



Review

Tumor Vaccines for the Management of Prostate Cancer

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Abstract. Prostate cancer is a significant health problem and one of the leading causes of cancer-related death among men. Given the typically long natural history of the disease, there is considerable interest in developing new therapies to treat or prevent metastatic disease, and cancer vaccines are a particularly attractive immune-based approach. Early clinical studies using non-specific immunomodulatory treatments have met with limited success, but also suggest that improved immunologic approaches might be useful in treating human prostate cancer. Over the last decade, the identification of immune cells responsible for actual destruction of prostate tissue and advances in immunologic and molecular techniques have led to a variety of vaccination approaches that are currently being evaluated in human clinical trials. The present article discusses the rationale in animal models for particular immunization strategies and describes the vaccines currently being used in patients with prostate cancer. The ongoing identification of tumor antigens and proteins involved in prostate cancer progression and the development of better immunologic animal models suggest a hopeful future for the design of effective prostate cancer vaccines.

Key words: prostate cancer; tumor vaccines; clinical trials; immunotherapy.

Prostate cancer is a significant health problem worldwide and currently second only to skin cancer as the most diagnosed male malignancy in the United States³. After appropriate local therapy, approximately 30% of patients will develop metastatic disease and approximately 10% of patients will die of their disease. At present there is no cure for metastatic prostate cancer and no accepted adjuvant therapy proven to reduce tumor progression. There is considerable interest in developing new therapies to treat or prevent metastatic disease. Recently, gene therapy and various immune-based strategies have been explored as possible treatments for prostate cancer^{21, 23, 24}. Given the gener-

ally long natural history of prostate cancer, tumor vaccines are a particularly attractive immune-based approach to consider for adjuvant therapy.

Background

Initial investigations performed nearly 20 years ago suggested prostate cancer is not an immunogenic tumor. Early reports suggested the prostate gland is devoid of afferent lymphatics⁵². In addition, non-specific tests of immune function, such as T cell rosette formation, T cell blastogenic response to mitogen

Abbreviations used: TIL – tumor-infiltrating lymphocytes, DTH – delayed type hypersensitivity, PSA – prostate specific antigen, GM-CSF – granulocyte-macrophage colony stimulating factor, PAP – prostatic acid phosphatase, PSMA – prostate specific membrane antigen, CTL – cytotoxic T lymphocytes, MHC – major histocompatibility complex, KLH – keyhole limpet hemocyanin.

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stimulation, and delayed type hypersensitivity (DTH) responses to common recall antigens, were all found to be reduced in patients with prostate cancer^{19, 56}. Other studies documented the reduction of major histocompatibility complex (MHC) class I molecules in metastatic prostate cancer lesions and disruption of the prostatic acid phosphatase TAP transporter machinery in human prostate cancer cell lines, suggesting possible mechanisms of escape from immune detection⁴⁶. 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 surveillance⁴³. Such findings led investigators to conclude that prostate cancer is not an immunogenic tumor⁶⁰.

More recent studies have challenged these assumptions. First, several reports have confirmed the presence of lymphocytic infiltrates in the prostate, suggesting the prostate is not immunologically privileged³⁴. Second, reported cases of granulomatous prostatitis demonstrate that inflammatory reactions occur within the prostate gland²⁸. Third, prostatic apoptosis associated with androgen ablative therapy has been shown to be, in part, mediated by an immune response in a rat model³⁰. Fourth, the presence of tumor-infiltrating lymphocytes (TIL) in prostate cancer specimens has been associated with higher 10-year survival than the absence of TIL, suggesting cancer-specific immune responses may play a role in tumor surveillance⁵⁹. Moreover, our group has demonstrated that patients with prostate cancer develop humoral immunity to prostate specific antigen (PSA) and HER-2/neu, and the prevalence of these immune responses is more common in patients with metastatic disease (McNEEL, submitted). Finally, we have also shown that patients with prostate cancer have preexistent T cell responses to PSA and PAP of a Th1-like phenotype, suggesting that potentially therapeutic immune responses already exist *in vivo*, albeit at low levels (McNEEL, submitted).

