of androgen deprivation therapy, 3 times, 30 days apart. In Protocol 2, men with hormone refractory disease received Ad5-PSA alone using the same three injection schedules, however Ad5-PSA was suspended in the gelfoam matrix. All of the patients in protocol 1 and 67 % of the patients in protocol 2 induced significant anti-PSA T cell responses; 64 % of the patients showed an increase in PSA doubling time [65].

AdV-tk including ProstAtak[™]

Intratumoral delivery of Ad vector encoding the herpes simplex virus enzyme thymidine kinase (AdV-tk) results in transduced tumor cells becoming susceptible to systemically administered prodrugs such as valacyclovir (VCV) or ganciclovir (GCV), which are selectively

converted by thymidine kinase to cytotoxic nucleotide analogs [66, 67]. These activated drugs can then lyse neighboring proliferating cells via immune activating bystander effects [68]. In fact, in a phase I/II clinical trial in 23 advanced PC patients, intraprostatic injections of Adv-tk followed by 2 weeks of GCV and prostatectomy 2-4 weeks later, induced a significant infiltration of CD8+ T cells within the tumor microenvironment compared to control patients [69]. Interestingly, Adv-tk+GCV preferentially induced cytotoxic effects to malignant (rather than benign) tissues, although there were no improvements in clinical outcome of patients and PSA levels. This lack of clinical efficacy prompted the use of combination treatments involving chemotherapy or radiotherapy and Adv-tk+GCV in a phase I/II study. Patients with PC received Adv-tk+GCV with radiotherapy and were grouped into 3 arms, (i) 29 low-risk PC patients (stage T1-T2a), (ii) 26 high-risk PC patients (stage T2b-T3) and (iii) 4 PC patients with stage D1 disease. The low-risk and high-risk groups showed good locoregional control and stabilization of PSA levels whilst those with D1 disease showed no such responses [70].

DNA vaccine formulations

Plasmid DNA encoding PAP: The pTVG-HP plasmid encoding the PAP protein has been well studied in preclinical and clinical settings [71]. In a phase I human clinical trial, pTVG-HP/PAP induces PAP-specific interferon (IFN)-gamma (IFN- γ) secreting T cells in males with recurrent PC, resulting in increased PSA doubling time [72]. Likewise, a phase I/II trial in patients with non-metastatic CRPC injection of pTVG-HP/PAP showed increased PSA doubling time from 6.5 months to 9.3 months [73]; no adverse reactions were reported. Interestingly, patients that did not respond to treatment had high levels of PAP specific IL-10 CD4+ and CD8+ T cells prior to vaccination, compared to those who responded with pre-existing IFN- γ and granzyme responses to androgen receptor, PSA and PAP [74]. Thus, determining pre-existing responses is important prior to PC vaccination trials, and is currently being evaluated in ongoing randomized clinical trials (NCT00849121). A list of selected ongoing clinical trials related to DNA vaccine are tabulated in Table 1.

Electroporation: Electroporation (EP) makes use of brief electrical pulses that generate transient “pores” in the cell membrane, allowing genes (DNA or RNA) to enter the cell’s

cytoplasm; antigen expression has been reported to increase up to 1000 fold [75, 76]. The use of EP has been translated into human clinical trials. In fact, in a phase I/II dose escalation trial, patients with biochemically recurrent PC were either immunized intramuscularly or EP with DNA encoding PSMA/tetanus toxin [77]. Both immunization schedules resulted in CD4+ and CD8+ T cell responses and increased PSA doubling time compared to baseline, however, a significant trend towards higher responses were noted in those treated with EP [77]. EP was well tolerated in this study, despite others reporting local pain, inflammation and bleeding at the site of injection [78, 79]. A current phase I clinical trial (NCT02514213) [80] is underway to determine the safety and immunogenicity of INO-5150 alone (DNA plasmids encoding PSA and PSMA) or in combination with INO-9012 (IL-2 plasmid) delivered intramuscularly followed by EP in males with biochemically relapsed PC. The trial is expected to be completed by mid-2017. It is clear that EP provides an encouraging platform for gene-based vaccine delivery.

