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# Entering a New Era – Prostate Cancer Immuno-Therapy After the FDA Approval for Sipuleucel-T

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## 1. Introduction

As cancer is estimated to cause 1,500,000 deaths in Europe and more than 500,000 alone in the US each year (Ferlay et al., 2007; Jemal et al., 2010) newer strategies are needed to improve current treatment success rates. The role of the immune system is designated cancer immunosurveillance as it limits tumor growth (Dunn et al., 2002). This biological principle has been deduced from clinical observations in human as if veterinary cancer patients and has also been proved experimentally in immunodeficient mice characterized by a high incidence of tumors (Shankanan et al., 2001). The evidence on the role of the immune system found in limiting tumor growth and progression is linked to observations showing a positive correlation between the presence of tumor infiltrating lymphocytes (TILs) and improved outcome in most - but not all - tumor entities studied so far; e.g. in colorectal cancer significantly higher levels of memory CD8+T-cell infiltrates are positively correlated with clinical benefit, defined as less advanced pathological stage, absence of metastatic invasion, and increased survival (Pages et al., 2005; Galon et al., 2006). Similarly, the presence of TILs has been associated with decreased tumor cells in the draining lymph nodes of cervical cancer patients limiting the risk of metastatic progression (Piersma et al., 2007). In lung carcinoma patients, increasing numbers of TILs have also been shown to significantly improve disease-specific survival (Al-Shibli et al., 2008). PCa glands are also frequent diffusely infiltrated (CD4+T-cells as if CD8+T-cells) so suggesting that PCa may be immunogenic but the correlation of these findings to clinical data is not that clear as in other tumor entities (McArdle et al., 2004; Zhang et al., 2006). Altogether, these observations support the immunosurveillance hypothesis and form the rationale to use the immune system to control tumor burden by vaccine-based interventions against cancer relying on the stimulation of an effective antitumor immune response in the cancer patient and resulted recently in labelling “avoiding immune destruction” as an emerging hallmark of cancer by the scientific community (Hanahan & Weinberg, 2011). The therapeutic cancer vaccine definition as given by the National Cancer Institute (NCI) is: vaccines, which are intended to treat already existing cancers by strengthening the body’s natural defences against cancer. But it is of great importance to notice the two paradoxical roles the immune system has in cancer: while at the one hand various components of the immune response - innate as if adaptive - are able to mediate cancer cell destruction, at the other hand specific types of

immune cells can also induce an environment that favours tumor growth as also the development of metastasis (DeNardo et al., 2008). Among the latter are, for example, tumor associated macrophages (TAM) (Mantovani et al., 2002; Luo et al., 2006), type 2 helper CD4+(TH2) T-cells (DeNardo et al., 2009; Ziegler et al., 2009), and last not least regulatory T(Treg)-cells (Curiel et al., 2004; Yamaguchi & Sakaguchi, 2006). These various immune cells have been shown to accumulate at tumor sites, negatively impacting the establishment of antitumor T-cell responses, and so creating an immunosuppressive tumor environment. Cancer cells themselves can also evolve mechanisms that allow them to evade immunosurveillance and to negatively affect the functionality of effector T-cells. These so called tumor-escape-mechanisms are: i) down regulation of antigen expression, components of the antigen-processing and presentation machinery, and expression of Major Histocompatibility Complex (MHC) molecules (Marincola et al., 2000), ii) decreased expression of co-stimulatory cytokines which are of crucial importance to T-cell activation (Sica et al., 2003), iii) enhanced surface expression of molecules that negatively regulate T-cell activation, so called “co-inhibitory signals”, such as PDL1/B7-H1 and B7-H4 (Dong et al., 2002; Driessens et al., 2009), and iv) secreting a milieu of soluble factors that ultimately inhibit the activation, proliferation, and differentiation of the various components of the immune response; e.g. TGF- $\beta$  (Thomas & Massague, 2005), IL-10 (Kurte et al., 2004), IL-13 (Terabe et al., 2000), and VEGF (Gabrilovich et al., 1996). And it was shown that the differential genetic and proteomic alterations of cancer cells accumulate in the course of disease from the localised tumor to lymph node positive state to end stage metastatic disease (Taylor et al., 2006). In order to design a successful anti-cancer immunostimulative strategy, it is important not to ignore the discoveries made by scientists working in the areas of immunity, infection and especially autoimmunity. For example, successful immunological clearance of viral as if bacterial infections are naturally accompanied by tremendous expansions of pathogen-reactive cytotoxic T-lymphocytes (CTLs), with up to 50 % of all circulating CD8+T-cells being antigen specific CTLs, which only subside after the infection has been defeated. On the other hand, most cancer vaccines which are in the field today generate poor T-cell responses with antigen-specific CTL rates beneath 1 % that often disappear soon after vaccination. Thus, one should not be surprised that insufficient tumor responses or regressions are observed (Cho & Celis, 2010).

The goal of vaccine-based cancer immunotherapy approaches is to induce a tumor-specific immune response that will reduce tumor burden by tipping the balance from a protumor to an antitumor immune environment - and from a clinicians point of view it is a success if tumor progression is stopped or if the tumor does not even metastasizes. So achieving cure is a high goal, which the so called therapeutic cancer vaccines might never reach, but we would save millions if we could force cancer into a chronic disease patients learn to live with but don't succumb from it. This chapter discusses strategies employed in the field of PCa vaccines aiming to enhance activation of an immune response that has shown impact in clinical trials.

