



Review – Prostate Cancer

Advances in Specific Immunotherapy for Prostate Cancer

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Abstract

Objectives: The absence of effective therapies for advanced prostate cancer has entailed an intensive search for novel treatments. This review presents an overview of specific immunotherapeutic strategies for prostate cancer.

Methods: Current literature was reviewed regarding the identification of tumor antigens and the design of T-cell- and antibody-based immunotherapy for prostate cancer. The PubMed database was searched using the key words antibodies, clinical trials, dendritic cells, immunotherapy, prostate cancer, and T cells.

Results: T cells and antibodies are powerful components of the specific anti-tumor immune response. CD8⁺ cytotoxic T lymphocytes (CTLs) efficiently destroy tumor cells. CD4⁺ T cells improve the antigen-presenting capacity of dendritic cells (DCs) and support the stimulation of tumor-reactive CTLs. Monoclonal antibodies exhibit their antitumor effects via antibody-dependent cellular cytotoxicity and complement activation. Consequently, much attention has been given to the identification of tumor antigens that represent attractive targets for specific immunotherapy. Several prostate cancer-related antigens were described and used in clinical trials. Such studies were based on the administration of peptides, proteins, or DNA. Furthermore, men with prostate cancer were vaccinated with peptide-, protein-, or RNA-loaded DCs, which display an extraordinary capacity to induce tumor-reactive T cells. Monoclonal antibodies directed against surface antigens were also used. Clinical trials revealed that immunotherapeutic strategies represent safe and feasible concepts for the induction of immunologic and clinical responses in men with prostate cancer.

Conclusions: Specific immunotherapy represents a promising treatment modality for prostate cancer. Further improvement of the current approaches is required and may be achieved by combining T-cell- and antibody-based vaccination strategies with radio-, hormone-, chemo-, or antiangiogenic therapy.

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1. Introduction

Prostate cancer (PCa) represents the most common noncutaneous cancer and the second leading cause of cancer-related deaths among American men with an estimated incidence of 218,890 cases and an estimated number of 27,050 deaths in 2007 [1]. Although the majority of patients are diagnosed with localized PCa and are successfully treated with radical prostatectomy or radiation therapy, 20–40% of patients develop recurrent disease [2,3]. Although androgen ablation represents an effective treatment modality for recurrent disease [4], most patients develop androgen-independent PCa [5]. In the management of metastatic hormone-refractory PCa (HRPC), chemotherapeutic trials involving docetaxel have demonstrated an improvement in median and progression-free survival [6]. Although promising palliative benefit and modest but real prolongation of survival have been achieved, additional treatment strategies are needed to prevent progression from localized to advanced disease and to further improve survival outcomes for patients with metastatic PCa.

2. T-cell-based and antibody-based immunotherapeutic strategies targeting PCa-associated antigens

Targeted immunotherapy of cancer aims to exploit cellular and humoral immune effector mechanisms for the specific recognition and elimination of tumor cells. The most promising arms of specific immunotherapy represent vaccination strategies attempting to stimulate effective antitumoral T-cell responses and the administration of antibodies.

T-cell-based immunotherapy of tumors has emerged with the observation that CD8⁺ cytotoxic T cells (CTLs) provide a high capability to recognize and destroy tumor cells that expose peptides derived from tumor-associated antigens (TAAs) in the complex with human leukocyte antigen (HLA) class I molecules [7]. Clinical studies focusing on the adoptive transfer of cytotoxic effector cells revealed tumor regression in cancer patients [8]. CD4⁺ T cells recognizing peptides in the context of HLA class II molecules also play an important role in antitumor immunity [9]. CD4⁺ T cells improve the capacity of dendritic cells (DCs) to induce CTLs by the interaction between CD40 on DCs and CD40 ligand on activated CD4⁺ T cells. Furthermore, CD4⁺ T cells provide help for the maintenance and expansion of CTLs by secreting cytokines such as interleukin 2 (IL-2) and can eradicate tumor cells directly.

Based on the crucial role of T cells in the elimination of tumor cells, much attention has been paid to the identification of tumor-associated proteins that may provide targets of tumor-reactive T cells and to the definition of peptide motifs within these proteins serving as T-cell epitopes when presented by HLA molecules [10]. In PCa, most of the targets for T-cell-mediated immunotherapy are differentiation antigens that are specifically expressed by normal and malignant prostate tissue. Some target structures are overexpressed in PCa as well as in other tumors. A summary of identified CD8⁺ T-cell epitopes is presented in Table 1.

For a variety of human hematopoietic malignancies and solid tumors, therapeutic strategies based on passive administration of antibodies have been successfully introduced into clinic [11,12]. The antibody-mediated effector mechanisms include the recruitment of immune effector cells for antibody-dependent cellular cytotoxicity (ADCC) and complement activation, and the functional interference of biologic pathways essential for tumor growth.

PCa can be considered as an attractive target for antibody-based therapies because the nonvital function of the prostate allows us to extend the spectrum of potential target molecules by tissue-specific markers not restricted to the tumor, and the usually small size of metastases is beneficial for antibody access and penetration [13]. So far, antibodies and antibody conjugates to toxins and radioisotopes targeting cell surface-associated molecules were shown to be effective in mouse models and are currently progressing to clinical evaluation.

