cancers

Review

Immunotherapy: Shifting the Balance of Cell-Mediated Immunity and Suppression in Human Prostate Cancer

Jo A. Tucker [1], Caroline Jochems [1], James L. Gulley [1,2], Jeffrey Schlom [1,*] and Kwong Y. Tsang [1]

[1] Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA;
E-Mails: joanne.tucker@nih.gov (J.A.T.); jochemscm@mail.nih.gov (C.J.); gulleyj@mail.nih.gov (J.L.G.); tsangkwo@mail.nih.gov (K.Y.T.)

[2] Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

* Author to whom correspondence should be addressed: E-Mail: js141c@nih.gov;
Tel.: +1-301-496-4343; Fax: +1-301-496-2756.

Abstract: Active immunotherapy is dependent on the ability of the immune system to recognize and respond to tumors. Despite overwhelming evidence to support a cell-mediated immune response to prostate cancer, it is insufficient to eradicate the disease. This is likely due to a high level of suppression at the tumor site from a variety of sources, including immunosuppressive cells. Immune cells entering the tumor microenvironment may be inhibited directly by the tumor, stromal cells or other immune cells that have been induced to adopt a suppressive phenotype. The resurgence of interest in immunotherapy following the approval of sipuleucel-T and ipilimumab by the Food and Drug Administration has brought about new strategies for overcoming tumor-mediated suppression and bolstering anti-tumor responses. Improved understanding of the immune response to prostate cancer can lead to new combination therapies, such as the use of vaccine with small molecule and checkpoint inhibitors or other immunotherapies.

Keywords: immunotherapy; prostate cancer; anti-tumor response; cell-mediated immunity

1. Introduction

Prostate cancer is the most prevalent cancer among U.S. men, and although the number of deaths from prostate cancer has been on the decline, it remains the second leading cause of cancer-related deaths in this population [1,2]. Localized prostate cancer can be treated with surgery or radiation, but about 30% of men develop recurrent disease. Recurrent or metastatic disease can often be controlled with androgen deprivation either through physical or chemical castration [3]. However, many patients become refractory to this treatment, developing castration-resistant prostate cancer (CRPC). Until recently, primary treatment for metastatic CRPC (mCRPC) consisted only of chemotherapeutic options. Now the U.S. Food and Drug Administration (FDA) has approved a therapeutic vaccine as a front-line treatment for mCRPC.

Prostate cancer presents an attractive model for immunotherapy based on several characteristics [4]. First, it is a relatively slow-growing tumor and second, it can be easily monitored by measuring serum levels of prostate-specific antigen (PSA). In addition to monitoring disease, the overexpression of PSA in prostate cancer serves as a target for immunotherapeutics. Prostate cancer also has several other well-described tumor-associated antigens (TAAs), including prostate-specific membrane antigen (PSMA) and prostatic acid phosphatase (PAP) [4]. As all of these antigens are tissue-specific, there is a decreased likelihood of any off-target side effects. In addition to these well-characterized antigens, several other novel TAAs overexpressed in prostate cancer have been described and evaluated as therapeutic targets. Prostate stem-cell antigen (PSCA) is overexpressed on the surface of primary androgen independent prostate tumor cells, as well as metastases [5]. Vaccination with dendritic cells loaded with a PSA/PSCA peptide has been shown to efficiently induce antigen-specific T cells *in vitro*, and in a Phase I/II clinical trial led to the complete regression of lymphadenopathy in 1/12 patients and stable disease in 6 others [6]. A peptide from the plasma membrane associated isoform of new gene expressed in prostate (NGEP) has been used to generate antigen-specific T cells *in vitro* that were capable of lysing NGEP-expressing human tumor cells [7]. Moreover, prostate cancer patients receiving a PSA-based vaccine had an increased frequency of NGEP-specific T cells post-vaccination. Another interesting prostate TAA is SLC45A3 (prostein). A common gene rearrangement in prostate cancer results in the formation of a fusion of prostein with the transcription factor ERG [8]. A prostein epitope was found to be capable of generating T cells that could kill prostate cancer cell lines [9], and a recent study reports that the loss of prostein correlated with gene rearrangement and shorter PSA-free survival time [10].

The presence of an immune response to prostate cancer can be seen in the form of tumor infiltrating lymphocytes (TILs) [11], particularly CD8+ T cells, which have been shown to be a positive prognostic factor in this disease and others [12–14]. However, cell-mediated anti-tumor responses are generally weak and inconsistent. This is likely because most TAAs are poorly immunogenic, in combination with a high level of immune suppression from the tumor and surrounding microenvironment. Utilizing the power and specificity of the immune system to fight tumors requires overcoming this inhibition to mount an effective response. The efficacy of active immunotherapies, such as therapeutic vaccines, may be improved by combining vaccines with treatments designed to alleviate suppression.

2. Cell-Mediated Immune Response to Prostate Cancer

As a component of the genitourinary tract, the prostate is part of the mucosal immune system. Prostate-associated lymphoid tissue is populated by T cells, natural killer cells (NK), dendritic cells (DC) and B cells, and is organized into two regions. The intraepithelial region consists of $CD3^+$ T cells, predominantly $CD8^+$, as well as NK, DC and B cells. The lymphoid aggregates form below the epithelial layer, arranged as B cell follicles, with parafollicular areas composed of mostly $CD4^+$ T cells and DCs [15]. Prostate tumors contain infiltrates of both effector and suppressor cell types, including T, B, NK, macrophages and regulatory T cells [16]. This infiltrate was shown to be hormonally regulated as patients treated with androgen deprivation therapy (ADT) had significant increases in the density of $CD3^+$ ($p < 0.001$) and $CD8^+$ T cells ($p < 0.001$), and $CD68^+$ macrophages ($p < 0.001$), as compared to patients receiving prostatectomy only. While a higher NK density correlated with lower risk of progression, a high density of macrophages was associated with risk of biochemical recurrence. Conversely, DC numbers have been reported to be significantly lower in prostate cancer than normal prostate tissue [17]. As DCs are primarily antigen presenting cells (APCs), a decrease in number could contribute to a lack of tumor-infiltrating lymphocyte activation. B cells can also act as APCs. Although intratumoral B cell numbers are not associated with clinical outcome [18], they could be acting as APCs in the absence of DCs [19].

