

T cell-based strategies for immunotherapy of prostate cancer

Review Article

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Summary

Prostate cancer is the most common noncutaneous cancer diagnosis and the second leading cause of cancer-related deaths among American men. The absence of effective curative therapies for advanced metastatic prostate cancer has entailed an intensive search for novel treatment modalities. T cells provide a powerful compartment of the adaptive immune system comprising important functions in antitumor immunity. Thus, CD8⁺ cytotoxic T lymphocytes (CTLs) are capable of efficient recognition and destruction of tumor cells. CD4⁺ T cells enhance the antigen-presenting capacity of dendritic cells (DCs) and provide help for the maintenance and expansion of tumor-reactive CTLs. Consequently, much attention has been paid to the identification of tumor-associated antigens that may serve as target structures for a T cell-based immunotherapeutic strategy. In this context, several prostate cancer-related proteins have been described which are capable of inducing antigen-specific and/or tumor-reactive T cells *in vitro*. Following the identification of suitable prostate cancer-associated antigens, several clinical trials were conducted which were based on the administration of selected peptides, recombinant proteins or DNA. In addition, prostate cancer patients were immunized with peptide-, protein-, or RNA-loaded DCs which display a unique capacity for the induction of primary T cell responses. These clinical trials provide evidence that the different immunotherapeutic strategies represent safe and feasible concepts for the induction of immunological and clinical responses in prostate cancer patients.

I. Introduction

Prostate cancer represents the most common noncutaneous cancer among American men with an estimated incidence of 232,090 cases in 2005 (Jemal et al, 2005). In addition, it is the second leading cause of cancer-related deaths in American men with an estimated number of 30,350 deaths in 2005 (Jemal et al, 2005). Although the majority of patients are diagnosed with localized prostate cancer and are treated with radical prostatectomy or radiation therapy, 20-40% of patients will develop recurrent disease (Coen et al, 2002; Han et al, 2003; Roehl

et al, 2004). Androgen ablation with either surgical orchiectomy or application of luteinizing hormone-releasing hormone agonists with or without antiandrogens represents an effective initial treatment modality for recurrent disease (Miyamoto et al, 2004; Sharifi et al, 2005). However, within several years, most patients develop androgen-independent prostate cancer (Feldman and Feldman, 2001). Recent clinical trials of docetaxel-based chemotherapy in patients with metastatic hormone-refractory prostate cancer (HRPC) have demonstrated a decrease of serum prostate-specific antigen (PSA) level, a

reduction in pain, an improvement of the quality of life and, for the first time, a prolonged survival (Petrylak et al, 2004; Tannock et al, 2004). 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 prostate cancer.

II. Prostate cancer-associated antigens recognized by T cells

Immunotherapy of tumors has advanced with the observation that CD8⁺ cytotoxic T cells (CTLs) provide a high capability to recognize and destroy tumor cells which expose peptides derived from tumor-associated antigens (TAAs) and bound to human leukocyte antigen (HLA) class I molecules (Rosenberg, 1997). In addition, clinical studies focussing on the adoptive transfer of cytotoxic effector cells revealed tumor regression in cancer patients (Dudley et al, 2002; Yee et al, 2002; Dudley and Rosenberg, 2003; Dudley et al, 2005; Vignard et al, 2005). CD4⁺ T cells recognizing peptide motifs in the context of HLA class II molecules also play an important role in antitumor immunity (Pardoll and Topalian, 1998; Toes et al, 1999; Wang, 2001). Thus, 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 (Bennett et al, 1998; Ridge et al, 1998; Schoenberger et al, 1998). CD4⁺ T cells also provide help for the maintenance and expansion of CTLs by secreting cytokines such as interleukin (IL)-2. Additional functions of CD4⁺ T cells were documented by several studies indicating that these cells can eradicate tumors and can contribute to the inhibition of angiogenesis (Mumberg et al, 1999; Qin and Blankenstein, 2000).

Since effector T cells play a major role in the elimination of tumor cells, much attention has been paid on the identification of tumor-associated proteins that may provide targets of tumor-reactive T cells and on the definition of concrete peptide motifs within these proteins serving as T cell epitopes when presented by HLA molecules (Stevanovic, 2002). In prostate cancer, most of the target molecules for T cell-mediated immunotherapy are differentiation antigens that are specifically expressed by normal and malignant prostate tissue. This group includes PSA, prostate-specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), prostate stem cell antigen (PSCA), prostein and transient receptor potential (trp)-p8. Some other potential target proteins as parathyroid hormone-related protein (PTH-rp), human telomerase reverse transcriptase (hTERT) and survivin are overexpressed in prostate cancer as well as in other tumors. A list of the so far identified T cell epitopes is given in **Table 1**.

Table 1. CD8⁺ and CD4⁺ T cell epitopes of prostate cancer-associated antigens

Antigen	HLA restriction element	Peptide position	Amino acid sequence	Reference	
PSA	HLA-A2	146-154	KLQCVDLHV	Xue et al, 1997; Perambakam et al, 2002	
		141-150	FLTPKKLQCV	Correale et al, 1997; Correale et al, 1998	
		154-163	VISNDVCAQV	Correale et al, 1997; Correale et al, 1998; Heiser et al, 2000	
	HLA-A3	154-163 (1Y) ^a	YISNDVCAQV	Terasawa et al, 2002	
		HLA-A24	162-170	QVHPQKVTK	Correale et al, 1998
	HLA-A1	248-257	68-77 ^b	HYRKWIKDTI	Gotoh et al, 2002; Harada et al, 2003
			49-63	VSHSFPPLY	Harada et al, 2003 Corman et al, 1998
		HLA-DR4	(6M, 10M) ^{b, c}	ILLGRMSLFMPEDTG	Corman et al, 1998
	PSMA	HLA-DR	64-78 ^b	QVFQVSHSFPPLYD	Corman et al, 1998
			HLA-DR B1*1501	171-190	LQCVDLHVISNDVCAQVHPQ
HLA-A2		221-240	GVLQGITSWGSEPCALPERP	Klyushnenkova et al, 2005	
		4-12 ^b	LLHETDSAV	Tjoa et al, 1996	
		711-719	ALFDIESKV	Murphy et al, 1996	
		27-35	VLAGGFLL	Lu and Celis, 2002	
		441-450	LLQERGVAYI	Harada et al, 2004	
HLA-A24	178-186	NYARTEDFF	Harada et al, 2002		
	227-235	LYSDPADYF	Horiguchi et al, 2002		
HLA-DR4	624-632	TSYVSFDSL	Kobayashi et al, 2003a		
	334-348	TGNFSTQKVKMHIHS	Kobayashi et al, 2003b		
HLA-DR9/ HLA-DR53	687-701	DPQSGAAVVHEIVRS	Kobayashi et al, 2003b		
	HLA-DR53	730-744	RQIYVAAFTVQAAAE	Kobayashi et al, 2003b	

PAP	HLA-A2	299-307	LLFGYPVYV	Peshwa et al, 1998
		112-120	TLMSAMTNL	Harada et al, 2004
	HLA-A*2404	213-221	LYCESVHNF	Inoue et al, 2001
	HLA class II	199-213	GQDLFGIWSKVYDPL	McNeel et al, 2001
PSCA	HLA class II	228-242	TEDTMTKLRLESELS	McNeel et al, 2001
	HLA-A*0201	14-22	ALQPGTALL	Dannull et al, 2000; Kiessling et al, 2002
Prostein	HLA-A2	105-113	AILALLPAL	Kiessling et al, 2002
		7-15	ALLMAGLAL	Matsueda et al, 2004a
		21-30	LLCYSCKAQV	Matsueda et al, 2004a
	HLA-A24	76-84	DYYVGGKNI	Matsueda et al, 2004b
	HLA-A*0201	31-39	CLAAGITYV	Kiessling et al, 2004
	HLA-B*5101	464-472	SACDVSVRV	Friedman et al, 2004
Trp-p8	HLA-Cw*0501	292-300	YTDFVGEGL	Friedman et al, 2004
		464-473	SACDVSVRVV	Friedman et al, 2004
	HLA-A*0201	187-195	GLMKYIGEV	Kiessling et al, 2003
PTH-rp	HLA-A*0201	59-68	FLHHLIAEIH	Francini et al, 2002
		165-173	TSTTSLEDL	Francini et al, 2002
	HLA-A2	59-67	FLHHLIAEI	Yao et al, 2005
		42-51	QLLHDKGKSI	Yao et al, 2005
hTERT	HLA-A24	36-44	RAVSEHQLL	Yao et al, 2004
		102-111	RYLTQETNKV	Yao et al, 2004
	HLA-A*0201	540-548	ILAKFLHWL	Vonderheide et al, 1999; Minev et al, 2000
Survivin		865-873	RLVDDFLLV	Minev et al, 2000
		572-580	RLFFYRKSV	Hernandez et al, 2002
		572-580 (1Y) ^d	YLFFYRKSV	Hernandez et al, 2002
	HLA-A3	973-981	KLFGVLRK	Vonderheide et al, 2001
	HLA-A24	324-332	VYAETKHFL	Arai et al, 2001
		461-469	VYGFVRACL	Arai et al, 2001
	HLA-A1	325-333	YAETKHFLY	Schreurs et al, 2005
	HLA-DR1/ HLA-DR7/ HLA-DR15	672-686	RPGLLGASVLGLDDI	Schroers et al, 2002; Schroers et al, 2003
	HLA-DR4/ HLA-DR11/ HLA-DR15	766-780	LTDLQPYMRQFVAHL	Schroers et al, 2003
	HLA-A*0201	95-104	ELTLGEFLKL	Schmitz et al, 2000; Andersen et al, 2001a
Survivin		5-14	TLPPAWQPFL	Schmitz et al, 2000; Siegel et al, 2004
		96-104 (2M) ^c	LLLGEFKLK	Andersen et al, 2001a; Anderson et al, 2001b
	HLA-A2	18-28	RISTFKNWPFL	Reker et al, 2004
	HLA-A1	92-101	QFEELTLGEF	Reker et al, 2004
		38-46 (9Y) ^f	MAEAGFIHY	Reker et al, 2004
		93-101 (2T) ^f	FTELTGEF	Reker et al, 2004
		47-56 (10Y) ^f	PTENEPDLAY	Reker et al, 2004
	HLA-A3	18-27 (10K) ^f	RISTFKNWPK	Reker et al, 2004
	HLA-A11	53-62	DLAQCFCK	Reker et al, 2004