3. PCa-associated target antigens for T-cell- and antibody-based immunotherapy

3.1. Prostate-specific antigens

The group of antigens typically expressed in prostate tissue includes prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), prostate stem cell antigen (PSCA), prostein, and transient receptor potential p8 (trp-p8).

PSA, a kallikrein-like serine protease, is almost exclusively expressed by prostate epithelial cells, can be detected in the majority of PCa tissues, and represents the most widely used serum marker for diagnosis and monitoring of PCa [14–17]. By in vitro stimulation using peptide-pulsed or RNA-transfected antigen-presenting cells (APCs), three HLA-A2-restricted PSA-derived peptides and a modified

peptide obtained by an amino acid replacement were demonstrated to stimulate tumor-reactive CTLs [18–22]. Correale et al developed a strategy to simultaneously induce PSA-specific CTL activities to different HLA-A2- and HLA-A3-restricted epitopes by applying an oligopeptide [23]. Two HLA-A24-binding PSA peptides were reported to generate peptide-specific CTLs from patients with PCa [24]. One of these epitopes induced HLA-A*2402-restricted CTLs in HLA-A*2402/K^b-transgenic mice [25]. Furthermore, immunogenic PSA peptides pre-

sented by other HLA class I molecules have been described [26,27].

The integral membrane glycoprotein PSMA represents a marker for normal prostate cells and is expressed in the majority of prostate tumors, particularly in undifferentiated, metastatic, and hormone-resistant cancer [28,29]. Four HLA-A2-restricted peptides were shown to induce antitumoral CTL responses in vitro [30–32]. Furthermore, three immunogenic HLA-A24-restricted peptides [33,34], and two peptides promiscuous for HLA-

Table 1 – CD8⁺ T-cell epitopes of prostate cancer-associated antigens

Antigen	HLA restriction element	Peptide position	Amino acid sequence	References	
Prostate-specific antigen (PSA)	HLA-A2	146–154	KLQCVDLHV	[18,20]	
		141–150	FLTPKKLQCV	[19,23]	
		154–163	VISNDVCAQV	[19,22,23]	
		154–163 (1Y) ^a	YISNDVCAQV	[21]	
	HLA-A3	162–170	QVHPQKVTK	[23]	
	HLA-A24	152–160	CYASGWGSI	[24,25]	
		248–257	HYRKWIKDTI	[24]	
	HLA-A1	68–77 ^b	VSHSFPHPHY	[26]	
	HLA-A11/ HLA-A31/ HLA-A33	16–24	GAAPLILSR	[27]	
	Prostate-specific membrane antigen (PSMA)	HLA-A2	4–12 ^b	LLHETDSAV	[30]
27–35			VLGGFFLL	[31]	
441–450			LLQERGVAYI	[32]	
178–186			NYARTEDFF	[33]	
227–235			LYSDPADYF	[33]	
HLA-A24		624–632	TSYVSFDSL	[34]	
HLA-A11/ HLA-A31/ HLA-A33		207–215	KVFRGNKVK	[27]	
		431–440	STEWAEENSR	[27]	
Prostatic acid phosphatase (PAP)		HLA-A*0201	135–143	ILLWQPIPV	[41,42]
		HLA-A2	299–307	LLFGYPVYV	[40]
		112–120	TLMSAMTNL	[32]	
	HLA-A*2404	213–221	LYCESVHNF	[43]	
	HLA-A11/ HLA-A31/ HLA-A33	155–163	YLPFRNCPR	[27]	
		248–257	GIHKQKEKSR	[27]	
Prostate stem cell antigen (PSCA)	HLA-A*0201	14–22	ALQPGTALL	[46,47]	
		105–113	AILALLPAL	[47]	
	HLA-A2	7–15	ALLMAGLAL	[48]	
		21–30	LLCYSCAQV	[48]	
	HLA-A24	76–84	DYYVGKKNI	[49]	
Prostein	HLA-A*0201	31–39	CLAAGITYV	[55]	
	HLA-B*5101	464–472	SACDVSVRV	[56]	
	HLA-Cw*0501	292–300	YTDFVGEGL	[56]	
		464–473	SACDVSVRVV	[56]	
Transient receptor potential-p8 (Trp-p8)	HLA-A*0201	187–195	GLMKYIGEV	[58]	
Six-transmembrane epithelial antigen of the prostate (STEAP)	HLA-A*0201	262–270	LLGTIHAL	[41,61]	
		86–94	FLYTLREV	[61]	
	HLA-A2	292–300	MIAVFLPIV	[60]	
		292–300 (2L) ^c	MLAVFLPIV	[60]	

Table 1 (Continued)

