

Review

Immune-based therapies for prostate cancer

Douglas G. McNeel^{a,b,1}, Miroslav Malkovsky^{b,c,*}

^a Department of Medicine, University of Wisconsin Medical School, 600 Highland Avenue, Madison, WI 53792, USA

^b University of Wisconsin Comprehensive Cancer Center, 600 Highland Avenue, Madison, WI 53792, USA

^c Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, 1300 University Avenue, Madison, WI 53706, USA

Received 21 June 2004; accepted 23 June 2004

Available online 23 July 2004

Abstract

Prostate cancer is a leading cause of cancer morbidity and mortality in the United States. Animal and clinical studies performed four decades ago suggested that immunological approaches might be useful for the treatment of prostate cancer. The wealth of information that has been learned over the last two decades has suggested many new directions to the immune-based therapy of prostate cancer, including passive and active immunotherapy approaches. The findings from current trials, and the likely combination of current immunotherapy approaches with conventional therapies, portends a hopeful future for the treatment of prostate cancer.

© 2004 Published by Elsevier B.V.

Keywords: Prostate cancer; Vaccines; Immunotherapy; Cytokine; $\gamma\delta$ T cells

1. Introduction

Prostate cancer is currently the most commonly diagnosed non-skin cancer in men in the United States and the second leading cause of cancer-related death in men in the United States [1]. Animal and clinical studies performed decades ago using non-specific immunomodulatory therapies suggested that improved immunological approaches might be useful for the treatment of prostate cancer. Over the last two decades there have been great strides in the fields of immunology and cancer biology that have suggested new directions to the immune-based therapy of prostate cancer. The following article will review several immunotherapeutic strategies being investigated as treatments for prostate cancer, focusing on passive (immunomodulatory and cytokine approaches) and active immunotherapies (vaccines targeting adaptive and innate immune effector functions).

2. Background and overview

There has been controversy over the role of the immune system in prostate cancer surveillance; many investigators decades ago concluded that prostate cancer is not an immunogenic tumor and the prostate gland is an “immunologically privileged” site [2,3]. For example, Smith initially reported that the prostate gland is devoid of afferent lymphatics [4]. Moreover, non-specific tests of immune function, such as T cell rosette formation, T cell blastogenic response to mitogen stimulation, and DTH responses to common recall antigens, were all found to be reduced in patients with prostate cancer [5,6]. Other studies documented the reduction of MHC class I molecules in metastatic prostate cancer lesions, the disruption of the TAP transporter machinery in human prostate cancer cell lines, and the overexpression of TGF β by prostate cancer cells as multiple possible mechanisms of prostate cancer escape from immune detection [7]. Finally, the absence of prostate tumors occurring in T cell deficient mice suggested that the presence of prostate cancer was not due to a defect in immune surveillance [8].

* Corresponding author. Tel.: +1 608 263 6316; fax: +1 608 263 6316.

E-mail addresses: dm3@medicine.wisc.edu (D.G. McNeel), mmalkovs@wisc.edu (M. Malkovsky).

¹ Tel.: +1 608 265 8131; fax: +1 608 265 8133.

More recent studies, however, have challenged these conclusions. For example, several reports have confirmed the presence of lymphocytic infiltrates in the prostate, suggesting the prostate is not immunologically privileged [9]. Our group and others have identified prostate antigen-specific T cells and IgG in patients with chronic prostatitis [10,11] and prostate cancer [12,13], suggesting that immune responses to prostate antigens occurs commonly in vivo. In patients with prostate cancer, the presence of tumor-infiltrating lymphocytes (TIL) in tumor specimens has actually been associated with higher 10-year survival than the absence of TIL, suggesting prostate-specific immune responses may play a role in tumor surveillance [14]. Finally, it is now generally assumed that the downregulation of MHC class I expression and expression of immunosuppressive cytokines by advanced prostate cancers are evidence that the immune system, in fact, does play a role in tumor surveillance that the cancer attempts to overcome.

Immunotherapeutic strategies can be broadly classified into passive therapies and active therapies. Passive immunotherapies include treatment with immunomodulatory agents, infusion of cytokines, or infusion of immune effector agents such as antibodies or lymphocytes. Active immunotherapies include vaccine strategies in which the goal is to elicit host-specific anti-tumor immune responses. Vaccines can be further classified as whole cell vaccines, antigen-specific vaccines, and non-antigen-specific vaccines. This article will briefly review several passive and active immunotherapy strategies currently being investigated in prostate cancer, focusing on cytokine and vaccine approaches.

3. Passive immunotherapy – cytokines

3.1. Granulocyte-macrophage colony stimulating factor (GM-CSF)

GM-CSF is a member of a large family of glycoprotein growth factors that regulate the growth and differentiation of hematopoietic progenitor cells and is known to act at several levels in the generation and propagation of immune responses. For example, it is known to prime neutrophils for enhanced arachidonic acid release and activate antibody-dependent cell-mediated cytotoxicity of neutrophils [15]. It also acts as a chemoattractant for eosinophils and has been demonstrated to enhance the cytotoxicity of eosinophils [16]. Finally, GM-CSF is known to induce the differentiation and promote the survival of peripheral blood dendritic cells (DC) [17].

