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Review

Inefficacy of Therapeutic Cancer Vaccines and Proposed Improvements. Casus of Prostate Cancer

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Abstract. *Prophylactic vaccination is arguably the most effective medical preventative method. After local inoculation, vaccines induce antigen-specific systemic immunity, protecting the whole body. Systemic antitumour immunity can cure advanced cancer, but will therapeutic vaccination suffice? A vaccine for castration-refractory prostate cancer (CRPC) was approved by regulatory authority, but its evidence is disputed. We critically reviewed the clinical efficacy of therapeutic cancer vaccines for prostate cancer, including the results of 31 clinical studies employing vaccines-only, and another 10 studies combining vaccines with immune co-stimulation. Vaccinations yielded immunological responses, but no study showed evidence for clinically relevant therapeutic improvement. Clinical failure of therapeutic vaccination is discussed in the light of immunological dogmas and mechanisms of antitumour therapies. We propose that cancer immunotherapy might be improved by immunological danger, i.e. disturbing tumour homeostasis by destroying the tumour tissue or inducing local inflammation. Such danger might override immunological tolerance, and thereby allow clinically relevant anticancer results.*

Curative treatment options for advanced cancer, *e.g.* disseminated disease, are very limited. Chemotherapy may prevent and reduce formation of metastases, but it rarely cures patients from advanced metastasized disease. In contrast, the immune system could mount antigen-specific responses against such cancerous lesions. Spontaneous tumour regression in patients with heavy infections has been documented for about a century (1). In the 1950s, the validity of the antitumour immunity concept was shown in animal experiments (2). In the 18th century, vaccines (*i.e.* weakened antigens) were already being used for efficient clinical protection. Therefore, it seemed logical to search for a vaccine against cancer. A decade ago, experts in the field concluded that half a century of antitumour vaccine research had not yielded any major clinical breakthroughs (3).

Classical vaccine technology holds the golden rule that vaccines should be applied in the prophylactic setting, *i.e.* prior inoculation with the pathogen. This is why children, but not diseased people, are vaccinated against bacterial and viral pathogens to prevent the development of disease. Prophylactic vaccination also yields good protection against infection with tumourigenic viruses (4-6). Therapeutic vaccination is a different chapter in immunology from prophylactic vaccination. Indeed, microbiologists have often tried therapeutic vaccination, but generally with little if any clinical efficacy. This is in sharp contrast to prophylactic vaccination which has been shown to have invaluable clinical efficacy. In therapeutic settings, the battle against micro-organisms does not include vaccination. Clinicians chose interference with pathogen reproduction by treatment with antibiotics. Such a dual strategy resembles prophylactic vaccination against tumourigenic viruses on the one hand, and applying chemotherapy in tumour-bearing patients. In light of the described efficacy of immunotherapy and the limited efficacy of chemotherapy in advanced disease, therapeutic vaccination for treatment of cancer might be evaluated on its clinical efficacy, despite the apparent lack of efficacy against microbes.

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Some commentators regard the approval of the first therapeutic cancer vaccine by the Food and Drug Administration (FDA) of the U.S.A. as clinical proof-of-concept for therapeutic vaccines. They have hailed this approval stating that immunotherapy has earned its spot in the ranks of cancer therapy (7). The studies with therapeutic vaccination using Sipuleucel-T in patients with castration-resistant prostate cancer (CRPC) showed statistically significant results (8, 9). However, the results did not match the criteria for clinically relevant improvement of anticancer therapy, *e.g.* at least six months prolonged survival compared to standard therapy (10, 11). Moreover, some serious concerns have been raised about the experimental design of these clinical studies. Patients in the control group were not subjected to standard treatment, but also received leukapheresis processing with 1.5-2 times the patient's blood volume. While patients in the Sipuleucel-T treatment group were reinfused with all their mononuclear cells, those in the 'control' group actually had a net removal of about 60% of their circulating mononuclear cells. Patients in the 'control' group had a decreased survival compared to literature, whereas the treatment group had 'normal' survival. Another unexpected finding was that elderly patients had an adverse prognosis in this study, in contrast to general expectations (12). In short, both the controversial study design and lack of clinically relevant results are a serious bloodletting to the evidence for clinical efficacy of therapeutic cancer vaccination.

The concept of therapeutic vaccination is not invalidated, however, by lack of evidence in a single study. Therapeutic vaccines have been extensively tested for CRPC using a dozen different approaches. From a tumour immunological viewpoint, CRPC is an ideal target since (i) prostate cancer is the most common cancer in men and the second most common cause of cancer-related death among men in Europe and North America (13, 14), (ii) no curative options exist for patients with advanced disease, *i.e.* disseminated CRPC (15-17), (iii) the overall survival is 28 to 36 months (18), implying sufficient time for effective immunological reactions to develop (iv) various prostate-specific antigens exist (19-22); and (v) the prostate it is not a vital organ, thus limiting the risk of life-threatening autoimmune complications. Keeping in mind, the massive clinical evidence of the efficacy of prophylactic vaccination, and the promises of cancer immunotherapy in general (23), therapeutic vaccination seems a reasonable approach for CRPC.

Forty-one clinical studies were performed that could have rejected the null hypothesis that there is no clinical-relevant effect of therapeutic vaccines. Most of these, 31/41, used therapeutic vaccines only, and 10 studies also used systemic injection of (antibodies against) co-stimulatory molecules (*e.g.* Cytotoxic T-Lymphocyte Antigen 4; CTLA4). We discuss these therapeutic results in light of cancer immunology, and anticancer therapies, including other immunotherapeutic approaches.

Therapeutic Vaccines Trigger Anticancer Immune Responses

The efficacy of prophylactic vaccination is often determined by measuring the increase in antigen-specific antibody or T-lymphocyte responses as a surrogate for immunological protection. Five studies measured antigen-specific immune responses by increasing levels of antibodies. The vast majority of treated patients, 85% (96 out of 113) had increased levels of tumour-associated antibodies (24-28). In three studies, cytotoxic T-lymphocytes and helper T-cells were counted, and vaccines stimulated antigen-specific T-cell proliferation in all 61 patients (24, 25, 27). This shows that these vaccines were technically effective in that they induced humoral and cellular immune responses in treated patients. No hard rules exist on immunological responses and clinical protection, but the immune responses are generally calibrated to their clinical efficacy. In prophylactic studies, immune protection is measured by pathogen challenge (*i.e.* in animal experiments) or the odds of developing disease after exposure to the pathogen. A major difference between prophylactic and therapeutic vaccination is that in the therapeutic setting no calibration of immune protection with surrogate immune parameters can be made. Thus the clinical efficacy must be measured directly, by monitoring disease regression and progression after vaccination.

Therapeutic Vaccination Yields No Clinically Relevant Anticancer Effects

Since patients with immunological response had a technically successful intervention, we focused on clinical responses in these patients. Only two out of 96 patients with antibody responses had a complete (CR) or a partial (PR) regression (Table I) (24-28). In addition, only one patient had a CR, and there were no PRs out of 61 patients with vaccine-stimulated antigen-specific T-cell proliferation (24, 25, 27). Although the vaccines were effective in inducing immune responses, they did not induce clinical responses. This therefore confirms earlier conclusions that antibody response and specific T-cell proliferation are not adequate predictors of clinical response to therapeutic vaccines (28, 29).

Clinically relevant parameters are the various measures of survival, *i.e.* disease-free, overall, and progression-free survival. Many studies also measure immune or biochemical parameters, but these are only important if they can be linked directly to survival of patients.

The Response Evaluation Criteria In Solid Tumours (RECIST) parameters also allow for measurement of tumour size (30). A surrogate parameter for the size of prostate carcinoma can be monitored by measuring Prostate-Specific Antigen (PSA) in serum. Prostate carcinomas shed PSA into the circulation, and a smaller tumour would shed less PSA

Table I. *Immunological responses compared to clinical responses in studies using therapeutic vaccination.*

	IR	CR	PR	PSA-R	Reference
Responses in antibody titers					
PA2024 antibodies					
	31/31	0	0	3	(24)
	13/21	1	0	1	(25)
	22/22	0	0	1	(26)
	11/11	0	0	3	(27)
LNCaP and PC-3 antibodies					
	19/28	0	1	1	(28)
Total antibodies	96/114	1	0	8	
Responses in T-cell proliferation					
	9/9	0	0	3	(27)
	31/31	0	0	3	(24)
	21/21	1	0	1	(25)
Total T-cell responses	61/61	1	0	7	

IR: Number of patients that demonstrated immunologic response, CR: complete regression, PR: partial regression, PSA-R: PSA response.

than a larger one. Changes in PSA are interpreted in two ways, as PSA response and changes in doubling time (DT). Changes in PSA DT are not considered to be a measure of therapeutic efficacy, since the DT implies that PSA is still high and rising (31). It is important to stress that from a clinical point of view, patients with a decrease in PSA DT still have progressive disease (PD).

