A Perspective of Immunotherapy for Prostate Cancer

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Academic Editor: Vita Golubovskaya
Received: 28 April 2016; Accepted: 1 July 2016; Published: 7 July 2016

Abstract: In cancer patients, the immune system is often altered with an excess of inhibitory factors, such as immunosuppressive cytokines, produced by regulatory T cells (Treg) or myeloid-derived suppressor cells (MDSC). The manipulation of the immune system has emerged as one of the new promising therapies for cancer treatment, and also represents an attractive strategy to control prostate cancer (PCa). Therapeutic cancer vaccines and immune checkpoint inhibitors have been the most investigated in clinical trials. Many trials are ongoing to define the effects of immune therapy with established treatments: androgen deprivation therapy (ADT) and chemotherapy (CT) or radiotherapy (RT). This article discusses some of these approaches in the context of future treatments for PCa.

Keywords: prostate cancer; immune system; regulatory T cells; myeloid-derived suppressor cells; tumor associated macrophages; dendritic cells; immune therapy

1. Introduction

Prostate cancer (PCa) continues to be a major hurdle, as it is the second most common cancer among men. According to the National Cancer Institute, there are estimated 137.9 new cases per 100,000 men per year, which accounts for one in five new diagnoses [1]. PCa is the second leading cause of cancer-related mortality among men associated with an estimated 27,540 deaths in 2015 [2].

Patients with localized PCa are often successfully treated via local therapies (surgery or radiotherapy) [3] and patients affected by metastatic PCa received androgen deprivation therapy (ADT), which still represents the most common form of treatment [3]. When patients develop a disease state known as castration-resistant prostate cancer (CRPC), which can include various clinical states ranging from asymptomatic or minimally symptomatic, non-metastatic disease to symptomatic, metastatic diseases (mCRPC), the time of progression may vary for each patient.

For this setting of patients, following docetaxel-based chemotherapy, the FDA recently approved four new agents for the treatment of PCa: cabazitaxel, a taxane chemotherapy agent; abiraterone [4] and enzalutamide [5], which target the androgen receptor (AR) axis [6]; and radium-223, an α-emitting radiopharmaceutical [7]. However, several patients have shown primary resistance to these agents, although the mechanisms of resistance are not fully understood [8].

Therefore, additional treatment strategies are needed to further improve the survival outcomes of patients with advanced and metastatic PCa [9]. The manipulation of the immune system represents...
a promising approach for cancer treatment therapies, and also an attractive strategy to control prostate cancer [10,11].

This article reviews the role of immune-based therapies that target both lymphoid as well as myeloid cells and vaccines for the treatment of PCa.

2. Introduction to Immunotherapy

Many studies have reported a continued interplay between tumor cells and the microenvironment that may prove decisive in disease outcomes [12]. Host antitumor reactions could be considered two sides of the same coin in terms of their consequences in tumor development. Persistent inflammatory reactions may be an important contributor to tumor progression, whereas immune response to tumor cells may inhibit disease progression [13].

Furthermore, several pieces of evidence have indicated that the tumor microenvironment alters myeloid and lymphoid cells, facilitating the suppression of the host immune response [14].

Actually an immune response that is primarily cell-mediated has been observed in the normal human prostate [15]. Then histological data have revealed the presence of CD4+ T cells, CD8+ T cells, natural killer (NK) cells, dendritic cells (DC), and macrophages within tumors [16–19]. Generally, a dense infiltration of lymphocytes has been correlated with longer patient survival, whereas a significant reduction of T cells is detected in high grade prostatic adenocarcinomas compared to benign nodular prostate hyperplasia [16–19], suggesting that tumor progression may be associated with alterations in cell-mediated immune responses [20,21]. Furthermore, high density of M2-polarized tumor-associated macrophages (TAM) is observed in both epithelial and stromal compartments and is statistically associated to poorer prognosis [22,23]. TAMs are a significant component of the inflammatory infiltrates in Prostate. Moreover, increased TAMs levels in biopsy are predictive of worse recurrence free survival in men treated with primary ADT [24]. An inverse correlation between total macrophage density and time to recurrence has also been reported from different analysis [24,25]. Similar results were observed for Tregs [18,26–28]. Another negative prognostic factor is represented by increasing myeloid-derived suppressor cells (MDSC) detected both at tumor site as well in the peripheral blood of patients. The increase of these cells correlated with other negative prognostic factors, such as lactate dehydrogenase, alkaline phosphatase, PSA, and anemia in PCa [29]. Finally, a strong correlation between the DCs and PCa characteristics has also been observed. Patients with metastatic disease showed fewer circulating myeloid DCs than their age-matched controls [30] and a lower number of DCs was parallel with a higher Gleason score while DCs are elevated in low risk cancer [31]. These results indicate that, in PCa patients, monocytes do not develop into myeloid DCs as efficiently as they do in healthy individuals. This idea is also supported by observations that serum of PCa patients inhibited monocytes differentiation into DCs and that the degree of inhibition correlated with higher PSA levels [32].

Crosstalk between these cells could promote synergy and amplify the immune suppressive effects of individual cell population in PCa as well as in many other tumors [33].

The goal of immunotherapy is to overcome such mechanisms in order to detect and destroy cancer cells or at least to induce the pathways that go back from “the escape phase to equilibrium phase” according to the definition of the cancer immunoediting [12].

Cancer immunotherapy has recently been introduced into the therapeutic field of metastatic PCa and mCPRC. Current immunologic approaches with particular relevance to mCPRC are discussed in a more detailed way in the following sections dividing into antibodies, vaccines, adoptive T cell therapy (ADT) while in the last section several immune functions are targeted by a chemical compound.

3. Immune Check Point Inhibitor

The immune check points are important for allowing the immune system to maintain homeostasis and self-tolerance. This is obtained by down regulating T cell activation or effector functions [34]. However, these check points may also represent a common mechanism of tumor cell escape from the
immune system [35]. There are currently several ongoing investigations but the most promising results for cancer therapy have been obtained by targeting the Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Programmed T cell death 1 (PD-1) receptors [34–37]. CTLA-4, expressed on activated T cells and Tregs, down-regulates the extent of T cells activation by determining the balance with CD28 signals [38]. Both CTLA-4 and CD28 bind the ligand B7-1 (CD80) and B7-2 (CD86) but CTLA-4 has a higher affinity than CD28 [39,40]. The binding with CTLA-4 limits T cells expansion by reducing the production of an important growth factor as IL-2, and also by inhibiting TCR-mediated induction and assembly of essential components of the cell cycle machinery [40]. Targeting CTLA-4 in order to remove inhibition signals for effector T cells, deplete suppressor Tregs and restore an immunological response against the tumors is a long story. It began with the first murine models in 1996 [35], passed through the first FDA approval in 2011 as a treatment for patients with advanced melanoma [41] and many clinical trials are currently ongoing on several different types of tumors [37] included PCa [42].

The other promising therapeutic strategy is directed to PD-1 and its ligands. PD-1 is an immune regulatory T cells agent that is expressed on a subset of thymic T cells; it is upregulated on activated NK, B and T cells [43]. PD-1 has two ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC) that are expressed on antigen presenting cells (APC) [44]. PD-1 is normally involved in promoting tolerance and preventing tissue damage in setting of chronic inflammation [45]. The interaction PD-1 with PDL-1 inhibits T cell receptor signaling and downregulates the expression of some antiapoptotic molecules and pro-inflammatory cytokines [45]. The interaction of PD-1/PD-L1 is likely the principal mediator of immunosuppression [46].

The expression of PD-L1 on tumor cells is thought to play a role in decreasing the immune responses against the tumor contributing to tumor progression [47]. PD-L1 is expressed on a variety of solid cancers and is correlated with a worse prognosis. Moreover, an over expression of PD-1 on tumor infiltrating lymphocytes (TIL) matched with compromised antitumor response [48]. Finally, preclinical data have reported that blocking PD-1 or PD-L1 could restore immune function resulting in a reduction of tumor load and metastatic spread [48]. To date, three monoclonal antibodies (mAb) against PD-1, and one against PD-L1 have been analyzed in Phase I trials [48]. All four agents have shown encouraging preliminary activity, and those that have been evaluated in larger patient populations appear to have also an acceptable safety profile [49,50]. Using mAbs that inhibit the interaction between PD-1 and its ligand has shown the most significant antitumor effects primarily in melanoma however there are interesting prospective for other types of tumors [51].

The challenges are not always without risks and targeting immune check point increased immune surveillance but could also break immune tolerance to self and cause autoimmune side effects. Such immune-related adverse effects (iAE) most commonly manifest as diarrhea, colitis, rash, and pruritus (grade 1–2) or hepatitis, hypophysitis, and thyroiditis (grade 3–4) and are generally manageable and responsive to corticosteroid therapy or other immune suppressive agents [52]. Remarkably, immunosuppressive therapy does not appear to moderate ongoing antitumor effects [53].

*Ipilimumab* (Yervoy), an anti-CTLA-4 mAb and fully human IgG1 (Bristol-Myers Squibb), was the first immune check point blocking compound to enter in oncology clinical trial. The clinical activity obtained in melanoma was very encouraging with significant improvement of overall survival (OS) among patients with metastatic melanoma [41] and manageable iAEs [41]. Long term follow-up showed that 19%–36% of patients with metastatic melanoma treated with ipilimumab had long-term survival, some with survival rates extending up to four years [54–57], besides patients non-responsive became responsive after a more long time [56]. These results have been used to evaluate the therapeutic potential in a variety of solid cancers. Ipilimumab is currently in trials for the treatment of advanced non-small cell lung cancer (NSCLC) [58], metastatic renal cancer [59] and ovarian cancer [60]. Regarding PCa, initial studies have shown that ipilimumab (3 mg/kg) administered every four weeks for a total of four doses had acceptable safety profile [42] but anticancer effects were obtained especially in combination therapy [61–63]. In some CRPC patients, combining CTLA-4 blockade with systemic granulocyte-macrophage colony-stimulating factor (GM-CSF) induced a decline in PSA
an expansion of activated circulating CD8+ T cells, presumably mediated by preexisting tumor-specific T cells that were primed by endogenous tumor-derived antigens and were receptive to the CTLA-4 blockade [61]. Synergistic antitumor activity was evaluated also by combining immunomodulatory therapy with radiotherapy. Data showed that not only radiotherapy is not immunosuppressive but works synergistically with immunotherapy for enhancing antitumor immune responses, inhibiting immunosuppression, and/or altering tumor cell phenotype in order to increase their susceptibility to immune-mediated killing [64]. In a phase I–II study [62], ipilimumab administered alone in increasing dosage or in addition to a single dose of radiation each day was associated with clinical antitumor activity and disease control in the mCRPC patients primarily in who received ipilimumab 10 mg/kg ± radiotherapy [63]. But at high dosage (10 mg/kg) independent from radiotherapy iAEs became more frequent (all grades, 80%; grade 3/4, 32%), and in some cases last longer [62,63].

