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Immunotherapy in prostate cancer: review of the current evidence

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Abstract Prostate cancer is the most common male malignancy in the Western world. Once it metastasizes, it is incurable. The current gold standard for metastatic disease is the combined docetaxel/prednisone regimen. Prostate cancer shows several characteristics that make it a suitable candidate for immunotherapy, as recently exemplified by the approval of sipuleucel-T, the first vaccine to treat any malignancy. Here, we review different tumor-associated antigen immunotherapy strategies currently being investigated, from a humanized radiolabeled monoclonal antibody (J-591) that targets radiation into tumor cells, moving on to vaccines and through to immunomodulator agents such as anti-CPLA-4 and anti-PD-1 monoclonal antibodies that activate T-cell responses via immune checkpoint inhibition. We explore different opinions on the best approach to integrate immunotherapy into existing standard therapies, such as androgen-deprivation therapy, radiotherapy or chemotherapy, and review different combination sequences, patient types and time points during the course of the disease to achieve a lasting immune response. We present data from recent phase III clinical trials that call for a change in trial endpoint design with immunotherapy agents, from the traditional tumor progression to overall survival and how such trials should include immune response measurements as secondary or intermediate endpoints to help identify patient clinical benefit in the earlier phases of treatment. Finally, we join in the recent questioning on the validity of RECIST criteria to measure response to immunotherapeutic agents, as initial increases in the size of tumors/lymph nodes, which are part of a normal immune response, could be categorized as disease progression under RECIST.

Keywords Prostate cancer · Immunotherapy · Metastatic castration-resistant prostate cancer (mCRPC) · Prostate-specific antigen (PSA) · Prostate-specific membrane antigen (PSMA) · Prostatic acid phosphatase (PAP)

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Introduction

Prostate cancer (PC) is the most common male malignancy in the Western world; it is expected to affect 233,000 men and cause 29,500 deaths [1] in the United States (US) in 2014.

Although the majority of patients are diagnosed with localized disease, about a third will relapse after successful local therapy and others will present as locally advanced or metastatic disease upfront. Androgen-deprivation therapy (ADT) is the first-line gold standard in advanced PC [2–4]. However, despite initial response rates of 80–90 %, all patients will eventually progress and develop metastatic

castration-resistant PC (mCRPC). Several compounds have demonstrated activity and improved overall survival (OS), gaining approval by regulatory authorities [5–15]. Among them, an autologous antigen presenting cell (APC)-based cancer vaccine, sipuleucel-T, was approved by the Food and Drug Administration (FDA) [16] in 2010 and by the European Medicine Agency (EMA) in 2014 for the treatment of patients with asymptomatic or minimally symptomatic mCRPC. Sipuleucel-T represents the first cell-based immunotherapy (IT) able to demonstrate an improvement in OS in cancer patients, opening a new treatment paradigm.

Different reasons make PC a suitable model for IT. Firstly, PC presents a variety of tumor-associated antigens (TAAs) such as prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) and prostate-specific membrane antigen (PSMA), all of which have been shown to produce a clinical response through immunogenicity and also have been classified as self-antigens, with the advantage of being able to regulate the normal mechanisms that develop autoimmunity. Additionally, PC has a relatively slow growth rate that may allow the immune system (IS) the necessary time to produce a response and it seems to be more immunogenic than previously thought [17, 18]. Also, the prostate is a dispensable organ, so any autoimmunity generated would have little consequences. Multiple IT agents have recently been tested and will progressively join the clinic.

The general principles of immunology in cancer, with a focus on recently developed immune-based treatment strategies for mCRPC, as well as other relevant topics such as the integration of IT with other treatment strategies, response assessment and the identification of predictive biomarkers of response, are reviewed here.

Immunity and cancer

Innate immunity acts as a first line of defense upon foreign antigen (Ag) (from an infectious agent or a tumor cell) detection, involves neutrophils, macrophages and natural killer (NK) cells, and results in opsonization, phagocytosis and cytokine, chemokine and other proteolytic enzymes release. Adaptive immunity involves B and T-lymphocytes/cells, responsible for humoral [i.e., antibody (Ab) mediated] and cellular IRs, respectively [19, 20]. Early tumor cells are believed to be "attacked" by both innate and adaptive immune responses (IRs). Cells that escape these mechanisms move into an "equilibrium" phase that can last the host's whole life and relies on the adaptive IS. However, tumor cells develop mechanisms that allow them to escape the host's adaptive IS and to grow into clinically detectable malignancies.

B cells express B-cell receptors (BCR) on their surface and induce Ab production upon recognition of a foreign Ag. Some B cells will produce and release a specific Ab for a given Ag throughout the host's life span (i.e., long-term immunity). Complex mechanisms avoid autoimmunity (prevention of auto-antigen recognition) by circulating B lymphocytes [21]. T cells originate in the bone marrow and migrate to the thymus, where, through a finely regulated gene re-arrangement process, will express a great variety of T-cell receptors (TCRs) on their surface. Once outside the thymus, T cells require two signals before they can recognize Ags specific to their TCRs. The first comes from the recognition of a human leukocyte antigen (HLA) receptor on the surface of an APC, a type of B cell. The second involves co-stimulatory molecules on the surface of APCs, known as B7 (CD80 and CD86) proteins [22] that recognize CD28 proteins on the surface of T cells. In parallel, a co-inhibitory signal mediated by cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1) (also known as "checkpoints") on the T cell and their respective ligands, will result in T-cell inhibition [23]. Other checkpoints, such as T-regulatory cells (Tregs), are activated by CTLA-4 [24]; hence, blocking CTLA-4 also leads to Treg inhibition. "Checkpoint" inhibition can be used as a strategy for activating T-cell-mediated IRs [25]. Activated T cells proliferate and generate two types of effector T cells: CD4+ T helper (Th) cells and CD8+ T cytotoxic (CTLs) cells. There are two types of CD4+ Th cells: Th1 cells are involved in cellular IRs and Th2 cells are involved in humoral IRs. CD8+ CTLs are mainly cytotoxic, recognize Ags bound to HLA receptors and destroy them either through the insertion of perforins in the target cell membrane (allowing the entrance of enzymes that kill the cell) or through binding the target cell Fas receptor (leading to intracellular caspase activation [19, 20] and death). Both CD4+ and CD8+ T cells have the ability to mount a rapid response upon subsequent exposure to the same Ag (i.e., immunological memory).

Passive immunotherapy

Passive IT uses antitumor agents generated in vitro, such as monoclonal antibodies (mAbs) or cytokines, with intrinsic immunological activity. To date, only a humanized radiolabeled mAb, J591, is used to target radiation directly to the tumor cells. A summary of phase I–II trials with J591 is presented in Table 1 [26–28].

Active immunotherapy

Active IT (immunization/vaccination) intends to generate an IR by the host by activating CTLs [23] against TAAs.