A PSA response, *i.e.* a significant reduction of PSA value, could be indicative of a clinical response. The drawback is that measuring serum PSA is an indirect measure of the therapeutic effect. PSA levels can rise due to prostatitis and other diseases (32-34). Most relevantly successful immunotherapy of regional cancer, *e.g.* bladder carcinoma, can significantly increase the PSA levels (35), possibly as a bystander effect of local inflammation. In line with this, current criteria of the Prostate Cancer Working Group (PCWG2) strongly diminish the importance of PSA levels, in favour of measurement of the primary tumour and metastases (36). Considering the effect of tumour load and inflammation, the intended immunological rejection of prostate cancer could cause an increase or a decrease in PSA. The surrogacy of PSA end-points makes it unsuitable as the primary end-point in clinical trials in prostate cancer, especially in testing non-cytotoxic agents such as immunotherapy (37-39).

Clinical relevance of a therapeutic intervention should be evaluated in terms of a clear therapeutic benefit for the patients, and not as a change of a surrogate marker (10, 11). Ideally the benefit should be clinically prolonged survival, however this is not measured in most studies. Therefore, we focused on direct measurement of the therapeutic efficacy, *e.g.* in terms of CR, PR, stable disease (SD), and PD. Depending on the stage of CRPC, overall or disease-free survival could be monitored. A PSA test is a highly sensitive biochemical measurement for the presence of prostate cells in the body. In this light, progression-free survival might be considered if the included patients only had non-clinically manifest, biochemical disease (*i.e.* 'PSA recurrence') and progression was defined as the first detected metastasis; technically this would be referred to as clinical progression.

Table II summarizes data of 41 clinical studies employing therapeutic vaccination against prostate cancer performed in 2000-2012. These vaccination approaches have used the entire spectrum of modern vaccine technology, including different types of antigen (prostate cancer cells, protein, peptides, DNA, and carbohydrates), different modes of delivery [virus, DNA, and dendritic cells (DCs)], and different adjuvants of co-stimulation (biological response modifiers, cytokines such as Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin 2 (IL2); co-stimulatory molecules or inhibition of CTLA4). Considering the importance of responses in clinical phase I, II and III studies, it may be assumed that any response, whether complete or partial would be reported. However the state-of-the art vaccination technologies led to only one CR and three PRs out of 1,100 treated patients.

Traditionally, clinical effects against CRPC are not measured by remissions (CRs and PRs), but by determining PSA level. As stated before, this may not be the optimal method since effective immune responses might also induce a rise in PSA. Table III shows the PSA responses for all vaccines together, vaccines-plus-docetaxel, docetaxel-alone and mitoxantrone. The PSA responses for all vaccines were low, with an average of about 2%. Even the best result in a single study yielded less than 13%. This is much lower than studies employing chemotherapy. A small study of the combination of vaccine with docetaxel yielded 21%, and docetaxel alone 48%. Another chemotherapy intervention, mitoxantrone yielded 30% PSA responses.

Taken together both the remissions and the PSA responses do not indicate clinical efficacy of the therapeutic vaccines.

Evaluation of the Lack of Clinical Efficacy

As stated before, prophylactic vaccination against pathogens effectively prevents disease by inducing immunity. Other immunotherapeutic approaches have produced results that suggest that the immune system can also cure cancer and protect from recurrent disease. Why are these results not reflected in the outcomes of 1,100 patients with prostate cancer treated with therapeutic vaccination? Many differences existed within vaccination philosophies and protocols used, and it could be argued that only a few vaccination studies used an effective protocol, blurring the data in the aggregate of 41 studies. One could further reason

Table II. *Summarized therapeutic results from studies employing different vaccination strategies.*

Principle	Number of		Response, n (%)		Reference
	Studies (*)	Patients	CR	PR	
hTERT vaccination	1 (–)	18	-	-	(116)
PSMA	2 (1)	43	-	-	(117, 118)
Peptide vaccination	4 (–)	110	-	1 (0.9%)	(119-122)
Carbohydrate	1 (–)	25	-	-	(123)
DNA vaccine	2 (–)	42	-	-	(119)
Viral prostate Ag	3 (–)	161	-	-	(124, 125)
BRM	2 (–)	54	-	-	(126, 127)
APC8015	7 (3)	182	1 (0.5%)	-	(24, 25, 27, 128-130)
GM-CSF	5 (1)	116	-	1 (0.9%)	(28, 131-134)
MVA-MUC-IL2	4 (–)	98	-	-	(135-138)
All vaccine only	31 (5)	849	1 (0.1%)	2 (0.2%)	All the above
Co-stimulation	11 (5)	251	-	1 (0.4%)	(8, 26, 132, 134, 139-144)

*Number of studies with metastasized disease. CR: Complete response; PR: partial response; hTERT: human Telomerase reverse transcriptase; PSMA: prostate-specific membrane antigen; Ag: antigen; BRM: biological response modifiers; APC8015: Sipuleucel-T (trade name Provenge); GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor; MVA-MUC-IL2: modified vaccinia virus Ankara (MVA) strain encoding human mucin 1 (MUC1) and interleukin-2 (IL-2).

that a putative effect of vaccination strategy in a small study of 10 to 50 patients would be too small to be seen. But do these arguments suffice for the lack of clinical efficacy that is found after vaccination?

First of all, the PSA responses were much lower, even in the vaccine study most responsive effects compared to docetaxel treatment, *e.g.* 13% *versus* 48%, respectively. Thus vaccines seem to be ineffective in inducing PSA response. Alternatively, a single study might induce CR or PR in a substantial group of patients. Various other cancer immunotherapies have yielded such results that are statistically significant and clinically relevant in groups of 10 to 50 patients (40). In order to obtain a p-value <0.05, at least three patients in a study should have a CR or PR. Besides the possibility of being statistically significant, this would also be considered as clinically relevant. We previously discussed the induction of immune responses in 85% to 100% of the patients treated, which correlates nicely with data from prophylactic studies showing that 90 to 100% of the vaccinated people were protected. If three CR or PR responses were obtained in a study with ten to fifty patients, it would be a clinical response of 6 to 30%. Thus, if therapeutic vaccination were clinically effective, such a response should be more than feasible. Reviews of clinical immunotherapeutic studies for eight different forms of both locally advanced and metastasized cancer showed that these yielded on average 50% CRs and PRs. All 15 studies with six to 35 patients had at least three clinical responses in patients with immunotherapy (41). From all these data it is more than reasonable to assume that if similar results had been obtained in any of these 41 studies, these would have arisen at the analysis of PSA responses, or CRs and PRs. Nevertheless, such a result was not found in any of the vaccination studies. It is hard to solely attribute the failure of success to a sub-optimal vaccination strategy, since these studies employed many different vaccination strategies, all being the state-of-the art vaccine technology.

Table III. *Comparing PSA responses (PSA-R) in studies employing different therapies against castration-resistant prostate cancer (CRPC)*

	n	% PSA-R	References
All vaccines	1100	2.3%	(see Table II)
Vaccine + docetaxel	14	21.4%	(134)
Docetaxel	1007	47.8%	(15, 145)
Mitoxantrone	673	29.5%	(15, 145)

N: Number of patients included in study; PSA-R: standard response in surrogate marker prostate-specific antigen (PSA), see text for discussion on PSA-R.

Cancer immunotherapy is not a strategy without clinical results. Although therapeutic vaccination has failed to show clinical efficacy, other forms of cancer immunotherapy did show clinically relevant results (40, 41). Thus the question might be asked is therapeutic vaccination a putative curative therapy of cancer? And if not, how could cancer immunotherapy be improved?

Cutting a long story short, vaccines are most effective in preventing, but not curing, disease. The next question is, can we explain the inefficacy of therapeutic vaccines using immunological dogmas?