Personalized peptide vaccination

Personalized peptide vaccination (PPV) uses multiple peptides based on the individuals pre-existing antibody and/or T cell immunity and has shown promise in boosting such immunity. Indeed, in phase I and II clinical trials in patients with CRPC, PPV was well tolerated with improved clinical outcomes [81]. In addition, 100 patients with progressive CRPC treated with PPV using 2-4 out of 31 candidate peptides showed improved PSA doubling time which correlated with antibody and T cell responses, and improved survival [82]. A phase II trial of PPV with or without low-dose cyclophosphamide showed no differences whether cyclophosphamide was used, however those who developed anti-peptide immune response showed longer improved survival regardless of the treatment arm [83]. Furthermore, a phase II trial (UMIN-CTR; 000000959) conducted in HLA-A2+, HLA-A24+ or HLA-A3+ asymptomatic patients with CRPC, were either injected with PPV (37 patients) or dexamethasone alone (35 patients). Those that received PPV showed significant longer progression-free survival than those receiving dexamethasone only (22 months vs 7 months); the improved survival was also longer in the PPV group (73.9 months vs 34.9 months) [84]. Moreover, BrightPath Biotherapeutics Co. Ltd is sponsoring a phase III, randomized, placebo-controlled double blind trial using PPV (ITK-1; 2-4 peptides) in HLA-

A24+ individuals with docetaxel-refractory mCRPC in Japan (UMIN-CTR; 000011308); 333 patients will be recruited, antibody, CD8+ T cell responses, frequency of adverse reactions and survival rate at 12 months will be assessed [71]. The last patient follow-up is expected to be completed by March 2018.

Checkpoint Inhibitors

Tumor-induced immunosuppressive environment plays a crucial role in the pathogenesis of cancer [85]. Targeting immune checkpoints such as CTLA-4 and PD1/PD-L1 pathways are gaining much attention, in the immunotherapy of cancer.

CTLA-4 checkpoint blockade

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) or CD152, is an immune checkpoint receptor responsible for suppressing CD8+ T-cell activation. CTLA-4 is also constitutively expressed on regulatory T cells where it mediates their immune suppressive effects [86]. Hence, CTLA-4 blockage could result in broad enhancement of antitumor immune responses, leading to the development of monoclonal antibodies that specifically inhibit CTLA-4. Indeed, ipilimumab and tremelimumab, are anti-CTLA-4 humanized monoclonal antibodies that have the potential to inhibit CTLA-4 ligand-driven immunosuppression. A number of ongoing studies are determining the efficacy of ipilimumab in early stage PC. Tables 2 and 3 summarize some selected completed clinical trials and the selected ongoing studies of checkpoint inhibitors in PC respectively.

PD-1 checkpoint inhibitors

The programmed cell death protein 1 (PD-1) or CD279, is expressed on activated T cells which binds to programmed death ligand 1 or 2 (PD-L1 or PD-L2) on tumor cells, resulting in inactivation and death of T cells. The absence of PD-1 expression on T cells has shown to significantly delay tumor growth and increase CD8+ T cells within the tumor microenvironment in mouse models [87]. Interestingly, tumor biopsies of 7 patients with PC noted that 90 % of the infiltrating CD8+ T cells had upregulated cell surface expression of PD-1 [88]. Nivolumab, a PD-1 inhibitor approved by the FDA for use in metastatic melanoma, showed no objective responses in a phase I trial of 17 patients with mCRPC [89].

However, in another trial, 36 % of those with PD-L1 positive tumors had an objective response to treatment. Thus, PD-L1 appears to be a viable biomarker which has significant correlation with response to nivolumab [90]. A Phase Ib, dose escalation ongoing study of nivolumab (MDX-1106) aims to determine its safety and efficacy in patients with certain types of cancer, including PC (NCT00730639).