2.1. T Cells

T cells, especially $CD8^+$ cells, have long been thought of as the dominant mediators of anti-tumor activity for their recognition of endogenous peptides via HLA Class I expression. IFNγ release by T cells also plays an important part by upregulating Class I antigen processing and presentation in tumor cells [20]. This is supported by the increased incidence of tumors in immunocompromised patients, particularly those with T cell deficits, such as AIDS or transplant patients [21]. Compared to normal prostate, the density of infiltrating immune cells in benign prostatic hyperplasia (BPH) is significantly higher and composed of 70 to 80% T cells [22]. However, these numbers return to nearly normal levels in high-grade prostatic adenocarcinoma. A study by Ebelt *et al.* shows the formation of lymphocyte clusters near cancerous tissues, but few tumor-infiltrating cells [23]. The majority of $CD3^+$ cells in both of these areas were $CD4^+$ and $CD69^+$. There was also a noted decrease in staining of both IFNγ and perforin in cancer tissue as compared to healthy prostate. TCR-Vβ analysis revealed a repertoire similar to that of normal prostate, indicating that there is an early T cell response to prostate cancer, but it appears nonspecific and dominated by $CD4^+$ cells. Although these cells display the activation marker CD69, they do not appear to be functional, and therefore are unlikely to prevent tumor growth. Elsässer-Beile *et al.* reported that $CD3^+$ TILs isolated from prostate carcinomas express significantly higher levels of IFNγ mRNA than those isolated from BPH [24]. This is in contrast to the previously mentioned decrease in IFNγ in carcinomas as measured by immunohistochemistry, possibly indicating a defect in protein production and thus impaired effector function.

In contrast to αβ T cells, γδ T cells do not kill in an antigen dependent manner, but have been shown to infiltrate some solid tumors and have lytic activity against cancer cells of epithelial origin [25]. Although little is known of the role of γδ T cells in human tumors, they have been shown to be

protective in the TRAMP model of prostate cancer [26]. These cells can be expanded *in vivo* and have been reported to have antitumor activity against human prostate cancer cell lines *in vitro* [27].

2.2. NK and NKT Cells

Despite the obvious importance of T cells, NKs also contribute considerably to anti-tumor immunity. Gannon *et al.* report that prostate cancer patients receiving ADT had an elevated frequency of NKs, which correlated with a lower risk of progression ($n = 75$, $p < 0.05$) [16]. NKs were the predominant infiltrating cells in a patient exhibiting spontaneous regression of metastatic pancreatic cancer. Once isolated, these cells were shown to lyse autologous tumor and both pancreatic and prostate tumor cell lines [28]. The anti-tumor activity of NKs against prostate cancer has also been reported to increase with the introduction of reovirus and Lactobacillus [29,30].

NKT cells, which express both T cell and NK receptors, can also play a role in tumor immunity. Although most solid tumors do not express the NKT target molecule CD1, NKT cells can indirectly promote or suppress the antitumor response by influencing myeloid cells, such as DCs and macrophages [31]. A positive correlation was found between levels of NKT cells and CD8^{+} T cells in the peripheral blood of 54 prostate cancer patients, supporting the regulatory role of these cells in human tumor immunity [32].

3. Cell-Mediated Suppression of the Anti-Tumor Response

Although tumors use many mechanisms to escape immune recognition, cell-mediated inhibition is particularly nefarious as it effectively turns the immune system against itself. Responding cells infiltrating the tumor site may be induced to adopt a suppressive phenotype either directly through contact with the tumor or indirectly by secreted factors and other tolerogenic immune cells within the tumor microenvironment. Inhibitory cells include regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs).

3.1. Regulatory T cells

Regulatory T cells are potent mediators of inhibition and can be induced at, or recruited to, the tumor site by TGF-β and CCL22 signaling [33–37]. As seen with many tumor types, the peripheral blood and tumor of prostate cancer patients are enriched for Tregs [38–40]. Yokokawa *et al.* previously reported that Tregs isolated from the peripheral blood of prostate cancer patients had increased suppressive function compared to healthy individuals (prostate cancer patients, 55.7 + 18.92%; healthy donors, 31.2 + 6.55%) [41]. Data also suggest that a decrease in Treg function post-vaccination with PSA-TRICOM may be associated with increased overall survival in mCRPC [42,43].

The induction of Tregs and T cell tolerance in tumors is likely tumor- or DC-mediated via molecules such as PD-1 and TGF-β [33,44,45]. Inflammatory factors at the tumor site promote tolerance and inhibit maturation of DCs, which in turn, may induce an immunosuppressive or tolerogenic phenotype in both naïve and effector T cells [11,46,47]. TGF-β signaling induces CD4^{+} T cells to produce prostaglandin E_2 (PGE_2), which contributes to Treg generation [48] and inhibits the ability of DCs to attract naïve T cells via CCL19 production [49]. It has been previously reported that

patients with localized prostate cancer showed increased serum levels of PGE_2 compared to patients with metastatic or biochemically progressive disease, or healthy donors [41]; this increase positively correlated with Treg function (R = 0.814). Treatment with 13, 26, 52 or 100 μM dimethyl PGE_2 enhanced the suppressive function of Tregs from healthy donors and patients with biochemically progressive or localized, but not metastatic, prostate cancer, ranging from a 1.6-fold increase at 13 μM to a 2.4-fold increase at 100 μM. This phenomenon is supported by another study in prostate cancer reporting that prostatectomy restored DC maturation and decreased Treg frequency in tumor-bearing patients [50]. Androgen deprivation therapy has been shown to increase Treg infiltration of the prostate; however, as CD8^{+} cytotoxic lymphocytes increased by the same ratio, no impact on disease-free survival was seen [51].

Several treatments aimed at depleting Tregs have been developed and tested in mouse models of prostate cancer [52]. However, as Tregs are not characterized by a specific cell surface marker, these methods are non-specific and often target activated lymphocytes as well. Human studies have focused on immunotoxins fused with either anti-CD25 antibodies or IL-2 [53–55]. Although some benefits have been reported, the effect seems to be transient as Tregs continue to be induced and quickly return to pre-treatment levels. Combining Treg depletion with vaccination seems promising, but will require optimizing dosage and scheduling in order to be effective. Another method of targeting Tregs is blockade of function. Ipilimumab, a monoclonal antibody against the activation marker CTLA4, has been extensively tested in humans and will be discussed at length below.