Tumour-bearing Patients Are Antigen-specific Tolerant

Patients that bear or bore tumours are not immunologically-naïve, but antigen-specific tolerant to the tumour. Immune tolerance is an active immunological process that involves various T-lymphocyte populations. Helper and cytotoxic T-lymphocytes can differentiate into a variety of effector and tolerogenic functions. Immunologists differentiate between type 1, type 2, type 17 (T_{H17}), and a whole range of suppressor and regulatory T-lymphocytes. Tumour-infiltrating T-cells can become immune suppressive or tolerogenic (42). Regulatory T-lymphocytes were shown to be involved in immune tolerance to murine tumours, and were also demonstrated in various human tumours (43). Tumours induce various kinds of regulatory T-lymphocytes (44). In the blood of patients with prostate cancer, cluster of differentiation 4 ($CD4^+$) T-cells co-expressing $CD25^{high}$ and forkhead box P3 ($FOXP3^+$), *e.g.* regulatory T-lymphocytes, are increased in number and function (45-47). Locally in the tumour, different types of tolerogenic T-lymphocytes are involved, *e.g.* T_{H17} and classical T_{Reg} (47, 48). Other studies have pinpointed the involvement of $CD8^+$ regulatory cells in prostate cancer by the markers $Foxp3^+$ or programmed death 1 ($PD\text{-}1^+$) (49, 50). The complex roles of different $CD4^+$ and $CD8^+$ T-lymphocytes were further confirmed (51, 52). In the most simplified model from a clinical perspective, two types of T-cells exist: immunogenic and tolerogenic lymphocytes. How does the presence of immunological tolerance affect the efficacy of therapeutic vaccination?

Therapeutic vaccination increases both effector T-lymphocytes and regulatory T-lymphocytes (53). Both types of antigen-specific T-lymphocytes respond to the tumour antigen they recognize and T-cell growth factors (*e.g.* cytokines such as IL2, and IL15). In the prophylactic setting, the immune system is naïve and has the plasticity to be easily instructed for immunogenic effectors. Any immunological adjuvants will steer the T-lymphocytes to immunogenic responses in the presence of antigen and cytokines. However, in diseased individuals the immune system is not naïve, but tolerant, *i.e.* 'instructed' for tumour tissue protecting responses. These memory T-lymphocytes have transcription factors that enable them to respond similarly to recall antigens as they did before (54-56). Thus once established, immune responses are rather rigid, and do not easily change their nature. Moreover, the antigen-specific tolerogenic T-lymphocytes mostly reside inside the tumour tissue (57). Thus tolerogenic cells do not differ from other memory T-lymphocytes that commonly circulate through the body, spending most time in their target tissue (*i.e.* tumour). If T-cell growth factors are produced due to the induction of an antitumour immune response, tolerogenic memory T-lymphocytes are activated in the presence of the tumour antigen. These cells divide and respond as determined by the transcriptions factors activated during immunological priming, and thus the recall antigens of vaccination result in more tolerogenic memory cells.

Figure 1 shows that therapeutic vaccination might amplify cells involved in both tumour tolerance and rejection, which might nullify the therapeutic efficacy. Some studies even suggest that therapeutic vaccination is detrimental (58). Because of the functional stability of (tolerogenic) T-lymphocytes (54, 55, 59), little is expected from subtle blocking of a single co-stimulatory molecule.

When clinical results are small, often the (antigenic) dose is increased or vaccination is repeated in order to induce more powerful responses. Increasing the number of responsive T-lymphocytes, might be accompanied by a higher level of tolerogenic T-cells, because of higher cytokine levels at the target tissue. Therapeutic cancer vaccines induce antibodies and cytotoxic T-lymphocytes against prostate cancer antigens. In the prophylactic setting, these immune responses are a good predictor of protection. However, in the therapeutic setting, vaccine-induced immune responses fail to be clinically effective, presumably because of the ongoing presence of antigen-specific tolerogenic lymphocytes that reside inside and nearby the tumour. A phase III study targeting CTLA4 to deplete regulatory T-cells systemically yielded minimal results with serious toxicity (60). The problem is not simply the presence of tumour at the moment of vaccination, since therapeutic vaccination strategy in patients with cleared tumours also yielded minimal results (61). Considering that tolerogenic lymphocytes are present that specifically are instructed at the immunological recall site, *i.e.* the vaccine depot, this might not be a complete surprise to immunologists.

In understanding the inefficacy of therapeutic vaccination, we would like to re-visit and deepen some questions. How can it be that vaccination is protective when applied in a prophylactic setting, but is not curative when applied in a therapeutic setting? In other words, what is the difference between immunological intervention in the prophylactic and the therapeutic settings? Tumour-bearing patients differ from healthy persons in two properties, their immunological status and, the presence of tumour as a '*de novo* organ' that maintains tissue homeostasis. In other words: How can this acquired immune-protected status of the tumour be broken?

Immunotherapy Becoming Effective by Destroying Tumour Homeostasis

Patients with cancer have acquired antigen-specific tolerance. Thus, how can we break tolerance and treat patients in a clinically effective way? Immunological tolerance to organs cannot be broken easily, since if tolerance against organs is lost it would cause autoimmune disorders incompatible with life. Thus, studies should focus on neutralizing antigen-specific tolerance. But how can we do this, knowing the wide spectrum

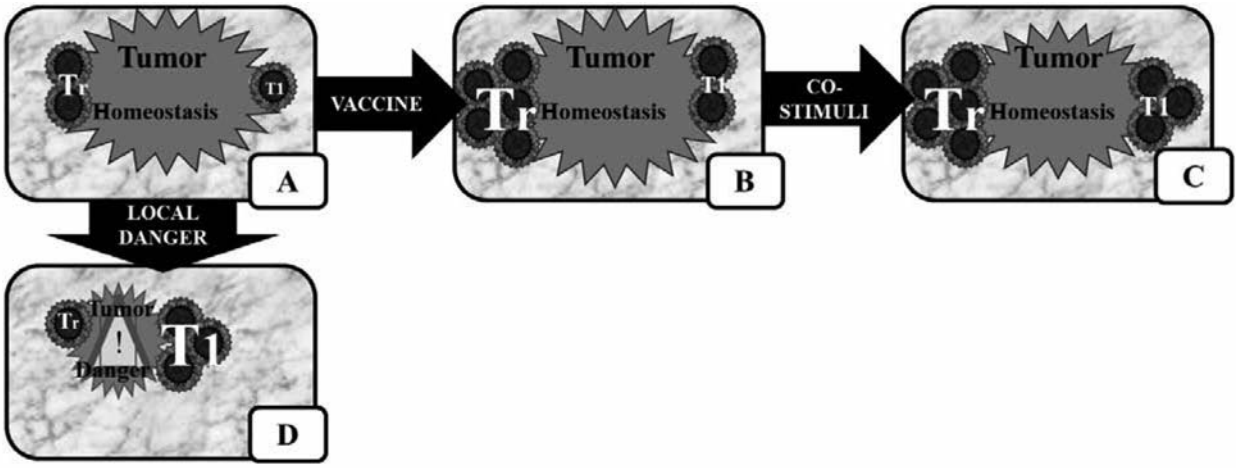

Figure 1. *A: Default immune status at a tumour. B: Immune status after vaccination. C: Immune status after vaccination and co-stimulation. D: Immune status after local 'danger' therapy. Tr, Regulatory or suppressive T (tolerogenic) lymphocytes; T1, type 1 helper or cytotoxic T-lymphocytes. Within tumour homeostasis regulatory T-lymphocytes actively protect the tissue from (immunological) harm (A). When a patient is vaccinated (B) tolerogenic and effector responses both increase. The effector responses are the direct result of the vaccination, but the presence of antigen plus T-cell growth factors (Interleukin-2, and - 15) also increases the number of tolerogenic cells. Since both tolerogenic and effector cells are increased in number and activity, the immunological awareness of the tumour is increased. Nevertheless, the tumour tissue remains actively protected. Similarly when co-stimulated, both tolerogenic and immunogenic lymphocytes are amplified to a similar extent, since both populations are activated in the same environment (i.e. at the tumour site) with the same T-cell growth factors (i.e. cytokines). Thus populations of both cells will increase (C). The effective option seems to be to induce local inflammation signals, either directly by cytokines, or indirectly by inducing local 'danger' in the tissue (D). Tumour cell killing releases antigen in the presence of danger signals, e.g. due to dying cells. The tolerogenic T-lymphocytes are destroyed or re-instructed to become effector cells, allowing the installation of tumour immunity.*

of tumour associated antigens? If only tolerance was broken of one or two antigens on the tumour, the tumour cells would be protected by the remaining tolerogenic T-lymphocytes.