Recent advances in molecular biology and basic immunology, including the identification of cytokines responsible for promoting specific types of immune responses, an identification of the immune cell subsets responsible for eradicating tumor cells, and the development of appropriate animal tumor models, have led to a variety of new approaches to generate tumor-specific immune responses by means of tumor vaccines. The present article will discuss the rationale in animal models for particular immunization strategies and describe the immunization strategies currently being used in patients with prostate cancer. These animal models and the human vaccine trials discussed are summarized in Table 1.

Whole Cell Vaccines

The goal of whole cell vaccines is to generate an anti-tumor immune response to a wide variety of tumor-associated antigens. Traditionally, the major difficulty with this approach has been that whole cell vaccines are typically only weakly immunogenic. Early studies in human vaccines with irradiated prostate cancer cells as vaccines showed modest evidence of an immunologic response when administered with foreign proteins^{5, 42}. These findings led researchers to attempt to increase the immunogenicity of cellular vaccines themselves. The Dunning rat model provided a good model for study, as the MatLyLu derived cell line forms anaplastic, androgen-independent tumors that spontaneously metastasizes to the lymph nodes and lung when injected orthotopically in rats²⁵. Studies showed that this tumor was poorly immunogenic; splenocytes from tumor-bearing animals demonstrated no cytolytic activity *in vitro* to MatLyLu cells and immunization of animals with irradiated MatLyLu cells generated no protection from subsequent exposure to live tumor cells⁶⁰. Studies conducted attempted to increase the immunogenicity of such cellular vaccines by transfecting them with immunomodulatory cytokines, similar to previous studies in animal models of melanoma^{11, 16}. Dunning rat prostate cancer cell lines, when transfected to express IL-2 and to a lesser extent granulocyte-macrophage colony stimulating factor (GM-CSF), and then used to immunize rats, protected immunized animals from subsequent challenge with live tumor cells⁶⁰. Splenocytes from immunized animals showed cytolytic activity toward non-transfected MatLyLu cells⁶⁰. Similar results were found by SANDA et al.⁴⁵ with GM-CSF transfected tumor vaccines in the Dunning rat model. This group went on to demonstrate that primary human prostate cancer cells could be transfected to express human GM-CSF⁴⁵. These findings were ultimately translated to human clinical trials in renal cell carcinoma⁴⁸, melanoma⁵³, and the first report of a GM-CSF-modified autologous prostate cancer cell vaccine trial has recently been reported by SIMONS et al.⁴⁹. In this study, 8 patients with large primary tumors undergoing radical prostatectomy had primary tumor lines established from specimens obtained at surgery. These lines were then transfected to express human GM-CSF, irradiated, and used to immunize patients 3 times over 3-week intervals. Eight of 11 patients were able to complete the vaccination series, 6 of whom had evidence of progressive disease, and 2 had transient clinical responses as determined by PSA serum levels. Three of the patients developed antibody responses to proteins expressed by

Table 1. Summary of preclinical animal models and recent human prostate cancer vaccine trials