3.2. Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells comprise various stages of differentiation along both the monocytic and granulocytic lineages, which may indicate that they can be induced at various early stages of development or that they originate from an early precursor [56,57]. Like Tregs, MDSCs accumulate in the peripheral blood of cancer patients [58]. Removal of these cells restored the ability of the DC fraction to activate allogeneic T cells *in vitro*. When isolated, MDSCs could not be matured to DCs, but were able to suppress T cell proliferation [58]. Cancer patients receiving standard treatment had a much higher percentage of MDSCs than either age-matched controls or untreated patients; this increase correlated with clinical cancer stage and metastatic tumor burden [58,59]. This indicates that the accumulation of MDSCs in cancer patients may be driven by the tumor, but can also be influenced by chemotherapies, such as cyclophosphamide. Thus, MDSCs represent another powerful mediator of immune suppression in prostate cancer patients, but due to their heterogeneous nature, may prove difficult to target for depletion.

3.3. Tumor-Associated Macrophages

Although TAMs are also of the myeloid lineage, they are a fully differentiated cell type. These resident macrophages act as part of the tumor stroma by promoting tumor growth and progression. Like other suppressive cells, TAMs are polarized to an M2 or tolerogenic phenotype by tumor-secreted factors [60,61]; however, there is evidence that their functions may differ based on location within or around the tumor (reviewed by Mantovani *et al.* [62]). In contrast to the classical M1 macrophages, which are efficient effectors, antigen presenters and produce T cell stimulating cytokines, M2

macrophages are beneficial to tumor growth and survival as they have poor antigen presenting ability, produce both pro-angiogenic and immunosuppressive factors, and promote tissue remodeling [63,64]. In human prostate cancer, increasing TAM size, number and area of infiltration were found to correlate with increasing Gleason score, but there is some disparity between studies as to the value of TAM quantification as a prognostic marker in this disease [65,66]. However, Satoh *et al.* showed that co-injection of mice with human prostate tumor cells and macrophages overexpressing IL-12 enhanced expression of MHC in TAMs increased T cell infiltration and reduced both the primary tumor and lung metastases [67]. TAMs not only suppress the immune response, but also directly support tumor growth, thus becoming an important factor for consideration in the development of future immunotherapies.

4. Immunotherapy for Prostate Cancer

Several different modes of immunotherapy for prostate cancer are currently being investigated in clinical trials. The mechanism of action for these therapies varies, aiming either to activate the immune system to better recognize and kill tumor cells, or to decrease suppression of the immune cells. Clinical trials combining therapeutic cancer vaccines with chemotherapy, radiation, and small molecule targeted therapeutics are in progress and may provide a new alternative for the treatment of prostate cancer.

One target of small molecule inhibitors that seems to be particularly promising for prostate cancer is c-Met. The c-Met pathway is dysregulated in many human cancers, including prostate, and is often associated with poor clinical outcome and shortened survival [68,69]. In prostate, c-Met signaling is mediated in a paracrine manner. Its ligand, hepatocyte growth factor (HGF), can be produced by stromal cells around the prostate, but also in bone, making c-Met a good target for localized and metastatic disease [70]. Expressed on a variety of tissues and organs, c-Met is mainly found on epithelial cells, but can also be expressed on monocytes and DCs [71]. Ligand activation of c-Met regulates the migration, but not the antigen presenting function, of DCs. Although further characterization of the immune response to c-Met blockade is needed, treatment with small molecule inhibitors to such targets should be a good candidate for combination with immunotherapy. Several c-Met inhibitors are in phase I or II clinical trials for prostate cancer and promising early results have been obtained with at least one, XL184 (cabozantinib) [72].

ADT, which is the first line of treatment for prostate cancer, has also been shown to affect the immune environment in several ways (reviewed by Aragon-Ching *et al.* [73]). T cell and macrophage infiltration of the prostate [16,74], and thymic regeneration with increasing T cell numbers, have been shown in humans after ADT [75]. Increasing the number of T cells may have a positive impact on immunotherapy. The following discussion highlights some of the recent advances and most promising immunotherapies for prostate cancer.

4.1. Sipuleucel-T (Provenge®)

Sipuleucel-T consists of autologous antigen-presenting cells that have been enriched and pulsed with a fusion protein of GM-CSF and PAP [4]. This product is given at three time points over the period of a month. Based on a phase III study in 512 patients, which showed increased overall survival (25.8 *versus* 21.7 months, $p = 0.01$) [76], the FDA approved sipuleucel-T for asymptomatic or minimally symptomatic mCRPC. The immune response to sipuleucel-T in patients was generally evaluated

2–3 months after initiation of therapy, and although median time to progression was 10–11 weeks [77], phase I/II trials reported significant T cell proliferation response to the target antigen, some decline in PSA, as well as evidence of antigen-specific T cell generation and antibody production [78,79]. Of 31 patients tested, 100% developed T cell proliferation responses upon three infusions with sipuleucel-T, after having little or no reaction pre-vaccination [78]. Serum PSA decreases were reported in six patients, three of whom experienced serum PSA decreases greater than 50%. The median time to disease progression was significantly greater for patients who developed an immune response (34 weeks, n = 20) than for those who did not (13 weeks, n = 11) ($p < 0.027$). A significant correlation between antigen-specific immune responses and overall survival (OS) ($p = 0.003$) was reported in a combined analysis of three phase III trials of men with mCRPC [80]. Although only antibody response independently correlated to OS, antigen-specific cellular responses were seen in 60% of patients receiving sipuleucel-T by T cell proliferation assay and 48% in IFNγ ELISPOT, while responses in controls subjects were 6% and 13%, respectively.

4.2. PROSTVAC®-VF

PROSTVAC is a poxviral vaccine (using a vaccinia vector for priming and fowlpox vector for boosting) containing PSA and three costimulatory molecules (B7.1, ICAM-1 and LFA-3, designated TRICOM) [81]. It is administered subcutaneously with GM-CSF, given on the same day as the vaccine and for three consecutive days. Recently, a multicenter randomized controlled phase II study of PROSTVAC in men with asymptomatic or minimally symptomatic mCRPC showed a 44% reduction in death rate and an 8.5 month improvement in overall survival compared to patients treated with control vectors [76]. Post-vaccination, 12/32 patients had decreases in serum PSA and 2/12 patients with soft tissue disease had decreases in index lesions [43]. Patients who had greater PSA-specific T cell responses by ELISPOT also showed a trend toward longer survival ($p = 0.055$), and 12/15 patients evaluated lived longer than predicted by the Halabi nomogram ($p = 0.035$) [82].