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

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

^cThe methionine residues in the positions 6 and 10 were substituted in place of histidines.

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

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

^fAs 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.

A. Prostate-specific antigen (PSA)

PSA is a kallikrein-like serin-protease showing a high degree of homology with human pancreatic kallikrein (Lundwell and Lilija, 1989). It represents the most widely

used serum marker for diagnosis and monitoring of prostate cancer and is nearly exclusively expressed by epithelial cells of the prostate (Balk et al, 2003). Furthermore, it is found in the majority of prostate cancer

tissues and can be detected in the cytoplasmic portion of these cells by immunoperoxidase staining (Oesterling, 1991).

Among prostate differentiation antigens, the T cell-mediated immune response to PSA has been studied most thoroughly to date. Xue et al, 1997 identified an HLA-A2-compatible peptide corresponding to amino acid (aa) residues 146-154 of PSA that was successfully used for the *in vitro*-stimulation of peptide-specific CTLs from a healthy donor by autologous peptide-pulsed peripheral blood mononuclear cells (PBMCs). In a complementary study, CTLs recognizing PSA peptide 146-154 were shown to specifically lyse HLA-A2-positive tumor cells endogenously expressing the PSA protein (Perambakam et al, 2002). Applying a similar stimulation protocol, two other HLA-A2-binding PSA-derived peptides consisting of aa 141-150 and 154-163 were defined as CD8⁺ T cell epitopes capable of inducing CTLs that were reactive against an HLA-A2- and PSA-positive prostate cancer cell line (Correale et al, 1997). PSA peptide 154-163 was additionally verified as a target structure of PSA-reactive CD8⁺ T effector cells from HLA-A2-positive donors that were generated by stimulation with PSA RNA-transfected autologous DCs (Heiser et al, 2000). In another study, modification of this PSA peptide by replacing the valine residue in the first position by a tyrosine led to a strong agonist peptide which markedly increased the efficiency to induce prostate cancer-reactive CTLs (Terasawa et al, 2002). Correale et al, 1998 developed a strategy to simultaneously induce PSA-restricted CTL activities to multiple epitopes. The authors constructed a 30-mer oligopeptide corresponding to aa 141-171 of the PSA protein that contained two immunogenic HLA-A2-binding peptides described previously (aa 141-150 and 154-163) and an additional HLA-A3-fitting peptide (aa 162-170). CD8⁺ T cell lines from HLA-A2- and HLA-A3-positive donors that were generated by stimulation with autologous PBMCs loaded with the oligopeptide reacted against target cells pulsed with the nonamer or decamer peptides and expressing the respective HLA molecule.

Two HLA-A24-binding PSA peptides were reported to generate peptide-specific CTLs. Gotoh et al, 2002 revealed that the PSA peptide spanning the aa 152-160 is immunogenic in HLA-A*2402/K^b-transgenic mice. Immunization with this peptide resulted in the induction of peptide-specific and HLA-A*2402-restricted CTLs. The same peptide as well as another one (aa 248-257) were demonstrated to function as HLA-A24-restricted CD8⁺ T cell epitopes by *in vitro*-activation of specific CTLs from HLA-A24-positive prostate cancer patients after stimulation with peptide-loaded PBMCs (Harada et al, 2003). Corman et al, 1998 described an HLA-A1-binding PSA-derived peptide (aa 68-77) with the capacity to induce CTLs specifically recognizing peptide-pulsed target cells. However, the endogenous generation and presentation of these motifs by prostate cancer cells was not analyzed by the authors.

T helper cell epitopes were defined by Corman et al, 1998 who identified HLA-DR4-binding peptides within the PSA protein (aa 49-63 with modifications in two positions and 64-78). Recently, two immunogenic HLA-

DRB1*1501-restricted 20-mer peptides (corresponding to aa 171-190 and 221-240) were found by immunization of HLA-DRB1*1501-transgenic mice with human PSA and subsequent screening a library of overlapping 20-mer peptides spanning the entire PSA protein for peptide-specific *in vitro*-proliferation (Klyushnenkova et al, 2005). These peptides led to the *in vitro*-generation of specific CD4⁺ T cell lines from HLA-DRB1*1501-positive patients with granulomatous prostatitis or prostate cancer when presented by autologous antigen-presenting cells (APCs). In addition, the peptide-specific CD4⁺ T cells responded to APCs pulsed with the whole PSA protein.

B. Prostate-specific membrane antigen (PSMA)

PSMA, an integral membrane glycoprotein that functions as protease and folate hydrolase, was identified using the monoclonal antibody 7E11.C5 (Israeli et al, 1993; Carter et al, 1996). Immunohistochemical findings indicate that PSMA is a marker of normal epithelial cells of the prostate (Murphy et al, 1998). In addition, its expression is increased in most prostate tumors, particularly in undifferentiated, metastatic and hormone-resistant cancer (Kawakami and Nakayama, 1997).

A number of studies has demonstrated the suitability of PSMA for T cell-based immunotherapy by the identification of immunogenic peptide epitopes. Tjoa et al, 1996 described an HLA-A2-binding peptide spanning the aa 4-12 that induced peptide-specific CTLs when PBMCs of prostate cancer patients were stimulated with peptide-pulsed DCs. Furthermore, Murphy and co-workers revealed that the HLA-A2-binding peptide comprising aa 711-719 had the potential to decrease the levels of PSA in prostate cancer patients following administration of peptide-pulsed DCs (Murphy et al, 1996). An additional HLA-A*0201-restricted PSMA peptide (aa 27-35) proved to be effective in triggering antitumoral CTL responses as demonstrated by the capacity of peptide-induced CTLs to lyse an HLA-A*0201-positive prostate cancer cell line (Lu and Celis, 2002). Recently, the HLA-A2-restricted peptide comprising the aa 441-450 of PSMA protein has not only been shown to induce HLA-A2-restricted and prostate cancer-reactive CTLs but was described to serve as target of humoral immune responses in prostate cancer patients (Harada et al, 2004). By the same strategy, Kobayashi et al, 2003a identified an immunogenic HLA-A24-restricted PSMA peptide (aa 624-632). Furthermore, the *in vitro*-stimulation of CD8⁺ T cells from a healthy HLA-A24-positive donor using DCs loaded with predicted HLA-A24-matching peptides revealed two additional peptides (aa 178-186 and 227-235) which originate from intracellular processing of PSMA protein in tumor cells (Horiguchi et al, 2002).

Recent approaches to identify PSMA-derived CD4⁺ T cell epitopes demonstrated that the peptide sequences comprising the aa positions 334-348, 687-701 and 730-744 were restricted to HLA-DR4, HLA-DR9 or HLA-DR53 and HLA-DR53, respectively and induced antigen-specific T cells which were capable of reacting with naturally processed antigen (Kobayashi et al, 2003b).