Antigen-specific T-lymphocytes home preferentially to their target tissue (62, 63). They are crucial in maintaining tissue homeostasis (56) and depend on tissue homeostasis and antigens. In the case of tumour-bearing patients the antigen-specific T-lymphocytes are tolerogenic cells. Thus if their target tissue is destroyed, these tolerogenic cells might be open for immunological reinstruction or destruction.

Many studies indicate that cancer immunotherapy other than therapeutic vaccination can lead to clinically relevant effects [reviewed in (40)]. The crux to understanding tumour immunology resides in understanding the mechanisms evoked by effective therapies. From a physiological point of view the tumour is a *de novo* organ, with quite normal homeostasis and absence of immunological danger (64, 65). The presence of danger is the key instruction for the immune system to discriminate between tolerogenic and immunogenic responses towards antigens. In the case of cancer the immune system recognizes danger by endogenous danger signals, *i.e.* the induction of cell death or inflammation. Thus immunological danger could be induced in two different ways.

Firstly, direct destruction of (a part of) the tumour would be read as immunological danger. Radiotherapy and chemotherapy are cytotoxic and destroy tumour homeostasis. Cell death induces the maturation of DCs *in vitro*, *ex vivo* and *in vivo* (66-68). DCs are crucial in initiating immune responses (69, 70). Animal models show that radiotherapy and chemotherapy may aid immunotherapy (71-76) and local radiotherapy can synergize with systemic anticancer treatment (77), as in prostate cancer (78). Chemotherapy, radiotherapy and hormone therapy induce antibodies and cellular immune responses against prostate cancer (76, 79, 80) and other tumours (74). A preliminary study has shown a therapeutic effect in preventing cancer recurrence by the combination of vaccination and irradiation (81). Induction of immunity could be important in the synergy between radiotherapy and chemotherapy with immunotherapy observed in the clinic for other tumour types (82-87). Research in an autologous animal model suggests that chemotherapy with cyclophosphamide could synergize with immunotherapy (76), this was confirmed by a phase II clinical trial (88).

Secondly, causing local inflammation can be seen as immunological danger. Local initiation is crucial in the normal development of immune responses. Many tumours deploy local immune inhibition to maintain physiology (89); inflammatory killing of tumour cells could break this *status quo* (90, 91). Deliberate induction of intra-tumoural inflammation can induce T-lymphocytes (92, 93) or reprogram

regulatory T-lymphocytes *in situ* (59). Thus inducing intra-tumoural inflammation can be clinically effective against cancer (94, 95). The principle of inducing inflammation against cancer has been successfully employed by Bacillus Calmette–Guérin (BCG) therapy for bladder carcinoma (96-100). Systemic BCG therapy would cause a life-threatening disease, thus BCG is only applied locally. BCG induces inflammation (101) by local mediators such as IL2 (102, 103). Local, rather than systemic IL2 is active against bladder cancer (104-107) and other forms of cancer (40, 41, 98, 108, 109). These effects of IL2 are not attributable to systemic IL2, since only local treatment shows clinically relevant efficacy against nasopharyngeal carcinoma (83, 110) and metastasized melanoma (111-115). Local, rather than systemic immune stimulation is effective, as systemic immune stimulation induces immunosuppressive feedback loops (40).

Conclusion

Vaccination has shown its clinical efficacy in the prophylactic setting, but not in the therapeutic setting. Therapeutic vaccination stimulates both immunogenic and tolerogenic responses, thereby nullifying its overall clinical efficacy. Thus, vaccination might have immunological effects, but little therapeutic effect. We hypothesize that immune protection might be due to tumour homeostasis, likely controlled by tolerogenic T-lymphocytes, such as regulatory and suppressor T-cells. Tumour homeostasis might be overruled by immunological danger in two different mechanisms. The first mechanism is by induction of tumour death by locally-applied cytotoxic anticancer therapy; the second is by induction of inflammation inside the tumour. Initiating local immune responses in the tumour seems to be required for effective anticancer immunotherapy. Several studies have shown that this is a possible approach in the pre-clinical and clinical setting for a variety of tumour types.

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References

1 Coley WB: The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of 10 original cases. 1893. Clin Orthopaed Rel Res *262*: 3-11, 1991.

2 Green HN: An immunological concept of cancer: a preliminary report. Br Med J *2*: 1374-1380, 1954.

3 Rosenberg SA, Yang JC and Restifo NP: Reply to "Cancer vaccines: pessimism in check". Nat Med *10*: 1279-1280, 2004.

4 Van der Sande MA, Waight PA, Mendy M, Zaman S, Kaye S, Sam O, Kahn A, Jeffries D, Akum AA, Hall AJ, Bah E, McConkey SJ, Hainaut P and Whittle HC: Long-term protection against HBV chronic carriage of Gambian adolescents vaccinated in infancy and immune response in HBV booster trial in adolescence. PLoS One *15*: e753, 2007.

5 Gissmann L and Nieto K: The therapeutic vaccine: is it feasible? Arch Med Res *40*: 493-498, 2009.

6 Stanley M: Prophylactic human papillomavirus vaccines: Will they do their job? J Internal Med *267*: 251-259, 2010.

7 Pardoll D and Drake C: Immunotherapy earns its spot in the ranks of cancer therapy. J Exp Med *209*: 201-209, 2012.

8 Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF, T A, A A, Arseneau J, Barth N, Bernstein G, Bracken B, Burch P, Caggiano V, Chin J, Chodak G, Chu F, Corman J, Curti B, Dawson N, Deeken JF, Dubernet T, Fishman M, Flanigan R, Gailani F, Garbo L, Gardner T, Gelmann E, George D, Godfrey T, Gomella L, Guerra M, Hall S, Hanson J, Israeli R, Jancis E, Jewett MA, Kassabian V, Katz J, Klotz L, Koeneman K, Koh H, Kratzke R, Lance R, Lech J, Leichman L, Lemon R, Liang J, Libertino J, Lilly M, Malik I, Martin SE, McCaffrey J, McLeod D, McNeel D, Miles B, Murdock M, Nabhan C, Nemunaitis J, Notter D, Pantuck A, Perrotte P, Pessis D, Petrylak D, Polikoff J, Pommerville P, Ramanathan S and Rarick M: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. New Engl J Med *363*: 411-422, 2010.

9 Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, Manson K, Panicali DL, Laus R, Schlom J, Dahut WL, Arlen PM, Gulley JL and Godfrey WR: 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 *28*: 1099-1105, 2010.

10 Jacobs JJL, Characiejus D, Scheper RJ, Stewart RJE, Tan JFV, Tomova R, Krastev Z and Den Otter W: The Amiens Strategy: Small Phase III Trials for Clinically Relevant Progress in the War Against Cancer. J Clin Oncol *27*: 3062-3063, 2009.

11 Stewart DJ and Kurzrock R: Comments and Controversies. Cancer: The road to Amiens. J Clin Oncol 27: 328-333, 2009.

12 Huber ML, Haynes L, Parker C, and Iversen P: Interdisciplinary Critique of Sipuleucel-T as Immunotherapy in Castration-Resistant Prostate Cancer. J Natl Cancer Inst *104*: 273-279, 2012.

13 Siegel R, Naishadham D, and Jemal A: Cancer Statistics, 2013. CA: Cancer J Clin *63*: 11-30, 2013.

14 Berrino F, De Angelis R, Sant M, Rosso S, Lasota MB, Coebergh JW, Santaquilani M, and the EUROCARE Working group: Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995–99: results of the EUROCARE-4 study. Lancet Oncol *8*: 773-783, 2007.

15 Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Théodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA, and TAX 327 Investigators: Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. New Engl J Med *351*: 205-206, 2004.

16 Calabrò F and Sternberg CN: Current indications for chemotherapy in prostate cancer patients. Eur Urol *51*: 17-26, 2007.

17 Mike S, Harrison C, Coles B, Staffurth J, Wilt TJ and Mason MD: Chemotherapy for hormone-refractory prostate cancer. Cochrane Database Syst Rev *18*: CD005247, 2006.

18 Sternberg CN: Systemic chemotherapy and new experimental approaches in the treatment of metastatic prostate cancer. Ann Oncol *19*: vii91-95, 2008.