Given all of these immunostimulatory properties, several groups have been investigating GM-CSF as a vaccine adjuvant [18–20]. Other groups have investigated administration of GM-CSF alone as a means of eliciting anti-tumor immunity. Dreicer et al. at the Cleveland Clinic reported the results of a small phase II trial in which 16 patients with

metastatic prostate cancer were treated with 250 μ g GM-CSF three times per week for up to six months [21]. While no objective disease responses were observed, several patients did experience a decline in serum PSA measurements [21]. Rini et al. reported similar findings from a separate phase II study of GM-CSF administered to patients with early micrometastatic prostate cancer (stage D0) [22]. In that study, patients were treated in 14-day cycles of daily GM-CSF, and three of 29 subjects achieved a reduction in serum PSA. Together, these findings would suggest that GM-CSF exerts a biological effect mediated by inflammatory cells. Unfortunately, however, neither group evaluated whether prostate-specific immune responses were affected by the treatment.

3.2. Flt3 ligand (FL)

Similar to GM-CSF, FL has been shown to be a growth and differentiation factor for DC [23]. Previous studies demonstrated that systemic administration of FL to mice or humans markedly increases circulating progenitor DC that retain antigen-presenting function and the capacity to stimulate the proliferation of antigen-specific T cells [24–28]. The ability of FL to stimulate the production of DC suggested to us that it might be useful as a systemic vaccine adjuvant, like GM-CSF, by increasing the number of circulating and tissue-resident antigen-presenting cells [29,30]. We have, in fact, demonstrated that treatment elicited antigen-specific immune responses [31].

The ability of FL to elicit functional DC suggested that direct systemic administration might elicit therapeutic anti-tumor immunity. This was first tested in animal models, and in fact shown in several tumor-bearing mice systems that treatment with cycles of FL resulted in tumor regression [24,32], including a transgenic mouse model of prostate cancer [33]. A similar study, recently reported by Higano and coworkers, was conducted in patients with early androgen-independent prostate cancer [34]. In that study of 31 patients, PSA serum responses were not reported, but a biological effect was implicated by an overall decrease in the rate of rise of serum PSA in the treated subjects [34].

4. Active immunotherapy – vaccines

4.1. Whole cell vaccines

Early experiments in infectious disease models demonstrated that vaccination of test animals with inactivated virus or bacteria would protect them from disease caused by subsequent exposure to the live pathogen. The field of tumor immunology arose largely from this same immunization concept, and a prevailing hypothesis was that a variety of antigens would be needed to elicit an effective anti-tumor immune response. In addition, if a specific tumor rejection antigen were not known, perhaps an approach that could simultaneously target multiple antigens would be of benefit.

Early clinical studies with a similar approach, immunizing cancer patients with irradiated autologous tumor cells, met with limited success. Consequently, recent investigations in whole cell vaccines have typically modified the tumor cells to express an immune-stimulating agent. Simons et al. reported the first trial in prostate cancer in which patients with high-volume local prostate cancer underwent immunization with autologous prostate cancer cells transfected with a retroviral construct to express GM-CSF [35]. This particular study, while not particularly feasible except in patients with very advanced localized disease, has led to the development of a generalized approach (GVAX[®], Cell Genesys) using inactivated allogeneic prostate cancer cell lines transfected to express GM-CSF. In unpublished results from a phase II trial of the GVAX vaccine (composed of the LNCaP and PC3 cell lines) conducted in patients with advanced metastatic prostate cancer, clinical responses were observed and there was a trend toward improved progression-free survival. A separate phase II trial was then initiated using a re-engineered vaccine secreting higher levels of GM-CSF [36]. Phase III studies are planned with this vaccination approach [37].

4.2. Antigen-specific vaccines

Prior to 1990, it was generally believed that the most suitable target antigens for anti-tumor vaccines must be mutated antigens not normally encountered by the immune system, or foreign antigens such as viral oncogenic proteins aberrantly expressed by the cancer. Studies in human melanoma, however, showed that the targets of tumor-infiltrating lymphocytes were non-mutated autologous antigens, including MAGE-1 [38] and tyrosinase [38]. These findings suggested that any tissue-specific protein might be an appropriate vaccine antigen. In the case of prostate cancer, for which many tissue-specific genes are known, this has led to the rapid preclinical evaluation of several prostate-specific proteins as vaccine antigens, including PSA, prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA). The development of these antigen-specific vaccines by a variety of delivery methods is detailed below.