Table 1 Selected overview of clinical trials with passive immunotherapy in mCRPC

Agent (MOA/target)	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Status
90-Y-J591 (humanized radiolabeled anti-PSMA mAb)	NA	Phase I trial of anti-PSMA mAb Y-90-J591 for androgen-independent PC	NA	MTD/RD determination	NA	PSA decline ($n = 2$) of 85 and 70 % (lasting 8 and 8.6 months, respectively), both had objective measurable disease responses; PSA stabilization ($n = 6$)	[26]	P
177-Lu-J591 (humanized radiolabeled anti-PSMA mAb)	NA	Phase I trial of anti-PSMA mAb 177-Lu-J591 in patients with androgen-independent PC	NA	MTD/RD determination	NA	≥50 % decline in PSA ($n = 4$) lasting from 3–8 months; PSA stabilization (for a median of 60 days) ($n = 16$)	[27]	P
177-Lu-J591 (humanized radiolabeled anti-PSMA mAb)	NA	Phase II study of anti-PSMA mAb 177-Lu-J591 for mCRPC	NA	Measurable disease RR and post-treatment PSA decline rate	NA	≥50 % PSA decline in 10.6 % patients; ≥30 % PSA decline in 36.2 % patients; evidence of dose response	[28]	P
177-Lu-J591 (humanized radiolabeled anti-PSMA mAb)	NA	A phase II trial of mAb 177-Lu- J591 in patients with metastatic androgen-independent PC	NA	PSA RR and measurable disease RR	NA	Data analysis pending	NCT00195039	C

Safety results for each trial are presented in the text

Ab antibody, *C* completed, *mAb* monoclonal antibody, *mCRPC* metastatic castration-resistant prostate cancer, *MOA* mechanism of action, *MTD* maximum-tolerated dose, *NA* not applicable, not available, *P* published, *PC* prostate cancer, *PSA* prostate-specific antigen, *PSMA* prostate-specific membrane antigen, *RD* recommended dose, *RR* response rate

Four types of vaccines, as well as immunomodulators (used to block immune "checkpoints") are able activate CTL responses and are currently being investigated for the treatment of mCRPC. B-cell Ab responses have also been observed in mCRPC patients who respond to CTLA-4 blockade treatment [29].

Autologous vaccines

Sipuleucel-T is designed against PAP. The vaccine process includes the collection of the patient's peripheral dendritic cells (DCs) (a type of APC) via leukapheresis and its incubation with a fusion protein (PA2024) composed of PAP (which targets the IR to PC cells) and granulocyte/macrophage-colony stimulating factor (GM-CSF) (which enhances the IR) [30, 31]. During a 36-h ex vivo incubation period, the patient's DCs break PA2024 into small peptides, later displayed by the HLA receptors. The activated DCs are then re-infused into the patient with the goal of generating a PAP-specific IR and the ensuing antitumor effect. A sipuleucel-T treatment means repeating this whole process three times at 2-week intervals. The quality of the reinfusion mix is ensured if it contains more than 40 million CD54+ DCs, as this has shown prolonged survival rates [32]. This approach has the advantages of activating APCs away from an immunosuppressive environment and directing the IR through an Ag-targeting process.

Three phase III clinical trials evaluated the efficacy of sipuleucel-T in mCRPC (Table 2). The first two trials [33, 34] (reported as a single integrated analysis) randomized patients to sipuleucel-T ($n = 147$) or placebo ($n = 78$) and had time-to-progression (TTP) as primary endpoint. A statistically significant OS benefit was demonstrated for patients treated with sipuleucel-T compared to those treated with placebo, translating into a 33 % reduction in the mortality risk. The most common adverse events (AEs) associated with treatment consisted of mostly mild-to-moderate chills, pyrexia, headache, asthenia, dyspnea, vomiting and tremor. Similar results were achieved in the third trial (IMPACT) [16] (Table 2), which randomly assigned patients to either sipuleucel-T (341 patients) or placebo (171 patients) and had OS as its primary endpoint. A relative reduction of 22 % in the mortality risk for patients treated with sipuleucel-T as compared to the placebo group translated into a statistically significant improvement in median OS. Results were unaltered after adjustment for the use of docetaxel after the study treatment (HR: 0.78; 95 % CI: 0.62–0.98; $P = 0.03$). IRs were reported in patients who received sipuleucel-T [32]. The AEs more frequently reported included chills, fever, and headache. Significantly, none of the trials showed a statistically significant advantage in the risk of disease progression (PD) for sipuleucel-T. Based on its survival advantage, sipuleucel-T was approved by the FDA in 2010 for the treatment of asymptomatic or minimally symptomatic mCRPC.

Cell-based vaccines

GVAX was developed by using an androgen-sensitive and an mCRPC cancer cell line (LNCaP and PC-3, respectively), and expressing GM-CSF [35–37].

Two phase III randomized trials tested GVAX in mCRPC (VITAL-1 and VITAL-2 in Tables 2, 5, respectively). VITAL-1 compared GVAX to D/P ($n = 626$) in asymptomatic CRPC patients, VITAL-2 compared a combination of GVAX with docetaxel against the gold standard D/P ($n = 408$) in symptomatic CRPC patients. Neither trial was able to show an OS advantage for GVAX. Both trials were prematurely terminated, VITAL-1 due to efficacy concerns (a futility analysis reported a less than 30 % chance of meeting an improved survival) and VITAL-2 due to an increased mortality rate in the GVAX plus docetaxel arm (67 deaths) compared to the D/P arm (47 deaths) [38, 39]. Although still unexplained, the increased death rate did not seem to be related to increased toxicity. The lack of a placebo-control group in the single-agent study (VITAL-1) has been used as an argument against the design of this trial [40], suggesting that, unless a placebo-control arm is used, CT should be standardized across treatment arms. Failure to optimize the dosing and timing of docetaxel in the combination regimen in a prior phase II study is also challenging the quality of VITAL-2 [40].

Another cell-based vaccine, Onyvax, has been made from three allogeneic PC cell lines, selected to contain elements from the major sites of the disease, OnyCap23 (similar to bone metastases), LnCaP (similar to lymph node metastasis) and P4E6 (derived from a primary PC biopsy). A phase II trial testing this vaccine in 26 mCRPC patients resulted in significant, prolonged reduction in PSA velocity (PSAV), no significant toxicity and an extended time to PD when compared to other standard treatments at a similar stage of the disease [41] (Table 2). PSAV-responding patients showed Th1 cytokine release in response to vaccine lysate re-stimulation and the immunologic profile correlated with PSAV response.

Cell-based vaccine therapies like GVAX and Onyvax have the potential to target multiple Ags with a favorable safety profile.

DNA-based vaccines

This type of vaccine uses plasmid DNA, which is taken up by the host's cells, which will subsequently express the proteins encoded within the plasmid and induce an IR.