In addition, a phase II trial of combined PD-1 and CTLA-4 blockade in patients with mCRPC is being tested (nivolumab and ipilimumab; NCT02601014). Furthermore, pembrolizumab, an anti-PD-1 monoclonal antibody is under evaluation as a single agent in mCRPC patients previously treated with enzalutamide (NCT02312557) in combination with pTVG-HP plasmid DNA vaccine (NCT02499835). CT-011, an anti-PD-1 antibody, is being assessed in a phase II trial in combination with sipuleucel-T and low dose cyclophosphamide in advanced patients with CRPC (NCT01420965) A phase II study of pembrolizumab in combination with enzalutamide in mCRPC patients upon progression on enzalutamide alone showed a PSA decline of over 50 % in 20 % of the patients of which some of them remained progression-free for up to 60 weeks [91].

Combination therapy strategy

Combination therapy is showing greater promise in cancer treatment. In addition to the vaccine platforms in early stage PC there is growing interest in combination therapy based on significant clinical and preclinical studies. In combination therapies, the addition of conventional medicine to the vaccine enhances the therapeutic efficacy through various mechanisms. Hormonal combination, chemotherapy combination, immune checkpoint inhibitor combination and radiation combination are studied in recent years. A summary of clinical trials in combination therapies for the management of PC are shown in Table 4.

Conclusion

Despite tremendous efforts in the last few decades, there is only one approved vaccine for PC with several others in clinical testing. Immune checkpoint inhibitors have shown some promise in improving immune based therapies, although it is suggested that single agent immune checkpoint inhibitors may have limited clinical utility. But a growing amount of preclinical and clinical data suggests that combining immune checkpoint inhibitors, either

with other immune checkpoint inhibitors or with therapeutic cancer vaccines, has the potential to improve immune response efficacy of vaccines. The improvements in vaccine delivery methods are significant to date, and we are well placed with a plethora of information and methods to determine the optimal regime for the treatment of mCRPC.

Future Perspectives

Identification of new PC antigens: Further studies of known PC antigens (MUC1, epidermal growth factor receptor, PSMA, prostate stem-cell antigen, platelet derived growth factor, metalloproteinase and urokinase plasminogen activator) and identification of new antigens will advance vaccination targeting strategies for PC. The six-transmembrane epithelial antigen of the prostate (STEAP1) is highly expressed by prostate cancer cells and it is thought to be involved in tumor initiation and progression and may be useful in the diagnosis of early disease. In addition, PC cells that express STEAP1 results in poorer clinical outcomes in patients with PC and is therefore, a potential target for immunotherapy studies [92]. In addition, in February 2017 Panacea Pharmaceuticals, Inc. reported that a vaccine based on the novel transmembrane protein, human aspartyl-asparaginyl- β -hydroxylase (HAAH) used to screen for upregulation of cancer proteins will be evaluated in patients with biochemically-relapsed PC. PAN-301-1 (NTC03120832) will be tested in an open-label, parallel-designed, multi-center phase I clinical trial to assess its safety and immunogenicity. The clinical trial vaccine is delivered via intradermal injection using 3M Drug Delivery Systems' hollow microstructured transdermal system (hMTS). It is delivered as "a quick injection" via the hMTS, which is a patient-friendly microneedle delivery solution [93]. The estimated completion date is December 2017. Other PC antigens such as, early prostate cancer antigen-1 and 2 (EPCA-1, EPCA-2), prostate cancer antigen-3 (PCA-3) and PSA isoforms are new and evolving PC antigens to be considered as targets for immunotherapy studies.