4.2.1. Protein-based vaccines

In a series of phase I pilot studies, PSA was delivered as a recombinant protein in a lipid emulsion adjuvant, with or without bacillus Calmette-Guérin (BCG), IL-2, cyclophosphamide, or GM-CSF as immunomodulatory agents [39]. Spitzer and coworkers reported the generation of PSA-specific CD4 T cells in some subjects treated [40]. No clinical benefit was described, however, and this approach is not being further pursued. Other groups have investigated protein-pulsed autologous dendritic cells as a means of eliciting antigen-specific cellular immunity. Fong et al., for example, demonstrated in a pilot clinical trial that autologous dendritic cells, loaded with the murine homologue of PAP, was effective in eliciting PAP-specific T cell immunity in

patients with prostate cancer [41,42]. Similarly, in a series of phase I/II trials for early metastatic and androgen independent disease, investigators at Dendreon Corporation have demonstrated that autologous antigen presenting cells, loaded *ex vivo* with a conjugate protein (PAP genetically fused with GM-CSF, “Provenge”), was capable of eliciting PAP-specific T cell immune responses [43]. In addition, patients that achieved a PAP-specific T cell proliferative response were found to have a significantly prolonged time to progression compared with patients that did not [44]. Based on these encouraging results, a multi-institutional, randomized, placebo-controlled phase III study is currently underway using this approach.

4.2.2. Cancer-associated carbohydrate vaccines

Several membrane-bound carbohydrate moieties, including MUC1, GM2, globo H and Thompson-Friedenreich antigen, have been found to be expressed preferentially on the surface of a variety of different tumor cell types, including prostate cancer, suggesting that they might be antigenic targets for tumor vaccines [45,46]. Slovin, Livingston and colleagues at Memorial Sloan-Kettering Cancer Center have conducted several phase I vaccine trials in patients with early metastatic prostate cancer targeting a variety of these carbohydrate moieties conjugated to keyhole limpet hemocyanin (KLH), and using the saponin derivative QS21 as an immunological adjuvant [47]. In the initial study targeting the globo H hexasaccharide, the investigators reported IgM antibody responses to globo H and stable PSA slopes (rate of change of serum PSA over time) compared with pre-treatment PSA slopes in specific patients over a 2-year period. The investigators have recently presented initial data from a phase II trial combining several carbohydrate-KLH conjugates into a multivalent vaccine and targeting the same population of prostate cancer patients [48]. Similar to the phase I trials, some patients were observed to have a decrease in the rate of serum PSA rise, suggesting possible clinical benefit.

4.2.3. Viral vaccines

Many viral pathogens are known to elicit potent cytotoxic T cell (CTL) responses [49], presumably mediated in part by cross presentation of antigen by infected MHC class I-expressing cells. Hodge et al. demonstrated that repetitive immunization of rhesus monkeys with recombinant vaccinia expressing PSA was capable of eliciting PSA-specific T cells [50]. They and others have conducted a series of phase I pilot studies using recombinant vaccinia expressing PSA (“Prostvac”) in patients with prostate cancer [51–53]. Given some concerns for repetitive immunizations with vaccinia that the immune response might be more directed against the viral proteins, current studies with the PSA-vaccinia vaccines (rV-PSA) have focused on “prime-boost” strategies in which the rV-PSA is given in sequence with other recombinant viruses, such as fowlpox, expressing PSA (rF-PSA) [54]. A multi-institutional phase II study was conducted in

the Eastern Cooperative Oncology Group using this strategy in patients with early metastatic prostate cancer [55]. In this particular trial, sixty-four patients were randomized to three treatment groups, with vaccinations occurring at six-week intervals. A trend toward improvement in serum PSA progression-free survival was observed in one group that received rV-PSA followed by rF-PSA, however the study was not powered to detect differences between the arms [55]. Further investigations in this area are now focused on either combining this approach with standard chemotherapy, or attempting to improve the efficacy of the subunit viral vaccines [56].

4.2.4. Peptide-based vaccines

T cells recognize antigens as processed peptides presented in the context of an appropriate MHC molecule. It was subsequently demonstrated that direct delivery of T cell peptide epitopes could be used as vaccines to elicit epitope-specific T cells [57,58]. Over the last several years, several groups have sought to identify MHC class I- and MHC class II-restricted T cell epitopes derived from prostate-specific proteins for potential peptide-based vaccines [59–69]. Peace and colleagues have reported preliminary immunological results from a phase I trial comparing direct peptide immunization with an epitope derived from PSA versus autologous dendritic cells loaded with this peptide [70]. Similarly, we conducted a pilot vaccine trial in which patients with advanced prostate cancer were vaccinated intradermally with an HLA-A2-restricted epitope (p369-377) from HER-2/neu [30]. In this particular study, few peptide-specific responses were detected. A recent phase I trial was conducted in Japan in which HLA-A24-expressing individuals with hormone-refractory prostate cancer were pre-screened for peptide-specific responses and administered peptide vaccines targeting only those peptides to which patients demonstrated a response. The authors report peptide-specific responses, and one of ten individuals achieved a serum PSA partial response [71].