Antigen	HLA restriction element	Peptide position	Amino acid sequence	References
Parathyroid hormone-related protein (PTH-rp)	HLA-A*0201	59–68	FLHHLIAEIH	[64]
		165–173	TSTTSLEDL	[64]
	HLA-A2	59–67	FLHHLIAEI	[65]
		42–51	QLLHDKGKSI	[65]
	HLA-A24	36–44	RAVSEHQLL	[66]
		102–111	RYLTQETNKV	[66]
Human telomerase reverse transcriptase (hTERT)	HLA-A*0201	540–548	ILAKFLHWL	[68,69]
		865–873	RLVDDFLLV	[69]
		572–580	RLFFYRKSIV	[70]
		572–580 (1Y) ^d	YLFFYRKSIV	[70]
		30–38	RLGPQGWRL	[71]
		30–38 (9V) ^e	RLGPQGWRV	[71]
	HLA-A1	325–333	YAETKHFLY	[72]
	HLA-A3	973–981	KLFGVLRLLK	[73]
	HLA-A24	324–332	VYAETKHFL	[74,75]
		461–469	VYGFVRACL	[74,75]
		167–175	AYQVCGPPL	[75]
		845–853	CYGD MENKL	[75]
		1088–1096	TYVPLLGSL	[75]
	HLA-B*0702	277–285	RPAAEATSL	[76]
		342–350	RPSFLLSSL	[76]
351–360		RPSLTGARRL	[76]	
Survivin	HLA-A*0201	95–104	ELTLGEFLKL	[79,81]
		5–14	TLPPAWQPFL	[79,80]
		96–104 (2M) ^f	LMLGEFKLK	[81]
	HLA-A2	18–28	RISTFKNWPFL	[82]
	HLA-A1	92–101	QFEELTLGEF	[82]
		38–46 (9Y) ^g	MAEAGFIHY	[82]
		93–101 (2T) ^g	FTELTGEF	[82]
		47–56 (10Y) ^g	PTENEPDLAY	[82]
	HLA-A3	18–27 (10K) ^g	RISTFKNWPK	[82]
	HLA-A11	53–62	DLAQCFCK	[82]

^a Agonist peptide in which valine at the first position was replaced by tyrosine.

^b Natural generation and presentation of this epitope by prostate cancer cells was not analyzed.

^c The isoleucine residue in position 2 was replaced by leucine leading to an increased immunogenicity.

^d The arginine residue in position 1 was replaced by tyrosine to increase immunogenicity.

^e The peptide contains a leucine to valine substitution at position 9.

^f The natural threonine at position 2 was changed to a methionine residue.

^g As compared to the native survivin protein sequence, cysteine was substituted by tyrosine at position 9 in peptide 38–46, glutamic acid by threonine at position 2 in peptide 93–101, glutamine by tyrosine at position 10 in peptide 47–56, and phenylalanine by lysine at position 10 in peptide 18–27, respectively.

A11, HLA-A31, and HLA-A33 [27], arising from intracellular processing of PSMA protein, have been identified. PSMA also represents an attractive target structure for antibody-based immunotherapy. Initial in vitro validation of different anti-PSMA monoclonal antibodies (mAbs) coupled to ricin A and the bismuth-conjugated mAb J591 binding to the extracellular PSMA portion revealed target-specific cytotoxicity against PSMA-expressing PCa cells [35,36]. In addition, ²¹³Bi-J591 and humanized ⁹⁰Y-chelate-J591 or ¹³¹I-J591 have been shown to markedly reduce the tumor volume in nude mice bearing LNCaP xenografts [35,37]. The use of an anti-PSMA x anti-CD3 bispecific diabody to recruit T cells to the tumor site revealed efficient inhibition of tumor growth in a xenograft model [38].

The expression of PAP is also highly restricted to prostate tissue [39]. Three HLA-A2-binding, naturally generated immunogenic peptides were identified [32,40] and resulted in specific tumor rejection in vivo [41,42]. Furthermore, CTL-inducing PAP-derived peptides fitting into other HLA class I molecules have been defined [27,43].

PSCA is a glycosylphosphatidylinositol-anchored cell surface glycoprotein that is mainly expressed in the prostate [44]. PSCA expression is detectable in >80% of primary PCa samples and bone metastases and is increased in both androgen-dependent and -independent prostate tumors when compared to the corresponding normal prostate tissues, particularly in carcinomas of high stages and Gleason scores [44,45]. We and others identified HLA-A2–

restricted PSCA peptides capable of generating tumor-reactive CTL responses *in vitro* [46–48]. We also detected increased frequencies of CD8⁺ T cells recognizing two of these peptides in the blood of PCa patients [47]. Furthermore, an HLA-A24-presented peptide that effectively stimulated CTLs from PCa patients was found [49]. PSCA has also been evaluated as target for antibodies. Anti-PSCA mAbs conjugated to the toxin maytansinoid were effectively internalized by PCa cells resulting in cytotoxicity and regression of xenografts in mice [50]. Furthermore, inhibited formation and retarded growth of established xenografts were observed in mice treated with unconjugated anti-PSCA [51]. Recently, several chimeric anti-PSCA antibody radioconjugates were shown to specifically target PSCA⁺ xenografts and to exhibit antitumor effects *in vivo* [52].

Prostein represents a transmembrane protein with unique specificity for normal and malignant prostate tissues [53,54]. Our group found abundant expression in malignant and normal prostate tissues and maintained or even elevated transcript levels in 87% of the primary tumors compared to autologous nonmalignant tissue samples [55]. *In vitro* stimulation of CD8⁺ T lymphocytes with peptide-loaded DCs we defined an autochthonously generated HLA-A*0201-presented CTL-activating peptide [55]. Immunogenic T cell epitopes presented by HLA-B*5101 and HLA-Cw*0501 were also identified [56].