New modes of PC antigen delivery: Vaccine development faces major challenges both technologically and economically. Newer vaccines that are stable, economical, require fewer doses and can be administered using needle free systems are a worldwide priority [94]. Hence, delivery of vaccines via oral, intranasal, transcutaneous and intradermal routes will

decrease the risk of needle-borne diseases and may eliminate the need for trained personnel and sterile equipment. As such, the Nanopatch™ has revolutionized vaccination delivery where vaccine is applied to the skin for 2 minutes and efficient transcutaneous antigen delivery ensures [95]. Although there are no studies of such technology for PC, data from other settings (influenza vaccine, poliovirus vaccine) are promising. It is speculated that in coming years there will be a number of exciting data in the application of the Nanopatch™ technology in the treatment of various cancers including PC. Additionally, various techniques involving new DNA delivery systems (receptor mediated, non-invasive ultrasound delivery), adjuvants, micro/nano particles, dendrimers, immunostimulatory complexes and transgenic plants are being developed and evaluated. Moreover, micellar suspensions, melt in mouth strips, nasal mucosal, polymeric nanoparticles and micro needle delivery strategies will take PC treatment options to a new level in coming years [94].

New generation checkpoint molecules: could there be a role in PC: Identification of new checkpoint proteins other than CTLA-4 and PD-1/PD-L1, expressed on T cells may further revolutionize immunotherapeutic approaches for cancer., B7 group of proteins are cell surface proteins expressed on activated antigen presenting cells (DC, macrophages) and interacts with either CD28 or CTLA-4 (CD152) on activated T cells. As a result, co-stimulatory or co-inhibitory signals are activated. In fact, the interaction between B7-1 (CD80) and B7-2 (CD86) costimulatory markers on antigen presenting cells with CD28 on T cells results in enhanced T-cell activation, whereas, B7-H1 (PD-L1 or CD274, expressed on antigen presenting cells and cancer cells) interaction with PD1 (on T cells) inhibits T cell functionality. In addition, B7-DC (PD-L2, CD273) also expressed on antigen presenting cells binds to PD-1 on T cells leading to their inhibition. The role of other B7 ligands (B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), B7-H6 (NCR3LG1) and B7-H7 (HLA2)) are not clear [96], although B7-H3 and B7-H4 are implicated in immune-modulatory functions within the tumor microenvironment promoting cancer development [97]. TIM3 expressed on Th1 CD4+ and CD8+ T cells (but not on Th2 cells) regulates the activation of macrophages. TIM3 also stimulates cancer progression, maintaining the tumor immunosuppressive microenvironment status by inducing T cell suppression [98]. Over-expression of TIM3 on infiltrating CD4 and CD8 T cells within the tumor microenvironment and on peripheral blood T cells of PC patients, correlates with advanced disease stage [99]. In various preclinical

models, inhibition of both PD-1 and TIM3 enhanced antitumor immune responses, support TIM3 as a new potential target for immunotherapy studies [100]. Understanding the role of B7 proteins and TIM3 may prove useful checkpoint inhibitors in future preclinical and clinical studies in the management of PC.

Executive summary

- The first autologous cellular immunotherapy approved by the FDA, sipuleucel-T shows promising results in patients with PC
- GVAX, PROSTVAC, TroVax, Ad5-PSA, AdV-tk including ProstAtak™ and tumor cells transfected with mRNA are emerging gene based strategies for PC
- DNA vaccine formulations including plasmid DNA encoding PAP or electroporation delivery induce immune responses in patients with PC
- Personalized peptide vaccination (PPV) uses multiple peptides based on the individuals pre-existing antibody and/or T cell immunity and has shown promise in boosting such immunity.
- The combination of immune checkpoint inhibitors and therapeutic cancer vaccines is of particular interest as it has the potential to increase efficacy compared to single agent immune checkpoint inhibition with minimal added toxicity.
- Identification of new checkpoint proteins other than CTLA-4 and PD-1/PD-L1 has revolutionized immunotherapeutic approaches for prostate cancer treatment.
- Phase III Clinical trial (NCT02111577) is recruiting patients for the study of DCVAC added to standard chemotherapy for men with mCRPC and the primary objective of the study is to evaluate overall survival. In this study DC will be used as biological intervention and docetaxel and taxotere as chemotherapeutic agents.
- A phase II clinical trial is recruiting patients for the study of PROSTVAC in combination with nivolumab and /or ipilimumab in males with PC (NCT02933255).