4.2.5. Plasmid DNA vaccines

The use of plasmid DNA alone, injected directly into muscle tissue, as a means of *in vivo* gene delivery was first described by Wolff et al. [72]. It was subsequently found that intramuscular or intradermal administration of DNA plasmids encoding genes elicited immune responses to the gene products [73–75]. We and other investigators have explored antigen-specific DNA vaccines for prostate cancer. In pre-clinical models, Kim et al. demonstrated that a DNA vaccine encoding human PSA was able to elicit PSA-specific immunity in mice and rhesus monkeys [76,77]. Our group has been exploring DNA vaccines targeting PAP, and have shown in a rat model that DNA vaccines expressing PAP are able to elicit PAP-specific CD4 and CD8 T cells, and prostate tissue inflammation [78]. A clinical trial using this approach is planned. Similarly, Wolchok et al. are conducting a phase I trial using DNA vaccines encoding either the human or

murine homologue of PSMA in a prime-boost immunization sequence [79].

4.3. Vaccines activating $\lambda\delta$ T cells

The vaccine strategies described above are designed to elicit adaptive immune responses to either a variety of tumor-specific antigens (in the case of whole cell vaccines) or antigen-specific responses. These immune responses are predominantly mediated by $\alpha\beta$ T cells, B cells and antibodies. However, approximately 1–5% of human peripheral blood lymphocytes express the $\gamma\delta$ T-cell receptor, the majority of these expressing the V γ 9V δ 2 variable segments. V γ 9V δ 2 T lymphocytes recognize nonpeptidic antigens (NpAgs) generated by the DOXP (many eubacteria, algae, plants, apicomplexa) and mevalonate (eukaryotes, archaeobacteria and certain eubacteria) pathways of isoprenoid synthesis. NpAgs are molecules structurally distinct from the typical peptidic antigens that interact with $\alpha\beta$ T-cell receptors. Also, the recognition of NpAgs by V γ 9V δ 2 T cells does not require ‘classical’ antigen processing and MHC class I or II presentation. It is believed that this pattern of recognition allows for a rapid primary immune response to antigen challenge, particularly by infectious agents [80–82]. In addition to NpAgs, it has also been demonstrated that certain nitrogen-containing bisphosphonates (N-BPs) such as pamidronate disodium or zoledronic acid are potent stimulators of V γ 9V δ 2 T cells [83,84]. Kunzmann et al. demonstrated that the anti-tumor effect of pamidronate on myeloma cells was correlated with its ability to elicit $\gamma\delta$ T cells *in vitro* using bone marrow from patients with multiple myeloma [85]. The investigators also reported that V γ 9V δ 2 T cells could be detected in the bone marrow of patients with multiple myeloma, and that cytoreduction of these cells permitted the outgrowth of malignant myeloma cells [85]. In addition, several human tumor cell lines became targets for V γ 9V δ 2 T cell-mediated lysis after exposure to pamidronate [86]. A recent clinical trial in patients with lymphoid malignancies has been reported in which $\gamma\delta$ T cells were augmented by means of pamidronate in combination with IL-2 [87]. Given these findings, we have begun to investigate the ability of $\gamma\delta$ T cells to be utilized as a prostate cancer therapy. We have demonstrated that $\gamma\delta$ T cells can specifically lyse prostate cancer cells *in vitro*. (Wallace et al., manuscript in preparation). Recently, we have shown that intravenous administration of N-BP or pyrophosphonoester drugs combined with low doses of IL-2 induces a large pool of CD27+ and CD27- effector/memory V γ 9V δ 2 T cells in the peripheral blood (Casetti et al., manuscript in preparation). Moreover, we have observed anecdotal cases of patients with hormone-refractory prostate cancer, who have been treated with pamidronate or zoledronic acid for palliative purposes and who have had evidence of a PSA decline and/or stabilization in the absence of other active therapies (Fig. 1). A clinical trial is currently underway to evaluate the ability to augment $\gamma\delta$ T cells *in vivo* in patients with prostate cancer.

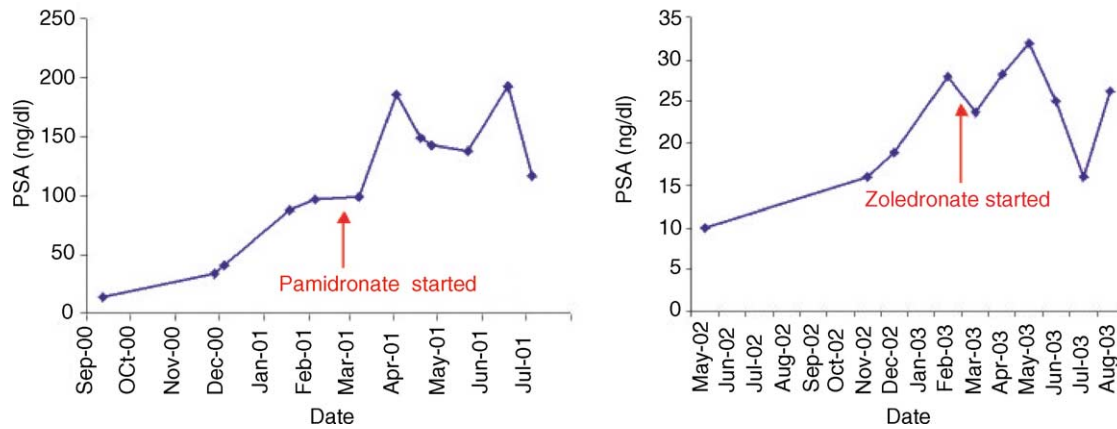


Fig. 1. PSA stabilization in patients with androgen-independent prostate cancer treated with bisphosphonates. The figure shows serum PSA levels in two patients with androgen-independent prostate cancer monthly treated with pamidronate (panel A) or zoledronate (panel B). No other prostate cancer therapies were introduced during the periods of time shown.