Further, a Phase III trial enrolled docetaxel-refractory men with mCRPC that were treated with radiation therapy to a bone metastasis followed by either ipilimumab (10 mg/kg every three weeks for a total of four doses) or placebo. In this trial, the patients lacking visceral disease and with favorable laboratory values treated with ipilimumab reported a prolonged OS [64,65].

Ongoing clinical trial is combining ipilimumab (3 mg/kg) and degarelix (a gonadotropin-releasing hormone (GnRH) receptor antagonist). This study provides two cohorts: one uses ipilimumab and degarelix prior to and following radical prostatectomy in men with newly mCRPC and the second cohort includes men who have already received definitive local therapy with radical prostatectomy but have since experienced biochemical and/or metastatic recurrence (NCT02020070). An overview of relevant ongoing clinical trials is provided in Table 1.

**Tremelimumab**, a fully human IgG2 (Pfizer, MedImmune) (CP-675,206) has been investigated in PCa both in neoadjuvant and in recurrent disease. In particular, in a phase I dose escalation trial [66], tremelimumab was combined with short-term ADT in patients with PSA-recurrent PCa. Three of 11 patients reported a prolongation in PSA doubling time detectable only several months after completing treatment while no favorable changes in PSA doubling time were observed in a shorter periods after completing treatment [66].

Tremelimumab was also included in a multicenter Phase I–II study combined with the PD-L1 antibody, durvalumab (MEDI4736), and the tumor microenvironment modulator polyICLC, a Toll like receptor-3 (TLR-3) agonist, in patients with several advanced, measurable, biopsy-accessible of cancers, including PCa (NCT02643303).

**Nivolumab** (Opdivo) an anti PD-1 mAb and fully human IgG4, approved by the FDA in 2014 for the treatment of malignant melanoma progressing after treatment with ipilimumab or after treatment with serine/threonine protein kinase B-Raf (BRAF) inhibitor [67–70]. Moreover nivolumab was also approved in 2015 for the treatment of refractory squamous NSCLC [70]. Recently, in December 2015, the FDA expanded the label to include the approval of Pembrolizumab (Keytruda) [71] for patients with metastatic melanoma who had not received prior ipilimumab.

A phase I study was performed to evaluate nivolumab in patients with a variety of malignancies [70]. This trial showed a favorable safety profile and a preliminary evidence of clinical activity, primarily in melanoma patients. In PCa, immune-histochemical analyses of PD-L1 expression have suggested an association of PD-L1 with a more aggressive tumor, indicating that the PD-1/PD-L1 pathway is correlated with the lower of antitumor immune response, promoting tumor proliferation and progression [72]. Further studies reported simultaneous high PD-1/PD-L1 expression in PCa patients enrolled to receive targeted anti-PD-1/PDL1 immunotherapy [73]. However only one of 17 patients with CRPC enrolled in the nivolumab trial reported a 28% reduction in measurable lesions and therefore no objective responses [74].

An approach to improve cancer immunotherapy has combined the two check point modulators. Indeed, ipilimumab and nivolumab have different biological characteristics that could result in synergic antitumor activity. Combining ipilimumab and nivolumab was analyzed in patients with metastatic melanoma to detect the maximum tolerated dose with the best clinical response [75]. The first clinical results
reported that the two immune check points work better in combination than when given separately. The melanoma patients experienced greater decrease in tumor size with longer progression-free survival (PFS) than either compound alone [76,77].

Table 1. An overview of relevant ongoing clinical trials in PCa.

| Therapy              | Molecule                        | Mechanism of Action                                         | Clinical Trial | Trial Identifier |
|----------------------|---------------------------------|-------------------------------------------------------------|----------------|-----------------|
| Ipilimumab (Yervoy®) | IgG1 Human monoclonal antibody  | Blocks the activity of CTLA-4 and T reg expression          | Phase II       | NCT01377389     |
|                      |                                 |                                                             | Phase I–II     | NCT01688492     |
|                      |                                 |                                                             | Phase II       | NCT01498978     |
|                      |                                 |                                                             | Phase II       | NCT02113657     |
|                      |                                 |                                                             | Phase I        | NCT00064129     |
|                      |                                 |                                                             | Phase II       | NCT02020070     |
|                      |                                 |                                                             | Phase II       | NCT02423928     |
|                      |                                 |                                                             | Phase II       | NCT02279862     |
| Tremelimumab (CP-675,206) | IgG2 Human monoclonal antibody  | Blocks the activity of CTLA-4 and T reg expression          | Phase I        | NCT02643303     |
|                      |                                 |                                                             | Phase I        | NCT02616185     |
| Nivolumab (Opdivo®)  | IgG4 Human monoclonal antibody  | Blocks the activity of PD-1                                 | Phase II       | NCT02601014     |
|                      |                                 |                                                             | Phase I–II     | NCT00064129     |
|                      |                                 |                                                             | Phase II       | NCT02020070     |
|                      |                                 |                                                             | Phase II       | NCT02423928     |
|                      |                                 |                                                             | Phase II       | NCT02279862     |
| Sipuleucel-T (Provenge®) | Autologous cellular immune-therapy | Stimulates a T cell immune response against cancer cells  | Phase II       | NCT01706458     |
|                      |                                 |                                                             | Phase II       | NCT02159950     |
|                      |                                 |                                                             | Phase II       | NCT01560923     |
|                      |                                 |                                                             | Phase II       | NCT01832870     |
|                      |                                 |                                                             | Phase II       | NCT02036918     |
|                      |                                 |                                                             | Phase II       | NCT01807065     |
|                      |                                 |                                                             | Phase II       | NCT02322230     |
|                      |                                 |                                                             | Phase II       | NCT01818986     |
|                      |                                 |                                                             | Phase II       | NCT02463799     |
|                      |                                 |                                                             | Phase II       | NCT01420965     |
|                      |                                 |                                                             | Phase II       | NCT01804465     |
|                      |                                 |                                                             | Phase II       | NCT01881867     |
|                      |                                 |                                                             | Phase II       | NCT01487863     |
|                      |                                 |                                                             | Phase II       | NCT01981122     |
| DCVAC/PCa            | Autologous Dcs pulsed with killed PSA-positive LnCap cells | Evocation of immune response                                | Phase III      | NCT02111577     |
| Prostvac-VF          | Viral based vaccine              | A recombinant viral vaccine based on combination of two viral particles. Promotes an immune response against PSA expressing cells | Phase III      | NCT01322140     |
|                      |                                 |                                                             | Phase II       | NCT02159918     |
|                      |                                 |                                                             | Phase II       | NCT01341652     |
|                      |                                 |                                                             | Phase II       | NCT00450463     |
|                      |                                 |                                                             | Phase II       | NCT01875250     |
|                      |                                 |                                                             | Phase II       | NCT02326805     |
|                      |                                 |                                                             | Phase II       | NCT02506114     |
| GVAX                 | Granulocyte-macrophage-colony stimulating factor tumor cell vaccine | Evocation of a strong immunoreaction by antigens expressed on human prostate cell lines modified by GM-CSF | Phase I–II     | NCT01696877     |
| CAR T cells therapy  | Chimeric antigen receptor T cells | Engineered patient’s T cells modified to recognize and destroy tumor cells | Phase I        | NCT0140373      |
| Tasquinimod (ABR-215050) | Quinolone-3-carboxamide analog | Inhibition of angiogenesis and immunomodulation             | Phase I        | NCT01513733     |

Combined trial is ongoing also in PCa, a phase II clinical trial (NCT02601014) is currently enrolling mCRPC patients with detectable androgen receptor-variant-7 (AR-V7), (tumor cells expressing AR-V7 has been shown to be resistant to hormone therapy and some chemotherapies). The outcome measures include a decline in PSA and PFS.
4. Therapeutic Cancer Vaccines

Therapeutic cancer vaccines have emerged as an attractive strategy to induce an antitumor immune response to shrink tumor and to protect against tumor recurrence or metastatic disease [78]. Preparing a successful cancer vaccine requires the selection of the opportune antigens and the suitable adjuvants to restore the immune response against the tumor, as well as the administration route [79,80]. Moreover, because immune responses may depend on presentation of the vaccine antigens by DCs, it is advantageous to administer APCs (i.e., DCs) with tumor antigens [79,80]. DCs have a critical role in preparation of vaccine, are routinely prepared ex vivo from peripheral blood mononuclear cells (PBMC) leukapheresis or buffy coats from CD34+ progenitor cells or CD14+ monocytes. Mature and immature DCs have been used, but mature DCs are superior in the induction of immune responses while immature DCs may induce tolerogenic responses [81]. The prostate represents an ideal target for cancer vaccines. Indeed several prostate-specific proteins have been identified to induce an immune response. In PCa, the immune response to specific antigens was approached by using both autologous and allogenic strategies [10,82,83].

*Sipuleucel-T* (Provenge) was the first autologous cellular immunotherapy approved by the FDA in 2010 and by the European Medicines Agency (EMA) for the treatment of asymptomatic or minimally symptomatic mCRPC [84], and to date it remains the only FDA-approved immunotherapy for PCa. This vaccine targets prostatic acid phosphatase (PAP), a secreted glycoprotein enzyme synthesized in prostate epithelium that significantly increases as cancer progresses; it is elevated in patients with bone metastasis and is associated with responsiveness to therapy and a shortened survival [85]. Rodent model and preclinical studies have determined that PAP elicits PAP-specific humoral and cellular immunity [86]. GM-CSF was added to stimulate APCs and obtain more mature DCs [87]. Autologous APC-containing peripheral blood mononuclear cells (PBMC) of PCa patients were harvested from a leukapheresis procedure, then transferred in a facility and incubated ex vivo for 36/48 h with a fusion protein (PA2024) combining PAP and GM-CSF. The fusion protein was washed out and finally the product was reinfused into the patient. This product contains at least $5 \times 10^7$ autologous activated CD54+ DCs and a variable number of T cells, B cells, NK cells, and other cells [87,88]. APCs should process the recombinant target antigen PAP-GM-CSF into small peptides that are presented to T cells [81].

*Sipuleucel-T* was routinely administered to patients via intravenous infusion. In a full course of therapy, this process is repeated twice at approximately two-week intervals. Phase I–II clinical trials have shown a good response to sipuleucel-T with few side effects that were primarily transient, low grade and infusion related [89,90]. After the treatment, the patients developed an appreciable antigen specific T-cells activation and a production of antibodies against the fusion protein. Moreover a PSA decline of more than 50% was reported in approximately 10% of patients [89,90].