The gene *trp-p8* encodes a seven-span transmembrane protein with significant homology to a family of Ca²⁺ channel proteins [57]. *Trp-p8* expression is mainly restricted to the prostate and is detected in the majority of prostate tumors [57]. Further analysis revealed overexpression in tumors of early stages and low grades when compared to the corresponding normal prostate tissue [58]. Recently, we identified an HLA-A*0201-binding peptide that was able to stimulate tumor-reactive CTLs *in vitro* [58].

3.2. Antigen overexpressed in various tumors including PCa

Several potential target structures for immunotherapy are overexpressed in different tumors of epithelial or hematopoietic origin, including PCa. This group comprises the six-transmembrane epithelial antigen of the prostate (STEAP), the parathyroid hormone-related protein (PTH-rp), the human telomerase reverse transcriptase (hTERT), survivin, Her-2/neu, epidermal growth factor receptor (EGFR), and tumor-associated glycoprotein 72 (TAG-72).

STEAP is a transmembrane protein with a prostate-specific expression in normal tissues that is overexpressed in a variety of tumor types including PCa [59]. Several naturally processed HLA-A2-restricted peptides capable of inducing CTLs *in vitro* and *in vivo* have been identified [41,60,61]. Recent data suggest that STEAP additionally represents an attractive target for therapeutic antibodies because two STEAP-specific mAbs significantly inhibited the growth of PCa xenografts in mice [62].

PTH-rp is a factor that binds receptors on osteoblasts and induces bone formation. It is highly overexpressed in PCa and other cancers of epithelial origin and is considered to be involved in the development of bone metastases [63]. Therefore, it might represent a promising immunotherapeutic target for men with PCa with bone metastases. Four HLA-A2-fitting epitopes induced tumor-reactive CTLs *in vitro*, and two of them additionally induced antitumoral CTL responses *in vivo* [64,65]. Furthermore, two HLA-A24-binding peptides were proven to be immunogenic *in vitro* [66].

hTERT protects tumor cells from telomere erosion. It is undetectable in most non-transformed somatic cells but is expressed in a variety of different tumor types including PCa [67]. Several naturally generated HLA-A*0201-restricted CTL epitopes have been identified effectively inducing peptide-specific and tumor-lysing CTLs *in vitro* and *in vivo* [68–71]. For two of these peptides immunogenicity was markedly increased by an amino acid substitution [70,71]. Furthermore, immunogenic hTERT-derived peptides fitting to other HLA class I molecules have been defined [72–76].

Survivin, an inhibitor of apoptosis, is highly overexpressed in many human tumors including PCa, and its expression correlates with poor prognosis of tumor disease [77,78]. The wide expression in cancer and the almost complete absence in differentiated adult tissues together with the functional role for tumor cell survival make survivin an interesting target for T-cell-based immunotherapy. We and others identified two naturally generated HLA-A*0201-restricted peptides that induced specific CTL responses *in vitro* [79,80]. Furthermore, CD8⁺ T cells reactive against one of the previously defined survivin peptides and a peptide modified at an anchor amino acid were found in the blood of patient with tumors [81]. A number of additional CD8⁺ T-cell epitopes restricted to other HLA class I molecules were defined by analyzing the peptide specificity of spontaneous CTL responses in cancer patients [82].

Several cell surface proteins overexpressed in different tumors including PCa have been evaluated as targets for therapeutic antibodies *in vitro* and *in vivo*. The tyrosine kinase receptor Her-2/neu is the target structure for the humanized mAb trastuzumab, which is used successfully for the treatment of breast cancer [83]. Recent findings revealed that Her-2/neu is important for the progression of PCa to an androgen-independent disease [84]. In androgen-dependent xenograft models, a growth of established tumors was significantly inhibited by trastuzumab administration [85]. EGFR was also involved in PCa progression providing a rationale for antibody-based immunotherapy [86]. Targeting of EGFR by the mAb cetuximab resulted in inhibition of PCa cell proliferation *in vitro* and tumor progression *in vivo* [87]. TAG-72, a mucin overexpressed in a variety of adenocarcinomas including PCa [88], has been targeted by the mAb CC49 in immunotherapeutic trials.

4. Vaccination of patients with PCa with peptides, proteins, DNA, or tumor cells

Following the identification of PCa-associated proteins that may be suitable targets of tumor-reactive T cells several clinical trials were conducted to determine feasibility, toxicity, and clinical responses. Furthermore, the efficiency of a particular vaccination strategy to induce or augment specific T-cell responses was analyzed. Methods for immune monitoring comprise limiting dilution assays, staining with peptide-loaded major histocompatibility complex (MHC) multimers, enzyme-linked immunospot analysis as well as proliferation and cytotoxicity assays [89]. Delayed-type hypersensitivity (DTH) testing has been used as an *in vivo* approach to demonstrate the induction of T-cell responses.

To determine the efficiency of individualized peptide vaccination in combination with estramustine for the treatment of HRPC patients a clinical phase 1/2 study was performed [90]. The administered peptides derived from several cancer-related antigens were selected based on the measurement of peptide-specific CD8⁺ T cells and peptide-reactive immunoglobulin G in the blood of patients before injection. Vaccination was well tolerated and augmentation of circulating peptide-specific CD8⁺ T cells was observed. All 13 patients treated with the combination therapy showed a decrease of serum PSA, including 6 men with a decrease of $\geq 50\%$.