Human Tregs are very heterogeneous phenotypically, but it has been demonstrated that the most active of them express high levels of CTLA4 [83]. Although there was no difference in $CD4^+CD25^+CD127^-FoxP3^+$ Treg levels pre- *versus* post-vaccination with PROSTVAC, there was a significant increase in the effector to $CTLA4^+$ Treg ratio in patients with longer than predicted survival and a significant decrease in patients with shorter than predicted survival [42]. Likewise, Treg suppressive function decreased post-vaccination in 10/13 patients with longer than predicted survival and increased in 6/8 patients with shorter than predicted survival [41]. Vergati *et al.* also found a significant correlation between the percentage of $CTLA4^+$ Tregs and suppressive function, and that patients with shorter than predicted survival had a significantly higher percentage of $CTLA4^+$ Tregs post-vaccination ($p = 0.019$) [42]. This study supports the evidence that patients with less disease burden may receive the most benefit from vaccine immunotherapy and that vaccination can have an impact on overall survival in prostate cancer patients. An international randomized controlled phase III trial of PROSTVAC opened in 2011 (NCT01322490) [84].

4.3. Ipilimumab (Yervoy™)

Ipilimumab is a fully humanized antibody that binds to cytotoxic T lymphocyte-associated antigen 4 (CTLA4) on activated T cells, preventing inactivation of these cells [85]. CTLA4 is also constitutively expressed on Tregs; thus blockade could alleviate Treg-mediated immune suppression [86]. However, Kavanagh *et al.* reported that treatment with anti-CTLA4 antibody resulted in an increase in both effector CD4$^+$ T cells and functional Tregs in the periphery [86]. Ipilimumab has been approved for first and second line treatment of melanoma, and is currently being investigated in phase III trials for prostate cancer and phase II trials for non-small cell lung cancer. In a phase I trial for mCRPC, the combination of ipilimumab with GM-CSF resulted in a higher frequency of activated circulating CD8$^+$ T cells and antibody responses to TAAs [87]. In the cohort receiving the highest dose, 50% (3/6) of patients had a PSA response and there was one partial response in visceral metastases. In a phase II trial in which ipilimumab was given with or without a single dose of docetaxel, 6/46 patients showed a PSA decline [88]. Along with anti-tumor responses, there have also been several immune-mediated adverse events reported with ipilimumab treatment [89,90]. However, in a small pilot trial of 14 mCRPC patients, a relatively low dose of ipilimumab (3 mg/kg) was well-tolerated and resulted in PSA declines of ≥50% in two patients [91]. Ipilimumab is currently being used in combination with vaccines and other drugs in several clinical trials. A clinical trial examining the combination of ipilimumab with GVAX®, a GM-CSF transfected allogeneic tumor cell vaccine, reported a median overall survival of 29.2 months (95% CI 9.6–48.8 months), while ipilimumab in combination with PROSTVAC® yielded a median overall survival of 34.4 months (95% CI 29.6–41 months) [92,93]. Both studies reported toxicities similar to treatment with ipilimumab alone. Although these were phase I dose-escalation trials, a greater than 50% decrease in PSA after treatment was reported in seven of 28 chemotherapy-naïve patients in the GVAX® trial and in six of 24 patients in the PROSTVAC® trial, thus warranting further research on the combination of ipilimumab and vaccine.

5. Conclusions

Although the human immune system is capable of recognizing and mounting a response to prostate cancer, this response is often circumvented by tumor-derived inhibition. This inhibition may include the induction of cells to a suppressive phenotype, effectively turning the immune response against itself. Despite recent developments and some limited successes in immunotherapy, it has yet to fulfill its potential. A multifaceted approach that combines vaccine with targeted therapies, such as small molecule and checkpoint inhibitors, may prove more successful by not only directing an effective immune response but also breaking the cycle of inhibition promoted by the tumor.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

References

1. U.S. Cancer Statistics Working Group. United States Cancer Statistics: 1999–2007 Incidence and Mortality Web-based Report. Available online: http://www.cdc.gov/uscs/ (accessed on 29 November 2012).
2. American Cancer Society. *Cancer Facts & Figures*; Atlanta, GA, USA, 2012.
3. Drake, C.G. Prostate cancer as a model for tumour immunotherapy. *Nat. Rev. Immunol.* **2010**, *10*, 580–593.
4. Sauce, D.; Larsen, M.; Abbott, R.J.; Hislop, A.D.; Leese, A.M.; Khan, N.; Papagno, L.; Freeman, G.J.; Rickinson, A.B. Upregulation of interleukin 7 receptor alpha and programmed death 1 marks an epitope-specific CD8$^+$ T-cell response that disappears following primary Epstein-Barr virus infection. *J. Virol.* **2009**, *83*, 9068–9078.
5. Li, Y.; Cozzi, P.J.; Russell, P.J. Promising tumor-associated antigens for future prostate cancer therapy. *Med. Res. Rev.* **2010**, *30*, 67–101.
6. Thomas-Kaskel, A.-K.; Zeiser, R.; Jochim, R.; Robbel, C.; Schultze-Seemann, W.; Waller, C.F.; Veelken, H. Vaccination of advanced prostate cancer patients with PSCA and PSA peptide-loaded dendritic cells induces DTH responses that correlate with superior overall survival. *Int. J. Cancer* **2006**, *119*, 2428–2434.
7. Cereda, V.; Poole, D.; Palena, C.; Das, S.; Bera, T.; Remondo, C.; Gulley, J.; Arlen, P.; Yokokawa, J.; Pastan, I.; *et al*. New gene expressed in prostate: A potential target for T cell-mediated prostate cancer immunotherapy. *Cancer Immunol. Immunother.* **2010**, *59*, 63–71.
8. Xu, J.; Kalos, M.; Stolk, J.A.; Zasloff, E.J.; Zhang, X.; Houghton, R.L.; Filho, A.M.; Nolasco, M.; Badaro, R.; Reed, S.G. Identification and characterization of prostein, a novel prostate-specific protein. *Cancer Res.* **2001**, *61*, 1563–1568.
9. Kiessling, A.; Stevanovic, S.; Fussel, S.; Weigle, B.; Rieger, M.A.; Temme, A.; Rieber, E.P.; Schmitz, M. Identification of an HLA-A*0201-restricted T-cell epitope derived from the prostate cancer-associated protein prostein. *Br. J. Cancer* **2004**, *90*, 1034–1040.
10. Perner, S.; Rupp, N.J.; Braun, M.; Rubin, M.A.; Moch, H.; Dietel, M.; Wernert, N.; Jung, K.; Stephan, C.; Kristiansen, G. Loss of SLC45A3 protein (prostein) expression in prostate cancer is associated with SLC45A3-ERG gene rearrangement and an unfavorable clinical course. *Int. J. Cancer* **2012**, doi:10.1002/ijc.27733.
11. Degl'Innocenti, E.; Grioni, M.; Capuano, G.; Jachetti, E.; Freschi, M.; Bertilaccio, M.T.S.; Hess-Michelini, R.; Doglioni, C.; Bellone, M. Peripheral T-cell tolerance associated with prostate cancer Is independent from CD4$^+$CD25$^+$ regulatory T cells. *Cancer Res.* **2008**, *68*, 292–300.