There are many attempts around to describe the different vaccine platforms systematically, but as for most approaches the molecular mode of action is not exactly known attempts to divide into active versus passive, specific versus unspecific vaccines are difficult. It is more practical to categorize each vaccine depending on the vaccine-delivery system used, and whether specific or multiple antigens are targeted (Palena & Schlom, 2010). In the multi-antigen vaccine formulations often known and unknown antigens are included. A list of the various types of vaccine-delivery systems under investigation in the field of PCa in clinical stages is presented in Table 1.

| 1. Immunisation against multiple (not specified) antigens          | Ref. (e.g.)  | 2. Immunisation against specified antigen(s)                     | Ref. (e.g.)  |
|--|--|--|--|
| 1.1 Cell-based   |  | 2.1 Cell-based   |  |
| 1.1.1 DCs pulsed ex vivo with allogeneic whole-tumor cell mRNA     | (Mu et al., 2005)  | 2.1.1 DCs pulsed ex vivo with a single peptide                   |  |
| 1.1.2 DCs pulsed ex vivo with autologous whole-tumor cell lysates  | (Pandha et al., 2004)  | human PAP as target (e.g. sipuleucel-T) xenogeneic PAP as target | (Higano et al., 2009a; Kantoff et al., 2010a) (Fong et al., 2001)  |
| 1.1.3 Allogeneic whole-tumor cells (modified ex vivo)              |  | PSMA as target   | (Tjoa et al., 1999; Fishman, 2009)                                 |
| e.g. GVAX  | (Simons et al., 2006; Small et al., 2007; Higano et al., 2008; Higano et al., 2009b) | PSA as target  | (Barrou et al., 2004) (Hildenbrand et al., 2007)                   |
| e.g. OnyP  | (Michael et al., 2005)   | PSCA as target   | (Thomas-Kaskel et al., 2006)                                       |
| e.g. LNCaP/IL2/IFN $\gamma$  | (Brill et al., 2007, 2009)   | Telomerase as target   | (Vonderheide et al., 2004; Su et al., 2005)                        |
| 1.1.4 whole tumor cells (modified in situ – “in situ vaccination”) |  | 2.1.2 DCs pulsed ex vivo with multiple peptides                  |  |
| Cell modification via viral vectors e.g. AdV-IL2                   | (Simons et al., 1999)  | DCs pulsed “peptide cocktail” diff. epitopes from prostate TAAs  | (Fuessel et al., 2006; Waeckerle-Men et al., 2006)                 |
|  |  | 2.2 Viral vector-based   |  |
|  |  | PSA in VV and FV (e.g. Prostvac-VF)                              | (Kaufman et al., 2004; Kantoff et al., 2010b; Gulley et al., 2010) |
|  |  | PSA in Adv   | (Lubaroff et al., 2009)  |
|  |  | MUC1 in MVA  | (Dreicer et al., 2009)   |
|  |  | 2.3 DNA based  |  |
|  |  | PSMA   | (Low et al., 2009)   |
|  |  | human PAP  | (McNeel et al., 2009)  |

| 1. Immunisation against multiple (not specified) antigens | Ref. (e.g.) | 2. Immunisation against specified antigen(s)                                       | Ref. (e.g.)               |
|---|-------------|--|---------------------------|
|   |             | 2.4 Peptide based  |                           |
|   |             | 2.4.1 Multiple peptides used: "peptide cocktail" diff. epitopes from prostate TAAs | (Feyerabend et al., 2009) |
|   |             | 2.4.2 Single peptide used: e.g. HER2/neu epitope (776-790)                         | (Perez et al., 2010)      |

AdV = Adenovirus; DCs = dendritic cells; diff. = different; FV = Fowl pox virus; mRNA = messenger RNA; PAP = prostatic acid phosphatase; PSMA = prostate-specific membrane antigen; PSCA = prostate stem cell antigen; TAA = tumor associated antigen; VV = Vaccinia virus

Table 1. Systematic list of vaccine-delivering systems in PCa vaccine approaches

Novel therapeutic options for patients of this stage of disease have been stated as an urgent medical need. Thus besides vaccine therapies a number of agents (e. g. endothelin receptor antagonists, receptor activator of nuclear factor  $\kappa$ B ligand inhibitors, anti-angiogenic drugs, cytochrome P17 enzyme inhibitors, vitamin D analogues) are now tested in phase III registration trials either alone or in combination with docetaxel for first- or second-line use in mCRPC patients (Antonarakis & Drake, 2010; Antonarakis & Eisenberger, 2011).

Clinical trails that have engrossed most interest include i) Dendritic Cell (DC)-based vaccines (with clinically meaningful outcome for sipuleucel-T), ii) DNA vaccines (e.g. viral vector-based Prostavac-VF) together with recombinant peptide vaccines, and iii) whole-tumor cell vaccines (e.g. GVAX).

## 2. DC-based vaccines

Since cancer in fact develops and evolves in the presence of an intact immune system TAAs are by definition inadequately immunogenic. This results in a suboptimal T-cell activation, induction of immune tolerance and thus an ineffective immune reaction. Cancer vaccines are intended to break this immune tolerance. Strong presentation of antigen by antigen-presenting cells (APCs) is essential. Dendritic cells (DCs), which are the most powerful APCs, are known to be deficient in number and function in cancer patients. Activating APCs that are able to appropriately process and present the TAA is pivotal to activating an adaptive immune response and breaking peripheral tolerance.

### 2.1 The sipuleucel-T (PROVENGE®) approach

The history of this new treatment option began with phase I and phase II clinical trials in the academic field back in the 1990th. In the early 2000th the first phase III accrual of patients started and first data published in 2006 and FDA approval was asked for. But the FDA concluded that further confirmation would be obligatory prior to approval. This judgment sparked protests from patients and advocates who urged the FDA to repeal its decision. In 2009 preliminary results of that phase III pivotal trial, named Immunotherapy for Prostate

Adenocarcinoma Treatment (IMPACT) study, were presented to the community at an AUA national conference and to the FDA (Schellhammer et al., 2009). After re-analysis of these facts the FDA finally permitted sipuleucel-T end of April 2010, so it is worth to have a closer look into the prescription information (FDA, 2010a) and the approval letter (FDA, 2010b) as this product is not just another in a row of existing PCa vaccines but – as the first active immune therapy granted with approval for use in human subjects – opens an entire new entity for curative intervention. Finally in July 2010 the IMPACT trial data were presented in a peer reviewed journal (Kanthoff et al., 2010b) and as a result the prostate panel of the National Comprehensive Cancer Network (NCCN) has changed its guidelines how to treat mCRPC patients by adding sipuleucel-T as a category 1 treatment recommendation (NCCN, 2010).