Meidenbauer et al [91] reported on a clinical trial enrolling 10 men with PCa, which was based on

JBT1001, a vaccine consisting of recombinant PSA with lipid A formulated in liposomes. Patients were vaccinated with JBT1001 emulsified in mineral oil or with the vaccine in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF represents an adjuvant that enhances recruitment, maturation, and antigen-presenting capacity of DCs leading to an efficient stimulation of tumor-reactive T cells. Whereas two patients had PSA-reactive T cells before vaccination, 8 of 10 patients showed detectable PSA-reactive T cells after vaccination.

Other clinical studies were conducted to determine the potential of a recombinant vaccinia virus expressing PSA. Eder et al [92] initiated a trial to investigate clinical and immunologic effects in 33 PCa patients treated with recombinant vaccinia-PSA (rV-PSA) with or without GM-CSF. Vaccination led to stabilization of serum PSA levels in 14 of 33 PCa patients for at least 6 mo after primary immunization. The number of PSA-reactive T cells increased at least 2-fold in 5 of 7 evaluated patients. Gulley et al [93] administered rV-PSA to patients with metastatic HRPC. Six of 42 patients had stable disease and 3 of 5 analyzed patients showed a vaccine-induced increase of PSA-specific T lymphocytes. Kaufman et al [94] conducted a phase 2 study evaluating a heterologous prime/boost vaccination protocol with vaccinia and fowlpox viruses expressing PSA in 64 PCa patients with biochemical progression after local therapy. Of the eligible patients, 45.3% remained free of PSA progression at 19.1 mo and 78.1% demonstrated clinical progression-free survival. An increase in PSA-specific T cells was found in 46% of patients. Gulley et al [95] reported on another phase 2 trial administering an admixture of rV-PSA plus recombinant vaccinia virus expressing the T-cell costimulatory molecule B7.1/CD80 followed by booster vaccinations with fowlpox virus containing PSA in combination with standard radiotherapy. Patients also received GM-CSF and IL-2. Thirteen of 17 evaluated patients with localized PCa treated by the combination therapy showed at least a 3-fold increase in PSA-specific T cells. Another clinical trial including patients with nonmetastatic androgen-independent PCa combined the same vaccination strategy with the antiandrogen nilutamide [96]. Median time to treatment failure was 9.9 mo in the vaccine arm and 7.6 mo in the antiandrogen arm. After 6 mo, eight patients in the antiandrogen arm received vaccine resulting in an additional median time to treatment failure of 5.2 mo and of 15.9 mo from onset of nilutamide. Twelve patients in the vaccine arm received nilutamide resulting in an additional

median time to treatment failure of 13.9 mo and of 25.9 mo from initiation of vaccination. In a further clinical study, 28 patients with metastatic androgen-independent PCa were randomized to receive either a combination of the same vaccine and docetaxel or vaccine alone [97]. Docetaxel did not impair the vaccine-induced increase of PSA-reactive T cells. In the combination therapy arm, 6 of 14 patients displayed a decline in serum PSA, whereas only 3 of 14 patients in the vaccine arm alone had a decline in serum PSA.

Other immunotherapeutic treatment modalities that were based on so-called “naked” DNA have also been explored. In a phase 1/2 trial, 26 men with PCa were immunized intradermally with DNA plasmids encoding either the extracellular domain of PSMA or the costimulatory molecule B7.2/CD86, a combined PSMA/CD86 plasmid, and a replication-deficient adenoviral vector expressing PSMA and GM-CSF [98]. Treatment was well tolerated. DTH reactions against PSMA were found in several patients including all those who were initially vaccinated with the adenoviral vector expressing PSMA. More recently, a phase 1 study investigating the administration of a DNA plasmid encoding PSA in combination with GM-CSF and IL-2 to HRPC patients was conducted [99]. Two of three patients receiving the highest dose developed a PSA-specific cellular immune response and a decrease in the slope of serum PSA.

Further clinical trials enrolling PCa patients were based on the administration of modified PCa cells. Simons et al [100] reported on a phase 1/2 study investigating the efficiency of immunotherapy with the irradiated, allogeneic PCa cell lines PC-3 and LNCaP transduced to secrete GM-CSF in patients with hormone-naïve PCa. Sixteen of 21 patients displayed a significant decrease in PSA velocity compared with prevaccination status. One patient had a partial PSA response of 7-mo duration. More recently, a phase 1/2 trial was conducted to evaluate the same vaccine in chemotherapy-naïve patients with metastatic HRPC [101]. The results suggest an advantage with a higher dose of the vaccine in patients with radiologically detectable metastases with regard to time to progression, PSA changes, and overall survival.

5. DC-based immunotherapy for PCa

DCs are professional APCs that display an extraordinary capacity to induce, sustain, and regulate T-cell responses [102]. Consequently, DCs evolved as promising candidates for vaccination protocols in

cancer therapy [103]. Animal models demonstrated that TAA-presenting DCs are capable of inducing protective and therapeutic antitumor responses [104,105]. Clinical trials revealed promising immunologic and antitumor responses of antigen-loaded DCs administered as a vaccine against cancer [106,107].