12. Sorrentino, C.; Musiani, P.; Pompa, P.; Cipollone, G.; di Carlo, E. Androgen deprivation boosts prostatic infiltration of cytotoxic and regulatory T lymphocytes and has no effect on disease-free survival in prostate cancer patients. *Clin. Cancer Res.* **2011**, *17*, 1571–1581.

13. Bronte, V.; Kasic, T.; Gri, G.; Gallana, K.; Borsellino, G.; Marigo, I.; Battistini, L.; Iafrate, M.; Prayer-Galetti, T.; Pagano, F.; *et al*. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J. Exp. Med.* **2005**, *201*, 1257–1268.

14. Kärjä, V.; Aaltomaa, S.; Lipponen, P.; Isotalo, T.; Talja, M.; Mokka, R. Tumour-infiltrating lymphocytes: A prognostic factor of PSA-free survival in patients with local prostate carcinoma treated by radical prostatectomy. *Anticancer Res.* **2005**, *25*, 4435–4438.

15. Di Carlo, E.; Magnasco, S.; D'Antuono, T.; Tenaglia, R.; Sorrentino, C. The prostate-associated lymphoid tissue (PALT) is linked to the expression of homing chemokines CXCL13 and CCL21. *Prostate* **2007**, *67*, 1070–1080.

16. Gannon, P.O.; Poisson, A.O.; Delvoye, N.; Lapointe, R.; Mes-Masson, A.M.; Saad, F. Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. *J. Immunol. Methods* **2009**, *348*, 9–17.

17. Troy, A.; Davidson, P.; Atkinson, C.; Hart, D. Phenotypic characterisation of the dendritic cell infiltrate in prostate cancer. *J. Urol.* **1998**, *160*, 214–219.

18. Flammiger, A.; Bayer, F.; Cirugeda-Kühnert, A.; Huland, H.; Tennstedt, P.; Simon, R.; Minner, S.; Bokemeyer, C.; Sauter, G.; Schlomm, T.; *et al*. Intratumoral T but not B lymphocytes are related to clinical outcome in prostate cancer. *APMIS* **2012**, *120,* 901–908.

19. Nelson, B.H. CD20^{+} B cells: The other tumor-Infiltrating lymphocytes. *J. Immunol.* **2010**, *185*, 4977–4982.

20. Dunn, G.P.; Koebel, C.M.; Schreiber, R.D. Interferons, immunity and cancer immunoediting. *Nat. Rev. Immunol.* **2006**, *6*, 836–848.

21. Dunn, G.P.; Bruce, A.T.; Ikeda, H.; Old, L.J.; Schreiber, R.D. Cancer immunoediting: From immunosurveillance to tumor escape. *Nat. Immunol.* **2002**, *3*, 991–998.

22. Hussein, M.-R.A.; Al-Assiri, M.; Musalam, A.O. Phenotypic characterization of the infiltrating immune cells in normal prostate, benign nodular prostatic hyperplasia and prostatic adenocarcinoma. *Exp. Mol. Pathol.* **2009**, *86*, 108–113.

23. Ebelt, K.; Babaryka, G.; Figel, A.M.; Pohla, H.; Buchner, A.; Stief, C.G.; Eisenmenger, W.; Kirchner, T.; Schendel, D.J.; Noessner, E. Dominance of CD4^{+} lymphocytic infiltrates with disturbed effector cell characteristics in the tumor microenvironment of prostate carcinoma. *Prostate* **2008**, *68*, 1–10.

24. Elsasser-Beile, U.; Przytulski, B.; Gierschner, D.; Grussenmeyer, T.; Katzenwadel, A.; Leiber, C.; Deckart, A.; Wetterauer, U. Comparison of the activation status of tumor infiltrating and peripheral lymphocytes of patients with adenocarcinomas and benign hyperplasia of the prostate. *Prostate* **2000**, *45*, 1–7.

25. Hayday, A.C. Gammadelta cells: A right time and a right place for a conserved third way of protection. *Ann. Rev. Immunol.* **2000**, *18*, 975–1026.

26. Liu, Z.; Eltoum, I.-E.A.; Guo, B.; Beck, B.H.; Cloud, G.A.; Lopez, R.D. Protective immunosurveillance and therapeutic antitumor activity of gammadeltaT cells demonstrated in a mouse model of prostate cancer. *J. Immunol.* **2008**, *180,* 6044–6053.

27. Liu, Z.; Guo, B.L.; Gehrs, B.C.; Nan, L.I.; Lopez, R.D. *Ex vivo* expanded human Vgamma9Vdelta2$^+$ gammadelta-T cells mediate innate antitumor activity against human prostate cancer cells *in vitro*. *J. Urol.* **2005**, *173*, 1552–1556.