Table 2 Selected overview of clinical trials with active immunotherapy in mCRPC

Agent (MOA/target)	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ ref.	Status
Autologous vaccines								
Sipuleucel-T (ex vivo autologous vaccine against PAP)	Placebo	Integrated data from two randomized, double-blind, placebo-controlled phase III trials of active cellular IT with sipuleucel-T in advanced PC	Sipuleucel-T vs. placebo	TTP	NA	Sipuleucel-T showed a 33 % reduction in the mortality risk (HR: 1.50; 95 % CI: 1.10–2.05; $P = 0.011$) compared to placebo (median OS: 23 vs. 18.9 mo.)	[34]	P
Sipuleucel-T (ex vivo autologous vaccine against PAP)	Placebo	Double-blind, placebo-controlled, multicenter phase III trial of active cellular IT with sipuleucel-T in CRPC	Sipuleucel-T vs. placebo	OS	Ag-specific IRs in 78.8 % patients and their presence correlated with OS ($P = 0.003$) [32]	Sipuleulcel-T showed a 22 % reduction in the mortality risk (HR: 0.78; 95 % CI: 0.61–0.98; $P = 0.03$) compared to placebo (median OS: 25.8 vs. 21.7 mo.)	[16]	P
Cell-based vaccines								
GVAX (cell-based allogeneic vaccine with two PC cell lines + GM-CSF)	Docetaxel plus prednisone	A phase III trial with a whole-tumor-cell mixture of two PC cell lines (GVAX) alone vs. standard CT (VITAL1)	GVAX vs. D/P	OS	NA	Median OS with GVAX was 20.7 mo. vs. 21.7 mo. with D/P (HR: 1.03, 95 % CI: 0.83–1.28; $p = 0.78$)	[38]	PT
Onyvax (cell-based allogeneic vaccine with three PC cell lines)	NA	A phase II trial with a whole-tumor-cell mixture of three PC cell lines with bacille Calmette–Guérin as vaccine adjuvant	NA	Clinical assessment: PSA levels, radiological scans and immune monitoring	Th1 cytokine release in PSAV-responding patients in response to vaccine lysate re-stimulation	Significant, prolonged reduction in PSAV in 42 % patients; extended time to PD (58 weeks) vs. other standard treatments (28 weeks)	[41]	P
DNA-based vaccines								
pVAX/PSA (plasmid vector expressing PSA with GM-CSF and IL-2 as adjuvants)	NA	A phase I trial of DNA vaccination with a plasmid expressing PSA in patients with hormone-refractory PC	NA	Safety and feasibility of the vaccine	PSA-specific cellular IR (IFN gamma production) at the highest dose; humoral IR (rise in anti-PSA IgG)	NA	[42]	P
PSMA/CD86 (PSMA in a viral vector + boosts of hPSMA/CD86 in a combined plasmid)	NA	Phase I/II clinical trial of naked DNA and adenoviral immunizations for IT in PC	NA	Safety	Immunization in all patients who received the viral vector vaccine followed by PSMA plasmid boosts	Several responders (change in local disease, distant metastases and PSA levels)	[43]	P

Table 2 continued

Agent (MOA/target)	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ ref.	Status
PSMA (genes encoding for mPSMA and hPSMA)	Xenogeneic (mouse)	Phase I trial of vaccination with a DNA encoding PSMA in patients with PC	Mouse vs. human (self) PSMA DNA followed by mouse DNA	Safety and immunogenicity	T-cell responses to PSMA-expressing fibroblasts at the highest dose	NA	[44]	AP
DNA fusion-gene vaccine (encodes DOM from tetanus toxin linked to a HLA-A2-binding epitope from PSMA)	NA	Phase I/II dose-escalation trial of a DNA fusion vaccine encoding a domain (DOM) from the tetanus toxin linked to an HLA-A2-binding epitope from PSMA	Intramuscular vaccination with or without electroporation	Immunogenicity and clinical response	Significant DOM-specific CD4+ ($p = 0.0223$) and PSMA-specific CD8+ T-cell responses ($p = 0.00248$)	PSA-DT increased significantly ($p = 0.0417$) (11.97 mo. pre-treatment vs. 16.82 mo. over the 72-week follow-up)	[45]	P
pTVG-HP (encoding hPAP and GM-CSF as adjuvant)	NA	Phase I/IIa trial conducted with a DNA vaccine encoding human PAP (hPAP) in patients with stage D0 PC	NA	Safety and immunological efficacy	PAP-specific IFN gamma-secreting CD8+ T-cell response in 14 % patients immediately after treatment and after 1-year follow-up [47]; PAP-specific CD4 + and/or CD8+ T-cell proliferation in 41 % patients	PSA-DT increased from 6.5 mo. pre-treatment to 8.5 mo. on-treatment ($P = 0.033$), and 9.3 mo. in the 1-year post-treatment period ($P = 0.054$)	[46]	P
pTVG-HP (encoding hPAP and GM-CSF as adjuvant)	GM-CSF alone	Randomized phase II trial of a DNA vaccine encoding PAP (pTVG-HP) vs. GM-CSF adjuvant in patients with non-metastatic PC	pTVG-HP vaccine with GM-CSF vs. GM-CSF alone	Two-year metastasis-free survival	NA	NA	01341652	R
pTVG-HP (encoding hPAP and GM-CSF as adjuvant)	Two different administration schedules	A pilot randomized two-arm study of a DNA vaccine encoding PAP in patients with non-metastatic CRPC	Vaccine q2wk for the first 12 weeks, then q12wk until PD vs. q2wk for the first 12 weeks, then q2wk, q4wk, or q3mo intervals as dictated by cellular IR	Safety and long-lived IRs	NA	NA	00849121	O
NY-ESO-1	NA	Phase I trial of a DNA vaccine encoding NY-ESO-1 in patients with solid tumors (including PC)	NA	Clinical outcome IR	NY-ESO-1-specific CD4+ T-cell and CD8+ T-cell responses	Despite IRs and a temporary increase in PS-DT, disease progressed in most cases	[48]	P