Clinical trials enrolling men with PCa revealed that DCs pulsed with TAA-derived peptide, protein, or mRNA were well tolerated, efficiently augmented antigen-specific T-cell responses, and exhibited clinical effects. Murphy et al [108,109] conducted a phase 1 trial to evaluate vaccination of DCs loaded with PSMA-derived peptides in patients with HRPC. Treatment was well tolerated by all 51 patients and antigen-specific cellular immune responses were observed in 7 partial responders based on National Prostate Cancer Project criteria and a 50% reduction of PSA level. Thereafter, the same group initiated a phase 2 trial to investigate the therapeutic efficiency of PSMA peptide-pulsed DCs. Nine partial responders were identified in a group of 33 HRPC patients who were already participants in the previous phase 1 study and were subsequently enrolled in the phase 2 trial [110].

In another trial, DCs pulsed with a hTERT-derived peptide and keyhole limpet hemocyanin were administered to five patients with metastatic HRPC [111]. Peptide-reactive T cells were induced in two patients after vaccination. All four evaluable patients had stabilization of disease. Recently, we conducted a clinical study to evaluate the potential of DCs loaded with a cocktail consisting of peptides derived from PSA, PSMA, survivin, prostein, and trp-p8 [112]. Four of eight patients showed a temporary decrease or stabilization of serum PSA levels. Three of these four PSA responders exhibited specific T-cell responses against prostein, survivin, or PSMA. Waeckerle-Men et al [113] used DCs pulsed with peptides derived from PSA, PSCA, PSMA, and PAP to treat six men with HRPC. Three patients displayed specific T-cell responses against all antigens and an increase in PSA doubling time. In another trial, DCs loaded with PSA- and PSMA-derived peptides were administered to 12 patients with hormone- and chemotherapy-refractory PCa [114]. Six patients had stable disease and five patients developed DTH reactions.

Small et al [115] conducted a phase 1/2 trial including 31 HRPC patients. Patients were treated with APCs pre-exposed *in vitro* to PA2024, a fusion protein consisting of human GM-CSF and PAP. Six patients showed a decline in PSA level and 38% of patients displayed immune responses to PAP. Burch et al [116] also administered PA2024-loaded APCs to

HRPC patients. These infusions were followed by subcutaneous applications of PA2024. Treatment induced antigen-specific cellular immunity and resulted in PSA level reduction in 3 of 19 evaluated patients. More recently, a phase 3 study enrolling 127 patients with metastatic HRPC was conducted to determine the safety and efficiency of PA2024-loaded APCs in a placebo-controlled trial [117]. Patients were randomized to receive the vaccine or placebo with primary end points of time to disease progression. The median for time to disease progression was not statistically significant at 11.7 wk in the vaccine group compared with 10.0 wk in the placebo group. However, a statistically significant increase in median overall survival was observed (25.9 mo in the vaccine group vs. 21.4 mo in the placebo group). The estimated survival rate for patients in the vaccine group was 34% compared with 11% in the placebo group at the 36-mo follow-up visit. Based on the clinical results obtained so far, the US Food and Drug Administration recently decided not to approve this vaccine for PCa therapy.

Another clinical trial including patients with metastatic PCa was based on the administration of DCs loaded with recombinant murine PAP [118]. Treatment was well tolerated. All patients developed T-cell immunity to mouse PAP and 11 of 21 patients to the homologous self-antigen. Six of 21 patients had evidence of clinical stabilization of their previously progressing PCa as determined by PSA level monitoring, computed tomography, and bone scans. Barrou et al [119] performed a clinical trial enrolling patients with PCa in biochemical relapse after radical prostatectomy to evaluate the efficiency of DCs pulsed with human recombinant PSA. Twenty-four patients received nine administrations of PSA-loaded DCs using intravenous, subcutaneous, and intradermal routes. No severe side effects were observed and 11 patients exhibited a transient PSA decrease.

Further clinical studies were conducted to investigate the potential of mRNA-transfected DCs for PCa therapy. Vaccination with PSA mRNA-transfected DCs was well tolerated and PSA-specific T cells were detected in all analyzed patients [120]. Six of seven evaluated patients had a significant decrease of PSA. Furthermore, administration of hTERT mRNA-transfected DCs resulted in an expansion of hTERT-specific T cells in 19 of 20 patients and was associated with a reduction of PSA velocity [121]. In an additional trial, patients were vaccinated with DCs transfected with allogeneic tumor mRNA. Thirteen of 19 patients that completed vaccination displayed a decrease in log slope PSA [122].

Another vaccination strategy for PCa is based on the administration of Flt3 ligand. This immunostimulatory agent efficiently promotes the differentiation and expansion of DCs *in vitro* and *in vivo*. Higano et al [123] conducted a trial evaluating the efficiency of Flt3 ligand in men with HRPC. Treatment was well tolerated. Flt3 ligand application resulted in a marked increase of DC number in the peripheral blood. Eleven of 31 patients showed a decrease or only a minor increase (<25%) in PSA levels.

6. Antibody-based clinical trials for PCa

Among the PCa-associated cell surface markers, PSA, Her-2/neu, and TAG-72 have progressed through the preclinical evaluation and are now clinically targeted by antibodies and antibody constructs.

