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ANTIANDROGEN, VACCINE AND COMBINATION THERAPY IN PATIENTS WITH NONMETASTATIC HORMONE REFRACTORY PROSTATE CANCER

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ABSTRACT

Purpose: There is no current standard treatment for patients with prostate cancer who have received hormonal therapy but have an increasing prostate specific antigen (PSA) without radiographic evidence of metastasis. This trial was designed to analyze toxicity, immunogenicity and time to treatment failure using vaccine, antiandrogen therapy or their sequential use.

Materials and Methods: A total of 42 patients were randomized to receive vaccine vs antiandrogen therapy with nilutamide. The vaccine consisted of recombinant vaccinia viruses containing the PSA and B7.1 costimulatory genes as prime vaccinations, and avipox-PSA as boosters. After 6 months patients with an increasing PSA and no metastasis may receive a combination of both treatments.

Results: Three patients on nilutamide were removed from study secondary to grade 3 toxicities but no grade 3 toxicities were attributed to vaccine. In the vaccine arm median time to treatment failure was 9.9 months with 13 of 21 decreases in PSA velocity vs 7.6 months with 16 of 21 decreases in PSA velocity in the nilutamide arm ($p = 0.28$). Of the patients in the nilutamide arm 8 had vaccine added at the time of PSA progression. Median time to treatment failure with combined therapy was 5.2 months, with a median duration from study entry of 15.9 months. Of the patients in the vaccine arm 12 had nilutamide added at the time of PSA progression. Median time to treatment failure with combined therapy was 13.9 months and a median of 25.9 months from initiation of therapy.

Conclusions: Further studies are merited to investigate the role of combining vaccine with antiandrogen therapy or vaccine followed by vaccine plus antiandrogen therapy in this patient population.

KEY WORDS: prostatic neoplasms, immunotherapy, clinical trials, vaccines

Widespread monitoring of serum prostate specific antigen (PSA) following definitive therapy has resulted in the diagnosis of a large number of patients who have only biochemical recurrence. Many of these patients will undergo androgen deprivation therapy. Unfortunately most patients eventually have an increase in PSA. Clinical symptoms and serum PSA may improve after antiandrogen withdrawal.[1–5] However, the majority of patients will eventually have an increase in PSA and confirmed metastatic disease on imaging. However, many patients will have an increase in PSA on hormonal therapy without radiographic evidence of disease (also called stage D0.5). Treatment options for these patients include additional hormonal manipulations, cytotoxic chemotherapy and enrollment in clinical trials with investigational agents. However, there is currently no standard of care.

Immunotherapy for prostate cancer is an active field of investigation using a wide variety of approaches. PSA and other tissue lineage antigens are potential targets for prostate cancer vaccine therapy. The fact that PSA is secreted and not membrane bound limits its use as a target for humoral immunity but not as a target of cellular immune attack.

Recent phase I clinical trials have evaluated the safety and biological effects of a vaccinia virus expressing human PSA in patients with prostate cancer.[6–8] Toxicity was minimal and dose limiting toxicity was not observed. The majority of patient assays had an increase in precursor T cells specific for PSA after vaccination and these T cells could lyse PSA expressing tumor cells in vitro. In some patients a stabilization of serum PSA levels for 6 to 11 or more months was observed.[7]

A total of 64 patients with an increasing PSA after definitive local therapy and with no evidence of disease on scans were randomized to receive 4 vaccines with rV-PSA (designated V) and/or the replication defective avipox (fowlpox) rF-PSA (designated F).[9] Thus the arms were VFFF, FFFF and FFFV. There was a substantial difference in PSA progression-free (PF) survival favoring the VFFF arm, lending support to the use of vaccinia priming and avipox vector boosting.[9]

In the clinical trial described here 4 different vaccine strat-

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See Editorial on page 415.

TABLE 1. *Demographics of randomized trial*

	Vaccine	Nilutamide	Vaccine Followed by Vaccine + Nilutamide	Nilutamide Followed by Nilutamide + Vaccine
No. pts	21	21	12	8
Age:				
Median	69	69	69	66.5
Mean	66.9	69.6	67	69.6
Range	51–87	52–87	51–79	61–87
Gleason:				
Mean	7	7.2	6.9	7
Range	3–9	4–10	5–8	5–9
No. pts				
2–4	1	1	0	0
5–7	11	9	7	4
8–10	8	9	4	4
Unknown	1	2	1	0
No. prior antiandrogens (bicalutamide, flutamide):				
0	3	4	2	1
1	13	10	8	4
2	5	7	2	3
Current testosterone decreasing treatment:				
Goserelin acetate	5	4	4	2
Leuprolide acetate	10	13	5	5
Orchiectomy	6	4	3	1
On study PSA:				
Median	8.74	16.51	13.31	18.66
Mean	35.1	19.32	24.83	21.12
Range	1.61–292.8	0.74–62.19	8.06–74.96	9.68–51.44