Sipuleucel-T is an antigen specific cellular immunotherapy based on autologous DCs (see Tab. 1) and indicated in metastatic but asymptomatic or minimally symptomatic patients who are in a hormone refractory and disease progressive stage. Sipuleucel-T consists of APCs and other cells of the peripheral blood mononuclear cells (PBMC) compartment, that have been activated during a defined ex vivo period with a recombinant human fusion protein combining PAP and GM-CSF. To obtain patient's PBMCs a standard leukapheresis procedure approximately 72 hours prior to the infusion date has to be performed. During ex vivo culture period the PAP protein can bind to and be processed by APCs into smaller TAA fragments, so the recombinant antigen should target the DCs, and is thought to direct the immune response to PAP (Kanthoff et al., 2010a).

Due to the autologous nature of sipuleucel-T and the individuality of this approach its final cellular composition (T-, B-, NK-, and other cells) depends on the cells obtained from the patient's leukapheresis and will vary from patient to patient and from dose to dose, but the ex vivo procedure is regulated in that a minimum of 50 million PAP-GM-CSF activated CD54+ cells are included, suspended in 250 mL of Lactated Ringer's solution, and reinfused intravenously to the patient (FDA, 2010a; Kanthoff et al., 2010a).

The IMPACT trial was randomized, placebo-controlled, double-blind, multicentered. A total of 512 patients were randomized (2:1 ratio) to receive sipuleucel-T (n = 341) or control (n = 171). The placebo material used in control subjects was peripheral blood mononuclear cells that had not been PAP-activated, but given back to the patients under equal clinical conditions. In case of disease progression control subjects were allowed to cross over to an open-label use of the vaccine. The effectiveness of sipuleucel-T showed an increase in OS of 4.1 months in the pivotal phase III trial (25.8 vs 21.7 months) and OS benefit (3-year OS of 31.7 % vs 23.0 %). Sipuleucel-T successfully reached the prespecified level of statistical significance and reduced the overall risk of death by 22 % compared to control ( $p < 0.05$ ) (Kanthoff et al., 2010b). Analyses of time to disease progression did not differ between verum and placebo patients and thus not meet statistical significance.

As sipuleucel-T is intended and produced solely for personalized use in a central laboratory there is no routine testing for transmissible infectious diseases so general precautions for handling blood products has to be employed. Due to the expiration time being as short as 18 h the product safety testing is challenging and sipuleucel-T has to be released for use based on the sterility and microbial results from a number of tests. If the sterility results show positive for microbial contamination after the use of sipuleucel-T, the manufacturer will inform the treating doctor. As, due to the character of the product, no cell filter can be used during the i.v. re-infusion of the ex vivo stimulated blood compounds, acute infusion reactions are the most common solely adverse event (AE) in patients receiving sipuleucel-T but also in control

subjects receiving the non-activated peripheral blood mononuclear cells. Such events included, but were not limited to, vomiting, fatigue, fever, chills, respiratory events (dyspnea, hypoxia, and bronchospasm), nausea, hypertension, and tachycardia. To minimize potential acute infusion reactions e.g. chills and/or fever, it is recommended to premedicate patients orally with an antihistamine prior to infusion of sipuleucel-T (Kanthoff et al., 2010b).

The safety evaluation of sipuleucel-T, released by the FDA, is based on 601 PCa patients who received at least one dose of sipuleucel-T reported in four different clinical trials (Small et al., 2006; Harzstark et al., 2009; Higano et al., 2009a; Kanthoff et al., 2010a). Almost all (98.3 %) individuals in the sipuleucel-T group and 96.0 % in the control group reported an adverse event. In 67.4 % of patients in the sipuleucel-T group, these adverse events were mild or moderate. Severe (grade 3) and life-threatening (grade 4) adverse events were reported in 23.6 % and 4.0 % of patients in the sipuleucel-T group compared with 25.1 % and 3.3 % of control group patients. Fatal (grade 5) adverse events were reported in 3.3 % of patients in the sipuleucel-T group compared with 3.6 % of patients in the control group. The FDA recommended the manufacturer to run a post-marketing study to assess the risk of cerebrovascular events in 1,500 PCa patients who receive sipuleucel-T and awaits completion of this study till December 31, 2015 (FDA, 2010b).

Each dose of sipuleucel-T requires a leukapheresis approximately three days prior to the infusion. AE's that were reported within one day following the leukapheresis procedure included citrate toxicity (14.2 %), oral paresthesia (12.6 %), general paresthesia (11.4 %), and fatigue (8.3 %) (FDA, 2010a).

Due to its novelty in the market as if the pricey production process and due to the high research and development costs sipuleucel-T as a pharmaceutical product is quite expensive - more than 90,000 USD. So there is a discussion whether or not these costs should be covered by the public. In comparison to other recently introduced drugs in other tumor entities (e.g. lung cancer or breast cancer) and based on the calculation of the achievable duration in life time the costs of sipuleucel-T has been calculate as about 10 times higher (Longo, 2010).

## 2.2 Other DC based vaccines

A wide variety of other approaches using DCs have been studied, including evaluation of DCs pulsed with defined proteins like PSMA (Tjoa et al., 1999; Fishman, 2009), PSA (Barrou et al., 2004; Hildenbrand et al., 2007), xenogeneic PAP (Fong et al., 2001), PSCA (Thomas-Kaskel et al., 2006), and telomerase (Vonderheide et al., 2004; Su et al., 2005). Strategies based on peptides included pulsing DCs with multiple but defined peptides (prostate stem cell antigen (PSCA14-22), prostatic acid phosphatase (PAP299-307), prostate-specific membrane antigen (PSMA4-12), Prostein (31-39), Survivin (95-104), Trp-p8 (187-195), and prostate-specific antigen (PSA154-163)) (Fuessel et al., 2006; Waeckerle-Men et al., 2006). To expand antitumor reactivity and prevent tumor escape from the immune system, researchers have used DCs genetically engineered to express an enlarged range of antigens by the use of tumor cell lysates (Pandha et al., 2004), and allogeneic PCa cell lines (DU145, LNCaP and PC-3) messenger RNA (Mu et al., 2005).