	Animal models	Investigators	Human clinical trials	Investigators
Whole cell vaccines				
GM-CSF transfected tumors	Dunning rat	SANDA et al. ⁴⁵ VIEWEG et al. ⁶⁰	phase I (autologous cell lines) phase II (LNCaP)	SIMONS et al. ⁴⁹
IFN- γ and IL-2 transfected LNCaP cell line	Dunning rat	VIEWEG et al. ⁶⁰	phase I/II	SIMONS Cell Genesys Inc. SLOVIN
Dendritic cell vaccines				
PSMA peptides PAP recombinant protein			phase II phase I	MURPHY et al. ³⁹ FONG et al. ¹⁴
Antigen-specific vaccines				
PAP	Dunning rat vaccinia-PAP	FONG et al. ¹⁵	phase I DC / protein	FONG et al. ¹⁴
PSA	Primate vaccinia-PSA	HODGE et al. ²²	phase I/II OncoVax-P phase I Vaccinia-PSA phase II Vaccinia/fowlpox	SPITLER ²¹ SANDA ⁴⁷ Kaufman ECOG
PSMA	Murine plasmid DNA	KIM et al. ²⁹	phase II DC / peptide	MURPHY ³⁹
Carbohydrates	Murine MUC1 – KLH	ZHANG et al. ⁶²	phase I globo H – KLH phase I Thompson- -Friedenreich – KLH	SLOVIN ⁵¹ SLOVIN ⁵⁰
HER-2/neu	Fischer rat MHC class II peptides neu transgenic mouse	DISIS et al. ¹⁰ DISIS	phase I MHC class I peptide	McNEEL

human prostate cancer cell lines⁴⁹. Of note, 2 of 8 patients had delayed type hypersensitivity (DTH) responses to autologous tumor cells prior to vaccine treatment, suggesting that patients develop either low-level or non-therapeutic immune responses to their tumors *in vivo*.

The whole cell vaccine approach has potential advantages as well as disadvantages. Given that appropriate tumor antigens have not really been identified for prostate cancer as they have for other human solid tumors, one potential advantage is that individual antigens are not necessarily targeted. The goal of cellular vaccine approaches is to generate an immune response against the most immunogenic proteins. On the other hand, this also represents a disadvantage, as immunizing with whole cells exposes the immune system to hundreds and perhaps thousands of irrelevant proteins, possibly swamping out a potentially therapeutic immune response. In addition, the transfected cytokine

itself may be immunogenic. Our group has demonstrated that immune responses to recombinant human GM-CSF can develop after vaccination³⁶. Moreover, whole cell vaccines are laborious, costly, and of necessity tailor-made for individual patients. Such vaccine strategies may not be feasible for large-scale studies. Likewise, similar to vaccines used for the prevention of infectious diseases, the role of prostate cancer vaccines, and tumor vaccines in general, will likely be in the adjuvant setting with minimal detectable disease⁵⁵. In the study reported by SIMONS et al.⁴⁹, the authors note that the production of transfected tumor vaccines necessitated large primary tumors, and therefore vaccinations were restricted to patients with large, high-risk tumors. For this reason, their approach in phase II studies, and the approach of others using similar cytokine-transfected prostate cancer cellular vaccines, is to use cytokine-transfected allogeneic prostate cancer cell lines⁴⁹.

Dendritic Cell Vaccines

Dendritic cells are potent antigen presenting cells⁵⁴. Investigations in murine models have shown that dendritic cells pulsed *ex vivo* with lysates from tumor cells can be used to vaccinate naive syngeneic mice, protecting animals from tumor challenge and reducing the number of metastases^{6, 12}. These studies have demonstrated that a tumor-specific immune response can be generated by dendritic cell immunization, and have led to the initiation of human clinical trials in prostate cancer. G. Murphy and colleagues initially reported that autologous dendritic cells from patients with prostate cancer could be pulsed *ex vivo* with either autologous tumor cell lysates or an HLA-A2-restricted peptide epitope from prostate specific membrane antigen (PSMA) to generate tumor-specific or peptide-specific cytotoxic T lymphocytes (CTL) *in vitro*⁵⁸. This group then went on to pioneer the use of dendritic cell vaccines for prostate cancer in an antigen-specific fashion using two putative HLA-A2-restricted MHC class I epitopes from the PSMA protein. To date, these investigators have observed some potential clinical responses in select patients, and perhaps some evidence of PSMA-specific cellular immune responses elicited by vaccinations^{39, 44}.