19 Novellino L, Castelli C and Parmiani G: A listing of human tumor antigens recognized by T-cells: March 2004 update. Cancer Immunol Immunother *54*: 187-207, 2005.

20 Weinschenk T, Gouttefangeas C, Schirle M, Obermayr F, Walter S, Schoor O, Kurek R, Loeser W, Bichler KH, Wernet D, Stevanović S and Rammensee HG: Integrated functional genomics approach for the design of patient-individual antitumor vaccines. Cancer Res *62*: 5818-5827, 2002.

21 Li Y, Cozzi PJ and Russel PJ: Promising tumor-associated antigens for future prostate cancer therapy. Med Res Rev *30*: 67-101, 2010.

22 Solin T, Kontturi M, Pohlmann R and Vihko P: Gene expression and prostate specificity of human prostatic acid phosphatase (PAP): evaluation by RNA blot analyses. Biochim Bioph Acta *1048*: 72-77, 1990.

23 Couzin-Frankel J: Cancer Immunotherapy. Science *342*: 1432-1433 2013.

24 Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV and Valone FH: Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol *18*: 3894-3903, 2000.

25 Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, Richardson RL, Valone FH and Vuk-Pavlović S: Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a phase II trial. Prost *60*: 197-204, 2004.

26 Rini BI, Weinberg V, Fong L, Conry S, Hershberg RM and Small EJ: Combination immunotherapy with prostatic acid phosphatase-pulsed antigen-presenting cells (provenge) plus bevacizumab in patients with serologic progression of prostate cancer after definitive local therapy. Cancer *107*: 67-74, 2006.

27 Burch PA, Breen JK, Buckner JC, Gastineau DA, Kaur JA, Laus RL, Padley DJ, Peshwa MV, Pitot HC, Richardson RL, Smits BJ, Sopapan P, Strang G, Valone FH and Vuk-Pavlović S: Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. Clin Cancer Res *6*: 2175-2182, 2000.

28 Small EJ, Sacks N, Nemunaitis J, Urba WJ, Dula E, Centeno AS, Nelson WG, Ando D, Howard C, Borellini F, Nguyen M, Hege K and Simons JW: Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin Cancer Res *13*: 3883-3891, 2007.

29 Hoos A, Eggermont AM, Janetzki S, Hodi FS, Ibrahim R, Anderson A, Humphrey R, Blumenstein B, Old L and Wolchok J: Improved endpoints for cancer immunotherapy trials. J Natl Cancer Inst *102*: 1388-1397, 2010.

30 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst *92*: 205-216, 2000.

31 Smith MR and Kantoff PW: Changes in PSA kinetics after DNA vaccine therapy – Not so fast! J Clin Oncol 28: 58, 2010.

32 Sindhwani P and Wilson CM: Prostatitis and serum prostate-specific antigen. Curr Urol Rep *6*: 307-312, 2005.

33 Kobayashi M, Nukui A, and Morita T: Serum PSA and percent free PSA value changes after antibiotic treatment. A diagnostic method in prostate cancer suspects with asymptomatic prostatitis. Urol Int *80*: 186-192, 2008.

34 Loeb S, Gashti SN and Catalona WJ: Exclusion of inflammation in the differential diagnosis of an elevated prostate-specific antigen (PSA). Urol Oncol *27*: 64-6, 2009.

35 Beltrami P, Ruggera L, Cazzoletti L, Schiavone D and Zattoni F: Are prostate biopsies mandatory in patients with prostate-specific antigen increase during intravesical immuno- or chemotherapy for superficial bladder cancer? Prost *68*: 1241-1247, 2008.

36 Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, Eisenberger MA, Higano C, Bubley GJ, Dreicer R, Petrylak D, Kantoff P, Basch E, Kelly WK, Figg WD, Small EJ, Beer TM, Wilding G, Martin A, Hussain M, and Prostate Cancer Clinical Trials Working Group: Design and endpoints of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol *26*: 1148-1159, 2008.

37 Collette L, Burzykowski T, Carroll KJ, Newling D, Morris T, Schröder FH, Cancer EOfRaTo, Centrum LU, and Pharmaceuticals. A: Is prostate-specific antigen a valid surrogate endpoint for survival in hormonally treated patients with metastatic prostate cancer? Joint research of the European Organisation for Research and Treatment of Cancer, the Limburgs Universitair Centrum, and AstraZeneca Pharmaceuticals. J Clin Oncol *23*: 6139-6148, 2005.

38 Collette L, Burzykowski T and Schröder FH: Prostate-specific antigen (PSA) alone is not an appropriate surrogate marker of long-term therapeutic benefit in prostate cancer trials. Eur J Cancer *42*: 1344-1350, 2006.

39 Collette L: Prostate-specific antigen (PSA) as a surrogate endpoint for survival in prostate cancer clinical trials. Eur Urol *53*: 6-9, 2008.

40 Jacobs JJL, Characiejus D, Tomova R, Baran J, Bubenik J, Zembala M, Krastev Z, Scheper RJ, Pawelec G and Den Otter W: Local, rather than systemic immunotherapy has therapeutic efficacy against metastasized cancer. Trends Cancer Res *7*: 1-14, 2011.

41 Den Otter W, Jacobs JJL, Battermann JJ, Hordijk GJ, Krastev Z, Moiseeva EV, Stewart RJE, Ziekman PGPM and Koten JW: Local therapy of cancer with free IL-2. Cancer Immunol Immunother *57*: 931-950, 2008.

42 Shafer-Weaver KA, Anderson MJ, Stagliano K, Malyguine A, Greenberg NM and Hurwitz AA: Cutting Edge: Tumor-specific $CD8^+$ T-cells infiltrating prostatic tumors are induced to become suppressor cells. J Immunol 183: 4848-4852, 2009.

43 Ha T-Y: Regulatory T cell in cancer. Immune Network *9*: 209-235, 2009.

44 Valzasina B, Piconese S, Guiducci C and Colombo MP: Tumor-induced expansion of regulatory T cells by conversion of $CD4^+CD25^+$ lymphocytes is thymus and proliferation independent. Cancer Res *66*: 4488-4495, 2006.

45 Yokokawa J, Cereda V, Remondo C, Gulley JL, Arlen PM, Schlom J and Tsang KY: Enhanced functionality of $CD4^+CD25^{high}FoxP3^+$ regulatory T cells in the peripheral blood of patients with prostate cancer. Clin Cancer Res *14*: 1032-1040, 2008.

46 Miller AM, Lundberg K, Ozenci V, Banham AH, Hellström M, Egevad L and Pisa P: CD4+CD25high T cells are enriched in the tumor and peripheral blood of prostate cancer patients. J Immunol *177*: 7398-7405, 2006.

47 Sfanos KS, Bruno TC, Maris CH, Xu L, Thoburn CJ, DeMarzo AM, Meeker AK, Isaacs WB and Drake CG: Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. Clin Cancer Res *14*: 3254-3261, 2008.

48 Poutahidis T, Rao VP, Olipitz W, Taylor CL, Jackson EA, Levkovich T, Lee CW, Fox JG, Ge Z and Erdman SE: CD4+ lymphocytes modulate prostate cancer progression in mice. Int J Cancer *125*: 868-878, 2009.

49 Sfanos KS, Bruno TC, Meeker AK, De Marzo AM, Isaacs WB and Drake CG: Human prostate-infiltrating CD8+ T lymphocytes are oligoclonal and PD-1+. Prost *69*: 1694-1703, 2009.

50 Kiniwa Y, Miyahara Y, Wang HY, Peng W, Peng G, Wheeler TM, Thompson TC, Old LJ and Wang RF: CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer. Clin Cancer Res *13*: 6947-6958, 2007.

51 Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, De Jong LA, Vyth-Dreese FA, Dellemijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA and Restifo NP: Tumor Regression and Autoimmunity after Reversal of a Functionally Tolerant State of Self-reactive CD8+ T-Cells. J Exp Med *198*: 569-580, 2003.

52 Degl'Innocenti E, Grioni M, Capuano G, Jachetti E, Freschi M, Bertilaccio MT, Hess-Michelini R, Doglioni C and Bellone M: Peripheral T-cell tolerance associated with prostate cancer is independent from CD4+CD25+ regulatory T cells. Cancer Res *68*: 292-300, 2008.