C. Prostatic acid phosphatase (PAP)

PAP was described as an isoenzyme of the heterogenous group of acid phosphatases specifically secreted by prostate cells (Gutman et al, 1936). The cDNA isolated by screening cDNA libraries with polyclonal antisera encodes a 386 aa protein which includes a 32 aa signal sequence (Yeh et al, 1987; Vihko et al, 1988). PAP expression was shown to be restricted to the prostate by RNA dot blot analysis (Solin et al, 1990) and by immunohistochemical staining with monoclonal antibodies (Kuciel et al, 1988; Lam et al, 1989).

Peshwa et al, 1998 identified an HLA-A2-restricted CTL epitope (aa position 299-307) within the PAP protein by stimulation of T cells from healthy donors with peptide-pulsed autologous DCs. Recently, an additional immunogenic HLA-A2-binding peptide (aa 112-120) activating peptide-specific and tumor-lysing CTLs from prostate cancer patients *in vitro* was defined (Harada et al, 2004). Inoue et al, 2001 revealed a PAP-derived, HLA-A*2402-binding peptide (aa position 213-221) that induced tumor-reactive CTLs from prostate cancer patients and healthy donors. In addition, two peptides (aa positions 199-213 and 228-242) were described as potential CD4⁺ T cell epitopes, although the HLA class II restriction elements were not determined (McNeel et al, 2001).

D. Prostate stem cell antigen (PSCA)

PSCA was identified by a PCR-based subtractive hybridization strategy as a gene specifically expressed in the prostate (Reiter et al, 1998). The encoded protein belongs to the Thy-1/Ly-6 family of glycosylphosphatidylinositol-anchored cell surface glycoproteins and its aa sequence shares 30% identity with stem cell antigen 2. By mRNA *in situ*-hybridization and immunohistochemistry, PSCA expression was detected in more than 80% of primary prostate carcinomas and in all bone metastases analyzed (Reiter et al, 1998; Gu et al, 2000). Its increased expression level in both androgen-dependent and -independent prostate tumors when compared to the corresponding normal prostate tissues and its upregulation in carcinomas of high stages and Gleason Scores make PSCA a promising target structure for the immunotherapy of hormone-refractory tumors. In addition, PSCA may also provide a candidate for the immunotherapy of tumors with different histological origin, as PSCA expression has also been found in transitional cell carcinomas of the bladder (Amara et al, 2001) and pancreatic cancer (Argani et al, 2001).

Different studies have pointed out the suitability of PSCA as a target antigen of CTL-mediated immunotherapy. An HLA-A*0201-restricted PSCA peptide comprising the aa 14-22 was reported to be capable of generating a peptide-specific and tumor-reactive CTL response from a patient with metastatic prostate cancer by an *in vitro*-stimulation protocol employing irradiated peptide-loaded PBMCs as APCs (Dannull et al, 2000). By enzyme-linked immunospot (ELISPOT) analyses, we detected increased frequencies of CD8⁺ T cells in the blood of HLA-A*0201-positive

prostate cancer patients that recognize the PSCA-derived HLA-A*0201-restricted peptides with the aa positions 14-22 and 105-113 (Kiessling et al, 2002). Moreover, these peptides had the capacity to induce peptide-specific and tumor-reactive CTLs from prostate cancer patients when loaded on autologous DCs for repetitive stimulations of CD8⁺ T cell cultures. Matsueda and colleagues identified two additional HLA-A2-restricted peptides (aa positions 7-15 and 21-30) and an HLA-A24-presented peptide (aa position 76-78) that effectively stimulated CTLs from prostate cancer patients (Matsueda et al, 2004a and 2004b).

E. Prostein

Prostein was identified by a combination of cDNA subtraction and microarray screening as a novel protein with a unique specificity for malignant and normal prostate tissues (Xu et al, 2001). Prostein is a protein of 553 aa that is predicted to contain eleven transmembrane domains and a cleavable signal sequence at the amino terminus. Xu et al, 2001 demonstrated the highly prostate-restricted expression pattern in normal human tissues by quantitative reverse-transcription PCR, Northern blot and cDNA microarray analyses as well as by immunohistochemical analysis. Determining the prostein mRNA level in paired samples of malignant and non-malignant prostate tissue from prostate cancer patients by real-time PCR our group found abundant expression in all tested samples (Kiessling et al, 2004). In addition, the transcript levels were maintained or even elevated in 87% of the primary tumors when compared to prostein expression in the autologous non-malignant tissue samples. In a recent study, a prostein-specific monoclonal antibody was used to determine prostein expression at the protein level in a high number of tumorous and non-tumorous human tissues of diverse histological origin (Kalos et al, 2004). In this study, prostein was detected in 94% of all non-malignant and malignant prostate samples including metastases, but in none of 4635 non-prostatic normal and tumor tissues. The tissue-specific expression profile of this molecule and the abundant expression in the great majority of prostate tumors are promising prerequisites for the use of this protein as target structure for specific immunotherapeutic strategies in prostate cancer.

To identify immunogenic CD8⁺ T cell epitopes from prostein, we selected six nonamer and decamer peptides from the aa sequence of prostein that were predicted to bind to HLA-A*0201 by a computer-based algorithm and verified the binding affinity to HLA-A*0201 by a competition assay (Kiessling et al, 2004). Using these peptides, exogenously loaded on DCs, for repetitive *in vitro*-stimulations of autologous CD8⁺ T lymphocytes from prostate cancer patients and healthy donors, we were able to activate cytotoxic T effector cells specifically recognizing a peptide comprising the aa positions 31-39 in the prostein protein. The peptide-specific CTLs that were raised from all T cell cultures stimulated with this peptide also efficiently lysed prostate tumor cells expressing both HLA-A*0201 and prostein. Recently, another group identified prostein-derived peptides, one of them presented

by HLA-B*5101 (aa position 464-472) and two presented by HLA-Cw*0501 (aa positions 292-300 and 464-473), that are recognized by tumor-reactive CTLs (Friedman et al, 2004). The authors used APCs infected with a prostein-expressing adenovirus for the stimulation of CD8⁺ T lymphocytes from two healthy donors and identified immunogenic peptides by the use of target cells expressing truncated prostein constructs or pulsed with synthetic prostein-derived peptides.

F. Transient receptor potential (trp)-p8

The gene *trp-p8* was recently identified by screening a prostate-specific subtracted cDNA library (Tsavaler et al, 2001). It encodes a protein of 1104 aa with seven putative transmembrane domains that shows significant homology to a family of Ca²⁺ channel proteins. By dot blot and Northern blot analyses as well as reverse transcription PCR, it has been demonstrated that *trp-p8*-mRNA expression in non-malignant human tissues is mainly restricted to the prostate (Tsavaler et al, 2001; Cunha et al, 2005). In addition, *trp-p8* transcripts were detected in all 16 analyzed prostate cancer specimens by *in situ*-hybridization (Tsavaler et al, 2001). Quantitative RT-PCR analyses of matched samples of malignant and non-malignant prostate tissues derived from prostatectomized patients revealed an abundant expression of the *trp-p8* mRNA in all specimens and a marked level of overexpression in tumors of early stages and low grades when compared to the corresponding normal prostate tissue (Kiessling et al, 2003).

In an approach to determine the potential of *trp-p8* as a target structure of specific CTLs, we used DCs pulsed with five HLA-A*0201-binding, *trp-p8*-specific peptides for the stimulation of autologous CD8⁺ T cells from prostate cancer patients (Kiessling et al, 2003). A peptide comprising the aa 187-195 was found to effectively induce CTLs and was demonstrated to be autochthonously presented on the surface of prostate cancer cells.

G. Parathyroid hormone-related protein (PTH-rp)

PTH-rp is an autocrine or paracrine factor that binds to receptors on osteoblasts and induces bone formation and reabsorption. It is highly overexpressed in prostate cancer and other cancers of epithelial origin and is considered to be involved in the development of bone metastases (Guise, 1997; Francini et al, 2002). Therefore, it might represent a promising immunotherapeutic target for prostate cancer patients with bone metastases.

Two HLA-A*0201-restricted peptides (aa 59-68 and 165-173) have been identified by *in vitro*-stimulation protocols using autologous peptide-pulsed PBMCs from healthy donors as APCs (Francini et al, 2002). The induced peptide-specific CTLs were able to kill PTH-rp- and HLA-A*0201-positive tumor cells. Recently, two other HLA-A2-fitting epitopes (aa 59-67 and 42-51) were defined inducing peptide-specific CTL responses in prostate cancer patients (Yao et al, 2005).

Furthermore, HLA-A24-binding peptides comprising the aa positions 36-44 and 102-111 were proved to be immunogenic in the activation of peptide-specific and

tumor-reactive CTLs when loaded on PBMCs from prostate cancer patients (Yao et al, 2004).