Table 2 continued

Agent (MOA/target)	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ ref.	Status
SSX2 (encoding two APLs)	NA	Phase I trial to determine whether modification in two HLA-A2-binding epitopes of SSX2 is able to enhance the efficacy of a DNA vaccine	NA	IR	Robust peptide-specific CD8+T cells and Th1 cytokines specific for each epitope	NA	[50]	P
Viral vector-based vaccines								
ProstVac (PSA-targeted poxviral vaccine including B7.1, ICAM-1 and LFA-3)	Control empty vectors plus saline injections	Phase II randomized controlled trial of a poxviral-based PSA-targeted IT in mCRPC	ProstVac plus GM-CSF vs. control empty vectors plus saline injections	PFS	No detectable Ab responses to PSA	Median OS significantly higher with ProstVac (25.1 vs. 16.6 mo.) (HR: 0.56; 95 % CI: 0.37–0.85; $P = 0.0061$); 3-year mortality reduced with ProstVac by a significant 44 %	[54]	P
ProstVac (PSA-targeted poxviral vaccine including B7.1, ICAM-1 and LFA-3)	ProstVac–GM-CSF	A randomized, double-blind, phase III efficacy trial of ProstVac ± GM-CSF in men with asymptomatic or minimally symptomatic mCRPC (Prospect)	ProstVacV/F + GM-CSF vs. ProstVacV/F–GM-CSF	OS	NA	NA	01322490	R
Adenovirus-PSA vaccine	NA	Phase I clinical trial of adenovirus/PSA vaccine in men with measurable mCRPC	NA	MTD determination	34 % of patients produced anti-PSA Abs; 68 % of patients produced anti-PSA T-cell responses	PSA-DT was increased in 48 % patients; 55 % patients survived longer than predicted	[56]	P
Adenovirus-PSA vaccine	Two different protocols are being explored	Phase II Study of adenovirus/PSA vaccine in men with hormone–refractory PC (APP22)	Newly recurrent PC treated with Ad5-PSA in a collagen matrix (as a single intervention or following ADT) vs. low CRPC disease burden treated with Ad5-PSA in a collagen matrix alone	Anti-PSA IR for patients with recurrent disease and PSA-DT, TTP and OS response for patients with low disease burden	100 % patients with recurrent disease and 67 % of patients with CRPC low disease burden have developed anti-PSA T-cell responses	NA	[58]	O

Safety results for each trial are presented in the text

Ab antibody, *ADT* androgen-deprivation therapy, *AP* abstract published, *APL* altered peptide ligand, *C* completed, *CI* confidence interval, *CT* chemotherapy, *D/P* docetaxel/prednisone, *h* human, *HR* hazard ratio, *hPSMA* human PSMA, *IR* immune response, *IT* immunotherapy, *mCRPC* metastatic castration-resistant prostate cancer, *mo.* months, *MOA* mechanism of action, *mPSMA* mouse PSMA, *MTD* maximum-tolerated dose, *NA* not applicable, not available, *O* ongoing, *OS* overall survival, *P* published, *PAP* prostatic acid phosphatase, *PC* prostate cancer, *PD* disease progression, *PSA* prostate-specific antigen, *PSA-DT* prostate-specific antigen doubling time, *PT* prematurely terminated, *q2wk* every 2 weeks, *q4wk* every 4 weeks, *q12wk* every 12 weeks, *q3mo* every 3 months, *R* recruiting, *TTP* time to progression

The first trial evaluating a DNA vaccine against CRPC was a phase I study with pVAX/PSA [42], targeting PSA. Vaccination with the plasmid vector pVAX/PSA plus GM-CSF and IL-2 as adjuvants, was tested at three different doses ($n = 8$). At the highest dose, the vaccine showed a PSA-specific cellular IR and a humoral IR. The vaccine had no AEs (Table 2). Other Ags targeted with this strategy include PSMA. A first phase I/II trial [43] ($n = 26$) could not conclude the effectiveness of the vaccine due to heterogeneity in the patient population and the concomitant use of hormone therapy in many patients. A second phase I trial targeting PSMA created a DNA vaccine encoding human PSMA and was followed by a DNA vaccine encoding mouse DNA (or vice versa) [44] under the hypothesis that a xenogeneic antigen was a more potent immunogen than self-Ags. T-cell responses to fibroblasts expressing PSMA were observed at the highest dose. In the next phase I/II trial, a DNA fusion vaccine encoded a domain from a fragment of the tetanus toxin (DOM) linked to a HLA-A2-binding epitope from PSMA [45]. The vaccine induced significant DOM-specific CD4+ and PSMA-specific CD8+ T-cell responses, PSA doubling time (PSA-DT) increased significantly over the 72-week follow-up, it was safe and well tolerated (Table 2). PAP has also been targeted in DNA vaccines. A phase I/IIa trial tested different doses of a DNA vaccine encoding human PAP plus GM-CSF (pTVG-HP) in patients with stage M0 PC [46] (patients with a diagnosis of PC and biochemical (serum PSA) recurrence after definitive surgery and/or radiation therapy with no evidence of suspected lymph node, bone, or visceral metastatic disease on bone scans or computed tomography scans). PAP-specific CD8+ T-cell responses were observed immediately after treatment and after 1-year patient follow-up [47] and PAP-specific CD4+ and/or CD8+ T-cell proliferation was also observed (Table 2). Humoral response was not detected and PSA-DT increased from 6.5 months pre-treatment to 8.5 months on-treatment and 9.3 months in the 1-year post-treatment period. A randomized phase II trial (NCT01341652) is currently evaluating the 2-year metastasis-free rate in patients receiving the vaccine and another trial (NCT00849121) is evaluating the safety of serial vaccinations and long-lived IRs and trying to find a better vaccination schedule. A cancer-testis Ag NY-ESO-1 has also been targeted with a DNA vaccine in a trial that included multiple solid tumor patients, including 10 patients with PC [48]. NY-ESO-1-specific CD4+ T-cell and CD8+ T-cell responses were reported after vaccination. However, responses were transient and disease progressed in most cases (despite a temporary increase in PSA-DT). In vitro depletion of Tregs restored detectable levels of Ag-specific effector T cells, an indication that Tregs down-regulate NY-ESO-1-specific T-cell responses [49].

Non-human DNA vaccines have already been approved in the US, preempting their potential in human IT. Among their advantages are the easier manufacturing, manipulation, storage and transport of the plasmid DNA as compared to peptides/proteins, bacterial and viral vectors, their cost-effectiveness and the fact that the bacterial backbone of the plasmid acts as an adjuvant. On the down side, they show weaker initial IRs than some of the alternatives already discussed, although this aspect can be overcome with repetitive immunizations [47] or with the alternative approach known as "altered peptide ligands (APLs)" consisting in the alteration of ligands in such a way that it results in an increased binding of the Ag to HLA receptors and an enhanced IR. One such case has been reported [50] (Table 2) in which two peptides within SSX2, a cancer-testis Ag expressed in 25 % of mCRPC lesions, were modified prior to insertion in a plasmid DNA vaccine in such a way that it increased their binding to HLA-A2, generated robust peptide-specific CD8+ T cells and produced Th1 cytokines specific for each epitope.

