Phase I Clinical Trial of an Adenovirus/Prostate-Specific Antigen Vaccine for Prostate Cancer: Safety and Immunologic Results

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Abstract

Purpose: We performed a phase I clinical trial of adenovirus/prostate-specific antigen (PSA) vaccine in men with measurable metastatic hormone-refractory disease.

Experimental Design: Men with measurable metastatic disease received one vaccine injection. Toxicity, immune responses, changes in PSA doubling times, and patient survival were assessed. Thirty-two patients with hormone-refractory metastatic prostate cancer were treated with a single s.c. vaccine injection at one of three dose levels, either as an aqueous solution or suspended in a Gelfoam matrix. All patients returned for physical and clinical chemistry examinations at regular intervals up to 12 months after injections.

Results: The vaccine was deemed safe at all doses in both administration forms. There were no serious vaccine-related adverse events; the most prevalent were localized erythema/ecchymoses and cold/flu-like symptoms. Anti-PSA antibodies were produced by 34% of patients and anti-PSA T-cell responses were produced by 68%. PSA doubling time was increased in 48%, whereas 55% survived longer than predicted by the Halabi nomogram.

Conclusions: The adenovirus/PSA vaccine was proven safe with no serious vaccine-related adverse events. The majority of vaccinated patients produced anti-PSA T-cell responses and over half survived longer than predicted by nomogram. Although the latter data are only derived from a small number of patients in this phase I trial, they are encouraging enough to pursue further studies. (Clin Cancer Res 2009;15(23):7375–80)

We have previously shown that immunizations with adenovirus (Ad) carrying the human prostate-specific antigen (PSA) gene can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a preclinical mouse model of prostate cancer (1, 2). Such active immunization against prostate cancer–associated antigens might be more effective than active nonspecific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an Ad that carries the gene for PSA. In preclinical studies, our group has shown that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines.[6] These include vaccinia viruses, both replication competent and deficient, and canarypox. The frequency of PSA-specific CD8+ T cells generated by the Ad/PSA vaccine was greater than were generated by any other vaccines tested. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam (Pharmacia and Upjohn), a collagen matrix, has been shown in preclinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (1). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high-titer anti-Ad antibodies (1). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-Ad antibodies as a result of prior natural exposure to the virus.

We initiated a phase I clinical trial of the Ad/PSA vaccine in men with measurable hormone-refractory prostate cancer (3). This was a dose escalation trial, with the vaccine injected s.c. in either an aqueous suspension or collagen matrix. Our primary

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Translational Relevance

We report the results of a phase I clinical trial using an adenovirus/prostate-specific antigen (Ad/PSA) vaccine for the treatment of prostate cancer. Preclinical studies showed the efficacy of the Ad/PSA vaccine by inducing anti-PSA responses and destruction of tumors. Our phase I clinical trial included 32 patients with hormone-refractory metastatic prostate cancer, who were treated with a single s.c. injection at one of three dose levels of the Ad/PSA vaccine (10^6, 10^7, 10^8 plaque-forming units) either in fluid phase or collagen matrix. The results of the trial established the safety of the vaccine with no serious adverse events. Examination of the immune response following vaccinations showed the presence of anti-PSA antibodies in 34% of patients and anti-PSA T-cell responses in 68% of patients. We showed an increase in PSA doubling times for 54% of study subjects and 55% of subjects survived longer than predicted by nomogram calculations.

objectives were to evaluate the development of toxicity to determine the maximum tolerated dose of vaccine in patients with both biochemical and clinical evidence of metastatic prostate cancer. Secondary objectives included the evaluation of development of anti-PSA immune responses in patients, and the assessment of any clinical impact of the vaccination such as changes in serum PSA levels, measurable disease, or survival. We report here (*a*) the absence of any substantive vaccine-related adverse events (AE), (*b*) the development of anti-PSA immune responses, and (*c*) in a subset of patients, an increase in PSA doubling time (PSADT), and (*d*) a prolonged survival.

Materials and Methods

This study was reviewed by the U.S. Food and Drug Administration (IND #9706), the University of Iowa Institutional Review Board, and was under surveillance by the Data Safety Monitoring Committee of the University of Iowa Holden Comprehensive Cancer Center and in accordance with an assurance filed with and approved by the Department of Health and Human Services. The study was an investigator-initiated trial as a direct extension of preclinical studies (1, 2).