H. Human telomerase reverse transcriptase (hTERT)

Whereas hTERT cannot be detected in most nontransformed somatic cells it is expressed in the majority of tumors of different histological origins including prostate cancer, (Kim et al, 1994) and is responsible for the protection of tumor cells from telomere erosion (Blasco and Hahn, 2003). Consequently, hTERT provides an attractive candidate for T cell-based immunotherapies of many tumors.

An immunogenic HLA-A*0201-restricted peptide comprising the aa 540-548 that is capable of inducing peptide-specific and tumor-reactive CTLs from healthy donors and prostate cancer patients was described by several groups (Vonderheide et al, 1999; Minev et al, 2000). Moreover, this peptide and an additional immunogenic HLA-A*0201-binding peptide (aa 865-873) were shown to induce peptide-specific CTLs in HLA-A*0201 transgenic mice (Minev et al, 2000). Hernandez et al, 2002 identified a third HLA-A*0201-matching peptide spanning aa 572-580 whose immunogenicity was markedly increased by substitution of the arginine residue at position one by tyrosine. Furthermore, an HLA-A3-fitting motif corresponding to aa 973-981 (Vonderheide et al, 2001), two HLA-A24-binding peptides (aa 324-332 and 461-469) (Arai et al, 2001) and an HLA-A1-restricted peptide (aa 325-333) (Schreurs et al, 2005) effectively inducing peptide-specific and tumor-lysing CTLs *in vitro* were described so far.

Schoers et al, 2002 identified an immunogenic HLA class II-restricted epitope (aa 672-686) by examining human T cell responses against synthetic peptides that had been selected by a prediction software. These authors demonstrated that the identified peptide is presented by HLA-DR7 molecules and derived from natural processing of hTERT in prostate cancer and other tumor cells. In a further study, the previously defined peptide comprising the aa 672-686 was demonstrated to be promiscuous and capable of inducing CD4⁺ T cell responses in the context of the HLA class II molecules HLA-DR1, HLA-DR7 and HLA-DR15 (Schroers et al, 2003). Moreover, these authors identified another CD4⁺ T cell epitope (aa 766-780) that is efficiently presented by HLA-DR4, HLA-DR11 and HLA-DR15 molecules, naturally generated by tumor cells and elicited antigen-specific CD4⁺ T cell responses when used for immunization of HLA-DR4 transgenic mice.

I. Survivin

Survivin is a member of the inhibitor of apoptosis protein family and is highly overexpressed in most human tumors of epithelial and hematopoietic origin including prostate cancer (Ambrosini et al, 1997; Altieri, 2003). Additionally, survivin expression correlates with poor prognosis of tumor disease (Swana et al, 1999). The wide expression in cancer and the almost complete absence of expression in differentiated adult tissues together with the functional role for the survival of tumor cells make

survivin an interesting target for the development of T cell-based immunotherapies.

Our group identified two HLA-A*0201-restricted peptides (aa 5-14 and 95-104) that induced peptide-specific CTL responses *in vitro* when presented by autologous DCs and one of these peptides (aa 95-104) was shown to evolve from intracellular processing of the protein as the CTLs effectively recognized Epstein-Barr virus-immortalized B cells transfected with survivin cDNA (Schmitz et al, 2000). By another group, the peptide spanning aa 5-14 was verified as target for immunotherapy by the lysis of survivin- and HLA-A*0201-positive tumor cells by peptide-specific CTLs (Siegel et al, 2004). Using ELISPOT assay to detect survivin-specific CD8⁺ T cells in the blood of tumor patients Anderson et al, 2001a found *in vivo* reactivities against one of the previously defined peptides (aa 95-104) and a modified peptide (aa 96-104) in which the native threonine at position 2 was replaced by the better anchor residue methionine. In an additional study, multimeric complexes of this modified peptide and HLA-A2 molecules were used to isolate CD8⁺ T lymphocytes from a melanoma-infiltrated lymph node that specifically recognized the native peptide as well as survivin- and HLA-A2-expressing tumor cells (Anderson et al, 2001b). A number of additional CD8⁺ T cell epitopes restricted to HLA-A1, HLA-A2, HLA-A3 and HLA-A11 were defined by Reker et al, 2004 based on spontaneous peptide-specific CTL responses of tumor-infiltrating lymphocytes determined by ELISPOT assay. The positions and sequences of these peptides can be learned from **Table 1**.

III. Vaccination of prostate cancer patients with TAA-derived peptides, proteins or DNA

Following the identification of prostate cancer-associated proteins that may be suitable targets of tumor-reactive T cells several clinical trials were conducted. Noguchi et al, 2005 performed a clinical phase I/II study to determine the feasibility, toxicity, immunological and clinical responses to individualized peptide vaccination in combination with estramustine phosphate for HRPC patients. The selection of the administered peptides derived from several prostate cancer-related and epithelial cancer-related antigens was based on the measurement of peptide-specific CD8⁺ T cells in the blood of patients before vaccination. Patients were immunized subcutaneously with only those peptides to which pre-existing CD8⁺ T cells could be detected. Vaccination was well tolerated and augmentation of peptide-specific CD8⁺ T cells was observed. All 13 patients treated with the combination therapy showed a decrease of serum PSA levels, including six patients with a decrease of more than 50%.

Meidenbauer et al, 2000 reported on a clinical trial enrolling 10 prostate cancer patients which was based on JBT1001, a vaccine consisting of recombinant PSA with lipid A formulated in liposomes. Patients were vaccinated with JBT1001 either in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) or

emulsified in mineral oil. Whereas two patients had PSA-reactive T cells before vaccination eight of 10 patients showed detectable PSA-reactive T cells after vaccination. However, the frequency of PSA-reactive T cells in the circulation of patients was low. In a follow up report, 10 patients treated with JBT1001 plus GM-CSF and eight additional patients receiving JBT1001 emulsified in mineral oil were tested for γ -chain expression in circulating T cells and spontaneous IL-10 secretion by PBMCs before and after vaccination (Meidenbauer et al, 2002). Prior to therapy, patients had lower γ -chain expression in circulating CD3⁺ T cells, a higher percentage of γ -chain negative CD3⁺ and CD4⁺ T cells and PBMCs producing more IL-10 than normal subjects. After vaccination, recovery of γ -chain expression was observed in 50% of all patients and IL-10 secretion decreased in patients treated with JBT1001 and GM-CSF.

Other clinical studies were conducted to evaluate the potential of a recombinant vaccinia virus expressing human PSA. Sanda and colleagues initiated a phase I clinical trial to determine the safety and biologic effects of recombinant vaccinia-PSA (rV-PSA) administered to six patients with recurrence of prostate cancer after radical prostatectomy (Sanda et al, 1999). Patients were treated with luteinizing hormone-releasing hormone agonist therapy until an undetectable PSA nadir was achieved and then vaccinated with rV-PSA. Treatment was well tolerated and one of six patients showed undetectable serum PSA for more than eight months after testosterone restoration. In another clinical trial, administration of rV-PSA led to stabilization of serum PSA levels in 14 of 33 prostate cancer patients for at least six months (Eder et al, 2000). Increases of at least twofold in the number of PSA-reactive T cells could be detected in five of seven evaluated patients. More recently, Gulley and colleagues administered rV-PSA to patients with metastatic androgen-independent prostate cancer (Gulley et al, 2002). Six of 42 patients had stable disease and three of five analyzed patients showed a vaccine-induced increase of PSA-specific T lymphocytes. Furthermore, *in vitro*-generated PSA-specific CTL lines of three patients were able to lyse PSA peptide-loaded APCs and prostate cancer cells.

Kaufman et al, 2004 conducted a clinical phase II study evaluating a heterologous prime/boost vaccination protocol with vaccinia and fowlpox viruses expressing PSA in prostate cancer patients with biochemical progression after local therapy. Of the eligible patients, 45.3% remained free of PSA progression at 19.1 months and 78.1% demonstrated clinical progression-free survival. An increase in PSA-specific T cells was found in 46% of patients. Gulley et al, 2005 reported on another phase II clinical 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. Thirteen of 17 evaluated patients with localized prostate cancer treated by the combination therapy showed an increase in PSA-specific T cells of at least threefold.

Other immunotherapeutic treatment modalities which were based on so-called “naked” DNA have also been explored. In a phase I/II clinical trial 26 prostate cancer patients with different stages of disease were immunized intradermally with varying combinations of separate 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 (Mincheff et al, 2000). Treatment was well tolerated. Delayed-type hypersensitivity reactions against the PSMA plasmid were found in several patients including all patients that were initially vaccinated with the adenoviral vector expressing PSMA. Six out of 12 patients who received immunotherapy only were regarded as responders. More recently, a phase I study investigating the administration of a DNA plasmid encoding PSA in combination with GM-CSF and IL-2 to HRPC patients was conducted (Pavlenko et al, 2004). Two of three patients who received the highest dose developed a significant PSA-specific cellular immune response and a decrease in the slope of serum PSA.