Viral vector-based vaccines

A gene is inserted into a recombinant virus vector, often a poxvirus (e.g., vaccinia, fowlpox). The Ags encoded in the viral vector (with or without co-stimulatory molecules) will then be lysed and taken up by APCs, which will present their peptides to CD4+ and CD8+ T cells. One of the intrinsic disadvantages of this type of vaccine is the development of host-induced Abs to the viral vector itself, which neutralizes the vaccine after several administrations and means most viral-based vaccines can be given only once [51]. A prime-boost approach has been used with ProstVac, a PSA-targeted poxviral vaccine, as an improved strategy that enhances the IR. It uses recombinant vaccinia for the prime vaccination and recombinant fowlpox for multiple booster injections that have been shown to induce non-neutralizing Abs in humans [52]. It also contains three T-cell co-stimulatory molecules (B7.1, ICAM-1 and LFA-3), designated as TRICOM [53]. Several studies have been conducted with ProstVac in mCRPC. A placebo-controlled phase II study [54] randomized (2:1) 125 patients to ProstVac plus GM-CSF or to control empty vectors plus saline injections. Median OS was significantly higher in the experimental arm, mortality at 3 years was significantly reduced with ProstVac and PD was similar in the two groups (Table 2). There were no detectable Ab responses to PSA and the vaccine was well tolerated. A global phase III study (NCT01322490) plans to recruit up to 1200 patients, who will be randomized to ProstVac, ProstVac plus GM-CSF or placebo and has OS as its primary endpoint.

Adenovirus type 5 (Ad5) vectors are also used for gen delivery and are useful adjuvants for the delivery of TAA-coding genes, due to their high affinity for DCs [55]. A phase I clinical trial tested Ad5-PSA in 32 mCRPC patients [56]. Antibodies against PSA were produced by 34 % of patients and anti-PSA T-cell responses were produced by 68 % of patients. PSA-DT was increased in 48 %, whereas 55 % survived longer than predicted (Table 2). A phase II study is in progress testing two vaccination protocols to determine if Ad5-PSA vaccines can yield therapeutic benefit. Patients with newly recurrent PC are being treated with Ad5-PSA/collagen matrix as a single intervention or following ADT (collagen matrixes have been shown pre-clinically to inhibit the production of anti-adenovirus Abs against the viral vector, resulting in more robust IRs than the use of Ad5-PSA alone) [57], while individuals with low CRPC disease burden are being treated with Ad5-PSA/collagen matrix alone. The development of anti-PSA IRs is the primary endpoint for patients with recurrent disease, while PSA-DT, time to progression and OS are the primary endpoints for CRPC patients with low disease burden. It has recently been reported [58] that 100 % of patients with recurrent disease and 67 % of patients with CRPC low disease burden have developed anti-PSA T-cell responses (Table 2).

The main advantage of viral vector-based vaccines lies on the fact that they retain their immunogenicity and lead to an increase in the TAA-specific T-cell IR, enhanced by the pro-inflammatory environment produced by the expression of viral proteins. Their main disadvantage is that most viral-based vaccines can be given only once to minimize Ab development to the viral vector.

Immunomodulator therapies

Co-inhibitory signaling result in T-cell inhibition and involves the following immune checkpoint molecules present on the T-cell surface: CD28, CTLA-4 and PD1. Immunomodulators are able to block co-inhibitory signals on the T cell, hence activating T-cell IRs.

Ipilimumab is a fully human monoclonal antibody that blocks CTLA-4, enhancing antitumor activity. Trials testing ipilimumab in PC are shown in Table 3. A phase I trial tested ipilimumab in mCRPC at increasing doses in combination with fixed doses of GM-CSF in order to see whether GM-CSF could enhance its antitumor efficacy. Three out of six patients treated at the highest dose had confirmed PSA declines of >50 %. Effector T-cell (CD25+ CD69+ CD8+) responses were of a higher magnitude at higher doses than with the same doses of either ipilimumab or GM-CSF alone [59]. A phase I/II trial is currently evaluating ipilimumab alone or in combination with radiotherapy (RT) in mCRPC [60] (Table 5). Results appeared to be in favor of the combination. Common AEs included fatigue, rash, pruritus, nausea, constipation and weight loss. Adrenal insufficiency, hepatitis and autoimmune colitis were some of the effects observed as a result of the activation of the IS. Two phase III trials are currently underway with ipilimumab in mCRPC. Patients with at least one bone metastasis from CRPC, were randomly assigned (1:1) in a phase II study to receive bone RT followed by either ipilimumab (10 mg/kg) or placebo every 3 weeks for up to four doses [61] after progressing to docetaxel. Non-progressing patients could continue to receive ipilimumab or placebo as maintenance therapy every 3 months until PD, unacceptable toxicity or death. Although OS (the primary endpoint) was not significantly different between the two arms, some signs of activity in favor of ipilimumab were observed. The most frequent grade 3–4 AEs included diarrhea (16 % in the ipilimumab group vs. 2 % in the placebo group), fatigue (11 vs. 9 %), anemia (10 vs. 11 %) and colitis (5 vs. 0 %). NCT01057810 is comparing ipilimumab with placebo in asymptomatic or minimally symptomatic CT-naïve mCRPC patients. Both have OS as their primary endpoint (Table 3).

PDL-1 is found on T-cells present in the prostate of men with mCRPC. Anti-PD-1 Abs block the PD1/PDL-1 interaction activating T-cell IRs. A phase I trial showed objective responses (complete or partial) in approximately 1:4–1:5 patients with other solid tumors with no significant AEs. No objective responses were observed in a group of 17 mCRPC patients [62]. To note, patients who did not respond had PDL-1-negative tumors.

Immunotherapy in neoadjuvancy in mCRPC

Neoadjuvancy aims at reducing the size of the tumor or the extent of the disease, increasing the probability of success of subsequent definitive procedures (surgical or RT) or decreasing the risk associated with such procedures when administered more extensively. Despite improvement in the reduction of prostate volumes and reduced serum PSA, published trials testing neoadjuvant ADT [63, 64], CT [65, 66] or targeted agents [67, 68] before radical prostatectomy (RP) surgery have not been able to prove a positive impact on OS, PFS or other clinically meaningful outcomes.

