Drugs of the Future 2011, 36(10): 771-775

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.10.1688500

REVIEW ARTICLE

THE USE OF TUMOR-ASSOCIATED ANTIGENS IN PROSTATE CANCER VACCINES

R.J. Amato[1] and M. Stepankiw[2]

[1]Division of Oncology, Department of Internal Medicine, University of Texas Health Science Center at Houston (Medical School), Memorial Hermann Cancer Center, Houston, Texas, USA; [2]Division of Oncology, Department of Internal Medicine, University of Texas Health Science Center at Houston (Medical School), Houston, Texas, USA

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SUMMARY

The lack of effective therapies for metastatic prostate cancer and the toxicity profiles of current prostate cancer therapies have created the need for continued development of immunogenic treatments with a favorable toxicity profile. Immune-based strategies target specific cancer antigens to help create a more personalized approach to achieve cancer control without significant toxicity. A variety of antigen-specific cancer vaccine trials of different phases have been conducted. Although the underlying mechanisms behind each vaccine vary, all immunogenic approaches have the same goal of eliciting a prostate tumor-associated antigen response. Currently, only one vaccine has been approved by the FDA; however, further developments may lead to the approval of more vaccines in the near future. This review provides an overview of vaccine-based strategies with details about their underlying mechanisms and relevant clinical trial data.

INTRODUCTION

Prostate cancer is the most common noncutaneous cancer in men and the second leading cause of cancer-related deaths in men in the U.S. (1). Approximately one in six men will be diagnosed with prostate cancer during their lifetime (2). Early-stage therapies such as prostatectomy and radiation therapy are successful in about 80% of patients. Patients who fail early-stage therapies or who have advanced stages of prostate cancer undergo hormonal therapies. Unfortunately, when hormonal therapy fails as well and patients reach the castration-resistant prostate cancer (CRPC) disease state, there are limited successful treatment options available. All of the current treatment options available for CRPC are palliative, with a median survival of less than 2 years (3).

The approval by the U.S. Food and Drug Administration (FDA) of docetaxel, which has been shown to extend life expectancy by 2 months, in April 2004 represented a significant milestone in the treatment of metastatic prostate cancer. Because docetaxel is a cell cycle-specific agent, it is cytotoxic to all dividing cells and not just tumor cells. This leads to some of the common side effects of docetaxel, including neutropenia, anemia, neuropathy, alopecia and nail damage. In addition to the side effects, some patients fail or are ineligible for docetaxel-based therapy. Therapeutic strategies with a more favorable toxicity profile are therefore needed for the management of disease progression. A prostate cancer vaccine is a promising alternative therapy that aims to eradicate tumors with tumor- or tissue-specific antigens, thereby targeting specific cancer cells (4).

Advancements in the molecular identification of prostate-specific or prostate cancer-associated antigens that can be recognized by T cells and recent studies on the regulation of immune responses and processes have facilitated the development of potent vaccination protocols. Cancer vaccines stimulate antitumor immune responses in patients by preparing the induction of T cells. These T cells bind to tumor-associated antigens (TAAs), which are sequences of 9-10 amino acids attached to proteins or carbohydrates. TAA epitopes are expressed on tumor cells but are not expressed or are expressed in reduced quantities on normal cells, and they contain the major histocompatibility complex (MHC). Each cytotoxic T-cell receptor recognizes and binds to its cognate MHC–antigen complex, which ensures that T cells are able to recognize subtle changes in the antigens expressed by somatic cells.

Prostate cancer is generally considered a slow-growing tumor, which may allow adequate time for a vaccine to activate the immune system by recognizing TAAs. Optimal TAAs are specific to the cancer and are either not expressed or are minimally expressed in healthy tissue and essential organs. Cancers contain many epitopes of particular interest for the development of anticancer vaccines. The antigens expected to work best on prostate cancer include prostatic acid phos-

Correspondence: Robert J. Amato, D.O., The University of Texas Memorial Hermann Cancer, 6410 Fannin St., Suite 830, Houston, TX 77030, USA. E-mail: robert.amato@uth.tmc.edu.

phatase (PAP), prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA; glutamate carboxypeptidase 2), which are expressed on endothelial cells, including tumor-associated endothelial cells, and have limited expression in normal tissues (5).

Because patients in the early stage of CRPC often present with low tumor burden and a long anticipated lifespan, they are ideal candidates for vaccine therapy. Cancer vaccines are currently in the stage of active preclinical and clinical investigation. To date, only one therapeutic cancer vaccine, sipuleucel-T (Provenge®), has been approved by the FDA for the treatment of prostate cancer.