Conflict of interest

There is no financial disclosure from any authors and no conflict of interest is reported.

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Table 1: Summary of some selected ongoing/completed clinical trials utilizing DNA vaccines for PC

Intervention	Phase & trial ID	Objectives of the study	Outcome/study completion date	Ref
PAP: Sipuleucel-T with or without pTVG-HP DNA booster vaccine	Phase II (NCT01706458)	Measurement of immune response	Ongoing/July 2021	[101]
PAP Plus rhGM-CSF with or without pTVG-HP	Phase II (NCT01341652)	Metastasis-free survival	Ongoing/March 2020	[102]
PROSTVAC V/F with or without GM-CSF	Phase III (NCT01322490)	Overall survival (OS)	Ongoing/June 2018	[103]
Flutamide with or without PROSTVAC/TRICOM	Phase II (NCT00450463)	Time to treatment failure	Study completed in June 2017. No results have been posted	[104]
Adenovirus/PSA Vaccine	Phase II (NCT00583024)	PSA-doubling time response	Ongoing/July 2017	[105]
PSA: Adenovirus/PSA with or without ADT	Phase II (NCT00583752)	PSA-doubling time response	Ongoing/July 2017	[105]

Table 2: Summary of completed trials of checkpoint inhibitors alone or in combination with other agents in prostate cancer

Study compound	Study details	Results	Reference
Ipilimumab	A Phase II study to evaluate OS, in chemotherapy naive mCRPC patients	Completed, No results available	NCT01057810
Ipilimumab	A phase II study to evaluate the Immunological variables measurements in patients with prostate cancer before radical prostatectomy	Completed, No results available	NCT01194271
Ipilimumab	A phase III study to determine if asymptomatic or minimally symptomatic patients with mCRPC who have not received chemotherapy live longer when treated with ipilimumab than those treated with a placebo	No significant difference in OS between treatment and placebo [28.6 months vs 29.73], PFS time was more in treatment group (5.59 months) than placebo (3.81 months)	NCT01057810
Ipilimumab with or without Radiation therapy (RT)	A phase I/II study to assess safety of ipilimumab alone or with RT in patients with mCRPC with or without prior chemotherapy	PSA decline >50%:16%	[106]
Ipilimumab plus ADT	A phase II study to compare a single dose of ipilimumab with ADT versus ADT alone in patients with mCRPC	Patients treated with ipilimumab plus ADT were more likely to have undetectable PSA levels by 3 months (55% vs 38%)	[107]
Ipilimumab with or without Docetaxel	A phase II study to compare the co-administration of ipilimumab alone or with docetaxel in chemotherapy-naive patients with mCRPC	Co-administration of docetaxel did not improve the activity of ipilimumab	[108]
Ipilimumab following RT	A randomized, phase III trial to compare ipilimumab vs placebo following RT in patients with mCRPC previously treated with docetaxel	The primary objective was not achieved [OS: 11.2 months vs 10 months; p=0.053]. Improvement PFS [4 months vs 3.1 months; p<0.0001] and in	[109]

		PSA response [13.1 % vs 5.2%]	
Ipilimumab plus PROSTVAC	A phase I dose-escalation trial to assess the safety of ipilimumab with and PROSTVAC in patients with mCRPC	The level of PSA was decreased by 58%	[110]
Ipilimumab plus GVAX	A phase I dose-escalation trial using one GVAX priming dose along with ipilimumab in patients with mCRPC	The level of PSA declined >50%	[111]
Ipilimumab plus GM-CSF	A phase I dose-escalation trial to assess the safety of ipilimumab with a fixed dose of GM-CSF	The level of PSA was decreased >50%	[112]
Tremelimumab plus ADT	A phase I dose-escalation trial to assess safety of tremelimumab in combination with bicalutamide	There was no significant increase in PSA doubling time	[113]

OS, overall survival; mCRPC, metastatic castration-resistant prostate cancer; PSA, prostate-specific antigen; ADT, androgen deprivation therapy; HR, hazard ratio; PFS, progression-free survival; RT, radiotherapy; GMCSF, granulocyte-macrophage colony-stimulating factor.