## 3. DNA vaccines

DNA vaccines are focused to the TAA which is used in a specific approach to switch the patient's immune system. Most DNA vaccines have focused on tissue-specific (PAP, PSMA,

PSCA and PSA) rather than tumor-specific antigens. The major advantages of DNA vaccines is, at least compared with DC-based vaccines, that they are easy and inexpensive to produce and that with some viral vectors used in this attempt there is a huge body of experience as they have been used in millions of persons for preventive vaccinations against infective disease (Fioretti et al., 2010).

### 3.1 Prostvac-VF approach

This approach has been tested clinically in a number of phase I studies demonstrating safety of the vectors (Sanda et al., 1999; DiPaola et al., 2006; Arlen et al., 2007), and three phase II studies. Prostvac-VF consists of two genetically engineered viruses (recombinant Vaccinia (V) virus and Fowl pox (F) virus) administered in a sequential regimen. The virus strain used in Prostvac-V is a to some extent attenuated version of the virus used for smallpox immunization. Fowl pox viruses are unable to replicate in human cells but have been shown to be an effective way of boosting cellular immune responses primarily initiated using Vaccinia virus. The viral vectors are engineered to contain a gene encoding human PSA which contains an alteration in the HLA-A2 specific epitope that is planned to enhance the immunogenicity of the expressed antigen. In addition, these viruses both contain the genes encoding three co-stimulatory molecules, B7.1, ICAM-1 and LFA-3 (together named TRICOM). The academic work to establish this vaccine platform was done in cooperation with industrial sponsorship initially with Therion Biologics Cambridge, MA and subsequently with BN ImmunoTherapeutics, Garcia Ave, CA (Madan et al., 2009). The first phase II trial conducted by the Eastern Cooperative Oncology group enrolled 64 eligible patients and assigned them randomly to receive i) four vaccinations with fowl pox-PSA (rF-PSA), ii) three rF-PSA vaccines followed by one vaccinia-PSA (rV-PSA) vaccine, or iii) one rV-PSA vaccine followed by three rF-PSA vaccines. In this trial the TRICOM-component was not included. The prime/boost schedule was well tolerated with a small amount of adverse events. Of the eligible patients, 45.3% of men remained free of PSA progression at 19.1 months and 78.1 % demonstrated clinical progression free survival. There was a trend favouring the treatment group that received a priming dose of rV-PSA (Kaufman et al., 2004).

So in further trials using this approach rV-PSA priming was always followed by rF-PSA boosting. In the second phase II trial (n = 125 patients) it could be shown that at 3 years post study analysis, Prostvac-VF patients (n = 82) had a better OS with 25 (30 %) of 82 alive versus 7 (17 %) of 40 controls, longer median survival by 8.5 months (25.1 vs 16.6 months for controls), an estimated hazard ratio of 0.56 (95% CI, 0.37-0.85), and stratified log-rank  $p < 0.01$ . There was a minor imbalance in favour of the Prostvac-VF arm in mean and median of some laboratory values. But integration of these factors plus performance status in the Halabi nomogram revealed a 1-month mean and 2-month median difference in predicted survival (mean and median of 20.4 months for controls vs mean of 21.4 months and median of 22.5 months for Prostvac-VF). The observed survival difference of 8.5 months far exceeds that predicted by the Halabi nomogram. So these data are – despite of OS being not the primary end point – considered clinically meaningful and strongly suggests that Prostvac-VF immunotherapy may produce an OS benefit, but still regarded as hypothesis generating data as the authors state in the discussion of there recently published work (Kantoff et al., 2010b). The National Cancer Institute (NCI) has also recently completed a third phase II study in 32 PCa bearing men in whom immune and regulatory T-cell responses were

evaluated. In that study, 13 of 28 evaluable patients had more than two-fold increases in PSA epitope specific immune responses, and four of five high responders (more than a six-fold increase) survived >40 months, while low or non-responders had a median OS of 20 months. The Halabi predicted survival of these metastatic CRPC patients was 17 months. Of interest is the Treg-cell course reported: Treg-cell suppressive function was shown to decrease following vaccine in patients surviving longer than predicted, and increase in patients surviving less than predicted (Gulley et al., 2010). Prostavac-VF immunotherapy is a promising approach, and a larger pivotal phase III trial is planned. If the data gained to date (OS benefit of 8.5 months) could be approved in a pivotal phase III trial – this platform is considered to be the next immunotherapy candidate for FDA approval. Main components of this approach (rV-PSA, rF-PSA and rV-B7.1) have also been tested in a two armed phase II trial with or without combining the vaccine and docetaxel (plus dexamethasone) showing that docetaxel can be administered safely with immunotherapy without inhibiting vaccine specific T-cell responses. The authors state that patients previously vaccinated with an anti-cancer vaccine respond longer to docetaxel compared with a historical control of patients receiving docetaxel alone (Arlen et al., 2006).

### 3.2 Other viral vector-based vaccines

A group at the University of Iowa, USA, used an adenovirus genetically engineered to carry the genetic information for PSA and injected one single amount of recombinant virus. They included 32 patients with measurable mCRPC in a phase I trial. Patients were treated with a single s.c. vaccine injection at one of three dose levels, either suspended in a Gelfoam matrix or as an aqueous solution. The vaccine was judged safe in both administration forms at all doses. Anti-PSA antibodies and anti-PSA T-cell responses were detected in the majority of individuals. As PSA doubling time (PSA-DT) was increased and half of the patients survived longer than predicted by the Halabi nomogram this vaccine could be stated safe and potentially clinical effective and should proceed to phase II (Lubaroff et al., 2009).