PEPTIDE AND PROTEIN VACCINES

Antigenic peptides and proteins can stimulate T-cell activation with or without immune-activating adjuncts. Peptide and protein vaccines primarily focus on the MHC human leukocyte antigen (HLA) class I-binding antigens PSA or PSMA. Many peptide and protein vaccines use a broad spectrum to target multiple epitopes. The best-characterized antigen for the creation of a prostate cancer vaccine is PSA. Several PSA-derived vaccines have demonstrated the ability to induce specific cytotoxic T lymphocytes in vitro (6) (Table I). However, the issue with PSA-derived vaccines is that PSA is a secreted protein and thus it is not exclusively present on tumor cells.

Some vaccines focus on the PAP antigen, which is an enzyme produced by the prostate, to target malignant cells. PAP is typically only expressed in normal and malignant prostate tissue. Previous studies have demonstrated in rats that genetic vaccines targeting PAP can result in antigen-specific $CD8^+$ T-cell activation (7, 8). One phase I/IIa trial found that the use of a DNA vaccine encoding PAP elicited an immune response, with an increase in PSA doubling time and no significant adverse events (7). Patients developed PAP-specific interferon gamma (IFN-γ)-secreting $CD8^+$ T cells, PAP-specific $CD4^+$ and/or $CD8^+$ T-cell proliferation.

Sipuleucel-T, the first prostate cancer vaccine to receive FDA approval, is comprised of autologous dendritic cells (DCs) that are activated in vitro with the recombinant fusion protein PA2024, consisting of PAP-linked to granulocyte–macrophage colony-stimulating factor (GM-CSF). The patient undergoes apheresis to collect the cells by co-culturing antigen-presenting cells (APCs) for 36-44 hours in media containing PA2024. The cellular product contains at least 50 million $CD54^+$ APCs and 60-70% lymphocytes and other mononuclear cells, which may contribute to the clinical activity. Higano et al. published a study in which 225 patients with CRPC were included in a randomized trial and treated with sipuleucel-T or placebo in a 2:1 ratio. Patients received three i.v. infusions approximately 2 weeks apart. The study found that patients who received the sipuleucel-T vaccine demonstrated a 33% reduction in the risk of death. Toxicity consisted primarily of grade 1 or 2 chills, pyrexia, headache, asthenia, dyspnea, vomiting and tremor, which lasted for a duration of 1-2 days (9).

A phase I/II study conducted by Waeckerle-Men et al. evaluated multi-epitope DC vaccination to determine if this approach is feasible and generates efficient cellular antitumor responses (3). Six patients with CRPC were enrolled in the study. The DCs were pulsed with influenza virus matrix protein (FluM) and the peptides prostate stem cell antigen (PSCA), PAP, PSMA and PSA. A total of six vaccinations were given biweekly followed by a monthly booster injection until the patient was taken off the study, which lasted approximately 6 months. Two patients were removed from the study —one for application of high-dose steroids for a suspected spinal compression and the other due to severe pyelonephritis. Of the remaining four patients, three showed significant antitumor immune responses

Table I. *Prostate cancer vaccine trials..*

Investigator	Patients (no.)	Type of vaccine	Response
Thomas-Kaskel et al. (6)	12	PSCA- and PSA-loaded DCs biweekly	6 patients SD, 1 CR Median OS of 13.4 months
McNeel et al. (7)	22	PAP vaccine	PSA doubling time increased from 6.5 months pretreatment to 8.5 months on treatment, and 9.3 months at 1 year
Higano et al. (9)	225	Sipuleucel-T or placebo	33% reduction in the risk of death
Feyerbend et al. (10)	19	Multi-epitope vaccine	PSA doubling time increased from 4.9 to 25.8 months
Fuessel et al. (11)	8	Multi-epitope vaccine (prostein, survivin and PSMA)	3 patients had $CD8^+$ T-cell activation against prostein, survivin and PSMA
Kantoff et al. (12)	122 (40 control)	PROSTVAC® or placebo	Median OS 8.4 months; 44% reduction in death rate
Amato et al. (13)	24	MVA-5T4 or MVA-5T4 + GM-CSF	All patients had a 5T4-specific response TTP 5.6 months
Arlen et al. (15)	28	MVA + 5T4 + docetaxel or docetaxel alone	Median PFS 6.1 months on docetaxel + MVA-5T4

SD, stable disease; CR, complete response; OS, overall survival; TTP, time to progression; PFS, progression-free survival; PSA, prostate-specific antigen; PAP, prostatic acid phosphatase; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; DCs, dendritic cells; GM-CSF, granulocyte–macrophage colony-stimulating factor.

after six vaccinations and the other discontinued therapy due to lack of general immune reactivity against the vaccine. The vaccine was essentially free of side effects. The maximal T-cell response against the tumor antigens was only 30-50% less than the control vaccine FluM. This study showed that simultaneous pulsing of multiple prostate-specific peptides on one DC population creates a vaccine that is capable of inducing a broad antitumor T-cell response.