Given its novelty, clinical trials investigating neoadjuvant IT in mCRPC are sparse, highlighting the need to continue to investigate this aspect (Table 4). Early reports of a phase II trial currently taking place (NCT00715104) with neoadjuvant sipuleucel-T have reported the recruitment of effector CD3+ T cells into the tumor edge, supporting the proposed MOA for sipuleucel-T [69]. A neoadjuvant trial of GVAX pre-RP (NCT01696877) is

Table 3 Selected overview of clinical trials with immunomodulators in mCRPC

Agent MOA/target	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Trial status
Ipilimumab (Ab anti-CPLA-4)	NA	Phase I trial of ipilimumab + GM−CSF in patients with mCRPC	NA	Safety	Effector T-cell (CD25+ CD69+ CD8+) responses were higher at higher doses than with the same doses of either ipilimumab or GM-CSF alone	Three out of six patients treated at the highest dose had confirmed PSA declines of >50 %	[59]	P
Ipilimumab (Ab anti-CPLA-4)	Placebo	Phase III study ipilimumab vs. placebo in advanced PC patients following RT and after docetaxel	Ipilimumab vs. placebo	OS	NA	No statistically significant OS differences between the two arms, but some signs of activity in favor of ipilimumab	[61]	O
Ipilimumab (Ab anti-CPLA-4)	Placebo	Phase III study ipilimumab versus placebo in advanced PC patients who have not received prior CT	Ipilimumab vs. placebo	OS	NA	NA	NCT01057810	O
Anti PD-1 Ab	NA	Phase I study of anti-PD-1 Ab in patients with advanced melanoma, NSCLC, CRPC, renal-cell or CRC	NA	MTD determination	NA	No objective responses in any of the 17 mCRPC patients included	[62]	P

Safety results for each trial are presented in the text

Ab antibody, *CT* chemotherapy, *mCRPC* metastatic castration-resistant prostate cancer, *MOA* mechanism of action, *MTD* maximum-tolerated dose, *NA* not applicable, not available, *O* ongoing, *OS* overall survival, *P* published, *PC* prostate cancer, *PD* disease progression, *RT* radiotherapy

Table 4 Selected overview of clinical trials with immunotherapy in neoadjuvancy in mCRPC

Neoadjuvant agent	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Trial status
Sipuleucel-T	NA	Open-label, phase II trial of IT with sipuleucel-T as neoadjuvant treatment prior to a scheduled RP surgery in men with localized PC	NA	IR within prostate tissue following neoadjuvant treatment with sipuleucel-T	Recruitment of effector CD3+ T-cells into the tumor edge [69]	NA	NCT00715104	O
GVAX	ADT	A neoadjuvant immunologic study of ADT combined with a GM-CSF-secreting allogeneic PC vaccine and low-dose CP in men with high-risk localized PC undergoing RP surgery	ADT vs. low-dose CP followed by GVAX followed by ADT	Intraprostatic CD8+ T-cell infiltration and number of patients with AEs	NA	NA	NCT01696877	R
GVAX	NA	Phase II trial of neoadjuvant docetaxel plus GVAX followed by RP surgery in patients with high-risk clinically localized PC	NA	Pathologic state of pT0, (defined as no evidence of PC)	NA	Median change in PSA following neoadjuvant therapy was 1.47 ng/ml; downgrade in Gleason scores in 4/5 patients completing RP; undetectable PSA in 3 patients (2 mo. after RP) and in 2 patients (3 years after RP)	[70]	P
Ipilimumab	NA	A neoadjuvant phase IIa study of ipilimumab plus ADT in men with PC followed by RP surgery	NA	Effector T-cell/Treg cell ratio (in blood and tumor), CD4+ ICOS+ T cells (in blood and tumor), CD8+ ICOS+ T cells (in blood and tumor), NY-ESO-1 antibodies (in blood) and absolute lymphocyte count (in blood)	NA	NA	NCT01194271	O

Safety results for each trial are presented in the text

ADT androgen-deprivation therapy, *IR* immune response, *IT* immunotherapy, *mo.* months, *NA* not applicable, not available, *O* ongoing, *P* published, *PC* prostate cancer, *PSA* prostate-specific antigen, *R* recruiting, *RP* radical prostatectomy

currently randomizing patients with localized PC to ADT alone or low-dose cyclophosphamide (CP) followed by GVAX and ADT. Primary endpoints are intraprostatic CD8+ T-cell infiltration and safety and tolerability of the vaccine (Table 4). Neoadjuvant docetaxel/GVAX has been studied in locally advanced disease prior to RP [70]. Patients ($n = 6$) received four cycles of docetaxel and 2–3 days later, four courses of GVAX IT (in a prime-boost modality) preoperatively; six additional courses were given post-operatively. The primary endpoint of the trial was a pathologic state of pT0 (defined as no evidence of PC). Median change in PSA following neoadjuvant therapy was 1.47 ng/ml and four of the five patients completing RP showed a downgrade in their Gleason score. Undetectable PSA was achieved in three patients (2 months after RP) and in two patients (3 years after RP) (Table 4). No serious drug-related AEs were observed. A phase II study of neoadjuvant ipilimumab (NCT01194271) in combination with ADT prior to RP is currently ongoing. Primary endpoints include the measurement of the ratio effector T cell/Treg cell (in blood and tumor), CD4+ ICOS+ T cells (in blood and tumor), CD8+ ICOS+ T cells (in blood and tumor), NY-ESO-1 antibodies (in blood) and absolute lymphocyte count (in blood).

Immunotherapy in combination treatments

ADT and immunotherapy

ADT has direct effects on the IS, such as the inhibition of immune tolerance (which commonly develops in PC due to the fact that many of the antigens on PC cells are also present on normal prostatic epithelium) to TAAs [71] and increased T-cell infiltration of the prostate [72]. Therefore, administration of ADT prior to a vaccine or other IT agent might offer a potential means of increasing the patient's response to the treatment [72] (Table 5). A randomized phase II trial [73] evaluated sipuleucel-T when administered either 2 weeks before or 3 weeks after standard ADT. Ag-specific responses (the primary end point of the trial) were similar across arms; however, cytokine responses and CD8+ T-cell activation were higher when sipuleucel-T was administered after ADT, suggesting more robust IRs when the vaccine is administered after the ADT. Conflicting with this report are the results from a trial evaluating a combination treatment of ProstVac followed by nilutamide vs. nilutamide followed by ProstVac [74]. Although non-statistically significant, the results show a trend in OS in favor of the vaccine alone or the vaccine followed by nilutamide over nilutamide alone or followed by the vaccine. Given the conflicting results, more trials are necessary before any suggestions can be made as to where IT should fit with current standard ADT treatment. Another phase II trial (NCT00450463) is currently underway and testing the combination treatment ProstVac plus flutamide vs. flutamide alone. The primary endpoint is time-to-treatment failure. Ipilimumab is also being evaluated in combination with neoadjuvant ADT [75], as well as in hormone-naïve mPC and in mCRPC in two phase II trials (NCT01377389 and NCT01498978).

Chemotherapy and immunotherapy

Although the traditional view is that of CT being an immunosuppressive treatment, it is now thought that CT might actually stimulate the IS through Treg inhibition, activation of effector T cells and B cells and cytotoxicity (which results in higher processing and presentation of TAAs by APCs) [76]. Therefore, it is possible that neoadjuvant CT might help tumor shrinking prior to IT, as exemplified in the ongoing trial (NCT01696877) evaluating ADT alone vs. neoadjuvant low-dose CP followed by GVAX and ADT in localized PC patients (Table 5). The design was supported by pre-clinical work showing that low-dose CP can inhibit immune tolerance by increasing CD8+ T-cell infiltration into the prostate and inhibiting Tregs. A phase I trial (NCT00916123) is currently comparing the standard D/P CT in combination with increasing doses of the anti-PSMA mAb 177Lu-J591 in mCRPC patients. A phase II trial (NCT01145508) is currently ongoing in slowly progressing mCRPC that compares standard D/P CT with ProstVac (during 12 weeks) followed by the standard CT. The primary endpoint is OS.