28. Frankel, T.L.; Burns, W.; Riley, J.; Morgan, R.A.; Davis, J.L.; Hanada, K.; Quezado, M.; Rosenberg, S.A.; Royal, R.E. Identification and characterization of a tumor infiltrating CD56($^+$)/CD16($^-$) NK cell subset with specificity for pancreatic and prostate cancer cell lines. *Cancer Immunol. Immunother.* **2010**, *59*, 1757–1769.

29. Gujar, S.A.; Pan, D.; Marcato, P.; Garant, K.A.; Lee, P.W.K. Oncolytic virus-initiated protective immunity against prostate cancer. *Mol. Ther.* **2011**, *19*, 797–804.

30. Horinaka, M.; Yoshida, T.; Kishi, A.; Akatani, K.; Yasuda, T.; Kouhara, J.; Wakada, M.; Sakai, T. Lactobacillus strains induce TRAIL production and facilitate natural killer activity against cancer cells. *FEBS Lett.* **2010**, *584*, 577–582.

31. Pilones, K.A.; Aryankalayil, J.; Demaria, S. Invariant NKT cells as novel targets for immunotherapy in solid tumors. *Clin. Dev. Immunol.* **2012**, *2012*, 11.

32. Schwemmer, B. Natural killer T cells in patients with prostatic carcinoma. *Urol. Int.* **2003**, *71*, 146–149.

33. Lu, X.; Liu, J.; Li, H.; Li, W.; Wang, X.; Ma, J.; Tong, Q.; Wu, K.; Wang, G. Conversion of intratumoral regulatory T cells by human gastric cancer cells is dependent on transforming growth factor-beta1. *J. Surg. Oncol.* **2011**, *104*, 571–577.

34. Mao, C.; Wang, S.; Jiang, Q.; Tong, J.; Ma, J.; Yang, M.; Xu, X.; Qiu, G.; Shao, Q.; Li, L.; Xu, H. Increased CD4CD25$^+$FOXP3$^+$ regulatory T Cells in cancer patients from conversion of CD4$^+$CD25$^-$ T cells through tumor-derived factors. *Onkologie* **2008**, *31*, 243–248.

35. Moo-Young, T.A.; Larson, J.W.; Belt, B.A.; Tan, M.C.; Hawkins, W.G.; Eberlein, T.J.; Goedegebuure, P.S.; Linehan, D.C. Tumor-derived TGF-beta mediates conversion of CD4$^+$Foxp3$^+$ regulatory T cells in a murine model of pancreas cancer. *J. Immunother.* **2009**, *32*, 12–21.

36. Olkhanud, P.B.; Damdinsuren, B.; Bodogai, M.; Gress, R.E.; Sen, R.; Wejksza, K.; Malchinkhuu, E.; Wersto, R.P.; Biragyn, A. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4 T cells to T-regulatory cells. *Cancer Res.* **2011**, *71*, 3505–3515.

37. Xu, L.; Xu, W.; Wen, Z.; Xiong, S. *In situ* prior proliferation of CD4$^+$ CCR6$^+$ regulatory T cells facilitated by TGF-beta secreting DCs is crucial for their enrichment and suppression in tumor immunity. *PLoS One* **2011**, *6*, e20282.

38. Miller, A.M.; Lundberg, K.; Ozenci, V.; Banham, A.H.; Hellstrom, M.; 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.

39. Valdman, A.; Jaraj, S.J.; Comperat, E.V.A.; Charlotte, F.; Roupret, M.; Pisa, P.; Egevad, L. Distribution of Foxp3-, CD4- and CD8-positive lymphocytic cells in benign and malignant prostate tissue. *APMIS* **2010**, *118*, 360–365.

40. Sfanos, K.S.; Bruno, T.C.; Maris, C.H.; Xu, L.; Thoburn, C.J.; DeMarzo, A.M.; Meeker, A.K.; Isaacs, W.B.; Drake, C.G. Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. *Clin. Cancer Res.* **2008**, *14*, 3254–3261.

41. Yokokawa, J.; Cereda, V.; Remondo, C.; Gulley, J.L.; Arlen, P.M.; Schlom, J.; Tsang, K.Y. Enhanced functionality of CD4$^+$CD25(^{high})FoxP3$^+$ regulatory T cells in the peripheral blood of patients with prostate cancer. *Clin. Cancer Res.* **2008**, *14*, 1032–1040.

42. Vergati, M.; Cereda, V.; Madan, R.; Gulley, J.; Huen, N.-Y.; Rogers, C.; Hance, K.; Arlen, P.; Schlom, J.; Tsang, K. Analysis of circulating regulatory T cells in patients with metastatic prostate cancer pre- *versus* post-vaccination. *Cancer Immunol. Immunother.* **2011**, *60*, 197–206.

43. Gulley, J.; Arlen, P.; Madan, R.; Tsang, K.-Y.; Pazdur, M.; Skarupa, L.; Jones, J.; Poole, D.; Higgins, J.; Hodge, J.; *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.

44. Wang, W.; Lau, R.; Yu, D.; Zhu, W.; Korman, A.; Weber, J. PD1 blockade reverses the suppression of melanoma antigen-specific CTL by CD4$^+$ CD25(Hi) regulatory T cells. *Int. Immunol.* **2009**, *21*, 1065–1077.

45. Adler, A.J. Mechanisms of T cell tolerance and suppression in cancer mediated by tumor-associated antigens and hormones. *Curr. Cancer Drug Targets* **2007**, *7*, 3–14.

46. Watkins, S.K.; Zhu, Z.; Riboldi, E.; Shafer-Weaver, K.A.; Stagliano, K.E.; Sklavos, M.M.; Ambs, S.; Yagita, H.; Hurwitz, A.A. FOXO3 programs tumor-associated DCs to become tolerogenic in human and murine prostate cancer. *J. Clin. Invest.* **2011**, *121*, 1361–1372.

47. Krempski, J.; Karyampudi, L.; Behrens, M.D.; Erskine, C.L.; Hartmann, L.; Dong, H.; Goode, E.L.; Kalli, K.R.; Knutson, K.L. Tumor-infiltrating programmed death receptor-1$^+$ dendritic cells mediate immune suppression in ovarian cancer. *J. Immunol.* **2011**, *186*, 6905–6913.

48. Baratelli, F.; Lee, J.M.; Hazra, S.; Lin, Y.; Walser, T.C.; Schaue, D.; Pak, P.S.; Elashoff, D.; Reckamp, K.; Zhang, L.; *et al*. PGE(2) contributes to TGF-beta induced T regulatory cell function in human non-small cell lung cancer. *Am. J. Transl. Res.* **2010**, *2*, 356–367.