Study patients. Patients had histologically confirmed adenocarcinoma of the prostate with evidence of metastatic disease. The pathology of the primary tumor or metastatic site of each patient was reviewed by the Department of Pathology at the University of Iowa Hospitals and Clinics. Protocol required that the disease be measurable as evidenced by one or more of the following positive results: bone scan, abdominal-pelvic computed tomography, chest X-ray or other standard radiologic techniques, as well as an increase in serum PSA levels. A PSA increase alone in the absence of other evidence of disease was insufficient for inclusion in this study. Evidence of hormonal independent growth and progression of disease was obtained by the detection of an increase in levels of serum PSA and progressive clinical features, such as a change in one or more radiologic exams. All patients had failed both first-line (radical prostatectomy or radiation) and second-line (radiation, androgen deprivation, and/or chemotherapy) treatments. Eligible patients had normal renal, hepatic, and hematologic functions; no unresolved infections; no parenteral antibiotics at least 7 d before study entry; no known clinical signs or symptoms of central nervous system metastases; no comorbid medical conditions that could result in a life expectancy of <1 y; no compromised immune system, either congenital or acquired, or immunosuppressive therapies; and no pre-existing malignancies that required treatment within the past 5 y except for basal or squamous cancers of the skin.

All patients were registered through the Clinical Trials Office of the University of Iowa Hospitals and Clinics. Patients who met eligibility criteria were enrolled and randomized to either the s.c. aqueous or s.c. matrix groups at each single dose of virus. This is similar to the method in a study by Conry et al. (4) in which the investigators compared two routes of injection for a vaccinia–carcinoembryonic antigen vaccine.

Vaccinations. The initial group of patients was randomized to receive 1×10^6 plaque-forming units (pfu). For the matrix-vaccine injections, the virus was suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per milliliter of virus suspension. All vaccines for injection were prepared by the University of Iowa Hospitals and Clinics Investigational Pharmacist and administered s.c. in the right thigh in a volume of 0.125 mL by the physician's assistant in the University of Iowa's General Clinical Research Center. The study groups consisted of patients that received vaccine doses that ranged from 10^6 to 10^8 pfu, administered either as an aqueous suspension or in a Gelfoam collagen matrix. Groups that received the lower doses of 10^6 or 10^7 pfu contained three patients each in the aqueous and matrix groups, whereas the 10^8 pfu groups contained 9 (aqueous) and 11 (matrix) patients. Each patient was housed and monitored overnight in the General Clinical Research Center to ascertain whether any acute AEs developed in the first 24 h of injection. Clinical evaluation before and for 24 h after injection consisted of monitoring vital signs, liver function, electrolytes, and complete blood counts. Each patient returned for further testing at 14 and 21 d, and 2, 4, 8, and 12 mo after vaccination. Sera and peripheral blood lymphocytes were collected at each evaluation for immunologic testing. Also, a series of tests were done to monitor for toxicity that included physical examination, complete blood count, liver and kidney function, diagnostic imaging, and electrocardiogram (3). If no significant toxicities were detected the initial dose group, the next group received the next highest dose (1×10^7 pfu), again randomized to s.c. aqueous or s.c. matrix, and the dose escalation and randomization continued until we either reached a maximum tolerated dose or the highest dose permitted by the Food and Drug Administration (1×10^8 pfu).

Antibody measurements. Serum was separated from clotted blood, stored at -80°C and tested for anti-PSA antibodies using a variation on the method of Cavacini et al. (5). Briefly, cells from the PSA-secreting E5 clone of the mouse prostate tumor RM11/PSA were incubated with serial dilutions of patients' sera, counterstained with FITC-conjugated anti-human immunoglobulin, and analyzed by flow cytometry for positive staining. Positive control serum was a polyclonal anti-PSA antibody (Dako North America, Inc.), and negative control serum was from pooled child samples (obtained from University of Iowa Department of Pediatrics). The last dilution of patient serum that showed positive staining was considered the antibody titer.

T-cell analysis. Anti-PSA T-cell immune responses were detected by ELISPOT analysis. Lymphocytes were separated from heparin anticoagulated peripheral blood using Fico/Lite-LymphoH (Atlanta Biological, Inc.), and the cells stored in cryopreservative solution (90% autologous serum, 10% DMSO) in liquid nitrogen. After all samples were collected, individual patient's samples for the entire 12-mo period were rapidly thawed and analyzed by ELISPOT for production of IFNγ. Briefly, ELISPOT plates (Whatman) were coated with the captured anti-IFNγ antibody (BD-Pharmingen). The plates were blocked and cells added at 5×10^5 cells per well in a volume of 100 μL. The following stimulants were added to appropriate wells: purified PSA (20 μg/mL), cytomegalovirus (CMV) extract (20 μg/mL; Microbix Biosystems), phorbol 12-myristate 13-acetate + ionomycin (7.5 ng/mL each; Sigma Aldrich), or medium alone. The phorbol 12-myristate 13-acetate + ionomycin stimulation acted as a control for the ability of cells to