A second randomized, single-center, open-label phase I/II study examined a multi-epitope cocktail in 19 HLA-positive patients who received 6 vaccinations with adjuvant therapies. A clinical response was observed in eight of nine patients, with PSA doubling time improving in four cases (10). A second study of a multi-epitope DC vaccine used a cocktail consisting of HLA-restricted peptides derived from PSA, PSMA, survivin (baculoviral IAP repeat-containing protein 5), prostein (solute carrier family 45 member 3) and transient receptor potential p8 (transient receptor potential cation channel subfamily M member 8). Eight patients received four vaccinations biweekly and no side effects were noted outside of local skin reactions. Four patients showed a PSA response, and three of these had antigen-specific $CD8^+$ T-cell activation against prostein, survivin and PSMA. The study suggests that the application of a multi-epitope peptide cocktail allows for genetic and biological heterogeneity of prostate cancer (11).

PROSTVAC® is a therapeutic prostate cancer vaccine that is currently entering the final stages of clinical development. Its underlying mechanism relies on a PSA in combination with two poxviruses plus three immune-enhancing co-stimulatory molecules. A randomized, controlled, blinded phase II study evaluated PROSTVAC® for safety and prolongation of progression-free survival and overall survival (12). The study involved 125 patients with minimally symptomatic castration-resistant metastatic prostate cancer, of whom 82 were randomly chosen as the PROSTVAC® group and 40 were randomly assigned to the control group. Although no association was found between the treatment group and progression-free survival, a strong association was found between the treatment group and overall survival. Median overall survival was 8.5 months and the treatment was associated with a 44% reduction in death rates. However, potential limitations of the study included the 2:1 ratio of treatment group versus control group and limited power for the secondary endpoint. The finding of improved overall survival without a difference in progression-free survival mirrors findings in the sipuleucel-T study described earlier.

Another focus of prostate cancer vaccines is a novel prostate-specific protein known as PSCA. PSCA has high tissue specificity and is expressed on more than 85% of prostate cancer specimens, with expression levels increasing with higher Gleason scores and androgen independence (6). Because PSCA is not shed from the cell membrane, it may be a promising option for a prostate cancer vaccine. Thomas-Kaskel et al. conducted a phase I/II trial involving 12 patients who received 4 vaccinations with peptide-loaded mature DCs in 2-week intervals to determine the safety of a PSCA-targeted vaccine. The patients experienced no evidence of toxicity. Six patients achieved stable disease. One patient had complete regression of retrovesical lymph node, but had rising PSA, and thus was counted as having achieved progressive disease. The median survival of patients with delayed-type hypersensitivity responses was 22 months. The PSCA vaccine has been shown to induce tumor-reactive cytotoxic T cells in vitro.

Modified vaccinia Ankara (MVA) induces potent immune responses with a favorable safety profile. TroVax® utilizes the 5T4 tumor antigen combined with vaccinia virus. The human oncofetal antigen 5T4 is expressed at high levels in the placenta and in a wide range of carcinomas, including prostate, renal and colorectal cancers, but is rarely expressed in healthy tissues (13). This restricted expression of 5T4 makes it an attractive target for cancer immunotherapy development. Preclinical studies show that TroVax® induced an immune response in mice, and phase I and II clinical trials in colorectal cancer patients have shown it to be safe and well tolerated when administered alone or in combination with chemotherapy (14).

A phase II trial conducted by Amato et al. involved 27 patients with CRPC —14 of whom received TroVax® alone and 13 TroVax® + GM-CSF (13). Only 24 patients were evaluable, but all 24 achieved 5T4-specific antibody responses with the peak titer/patient ranging from 80 to 10,240. Three patients had shown a weak 5T4 antibody response before TroVax® was administered, but all showed significant increases after vaccination, with peak titers ranging from 640 to 10,240. There were no grade 3 or 4 toxicities, and patients injected with GM-CSF experienced myalgia, bone discomfort, low-grade temperature elevation and injection-site irritation. This study demonstrated that TroVax® can be given safely alone or in combination with GM-CSF, and 5T4-specific immune responses were induced in all patients after vaccination; however, the addition of GM-CSF to TroVax® had no demonstrable overall benefit.