Table 3: Summary of selected ongoing checkpoint inhibitors in patients with mCRPC

Study compound and study Phase	Primary endpoints	Trial Status	Clinicaltrials.gov ID
Nivolumab and/or Ipilimumab, phase I/II	To evaluate changes in T-cell infiltration in the tumor after neoadjuvant treatment	Estimated study completion date August 2021	NCT02933255
Ipilimumab/early phase I	To assess the impact of Ipilimumab on T cell responses to new antigens	Active, estimated study completion date, August 2018	NCT02113657
Ipilimumab plus AA, phase I/II	PFS and safety	Active, estimated completion date September 2017	NCT01688492
Ipilimumab with Nivolumab, phase II	To assess the changes in PSA response (> 50% PSA decline) using PCWG2 guidelines	Active, estimated completion date February 2019	NCT02601014
Ipilimumab with Degarelix, phase II	To evaluate an undetectable PSA at 12 and 20 months (weeks 52 and 84, respectively) from the start of treatment among patients with non-castrate (> 150 ng/ml) levels of testosterone.	Active, estimated completion date December 2018	NCT02020070

Table 4: Selected combination strategy in clinical trial phases for the management of PC

Title	Clinical trial ID number and phase	Objective & present status of trial
Sequencing of Sipuleucel-T and ADT in males with non-metastatic PC	NCT01431391, phase II	To determine whether ADT started before or after sipuleucel-T leads to a better immune system response. Evaluation of the safety of sipuleucel-T treatment, immune system responses over time, the characteristics of sipuleucel-T, and changes in PSA values over time. Study completed. Immune response was more in Sipuleucel-T followed by ADT than ADT followed by Sipuleucel-T in regards to T cell stimulation
Enzalutamide in combination with PSA-TRICOM in patients with non-mCRPC	NCT01875250, phase II	To compare the safety and effectiveness of enzalutamide with and without vaccine therapy for advanced PC. Estimated completion date January 2019
Enzalutamide +/- vaccine therapy for advanced PC	NCT01867333, phase II	A comparative study on the safety and effectiveness of enzalutamide with and without vaccine therapy for advanced PC. Estimated completion date January 2019
Sipuleucel-T +/- radiation therapy in treating patients with hormone-resistant metastatic PC	NCT01807065, phase II	A feasibility study, based on the % able or willing to receive all 3 infusions of sipuleucel-T immunotherapy, when combining sipuleucel-T with radiation therapy to a single site of metastasis delivered one week prior to beginning of sipuleucel-T therapy. Estimated completion date January 2018
Sipuleucel-T and Stereotactic Ablative Body Radiation in mCRPC patients.	NCT01818986, phase II	To evaluate the synergistic effects of immunotherapy and stereotactic ablative body radiation which is expected to improve the treatment outcome for mCRPC. Estimated completion date December 2018
Vaccine therapy with pembrolizumab in treating patients with hormone-resistant, metastatic PC	NCT02499835, phase I and II	A randomized pilot trial to study vaccine therapy and pembrolizumab in treating patients with PC that do not respond to treatment with hormones (hormone-resistant) and have spread to other places in the body (metastatic). Monoclonal antibodies, such as pembrolizumab, may find tumor cells and help kill them. Giving pTVG-HP plasmid DNA vaccine and pembrolizumab may kill more tumor cells. Estimated completion date April 2019