A multicenter phase II trial in 40 patients with PSA progression used TG4010, a recombinant MVA vector expressing the tumor-associated antigen mucin 1 (MUC1) and Interleukin-2 (IL-2) as an adjuvant. Despite the primary endpoint of a 50 % decrease in PSA values from baseline was not observed, in 13 of 40 patients a more than two fold improvement in PSA-DT could be observed ( $p < 0.01$  for all 40 patients) and ten patients had a PSA plateau for over 8 months demonstrating evidence of biologic activity (Dreicer et al., 2009).

### 3.3 Other DNA-vaccines

A phase I/II, dose escalation, DNA vaccination trial with plasmid DNA, coding for PSMA, fused to a domain (DOM1) of the C fragment of tetanus toxin was performed in patients with recurrent PCa. The DNA, was delivered either by i.m. injection without further manipulation or i.m. followed by electroporation. The PSMA epitope used in this study is a short stretch of 9 amino acids. Preliminary analysis of CD8+ T-cell reactivity against the PSMA target peptide indicated significant responses in three out of three patients and CD4+ T-cell responses against the DOM1. These data suggest electroporation as an effective method for stimulating the humoral system induced by DNA vaccination in humans (Low et al., 2009).

Results of a phase I/II trial, conducted with DNA vaccine encoding human PAP co-administered intradermally with GM-CSF, in PCa patients (stage D0) showed an

increased PSA-DT, 6.5 months pretreatment versus 9.3 months in the 1 year post treatment (McNeel et al., 2009).

In a phase I/II trial a composition of 13 prostate-associated synthetic peptides (e.g. PSA, PSCA, PSMA, Survivin and Prostein) was used to counteract tumor escape mechanisms by genetic mutations or antigen loss. The TAA-epitopes were presented on HLA-A2 and on HLA-DR molecules, with the aim to activate a broad spectrum of CD8+ and CD4+ specific T-cells. The peptides were applied s.c. with or without immune stimulants (imiquimod, GM-CSF, MUC1-protamin complex). Four out of 19 men had a fivefold elevation of PSA-DT. Four individuals had minor PSA changes during vaccination and 11 patients showed progressive disease. In four men the vaccination was discontinued due to adverse events graded moderate. Although underpowered to draw definitive results imiquimod as an adjuvant seems beneficial (Feyerabend et al., 2009).

In contrast to the later approach using a peptide cocktail it is also possible to induce immune responses (elevated DTH-reaction, increased IFN $\gamma$  ELISPOT activity and decreased Treg-cell frequency) by only using a short active sequence existing of fourteen amino acids of the TAA HER2/neu. Such synthetic fragments (500  $\mu$ g) were administered together with GM-CSF in a phase I trial using a schedule of six intradermal vaccinations (Perez et al., 2010).

A slightly different but unique approach is used by a group at the university of Kurume, Japan. They tested an individualized method of peptide vaccination based on preexisting cytotoxic T-cell and immunoglobulin (IgG) reactivity. Each patient was tested for reactivity among 16 immunogenic peptides known to bind to HLA-A24. Peptides were derived from a number of targets, including PAP, PSMA, multidrug resistance protein, PSA, and a variety of other epithelial tumor antigens. Each patient was immunized with four peptides on the basis of his reactivity panel. This personalized medicine approach was tested as monotherapy and in combination with chemotherapy. As the peptides were injected s.c. vaccines were well tolerated and showed reactivity (DTH-reactions, CTL and IgG responses) and clinical activity (PSA declines up to 30 % in four out of 17 patients (Uemura et al., 2010). In a second trial the combination with estramustine showed a better PFS as estramustine alone ( $p < 0.05$ ) (Noguchi et al., 2010).

#### **4. Whole-tumor-cell vaccines**

While autologous whole-tumor-cell vaccines are derived from the patient's own tumor cells in an often lengthy and pricey process, allogeneic whole-tumor-cell vaccines originate from various tumor cell lines and are easier to set up (Risk & Corman, 2009).

##### **4.1 Prostate GVAX®**

The GM-CSF-secreting cancer cell immunotherapy platform (GVAX®) (managed by Cell Genesys, South San Francisco, CA) was set up to be used in diverse types of carcinomas. The prostate GVAX® form uses two different PCa cell lines (PC3 and LNCaP) which have been modified through adenoviral transfer to secrete GM-CSF (Ward & McNeel, 2007). Analyses of these two cell lines showed up many genes well-known in human PCa metastases, including previously described prostate TAAs. The PC-3 cell line was derived from a PCa bone metastasis, and LNCaP was derived from a PCa metastasis to a lymph node. LNCaP was shown to express PAP, PSMA, PSA, urokinase-type plasminogen activator and prostate stem cell antigen (PSCA) (Simons et al., 1999; Kiessling et al., 2002; Lu & Celis, 2002). PC-3

was shown to express glutathione S-transferase, mutant p53, CEA, and urokinase-type plasminogen activator (Warren & Weiner, 2000). GM-CSF – the cytokine the GVAX inventors have chosen as to further augment their vaccine – has shown some impact in PCa patients (PSA modulations) if administered as a solely therapeutic agent (Small et al., 1999; Dreicer et al., 2001; Schwaab et al., 2006). However, the use of GM-CSF might be challenged by counterregulatory immune responses that aim to reduce the expansion of cytotoxic T cells, thereby limiting antitumor activity. The use of GM-CSF for anti-cancer immunostimulation has caused some concerns as GM-CSF is associated with the presence of CD34+myeloid suppressor cells. Besides that it has been shown that GM-CSF is secreted by some carcinomas (Bronte et al., 1999) with a clinically relevant worse outcome (e. g. higher rate of recurrence) as in tumors with lesser CD34+cells which release Transforming Growth Factor (TGF)  $\beta$  inhibiting T-cell functions (Young, et al., 1997). This knowledge is important to determine the best use of GM-CSF and generally, low doses of GM-CSF are associated with greater stimulation of the immune response than higher doses which might create a counterproductive immune response via inducible nitric oxide synthase (iNOS) in well designed mouse data. This immunosuppression could be abandoned by the specific iNOS inhibitor, L-NMMA, resulting in restored antigenspecific T-cell responsiveness in vitro (Serafini et al., 2004). Therefore, it is critical to optimize the use of GM-CSF, in order to improve, rather than hamper, the immune response (Harzstark et al., 2009).