IV. Vaccination of prostate cancer patients with dendritic cells pulsed with prostate cancer-associated antigens

DCs are professional APCs which display an extraordinary capacity to induce, sustain and regulate T cell responses (Banchereau and Steinman, 1998; Banchereau et al, 2000; Steinman, 2003). DCs circulate through the blood and become resident in peripheral tissues, where they continuously monitor invading pathogens. These immature DCs are particularly efficient in antigen capture but are rather ineffective in antigen-processing and in stimulating antigen-specific T cells. DC maturation is induced by pathogens or proinflammatory cytokines. Its hallmark is the acquisition of the capacity to efficiently process and present antigens. During maturation DCs migrate from the peripheral tissues to the T cell-rich areas of secondary lymphoid organs, where they initiate antigen-specific T cell responses. Owing to their unique ability to activate naive T cells DCs evolved as promising candidates for vaccination protocols in cancer therapy (Fong and Engleman, 2000; Banchereau and Palucka, 2005; Nestle et al, 2005). The ability of TAA-loaded DCs to induce both protective and therapeutic antitumor responses has been documented in animal models (Mayordomo et al, 1995; Celluzzi et al, 1996; Nair et al, 2000). Also in human, clinical trials revealed promising immunologic and clinical effects of antigen-loaded DCs administered as a vaccine against cancer (Hsu et al, 1996; Nestle et al, 1998; Thurner et al, 1999).

In the setting of prostate cancer, clinical trials have shown that DCs pulsed with TAA-derived peptide, protein or mRNA were well tolerated, efficiently augmented antigen-specific T cell responses and exhibited partial or complete clinical effects. Thus, Murphy and colleagues conducted a phase I trial to determine the safe administration of DCs and HLA-A*0201-restricted

PSMA-derived peptides to HRPC patients (Murphy et al, 1996; Tjoa et al, 1997). Treatment was well tolerated by all 51 patients and favourable antigen-specific cellular immune responses were observed in seven partial responders based on National Prostate Cancer Project criteria and a 50% reduction of PSA level. Following the phase I study, the same group initiated a phase II clinical trial to investigate the therapeutic efficiency of infused DCs loaded with two HLA-A*0201-restricted PSMA-derived peptides. Nine partial responders were identified in a group of 33 HRPC patients which were already participants in the previous phase I study and were subsequently enrolled in the phase II trial (Tjoa et al, 1998). In addition, two complete and six partial responders were observed in a group of 25 evaluated patients with no previous immunotherapy experience (Murphy et al, 1999a). Furthermore, one complete and 10 partial responders were identified from 37 patients with presumed local recurrence of prostate cancer after primary treatment failure (Murphy et al, 1999b).

An additional clinical phase I trial which was based on the administration of peptide-loaded DCs to patients with metastatic HRPC was performed (Vonderheide et al, 2004). Five patients were vaccinated with DCs pulsed with an HLA-A*0201-restricted hTERT-derived peptide and keyhole limpet hemocyanin. No significant side effects were observed. T cells reactive against the hTERT-derived peptide were induced in two patients after vaccination. All four evaluable patients had stabilization of disease. More recently, we conducted a phase I clinical trial to evaluate the potential of DCs loaded with a cocktail consisting of HLA-A*0201-restricted peptides derived from PSA, PSMA, survivin, prostein and trp-p8 (unpublished data). No severe side effects were noted. Four out of eight patients had a temporary decrease or stabilization of serum PSA level. In addition, three out of these four PSA responders exhibited antigen-specific T cell responses against prostein, survivin or PSMA.

Small et al, 2000 reported on a clinical phase I/II trial including 31 patients with HRPC. Patients were treated with enriched DC precursors preexposed *in vitro* to PA2024, a fusion protein consisting of human GM-CSF and PAP. Treatment was well tolerated. All patients developed immune responses to the fusion protein and 38% displayed immune responses to PAP. Six patients showed a decline in PSA level. Burch and colleagues also administered PA2024-loaded DCs to HRPC patients. These infusions were followed by subcutaneous applications of PAP2024 without cells. Treatment was safe, induced antigen-specific cellular immunity and resulted in PSA level reduction in three out of 12 evaluated patients (Burch et al, 2000). A subsequent phase II study demonstrated a decline in PSA level in three out of 19 evaluated patients (Burch et al, 2004).

Another clinical trial including patients with metastatic prostate cancer was based on the administration of DCs loaded with recombinant murine PAP. Minimal treatment-associated side effects were observed. 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 prostate cancer as determined by PSA level monitoring, computerized tomography and bone scans (Fong et al, 2001).

Barrou et al, 2004 performed a clinical trial enrolling prostate cancer patients in biochemical relapse after radical prostatectomy to assess the feasibility, safety and immunogenicity of vaccination with DCs pulsed with human recombinant PSA. Twenty-four patients received nine administrations of PSA-loaded DCs by combined intravenous, subcutaneous and intradermal routes. No severe side effects were observed, PSA-specific T cells were detected and 11 patients exhibited a transient PSA decrease.

Two other clinical phase I studies were conducted to evaluate the potential of DCs transfected with mRNA encoding TAAs. In the first trial, 13 patients with metastatic prostate cancer received PSA mRNA-transfected DCs (Heiser et al, 2002). Vaccination was well tolerated and PSA-specific T cells were detected in all patients. Six of seven evaluated patients had a significant decrease of PSA and three patients exhibited a transient molecular clearance of circulating tumor cells. In the second trial, hTERT mRNA-transfected DCs were administered to 20 patients with metastatic prostate cancer (Su et al, 2005). Expansion of hTERT-specific T cells was detected in 19 of 20 patients. Vaccination was associated with a reduction of PSA velocity and molecular clearance of circulating tumor cells.

V. Conclusion

Current therapeutic approaches revealed only modest impact on survival outcomes for patients with metastatic prostate cancer. Recent advances in the identification of TAA-derived T cell epitopes and in the successful activation of tumor-reactive CTLs and CD4⁺ T cells paved the way for new treatment modalities for prostate cancer. Clinical trials which were based on the *in vivo*-stimulation of effector T cells by the administration of peptides, proteins, DNA or TAA-pulsed DCs provide evidence that these concepts were safe and feasible. In addition, they led to the induction of antigen-specific T cells as well as clinical responses in prostate cancer patients. However, further improvement of prostate cancer therapy is required and may be achieved by combining T cell-based vaccination strategies with radio-, hormone-, chemo-, antibody- or anti-angiogenic therapy.

References

- Altieri DC (2003) Validating survivin as a cancer therapeutic target. **Nat Rev Cancer** 3, 46-54.
- Amara N, Palapattu GS, Schrage M, Gu Z, Thomas GV, Dorey F, Said J and Reiter RE (2001) Prostate stem cell antigen is overexpressed in human transitional cell carcinoma. **Cancer Res** 61, 4660-4665.
- Ambrosini G, Adida C and Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. **Nat Med** 3, 917-921.
- Andersen MH, Pedersen LO, Becker JC and thor Straten P (2001a) Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. **Cancer Res** 61, 869-872.
- Andersen MH, Pedersen LO, Capeller B, Brocker EB, Becker JC and thor Straten P (2001b) Spontaneous cytotoxic T cell responses against survivin-derived MHC class I-restricted T cell epitopes *in situ* as well as *ex vivo* in cancer patients. **Cancer Res** 61, 5964-5968.
- Arai J, Yasukawa M, Ohminami H, Kakimoto M, Hasegawa A and Fujita S (2001) Identification of human telomerase reverse transcriptase-derived peptides that induce HLA-A24-restricted antileukemia cytotoxic T lymphocytes. **Blood** 97, 2903-2907.
- Argani P, Rosty C, Reiter RE, Wilentz RE, Murugesan SR, Leach SD, Ryu B, Skinner HG, Goggins M, Jaffee EM, Yeo CJ, Cameron JL, Kern SE and Hruban RH (2001) Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. **Cancer Res** 61, 4320-4324.
- Balk SP, Ko YJ and Bubley GJ (2003) Biology of prostate-specific antigen. **J Clin Oncol** 21, 383-391.
- Banchereau J and Steinman RM (1998) Dendritic cells and the control of immunity. **Nature** 392, 245-252.
- Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B and Palucka K (2000) Immunobiology of dendritic cells. **Annu Rev Immunol** 18, 767-811.
- Banchereau J and Palucka AK (2005) Dendritic cells as therapeutic vaccines against cancer. **Nat Rev Immunol** 5, 296-306.
- Barrou B, Benoit G, Ouldacaci M, Cussenot O, Salcedo M, Agrawal S, Massicard S, Bercovici N, Ericson ML and Thiounn N (2004) Vaccination of prostatectomized prostate cancer patients in biochemical relapse, with autologous dendritic cells pulsed with recombinant human PSA. **Cancer Immunol Immunother** 53, 453-460.
- Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF and Heath WR (1998) Help for cytotoxic T cell responses is mediated by CD40 signalling. **Nature** 393, 478-480.
- Blasco MA and Hahn WC (2003) Evolving views of telomerase and cancer. **Trends Cell Biol** 13, 289-294.
- 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-Pavlovic S (2000) Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. **Clin Cancer Res** 6, 2175-2182.
- Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, Richardson RL, Valone FH and Vuk-Pavlovic S (2004) Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a phase 2 trial. **Prostate** 60, 197-204.
- Carter RE, Feldman AR and Coyle JT (1996) Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. **Proc Natl Acad Sci USA** 93, 749-753.
- Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT and Falo LD, Jr. (1996) Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. **J Exp Med** 183, 283-287.
- Coen JJ, Zietman AL, Thakral H and Shipley WU (2002) Radical radiation for localized prostate cancer: local persistence of disease results in a late wave of metastases. **J Clin Oncol** 20, 3199-3205.
- Corman JM, Sercarz EE and Nanda NK (1998) Recognition of prostate-specific antigenic peptide determinants by human CD4 and CD8 T cells. **Clin Exp Immunol** 114, 166-172.
- Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Schlom J and Tsang KY (1997) *In vitro* generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. **J Natl Cancer Inst** 89, 293-300.