Table 1. Summary of patient population

No. patients	32
Mean age, y (range)	71 (52-89)
Mean enrollment PSA, ng/mL (range)	128 (1.31-3,110)
Median follow-up, mo (range)	12 (2-12)
Median survival, mo (range)	18 (2.5-35.5)

respond to a nonspecific stimulus, the CMV as a positive control for a response to an antigen receptor–mediated stimulus, and the PSA was the experimental stimulus. A positive control of lymphocytes from a male volunteer who was CMV positive and a negative control from a female volunteer who was CMV negative were used in all assays. The ELISPOT plates were incubated in a 37°C incubator for 48 h, washed, and incubated first with biotin anti-human IFNγ followed by strepatvidin–horseradish peroxidase (Zymed Laboratories), and then AEC substrate solution (Vector Laboratories). After incubations, the plates were washed, air-dried, and analyzed in an ImmunoSpot Analyzer (Cellular Technologies).

Clinical assessment. Serum PSA levels were analyzed at the University of Iowa Department of Pathology clinical laboratories. PSADTs were calculated for each patient using the following equation: PSADT = $log2 \times dT/(logB - logA)$; A and B are the initial (A) and final (B) PSA measurements, and dT is the time difference between the calendar dates of the two PSA measurements. One of our initial objectives was to determine the effect of the vaccination on the prostate cancer of each patient by the use of computed tomography and bone scans, but these were not quantitative enough for meaningful data. Therefore, we determined the effect of vaccination on patient survival. We calculated expected survival in months using an accepted nomogram (6) and compared the value for each patient to actual survival.

Results

Patient characteristics. Thirty-two patients with measurable metastatic hormone-refractory disease were treated with one dose each, in groups with escalating doses of Ad/PSA vaccine as an aqueous suspension or collagen matrix (Table 1). The mean age of all patients was 71 years (range, 52-89), mean serum PSA level at enrollment was 128 ng/mL (range, 1.31-3,110 ng/mL), mean follow-up was 12 months (range, 2-12), and mean survival was 18 months (range, 2.5-35.5).

Treatment-related toxicities. No serious AEs were reported (Table 2). Ecchymoses, erytherma, and pain at the injection site were noted in nine patients and constituted 28.1% of AEs, all of which were grade 1. The next most frequent vaccine-related events included decrease in WBCs, either lymphocytes or neutrophils (one grade 1 each), cold/flu-like symptoms (one grade 1 and one grade 2), fatigue (two grade 1), and proteinuria (two grade 1). All other events were only observed in one patient each. No vaccine-related grades 3 to 5, deaths, or irreversible AEs were observed, and most were resolved within 48 hours.

Anti-PSA immune responses. Sera from patients were assayed for the presence of anti-PSA antibodies using a modification of the flow cytometry method of Cavacini et al. (5). Dilutions of 1:2, 1:20, and 1:200 were run with a negative control of the secondary fluorochrome-conjugated antibody. Responses were deemed positive if any of the dilutions showed a shift to the right of the flow cytometry peak. Table 3 contains the results of our antibody study.

Although the number of patients in this phase I study is small, it is interesting to note that a larger number of patients injected with the vaccine as an aqueous suspension developed measurable anti-PSA antibodies than did patients injected with the vaccine in the collagen matrix. Fifty-eight percent of the aqueous vaccine patient population had positive responses, compared with 10% of the collagen matrix vaccine patient population. Overall, 34% of all patients had measurable anti-PSA antibody levels above those detected before vaccination.

T-cell immune responses were analyzed by ELISPOT, measuring the number of IFNγ-secreting cells. Stimulation with phorbol 12-myristate 13-acetate and ionomycin was used in all assays to determine the ability of cells from each patient, previously cryopreserved, to respond to polyclonal stimuli. The ability of patient cells to respond to receptor-mediated signals was tested by the response to CMV. PSA-specific responses were tested using 20 μg of purified PSA. Lymphocytes from a volunteer with known anti-CMV activity were used as positive reactive cells and cells from a CMV-negative female volunteer were used as negative reactive cells in all assays. Table 4 shows T-cell responses of all evaluated patients. In contrast to the antibody data, more positive T-cell responses were seen in patients receiving

Table 2. AEs, judged to be related or possibly related to Ad/PSA vaccine

Event	Grade 1	Grade 2	Grades 3-5	Total
Anemia	2	0	0	2
Injection site irritation, pain	11	0	0	11
Flu, cold-like symptoms	1	1	0	2
Decreased WBC (lymphocytes, neutrophils)	2	0	0	2
Fatigue	2	0	0	2
Fever	1	0	0	1
Hyperglycemia	0	1	0	1
Hyponatremia	1	0	0	1
Hypotension	1	0	0	1
Increased alkaline phosphatase	1	0	0	1
Increased AST	1	0	0	1
Ketonuria	0	1	0	1
Inguinal pain	1	0	0	1
Proteinuria	2	0	0	2

Abbreviation: AST, aspartate aminotransferase.