Acknowledgements

The authors are grateful for grant support from NIH, the Howard Hughes Medical Institute, and the Prostate Cancer Research Foundation (CaPCURE).

References

- [1] Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics 2003;53:5–26.
- [2] Gittes RF, McCullough DL. Occult carcinoma of the prostate: an oversight of immune surveillance—a working hypothesis. *J Urol* 1974;112:241–4.
- [3] Vieweg J, Rosenthal FM, Bannerji R, Heston WD, Fair WR, Gansbacher B, Gilboa E. Immunotherapy of prostate cancer in the Dunning rat model: use of cytokine gene modified tumor vaccines. *Cancer Res* 1994;54:1760–5.
- [4] Smith M. The lymphatics of the prostate. *Invest Urol* 1996;3:439–44.
- [5] Guinan P, Bhatti R, Sharifi R, Sundar B, Nagubadi S, Baumgartner G. The immune response to prostate cancer: an evaluation and a review. *J Surg Oncol* 1980;15:309–18.
- [6] Thomas JW, Jerkins G, Cox C, Lieberman P. Defective cell-mediated immunity in carcinoma of the prostate. *Invest Urol* 1976;14:72–5.
- [7] Sanda MG, Restifo NP, Walsh JC, Kawakami Y, Nelson WG, Pardoll DM, Simons JW. Molecular characterization of defective antigen processing in human prostate cancer. *J Natl Cancer Inst* 1995;87:280–5.
- [8] Rygaard J, Povlsen CO. The nude mouse vs. the hypothesis of immunological surveillance. *Transplant Rev* 1976;28:43–61.
- [9] McClinton S, Miller ID, Eremin O. An immunohistochemical characterisation of the inflammatory cell infiltrate in benign and malignant prostatic disease. *Br J Cancer* 1990;61:400–3.
- [10] Alexander RB, Brady F, Ponniah S. Autoimmune prostatitis: evidence of T cell reactivity with normal prostatic proteins. *Urology* 1997;50:893–9.
- [11] Dunphy EJ, Eickhoff JC, Muller CH, Berger RE, McNeel DG. Identification of antigen-specific IgG in sera from patients with chronic prostatitis. *J Clin Immunol* (in press).
- [12] McNeel DG, Nguyen LD, Storer BE, Vessella R, Lange PH, Disis ML. Antibody immunity to prostate cancer-associated antigens can be detected in the serum of patients with prostate cancer. *J Urol* 2000;164:1825–9.
- [13] McNeel DG, Nguyen LD, Ellis WJ, Higano CS, Lange PH, Disis ML. Naturally occurring prostate cancer antigen-specific T cell responses of a Th1 phenotype can be detected in patients with prostate cancer. *Prostate* 2001;47:222–9.
- [14] Vesalainen S, Lipponen P, Talja M, Syrjanen K. Histological grade, perineural infiltration, tumour-infiltrating lymphocytes and apoptosis as determinants of long-term prognosis in prostatic adenocarcinoma. *Eur J Cancer* 1994;1797–803.
- [15] Weisbart RH, Golde DW, Clark SC, Wong GG, Gasson JC. Human granulocyte-macrophage colony stimulating factor is a neutrophil activator. *Nature* 1985;314:361–3.
- [16] Weller PF. Cytokine regulation of eosinophil function. *Clin Immunol Immunopathol* 1992;62:55s–9s.
- [17] Markowicz S, Engleman EG. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *J Clin Invest* 1990;85:955–61.
- [18] Disis ML, Bernhard H, Shiota FM, Hand SL, Galow JR, Huseby ES, Gillis S, Cheever MA. Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines. *Blood* 1996;88:202–10.
- [19] Simmons SJ, Tjoa BA, Rogers M, Elgamil A, Kenny GM, Ragde H, Troychak MJ, Boynton AL, Murphy GP. GM-CSF as a systemic adjuvant in a phase II prostate cancer vaccine trial. *Prostate* 1999;39:291–7.
- [20] Hess G, Kreiter F, Kusters W, Deusch K. The effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on hepatitis B vaccination in haemodialysis patients. *J Viral Hepat* 1996;3:149–53.
- [21] Dreicer R, See WA, Klein EA. Phase II trial of GM-CSF in advanced prostate cancer. *Invest New Drugs* 2001;19:261–5.
- [22] Rini BI, Weinberg V, Bok R, Small EJ. Prostate-specific antigen kinetics as a measure of the biologic effect of granulocyte-macrophage colony-stimulating factor in patients with serologic progression of prostate cancer. *J Clin Oncol* 2003;21:99–105.
- [23] Brasel K, McKenna HJ, Morrissey PJ, Charrier K, Morris AE, Lee CC, Williams DE, Lyman SD. Hematologic effects of flt3 ligand in vivo in mice. *Blood* 1996;88:2004–12.
- [24] Chen K, Braun S, Lyman S, Fan Y, Traycoff CM, Wiebke EA, Gaddy J, Sledge G, Broxmeyer HE, Cornetta K. Antitumor activity and immunotherapeutic properties of Flt3-ligand in a murine breast cancer model. *Cancer Res* 1997;57:3511–6.
- [25] Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus WJ, Maraskovsky E, Lotze