In a first phase I/II trial (coded G9802) a fixed total cell dose of  $1.2 \times 10^8$  cells ( $6 \times 10^7$  per cell line) was used in hormone therapy-naïve patients with PSA recurrence following radical prostatectomy and absence of radiologic metastases. GM-CSF secretion from the clinical lots used in this trial was 150 ng/ $10^6$  cells/24 h (LNCaP) and 450 ng/ $10^6$  cells/24 h (PC-3). Patients were vaccinated weekly via intradermal injections for 8 weeks and resulted in one patient having a partial PSA response of 7 month duration. The injection sites were found to have invasion of inflammatory cells and APCs on histopathology. At 20 weeks after the first treatment, 16 of 21 treated patients showed a statistically significant decrease in PSA velocity (slope) compared with prevaccination PSA course (Simons et al., 2006) (Urba et al., 2008). Two further uncontrolled single-arm phase II studies included asymptomatic CRPC patients with (n = 34) or without (n = 21) metastases (G9803 trial) and only men with metastases (n = 80) (G0010 trial). The two trials have shown anti-tumor effects of prostate GVAX®, the first one (G9803 trial) demonstrating an overall survival benefit of 34.9 versus 26.2 months in the mCRPC subgroup (n = 34) (Small et al., 2007) and the other (G0010 trial), a study in which the vaccine was re-engineered to secrete a higher dose of GM-CSF, showing an OS ranging from 20.0 to 29.1 months (n = 80) depending on dosing regimen. Dose levels ranged from  $1 \times 10^8$  cells q28d  $\times$  6 to as many as  $5 \times 10^8$  cells prime/ $3 \times 10^8$  cells boost q14d  $\times$  11. Besides the differences in OS also the proportion of men who generated an antibody response to one or both cell lines increased with dose and included 10 of 23 in the low-dose up to 16 of 18 in the highest dose group (p < 0.01; Cochran-Armitage trend test) (Higano et al., 2008). A combined expanded retrospectively analyses of antibody response using the data from the three above mentioned trials indicated a significant (p < 0.05) association of alternative reading frame protein (TARP) antibody induction and median survival time (Nguyen et al., 2010). No dose-limiting or autoimmune toxicities were seen. The most common adverse events in both studies were injection-site erythema, myalgias, fatigue, malaise, and arthralgias. Based on these promising findings, two powerful sized randomized phase III studies of GVAX immunotherapy (VITAL-1 and VITAL-2) were set

up. VITAL-1 involved 626 men with asymptomatic chemotherapy-naïve CRPC, and randomized them to GVAX or docetaxel/prednisone, with OS as the primary endpoint. The trial was terminated in October 2008 based on the results of a previously unplanned futility analysis conducted by the study's Independent Data Monitoring Committee (IDMC), which indicated that the trial had a less than 30 % chance of showing OS (predefined primary endpoint) (Higano et al., 2009b). VITAL-2 was designed initially to enrol 600 men with symptomatic mCRPC, randomizing them to docetaxel/prednisone or docetaxel/GVAX. It was halted after having enrolled patients for two years (n = 408) in August 2008 as mortality appeared to be higher in men on the investigational arm receiving docetaxel/GVAX (67 vs. 47 respectively). Preliminary analysis revealed no significant difference in the patients baseline characteristics of toxic effect that could explain the unexpected discrepancy in death rate. A survival advantage (14.1 vs 12.2 moths; HR 1.7, 95 % CI 1.15-2.53) was seen in the control arm (doxetacel-prednisone) over the experimental (GVAX-doxetacel) arm (Small et al., 2009). Further evaluation has not yet been released and due to this contradictory data the future of GVAX is unclear.

Two other whole-tumor-cell vaccines (ONY-P1 and LNCaP-IL2-IFN $\gamma$ ) have finished phase I/II trials and released results.

#### **4.2 ONY-P1**

ONY-P1 (managed by Onyvax, Ltd, London, UK) consists of three irradiated PCa cell lines given to 26 patients with nonmetastatic CRPC intradermally ( $2.4 \times 10^7$  cells per injection), once a month for up to 12 month. In total 11 of the 26 patients demonstrated a prolonged decrease in their PSA-velocity (PSAV). None of the treated patients experienced any significant toxicity. Median time to disease progression was 58 weeks. PSAV-responding patients showed a titratable TH1 cytokine release profile in reply to restimulation with a vaccine lysate, while non-responders showed a mixed TH1 and TH2 response. Furthermore, immunologic profile correlated with PSAV response by artificial neural network analysis (Michael et al., 2005).

#### **4.3 LNCaP-IL2-IFN $\gamma$**

This approach uses only LNCaP cells retrovirally transduced with a N2/huIL2/huIFN $\gamma$ -vector, resulting in IL-2 and IFN $\gamma$  secretion. The two cytokines chosen for this approach have been used for immunostimulation solely and in combination in a variety of tumors (Brill et al., 2007; Dieli et al., 2007) and both substances are FDA approved single agents. Expression of tumor-associated antigens is upregulated after treatment with IFN $\gamma$  via IFN $\gamma$ -inducible genes, thereby increasing the susceptibility of tumors to MHC restricted CD8+CTL-mediated killing (Gansbacher et al., 1990a; Shankanan et al., 2001; Propper et al., 2003; Dunn et al., 2005). IL-2 is a well-known T cell growth factor, which is traditionally implicated in the agonistic stimulation of immune responses (Gansbacher et al., 1990b; Rosenthal et al., 1994) and FDA approved for systemic application against metastatic renal cell cancer. IL-2 is the only cytokine to date not been detected to be produced by any cancer. LNCaP cells, as mentioned above (see GVAX) express some relevant TAAs but in contrast to other PCa cell lines used as cancer vaccines, do not express transforming growth factor (TGF)- $\beta$ . After detailed investigation of the safety profile with special attention to induction of autoimmunity (n = 3 patients at a dose level of  $7.5 \times 10^6$  cells) (Brill et al., 2007), further patients (n = 27) were scheduled to receive four intradermal vaccine injections (dose level of