Radiotherapy and immunotherapy

RT also has a cytotoxic effect that leads to both a higher processing and presentation of TAAs by APCs and an increase in the host's IR. Efforts to combine RT with IT in order to improve the efficacy of IT treatments [77] are being done. A phase II trial carried out in 30 patients randomized to either RT alone or in combination with a poxviral PSA vaccine showed that 13 out of 17 patients receiving the combined treatment had a threefold increase in PSA-specific T cells ($p < 0.0005$) [78] (Table 5). A phase I/II [60] and more recently a phase III [79] trial testing ipilimumab plus RT versus ipilimumab alone in mCRPC showed clinical antitumor activity with disease control and manageable AEs in the combination arm.

Combination of immunotherapies

Given all the different MOAs discussed, it might be expected that the combination of two or more ITs would result in more robust IRs. A phase I trial tested ipilimumab

Table 5 Selected overview of clinical trials with immunotherapy in combination treatments in mCRPC

Combination	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Trial status
ADT and immunotherapy								
ADT followed by sipuleucel-T	Sipuleucel-T followed by ADT	Phase II trial evaluating the optimal sequencing of sipuleucel-T and ADT by examining IR markers in patients with BRPC at high risk for metastases	Sipuleucel-T followed by ADT vs. ADT followed by sipuleucel-T	Ag-specific IRs to PA2024	Ag-specific responses similar across arms; cytokine responses and CD8+ T-cell activation higher when sipuleucel-T was administered after ADT	NA	[73]	P
ProstVac followed by nilutamide	Nilutamide followed by ProstVac	Randomized phase II study of ProstVac, nilutamide, and the combined therapy (crossover for each arm) in patients with non-metastatic CRPC	ProstVac followed by nilutamide vs. nilutamide followed by ProstVac	OS at 6.5 years from the initiation of therapy	NA	Non-statistically significant improvement in median survival for patients initially randomized to vaccine (5.1 vs. 3.4 years; $P = 0.13$) vs. patients initially randomized to nilutamide (6.2 vs. 3.7 years; $P = 0.045$)	[74]	P
ProstVac plus flutamide	Flutamide alone	A randomized phase II trial combining vaccine with ProstVac and flutamide vs. flutamide alone in men with androgen insensitive, non-metastatic (D0.5) PC	ProstVac and flutamide vs. flutamide alone	Time to treatment failure	NA	NA	NCT00450463	O
ADT followed by ipilimumab	NA	A phase II trial testing ADT followed by ipilimumab for the treatment of locally or systemically advanced PC prior to RP	NA	NA	NA	Dramatic disease down-staging in 2/5 patients; one disease-free patient (undetectable PSA for 19 mo. following adjuvant RT), the other patient awaiting f/u	[75]	AP
Ipilimumab plus ADT	NA	A phase II study of ipilimumab plus ADT in castrate-sensitive PC	NA	Proportion of participants achieving a PSA $\leq$0.2 ng/mL at month 7	NA	NA	NCT01377389	O
Ipilimumab plus ADT	NA	A phase II study of CTLA blockade by ipilimumab plus ADT in patients with an incomplete response to ADT alone for mPC	NA	Proportion of participants achieving a PSA $\leq$0.2 ng/mL	NA	NA	NCT01498978	O

Table 5 continued

Combination	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Trial status
Chemotherapy and immunotherapy								
Low-dose CP followed by GVAX followed by ADT	ADT	A neoadjuvant immunologic study of ADT plus GM-CSF-secreting allogeneic PC vaccine and low-dose CP in men with high-risk localized PC undergoing RP surgery	ADT vs. low-dose CP followed by GVAX followed by ADT	Intraprostatic CD8+ T-cell infiltration and number of patients with AEs	NA	NA	NCT01696877	R
Docetaxel/ prednisone plus fractionated 177Lu–J591	NA	A phase I trial of D/P plus fractionated 177Lu-J591 anti-PSMA mAb in patients with mCRPC	NA	MTD of fractionated 177Lu–J591	NA	NA	NCT00916123	U
ProstVac followed by D/P	D/P	Randomized phase II trial of docetaxel with or without PSA-TRICOM vaccine in patients with CRPC	ProstVac followed by D/P vs. D/P	OS	NA	NA	NCT01145508	O
GVAX plus docetaxel	Docetaxel plus prednisone	A phase III trial of GVAX IT in combination with docetaxel vs. D/P in symptomatic CRPC	GVAX plus docetaxel vs. D/P	OS	NA	OS of 12.2 mo. with GVAX plus docetaxel vs. 14.1 mo. with D/P (HR: 1.70, 95 % CI: 1.15–2.53; $p = 0.0076$)	[39]	PT
Radiotherapy and immunotherapy								
Poxviral PSA vaccine in combination with RT	RT alone	A randomized phase II trial designed with a poxviral vaccine encoding PSA combined with RT in patients with clinically localized PC	Poxviral PSA vaccine in combination with RT vs. RT alone	T-cell response	Threefold increases in PSA-specific T cells ($P < 0.0005$) in 13/17 patients on combination treatment	NA	[78]	P
Ipilimumab plus RT	Ipilimumab alone	Phase I/II study in patients with mCRPC exploring ipilimumab as monotherapy and in combination with RT	Ipilimumab as monotherapy vs. ipilimumab in combination with RT	Safety, PSA decline and tumor response	NA	Ipilimumab + RT suggested clinical antitumor activity with disease control (PSA declines of ≥50 % (3−13 + mo.) in eight patients, CR (11.3 + mo.) in 1 patient, SD (2.8−6.1 mo.) in six patients	[60]	P

Safety results for each trial are presented in the text

AP abstract published, *BRPC* biochemically recurrent prostate cancer, *CRPC* castration-resistant PC, *D/P* docetaxel/prednisone, *f/u* follow-up, *IR* immune response, *mo.* months, *O* ongoing, *P* published, *PT* prematurely terminated, *RT* radiotherapy, *U* unknown

Table 6 Overview of selected clinical trials with multiple immunotherapies in mCRPC