- MT. FLT3 ligand induces the generation of functionally active dendritic cells in mice. *Cell Immunol* 1997;179:174–84.
- [26] Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K, McKenna HJ. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med* 1996;184:1953–62.
- [27] Siena S, Di Nicola M, Bregni M, Mortarini R, Anichini A, Lombardi L, Ravagnani F, Parmiani G, Gianni AM. Massive ex vivo generation of functional dendritic cells from mobilized CD34+ blood progenitors for anticancer therapy. *Exp Hematol* 1995;23:1463–71.
- [28] Maraskovsky E, Daro E, Roux E, Teepe M, Maliszewski CR, Hoek J, Caron D, Lebsack ME, McKenna J. In vivo generation of human dendritic cell subsets by Flt3 ligand. *Blood* 2000;96:878–84.
- [29] Disis ML, Rinn K, Knutson KL, Davis D, Caron D, dela Rosa C, Schiffman K. Flt3 ligand as a vaccine adjuvant in association with HER-2/neu peptide-based vaccines in patients with HER-2/neu-overexpressing cancers. *Blood* 2002;99:2845–50.
- [30] McNeel DG, Knutson KL, Schiffman K, Davis DR, Caron D, Disis ML. Pilot study of an HLA-A2 peptide vaccine using Flt3 ligand as a systemic vaccine adjuvant. *J Clin Immunol* 2003;23:62–72.
- [31] Dunphy EJ, McNeel DG. SEREX methodology to identify immunological responses elicited during immunomodulatory therapy. *Proc Am Assn Canc Res* 2003;44:1845a.
- [32] Esche C, Subbotin VM, Maliszewski C, Lotze MT, Shurin MR. FLT3 ligand administration inhibits tumor growth in murine melanoma and lymphoma. *Cancer Res* 1998;58:380–3.
- [33] Ciavarra RP, Somers KD, Brown RR, Glass WF, Consolvo PJ, Wright GL, Schellhammer PF. Flt3-ligand induces transient tumor regression in an ectopic treatment model of major histocompatibility complex-negative prostate cancer. *Cancer Res* 2000;60:2081–4.
- [34] Higano CS, Vogelzang NJ, Sosman JA, Feng A, Caron D, Small EJ. Safety and biological activity of repeated doses of recombinant human Flt3 ligand in patients with bone scan-negative hormone-refractory prostate cancer. *Clin Cancer Res* 2004;10:1219–25.
- [35] Simons JW, Mikhak B, Chang JF, DeMarzo AM, Carducci MA, Lim M, Weber CE, Baccala AA, Goemann MA, Clift SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S, Marshall FF, Nelson WG. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. *Cancer Res* 1999;59:5160–8.
- [36] Simons J, Higano C, Corman J, Hudes G, Centeno A, Dula E, Smith D, Steidle C, Sacks N, Small E. A phase I/II study of high dose allogeneic GM-CSF gene-transduced prostate cancer cell line vaccine in patients with metastatic hormone-refractory prostate cancer. *Proc Am Soc Clin Oncol* 2003;22:667.
- [37] Cell genesys reports long-term survival data in Phase II trial of GVAX. *Expert Rev Anticancer Ther* 2002;2:245–6.
- [38] Brichard V, Van Pel A, Wolfel T, Wolfel C, De Plaen E, Lethe B, Coulie P, Boon T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 1993;178:489–95.
- [39] Harris DT, Matyas GR, Gomella LG, Talor E, Winship MD, Spittle LE, Mastrangelo MJ. Immunologic approaches to the treatment of prostate cancer. *Semin Oncol* 1999;26:439–47.
- [40] Meidenbauer N, Harris DT, Spittle LE, Whiteside TL. Generation of PSA-reactive effector cells after vaccination with a PSA-based vaccine in patients with prostate cancer. *Prostate* 2000;43:88–100.
- [41] Fong L, Brockstedt D, Benike C, Wu L, Engleman EG. Dendritic cells injected via different routes induce immunity in cancer patients. *J Immunol* 2001;166:4254–9.