- Correale P, Walmsley K, Zaremba S, Zhu M, Schlom J and Tsang KY (1998) Generation of human cytolytic T lymphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligopeptide peptide. **J Immunol** 161, 3186-3194.
- Cunha AC, Weigle B, Kiessling A, Bachmann M and Rieber EP (2005) Tissue-specificity of prostate specific antigens: comparative analysis of transcript levels in prostate and non-prostatic tissues. **Cancer Lett**, in press.
- Dannull J, Diener PA, Prikler L, Furstenberger G, Cerny T, Schmid U, Ackermann DK and Groettrup M (2000) Prostate stem cell antigen is a promising candidate for immunotherapy of advanced prostate cancer. **Cancer Res** 60, 5522-5528.
- Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE and Rosenberg SA (2002) Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. **Science** 298, 850-854.
- Dudley ME and Rosenberg SA (2003) Adoptive cell transfer therapy for the treatment of patients with cancer. **Nat Rev Cancer** 3, 666-675.
- Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A and Rosenberg SA (2005) Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. **J Clin Oncol** 23, 2346-2357.
- Eder JP, Kantoff PW, Roper K, Xu GX, Bublej GJ, Boyden J, Gritz L, Mazzara G, Oh WK, Arlen P, Tsang KY, Panicali D, Schlom J and Kufe DW (2000) A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. **Clin Cancer Res** 6, 1632-1638.
- Feldman BJ and Feldman D (2001) The development of androgen-independent prostate cancer. **Nat Rev Cancer** 1, 34-45.
- Fong L and Engleman EG (2000) Dendritic cells in cancer immunotherapy. **Annu Rev Immunol** 18, 245-273.
- Fong L, Brockstedt D, Benike C, Breen JK, Strang G, Ruegg CL and Engleman EG (2001) Dendritic cell-based xenotigen vaccination for prostate cancer immunotherapy. **J Immunol** 167, 7150-7156.
- Francini G, Scardino A, Kosmatopoulos K, Lemonnier FA, Campoccia G, Sabatino M, Pozzessere D, Petrioli R, Lozzi L, Neri P, Fanetti G, Cusi MG and Correale P (2002) High-affinity HLA-A(*):02.01 peptides from parathyroid hormone-related protein generate *in vitro* and *in vivo* antitumor CTL response without autoimmune side effects. **J Immunol** 169, 4840-4849.
- Friedman RS, Spies AG and Kalos M (2004) Identification of naturally processed CD8 T cell epitopes from prostein, a prostate tissue-specific vaccine candidate. **Eur J Immunol** 34, 1091-1101.
- Gotoh M, Takasu H, Harada K and Yamaoka T (2002) Development of HLA-A2402/K(b) transgenic mice. **Int J Cancer** 100, 565-570.
- Gu Z, Thomas G, Yamashiro J, Shintaku IP, Dorey F, Raitano A, Witte ON, Said JW, Loda M and Reiter RE (2000) Prostate stem cell antigen (PSCA) expression increases with high Gleason score, advanced stage and bone metastasis in prostate cancer. **Oncogene** 19, 1288-1296.
- Guise TA (1997) Parathyroid hormone-related protein and bone metastases. **Cancer** 80, 1572-1580.
- Gulley J, Chen AP, Dahut W, Arlen PM, Bastian A, Steinberg SM, Tsang K, Panicali D, Poole D, Schlom J and Michael HJ (2002) Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. **Prostate** 53, 109-117.
- Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, Tsang KY, Yokokawa J, Hodge JW, Menard C, Camphausen K, Coleman CN, Sullivan F, Steinberg SM, Schlom J and Dahut W (2005) Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. **Clin Cancer Res** 11, 3353-3362.
- Gutman E, Sproul E and Gutman A (1936) Significance of increased phosphatase activity of bone at the site of osteoblastic metastases secondary to carcinoma of the prostate gland. **Am J Cancer** 28, 485.
- Han M, Partin AW, Zahurak M, Piantadosi S, Epstein JI and Walsh PC (2003) Biochemical (prostate specific antigen) recurrence probability following radical prostatectomy for clinically localized prostate cancer. **J Urol** 169, 517-523.
- Harada M, Kobayashi K, Matsueda S, Nakagawa M, Noguchi M and Itoh K (2003) Prostate-specific antigen-derived epitopes capable of inducing cellular and humoral responses in HLA-A24⁺ prostate cancer patients. **Prostate** 57, 152-159.
- Harada M, Matsueda S, Yao A, Ogata R, Noguchi M and Itoh K (2004) Prostate-related antigen-derived new peptides having the capacity of inducing prostate cancer-reactive CTLs in HLA-A24⁺ prostate cancer patients. **Oncol Rep** 12, 601-607.
- Heiser A, Dahm P, Yancey DR, Maurice MA, Boczkowski D, Nair SK, Gilboa E and Vieweg J (2000) Human dendritic cells transfected with RNA encoding prostate-specific antigen stimulate prostate-specific CTL responses *in vitro*. **J Immunol** 164, 5508-5514.
- Heiser A, Coleman D, Dannull J, Yancey D, Maurice MA, Lallas CD, Dahm P, Niedzwiecki D, Gilboa E and Vieweg J (2002) Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. **J Clin Invest** 109, 409-417.
- Hernandez J, Garcia-Pons F, Lone YC, Firat H, Schmidt JD, Langlade-Demoyen P and Zanetti M (2002) Identification of a human telomerase reverse transcriptase peptide of low affinity for HLA A2.1 that induces cytotoxic T lymphocytes and mediates lysis of tumor cells. **Proc Natl Acad Sci USA** 99, 12275-12280.
- Horiguchi Y, Nukaya I, Okazawa K, Kawashima I, Fikes J, Sette A, Tachibana M, Takesako K and Murai M (2002) Screening of HLA-A24-restricted epitope peptides from prostate-specific membrane antigen that induce specific antitumor cytotoxic T lymphocytes. **Clin Cancer Res** 8, 3885-3892.
- Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, Engleman EG and Levy R (1996) Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. **Nat Med** 2, 52-58.
- Inoue Y, Takaue Y, Takei M, Kato K, Kanai S, Harada Y, Tobisu K, Noguchi M, Kakizoe T, Itoh K and Wakasugi H (2001) Induction of tumor specific cytotoxic T lymphocytes in prostate cancer using prostatic acid phosphatase derived HLA-A2402 binding peptide. **J Urol** 166, 1508-1513.
- Israeli RS, Powell CT, Fair WR and Heston WD (1993) Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. **Cancer Res** 53, 227-230.
- Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghaffour A, Feuer EJ and Thun MJ (2005) Cancer statistics, 2005. **CA Cancer J Clin** 55, 10-30.
- Kalos M, Askaa J, Hylander BL, Repasky EA, Cai F, Vedvick T, Reed SG, Wright GL, Jr. and Fanger GR (2004) Prostein