Three phase III clinical trials have been completed. The two first studies analyzed sipuleucel-T versus placebo in asymptomatic mCRPC patients. The time to progression did not differ but there was a significant increase of OS (25.9 months versus 21.4 and 19.0 months versus 15.7) in patients treated with Sipuleucel-T [87,91]. The Phase III clinical trial, known as the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT), enrolled asymptomatic and symptomatic or minimally symptomatic mCRPC patients and reported a 4.1 months improvement in the median OS and at 36 months the survival rate was 31.7% for treated patients compared to 23.0% for patients treated with the placebo [92], no significant difference in the time to disease progression (14.6 weeks vs. 14.4 weeks).

Data from the IMPACT study showed that the greatest benefit occurred in patients with a lower disease burden [92,93]. Studies addressed for evaluating immune responses showed that sipuleucel-T induces the antigen-specific immune response as well as promotes the recruitment of activated effector T cells in the prostate tumor microenvironment [94,95]. Early screening and diagnosis are important for identifying patients who may benefit most from sipuleucel-T treatment. Patients with lower PSA levels indicative of an early stage of mCRPC appear to get better clinical outcomes [95,96]. Many studies are aimed at clarifying the mechanism of action of sipuleucel-T, at the identification of new biomarkers to
predict survival benefit and at optimizing the doses and the schedules of treatment of sipuleucel-T when combined with other therapies in clinical [95,96].

In fact this treatment has short duration (~4 week), providing an opportunity for patients to receive subsequent treatment. Most patients in the IMPACT study went on to receive other therapies [83]. Sipuleucel-T is also trialed combined with chemotherapy. Experimental data obtained in mice and humans have also contradicted the traditional thinking that taxanes suppress immune-cell functions. The same concepts are for radiotherapy [88].

A recent study has evaluated abiraterone in combination with sipuleucel-T and no alteration in immune parameters that correlates with sipuleucel-T was detected. A long-term follow-up for OS is ongoing in the STAMP study [97].

A case report described that the treatment with sipuleucel-T following to enzalutamide in a patient with mCRPC resulted in a complete and durable biochemical response [98].

Another approach of combining therapy with activated DCs is DCVAC/PCa.

DCVAC/PCa is an autologous immunotherapy. Immature DCs were harvested from leukapheresis and pulsed with killed LnCaP, a PSA-positive prostate cancer cell line. Tumor cell-pulsed DCs were then matured with 25 µg/mL of Poly I:C. Such vaccine was injected in patients with mCRPC that have already received docetaxel. From this Phase I/II clinical trial is resulted that the therapy was well tolerated and iAEs were low grade [99]. Actually, a phase III clinical trial (NCT02111577) is ongoing to evaluate the efficacy and the safety of DCVAC/PCa versus placebo in men with mCRPC eligible for first line chemotherapy. Lastly, a similar approach is in BDCA/1 BDC-01.

BDCA/1 BDC-01 is an autologous vaccine prepared by pulsing autologous CD1c+ BDC from leukapheresed PBMCs with a cocktail of HLA-A*0201-restricted peptides (PSA, PAP, prostate specific membrane antigen, and control influenza peptide) and keyhole limpet hemocyanin [100]. It was injected in 12 patients with mCRPC. Until now, no acceleration of disease was noted, PSA levels remained unchanged and iAEs were of low grade [100]. Other trials are necessity to evaluate its efficacy.

Prostvac-VF (viral-based vaccine) is a recombinant viral vaccine currently studied as an immunotherapy for PCa. Prostat-VF (TRICOM or PSA TRICOM) is based on a combination of two viral particles, vaccinia that is a potent immunologic priming agent, followed by fowlpox that is minimally or nonreactive to vaccinia that is used as a boosting agent [101]. Prostvac-VF targets PSA, one of the first antigens discovered to be expressed at substantial levels by most patients with PCa. It is known that PSA is released by the ductal and acinar epithelial cells of the prostate gland in males [85].

Both recombinant viruses were engineered to encode the entire PSA gene with a modified agonist epitope [101–103]. In addition, DNA encoding costimulatory molecules was incorporated in order to further enhance the immune response: B7-1 (facilitates T cell activation), lymphocyte function-associated antigen 3 (LFA-3; CD58, enhances signaling through the T cells receptor for antigen), and intercellular adhesion molecule-1 (ICAM-1; CD54, a cell surface adhesion molecule which plays a prominent role in regulating the migration and activation of both DCs and T cells) [101,103]. APCs should be activated directly by viral vectors and mainly by cellular debris containing encoded antigens derived from infected epithelial cells. Activated APCs present antigens to CD4+ and CD8+ T cells and induce T cell mediated immune response that detect and destroy PSA expressing cells [101,104].

Prostvac-VF does not require complex individualized therapy and is manufactured and reproduced easily. Negligible side effects were observed in treated patients; most iAEs were injection site related, that were identified in only a subset of patients and associated with symptoms of fatigue fevers, and nausea (grade 2 toxicity) and some flu-like symptoms [101].

Prostvac-VF was evaluated in a randomized phase II clinical trial in men with mCPRC. Comparing men who received Prostvac-VF and GM-CSF with men who received an empty vector with a placebo, the study showed positive results in the median OS with a difference of eight months in the treatment groups. The medians OS for the control group was 16.6 compared to 25.1 months for the Prostvac-VF group [105]. Additionally, an increase in T cell response, greater than six-fold, and a lower Tregs was observed in patients who survived longer than expected according the Halabi prognostic
model. No difference was detected for the GM-CSF combination [106]. In addition, for Prostvac-VF treatment, patients with less aggressive or early stage disease exhibited greater benefits [105,106].

A study addressed to understand the impact of this vaccine on generating tumor-specific T cells determined that a T-cell response, mostly CD8+, appeared predominant, with no evidence of B-cell response. This is a promising data considering the depletion of CD8+ at prostate cancer sites. Furthermore, the absence of antibody against PSA allows for the use of PSA levels to assess disease kinetics in vaccine-treated patients [107].

An international phase III trial of asymptomatic or minimally symptomatic men with mCRPC for treatment with and without GM-CSF is currently ongoing (NCT01322490). More than this an open label, randomized trial of PROSTVAC in combination of PROSTVAC with ipilimumab in localized PCa (NCT02506114).

GVAX, granulocyte-macrophage colony-stimulating factor tumor cell vaccine, represents a whole-cell based immunotherapy. In this approach, whole autologous or allogeneic tumor cells as source of immunogens are genetically modified to express GM-CSF. This growth factor induces an advantageous microenvironment for tumor antigen presentation through the recruitment of APCs, a critical step in the induction of an optimal immune response to any immunotherapy [10]. Because the small number of cells that can be obtained from surgically removed tumors limits autologous approach, GVAX for PCa is composed of two human prostate cell lines, LnCaP (androgen sensitive derived from a lymph node metastasis) and PC3 (androgen insensitive derived from bone metastasis) as antigens source, which are transfected with GM-CSF, and then irradiated for safety [107,108]. Phase I–II trials enrolling patients with CRPC, chemotherapy-naïve, received an intradermal priming vaccination with GVAX-Pca (5 × 10⁸ cells, half quantity of each cell line) followed by 12 weekly boost for six months [107] or ranged doses (1 × 10⁸ cells to 5 × 10⁸ cells) [108]. This immunotherapy was well tolerated and immunogenic for several patients in terms of dose and time treatment and was associated with an encouraging OS rates. These data supported to perform two phase III trials to confirm the survival benefits. The first phase III trial, Vaccine Immunotherapy with Allogeneic Prostate Cancer Cell Lines (VITAL)-1, was designed to compare GVAX to docetaxel plus prednisone in asymptomatic CRPC [109,110]. VITAL-2 was conducted in symptomatic CRPC patients [109,110]. The VITAL-2 study was early stopped due to increased mortality in the vaccine arm. The VITAL-1 study was also stopped based on a futility analysis because its primary end point was indicated as less than a 30% [109].

Preclinical studies have shown that GVAX combined with ipilimumab has a potent synergic effect evidenced by both biochemical and radiological responses in mCRPC patient [110]. The safety profile resulted in this study warrant further research.

5. Adoptive T Cell Therapy

Another strategy to induce the immune response against the tumor is directed to the adoptive T cell therapy (ACT). This therapy involves the isolation and the expansion of T cells, activated ex vivo, then reinjected into cancer patients with the goal of recognizing, targeting, and destroying tumor cells [111].

It has long been known that ACT based on tumor-infiltrating lymphocytes (TILs) can induce tumor regression [112]. Currently the best clinical responses are achieved in patients with metastatic melanoma [112]. Some treatments, such as lymphodepletion prior the reinfusion of TILs further enhance the responsivity [113]. The optimization of the treatment required also the co-administered of high dose of IL-2, which on the other hand can cause toxicity [114]. Probably, these conditions by removing Tregs and by stimulating T cells can overcome immune suppressive microenvironment [115] and mediate tumor regression, as observed in 50%–70% of patients with metastatic melanoma [116].

TILs can be grown from several cancer types, such as kidney, breast and colon but until now they have not shown cytolytic activity against autologous tumor cells [117]. Ongoing studies are addressed to other cancers that could express targeting antigens to TILs. Somatic mutations are often detected in tobacco related tumors, such as lung carcinomas and head and neck cancer [117]. Furthermore viral proteins expressed in human papilloma virus (HPV) primarily associated to oropharyngeal and
cervical cancers, as well as Epstein-Barr viral (EBV) proteins expressed in EBV-related cancers, included most Burkitt’s lymphoma, undifferentiated nasopharyngeal carcinoma, and lymphoproliferative disorders, some Hodgkin’s disease and non-Hodgkin’s lymphoma, and gastrointestinal lymphoma [117].

Since TILs with tumor-specific receptors can only be generated from some cancer patients, ADT has been improved by introducing antigen receptors into circulating lymphocytes.

**CAR T cells**, chimeric antigen receptors T cells, represent a therapy based on engineering patient’s T cells, specifically modified to recognize and destroy the tumor cells [118]. T cells can be reprogrammed to express chimeric antigen receptors (CAR) so that the specificity of the antibodies are combined with the cytotoxic functions of T cells in order to target several tumor antigens [119]. As above describe, PBMC must first be collected by leukapheresis and grown under conditions that will support the expansion and the stimulation of T cells.

CARs are generally composed of an extracellular single-chain antibody variable fragment (scFv) directed to TAA that is linked, via hinge and transmembrane domains, to intracellular signaling domains [120,121]. Genetic material is transferred into the patient’s T cells using either viral or non-viral vectors [121,122]. Because they are derived from antibodies, the recognition of target TAAs is not MHC-restricted and this technique can be applied to all individuals irrespective of their HLA type, and it can also recognize carbohydrate and glycolipid antigens.