53 Zhou G, Drake CG and Levitsky HI: Amplification of tumor-specific regulatory T-cells following therapeutic cancer vaccines. Blood *107*: 629-636, 2006.

54 Lees JR and Farber DL: Generation, persistence and plasticity of CD4 T-cell memories. Immunology *130*: 463-470, 2010

55 Ahmadzadeh M and Farber DL: Functional plasticity of an antigen-specific memory CD4 T cell population. Proc Nat Acad Sci USA *99*: 11802-11807, 2002

56 Oleinika K, Nibbs RJ, Graham GJ and Fraser AR: Suppression, subversion and escape: the role of regulatory T-cells in cancer progression. Clin Exp Immunol *171*: 36-45, 2013

57 Huang Y, Shah S and Qiao L: Tumor resistance to CD8+ T cell-based therapeutic vaccination. Arch Immunol Ther Exp (Warsz) *55*: 205-217, 2007.

58 Ricupito A, Grioni M, Calcinotto A, Michelini RH, Longhi R, Mondino A and Bellone M: Booster Vaccinations against Cancer Are Critical in Prophylactic but Detrimental in Therapeutic Settings. Cancer Res *73*: 3545-3554, 2013.

59 da Silva Martins M and Piccirillo CA: Functional stability of FoxP3+ regulatory T cells. Trends Mol Med *18*: 454-462, 2012.

60 Gerritsen WR, Kwon ED, and Fizazi K: Ipilimumab does not significantly improve survival in patients with advanced CRPC but may have most benefit on patients with lower disease burden. Oncol Ex *12*: 30-31, 2013.

61 Gates JD, Benavides LC, Carmichael MG, Holmes JP, Hueman MT, Mittendorf EA, McLeod DG, Ponniah S and Peoples GE: Long-term follow-up assessment of a HER-2/neu peptide (E75) vaccine for the prevention of recurrence in high-risk prostate cancer patients. J Clin Oncol *26*: (15S) 3067, 2008.

62 Kantele A, Kantele JM, Savilahti E, Westerholm M, Arvilommi H, Lazarovits A, Butcher EC and Mäkelä PH: Homing potentials of circulating lymphocytes in humans depend on the site of activation: oral, but not parenteral, typhoid vaccination induces circulating antibody-secreting cells that all bear homing receptors directing them to the gut. J Immunol *158*: 574-579, 1997.

63 Kantele A, Zivny J, Häkkinen M, Elson CO and Mestecky J: Differential Homing Commitments of Antigen-Specific T Cells After Oral or Parenteral Immunization in Humans. J Immunol *162*: 5173-5177, 1999.

64 Matzinger P: Tolerance, danger, and the extended family. Ann Rev Immunol *12*: 991-1045, 1994.

65 Fuchs EJ and Matzinger P: Is cancer dangerous to the immune system? Sem Immunol *8*: 271-280, 1996.

66 Gallucci S, Lolkema M and Matzinger P: Natural adjuvants: endogenous activators of dendritic cells. Nat Med *5*: 1249-1255, 1999.

67 Jacobs JJL, Lehé CL, Hasegawa H, Elliott GR and Das PK: Skin irritants and contact sensitizers induce Langerhans cell migration and maturation at irritant concentration. Exp Derm *15*: 432-440, 2006.

68 Drexhage HA, Mullink H, de Groot J, Clarke J and Balfour BM: A study of cells present in peripheral lymph of pigs with special reference to a type of cell resembling the Langerhans cell. Cell Tissue Res *202*: 407-430, 1979.

69 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, and Palucka K: Immunobiology of dendritic cells. Ann Rev Immunol *18*: 767-811, 2000.

70 Ridge JP, Di Rosa F and Matzinger P: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helperand a T-killer cell. Nature *393*: 474-478, 1998.

71 Everse LA, Bernsen MR, Dullens HFJ and Den Otter W: Priming of the antitumor response promotes efficacy of interleukin-2 therapy. Cancer Immunol Immunother *44*: 221-229, 1997.

72 Everse LA, Renes IB, Jürgenliemk-Schulz IM, Rutgers DH, Bernsen MR, Dullens HFJ, Den Otter W and Battermann JJ: Local low-dose interleukin-2 induces systemic immunity when combined with radiotherapy of cancer. A pre-clinical study. Int J Cancer *72*: 1003-1007, 1997.

73 Harris TJ, Hipkiss EL, Borzillary S, Wada S, Grosso JF, Yen HR, Getnet D, Bruno TC, Goldberg MV, Pardoll DM, DeWeese TL and Drake CG: Radiotherapy augments the immune response to prostate cancer in a time-dependent manner. Prost *68*: 1319-1329, 2008.

74 Perez CA, Fu A, Onishko H, Hallahan DE and Geng L: Radiation induces an antitumour immune response to mouse melanoma. Int J Rad Biol *85*: 1126-1136, 2009.

75 Chu Y, Wang LX, Yang G, Ross HJ, Urba W and J PR: Efficacy of GM-CSF-producing tumor vaccine after docetaxel chemotherapy in mice bearing established Lewis lung carcinoma. J Immunother *29*: 367-380, 2006.

76 Wada S, Yoshimura K, Hipkiss EL, Harris TJ, Yen HR, Goldberg MV, Grosso JF, Getnet D, Demarzo AM, Netto GJ, Anders R, Pardoll DM and Drake CG: Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. Cancer Res *69*: 4309-4318, 2009.

77 Formenti SC and Demaria S: Systemic effects of local radiotherapy. Lancet Oncol *10*: 718-726, 2009.

78 Verhagen PCMS, Schröder FH, Collette L and Bangma CH: Does local treatment of the prostate in advanced and/or lymph node metastatic disease improve efficacy of androgen-deprivation therapy? A systematic review. Eur Urol *58*: 261-269, 2010.

79 Nesslinger NJ, Sahota RA, Stone B, Johnson K, Chima N, King C, Rasmussen D, Bishop D, Rennie PS, Gleave M, Blood P, Pai H, Ludgate C and Nelson BH: Standard treatments induce antigen-specific immune responses in prostate cancer. Clin Cancer Res *13*: 1493-1502, 2007.

80 Fujita T, Teh BS, Timme TL, Mai WY, Satoh T, Kusaka N, Naruishi K, Fattah EA, Aguilar-Cordova E, Butler EB and Thompson TC: Sustained long-term immune responses after *in situ* gene therapy combined with radiotherapy and hormonal therapy in prostate cancer patients. Int J Rad Oncol Biol Physiol *65*: 84-90, 2006.

81 Aguilar LK, Teh B, Mai W, Caillouet J, Ayala G, Aguilar-Cordova E and Butler E: Five year follow up of a phase II study of cytotoxic immunotherapy combined with radiation in newly diagnosed prostate cancer. J Clin Oncol *24*: (18S) 4635, 2006.

82 Spanos WC, Nowicki P, Lee DW, Hoover A, Hostager B, Gupta A, Anderson ME and Lee JH: Immune response during therapy with cisplatin or radiation for human papillomavirus-related head and neck cancer. Arch Otolaryngol Head Neck Surg *135*: 1137-1146, 2009.

83 Jacobs JJL, Hordijk GJ, Jürgenliemk-Schulz IM, Terhaard CHJ, Koten JW, Battermann JJ and Den Otter W: Treatment of stage III-IV nasopharyngeal carcinomas by external beam irradiation and local low doses of IL-2. Cancer Immunol Immunother *54*: 792-798, 2005.

84 Jourdier TM, Moste C, Bonnet MC, Delisle F, Tafani JP, Devauchelle P, Tartaglia J and Moingeon P: Local immunotherapy of spontaneous feline fibrosarcomas using recombinant poxviruses expressing interleukin 2 (IL2). Gene Ther *10*: 2126-2132, 2003.

85 Weide B, Eigentler TK, Pflugfelder A, Leiter U, Meier F, Bauer J, Schmidt D, Radny P, Pföhler C and Garbe C: Survival after intratumoral interleukin-2 treatment of 72 melanoma patients and response upon the first chemotherapy during follow-up. Cancer Immunol Immunother *60*: 487-493., 2011.

86 Spoormakers TJ, Klein WR, Jacobs JJL, Van Den Ingh TS, Koten JW and Den Otter W: Comparison of the efficacy of local treatment of equine sarcoids with IL2 or cisplatin/IL2. Cancer Immunol Immunother *52*: 179-184, 2003.