- [42] Fong L, Brockstedt D, Benike C, Breen JK, Strang G, Ruegg CL, Engleman EG. Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. *J Immunol* 2001;167:7150–6.
- [43] 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, Vuk-Pavlovic S. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res* 2000;6:2175–82.
- [44] Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, Valone FH. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol* 2000;18:3894–903.
- [45] Livingston P. Ganglioside vaccines with emphasis on GM2. *Semin Oncol* 1998;25:636–45.
- [46] Zhang S, Zhang HS, Reuter VE, Slovin SF, Scher HI, Livingston PO. Expression of potential target antigens for immunotherapy on primary and metastatic prostate cancers. *Clin Cancer Res* 1998;4:295–302.
- [47] Slovin SF. Vaccines as treatment strategies for relapsed prostate cancer: approaches for induction of immunity. *Hematol Oncol Clin North Am* 2001;15:477–96.
- [48] Slovin S, Ragupathi G, Clarke T, Fernandez C, Diani M, Heller G, Danishefsky S, Livingston P, Scher HI. Multivalency in a phase II prostate cancer (PC) vaccine trial: are more antigens better? *Proc Am Soc Clin Oncol* 2003;22:671.
- [49] Doherty PC, Biddison WE, Bennink JR, Knowles BB. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. *J Exp Med* 1978;148:534–43.
- [50] Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS, Gritz L, Panicali D, Kantor JA. A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer* 1995;63:231–7.
- [51] Sanda MG, Smith DC, Charles LG, Hwang C, Pienta KJ, Schlom J, Milenic D, Panicali D, Montie JE. Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. *Urology* 1999;53:260–6.
- [52] Eder JP, Kantoff PW, Roper K, Xu GX, Bublely GJ, Boyden J, Gritz L, Mazzara G, Oh WK, Arlen P, Tsang KY, Panicali D, Schlom J, Kufe DW. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. *Clin Cancer Res* 2000;6:1632–8.
- [53] Gulley J, Chen AP, Dahut W, Arlen PM, Bastian A, Steinberg SM, Tsang K, Panicali D, Poole D, Schlom J, Michael Hamilton J. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. *Prostate* 2002;53:109–17.
- [54] Cavacini LA, Duval M, Eder JP, Posner MR. Evidence of determinant spreading in the antibody responses to prostate cell surface antigens in patients immunized with prostate-specific antigen. *Clin Cancer Res* 2002;8:368–73.
- [55] Kaufman HL, Wang W, Manola J, DiPaola RS, Ko Y-J, Williams SD, Whiteside T, Schlom J, Wilding G, Weiner LM. Prime-boost vaccination using poxviruses expressing PSA in D0 prostate cancer: preliminary results of ECOG 7897, a randomized phase II clinical trial 2002;21:12.
- [56] Arlen PM, Gulley JL, Parker C, Skarupa L, Panicali D, Beetham P, Palena C, Tsang KY, Schlom J, Dahut W. A pilot study of concurrent docetaxel plus PSA pox-vaccine versus vaccine alone in metastatic androgen independent prostate cancer (AIPC). *Proc Am Soc Clin Oncol* 2003;22:1701.
- [57] Celis E, Ou D, Otvos Jr L. Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferative and cytotoxic responses to a major antigenic determinant defined by synthetic peptides. *J Immunol* 1998;140:1808–15.
- [58] Celis E, Sette A, Grey HM. Epitope selection and development of peptide based vaccines to treat cancer. *Semin Cancer Biol* 1995;6:329–36.
- [59] Tjoa B, Boynton A, Kenny G, Ragde H, Misrock SL, Murphy G. Presentation of prostate tumor antigens by dendritic cells stimulates T-cell proliferation and cytotoxicity. *Prostate* 1996;28:65–9.