IT combination	Comparator	Trial description	Randomization	Primary endpoint	Immunological response	Clinical response	Trial ID/ref.	Trial status
Ipilimumab plus GVAX	NA	Open-label, single-center, dose-escalation phase I study of ipilimumab concurrent with a fixed dose of GVAX in patients with mCRPC	NA	Safety	NA	≥50 % declines in PSA from baseline in 25 % patients	[80]	Published
Ipilimumab plus ProstVac	NA	Phase I trial of a PSA-based vaccine and an anti-CTLA-4 Ab in adults with androgen-independent mPC	NA	Safety and tolerability of a combination of a fixed dose ProstVac vaccine and escalated doses of ipilimumab	NA	NA	NCT00113984	Completed
IL-21 plus an anti-PD1 Ab	NA	A phase I dose-escalation study of rIL-21 in combination with an anti-PD-1 Ab in subjects with advanced or metastatic solid tumors	NA	Safety	NA	NA	NCT01629758	Recruiting
Sipuleucel-T plus an anti-PD-1 mAb	Sipuleucel-T	A pilot study to test the feasibility and immunologic impact of sipuleucel-T administered with or without anti-PD-1 mAb (CT-011) and low-dose CP in men with advanced CRPC	Sipuleucel-T vs. sipuleucel-T plus an anti-PD-1 mAb vs. sipuleucel-T plus low-dose CP plus an anti-PD-1 mAb	Feasibility of sipuleucel-T plus low-dose CP as well as the immune efficacy of sipuleucel-T alone vs. sipuleucel-T plus low-dose CP and anti-PD1 mAb on the change in specific IR	NA	NA	NCT01420965	Recruiting
mAb J591 plus IL-2	NA	Open-label, non-randomized phase II trial of mAb J591 in combination with low-dose IL-2 in patients with recurrent hormone-refractory PC	NA	Safety/efficacy	NA	NA	NCT00040586	Completed

Safety results for each trial are presented in the text

Ab antibody, *CT* chemotherapy, *IR* immune response, *mAb* monoclonal antibody, *IL* interleukin, *mCRPC* metastatic castration-resistant prostate cancer, *NA* not applicable, not available, *PSA* prostate-specific antigen

in combination with GVAX [80], which appeared to be well tolerated and showed a decrease ≥50 % in PSA levels in 25 % of patients (Table 6). The safety and tolerability of a combination of ipilimumab and ProstVac are being analyzed in a phase I trial (NCT00113984), the safety and efficacy of IL-21 and anti-PD-1 are being tested in NCT01629758, the feasibility of treatment with sipuleucel-T with or without an anti-PD-1 mAb (CT-011) and low-dose CP in advanced CRPC is under review in clinical trial NCT01420965 and the mAb J591 is being investigated in combination with recombinant IL-2 in a phase II trial (NCT00040586).

The evaluation of response in immunotherapy

Measurable disease is infrequent in PC, yet Response Evaluation Criteria in Solid Tumors (RECIST) [81] continue to be the main guide to assess tumor response to therapeutic agents. The PC Clinical Trials Working Group (PC-CTWG) [82], which led the change from traditional trial objectives (early PSA decline and regression of target lesions) to time-to-event endpoints to ensure that a drug was not discontinued before it had had time to work, is now proposing two routes in drug evaluation, one for cytotoxic agents and a different route for non-cytotoxic drugs that work more on the basis of slow tumor growth. It advises to ignore early changes (within the first 12 weeks) in serum PSA, pain and bone scans, and recommends that disease assessments be performed at fixed intervals and at the time of study end. Moreover, it suggests that categorizations such as complete, partial or stable response be dropped in favor of "time-to-treatment failure" measures. Since the last PC-CTWG publication, three phase III clinical trials resulted in the approval of sipuleucel-T after showing a statistically significant OS benefit [16, 33, 34] despite showing no PFS improvement. These trials illustrate how OS, and not PFS, should be used as a more robust clinical trial endpoint in IT trials, as maximal antitumor IR may not occur until 12 weeks or longer after initiation of therapy [83]. OS benefit despite no PFS benefit in IT can be explained [84] because IT agents do not target the tumor itself, but the host's IS and it takes weeks to months to mount a clinically significant IR after immunization. Using OS as a clinical trial endpoint would mean, however, that trials would take years to complete; introducing measures of IR as secondary/intermediate endpoints could help solve this issue. Biomarkers of an IR could identify patient benefit in the earlier treatment phases and guide decisions to continue/discontinue therapy. Most biomarkers used to date are based on measuring CTL responses to specific TAAs [85] (production of gamma IFN ex vivo in an ELISPOT assay). However, results vary from institution to institution and the test is not able to assess the expansion of a T-cell response to TAAs not present in the vaccine via the "antigen cascade" mechanism (presentation by APCs of TAAs derived from dying tumor cells). Therefore, intermediate endpoints of response to IT need to be defined further.

In essence, while RECIST guidelines (originally designed for cytotoxic agents) assumed that an early increase in tumor growth and/or the appearance of new lesions signaled PD and resulted in treatment discontinuation, these criteria may not be sufficient to fully characterize the outcomes of IT [86], where responses may occur after conventional PD. Therefore, "clinically insignificant" PD (e.g., small new lesions in the presence of other responsive lesions) and durable SD may both represent antitumor activity and therapy discontinuation may not be appropriate unless PD is confirmed (as is usually done for response). Recent attempts to create new immune-related response criteria (irRC) [86, 87] have been done exclusively on ipilimumab phase II clinical data. Therefore, it is not clear whether this system could be extended to other ITs, such as vaccines, and how it would fit with the OS endpoint proposal. Another aspect to be taken into account in IT response assessment is that statistical methods need to be modified, as hazard ratios (i.e., the difference in death rates between active treatment and control changes with time) in their traditional way have no meaning [88], since there is a delayed separation of the Kaplan–Meier survival curves between control and treatment arms [85].

Conclusions

Due to its particular nature, PC often shows resistance to cytotoxic drugs, hence new therapeutic approaches that do not rely on high cell proliferation, such as IT, are now a welcome reality. Different IT agents have shown to be well tolerated and less toxic than traditional CT, as well as extending patient survival significantly in some cases.

The combination of one or more IT strategies with other standard PC treatments such as ADT, RT or CT seems to be the most effective way of inducing a lasting IR. The nature and sequencing of these combinations, their integration with current standard treatments as well as the type of patient (low tumor burden vs. heavily pre-treated mCRPC) and the best time (neoadjuvancy vs. adjuvancy) during the patient's clinical history remain to be characterized.

Given the time required for an IR to develop, and in view of the lack of association between OS and PFS, the former is now accepted as the more robust endpoint in IT trials. IR measurement should be introduced as a secondary endpoint to identify clinical benefit in the earlier phases of treatment. In addition to the CTL response measurement,

humoral responses, Treg depletion, tissue-based biomarkers and recruitment of immune cells to the tumor microenvironment are suggested as predictive biomarkers.

The value of RECIST in IT is challenged, as lymph node or tumor mass size increase as a result of the IR could be categorized as PD. If IT is to play a significant role in the future of PC, new clinically validated, standardized response criteria must be developed.

Conflict of interest EM Fernández-García, FE Vera-Badillo, B Perez-Valderrama, A Soto Matos-Pita and I Duran have no conflicts of interest related to this article.

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