In principle, using the antigen presenting cells that most directly promote cellular immune responses is a very reasonable approach. As an example, DHODAPKAR et al.⁷ demonstrated that healthy subjects could be effectively immunized, as assessed clinically by DTH testing and by quantitative *in vitro* assays of antigen-specific T cells, with autologous dendritic cells pulsed with 1 of 3 well-characterized immunogenic proteins or peptide epitopes in a single immunization. Animal studies using dendritic cells pulsed with tumor cell extracts have demonstrated that knowledge of the specific antigens recognized need not be known, as animals immunized in this fashion develop an anti-tumor immune response¹². Like whole cell vaccines, however, the generation of dendritic cells is a labor-intensive, individualized therapy that may be difficult to extrapolate to larger pools of patients. Nonetheless, this strategy is entering phase II/III studies for the treatment of advanced melanoma, and shows promise for future development of prostate cancer vaccines.

Antigen-Specific Vaccines

Given the disadvantages and individualized therapy inherent in cellular vaccines, several investigators have focused on antigen-specific vaccines. The theoretical

advantages of targeting a specific antigen include the ability to concentrate the immune response against one specific, immunogenic target and thereby avoid exposure to extraneous or potentially disadvantageous antigens. Certainly in the case of vaccines for infectious diseases, an immune response directed against single, immunogenic proteins may be advantageous^{31, 40}. In addition, this approach does not generally rely on culturing cells from individual patients, and therefore may be more amenable to treating larger numbers of patients in a reproducible fashion. Finally, the identification of proteins specifically involved in the metastatic progression of prostate cancer may permit vaccines to interfere directly with the progression of the disease. The identification of several prostate-specific proteins over the last several years and the ongoing elucidation of the pathways involved in prostate cancer progression, metastasis, and androgen independence make antigen-specific vaccines an attractive strategy for the future development of prostate cancer vaccines.

Several delivery systems for antigen-specific vaccines are being investigated, including recombinant bacterial and viral delivery systems, recombinant proteins in adjuvant, MHC-binding peptide epitopes, the use of various cytokines as immunomodulatory adjuvants, and bacterial plasmid DNA encoding the target antigen. Each of these approaches will be discussed in the context of the specific antigens currently being targeted in human prostate clinical trials.

Prostatic Acid Phosphatase

The identification over the last 10 years of several proteins whose expression is essentially limited to the prostate has provided several candidate antigens for vaccine trials. PAP was first identified in 1938, and was initially used as a serum marker for the detection of prostate cancer^{17, 20}. Given the early identification of a rat homolog, our group and others have used a rodent model to study vaccination strategies targeting this prostate-specific protein. FONG et al.¹⁵ reported that immunizing rats with recombinant vaccinia virus engineered to express rat PAP was not effective in generating an immune response to PAP. By immunizing rats with vaccinia virus expressing the human homologue, however, rats generated a cross-reactive immune response with the generation of PAP-specific CTL and destruction of prostate tissue. In similar studies, we have found that immunizing rats with vaccinia-PAP constructs repeatedly does not generate a PAP-specific response, but immunizing first with vaccinia-PAP and then boost-

ing with PAP protein in adjuvant leads to a Th1 biased cellular response with the generation of destructive prostatitis³⁵. The results of these studies have provided important information for the design of human prostate cancer vaccine strategies. First, an antigen-specific immune response is capable of destroying prostate tissue *in vivo*. Second, the effector cells are predominantly Th1-like with antigen-specific CTL; antigen-specific antibody responses did not result in destructive prostatitis. Third, repeated immunizations with a viral vector do not necessarily lead to an antigen-specific immune response in the case where a "self" protein is targeted most likely because of the overwhelming majority of foreign, more immunogenic proteins introduced by means of viral vaccination. FONG et al.¹⁴ have used this information to initiate a clinical trial targeting PAP by using autologous dendritic cells pulsed *ex vivo* with the recently identified mouse PAP homolog. By this strategy, they preliminarily report that patients develop T cell responses to PAP.