- expression is highly restricted to normal and malignant prostate tissues. **Prostate** 60, 246-256.
- Kaufman HL, Wang W, Manola J, DiPaola RS, Ko YJ, Sweeney C, Whiteside TL, Schlom J, Wilding G and Weiner LM (2004) 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.
- Kawakami M and Nakayama J (1997) Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by *in situ* hybridization. **Cancer Res** 57, 2321-2324.
- Kiessling A, Schmitz M, Stevanovic S, Weigle B, Holig K, Fussel M, Fussel S, Meye A, Wirth MP and Rieber EP (2002) Prostate stem cell antigen: Identification of immunogenic peptides and assessment of reactive CD8⁺ T cells in prostate cancer patients. **Int J Cancer** 102, 390-397.
- Kiessling A, Fussel S, Schmitz M, Stevanovic S, Meye A, Weigle B, Klenk U, Wirth MP and Rieber EP (2003) Identification of an HLA-A*0201-restricted T cell epitope derived from the prostate cancer-associated protein trp-p8. **Prostate** 56, 270-279.
- Kiessling A, Stevanovic S, Fussel S, Weigle B, Rieger MA, Temme A, Rieber EP and Schmitz M (2004) Identification of an HLA-A*0201-restricted T cell epitope derived from the prostate cancer-associated protein prostein. **Br J Cancer** 90, 1034-1040.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL and Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. **Science** 266, 2011-2015.
- Klyushnchenkova EN, Link J, Oberle WT, Kodak J, Rich C, Vandenbark AA and Alexander RB (2005) Identification of HLA-DRB1*1501-restricted T cell epitopes from prostate-specific antigen. **Clin Cancer Res** 11, 2853-2861.
- Kobayashi K, Noguchi M, Itoh K and Harada M (2003a) Identification of a prostate-specific membrane antigen-derived peptide capable of eliciting both cellular and humoral immune responses in HLA-A24⁺ prostate cancer patients. **Cancer Sci** 94, 622-627.
- Kobayashi H, Omiya R, Sodey B, Yanai M, Oikawa K, Sato K, Kimura S, Senju S, Nishimura Y, Tateno M and Celis E (2003b) Identification of naturally processed helper T cell epitopes from prostate-specific membrane antigen using peptide-based *in vitro* stimulation. **Clin Cancer Res** 9, 5386-5393.
- Kuciel R, Mazurkiewicz A, Ostrowski WS, Stachura J, Studen I, Szkudlarek J and Radzikowski C (1988) Characterization of anti-prostatic acid phosphatase monoclonal antibody and its medical significance. **Biotechnol Appl Biochem** 10, 257-272.
- Lam KW, Li CY, Yam LT, Sun T, Lee G and Ziesmer S (1989) Improved immunohistochemical detection of prostatic acid phosphatase by a monoclonal antibody. **Prostate** 15, 13-21.
- Lu J and Celis E (2002) Recognition of prostate tumor cells by cytotoxic T lymphocytes specific for prostate-specific membrane antigen. **Cancer Res** 62, 5807-5812.
- Lundwell A and Liliya H (1989) Molecular cloning of human prostate specific antigen. **FEBS Lett** 214, 317-320.
- Matsueda S, Kobayashi K, Nonaka Y, Noguchi M, Itoh K and Harada M (2004a) Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2⁺ patients with hormone-refractory prostate cancer. **Cancer Immunol Immunother** 53, 479-489.
- Matsueda S, Yao A, Ishihara Y, Ogata R, Noguchi M, Itoh K and Harada M (2004b) A prostate stem cell antigen-derived peptide immunogenic in HLA-A24⁺ prostate cancer patients. **Prostate** 60, 205-213.
- Mayordomo JI, Zorina T, Storkus WJ, Zitvogel L, Celluzzi C, Falo LD, Melief CJ, Ildstad ST, Kast WM, Deleo AB, Lotze MT (1995) Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. **Nat Med** 1, 1297-1302.
- McNeel DG, Nguyen LD and Disis ML (2001) Identification of T helper epitopes from prostatic acid phosphatase. **Cancer Res** 61, 5161-5167.
- Meidenbauer N, Harris DT, Spitler LE and Whiteside TL (2000) Generation of PSA-reactive effector cells after vaccination with a PSA-based vaccine in patients with prostate cancer. **Prostate** 43, 88-100.
- Meidenbauer N, Gooding W, Spitler L, Harris D and Whiteside TL (2002) Recovery of zeta-chain expression and changes in spontaneous IL-10 production after PSA-based vaccines in patients with prostate cancer. **Br J Cancer** 86, 168-178.
- Mincheff M, Tchakarov S, Zoubak S, Loukinov D, Botev C, Altankova I, Georgiev G, Petrov S and Meryman HT (2000) Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a phase I/II clinical trial. **Eur Urol** 38, 208-217.
- Minev B, Hipp J, Firat H, Schmidt JD, Langlade-Demoyen P and Zanetti M (2000) Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. **Proc Natl Acad Sci USA** 97, 4796-4801.
- Miyamoto H, Messing EM and Chang C (2004) Androgen deprivation therapy for prostate cancer: current status and future prospects. **Prostate** 61, 332-353.
- Mumberg D, Monach PA, Wanderling S, Philip M, Toledano AY, Schreiber RD and Schreiber H (1999) CD4⁺ T cells eliminate MHC class II-negative cancer cells *in vivo* by indirect effects of IFN-gamma. **Proc Natl Acad Sci USA** 96, 8633-8638.
- Murphy G, Tjoa B, Ragde H, Kenny G and Boynton A (1996) Phase I clinical trial: T cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201-specific peptides from prostate-specific membrane antigen. **Prostate** 29, 371-380.
- Murphy GP, Elgamal AA, Su SL, Bostwick DG and Holmes EH (1998) Current evaluation of the tissue localization and diagnostic utility of prostate specific membrane antigen. **Cancer** 83, 2259-2269.
- Murphy GP, Tjoa BA, Simmons SJ, Jarisch J, Bowes VA, Ragde H, Rogers M, Elgamal A, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Salgaller ML and Boynton AL (1999a) Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: a phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. **Prostate** 38, 73-78.
- Murphy GP, Tjoa BA, Simmons SJ, Ragde H, Rogers M, Elgamal A, Kenny GM, Troychak MJ, Salgaller ML and Boynton AL (1999b) Phase II prostate cancer vaccine trial: report of a study involving 37 patients with disease recurrence following primary treatment. **Prostate** 39, 54-59.
- Nair SK, Heiser A, Boczkowski D, Majumdar A, Naoe M, Lebkowski JS, Vieweg J and Gilboa E (2000) Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. **Nat Med** 6, 1011-1017.
- Nestle FO, Aljagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G and Schadendorf D (1998) Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. **Nat Med** 4, 328-332.
- Nestle FO, Farkas A and Conrad C (2005) Dendritic cell-based therapeutic vaccination against cancer. **Curr Opin Immunol** 17, 163-169.
- Noguchi M, Itoh K, Yao A, Mine T, Yamada A, Obata Y, Furuta M, Harada M, Suekane S and Matsuoka K (2005)