egies that have been shown to enhance T-cell responses and antitumor activity in preclinical models have been combined. These strategies include 1) poxvirus vector presentation of a tumor associated antigen, 2) a diversified prime (vaccinia) and boost (fowlpox) regimen, 3) the use of a T-cell costimulatory molecule (B7.1) to activate T-cells better, and 4) the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) to enhance dendritic cell recruitment. In addition, this is the first report to our knowledge of a randomized trial looking at the role of nilutamide exclusively in the nonmetastatic hormone refractory population. It is also the first time that vaccine therapy has been studied in combination with an antiandrogen.

PATIENTS AND METHODS

Patient selection and trial design. This trial was designed to evaluate primarily the efficacy, and secondarily the immunological effects, of a vaccination regimen composed of priming with rV-PSA admixed with rV-B7.1 followed by boosting with rF-PSA, relative to the efficacy of antiandrogen therapy with nilutamide in patients with hormone refractory prostate cancer and increasing PSA levels but with no radiographic evidence of metastatic disease. Patients were required to discontinue bicalutamide at least 6 weeks or discontinue flutamide 4 weeks before enrollment. A total of 42 patients were enrolled in this randomized phase II trial approved by the National Cancer Institute (NCI) Institutional Review Board and conducted at the NCI.

Patients must have had castrate levels of serum testosterone (less than 50 ng/dl). Patients in whom prior androgen deprivation therapy failed were required to have 2 consecutive increasing serum PSA levels 1 week apart, measured at least 6 weeks after bicalutamide withdrawal or 4 weeks after flutamide withdrawal. Patients needed to be Zubrod performance status 0 or 1, and have adequate hematological, hepatic and renal function. In addition, patients were required to have no evidence of an immunocompromised state as defined by nonreactive HIV testing, no diagnosis of altered immune function, no prior radiotherapy to more than 50% of nodal groups, no prior splenectomy and no concurrent steroid use. Prior vaccinia exposure (for smallpox vaccination) was required.

Exclusion criteria were known egg allergy, active cases or history of skin disorders (such as eczema, extensive psoriasis, varicella zoster, impetigo or burns), history of seizures, serious intercurrent illnesses, a noncutaneous malignant process and close contact with immunocompromised individuals, those with the previously mentioned skin conditions or children younger than 5 years old. Prior nilutamide therapy was also an exclusion criterion for this study. All patients gave written informed consent in accordance with federal, state and institutional guidelines, and the principles embodied in the Declaration of Helsinki.

The trial was originally designed to enroll a minimum of 28 patients per arm. However, after an informal analysis ac-

TABLE 2. *Toxicity*

	No. Grade 2 (%)	No. Grade 3 (%)
Vaccine		
Injection site reaction*	13 (37.9)	0 (0)
GM-CSF		
Arthralgia	2.2 (13.8)	0 (0)
Cardiac ischemia	0.0 (0)	1.5 (3.4)
Dyspnea	0.6 (6.9)	0 (0)
Fatigue	1.5 (10.3)	0 (0)
IL-2		
Constitutional symptoms:		
Fatigue	13.6 (48.3)	1.8 (10.3)
Fever	4.7 (13.8)	1.2 (6.9)
Arthralgias	2.4 (6.9)	0 (0)
Metabolic/laboratory: Hyperglycemia	7.1 (20.7)	1.2 (6.9)
Blood/bone marrow: lymphopenia	4.7 (13.8)	0.6 (3.4)
Gastrointestinal:		
Dehydration/anorexia	2.4 (10.3)	0.6 (3.4)
Diarrhea	2.4 (10.3)	0 (0)
Nilutamide		
Dyspnea	1.9 (15.2)	0.2 (3.0)
Fatigue	1.9 (15.2)	0.2 (3.0)
Hot flashes	2.2 (15.2)	0 (0)

There were a number of grade 3 toxicities attributed to IL-2 requiring dose reduction and eventual discontinuation of the drug in some patients with subsequent vaccinations. Grade 3 ischemia developed in 1 patient following GM-CSF administration. However, he had a long-standing history of coronary artery disease and it was unclear if the GM-CSF precipitated this episode. We discontinued the use of GM-CSF with the vaccine in this individual. No patients were taken off study secondary to vaccine related toxicity. Dyspnea developed in 1 patient following nilutamide therapy, and was diagnosed by CT and bronchoscopy with interstitial pneumonitis. He was taken off study and placed on steroid therapy with marked improvement in symptoms.