49. Muthuswamy, R.; Mueller-Berghaus, J.; Haberkorn, U.; Reinhart, T.A.; Schadendorf, D.; Kalinski, P. PGE2 transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naive T cells. *Blood* **2010**, *116*, 1454–1459.

50. Brusa, D.; Carletto, S.; Cucchiarale, G.; Gontero, P.; Greco, A.; Simone, M.; Ferrando, U.; Tizzani, A.; Matera, L. Prostatectomy restores the maturation competence of blood dendritic cell precursors and reverses the abnormal expansion of regulatory T lymphocytes. *Prostate* **2011**, *71*, 344–352.

51. Sorrentino, C.; Musiani, P.; Pompa, P.; Cipollone, G.; di Carlo, E. Androgen Deprivation Boosts Prostatic Infiltration of Cytotoxic and Regulatory T Lymphocytes and Has No Effect on Disease-Free Survival in Prostate Cancer Patients. *Clin. Cancer Res.* **2011**, *17*, 1571–1581.

52. Nizar, S.; Copier, J.; Meyer, B.; Bodman-Smith, M.; Galustian, C.; Kumar, D.; Dalgleish, A. T-regulatory cell modulation: The future of cancer immunotherapy? *Br. J. Cancer* **2009**, *100*, 1697–1703.

53. Powell, D.J.J.; Attia, P.; Ghetie, V.; Schindler, J.; Vitetta, E.S.; Rosenberg, S.A. Partial reduction of human FOXP3$^+$ CD4 T cells *in vivo* after CD25-directed recombinant immunotoxin administration. *J. Immunother.* **2008**, *31*, 189–198.

54. Powell, D.J.; Felipe-Silva, A.; Merino, M.J.; Ahmadzadeh, M.; Allen, T.; Levy, C.; White, D.E.; Mavroukakis, S.; Kreitman, R.J.; Rosenberg, S.A.; *et al.* Administration of a CD25-directed immunotoxin, LMB-2, to patients with metastatic melanoma induces a selective partial reduction in regulatory T cells *in vivo*. *J. Immunol.* **2007**, *179*, 4919–4928.

55. Hobeika, A.C.; Morse, M.A.; Osada, T.; Peplinski, S.; Lyerly, H.K.; Clay, T.M. Depletion of human regulatory T cells. In *Regulatory T Cells: Methods and Protocols*; Kassiotis, G., Liston, A., Eds.; Humana Press: New York, NY, USA, 2011; Volume 707, pp. 219–231.

56. Solito, S.; Falisi, E.; Diaz-Montero, C.M.; Doni, A.; Pinton, L.; Rosato, A.; Francescato, S.; Basso, G.; Zanovello, P.; Onicescu, G.; *et al.* A human promyelocytic-like population is responsible for the immune suppression mediated by myeloid-derived suppressor cells. *Blood* **2011**, *118*, 2254–2265.

57. Ostrand-Rosenberg, S. Myeloid-derived suppressor cells: More mechanisms for inhibiting antitumor immunity. *Cancer Immunol. Immunother.* **2010**, *59*, 1593–1600.

58. Almand, B.; Clark, J.I.; Nikitina, E.; van Beynen, J.; English, N.R.; Knight, S.C.; Carbone, D.P.; Gabrilovich, D.I. Increased production of immature myeloid cells in cancer patients: A mechanism of immunosuppression in cancer. *J. Immunol.* **2001**, *166*, 678–689.

59. Diaz-Montero, C.M.; Salem, M.L.; Nishimura, M.I.; Garrett-Mayer, E.; Cole, D.J.; Montero, A.J. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol. Immunother.* **2009**, *58*, 49–59.

60. Lewis, C.E.; Pollard, J.W. Distinct role of macrophages in different tumor microenvironments. *Cancer Res.* **2006**, *66*, 605–612.

61. Sica, A.; Schioppa, T.; Mantovani, A.; Allavena, P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: Potential targets of anti-cancer therapy. *Eur. J. Cancer* **2006**, *42*, 717–727.

62. Mantovani, A.; Sozzani, S.; Locati, M.; Allavena, P.; Sica, A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* **2002**, *23*, 549–555.

63. Lin, E.Y.; Li, J.-F.; Gnatovskiy, L.; Deng, Y.; Zhu, L.; Grzesik, D.A.; Qian, H.; Xue, X.-N.; Pollard, J.W. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res.* **2006**, *66*, 11238–11246.

64. Dirkx, A.E.M.; oude Egbrink, M.G.A.; Wagstaff, J.; Griffioen, A.W. Monocyte/macrophage infiltration in tumors: Modulators of angiogenesis. *J. Leukoc. Biol.* **2006**, *80*, 1183–1196.

65. Lissbrant, I.; 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*, 445–496.

66. Shimura, S.; Yang, G.; Ebara, S.; Wheeler, T.M.; Frolov, A.; Thompson, T.C. Reduced infiltration of tumor-associated macrophages in human prostate cancer: Association with cancer progression. *Cancer Res.* **2000**, *60*, 5857–5861.

67. Satoh, T.; Saika, T.; Ebara, S.; Kusaka, N.; Timme, T.L.; Yang, G.; Wang, J.; Mouraviev, V.; Cao, G.; Fattah, E.M.A.; *et al*. Macrophages transduced with an adenoviral vector expressing Interleukin 12 suppress tumor growth and metastasis in a preclinical metastatic prostate cancer model. *Cancer Res.* **2003**, *63*, 7853–7860.

68. Pisters, L.L.; Troncoso, P.; Zhau, H.E.; Li, W.; von Eschenbach, A.C.; Chung, L.W.K. C-met proto-oncogene expression in benign and malignant human prostate tissues. *J. Urol.* **1995**, *154*, 293–298.

69. Liu, X.; Newton, R.C.; Scherle, P.A. Development of c-MET pathway inhibitors. *Expert Opin. Investig. Drugs* **2011**, *20*, 1225–1241.