- Immunological evaluation of individualized peptide vaccination with a low dose of estramustine for HLA-A24⁺ HRPC patients. **Prostate** 63, 1-12.
- Oesterling JE (1991) Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. **J Urol** 145, 907-923.
- Pardoll DM and Topalian SL (1998) The role of CD4⁺ T cell responses in antitumor immunity. **Curr Opin Immunol** 10, 588-594.
- Pavlenko M, Roos AK, Lundqvist A, Palmborg A, Miller AM, Ozenci V, Bergman B, Egevad L, Hellstrom M, Kiessling R, Masucci G, Wersall P, Nilsson S and Pisa P (2004) A phase I trial of DNA vaccination with a plasmid expressing prostate-specific antigen in patients with hormone-refractory prostate cancer. **Br J Cancer** 91, 688-694.
- Perambakam S, Xue BH, Sosman JA and Peace DJ (2002) Induction of Tc2 cells with specificity for prostate-specific antigen from patients with hormone-refractory prostate cancer. **Cancer Immunol Immunother** 51, 263-270.
- Peshwa MV, Shi JD, Ruegg C, Laus R and van Schooten WC (1998) Induction of prostate tumor-specific CD8⁺ cytotoxic T lymphocytes *in vitro* using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. **Prostate** 36, 129-138.
- Petrylak DP, Tangen CM, Hussain MH, Lara PN, Jr., Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D and Crawford ED (2004) Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. **N Engl J Med** 351, 1513-1520.
- Qin Z and Blankenstein T (2000) CD4⁺ T cell-mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFN gamma receptor expression by nonhematopoietic cells. **Immunity** 12, 677-686.
- Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, Wahl M, Nisitani S, Yamashiro J, Le Beau MM, Loda M and Witte ON (1998) Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. **Proc Natl Acad Sci USA** 95, 1735-1740.
- Reker S, Meier A, Holten-Andersen L, Svane IM, Becker JC, thor Straten P and Andersen MH (2004) Identification of novel survivin-derived CTL epitopes. **Cancer Biol Ther** 3, 173-179.
- Ridge JP, Di Rosa F and Matzinger P (1998) A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell. **Nature** 393, 474-478.
- Roehl KA, Han M, Ramos CG, Antenor JA and Catalona WJ (2004) Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. **J Urol** 172, 910-914.
- Rosenberg SA (1997) Cancer vaccines based on the identification of genes encoding cancer regression antigens. **Immunol Today** 18, 175-182.
- Sanda MG, Smith DC, Charles LG, Hwang C, Pienta KJ, Schlom J, Milenic D, Panicali D and Montie JE (1999) Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. **Urology** 53, 260-266.
- Schmitz M, Diestelkoetter P, Weigle B, Schmachtenberg F, Stevanovic S, Ockert D, Rammensee HG and Rieber EP (2000) Generation of survivin-specific CD8⁺ T effector cells by dendritic cells pulsed with protein or selected peptides. **Cancer Res** 60, 4845-4849.
- Schoenberger SP, Toes RE, van der Voort EI, Offringa R and Melief CJ (1998) T cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. **Nature** 393, 480-483.
- Schreurs MW, Kueter EW, Scholten KB, Kramer D, Meijer CJ and Hooijberg E (2005) Identification of a potential human telomerase reverse transcriptase-derived, HLA-A1-restricted cytotoxic T lymphocyte epitope. **Cancer Immunol Immunother** 54, 703-712.
- Schroers R, Huang XF, Hammer J, Zhang J and Chen SY (2002) Identification of HLA DR7-restricted epitopes from human telomerase reverse transcriptase recognized by CD4⁺ T-helper cells. **Cancer Res** 62, 2600-2605.
- Schroers R, Shen L, Rollins L, Rooney CM, Slawin K, Sonderstrup G, Huang XF and Chen SY (2003) Human telomerase reverse transcriptase-specific T-helper responses induced by promiscuous major histocompatibility complex class II-restricted epitopes. **Clin Cancer Res** 9, 4743-4755.
- Sharifi N, Gulley JL and Dahut WL (2005) Androgen deprivation therapy for prostate cancer. **JAMA** 294, 238-244.
- Siegel S, Steinmann J, Schmitz N, Stuhlmann R, Dreger P and Zeis M (2004) Identification of a survivin-derived peptide that induces HLA-A*0201-restricted antileukemia cytotoxic T lymphocytes. **Leukemia** 18, 2046-2047.
- Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV and Valone FH (2000) Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. **J Clin Oncol** 18, 3894-3903.
- Solin T, Kontturi M, Pohlmann R and Vihko P (1990) Gene expression and prostate specificity of human prostatic acid phosphatase (PAP): evaluation by RNA blot analyses. **Biochim Biophys Acta** 1048, 72-77.
- Steinman RM (2003) Some interfaces of dendritic cell biology. **APMIS** 111, 675-697.
- Stevanovic S (2002) Identification of tumour-associated T cell epitopes for vaccine development. **Nat Rev Cancer** 2, 514-520.
- Su Z, Dannull J, Yang BK, Dahm P, Coleman D, Yancey D, Sichi S, Niedzwiecki D, Boczkowski D, Gilboa E and Vieweg J (2005) Telomerase mRNA-transfected dendritic cells stimulate antigen-specific CD8⁺ and CD4⁺ T cell responses in patients with metastatic prostate cancer. **J Immunol** 174, 3798-3807.
- Swana HS, Grossman D, Anthony JN, Weiss RM and Altieri DC (1999) Tumor content of the antiapoptosis molecule survivin and recurrence of bladder cancer. **N Engl J Med** 341, 452-453.
- Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA and Eisenberger MA (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. **N Engl J Med** 351, 1502-1512.
- Terasawa H, Tsang KY, Gulley J, Arlen P and Schlom J (2002) Identification and characterization of a human agonist cytotoxic T lymphocyte epitope of human prostate-specific antigen. **Clin Cancer Res** 8, 41-53.
- Turner B, Haendle I, Roder C, Dieckmann D, Keikavoussi P, Jonuleit H, Bender A, Maczek C, Schreiner D, von den Driesch P, Brocker EB, Steinman RM, Enk A, Kampgen E and Schuler G (1999) Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. **J Exp Med** 190, 1669-1678.
- Tjoa B, Boynton A, Kenny G, Ragde H, Misrock SL and Murphy G (1996) Presentation of prostate tumor antigens by dendritic cells stimulates T cell proliferation and cytotoxicity. **Prostate** 28, 65-69.
- Tjoa B, Erickson SJ, Bowes VA, Ragde H, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Boynton AL and Murphy GP (1997) Follow-up evaluation of prostate cancer patients

- infused with autologous dendritic cells pulsed with PSMA peptides. **Prostate** 32, 272-278.
- Tjoa BA, Simmons SJ, Bowes VA, Ragde H, Rogers M, Elgamil A, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Salgaller ML, Boynton AL and Murphy GP (1998) Evaluation of phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides. **Prostate** 36, 39-44.
- Toes RE, Ossendorp F, Offringa R and Melief CJ (1999) CD4 T cells and their role in antitumor immune responses. **J Exp Med** 189, 753-756.
- Tsavaler L, Shapero MH, Morkowski S and Laus R (2001) Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. **Cancer Res** 61, 3760-3769.
- Vignard V, Lemercier B, Lim A, Pandolfino MC, Guilloux Y, Khammari A, Rabu C, Echasserieau K, Lang F, Gougeon ML, Dreno B, Jotereau F and Labarriere N (2005) Adoptive transfer of tumor-reactive Melan-A-specific CTL clones in melanoma patients is followed by increased frequencies of additional Melan-A-specific T cells. **J Immunol** 175, 4797-4805.
- Vihko P, Virkkunen P, Henttu P, Roiko K, Solin T and Huhtala ML (1988) Molecular cloning and sequence analysis of cDNA encoding human prostatic acid phosphatase. **FEBS Lett** 236, 275-281.
- Vonderheide RH, Hahn WC, Schultze JL and Nadler LM (1999) The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. **Immunity** 10, 673-679.
- Vonderheide RH, Anderson KS, Hahn WC, Butler MO, Schultze JL and Nadler LM (2001) Characterization of HLA-A3-restricted cytotoxic T lymphocytes reactive against the widely expressed tumor antigen telomerase. **Clin Cancer Res** 7, 3343-3348.
- Vonderheide RH, Domchek SM, Schultze JL, George DJ, Hoar KM, Chen DY, Stephans KF, Masutomi K, Loda M, Xia Z, Anderson KS, Hahn WC and Nadler LM (2004) Vaccination of cancer patients against telomerase induces functional antitumor CD8⁺ T lymphocytes. **Clin Cancer Res** 10, 828-839.
- Wang RF (2001) The role of MHC class II-restricted tumor antigens and CD4⁺ T cells in antitumor immunity. **Trends Immunol** 22, 269-276.
- Xu J, Kalos M, Stolk JA, Zasloff EJ, Zhang X, Houghton RL, Filho AM, Nolasco M, Badaro R and Reed SG (2001) Identification and characterization of prostein, a novel prostate-specific protein. **Cancer Res** 61, 1563-1568.
- Xue BH, Zhang Y, Sosman JA and Peace DJ (1997) Induction of human cytotoxic T lymphocytes specific for prostate-specific antigen. **Prostate** 30, 73-78.
- Yao A, Harada M, Matsueda S, Ishihara Y, Shomura H, Noguchi M, Matsuoka K, Hara I, Kamidono S and Itoh K (2004) Identification of parathyroid hormone-related protein-derived peptides immunogenic in human histocompatibility leukocyte antigen-A24⁺ prostate cancer patients. **Br J Cancer** 91, 287-296.
- Yao A, Harada M, Matsueda S, Ishihara Y, Shomura H, Takao Y, Noguchi M, Matsuoka K, Hara I, Kamidono S and Itoh K (2005) New epitope peptides derived from parathyroid hormone-related protein which have the capacity to induce prostate cancer-reactive cytotoxic T lymphocytes in HLA-A2⁺ prostate cancer patients. **Prostate** 62, 233-242.
- Yee C, Thompson JA, Byrd D, Riddell SR, Roche P, Celis E and Greenberg PD (2002) Adoptive T cell therapy using antigen-specific CD8⁺ T cell clones for the treatment of patients with metastatic melanoma: *in vivo* persistence, migration and antitumor effect of transferred T cells. **Proc Natl Acad Sci USA** 99, 16168-16173.
- Yeh LC, Lee AJ, Lee NE, Lam KW and Lee JC (1987) Molecular cloning of cDNA for human prostatic acid phosphatase. **Gene** 60, 191-196.



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