PSMA has been used in several clinical studies for imaging and treatment of PCa. In this context, radioisotope-coupled humanized J591 was successfully applied for imaging of PCa with absence of toxicity and excellent specific targeting to bone and soft tissue metastatic lesions as prerequisite for therapeutic purposes [124]. Two subsequently initiated phase 1 trials using humanized ⁹⁰Y- or ¹⁷⁷Lu-labeled J591 in patients with androgen-independent PCa revealed tolerable toxicity and declines of PSA serum levels of $\geq 50\%$ in 2 of 29 patients or 4 of 35 patients, and stabilized PSA levels in 6 of 29 patients or 16 of 35 patients, respectively [125,126]. In another phase 1 study, treatment of patients with metastatic PCa with uncoupled or with ¹¹¹In-labeled J591 was well tolerated [127]. One of 14 patients showed a PSA decline of $>50\%$.

Therapeutic approaches using Her-2/neu as target for trastuzumab in advanced breast cancer have clearly demonstrated a prolonged time to disease progression and a longer survival [83]. In contrast, several clinical trials using trastuzumab for the treatment of Her-2/neu⁺ PCa showed only limited therapeutic effects [128]. The bispecific antibody construct MDXH210 (anti-HER-2/neu x anti-Fc γ RI) was developed to recruit monocytes, macrophages, and neutrophils to Her-2/neu⁺ tumor cells. In a phase 1 study, treatment of PCa patients with MDXH210 was well tolerated and resulted in stable PSA levels in five of six patients for at least 40 d [129].

TAG-72 has been targeted by the mAb CC49 in several clinical studies. Application of ¹³¹I-conjugated CC49 to patients with HRPC failed to induce objective responses [130]. Combination therapy with ¹³¹I-conjugated CC49 and interferon as a positive regulator of TAG-72 expression resulted in a PSA

stabilization in 2 of 14 patients [131] or minor radiographic responses in 2 patients [132].

In addition to the antibody targeting of PCa-associated surface antigens, mAbs have been generated to inhibit tumor angiogenesis or to improve antitumor immunity. Vascular endothelial growth factor (VEGF), a potent angiogenic factor that promotes tumor growth, is overexpressed in PCa and shows increased plasma levels in patients with metastatic PCa [133,134]. Treatment of mice with anti-VEGF-neutralizing antibodies both suppressed the primary tumor growth and the progression of established PCa xenografts [135,136]. Recently, a clinical study was conducted evaluating the combination of the humanized anti-VEGF mAb bevacizumab with PA2024-loaded APCs in PCa patients with

biochemical relapse [137]. Nine of 21 analyzed patients had a decrease in PSA level.

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is expressed on activated T cells and competes with CD28 for binding to B7 costimulatory molecules on APCs thereby preventing costimulation and promoting down-regulation of T-cell activity. Treatment with anti-CTLA-4 mAbs alone or in combination with other immunotherapies has been demonstrated to facilitate antitumor immunity and tumor regression in mouse models and clinical trials in PCa [138]. Recently, Small et al [139] performed a clinical trial to determine the efficacy of the humanized mAb ipilimumab in 14 HRPC patients. Treatment was well tolerated with clinical autoimmunity limited to one patient. Two patients