- [60] Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Schlom J, Tsang KY. In vitro generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. *J Natl Cancer Inst* 1997;89:293–300.
- [61] Xue BH, Zhang Y, Sosman JA, Peace DJ. Induction of human cytotoxic T lymphocytes specific for prostate-specific antigen. *Prostate* 1997;30:73–8.
- [62] Corman JM, Sercarz EE, Nanda NK. Recognition of prostate-specific antigenic peptide determinants by human CD4 and CD8 T cells. *Clin Exp Immunol* 1998;114:166–72.
- [63] Peshwa MV, Shi JD, Ruegg C, Laus R, van Schooten WC. Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. *Prostate* 1998;36:129–38.
- [64] McNeel DG, Knutson KL, Disis ML. Identification of PAP-specific MHC class I peptide epitopes by screening patients with prostate cancer by IFN-gamma ELISPOT. *Proc Am Assn Cancer Res* 2001;42:277.
- [65] McNeel DG, Nguyen LD, Disis ML. Identification of T helper epitopes from prostatic acid phosphatase. *Cancer Res* 2001;61:5161–7.
- [66] Lu J, Celis E. Recognition of prostate tumor cells by cytotoxic T lymphocytes specific for prostate-specific membrane antigen. *Cancer Res* 2002;62:5807–12.
- [67] Schroers R, Shen L, Rollins L, Xiao Z, Sonderstrup G, Slawin K, Huang XF, Chen SY. Identification of MHC class II-restricted T-cell epitopes in prostate-specific membrane antigen. *Clin Cancer Res* 2003;9:3260–71.
- [68] Hural JA, Friedman RS, McNabb A, Steen SS, Henderson RA, Kalos M. Identification of naturally processed CD4 T cell epitopes from the prostate-specific antigen kallikrein 4 using peptide-based in vitro stimulation. *J Immunol* 2002;169:557–65.
- [69] Kiessling A, Schmitz M, Stevanovic S, Weigle B, Holig K, Fussel M, Fussel S, Meye A, Wirth MP, Rieber EP. Prostate stem cell antigen: Identification of immunogenic peptides and assessment of reactive CD8+ T cells in prostate cancer patients. *Int J Cancer* 2002;102:390–7.
- [70] Reddy SL. A phase I trial of a PSA peptide vaccine for patients with prostate cancer. *Proc Am Soc Clin Oncol* 2003;22:757.
- [71] Noguchi M, Kobayashi K, Suetsugu N, Tomiyasu K, Suekane S, Yamada A, Itoh K, Noda S. Induction of cellular and humoral immune responses to tumor cells and peptides in HLA-A24 positive hormone-refractory prostate cancer patients by peptide vaccination. *Prostate* 2003;57:80–92.
- [72] Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. *Science* 1990;247:1465–8.
- [73] Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992;356:152–4.
- [74] Wang B, Ugen KE, Srikantan V, Agadjanyan MG, Dang K, Refaeli Y, Sato AI, Boyer J, Williams WV, Weiner DB. *Proc. Natl. Acad. Sci. USA*. Gene inoculation generates immune responses against human immunodeficiency virus type 1 1993;90:4156–60.
- [75] Raz E, Carson DA, Parker SE, Parr TB, Abai AM, Aichinger G, Gromkowski SH, Singh M, Lew D, Yankauckas MA, Baird SM, Rhodes GH. Intradermal gene immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses 1994;91:9519–23.
- [76] Kim JJ, Trivedi NN, Wilson DM, Mahalingam S, Morrison L, Tsai A, Chattergoon MA, Dang K, Patel M, Ahn L, Boyer JD, Chalian AA, Shoemaker H, Kieber-Emmons T, Agadjanyan MA, Weiner DB. Molecular and immunological analysis of genetic prostate specific antigen (PSA) vaccine. *Oncogene* 1998;17:3125–35.
- [77] Kim JJ, Yang JS, Dang K, Manson KH, Weiner DB. Engineering enhancement of immune responses to DNA-based vaccines in a prostate cancer model in rhesus macaques through the use of cytokine gene adjuvants. *Clin Cancer Res* 2001;7:882s–9s.
- [78] Johnson LE, McNeel DG. DNA vaccine encoding a prostate-specific protein elicits CD8 T cell immunity and tissue-specific inflammation. *Proc Am Assn Canc Res* 2004;45:1244.
- [79] Wolchok JD, Gregor PD, Nordquist LT, Slovin SF, Scher HI. DNA vaccines: an active immunization strategy for prostate cancer. *Semin Oncol* 2003;30:659–66.
- [80] Poccia F, Agrati C, Ippolito G, Colizzi V, Malkovsky M. Natural T cell immunity to intracellular pathogens and nonpeptidic immunoregulatory drugs. *Curr Mol Med* 2001;1:137–251.
- [81] Poccia F, Malkovsky M, Pollak A, Colizzi V, Sireci G, Salerno A, Dieli F. In vivo $\gamma\delta$ T cell priming to mycobacterial antigens by primary *Mycobacterium tuberculosis* infection and exposure to nonpeptidic ligands. *Mol Med* 1999;5:471–6.
- [82] Poccia F, Gougeon ML, Agrati C, Montesano C, Martini F, Pauza CD, Fisch P, Wallace M, Malkovsky M. Innate T-cell immunity in HIV infection: the role of V γ 9V δ 2 T lymphocytes. *Curr Mol Med* 2002;2:769–78.
- [83] Das H, Wang L, Kamath A, Bukowski JF. V γ 2V δ 2 T-cell receptor-mediated recognition of aminobisphosphonates. *Blood* 2001;98:1616–8.
- [84] Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, Arcara C, Valerio MR, Meraviglia S, Di Sano C, Sireci G, Salerno A. Induction of $\gamma\delta$ T-lymphocyte effector functions by bisphosphonate zolendronic acid in cancer patients in vivo. *Blood* 2003;102:2310–1.
- [85] Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of $\gamma\delta$ T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood* 2000;96:384–92.
- [86] Kato Y, Tanaka Y, Miyagawa F, Yamashita S, Minato N. Targeting of tumor cells for human $\gamma\delta$ T cells by nonpeptide antigens. *J Immunol* 2001;167:5092–8.
- [87] Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, Tony HP. $\gamma\delta$ T cells for immune therapy of patients with lymphoid malignancies. *Blood* 2003;102:200–6.