87 Kaasinen E, Rintala E, Hellström P, Viitanen J, Juusela H, Rajala P, Korhonen H and Liukkonen T: Factors explaining recurrence in patients undergoing chemoimmunotherapy regimens for frequently recurring superficial bladder carcinoma. Eur Urol *42*: 167-174, 2002.

88 Antonarakis ES and Carducci MA: Combining low-dose cyclophosphamide with GM-CSF-secreting prostate cancer immunotherapy enhances antitumor immune effects. Exp Op Invest Drugs *19*: 311-314, 2010.

89 Miller AM and Pisa P: Tumor escape mechanisms in prostate cancer. Cancer Immunol Immunother *56*: 81-87, 2007.

90 Wang R-F: Regulatory T-cells and innate immune regulation in tumor immunity. Springer Sem Immunol *28*: 17-23, 2006.

91 Daniels GA, Sanchez-Perez L, Diaz RM, Kottke T, Thompson J, Lai M, Gough M, Karim M, Bushell A, Chong H, Melcher A, Harrington K and Vile RG: A simple method to cure established tumors by inflammatory killing of normal cells. Nat Biotech *22*: 1129-1132, 2004.

92 Jackaman C, Bundell CS, Kinnear BF, Smith AM, Filion P, van Hagen D, Robinson BW, Nelson DJ and 171: 5051 JI: IL-2 intratumoral immunotherapy enhances CD8+ T cells that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2. J Immunol *171*: 5051-5063, 2003.

93 Ruffini PA, Morandi P, Cabioglu N, Altundag K and M. Cristofanilli: Manipulating the Chemokine-Chemokine Receptor Network to Treat Cancer. Cancer *109*: 2392-2404, 2007.

94 Jackaman C, Lew AM, Zhan Y, Allan JE, Koloska B, Graham PT, Robinson BW and Nelson DJ: Deliberately provoking local inflammation drives tumors to become their own protective vaccine site. Int Immunol *20*: 1467-1479, 2008.

95 Jacobs JJL, Sparendam D and Den Otter W: Local interleukin 2 therapy is most effective against cancer when injected intra-tumourally. Cancer Immunol Immunother *54*: 647-654, 2005.

96 Kurth KH, Bouffioux C, Sylvester R, van der Meijden AP, Oosterlinck W and Brausi M: Treatment of superficial bladder tumors: achievements and needs. The EORTC Genitourinary Group. Eur Urol *37(Suppl 3)*: 1-9, 2000.

97 Malmström PU, Sylvester RJ, Crawford DE, Friedrich M, Krege S, Rintala E, Solsona E, Di Stasi SM and Witjes JA: An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C *versus* bacillus Calmette-Guérin for non-muscle-invasive bladder cancer. Eur Urol *56*: 247-256, 2009.

98 Kresowik TP and Griffith TS: Bacillus Calmette-Guerin immunotherapy for urothelial carcinoma of the bladder. Immunotherapy *1*: 281-288, 2009.

99 Morales A: Evolution of intravesical immunotherapy for bladder cancer: mycobacterial cell wall preparation as a promising agent. Exp Op Invest Drugs *17*: 1265, 2008.

100 Shelley MD, Mason MD and Kynaston H: Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses. Cancer Treatm Rev *36*: 195-205, 2010.

101 Totterman TH, Loskog A and Essand M: The immunotherapy of prostate and bladder cancer. BJU international *96*: 728-735, 2005.

102 Böhle A and Brandau S: Immune mechanisms in bacillus Calmette-Guerin immunotherapy for superficial bladder cancer. J Urol *170*: 964-969, 2003.

103 Saint F, Kurth N, Maille P, Vordos D, Hoznek A, Soyeux P, Patard JJ, Abbou CC and Chopin DK: Urinary IL-2 assay for monitoring intravesical Bacillus Calmette-Guérin response of superficial bladder cancer during induction course and maintenance therapy. Int J Cancer *107*: 434-440, 2003.

104 Tubaro A, Stoppacciaro A, Velotti F, Bossola PC, Cusumano G, Vicentini C, De Carli P, Ruco L, Santoni A and Cancrini A: Local immunotherapy of superficial bladder cancer by intravesical instillation of recombinant interleukin-2. Eur Urol *28*: 297-303, 1995.

105 Ferlazzo G, Magno C, Iemmo R, Rizzo M, Lupo G, Semino C, Bruno S and Melioli G: Treatment of superficial bladder cancer with intravesical perfusion of rIL-2: a follow-up study. Anticanc Res *16*: 979-980, 1996.

106 Den Otter W, Dobrowolski Z, Bugajski A, Papla B, Van Der Meijden APM, Koten JW, Boon TA, Siedlar M and Zembala M: Intravesical interleukin-2 in T1 papillary bladder carcinoma: regression of marker lesion in 8 of 10 patients. J Urol *159*: 1183-1186, 1998.

107 Grasso M, Torelli F, Scannapieco G, Franzoso F and Lania C: Neoadiuvant treatment with intravesical interleukin-2 for recurrent superficial transitional bladder carcinoma Ta-T1/G1-2. J Immunother *24*: 184-187, 2001.

108 Shaker MA and Younes HM: Interleukin-2: Evaluation of routes of administration and current delivery systems in cancer therapy. J Pharm Sci *98*: 2268-2298, 2009.

109 Lichtor T, Glick RP, Awa G, Hardman J and Feldman LA: Advantages of intracerebral *versus* systemic administration of a DNA-based vaccine in treatment of an intracerebral tumor Gene Ther Mol Biol *12A*: 1-6, 2008.

110 Chi K, Myers J, Chow K, Chan W, Tsang Y, Chao Y, Yen S and Lotze M: Phase II trial of systemic recombinant interleukin-2 in the treatment of refractory nasopharyngeal carcinoma. Oncology *60*: 110-115, 2001.

111 Atallah E and Flaherty L: Treatment of metastatic malignant melanoma. Curr Treatm Opt Oncol *6*: 185-193, 2005.

112 Atkins M, Lotze M, Dutcher J, Fisher R, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L and SA R: High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. J Clin Oncol *17*: 2105-2116, 1999.

113 Radny P, Caroli UM, Bauer J, Paul T, Schlegel C, Eigentler TK, Weide B, Schwarz M and Garbe C: Phase II trial of intralesional therapy with interleukin-2 in soft-tissue melanoma metastases. Br J Cancer *89*: 1620-1626, 2003.

114 Smith FO, Downey SG, Klapper JA, Yang JC, Sherry RM, Royal RE, Kammula US, Hughes MS, Restifo NP, Levy CL, White DE, Steinberg SM and Rosenberg SA: Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res *14*: 5610-5618, 2008.

115 Weide B, Derhovanessian E, Pflugfelder A, Eigentler TK, Radny P, Zelba H, Pföhler C, Pawelec G and Garbe C: High response rate after intratumoral treatment with interleukin-2: results from a phase 2 study in 51 patients with metastasized melanoma. Cancer *116*: 4139-4146, 2010.

116 Su Z, Vieweg JW, Dannull J and Dahm P: Vaccination of metastatic prostate cancer patients using mature dendritic cells transfected with mRNA encoding hTERT or an MHC class II targeted hTERT/LAMP fusion protein: Results from a phase I clinical trial. J Clin Oncol *22*: (14S) 2507, 2004.

117 Valdagni R, Marrari A, Squarcina P, Villa S, Filipazzi P, Salvioni R, Rancati T, Asioli M, Parmiani G and Rivoltini L: Vaccination with survivin and PSMA-derived peptides for controlling biochemical recurrence in prostate cancer: A pilot study J Clin Oncol *27*: (15S) e16042 2009.

118 Tanaka M, Uemura H, Uejima S, Fujimoto K, Hirao Y and Itoh K: Phase I/II study of individualized peptide vaccines for HLA-A2/A24-positive patients with hormone-refractory prostate cancer. J Clin Oncol *26*: (15S) 5155, 2008.

119 McNeel DG, Dunphy EJ, Davies JG, Frye TP, Johnson LE, Staab MJ, Horvath DL, Straus J, Alberti D, Marnocha R, Liu G, Eickhoff JC and Wilding G: Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer. J Clin Oncol *27*: 4047-4054, 2009.

120 Perambakam S, Xie H, Edassery S and Peace DJ: Long-Term Follow-Up of HLA-A2+ Patients with High-Risk, Hormone-Sensitive Prostate Cancer Vaccinated with the Prostate Specific Antigen Peptide Homologue (PSA146-154). Clin Dev Immunol *2010*: 473453, 2010.