Table 3. Summary of anti-PSA antibody analysis

Dose	Vehicle	Positive	Antibody titers
10^6	Aqueous	67%	1:20; 1:100
10^6	Matrix	0%	—
10^7	Aqueous	50%	1:200
10^7	Matrix	0%	—
10^8	Aqueous	57%	1:20-1:200
10^8	Matrix	30%	1:20-1:200
Aqueous—all doses		58%	
Matrix—all doses		10%	
All patients		34%	

the Ad/PSA vaccine in collagen matrix (77%) than in patients receiving the vaccine as an aqueous suspension (57%).

Clinical responses to vaccination. Analysis of the effect of Ad/PSA vaccination was accomplished by examining changes in PSADT and by calculating the change in patient survival compared with predicted survival using the Halabi nomogram (Table 5; ref. 6). Although the number and percent of patients with increased or decreased PSADT were virtually identical, the number and percent were quite different for patients receiving the vaccine in collagen matrix versus aqueous suspension. More patients vaccinated in collagen matrix had increased PSADT (57%) than did patients vaccinated in aqueous suspension (36%), although with few patients in each group, statistical significance is not possible to ascertain. When analyzing any change in patient survival (Table 6) in the cohorts vaccinated with Ad/PSA in either administration, the data show that about half of all patients survived longer than predicted by nomogram, with about equal numbers of patients in the two administration groups (8 of 16 versus 9 of 15, respectively). Three patients survived almost 4 years longer than the prediction (45, 46, and 47 months), whereas the shortest survival time was 12.5 months shorter than predicted.

Immunologic and clinical data correlation. In an attempt to determine whether any of the measurements correlated in this phase I trial, we compared the data for PSADT, survival, anti-PSA antibody, and T-cells responses. It seems that antibody responses correlated more with increases in PSADT than did T-cell responses, where 55% of the patients that developed anti-PSA antibodies had increases in their PSADT, whereas only 32% of the patients that developed positive anti-PSA T-cell responses had increases. In contrast, increased survival of patients correlated more with the production of anti-PSA T-cell responses, where 60% of the patients with positive anti-PSA T-cells responses survived longer than predicted, whereas 44% of patients with positive anti-PSA antibodies had a longer survival time. Although these data are certainly encouraging, they are based on a small number of patients who were typically treated in a phase I toxicity study and only received one dose of the vaccine. Further testing in a larger patient cohort will be required to validate these findings.

Table 4. Summary of anti-PSA T-cell responses by ELISPOT

Dose	Medium	No. evaluated	No. positive	% Positive
10^6	aqueous	3	1	33
10^6	matrix	3	3	100
10^7	aqueous	3	1	33
10^7	matrix	3	2	67
10^8	aqueous	8	6	75
10^8	matrix	11	8	73
Aqueous—all doses		14	8	57
Matrix—all doses		17	13	77
All patients		31	21	68

Table 5. Summary of changes in PSADTs

Dose	Vehicle	Percent with decreased PSADT	Percent with increased PSADT
10^6	Aqueous	2/3 (67%)	1/3 (33%)
10^6	Matrix	2/3 (67%)	1/3 (33%)
10^7	Aqueous	1/3 (33%)	2/3 (67%)
10^7	Matrix	1/3 (33%)	2/3 (67%)
10^8	Aqueous	6/8 (75%)	2/8 (25%)
10^8	Matrix	3/8 (38%)	5/8 (63%)
Aqueous—all doses		9/14 (64%)	5/14 (36%)
Matrix—all doses		6/14 (43%)	8/14 (57%)
All patients		15/28 (54%)	13/28 (46%)

Discussion

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (5, 7–14), prostatic acid phosphatase (15–18), prostate-specific membrane antigen (19–21), telomerase (22, 23), Thomsen-Friedenreich antigens (24), mucins (25), carbohydrates (26), and HLA-associated peptides (27). A variety of vectors have been used in the immunization process: dendritic cells (10, 15–23, 28), vaccinia virus (5, 7, 8, 12, 14), fowlpox virus (5, 12), liposomes (9), plasmids, (13), and chemical conjugates (24–26).