Prostate Specific Antigen

The essentially prostate-specific expression of PSA has made it a natural target for antigen-specific vaccine strategies. We have demonstrated that patients with prostate cancer, and particularly the subgroup of patients with metastatic prostate cancer, develop both a humoral and cellular immune response to PSA³⁷ (McNEEL, submitted). In a series of phase I/II clinical trials, Jenner Biotherapies treated 45 patients with prostate cancer with recombinant human PSA in a lipophilic adjuvant, OncoVax-P. Patients were immunized by a variety of routes (reviewed in²¹), including i.m., i.v., s.c. and i.d. Vaccines were also given in an oil emulsion or with a variety of immunomodulatory agents (BCG, GM-CSF, IL-2 and cyclophosphamide). To date, preliminary results suggest that patients treated develop antibodies to PSA and DTH responses to PSA^{21, 38}.

The success of viral vectors for generating cellular immune responses has led other investigators to use vaccinia or fowlpox as a means of immunizing patients against PSA. HODGE et al.²² reported the construction of a recombinant vaccinia virus expressing human PSA and its safety and efficacy in non-human primates in generating a PSA-specific antibody response. These results have led to a phase I/II trial evaluating the safety and efficacy of a recombinant vaccinia-PSA (PROST-VAC) vaccine construct in patients with stage D0 prostate cancer. SANDA et al.⁴⁷ have recently reported the results of this trial, in which 1 of 6 patients developed

an IgG antibody response to PSA, and one patient had stable serum PSA levels following immunization. The investigators do not report whether patients generate T cell immune responses. Given the limited number of immunizations that can be performed with vaccinia virus due to the overwhelming immune response to the vector itself, investigators have initiated a phase II cooperative study using either recombinant fowlpox-PSA or fowlpox-PSA with vaccinia-PSA in a prime-boost strategy.

There are multiple reports in animal models that immunization strategies using bacterial plasmid DNA alone as the delivery system is a potent means of generating cellular immune responses, particularly CTL responses⁵⁷. Advantages of DNA immunization are that it essentially removes exposure to other competing antigens expressed in viral immunization strategies and is not MHC-restricted as are peptide vaccination strategies. In an animal model, KIM et al.²⁹ have used plasmid DNA encoding human PSA under a eukaryotic expression promoter as a means of immunizing mice. This strategy was effective at generating both humoral and CTL responses specific for PSA. Human prostate cancer vaccine trials using DNA as the antigen delivery system are being contemplated.

One of the limitations to vaccine strategies targeting PSA is that serum PSA levels are increasingly becoming accepted as a surrogate clinical endpoint in prostate cancer treatment trials². Monitoring PSA levels may not be a suitable clinical endpoint in vaccine trials in which immune responses are generated to this protein. Another limitation has been the absence of relevant animal models, since mice and rats do not have a PSA homologue. WEI et al.⁶¹ have reported the development of a transgenic mouse model that expresses human PSA in a prostate-restricted fashion. This model, and similar transgenic animal models, will provide valuable systems to evaluate the safety and potential efficacy of different vaccine strategies targeting PSA and other antigens in a preclinical fashion.

Prostate Specific Membrane Antigen

PSMA was originally identified and cloned as the protein recognized by a monoclonal antibody raised against the human metastatic prostate cancer cell line LNCaP²⁷. Ribonuclease protection studies demonstrated that expression of the protein is nearly restricted to the prostate, and malignant prostate tissue in general highly expresses the protein²⁶. The presence of a membrane-bound, prostate-specific protein suggested it

might be a good protein for diagnostic imaging studies to detect prostate cancer, as well as a target antigen for immunotherapeutic strategies. As described above, G. Murphy and colleagues reported that dendritic cells from prostate cancer patients could be pulsed *ex vivo* with an HLA-A2-restricted peptide epitope from PSMA to generate peptide-specific CTL⁵⁸ and are conducting several clinical trials using dendritic cells to generate immunity to PSMA.