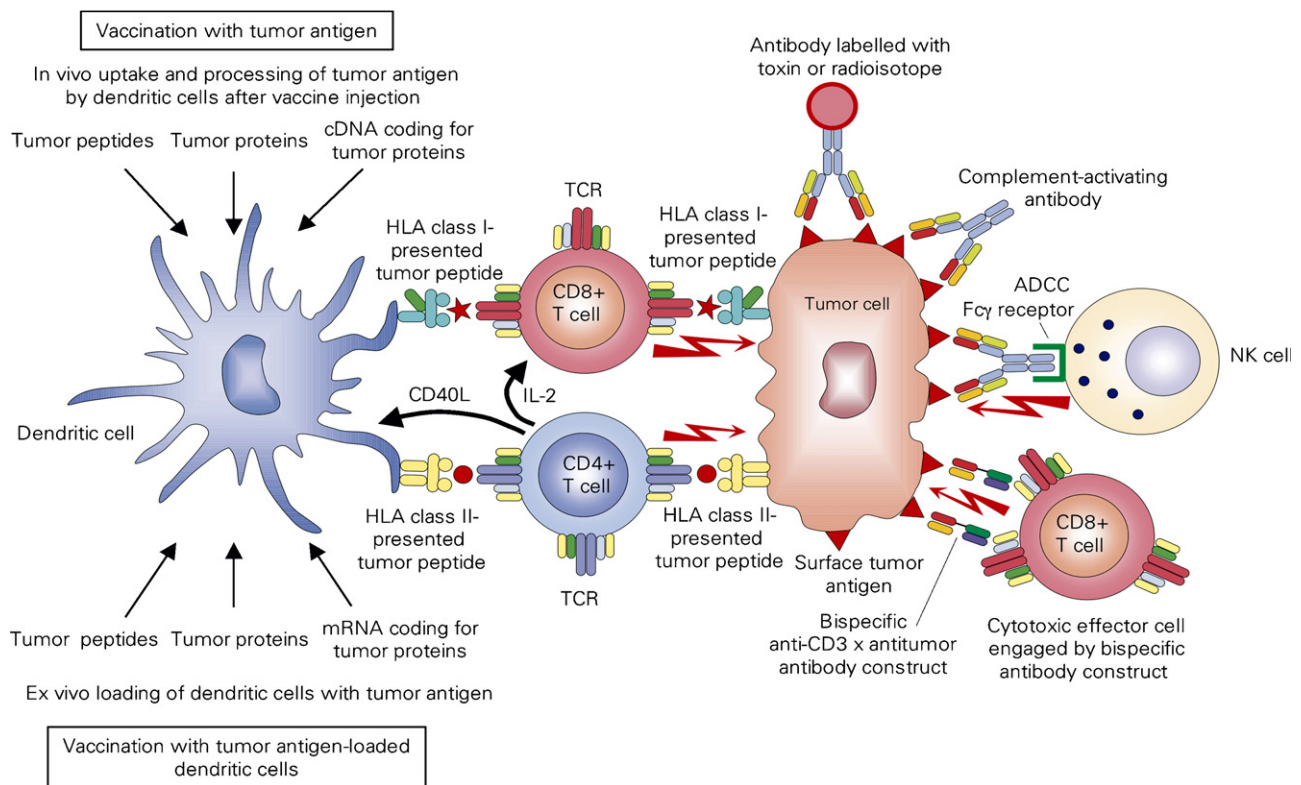


Fig. 1 – Current T-cell- and antibody-based strategies in immunotherapy of prostate cancer. Tumor-specific T-cell responses can be induced by vaccination. Tumor peptides, tumor proteins, or cDNA coding for tumor antigens are administered to the patient. Being taken up by DCs in vivo they are presented to T cells in regional lymph nodes. Alternatively, DCs are loaded ex vivo with the respective tumor antigen preparation and injected into the patient as DC vaccine. DCs can efficiently activate CD8⁺ CTLs, which are capable of recognizing and destroying tumor cells. They can also stimulate CD4⁺ T cells, which increase the capacity of DCs to induce CD8⁺ CTLs through the interaction of CD40 ligand on CD4⁺ cells and the CD40 receptor on DCs. In addition, CD4⁺ T cells support the expansion and maintenance of CD8⁺ CTLs by secreting cytokines such as IL-2 and are themselves capable of killing tumor cells. Monoclonal antibodies directed against tumor surface antigens can mediate cytotoxicity either by engaging cytotoxic effector cells such as NK cells via Fc receptors (ADCC) or by complement activation. Toxins or radioisotopes when coupled to monoclonal antibodies can specifically be targeted to tumor cells. Recombinant bispecific antibody constructs directed against a tumor cell surface antigen and an activating receptor on immune effector cells can polyclonally engage these cells into tumor eradication. ADCC = antibody-dependent cellular cytotoxicity; CTLs = cytotoxic T cells; DCs = dendritic cells; HLA = human leukocyte antigen; IL = interleukin; NK = natural killer; TCR = T-cell receptor.

showed PSA declines of $\geq 50\%$. An additional study was conducted evaluating the combination of ipilimumab and GM-CSF for the treatment of HRPC in 24 patients [140]. Of six patients treated with the highest dose level (4×3 mg/kg ipilimumab), three displayed a PSA decline of $>50\%$, and one of these patients had a partial response in hepatic metastases. A further clinical trial investigated the combination of ipilimumab and irradiated, allogeneic PCa cell lines transduced to secrete GM-CSF in men with HRPC [141]. Five of six patients treated on the higher ipilimumab doses (3 and 5 mg/kg) developed endocrinopathy consistent with hypophysitis manifested by adrenal insufficiency or hypothyroidism (or both). Furthermore, these higher ipilimumab doses resulted in a PSA decline of $>50\%$ in five of six patients with median response duration of 4.9 mo.

7. Conclusions

Current therapeutic approaches revealed only modest impact on survival outcomes for patients with advanced PCa. Recent progress in the identification of TAAs and derived T-cell epitopes paved the way for the design of novel T- cell- or antibody-based immunotherapeutic strategies (Fig. 1). Clinical trials that aimed at the *in vivo* activation of CD8⁺ CTLs and CD4⁺ T-helper cells by vaccination with peptides, proteins, DNA, tumor cells, or TAA-pulsed DCs provide evidence that these concepts were safe and feasible. Furthermore, immunologic and clinical responses were induced in patients with PCa. Clinical studies including mAbs directed against PCa surface antigens were also conducted and resulted in some clinical responses. Despite these promising effects the clinical efficiency of the different immunotherapeutic strategies for the majority of patients with advanced PCa is still limited owing to various immune evasion mechanisms mediated by tumors. These mechanisms include down-regulation of different components of the MHC class I processing and presentation machinery, generation of antigen loss variants, production of inhibitory cytokines such as transforming growth factor- β and IL-10, and expression of apoptosis-inducing molecules [142,143]. Thus, further improvement of current treatment modalities for advanced PCa is required, which may be achieved by combining T-cell- or antibody-based vaccination strategies with radio-, hormone-, chemo-, or antiangiogenic therapy. Immunotherapeutic approaches should also be evaluated in patients with PCa at earlier stages of disease.

Conflicts of interest

The authors have nothing to disclose.

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