70. Varkaris, A.; Corn, P.G.; Gaur, S.; Dayyani, F.; Logothetis, C.J.; Gallick, G.E. The role of HGF/c-Met signaling in prostate cancer progression and c-Met inhibitors in clinical trials. *Expert Opin. Investig. Drugs* **2011**, *20*, 1677–1684.

71. Kurz, S.M.; Diebold, S.S.; Hieronymus, T.; Gust, T.C.; Bartunek, P.; Sachs, M.; Birchmeier, W.; Zenke, M. The impact of c-met/scatter factor receptor on dendritic cell migration. *Eur. J. Immunol.* **2002**, *32*, 1832–1838.

72. Smith, D.C.; Smith, M.R.; Small, E.J.; Sweeney, C.; Kurzrock, R.; Gordon, M.S.; Vogelzang, N.J.; Scheffold, C.; Ballinger, M.D.; Hussain, M. Phase II study of XL184 in a cohort of patients with castration-resistant prostate cancer (CRPC) and measurable soft tissue disease. *J. Clin. Oncol.* **2011**, *29*, Abstract 2127.

73. Aragon-Ching, J.B.; Williams, K.M.; Gulley, J.L. Impact of androgen-deprivation therapy on the immune system: Implications for combination therapy of prostate cancer. *Front. Biosci.* **2007**, *12*, 4957–4971.

74. Mercader, M.; Bodner, B.K.; Moser, M.T.; Kwon, P.S.; Park, E.S.; Manecke, R.G.; Ellis, T.M.; Wojcik, E.M.; Yang, D.; Flanigan, R.C.; *et al*. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc. Natl Acad. Sci. USA* **2001**, *98*, 14565–14570.

75. Sutherland, J.S.; Goldberg, G.L.; Hammett, M.V.; Uldrich, A.P.; Berzins, S.P.; Heng, T.S.; Blazar, B.R.; Millar, J.L.; Malin, M.A.; Chidgey, A.P.; *et al*. Activation of thymic regeneration in mice and humans following androgen blockade. *J. Immunol.* **2005**, *175*, 2741–2753.

76. Kantoff, P.W.; Higano, C.S.; Shore, N.D.; Berger, E.R.; Small, E.J.; Penson, 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.

77. So-Rosillo, R.; Small, E.J. Sipuleucel-T (APC8015) for prostate cancer. *Expert Rev. Anticancer Ther.* **2006**, *6*, 1163–1167.

78. 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.

79. 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.

80. Sheikh, N.; Petrylak, D.; Kantoff, P.; dela Rosa, C.; Stewart, F.; Kuan, L.-Y.; Whitmore, J.; Trager, J.; Poehlein, C.; Frohlich, M.; *et al*. Sipuleucel-T immune parameters correlate with survival: An analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. *Cancer Immunol. Immunother*. **2012**, PMID 22865266.

81. Gulley, J.; Chen, A.P.; Dahut, W.; Arlen, P.M.; Bastian, A.; Steinberg, S.M.; Tsang, K.; Panicali, D.; Poole, D.; Schlom, J.; *et al*. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. *Prostate* **2002**, *53*, 109–117.

82. Halabi, S.; Small, E.J.; Kantoff, P.W.; Kattan, M.W.; Kaplan, E.B.; Dawson, N.A.; Levine, E.G.; Blumenstein, B.A.; Vogelzang, N.J. Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. *J. Clin. Oncol.* **2003**, *21*, 1232–1237.

83. Miyara, M.; Yoshioka, Y.; Kitoh, A.; Shima, T.; Wing, K.; Niwa, A.; Parizot, C.; Taflin, C.; Heike, T.; Valeyre, D.; *et al*. Functional delineation and differentiation dynamics of human CD4$^+$ T cells expressing the FoxP3 transcription factor. *Immunity* **2009**, *30*, 899–911.

84. ClinicalTrials.gov. A Phase 3 efficacy study of a recombinant vaccinia virus vaccine to treat metastatic prostate cancer (Prospect). Available online: http://clinicaltrials.gov/ct2/show/NCT01322490/ (accessed on 14 June 2012).

85. Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C.; *et al*. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **2010**, *363*, 711–723.

86. Kavanagh, B.; O'Brien, S.; Lee, D.; Hou, Y.; Weinberg, V.; Rini, B.; Allison, J.P.; Small, E.J.; Fong, L. CTLA4 blockade expands FoxP3$^+$ regulatory and activated effector CD4$^+$ T cells in a dose-dependent fashion. *Blood* **2008**, *112*, 1175–1183.

87. 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.

88. Small, E.J.; Higano, C.S.; Tchekmedyian, N.; Sartor, O.; Stein, B.; Young, R.; Vestal, J.; Moseley, W.; Fischkoff, S.; Lowy, I. Randomized phase ii study comparing 4 monthly doses of ipilimumab (mdx-010) as a single agent or in combination with a single dose of docetaxel in patients with hormone-refractory prostate cancer. *J. Clin. Oncol.* **2006**, *24*, Abstract 4609.

89. Attia, P.; Phan, G.Q.; Maker, A.V.; Robinson, M.R.; Quezado, M.M.; Yang, J.C.; Sherry, R.M.; Topalian, S.L.; Kammula, U.S.; Royal, R.E.; *et al*. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J. Clin. Oncol.* **2005**, *23*, 6043–6053.

90. Sanderson, K.; Scotland, R.; Lee, P.; Liu, D.; Groshen, S.; Snively, J.; Sian, S.; Nichol, G.; Davis, T.; Keler, T.; *et al*. Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and montanide ISA 51 for patients with resected stages III and IV melanoma. *J. Clin. Oncol.* **2005**, *23*, 741–750.

91. Small, E.J.; Tchekmedyian, N.S.; Rini, B.I.; Fong, L.; Lowy, I.; Allison, J.P. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin. Cancer Res.* **2007**, *13*, 1810–1815.

92. Madan, R.A.; Mohebtash, M.; Arlen, P.M.; Vergati, M.; Rauckhorst, M.; Steinberg, S.M.; Tsang, K.Y.; Poole, D.J.; Parnes, H.L.; Wright, J.J.; *et al*. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: A phase 1 dose-escalation trial. *Lancet Oncol.* **2012**, *13*, 501–508.

93. Van den Eertwegh, A.J.; 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 castration-resistant prostate cancer: A phase 1 dose-escalation trial. *Lancet Oncol.* **2012**, *13*, 509–517.