TroVax® is currently in phase II development in an open-label study at the NYU Clinical Cancer Center in 80 patients with metastatic CRPC that will assess its activity in combination with docetaxel versus docetaxel alone. One study conducted by Arlen et al. found that docetaxel can be safely administered with a weekly vaccinia-based vaccine (15). Patients experienced grade 2 or less toxicity, with only one instance of grade 3 lymphopenia. The study also found that patients who have previously received anticancer vaccinations may respond longer to docetaxel in comparison to patients who received docetaxel alone.

Research indicates that peptide and protein vaccines are viable options for creating a vaccine for CRPC. Further research will help identify appropriate proteins and peptides, as well as the dose level that will be most effective in targeting tumor cells.

DNA

Another focus of vaccines is to inject recombinant plasmid DNA encoding the antigen of choice. Direct intradermal or intramuscular delivery of plasmid DNA leads to the generation of both humoral and cellular immune responses and protective immunity. DNA vaccines are antigen-specific yet cost little to produce, making them a viable alternative; however, DNA vaccines tend to generate a relatively poor immune response in comparison to other vaccine approaches (14). DNA prostate cancer vaccines tend to target PSA or PSMA to induce an immunogenic response. DNA vaccines can be prepared relatively quickly and have been shown to be safe in several murine and human trials.

MULTIPLE ANTIGEN TARGETING

DCs are a crucial component in the induction of a potent antitumor response, as these cells present tumor antigens to T cells. All class

I- or class II-restricted epitope antigens require processing by DCs to activate the T-cell-mediated immune response (16). DCs prime and activate both $CD4^+$ and $CD8^+$ cells. To date, no significant adverse events have been experienced during DC vaccine trials, and an immunogenic response has been demonstrated. DC-based vaccines have been shown to elicit a significant immune response against PSMA, PSA, PAP or telomerase reverse transcriptase. This multi-epitope approach may be able to overcome the ability of tumor cells to evade immune recognition by downregulating antigen expression (17) or altering antigen processing (18). DCs may be the most promising strategy because of their ability to activate innate and adaptive immunity. However, only a fraction of patients enrolled in clinical trials using DCs have demonstrated a potent immune response. Since whole tumor cells express an array of antigens, a multi-epitope approach may circumvent the MHC restriction and the need for patients to have antigen-specific vaccines. However, this approach may have limited effectiveness due to the immune system's inherent tolerance to several tumor antigens and the expression of these antigens on healthy tissue.

CONCLUSION

Currently, the only prostate cancer vaccine so far to have gained FDA approval is sipuleucel-T (Provenge®). TroVax® is currently in phase II trials for treatment of prostate cancer, and PROSTVAC® is in phase III trials and FDA approval is being sought. Cancer vaccines are a powerful potential treatment for prostate cancer of all levels. These vaccines offer an immunogenic response with less toxicity than other treatments and are tumor-specific. New advancements in the identification of specific antigens and research into immunogenic treatments that target epitopes for use in vaccines is bringing us one step closer to achieving tumor-specific vaccines that will not target healthy cells. However, further research on the molecular and cellular mechanisms that regulate antitumor immunity and the malignant process will allow for further development of vaccines using more potent PSAs and new protocols to use as vaccines alone or in combination with adjuvant therapies.

FUTURE PERSPECTIVES

Immunotherapy for human cancers such as prostate cancer has largely focused on immunization with peptides or whole antigens, intact tumor cells or DCs pulsed with antigenic peptides under normal immune surveillance. The immune system recognizes and destroys tumor cells through the coordinated actions of DCs and lymphocytes. DCs are professional APCs that recognize TAAs, process them into small peptides and then present these peptides to T cells within the context of MHC. If this presentation is made in the presence of appropriate co-stimulatory molecules, it leads to clonal expansion of activated T cells and generation of cytotoxic T cells that mediate specific tumor immunity.

Unfortunately, tumor cells are able to evade immune surveillance by creating a microenvironment that suppresses cytotoxic T-cell mechanisms or by altering or masking the expression of TAAs. Prostate cancer vaccines have been developed and evaluated in an effort to make tumor cells more immunogenic and thereby overcome their defense mechanisms. Immunological strategies such as the use of blocking antibodies to cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or GM-CSF, or cyclophosphamide, are being combined with these immunization approaches to influence the immune system and potentially reveal greater antitumor activity in comparison to the vaccine alone. Furthermore, trials in combination with chemotherapy and prostate-related vaccines are in progress based on the hypothesis that the chemotherapy will influence the suppression of T regulatory cells and allow more antitumor activity associated with the vaccine approach.

DISCLOSURES

The authors state no conflicts of interest.

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