Currently three generations of CARs have been described. First-generation contained only T cell CD3ζ chain and antigen recognition domains while subsequent second- and third-generation additional costimulatory molecules, such as: CD28, 4-1BB, CD27, ICOS or OX40, were added to increase the antitumor effects and to improve the proliferation and the survival of CAR T cells [123–127].

The clinical trials using first generation CAR T cells reported no objective responses [111]. Afterwards, the most clinical positive data have been achieved in the treatment of hematological malignancies [128], particularly with CD19-specific CAR T cells in patients with relapsed or refractory B-cell malignancies [129–132]. These results have raised strong interest in development of CARs for solid tumors as well and many studies are rapidly evolving in order to design an optimal CAR for the best results in human trials.

Indeed, upon infusion into a patient, CAR T cells must overcome a number of obstacles before attacking tumor cells and for this reason many strategies are still under investigation to improve the different steps.

Critical step is to determine the conditions leading to an optimal CAR T cells persistence [133] and selectively reducing Tregs [134]. It is also important to define those cytokines to include into CAR T cell gene in order to induce the physiologic mechanisms, memory formation and antigen-driven expansion [135]. Additionally, because CAR T cells are “living drugs” some imperfection may result in T cells that target and damage undesirable tissue or in over-proliferation. Thus, suicide genes are also necessary [136,137]. In solid tumors, other critical points are represented from tumor vasculatures, vascular normalization resulted after the treatment with low doses of angiogenesis inhibitor improved CAR T cells response [138,139]. As well as engineering with ligands of specific chemokines allows CAR T cells to more easily reach primary tumor and specially the metastasis [140,141]. Studies are also aimed to ameliorate CAR using humanized antibodies binding multiple antigens [142,143].

Until now, the most relevant results come from clinical studies with such therapy based on anti-CD19 CARs against a variety of B-cell malignancies. Treatment is associated with transient but frequently severe iAE related to elevated serum cytokine levels [117].

The greatest challenge is the identification of antigens in solid tumors target for CAR T cells therapy. Many tumor cell surface antigens are also expressed at low level on healthy tissue so that an immune response could be activated against vital organs. Mesothelin [144] and Epidermal growth Factor variant III (EGFvIII) [145,146] are presently being studied in a phase I clinical trial with careful dose escalation [117].
In PCa, CAR T cells can be engineered to target PSCA and PSMA. Slovin et al have established an ex vivo transduction, expansion and therapeutic protocol for the generation and to testing the safety, clinical-grade, PSMA targeted T cells in PCa [147].

There are only few clinical trials, and thus much more research is needed. For most patients, iAE are mild enough and can be managed with standard supportive therapies, including steroids. Cytokine-release syndrome is the most troublesome effect also induced in patients treated with Car T cells.

6. Tasquinimod

One compound under clinical investigation for the treatment of PCa as well as several solid tumors is tasquinimod (TasQ) [148]. Chemically, it is a second-generation quinoline-3-carboxamide compound with lack of pro-inflammatory effects and high efficacy against antiangiogenic activity [149]. The antiangiogenic property of this molecule is important because tumor growth inhibition induced by TasQ was associated with reduced microvasculature density, increased expression, and secretion of the angiogenesis inhibitor thrombospondin-1 (TSP-1), and downregulation of VEGF and hypoxia-inducible factor-1α (HIF) [150].

This orally agent has shown efficacy and favorable safety profile in Phase I–II clinical trial in PCa. At present time, phase III clinical trial on CRPC patients is in progress. Preclinical studies show that TasQ suppresses reciprocal cross-talk between cancer and tumor infiltrating host cells such as endothelial cells, MDSC, and macrophages [151,152] contributing to its pleiotropic effects, including anti-angiogenesis, immunomodulation, and inhibition of metastasis [153,154]. Although its mechanism of action is still under investigation, some target results seem to have an important role.

In this regard, the calcium binding protein, S100A9 has been identified as a potential target of TasQ [155,156]. S100A9 interacts with pro-inflammatory receptors: Toll like receptor (TLR)-4 and receptor of advanced glycation end products (RAGE) [155–157]. These receptors are expressed on MDSC and S100A9 regulates the recruitment from bone marrow [158] and the accumulation of these cells in tumor microenvironment inhibits the immune response [33]. The modulation of MDSCs represents a critical biologic mechanism of action of TasQ, indeed MDSCs induce an immune suppressive microenvironment and promote the M2-polarized TAMs that support angiogenesis and metastasis [33,159,160]. In prostate cancer, S100A9 is upregulated in both prostatic intraepithelial neoplasia and adenocarcinoma, whereas benign prostatic tissue showed minimal to no expression [161]. It is probably that antitumor activity of TasQ, in prostate as well as in other tumors, is attributable to the suppression of MDSCs [157].

In addition, results from other studies have reported histone deacetylase (HDAC)-4 as a molecular target for TasQ. TasQ binds and blocks HDAC4 in an inactive conformation, preventing epigenetic reprogramming needed for the angiogenic switch induced by HIF-1α [162]. HDAC-4 is overexpressed in CRPC and in vitro studies have shown a growth inhibition induced by suppression of HDAC-4 [162].

At dosage of 0.5–1 mg per day, TasQ is well tolerated and only common iAEs are reported in a phase I trial. A phase II trial, TasQ doubled the PFS at six months and prolonged survival of patients with mCRPC compared to placebo [152,163,164]. Exploratory biomarker analyses indicate that TasQ treatment had a more pronounced effect on OS in men with low levels of biomarkers, indicating a greater impact in men with a lower disease burden, similar to the observations for other immunotherapies. The systemic TSP-1 levels below the median at baseline correlated with a survival benefit with TasQ versus placebo [164]. A subsequent analysis of bone scan index (BSI) suggested a modest, short-term delay in objective radiographic bone scan progression [165]. Based on these preclinical and clinical observations, a phase III double-blind, placebo-controlled international trial of men with mCRPC and bone metastases was conducted (NCT01234311). Although the final manuscript and presentation are not yet available, the effects of TasQ are not consistent with the phase II observations [166]. It may be that TasQ, similar to other compounds, as a single agent has a modest activity, and only in combination can improve clinical outcome.
In this regards, a phase I non-randomized trial study is focused on evaluating in mCRPC men with chemorefractory the combination of prednisone and cabazitaxel with TasQ at the administration of 0.25 mg, 0.5 mg, and 1.0 mg (NCT01513733).

7. Conclusions

Immunotherapy can trigger a dynamic immune response that can kill tumor cells for an extended period time. Progress in immunotherapy has been a result of significant advances in our understanding of the complex nature of the regulatory events in cytotoxic T cell-mediated immune responses, particularly antigen presentation, activation and immuno-editing in the cancer microenvironment. Despite the success of these agents, their efficacies do not appear to be equal for all solid tumors.

Substantial evidence has suggested that combining different immunotherapeutic approaches, as well as combining other local or systemic cancer therapies, may likely be required to realize synergistic benefits. This could be a daunting task, given the non-classic cancer responses to immunotherapy and co-evolving immune escape mechanisms. Knowledge-based trials to help inform dosing, timing, and sequencing and the development of precise criteria for patient selection are needed. Studies regarding possible combinations of immunotherapies are ongoing to better establish safety and toxicity in addition to the efficacy of such treatments. We believe that identifying the doses and timing and sequences of combined treatments are crucial for gaining synergic effects.

Acknowledgments: The authors would like to thank Tiziano Pergolizzi for his assistance in drafting and preparing the manuscript.

Author Contributions: Conceived the concepts: Ida Silvestri, Susanna Cattarino, Sabrina Giantulli, Cristina Nazzari, Giulia Collalti, and Alessandro Sciarra. Evaluated the literature: Ida Silvestri, Susanna Cattarino, Sabrina Giantulli, Cristina Nazzari, Giulia Collalti, and Alessandro Sciarra. Wrote the first draft of the manuscript: Ida Silvestri, Susanna Cattarino, Cristina Nazzari and Giulia Collalti. Agree with manuscript results and conclusions: Ida Silvestri, Cristina Nazzari, Giulia Collalti and Alessandro Sciarra. Jointly developed the structure and arguments for the paper: Susanna Cattarino, Sabrina Giantulli, Giulia Collalti, and Alessandro Sciarra. Made critical revisions and approved final version: Ida Silvestri and Alessandro Sciarra. All authors reviewed and approved of the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2016. CA Cancer J. Clin. 2016, 66, 7–30. [CrossRef] [PubMed]
2. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2015. CA Cancer J. Clin. 2015, 65, 5–29. [CrossRef] [PubMed]
3. Heidenreich, A.; Bellmunt, J.; Bolla, M.; Joniau, S.; Mason, M.; Matveev, V.; Mottet, N.; Schmid, H.P.; van der Kwast, T.; Wiegel, T.; et al. EAU guidelines on prostate cancer. Part 1: Screening, Diagnosis, and treatment of clinically localized disease. Eur. Urol. 2011, 59, 61–71. [CrossRef] [PubMed]
4. De Bono, J.S.; Logothetis, C.J.; Molina, A.; Fizazi, K.; North, S.; Chu, L.; Chi, K.N.; Jones, R.J.; Goodman, O.B., Jr.; Saad, F.; et al. Abiraterone and increased survival in metastatic prostate cancer. N. Engl. J. Med. 2011, 364, 1995–2005. [CrossRef] [PubMed]
5. Scher, H.I.; Fizazi, K.; Saad, F.; Taplin, M.E.; Sternberg, C.N.; Miller, K.; de Wit, R.; Mulders, P.; Chi, K.N.; Shore, N.D.; et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N. Engl. J. Med. 2012, 367, 1187–1197. [PubMed]
6. Mohler, L.; Gregory, C.W.; Ford, O.H., 3rd; Kim, D.; Weaver, C.M.; Petrusz, P.; Wilson, E.M.; French, F.S. The androgen axis in recurrent prostate cancer. Clin. Cancer Res. 2004, 10, 440–448. [CrossRef] [PubMed]
7. Parker, C.; Nilsson, S.; Heinrich, D.; Helle, S.I.; O’Sullivan, J.M.; Fossà, S.D.; Chodacki, A.; Wiechno, P.; Logue, J.; Seke, M.; et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N. Engl. J. Med. 2013, 369, 213–223. [CrossRef] [PubMed]
8. Buttiglieri, C.; Tucci, M.; Bertaglia, V.; Vignani, F.; Bironzo, P.; di Maio, M.; Scagliotti, G.V. Understanding and overcoming the mechanisms of primary and acquired resistance to abiraterone and enzalutamide in castration resistant prostate cancer. Cancer Treat. Rev. 2015, 41, 884–892. [CrossRef] [PubMed]
9. Heidenreich, A.; Bastian, P.J.; Bellmunt, J.; Bolla, M.; Joniau, S.; van der Kwast, T.; Mason, M.; Matveev, V.; Wiegel, T.; Zattoni, F.; et al. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. *Eur. Urol.* 2014, 65, 467–479. [CrossRef] [PubMed]