$1.5 \times 10^7$  cells) on days 1, 15, 29, and 92. In the absence of disease progression, patients received further vaccinations every three months – resulting in a total amount of more or less 100 million cells/year – as compared to 4,600 million used in other trials (see GVAX). The primary study criteria were safety and the difference in PSA-DT, determined in the pretreatment phase (before the start of vaccination) and in the trial treatment phase (during vaccination). During vaccination there was a significant prolongation of the PSA-DT compared with the prevaccination period (from 63 to 114 days ( $p < 0.01$ ; intention to treat analyses)). The median overall survival time from first vaccination was 32 months – the median Halabi-predicted survival in these thirty heavily pretreated mCRPC-patients was calculated as low as 15 months. Despite such comparisons of assessed and Halabi-predicted survival are widely accepted to compare patients groups and even different approaches (Lubaroff et al., 2009; Gulley et al., 2010; Kantoff et al., 2010b) these data are to be categorized somehow as speculative. ELISPOT based immune monitoring revealed T-cell stimulation in the majority of patients and artificial network analysis showed best predictive value for a response to the vaccination for survivin-reactivity at day 36 (Brill et al., 2009).

## 5. Approaches to overcome tumor induced immunosuppression

Three strategies have reached clinical phases in this respect: i) Inhibition of CTLA-4, ii) depletion of Treg-cells, and blocking programmed death-1 (PD-1) checkpoint.

CTLA-4 is a T-cell surface glycoprotein that is up regulated following T-cell activation to restrain the immune response. Its main task is to prevent autoimmunity by regulating the body's immune response to self antigens. Two fully human mAbs focussed to block CTLA-4 are at this time in clinical development: ipilimumab (MDX-010; Medarex/Bristol-Myers Squibb, Princeton, NJ) and tremelimumab (CP-675,206; Pfizer, New York, NY). The later is an immunoglobulin G2 antibody and ipilimumab is an immunoglobulin G1k antibody; both bind to CTLA-4 with affinities less than 1 nmol/L. T-cells express two counteracting receptors on their cell surface – CD28 and CTLA-4. Both bind to the same ligands or co-stimulatory molecules on the outside of APCs (B7.1 and B7.2). Binding to CD28 results in activating T-cells, while interacting with CTLA-4 inhibits T-cell stimulation. In the first human phase I trial the use of an anti-CTLA-4 antibody alone in men with PCa, 14 patients with progressive mCRPC, seven of whom had received prior chemotherapy, were given one single dose of ipilimumab. Two patients demonstrated PSA declines of  $> 50\%$  and two patients established prolongation of their PSA-DT (Small et al., 2007b). These data suggest – as these end stage patients got no other therapy besides anti-CTLA-4 mAb – that some degree of immune “autoprimering” exists even in a difficult stage of disease, with an ongoing low-level presentation of antigen, perhaps from basal apoptotic rates, which can then be enhanced with CTLA-4 blockade (Harzstark et al., 2009). Blocking CTLA-4 has been shown to prolong and potentiate immune responses to vaccine in two phase I trials (one as combination with GVAX; the other as combination with Prostavac-VF) (Arlen et al., 2009). Combinations of ipilimumab with chemotherapy as if vaccine has been successful in malignant melanoma and ipilimumab was recently approved for this purpose by the FDA (Hodi et al., 2010; Robert et al., 2011).

Treg-cells are induced during vaccination and the expansion of Treg-cells already present in the patient are possible factors causative to impaired vaccine efficacy by suppressing CD4+ and CD8+ effector T-cells through IL-2 consumption, the secretion of suppressive cytokines,

and cytotoxicity. Therefore, combining the elimination of Treg-cells – achieved in specific (via mAb) or unspecific (via low dose chemotherapy) manner – with therapeutic immunotherapy approaches seems promising. When comparing DC vaccination in patients with renal cell carcinoma (RCC) with/without previous elimination of Treg-cells by an IL-2 diphtheria toxin conjugate (Denileukin diftitox; ONTAK®), a higher immune response rate was achieved in the Denileukin diftitox group (Dannull et al, 2005). Low, i.e. not tumoricidal doses of chemotherapy can also synergize with vaccination: It could be shown that low dose cyclophosphamide selectively diminishes quantity and function of Treg-cells with little effect on other lymphocyte subpopulations (Ghiringhelli et al., 2007). Besides low dose chemotherapy it has been noticed in individuals with RCC that the multi-kinase inhibitor sunitinib shows depletion of Treg-cells (Finke et al, 2008; Kusmartsev & Vieweg, 2009). Besides Treg-cells (CD4+CD25<sup>high</sup>FoxP3+T-cell) other subsets of immune cells with suppressive function are focused in preclinical studies (e.g. CD4+NKT-cells, MDSCs, tumor associated Macrophages, and CD4+TH2-cells) (Palena & Schlom, 2010).

PD-1 is another co-inhibitory receptor molecule expressed on activated T-lymphocytes and functioning as an immune checkpoint. When PD-1 is bound by its ligand, T-cell proliferation and activation are inhibited, resulting in inhibition of anti-tumor immune responses (Blank & Mackensen, 2007). Expression of the PD-1 ligand has been described on a variety of human tumor cells including PCa, leading to decreased tumor-specific immunogenicity (Ebelt et al., 2009). In addition, expression of the PD-1 ligand correlates with a poorer prognosis in some human malignancies. MDX-1106, a fully human anti-PD-1 blocking antibody, is the first agent in its class to reach human clinic. Results of a phase I trial using MDX-1106 in patients with refractory metastatic solid tumors (including metastatic CRPC) were recently published and shows signs of immunological impact and clinical response – but not just in the included PCa patients (Brahmer et al., 2010). Common side effects of this agent – but also seen in trials blocking CTLA-4 and after depleting Treg-cells – include subclinical hypothyroidism, colitis and autoimmune arthritis.