* Percent cycles with toxicity (% patients with toxicity).

TABLE 3. *T-cell responses to PSA peptide before and after vaccination*

Treatment	Mos	Flu Peptide	PSA3 Peptide
LS2149:			
Vaccinia-PSA + vaccinia-B7.1 + GM-CSF + IL-2	Before treatment	1/41,666	1/83,333
Fowlpox-PSA + GM-CSF	1	1/16,667	Less than 1/200,000
Fowlpox-PSA + GM-CSF	2	1/20,000	Less than 1/200,000
Fowlpox-PSA + GM-CSF	3	1/50,000	1/50,000
Fowlpox-PSA + GM-CSF	4	1/25,000	1/50,000
Fowlpox-PSA + GM-CSF	5	1/16,667	1/33,333
Fowlpox-PSA + GM-CSF	7	1/62,500	1/83,333
Fowlpox-PSA + GM-CSF	9	1/33,333	1/71,429
Fowlpox-PSA + GM-CSF	10	1/16,216	1/20,000
Fowlpox-PSA + GM-CSF	11	1/42,857	1/17,143
No treatment	12	1/25,000	1/11,321
No treatment	13	1/33,333	1/9,231
Fowlpox-PSA + GM-CSF + nilutamide	14	1/15,385	1/4,938
Nilutamide	16	1/26,087	1/14,286
Nilutamide	18	1/18,750	1/66,667
Nilutamide	19	1/42,857	1/30,000
Nilutamide	20	1/20,000	1/50,000
DS7075:			
Vaccinia-PSA + vaccinia-B7.1 + GM-CSF + IL-2	Before treatment	1/28,571	Less than 1/200,000
Fowlpox-PSA + GM-CSF + IL-2	1	1/20,000	1/54,545
Fowlpox-PSA + GM-CSF + IL-2	2	1/19,355	1/40,000
Fowlpox-PSA + GM-CSF + IL-2	3	1/12,500	1/21,429
Fowlpox-PSA + GM-CSF + IL-2	6	1/17,647	1/14,634
Fowlpox-PSA + GM-CSF + IL-2	7	1/25,000	1/10,345
Fowlpox-PSA + GM-CSF + IL-2	8	1/28,571	1/20,690
Fowlpox-PSA + GM-CSF + IL-2	9	1/15,385	1/30,000
Fowlpox-PSA + GM-CSF	10	1/22,222	1/13,636
Fowlpox-PSA + GM-CSF + IL-2	11	1/18,750	1/13,954
No treatment	12	1/10,714	1/26,087
No treatment	13	1/9,524	1/25,000
Fowlpox-PSA + GM-CSF + IL-2	14	1/10,000	1/60,000
Nilutamide	15	1/13,044	1/85,714
Nilutamide	16	1/10,000	1/54,545
Fowlpox-PSA + GM-CSF + IL-2 + nilutamide	17	1/15,789	Less than 1/200,000

Patients LS2149 and DS7075 who were tested to be HLA-A2+ had peripheral blood mononuclear cells from 60 ml of blood collected in heparinized tubes at monthly intervals. The mononuclear fraction was separated as described.[11] T-cell responses to the flu peptide were measured as an internal control for the assay. Only minor variations were noted in the T-cell responses to a flu peptide. It should be noted that after 12 monthly vaccines patients received vaccine on an every 3-month basis while on study.

crual ended early after 21 patients had enrolled in each arm because no statistical difference was observed in the primary end point. Patients randomized to the vaccine arm also received GM-CSF and interleukin-2 (IL-2) as part of their vaccination schedule. Patients continued therapy monthly until there was radiographic evidence of metastatic disease, toxicity, or refusal of further treatment. Serum immunological markers and PSA levels were followed as secondary end points while patients continued to receive treatment on the protocol. Patients who were tested to be HLA-A2 positive had peripheral blood mononuclear cells collected for immunological testing using the ELISPOT assay as a readout.

Vaccine formulation. Each of the 3 viral vaccine productions was constructed and manufactured by Therion Biologics Corporation, Cambridge, Massachusetts, and was provided by the Cancer Therapy Evaluation Program, NCI. rV-PSA (National Service Center [NSC] #697729) and rV-B7.1 (NSC #699018) were prepared from virus derived from the Wyeth (New York City Board of Health) strain of vaccinia. rV-PSA was constructed by the insertion of the entire human PSA gene into the viral genome while rV-B7.1 was constructed by the insertion of the entire human B7.1 costimulatory molecule gene into the viral genome. The priming vaccine comprised 3.51×10^8 PFU of rV-PSA admixed with 1.17×10^8 PFU of rV-B7.1 (3:1 ratio) administered subcutaneously. A sterile nonadherent dressing (ie TELFA™) was used to cover the site. rF-PSA (NSC #694450) also contained the entire gene for human PSA inserted into the fowlpox virus. This vector, used for each of the vaccine boosts, was injected subcutaneously in alternating sites at 1.5×10^9 PFU.