10. Drake, C.G. Prostate cancer as a model for tumour immunotherapy. *Nat. Rev. Immunol.* 2010, 10, 580–593. [CrossRef] [PubMed]

11. Saad, F.; Miller, K. Current and Emerging Immunotherapies for Castration-resistant Prostate Cancer. *Urology* 2015, 85, 976–986. [CrossRef] [PubMed]

12. Mittal, D.; Hubert, J.; Saint, F.; Mottet, N. High-grade inflammation in prostate cancer as a prognostic factor for biochemical recurrence after radical prostatectomy. *Urology* 1999, 54, 467–472. [CrossRef] [PubMed]

13. Goujon, J.; Fuchs, C.S.; Dranoff, G. Cancer immunology-analysis of host and tumor factors for personalized medicine. *Rev. Clin. Oncol.* 2011, 8, 711–719. [CrossRef] [PubMed]

14. Gabrilovich, D.I.; Ostrand-Rosenberg, S.; Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* 2013, 12, 253–268. [CrossRef] [PubMed]

15. Mcardle, P.A.; Canna, K.; McMillan, D.C.; Mcnicol, A.M.; Campbell, R.; Underwood, M.A. The relationship between T-lymphocyte subset infiltration and survival in patients with prostate cancer. *Br. J. Cancer* 2004, 91, 541–543. [CrossRef] [PubMed]

16. Vesalainen, S.; Lipponen, P.; Talja, M.; Syrjänen, K. Histological grade, perineural infiltration, tumour-infiltrating lymphocytes and apoptosis as determinants of long-term prognosis in prostatic adenocarcinoma. *Exp. Mol. Pathol.* 2009, 86, 108–113. [CrossRef] [PubMed]

17. San, S.; Galon, J.; Fuchs, C.S.; Dranoff, G. Cancer immunology-analysis of host and tumor factors for personalized medicine. *Rev. Clin. Oncol.* 2011, 8, 711–719. [CrossRef] [PubMed]

18. Mantovani, A.; Allavena, P.; Balkwill, F. Cancer-related inflammation. *Nature* 2008, 454, 436–444. [CrossRef] [PubMed]

19. Lissbrant, I.F.; Stattin, P.; Wikstrom, P.; Damber, J.E.; Egevad, L.; Bergh, A. Tumor associated macrophages in human prostate cancer: Relation to clinicopathological variables and survival. *Int. J. Oncol.* 2000, 17, 445–451. [CrossRef] [PubMed]

20. Nonomura, N.; Takayama, H.; Nakayama, M.; Nakai, Y.; Kawashima, A.; Mukai, M.; Nagahara, A.; Aozasa, K.; Tsujimura, A. Infiltration of tumor-associated macrophages in prostate biopsy specimens is predictive of disease progression after hormonal therapy for prostate cancer. *BJU Int.* 2000, 107, 1918–1922. [CrossRef] [PubMed]

21. Shimura, S.; Aozasa, K.; Tsujimura, A. Infiltration of tumor-associated macrophages in human prostate cancer: Association with cancer progression. *Cancer Res.* 2000, 60, 5857–5861. [PubMed]

22. Wilke, C.M.; Wu, K.; Zhao, E.; Wang, G.; Zou, W. Prognostic significance of regulatory T cells in tumor. *Int. J. Cancer* 2010, 127, 748–758. [CrossRef] [PubMed]

23. Miller, A.M.; Lundberg, K.; Özençi, V.; Banham, A.H.; Egevad, L.; Pisa, P. CD4+CD25 high T cells are enriched in the tumor and peripheral blood of prostate cancer patients. *J. Immunol.* 2006, 177, 7398–7405. [CrossRef] [PubMed]
28. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

29. Idorn, M.; Køllgaard, T.; Kongsted, P.; Sengeløv, L.; Thor Straten, P. Correlation between frequencies of blood monocytes and myeloid-derived suppressor cells, regulatory T cells and negative prognostic markers in patients with castration-resistant metastatic prostate cancer. *Cancer Immunol. Immunother.* **2014**, *63*, 1177–1187. [CrossRef] [PubMed]

30. Aalamian-Matheis, M.; Chatta, G.S.; Shurin, M.R.; Huland, E.; Huland, H.; Shurin, G.V. Inhibition of dendritic cell generation and function by serum from prostate cancer patients: Correlation with serum-free PSA. *Adv. Exp. Med. Biol.* **2007**, *601*, 173–182. [PubMed]

31. Liu, Y.; Sæter, T.; Vlatkovic, V.; Servoll, E.; Waaler, G.; Axén, U.; Giercksky, K.E.; Nesland, J.M.; Suo, Z.H.; Axén, K. Dendritic and lymphocytic cell infiltration in prostate carcinoma. *Histol. Histopathol.* **2013**, *28*, 1621–1628. [PubMed]

32. Ostrand-Rosenberg, S.; Sinha, P.; Beury, D.W.; Clements, V.K. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Semin. Cancer Biol.* **2012**, *22*, 275–281. [CrossRef] [PubMed]

33. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

34. Rudd, C.E.; Taylor, A.; Schneider, H. CD28 and CTLA-4 coreceptor expression and signal transduction. *Annu. Rev. Immunol.* **2008**, *26*, 195–201. [CrossRef] [PubMed]

35. Chambers, C.A.; Kuhns, M.S.; Egen, J.G.; Allison, J.P. CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu. Rev. Immunol.* **2001**, *19*, 565–594. [CrossRef] [PubMed]

36. Chambers, C.A.; Kuhns, M.S.; Egen, J.G.; Allison, J.P. CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu. Rev. Immunol.* **2001**, *19*, 565–594. [CrossRef] [PubMed]

37. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

38. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

39. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

40. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

41. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

42. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

43. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

44. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

45. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

46. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]

47. Flammiger, A.; Weisbach, L.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schömig, T.; Trepel, M. High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. *Eur. J. Cancer* **2013**, *49*, 1273–1279. [CrossRef] [PubMed]
48. McDermott, D.F.; Atkin, M.B. PD-1 as a potential target in cancer therapy. Cancer Med. 2013, 2, 662–673. [CrossRef] [PubMed]
49. Hamid, O.; Carvajal, R.D. Anti-programmed death-1 and anti-programmed death-ligand 1 antibodies in cancer therapy. Expert Opin. Biol. Ther. 2013, 13, 847–861. [CrossRef] [PubMed]
50. Mahoney, K.M.; Freeman, G.J.; McDermott, D.F. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. Clin. Ther. 2015, 37, 764–782. [CrossRef] [PubMed]
51. Brahmer, J.R.; Tykodi, S.S.; Chow, L.Q.; Hwu, W.J.; Topalian, S.L.; Hwu, P.; Drake, C.G.; Camacho, L.H.; Kauh, J.; Odunsi, K.; et al. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. N. Engl. J. Med. 2012, 366, 2455–2465. [CrossRef] [PubMed]
52. Redman, J.M.; Gibney, G.T.; Atkins, M.B. Advances in immunotherapy for melanoma. BMC Med. 2016, 14, 20–31. [CrossRef] [PubMed]
53. Horvat, T.Z.; Adel, N.G.; Dang, T.O.; Postow, M.A.; Callahan, M.K.; Carvajal, R.D.; Dickson, M.A.; D’Angelo, S.P.; Woo, K.M.; et al. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. J. Clin. Oncol. 2015, 33, 3193–3198. [CrossRef] [PubMed]
54. Lebbé, C.; Weber, J.S.; Maio, M.; Neyns, B.; Harmankaya, K.; Hamid, O.; O’Day, S.J.; Konto, C.; Cykowski, L.; McHenry, M.B.; et al. Survival follow-up and ipilimumab retreatment of patients with advanced melanoma who received ipilimumab in prior phase II studies. Ann. Oncol. 2014, 25, 2277–2284. [CrossRef] [PubMed]
55. Wolchok, J.D.; Neyns, B.; Linette, G.; Negrier, S.; Lutzky, J.; Thomas, L.; Waterfield, W.; Schadendorf, D.; Smylie, M.; Guthrie, T., Jr.; et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: A randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol. 2010, 11, 155–164. [CrossRef]
56. Schadendorf, D.; Hodi, F.S.; Robert, C.; Weber, J.S.; Margolin, K.; Hamid, O.; Patt, D.; Chen, T.T.; Berman, D.M.; Wolchok, J.D. Pooled analysis of longterm survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J. Clin. Oncol. 2015, 33, 1889–1894. [CrossRef] [PubMed]
57. Prieto, P.A.; Yang, J.C.; Sherry, R.M.; Hughes, M.S.; Kammula, U.S.; White, D.E.; Levy, C.L.; Rosenberg, S.A.; Phan, G.Q. CTLA-4 blockade with ipilimumab: Long-term follow-up of 177 patients with metastatic melanoma. Clin. Cancer Res. 2012, 18, 2039–2047. [CrossRef] [PubMed]
58. Garon, E.B. Current perspectives in immunotherapy for non-small cell lung cancer. Semin. Oncol. 2015, 42, 11–18. [CrossRef] [PubMed]
59. Parekh, H.; Rini, B.I. Emerging therapeutic approaches in renal cell carcinoma. Expert Rev. Anticancer Ther. 2015, 15, 1305–1314. [CrossRef] [PubMed]
60. Tse, B.W.; Collins, A.; Oehler, M.K.; Zippelius, A.; Heinzelmann-Schwarz, V.A. Antibody-based immunotherapy for ovarian cancer: Where are we at? Ann. Oncol. 2014, 25, 322–331. [CrossRef] [PubMed]
61. Fong, L.; Kwek, S.S.; O’Brien, S.; Kavanagh, B.; McNeel, D.G.; Weinberg, V.; Lin, A.M.; Rosenberg, J.; Ryan, C.J.; Rini, B.I.; et al. Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. Cancer Res. 2009, 69, 609–615. [CrossRef] [PubMed]
62. Slovin, S.F.; Higano, C.S.; Hamid, O.; Tejwani, S.; Harzstark, A.; Alumkal, J.J.; Scher, H.I.; Chin, K.; Gagnier, P.; McHenry, M.B.; et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration resistant prostate cancer: Results from an open-label, multicenter phase I/II study. Ann. Oncol. 2013, 24, 1813–1821. [CrossRef] [PubMed]
63. Kwon, E.D.; Drake, C.G.; Scher, H.I.; Fizazi, K.; Bossi, A.; van den Eertwegh, A.J.; Krainer, M.; Houede, N.; Santos, R.; Mahammedi, H.; et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184–043): A multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014, 15, 710–712. [CrossRef]
64. Kwilas, A.R.; Donahue, R.N.; Bernstein, M.B.; Hodge, J.W. In the field: Exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. Front. Oncol. 2012, 2, 104–115. [CrossRef] [PubMed]
65. Finkelstein, S.E.; Salienius, S.; Mantz, C.A.; Shore, N.D.; Fernandez, E.B.; Shulman, J.; Myslicki, F.A.; Agassi, A.M.; Rotterman, Y.; DeVries, T.; et al. See comment in PubMed Commons below combining immunotherapy and radiation for prostate cancer. Clin. Genitourin. Cancer 2015, 13, 1–9. [CrossRef] [PubMed]
66. McNeel, D.G.; Smith, H.A.; Eickhoff, J.C.; Lang, J.M.; Staab, M.J.; Wilding, G.; Liu, G. Phase I trial of tremelimumab in combination with short-term androgen deprivation in patients with PSA-recurrent prostate cancer. *Cancer Immunol. Immunother.* 2012, 61, 1137–1147. [CrossRef] [PubMed]