Cancer-Associated Membrane Carbohydrates

Specific membrane-bound carbohydrate moieties, such as GM2, MUC1, globo H and Thompson-Friedenreich antigen, have been found to be expressed preferentially on the surface of a variety of different tumor cells, suggesting that they may be candidate targets for immunotherapeutic approaches³². Investigators at Memorial Sloan-Kettering Cancer Center screened primary and metastatic human prostate tissues compared with a panel of normal tissues by immunohistochemistry and identified several membrane-bound carbohydrate antigens that are overexpressed on malignant prostate tissue compared with normal tissues⁶³. They went on to initiate a phase I vaccine trial in prostate cancer targeting the globo H hexasaccharide, a membrane-bound carbohydrate molecule⁵¹. In this study, patients were immunized subcutaneously monthly for 5 months with globo H hexasaccharide conjugated to the keyhole limpet hemocyanin (KLH) carrier antigen in an immunologic adjuvant, QS-21. A similar immunization strategy had been used previously in patients with melanoma targeting the GM2 ganglioside antigen, with evidence of IgG antibody responses being generated to GM2 with this approach³³. In the globo H study, the investigators report IgM antibody responses to globo H and stable serum PSA slopes compared with pre-treatment PSA slopes in specific patients over a 2-year period, suggesting that such treatment may have a clinical effect. A new study targeting another carbohydrate antigen, the Thompson-Friedenreich antigen, conjugated to KLH in QS-21 adjuvant, is underway, but no results are yet available⁵⁰.

HER-2/neu

HER-2/neu is well documented as a tumor-associated antigen in human breast, ovarian, and colon cancer^{1, 8, 13}. Our group has previously reported that patients with early stage breast cancer have preexistent

antibody and T cell responses to HER-2/neu that are not detected in a control population⁸. These responses are low-level, but suggest that immune responses to HER-2/neu can be generated *in vivo*. Relevant animal models demonstrated that immunization of rats with either the human homologue or with MHC class II-binding peptides derived from rat PAP are capable of eliciting rat *neu*-specific T cell responses¹⁰. In a *neu* transgenic mouse model of human breast cancer, immunization of mice with MHC class II-binding peptides with GM-CSF as an adjuvant is not only capable of eliciting anti-*neu* protein-specific T cell responses, but in protecting immunized animals from developing tumors (unpublished data). These results have led to a human clinical trial targeting HER-2/neu using MHC class II-binding peptides with GM-CSF as an adjuvant. In an interim summary, 8 of 8 patients developed peptide-specific T cell immunity to the immunizing peptides and 6 of 8 developed HER-2/neu-specific T cell immunity⁹.

The role of HER-2/neu in prostate cancer progression has been unclear, with different groups reporting differing findings about the expression of HER-2/neu in primary prostate cancers^{18, 41}. Recent data, however, suggests that HER-2/neu overexpression may permit androgen-independent growth of prostate cancer cells⁴. We have reported that patients with prostate cancer, and in particular the subgroup of patients with androgen-independent prostate cancer, have preexisting antibody immunity to HER-2/neu, similar to HER-2/neu-overexpressing breast cancer (McNEEL, submitted). Given these findings, and based on our animal model data that a CTL response is critical for mediating destruction of prostate tissue, we have initiated a phase I vaccine trial using a well-characterized HLA-A2-restricted 9-mer MHC class I-binding epitope (p369) derived from the amino acid sequence of HER-2/neu^{1, 13}. In this trial, peptide is administered intradermally with either GM-CSF as a vaccine adjuvant, or in the course of flt3 ligand stimulated dendritic cell mobilization. Flt3 ligand is a potent growth and differentiation factor for dendritic cells, and consequently the purpose of the study is to evaluate whether dendritic cells expanded *in vivo* are capable of properly presenting peptide antigen to generate an antigen-specific CTL response.

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