Table 6. Summary of survival times compared with expected

Dose	Vehicle	Number with longer survival	Percent with longer survival
10^6	Aqueous	1/3	33
10^6	Matrix	2/3	67
10^7	Aqueous	3/3	100
10^7	Matrix	1/3	33
10^8	Aqueous	5/9	56
10^8	Matrix	5/10	50
Aqueous—all doses		9/15	60
Matrix—all doses		8/16	50
All patients		17/31	55

NOTE: Expected survival times calculated using Halabi nomogram (6).

The results from previous trials vary in terms of patient populations studied (hormone dependent versus hormone independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses (29–31). To date, no single vaccine immunotherapy has proven definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the possibility that an increase in PSADT may represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al. (31). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine.

Anti-PSA immune responses were detected in 50% or more of our patients, including antibody and/or T-cell responses. An interesting association of injection vehicle and immune response was noted. A higher number of patients vaccinated with aqueous vaccine developed anti-PSA antibody responses compared with patients vaccinated with the matrix vaccine. The opposite seemed true for anti-PSA T-cell responses, with matrix-injected patients demonstrating more cellular responses than did aqueous-injected patients. Also interesting is the finding that antibody responses correlated more with increases in PSADT than with patient survival, whereas T-cell responses correlated with survival. Conclusions from the secondary objectives of generating anti-PSA antibody and T-cell reactivity and from clinical responses as measured by changes in PSADT and survival times are tenuous due to the small number of patients enrolled in this phase I study. Any verification of the observations must wait for the completion of additional studies.

It is important to keep in mind that the generation of antitumor immune responses that may have therapeutic benefits are not only dependent on the use of strong immunogens such as a viral vaccine carrying the transgene for a tumor-associated antigen, but also on the ability to overcome negative regulatory elements. These latter conditions include the breaking of immune tolerance to the antigen as well as the effects of regulatory cells and molecules that include, but not confined to, regulatory T cells (32), myeloid-derived suppressor cells (33), indolamine dioxygenase (34), and arginase (35).

To determine whether vaccination of prostate cancer patients with the Ad/PSA vaccine will result in a therapeutic benefit, we have recently initiated a phase II trial of the vaccine in men with recurrent prostate cancer. Two different patient populations will be enrolled into one of two protocols in the phase II study. In the first protocol patients with newly recurrent prostate cancer, as determined by a continuous increase in serum PSA, will be enrolled into one of two arms (A and B). The ideal patient population to determine a therapeutic benefit of a new treatment, particularly immunotherapy, is one with minimal disease burden. The low tumor burden should allow therapies, particularly those relying on antigen-specific effector T lymphocytes, to destroy all of the cancerous tissues and cells. The first therapeutic arm (arm A) will enroll men with recent evidence of recurrence following surgery or radiation therapy for their primary tumor, and receive the Ad/PSA vaccine alone in three separate injections each 30 days apart. The second therapeutic arm (arm B) will enroll men with recurrent disease, and these men will undergo androgen depletion therapy. The choice of this additional patient population is based on published documentation that inflammation and the generation of immune responses are augmented by hormone withdrawal (36–38). Mercader et al. (36), in attempts to show an enhanced termination of tolerance to prostate-associated antigens, documented CD4+ and CD8+ T-cell infiltrates in benign prostates and in prostate tumors of men undergoing androgen withdrawal. Roden and coworkers (37) published data demonstrating that T-cell levels and T-cell proliferation were increased in mice following castration, whereas Drake et al. (38) reported breaking tolerance to antigens associated with the TRAMP prostate tumors in mice. Therefore, we propose to vaccinate men beginning 14 days after the initiation of androgen depletion therapy using the same three-injection protocol. Patients deemed eligible for entry into protocol 1 will be randomized into arm A or arm B using a card selection method. In the second protocol, we plan to enroll prostate cancer patients with hormone-refractory metastatic disease. This group of patients is similar to the population that constituted the majority of patients in the phase I toxicity trial reported in this publication. Patients in this trial will have a low burden of disease, despite the fact that they are hormone refractory, i.e., have negative bone scans and/or low serum PSA.

In summary, we report here the absence of serious AEs in patients injected with a single dose of an Ad/PSA vaccine, either delivered as an aqueous suspension or in a collagen matrix, even at the highest doses possible with the current vaccine preparation. In addition, anti-PSA immune responses were detected in a percentage of patients, with the highest percentage (68%) found in T-cell responses. A phase II study is in progress to verify the immunologic and clinical observations from this phase I study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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