67. Weber, J.S.; D’Angelo, S.P.; Minor, D.; Hodi, F.S.; Gutzmer, R.; Neyns, B.; Hoeller, C.; Khushalani, N.I.; Miller, W.H., Jr.; Lao, C.D.; et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2015, 16, 375–384. [CrossRef]

68. Robert, C.; Long, G.V.; Brady, B.; Dutriaux, C.; Maio, M.; Mortier, L.; Hassel, J.C.; Rutkowsk, P.; McNeil, C.; Kalinka-Warzocha, E.; et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* 2015, 372, 320–330. [CrossRef] [PubMed]

69. Rizvi, N.A.; Mazières, J.; Planchar, D.; Stinchcombe, T.E.; Dy, G.K.; Antonia, S.J.; Horn, L.; Lena, H.; Minenza, E.; Menencier, B.; et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): A phase 2, single-arm trial. *Lancet Oncol.* 2015, 16, 257–265. [CrossRef]

70. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B.; et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 2012, 366, 2443–2454. [CrossRef] [PubMed]

71. Sullivan, R.J.; Flaherty, K.T. Pembrolizumab for treatment of patients with advanced or unresectable melanoma. *Clin. Cancer Res.* 2015, 21, 2892–2897. [CrossRef] [PubMed]

72. Gevensleben, H.; Dietrich, D.; Golletz, C.; Steiner, S.; Jung, M.; Thiesler, T.; Majoizes, M.; Stein, J.; Uhl, B.; Müller, S.; et al. The Immune checkpoint regulator PD-L1 is highly expressed in aggressive primary prostate cancer. *Clin. Cancer Res.* 2015, 22, 1969–1977. [CrossRef] [PubMed]

73. Massari, F.; Ciccarese, C.; Calìò, A.; Munari, E.; Cima, L.; Porcaro, A.B.; Novella, G.; Artibani, W.; Sava, T.; Eccher, A.; et al. Magnitude of PD-1, PD-L1 and T lymphocyte expression on tissue from castration-resistant prostate adenocarcinoma: An exploratory analysis. *Target Oncol.* 2015, 11, 1–7. [CrossRef] [PubMed]

74. Bracarda, S.; Altavilla, A.; Hamzaj, A.; Sisani, M.; Marrococo, F.; Del Buono, S.; Danielli, R. Immunologic checkpoints blockade in renal cell, prostate, and urothelial malignancies. *Semin. Oncol.* 2015, 42, 495–505. [CrossRef] [PubMed]

75. Melero, I.; Berman, D.M.; Aznar, M.A.; Korman, A.J.; Pérez Gracia, J.L.; Haanen, J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nat. Rev. Cancer* 2015, 15, 457–472. [CrossRef] [PubMed]

76. Postow, M.A.; Chesney, J.; Pavlick, A.C.; Robert, C.; Grossmann, K.; McDermott, D.; Linette, G.P.; Meyer, N.; Giguere, J.K.; Agarwala, S.S.; et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N. Engl. J. Med.* 2015, 372, 2006–2017. [CrossRef] [PubMed]

77. Larkin, J.; Hodi, F.S.; Wolchok, J.D. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* 2015, 373, 1270–1271. [CrossRef] [PubMed]

78. Muenst, S.; Läubli, H.; Soysal, S.D.; Zippelius, A.; Tzankov, A.; Hoeller, C.; Khushalani, N.I.; Miller, W.H., Jr.; Lao, C.D.; et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2015, 16, 375–384. [CrossRef]

79. Muenst, S.; Läubli, H.; Soysal, S.D.; Zippelius, A.; Tzankov, A.; Hoeller, C.; Khushalani, N.I.; Miller, W.H., Jr.; Lao, C.D.; et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2015, 16, 375–384. [CrossRef]

80. Obeid, J.; Hu, Y.; Singluff, C.L., Jr. Vaccines, Adjuvants, and dendritic cell activators-current status and future challenges. *Semin. Oncol.* 2015, 42, 549–561. [CrossRef] [PubMed]

81. Joniau, S.; Abrahamsson, P.A.; Bellmunt, J.; Figdor, C.; Hamdy, F.; Verhagen, P.; Vogelzang, N.J.; Wirth, M.; van Poppel, H.; Osanto, S. Current vaccination strategies for prostate cancer. *Eur. Urol.* 2012, 61, 290–306. [CrossRef] [PubMed]

82. Fernandez-Garcìa, E.M.; Vera-Badillo, F.E.; Perez-Valderrama, B.; Matos-Pita, A.S.; Duran, I. Immunotherapy in prostate cancer: Review of the current evidence. *Clin. Transl. Oncol.* 2014, 17, 339–357. [CrossRef] [PubMed]

83. Wei, X.X.; Fang, L.; Small, E.J. Prostate cancer immunotherapy with sipuleucel-t: Current standards and future directions. *Expert Rev. Vaccines* 2015, 14, 1529–1541. [CrossRef] [PubMed]

84. Sims, R.B. Development of sipuleucel-T: Autologous cellular immunotherapy for the treatment of metastatic castrate resistant prostate cancer. *Vaccine* 2012, 30, 4394–4397. [CrossRef] [PubMed]
85. Matera, L. The choice of the antigen in the dendritic cell-based vaccine therapy for prostate cancer. *Cancer Treat. Rev.* 2010, 36, 131–141. [CrossRef] [PubMed]

86. Johnson, L.E.; Frye, T.P.; Arnott, A.R.; Marquette, C.; Couture, L.A.; Gendron-Fitzpatrick, A.; McNeel, D.G. Safety and immunological efficacy of a prostate cancer plasmid DNA vaccine encoding prostatic acid phosphatase (PAP). *Vaccine* 2006, 24, 293–303. [CrossRef] [PubMed]

87. Small, E.J.; Schellhammer, P.F.; Higano, C.S.; Redfern, C.H.; Nemunaitis, J.J.; Valone, F.H.; Verjee, S.S.; Jones, L.A.; Hershberg, R.M. Placebo-controlled phase III trial of immunologic therapy with Sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J. Clin. Oncol.* 2006, 24, 3089–3094. [CrossRef] [PubMed]

88. Tse, B.W.; Jovanovic, L.; Nelson, C.C.; de Souza, P.; Power, C.A.; Russell, P.J. From bench to bedside: Immunotherapy for prostate cancer. *Biomed. Res. Int.* 2014, 1–11. [CrossRef] [PubMed]

89. Small, E.J.; Fratesi, P.; Reese, D.M.; Strang, G.; Laus, R.; Peshwa, M.V.; Valone, F.H. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J. Clin. Oncol.* 2000, 18, 3894–3903. [PubMed]

90. Burch, P.A.; Breen, J.K.; Buckner, J.C.; Gastineau, D.A.; Kaur, J.A.; Laus, R.L.; Padley, D.J.; Peshwa, M.V.; Pitot, H.C.; Richardson, R.L.; et al. Priming tissue specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin. Cancer Res.* 2000, 6, 2175–2182. [PubMed]

91. Higano, C.S.; Schellhammer, P.F.; Small, E.J.; Burch, P.A.; Nemunaitis, J.; Yuh, L.; Provost, N.; Frohlich, M.W. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer* 2009, 115, 3670–3679. [CrossRef] [PubMed]

92. Kantoff, P.W.; Higano, C.S.; Shore, N.D.; Berger, E.R.; Small, E.J.; Pennon, D.F.; Redfern, C.H.; Ferrari, A.C.; Dreicer, R.; Sims, R.B.; et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N. Engl. J. Med.* 2010, 363, 411–422. [PubMed]

93. Schellhammer, P.F.; Chodak, G.; Whitmore, J.B.; Sims, R.; Frohlich, M.W.; Kantoff, P.W. Lower baseline prostate-specific antigen is associated with a greater overall survival benefit from sipuleucel-T in the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial. *Urology* 2013, 81, 1297–1302. [CrossRef] [PubMed]

94. Wesley, J.D.; Whitmore, J.; Trager, J.; Sheikh, N. An overview of sipuleucel-T: Autologous cellular immunotherapy for prostate cancer. *Hum. Vaccines Immunother.* 2012, 8, 520–527. [CrossRef] [PubMed]

95. Agarwal, N.; Padmanabh, S.; Vogelzang, N.J. Development of novel immune interventions for prostate cancer. *Clin. Genitourin. Cancer* 2012, 10, 84–92. [CrossRef] [PubMed]

96. Pieczonka, C.M.; Telonis, D.; Mouraviev, V.; Albala, D. Sipuleucel-T for the treatment of patients with metastatic castrate-resistant prostate cancer: Considerations for clinical practice. *Rev. Urol.* 2015, 17, 203–210. [PubMed]

97. Small, E.J.; Lance, R.S.; Gardner, T.A.; Karsh, L.I.; Fong, L.; McCoy, C.; DeVries, T.; Sheikh, N.A.; GuhaThakurta, D.; Chang, N.; et al. Randomized phase II trial of sipuleucel-T with concurrent versus sequential abiraterone acetate plus prednisone in metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* 2015, 21, 3862–3869. [CrossRef] [PubMed]

98. Graff, J.N.; Drake, C.G.; Beer, T.M. Complete biochemical (prostate-specific antigen) response to sipuleucel-T with enzalutamide in castration-resistant prostate cancer: A case report with implications for future research. *Urology* 2013, 81, 381–383. [CrossRef] [PubMed]