121 Perez SA, Kallinteris NL, Bisias S, Tzonis PK, Georgakopoulou K, Varla-Leftherioti M, Papamichail M, Thanos A, von Hofe E and Baxevanis CN: Results from a phase I clinical study of the novel Ii-Key/HER-2/neu(776-790) hybrid peptide vaccine in patients with prostate cancer. Clin Cancer Res *16*: 3495-3506, 2010.

122 Uemura H, Fujimoto K, Mine T, Uejima S, de Velasco MA, Hirao Y, Komatsu N, Yamada A and Itoh K: Immunological evaluation of personalized peptide vaccination monotherapy in patients with castration-resistant prostate cancer. Cancer Sci *101*: 601-608, 2010.

123 Slovin SF, Ragupathi G, Musselli C, Olkiewicz K, Verbel D, Kuduk SD, Schwarz JB, Sames D, Danishefsky S, Livingston PO and Scher HI: Fully Synthetic Carbohydrate-Based Vaccines in Biochemically Relapsed Prostate Cancer: Clinical Trial Results With α-N-Acetylgalactosamine-O-Serine/ Threonine Conjugate Vaccine. J Clin Oncol *23*: 4292-4298, 2003.

124 Aena DA, Joudi F, Williams RD, Eastman J, Flanders E, Zehr P, Griffith K and Lubaroff D: Adenovirus-PSA vaccination in recurrent and castration-resistant prostate cancer: Phase II trial interim results. J Clin Oncol 29: (15S) e15070, 2010

125 Kaufman HL, Wang W, Manola J, DiPaola RS, Y.-J. Ko, Sweeney C, Whiteside TL, Schlom J, Wilding G and Weiner LM: Phase II Randomized Study of Vaccine Treatment of Advanced Prostate Cancer (E7897): A Trial of the Eastern Cooperative Oncology Group. J Clin Oncol *22*: 2122-2132, 2004.

126 Pandha HS, Michael A, Quatan N, Wushishi F, Russell N, Whelan J and Whelan M: The effect of whole cell allogeneic vaccination on the progression of hormone-relapsed prostate cancer. J Clin Oncol *22*: (14S) 4735, 2004.

127 Dalgleish AG, Quatan N, Michael A, Wushishi F and Pandha H: Increased time to progression and sustained PSA velocity responses in a phase II trial in advanced metastatic prostate cancer following treatment with ONY-P1, an allogeneic whole cell vaccine. J Clin Oncol *23*: (16S) 4726, 2005.

128 Beinart G, Rini BI, Weinberg V and Small EJ: Antigen-presenting cells 8015 (Provenge) in patients with androgen-dependent, biochemically relapsed prostate cancer. Clin Prost Cancer *4*: 55-60, 2005.

129 Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, Verjee SS, Jones LA and Hershberg RM: Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone-refractory prostate cancer. J Clin Oncol *24*: 3089-3094, 2006.

130 Dueland S, Mu L-J, Kvalheim G, Hauser M, Waehre H, Aamdal S and Gaudernack G: Dendritic cells transfected with allo-tumor mRNA as cancer vaccine in treatment of hormone resistant prostate cancer patients. J Clin Oncol *23*: (16S) 2541, 2005.

131 Higano CS, Corman JM, Smith DC, Centeno AS, Steidle CP, Gittleman M, Simons JW, Sacks N, Aimi J and Small EJ: Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 113: 975-984, 2008.

132 Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I and Allison JP: A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. Clin Cancer Res *13*: 1810-1816, 2007.

133 Simons JW, Carducci MA, Mikhak B, Lim M, Biedrzycki B, Borellini F, Clift SM, Hege KM, Ando DG, Piantadosi S, Mulligan R and Nelson WG: Phase I/II trial of an allogeneic cellular immunotherapy in hormone-naïve prostate cancer. Clin Cancer Res *12*: 3394-3401, 2006.

134 Arlen PM, Gulley JL, Parker C, Skarupa L, Pazdur M, Panicali D, Beetham P, Tsang KY, Grosenbach DW, Feldman J, Steinberg SM, Jones E, Chen C, Marte J, Schlom J and Dahut W: A randomized phase II study of concurrent docetaxel plus vaccine *versus* vaccine alone in metastatic androgen-independent prostate cancer. Clin Cancer Res *12*: 1260-1269, 2006.

135 Pantuck AJ, van Ophoven A, Gitlitz BJ, Tso CL, Acres B, Squiban P, Ross ME, Belldegrun AS and Figlin RA: Phase I trial of antigen-specific gene therapy using a recombinant vaccinia virus encoding MUC-1 and IL-2 in MUC-1-positive patients with advanced prostate cancer. J Immunother *27*: 240-253, 2004.

136 Dreicer R, Stadler WM, Ahmann FR, Whiteside T, Bizouarne N, Acres B, Limacher JM, Squiban P and Pantuck A: MVA-MUC1-IL2 vaccine immunotherapy (TG4010) improves PSA doubling time in patients with prostate cancer with biochemical failure. Invest New Drugs *27*: 379-386, 2009.

137 Gansbacher B, Brill T, Eisele B, Kuebler H, Randenborgh Hv, Paul R, Hartung R, Fend F, Pohla H and Schendel D: Cancer gene therapy with a retrovirally transduced IL2-IFN{gamma}-secreting allogeneic tumorvaccine in patients with progressive hormone refractory prostate cancer–A phase I/II trial. J Clin Oncol *25*: (18S) 5127, 2007.

138 Brill TH, Kübler HR, Pohla H, Buchner A, Fend F, Schuster T, van Randenborgh H, Paul R, Kummer T, Plank C, Eisele B, Breul J, Hartung R, Schendel DJ and Gansbacher B: Therapeutic vaccination with an interleukin-2-interferon-gamma-secreting allogeneic tumor vaccine in patients with progressive castration-resistant prostate cancer: a phase I/II trial. Human Gene Ther *20*: 1641-1651, 2009.

139 Madan RA, Mohebtash M, Arlen PM, Vergati M, Steinberg SM, Tsang KY, Dahut WL, Schlom J and Gulley JL: Overall survival (OS) analysis of a phase l trial of a vector-based vaccine (PSA-TRICOM) and ipilimumab (Ipi) in the treatment of metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol *28*: (15S) 2550, 2010.

140 Gerritsen W, Van Den Eertwegh AJ, De Gruijl T, Giaccone G, Scheper RJ, Lowy I, Levy E, Hege K and Sacks N: A dose-escalation trial of GM-CSF-gene transducted allogeneic prostate cancer cellular immunotherapy in combination with a fully human anti-CTLA4 antibody (MDX-010, ipilimumab) in patients with metastatic hormone-refractory prostate cancer (mHRPC). J Clin Oncol *24*: S2500, 2006.

141 Fong L, Kwek SS, O'Brien S, Kavanagh B, McNeel DG, Weinberg V, Lin AM, Rosenberg J, Ryan CJ, Rini BI and Small EJ: Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. Cancer Res *69*: 609-615, 2009.

142 Mohebtash M, Madan RA, Arlen PM, Rauckhorst M, Tsang KY, Cereda V, Vergati M, Poole DJ, Dahut WL, Schlom J, and Gulley JL: Phase I trial of targeted therapy with PSA-TRICOM vaccine (V) and ipilimumab (ipi) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol *2009*: (15S) 5144, 2009.

143 Arlen PM, Skarupa L, Pazdur M, Seetharam M, Tsang KY, Grosenbach DW, Feldman J, Poole DJ, Litzinger M, Steinberg SM, Jones E, Chen C, Marte J, Parnes H, Wright J, Dahut W, Schlom J and Gulley JL: Clinical safety of a viral vector based prostate cancer vaccine strategy. J Urol *178*: 1515-1520, 2007.

144 DiPaola RS, Plante M, Kaufman H, Petrylak DP, Israeli R, Lattime E, Manson K and Schuetz T: A phase I trial of pox PSA vaccines (PROSTVAC-VF) with B7-1, ICAM-1, and LFA-3 co-stimulatory molecules (TRICOM) in patients with prostate cancer. J Transl Med *4*: 1-5, 2006.

145 Petrylak DP, Tangen CM, Hussain MH, Lara PNJ, Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D and Crawford ED: Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. New Engl J Med *351*: 1513-1520, 2004.

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