Other targets but to date only in preclinical research are TH17 cells and B7-inhibitors. For TH17 cells it is known that TGF- $\beta$  together with IL-6 in addition to IL-1- $\beta$ , IL-21 or IL-23 promotes development of TH17 cells (O'Connor et al., 2010). The role of that TH-subtype in cancer vaccine settings is not clear yet. They have a well defined function in autoimmune or autoinflammatory-associated pathology as they at least in some aspects (e.g. production of IL-10) act like Treg-cells. The frequency of IL-17 producing T-cells might correlate with clinical response to therapeutic vaccination in CRPC patients (Derhovanessian et al., 2009). B7 is a co-signal important in T-cell development (see Prosvac-VF) and B7-inhibitors are constitutively expressed in numerous types of human tumors and had been shown to support evasion of tumor immunity by promoting apoptosis of activated effector T-cells, inhibiting IL-2 production and tumor resistance to T-cell mediated lysis. B7-inhibitors can be blocked by new drugs tested in preclinical models resulting in enhanced cytotoxic T-cell responses against TAAs (Palena & Schlom, 2010).

## 6. Points to consider: Safety, regulatory and ethical issues

Immunostimulatory trials in various cancer entities has often been judged as limited in the impact but have always been seen as at least non toxic and so not harming the patients. AEs seen in vaccine trials have in most cases be limited to local injection side reactions. Vaccines which use a cytokine in addition reported flu-like symptoms but not above grade 2. If

CTLA4-antibodies are used or Tregs-cells depleted some aspects of autoimmunity and other immunological break through events has been seen up to grade 3. In a combination of chemotherapy with vaccine (see GVAX) there were more deaths and diminished OS noticed in the combination arm.

AEs seen in the sipuleucel-T trials could be explained by the i.v. re-infusion of cells which had been manipulated outside the body as the AEs were as common in the verum group (stimulated T-cell re-infusion) as in the control group (unstimulated T-cell re-infusion). Also in other clinical trials severe up to fatal acute infusion reactions had caused concerns after re-infusion of immune cells. While for sipuleucel-T the total amount of cells is unknown - only the total amount of CD54+ cells is adjusted to a minimum of  $5 \times 10^8$  APCs - patients with fatal AE received  $2 \times 10^9$  and  $10 \times 10^{10}$  gene modified T-cells respectively (Brentjens et al., 2010; Morgan et al., 2010). So one could state that for vaccines based on DCs the i.v. application route seems not to be ideal and even more doubtful as other routes of application with a better safety profile are well established (e. g. intranodal (inguinal lymph node) (Mu et al., 2005), subcutaneous (Vonderheide et al., 2004; Hildenbrand et al., 2007) or intradermal re-infusion of DCs (Mu et al., 2005; Su et al., 2005; Waeckerle-Men et al., 2006; Hildenbrand et al., 2007; Sampson et al., 2009; Toh et al., 2009)) in urological and other tumor entities.

Cancer vaccines are a diverse array of therapeutics with several mechanisms of action - and some times the mode of action is not well known even for products having succeeded in late-stage trials. So cancer vaccines are a challenge for the regulators, researchers and sponsors in several aspects: i) preclinical testing and the need for relevant animal models, ii) finding the proper dose, iii) trial design especially assessment of the right end point, and iv) attempts to conduct cancer vaccine trials in early state patients.

Besides some basic ethical questions - e.g. is it ethical to do a placebo treatment in a cancer patient at all - another challenge in cancer vaccine research is to investigate what healthcare strategies and interventions offer the greatest benefits to individual patients and the population as a whole, that means to do comparative effectiveness research (CER) which to date has not been performed in the cancer vaccine field at all (Stewart et al., 2010). Undertaking any type of CER in oncology, calls for an outcomes measurement that can capture quality-of-life (QOL) measures. Most treatment decisions in cancer care are made with palliative intent or with probabilities of cure that must be weighed against the toxicity side-effects of the treatment. Although great strides have been made in the use of targeted therapies with more favourable side-effect profiles, oncologists remain extremely aware of the clinical tradeoffs between length of life and quality of life. Last points to mention are attempts to use earlier patients or even to test cancer vaccines in the preventive setting (Gray et al., 2008) and to define special vaccine adjuvants tailored to be used in the new therapeutic area of therapeutic cancer vaccines (Dubensky & Reed, 2010; Young, 2010).

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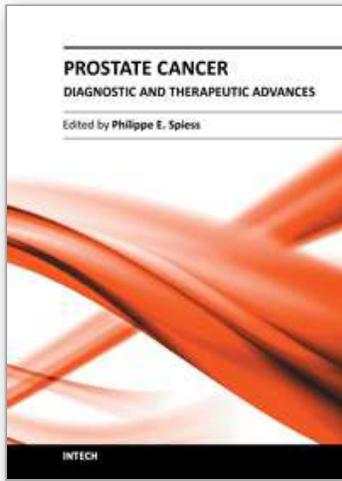
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In this book entitled "Prostate Cancer - Diagnostic and Therapeutic Advances", we highlight many of the significant advances made in our treatment armamentarium of prostate cancer. The book is subdivided into four sections termed: 1) novel diagnostic approaches, 2) surgical treatments options, 3) radiation therapy and its potential sequelae, and 4) medical management and its treatment complications. After reading the present book, readers will be very familiar with the major clinical advances made in our multifaceted treatment approach to prostate cancer over the past decade. This book is a tribute to our pioneering urologists and allied healthcare professionals who have continually pushed forward our traditional therapeutic envelope.

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