99. Podrazil, M.; Horvath, R.; Becht, E.; Rozkova, D.; Bilkova, P.; Sochorova, K.; Hromadkova, H.; Kayserova, J.; Vavrova, K.; Lastovicka, J.; et al. Phase I/II clinical trial of dendritic-cell based immunotherapy (DCVAC/PCa) combined with chemotherapy in patients with metastatic, castration-resistant prostate cancer. *Oncotarget* 2015, 6, 18192–18205. [CrossRef] [PubMed]

100. Prue, R.L.; Vari, F.; Radford, K.; Tong, H.; Hardy, M.Y.; D’Rozario, R.; Waterhouse, N.J.; Rossetti, T.; Coleman, R.; Tracey, C.; et al. A phase I clinical trial of CD1c (BDCA-1)+ dendritic cells pulsed with HLA-A*0201 peptides for immunotherapy of metastatic hormone refractory prostate cancer. *J. Immunother.* 2015, 38, 71–76. [CrossRef] [PubMed]

101. Madan, R.A.; Arlen, P.M.; Mohebtash, M.; Hodge, J.W.; Gulley, J.L. Prostvac-VF: A vector-based vaccine targeting PSA in prostate cancer. *Expert Opin. Investig. Drugs* 2009, 18, 1001–1011. [CrossRef] [PubMed]

102. Kim, J.W.; Gulley, J.L. Poxviral vectors for cancer immunotherapy. *Expert Opin. Biol. Ther.* 2012, 12, 463–467. [CrossRef] [PubMed]
103. Mandl, S.J.; Rountree, R.B.; Dela Cruz, T.B.; Foy, S.P.; Cote, J.J.; Gordon, E.J.; Trent, E.; Delcayre, A.; Franzusoff, A. Elucidating immunologic mechanisms of PROSTVAC cancer immunotherapy. J. Immunother. Cancer 2014, 2, 1–13. [CrossRef] [PubMed]

104. Fernández-Garcia, E.M.; Vera-Badillo, F.E.; Perez-Valderrama, B.; Matos-Pita, A.S.; Duran, I. Immunotherapy in prostate cancer: Review of the current evidence. Clin. Transl. Oncol. 2014, 17, 339–357. [CrossRef] [PubMed]

105. Kantoff, P.W.; Schuetz, T.J.; Blumenstein, B.A.; Glode, L.M.; Bilhartz, D.L.; Wyand, M.; Manson, K.; Panicali, D.L.; Laus, R.; Schlom, J.; et al. Overall survival analysis of a phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J. Clin. Oncol. 2010, 28, 1099–1105. [CrossRef] [PubMed]

106. Gulley, J.L.; Arlen, P.M.; Madan, R.A.; Tsang, K.Y.; Pazdur, M.P.; Skarupa, L.; Jones, J.L.; Poole, D.J.; Higgins, J.P.; Hodge, J.W.; et al. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer Immunol. Immunother. 2010, 59, 663–674. [CrossRef] [PubMed]

107. Gulley, J.L.; Madan, R.A.; Tsang, K.Y.; Jochems, C.; Marté, J.L.; Farsaci, B.; Tucker, J.A.; Hodge, J.W.; Lieber, D.J.; Steinberg, S.M.; et al. Immune impact induced by PROSTVAC (PSA-TRICOM), a therapeutic vaccine for prostate cancer. Cancer Immunol. Res. 2014, 2, 133–141. [CrossRef] [PubMed]

108. Small, E.J.; Sacks, N.; Nemunaitis, J.; Urba, W.J.; Dula, E.; Centeno, A.S.; Nelson, W.G.; Ando, D.; Howard, C.; Borellini, F.; et al. Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin. Cancer Res. 2007, 13, 3883–3891. [CrossRef] [PubMed]

109. Higano, C.S.; Corman, J.M.; Smith, D.C.; Centeno, A.S.; Steidle, C.P.; Gittleman, M.; Simons, J.W.; Sacks, N.; Aimi, J.; Small, E.J. Phase 1/2 dose escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. J. Immunol. 2015, 194, 59–67. [CrossRef] [PubMed]

110. Drake, C.G. Immunotherapy for prostate cancer: Walk, don’t run. J. Clin. Oncol. 2009, 27, 4035–4037. [CrossRef] [PubMed]

111. Rosenberg, S.A.; Restifo, N.P. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 2015, 348, 62–68. [CrossRef] [PubMed]

112. Rosenberg, S.A.; Yannelli, J.R.; Yang, J.C.; Topalian, S.L.; Schwartzentruber, D.J.; Weber, J.S.; Parkinson, D.R.; Seipp, C.A.; Einhorn, J.H.; White, D.E. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J. Natl. Cancer Inst. 1994, 86, 1159–1166. [CrossRef] [PubMed]

113. Dudley, M.E.; Wunderlich, J.R.; Yang, J.C.; ogers, L.J.; Gracia, G.J.; Jones, S.A.; Mangiameli, D.P.; Pelletier, M.M.; Gea-Banacloche, J.; Robinson, M.R.; et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J. Clin. Oncol. 2005, 23, 2346–2357. [CrossRef] [PubMed]

114. Dudley, M.E.; Wunderlich, J.R.; Robbins, P.E.; Yang, J.C.; Hwu, P.; Schwartzentruber, D.J.; Topalian, S.L.; Sherry, R.; Restifo, N.P.; Hubicki, A.M.; et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002, 298, 850–854. [CrossRef] [PubMed]

115. Restifo, N.P.; Dudley, M.E.; Rosenberg, S.A. Adoptive immunotherapy for cancer: Harnessing the T cell response. Nat. Rev. Immunol. 2012, 12, 269–281. [CrossRef] [PubMed]

116. Rosenberg, S.A. Cell transfer immunotherapy for metastatic solid cancer-what clinicians need to know. Nat. Rev. Clin. Oncol. 2011, 8, 577–585. [CrossRef] [PubMed]

117. Hinrichs, C.S.; Rosenberg, S.A. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol. Rev. 2014, 257, 56–71. [CrossRef] [PubMed]

118. Kershaw, M.H.; Westwood, J.A.; Darcy, P.K. Gene-engineered T cells for cancer therapy. Nat. Rev. Cancer 2013, 13, 525–541. [CrossRef] [PubMed]

119. Frigault, M.J.; Maus, M.V. Chimeric antigen receptor-modified T cells strike back. Int. Immunol. 2016, 6, 1–9. [CrossRef] [PubMed]

120. Finney, H.M.; Lawson, A.D.; Bebbington, C.R.; Weir, A.N. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. J. Immunol. 1998, 161, 2791–2797. [PubMed]

121. Srivastava, S.; Riddell, S.R. Engineering CAR-T cells: Design concepts. Trends Immunol. 2015, 36, 494–502. [CrossRef] [PubMed]
122. Maus, M.V.; Grupp, S.A.; Porter, D.L.; June, C.H. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. Blood 2014, 123, 2625–2635. [CrossRef] [PubMed]

123. Savoldo, B.; Ramos, C.A.; Liu, E.; Mims, M.P.; Keating, M.J.; Carrum, G.; Kamble, R.T.; Bollard, C.M.; Gee, A.P.; Mei, Z.; et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J. Clin. Investig. 2011, 121, 1822–1826. [CrossRef] [PubMed]

124. Zhong, X.S.; Matsushita, M.; Plotkin, J.; Riviere, I.; Sadelain, M. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI3kinase/AKT/Bcl XL activation and CD8+ T cell-mediated tumor eradication. Mol. Ther. 2010, 18, 413–420. [CrossRef] [PubMed]

125. Long, A.H.; Haso, W.M.; Shern, J.F.; Wanhainen, K.M.; Murgai, M.; Ingaramo, M.; Smith, J.P.; Walker, A.J.; Kohler, M.E.; Venkateshvara, V.R.; et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat. Med. 2015, 21, 581–590. [CrossRef] [PubMed]

126. Koehler, H.; Kofler, D.; Hombach, A.; Abken, H. CD28 costimulation overcomes transforming growth factor-beta-mediated repression of proliferation of redirected human CD4+ and CD8+ T cells in an antitumor cell attack. Cancer Res. 2007, 67, 2265–2273. [CrossRef] [PubMed]

127. Milone, M.C.; Fish, J.D.; Carpenito, C.; Carroll, R.G.; Binder, G.K.; Teachey, D.; Samanta, M.; Lakhal, M.; Gloss, B.; Danet-Desnoyers, G.; et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol. Ther. 2009, 17, 1453–1464. [CrossRef] [PubMed]

128. Kalos, M.; Levine, B.L.; Porter, D.L.; Katz, S.; Grupp, S.A.; Bagg, A.; June, C.H. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci. Transl. Med. 2011, 3. [CrossRef] [PubMed]

129. Kochenderfer, J.N.; Dudley, M.E.; Feldman, S.A.; Wilson, W.H.; Spaner, D.E.; Maric, I.; Stetler-Stevenson, M.; Phan, G.Q.; Hughes, M.S.; Sherry, R.M.; et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 2012, 119, 2709–2720. [CrossRef] [PubMed]

130. Brentjens, R.J.; Rivière, I.; Park, J.H.; Davila, M.L.; Wang, X.; Stefanski, J.; Taylor, C.; Yeh, R.; Bartido, S.; Borquez-Ojeda, O.; et al. Safety and persistence of adoptively transferred autologous CD19 targeted T cells in patients with relapsed or chemotherapy refractory B cell leukemias. Blood 2011, 118, 4817–4828. [CrossRef] [PubMed]

131. Maude, S.L.; Frey, N.; Shaw, P.A.; Aplenc, R.; Barrett, D.M.; Bunin, N.J.; Chew, A.; Gonzalez, V.E.; Zheng, Z.; Lacey, S.F.; et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N. Engl. J. Med. 2014, 371, 1507–1517. [CrossRef] [PubMed]

132. Davila, M.L.; Riviere, I.; Wang, X.; Bartido, S.; Park, J.; Curran, K.; Chun, S.S.; Stefanski, J.; Borquez-Ojeda, O.; Olszewska, M.; et al. Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci. Transl. Med. 2014, 6. [CrossRef] [PubMed]

133. Rapoport, A.P.; Stadtmauer, E.A.; Aqui, N.; Vogl, D.; Chew, A.; Fang, H.B.; Janofsky, S.; Yager, K.; Veloso, E.; et al. Rapid immune recovery and graft-versus-host disease-like engraftment syndrome following adoptive transfer of costimulated autologous T cells. Clin. Cancer Res. 2009, 15, 4499–4507. [CrossRef] [PubMed]

134. Yao, X.; Ahmadzadeh, M.; Lu, Y.C.; Lievehr, D.J.; Dudley, M.E.; Liu, F.; Schrump, D.S.; Steinberg, S.M.; Rosenberg, S.A.; Robbins, P.F. Levels of peripheral CD4(+)/FoxP3(+) regulatory T cells are negatively associated with clinical response to adoptive immunotherapy of human cancer. Blood 2012, 119, 5688–5696. [CrossRef] [PubMed]

135. Dotti, G.; Gottschalk, S.; Savoldo, B.; Brenner, M.K. Design and development of therapies using chimeric antigen receptor-expressing T cells. Immunol. Rev. 2014, 257, 107–126. [CrossRef] [PubMed]

136. Hoyos, V.; Savoldo, B.; Quintarelli, C.; Mahendravada, A.; Zhang, M.; Vera, J.; Heslop, H.E.; Rooney, C.M.; Brenner, M.K.; Dotti, G. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 2010, 24, 1160–1170. [CrossRef] [PubMed]

137. Di Stasi, A.; Tey, S.K.; Dotti, G.; Fujita, Y.; Kennedy-Nasser, A.; Martinez, C.; Straathof, K.; Liu, E.; Durett, A.G.; Grilley, B.; et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N. Engl. J. Med. 2011, 365, 1673–1683. [CrossRef] [PubMed]

138. Shi, S.; Chen, L.; Huang, G. Antiangiogenic therapy improves the antitumor effect of adoptive cell immunotherapy by normalizing tumor vasculature. Med. Oncol. 2013, 30. [CrossRef] [PubMed]
139. Huang, Y.; Yuan, J.; Righi, E.; Ancukiewicz, M.; Nezivar, J.; Santosuosso, M.; Martin, J.D.; Martin, M.R.; Vianello, F.; Leblanc, P.; et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc. Natl. Acad. Sci. USA 2012, 109, 17561–17566. [CrossRef] [PubMed]

140. Di Stasi, A.; De Angelis, B.; Rooney, C.M.; Zhang, L.; Mahendravada, A.; Foster, A.E.; Heslop, H.E.; Brenner, M.K.; Dotti, G.; Savoldo, B. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 2009, 113, 6392–6402. [CrossRef] [PubMed]

141. Craddock, J.A.; Lu, A.; Bear, A.; Pule, M.; Brenner, M.K.; Rooney, C.M.; Foster, A.E. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J. Immunother. 2010, 33, 780–788. [CrossRef] [PubMed]

142. Morgan, R.A.; Johnson, L.A.; Davis, J.L.; Zheng, Z.; Woolard, K.D.; Reap, E.A.; Feldman, S.A.; Chinnasamy, N.; Ahmed, N.; Brawley, V.S.; Hegde, A.; Robertson, C.; Ghazi, A.; Gerken, C.; Liu, E.; Dakhova, O.; Ashoori, A.; Corder, A.; et al. Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. Clin. Cancer Res. 2015, 21, 6392–6402. [CrossRef] [PubMed]

143. Kloss, C.C.; Condomines, M.; Cartellieri, M.; Bachmann, M.; Sadelain, M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat. Biotechnol. 2013, 31. [CrossRef] [PubMed]

144. Bonini, C.; Mondino, A. Adoptive T-cell therapy for cancer: The era of engineered T cells. Eur. J. Immunol. 2015, 45, 2457–2469. [CrossRef] [PubMed]

145. Kelly, R.J.; Sharon, E.; Pastan, I.; Hassan, R. Mesothelin-targeted agents in clinical trials and in preclinical development. Mol. Cancer Ther. 2012, 11, 517–525. [CrossRef] [PubMed]

146. Ahmed, N.; Brawley, V.S.; Hegde, M.; Robertson, C.; Ghazi, A.; Gerken, C.; Liu, E.; Dakhova, O.; Ashoori, A.; Corder, A.; et al. Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J. Clin. Oncol. 2015, 33, 1688–1696. [CrossRef] [PubMed]

147. Slovin, S.F.; Wang, X.; Hullings, M.; Santegoets, S.J.; van Moorselaar, R.J.; van der Sluis, T.M.; Gall, H.E.; Harding, T.C.; Jooss, K.; Lowy, I.; et al. Chimeric antigen receptor (CARþ) modified T cells targeting prostate-specific membrane antigen (PSMA) in patients (pts) with castrate metastatic prostate cancer (CMPC). J. Clin. Oncol. 2013, 31, Abstract 72.

148. Van den Eertwegh, A.J.M.; Versluis, J.; van den Berg, H.P.; Santegoets, S.J.; van Moorselaar, R.J.; van der Sluis, T.M.; Gall, H.E.; Harding, T.C.; Jooss, K.; Lowy, I.; et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castrationresistant prostate cancer: A phase 1 dose-escalation trial. Lancet Oncol. 2012, 13, 509–517. [CrossRef]

149. Isaacs, J.T.; Pili, R.; Qian, D.Z.; Dalrymple, S.L.; Garrison, J.B.; Kyprianou, N.; Björk, A.; Olsson, A.; Leanderson, T. Identification of ABR-215050 as lead second generation quinoline-3-carboxamide anti-angiogenic drug for the treatment of prostate cancer. Expert Opin. Investig. Drugs 2010, 19, 1235–1243. [CrossRef] [PubMed]

150. Isaacs, J.T.; Pili, R.; Qian, D.Z.; Dalrymple, S.L.; Garrison, J.B.; Kyprianou, N.; Björk, A.; Olsson, A.; Leanderson, T. Quinoline-3-carboxamide anti-angiogenic agent, modulates the expression of thrombospondin-1 in human prostate tumors. Mol. Cancer 2010, 9, 107–124.

151. Björk, O.A.; Vallon-Christersson, J.; Isaacs, J.T.; Dalrymple, S.L.; Garrison, J.B.; Kyprianou, N.; Björk, A.; Olsson, A.; Leanderson, T. Quasinimod (ABR-215050), a quinoline-3-carboxamide anti-angiogenic agent, modulates the expression of thrombospondin-1 in human prostate tumors. Mol. Cancer 2010, 9, 107–124.

152. Shen, L.; Sundstedt, A.; Ciesielski, M.; Miles, K.M.; Celander, M.; Adelaiyi, R.; Orillion, A.; Ciamporcaro, E.; Ramakrishnan, S.; Ellis, L.; et al. Quasinimod modulates suppressive myeloid cells and enhances cancer immunotherapies in murine models. Cancer Immunol. Res. 2015, 3, 136–148. [CrossRef] [PubMed]

153. Dalrymple, S.L.; Becker, R.E.; Isaacs, J.T. The quinoline-3-carboxamide anti-angiogenic agent, tasquinimod, enhances the anti-prostate cancer efficacy of androgen ablation and taxotere without effecting serum PSA directly in human xenografts. Prostate 2007, 67, 790–797. [CrossRef] [PubMed]
155. Dalrymple, S.L.; Becker, R.E.; Zhou, H.; DeWeese, T.L.; Isaacs, J.T. Tasquinimod prevents the angiogenic rebound induced by fractionated radiation resulting in an enhanced therapeutic response of prostate cancer xenografts. *Prostate* **2012**, *72*, 638–648. [CrossRef] [PubMed]

156. Bjork, P.; Bjork, A.; Vogl, T.; Liberg, D.; Olsson, A.; Roth, J.; Ivars, F.; Leanderson, T. Identification of human S100A9 as a novel target for treatment of autoimmune disease via binding to quinoline-3-carboxamides. *PLoS Biol.* **2009**, *7*, 800–812. [CrossRef] [PubMed]

157. Srikrishna, G. S100A8 and S100A9: New insights into their roles in malignancy. *J. Innate Immun.* **2012**, *4*, 31–40. [CrossRef] [PubMed]

158. Mehta, A.R.; Armstrong, A.J. Tasquinimod in the treatment of castrate-resistant prostate cancer-current status and future prospects. *Ther. Adv. Urol.* **2016**, *8*, 9–18. [CrossRef] [PubMed]

159. Cheng, P.; Corzo, C.A.; Luetteke, N.; Yu, B.; Nagaraj, S.; Bui, M.M.; Ortiz, M.; Nacken, W.; Sorg, C.; Vogl, T.; et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J. Exp. Med.* **2008**, *205*, 2235–2249. [CrossRef] [PubMed]

160. Mosser, D.M.; Edwards, J.P. Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* **2008**, *8*, 958–969. [CrossRef] [PubMed]

161. Locati, M.; Mantovani, A.; Sica, A. Macrophage activation and polarization as an adaptive component of innate immunity. *Adv. Immunol.* **2013**, *120*, 163–184. [PubMed]

162. Hermani, A.; Hess, J.; De Servi, B.; Medunjanin, S.; Grobholz, R.; Trojan, L.; Angel, P.; Mayer, D. Calcium binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. *Clin. Cancer Res.* **2005**, *11*, 5146–5152. [CrossRef] [PubMed]

163. Bratt, O.; Häggman, M.; Ahlgren, G.; Nordle, O.; Björk, A.; Damber, J.E. Open-label, clinical phase I studies of tasquinimod in patients with castration-resistant prostate cancer. *Br. J. Cancer* **2009**, *101*, 1233–1240. [CrossRef] [PubMed]

164. Pili, R.; Häggman, M.; Stadler, W.M.; Gingrich, J.R.; Assikis, V.J.; Björk, A.; Nordle, O.; Forsberg, G.; Carducci, M.A.; Armstrong, A.J. Phase II randomized, double-blind, placebo-controlled study of tasquinimod in men with minimally symptomatic metastatic castrate-resistant prostate cancer. *J. Clin. Oncol.* **2011**, *29*, 4022–4028. [CrossRef] [PubMed]

165. Armstrong, A.J.; Häggman, M.; Stadler, W.M.; Gingrich, J.R.; Assikis, V.; Polikoff, J.; Damber, J.E.; Belkoff, L.; Nordle, Ö.; Forsberg, G.; et al. Longterm survival and biomarker correlates of tasquinimod efficacy in a multicenter randomized study of men with minimally symptomatic metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* **2013**, *19*, 6891–6901. [CrossRef] [PubMed]

166. Armstrong, A.J.; Kaboteh, R.; Carducci, M.A.; Damber, J.E.; Stadler, W.M.; Hansen, M.; Edénbrandt, L.; Forsberg, G.; Nordle, Ö.; Pili, R.; et al. Assessment of the bone scan index in a randomized placebo-controlled trial of tasquinimod in men with metastatic castration-resistant prostate cancer (mCRPC). *Urol. Oncol.* **2014**, *32*, 1308–1316. [CrossRef] [PubMed]