Treatment plan. Patients were randomized to receive either a prime and boost PSA vaccination strategy, or second line antiandrogen therapy with nilutamide. The rV-PSA/ rV-

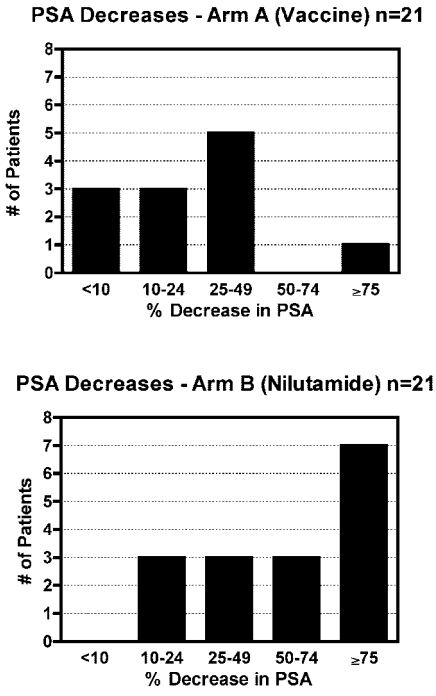

FIG. 1. Patient serum PSA levels were measured before randomization and after each monthly treatment. PSA decreases were shown as maximum percentage decrease during first 6 months. In vaccine arm 12 patients had stabilization or decrease in serum PSA with 1 patient having greater than 75% decrease. Total of 16 patients had decrease in PSA on nilutamide with 3 experiencing decrease of 50% to 74% and 7 patients having decrease of 75% or more.

TABLE 4. *Slopes of serum PSA velocity before and after therapy*

Arm A (vaccine)				Arm B (nilutamide)			
Pt No.	Before Treatment	After Treatment	Change in Velocity	Pt No.	Before Treatment	After Treatment	Change in Velocity
1	0.70	0.35	Decrease	1	0.22	−0.05	Decrease
2	0.24	0.16	Decrease	2	1.75	0.18	Decrease
3	27.44	21.80	Decrease	3	0.39	−0.35	Decrease
4	6.87	1.38	Decrease	4	4.68	−1.38	Decrease
5	0.99	0.10	Decrease	5	1.64	−1.68	Decrease
6	8.40	1.69	Decrease	6	1.86	0.30	Decrease
7	2.00	−0.71	Decrease	7	3.67	−0.27	Decrease
8	36.11	25.47	Decrease	8	3.90	−0.83	Decrease
9	3.01	−3.81	Decrease	9	4.15	2.44	Decrease
10	24.43	10.63	Decrease	10	23.20	4.88	Decrease
11	0.93	0.36	Decrease	11	4.09	1.61	Decrease
12	0.48	0.06	Decrease	12	10.13	−0.94	Decrease
13	6.27	1.71	Decrease	13	0.90	−0.50	Decrease
14	2.85	2.61	None	14	0.54	−0.02	Decrease
15	0.24	0.23	None	15	0.68	−0.01	Decrease
16	0.95	2.95	Increase	16	0.41	−0.02	Decrease
17	0.37	0.53	Increase	17	−1.33	−0.02	Increase
18	6.78	12.02	Increase	18	0.23	1.76	Increase
19	5.24	6.66	Increase	19	0.78	4.42	Increase
20	3.59	10.00	Increase	20	−0.08	0.81	Increase
21	0.10	0.57	Increase	21	4.05	Not available	
Overall			13/21				16/21

Slopes were determined as defined by statistical considerations previously mentioned. Pretreatment PSA levels were obtained from patient records before enrollment as part of the eligibility criteria.

B7.1 admix was given as a priming vaccination and rF-PSA was given for each of 2 subsequent monthly boosts. Computerized tomography (CT) and bone scans were performed every 3 months. If there was no radiographic evidence of metastatic disease, patients could continue monthly rF-PSA boosts for a total of 12 months and then every 12 weeks thereafter. All vaccines were given on day 2 of each 28-day cycle. In addition, 100 μg per day sargramostim (GM-CSF) were given subcutaneously at the same site as the vaccination on days 1 to 4 and 6 MIU/M^2 aldesleukin (IL-2) were given subcutaneously in the abdomen on days 8 to 12. If a patient experienced grade III toxicity attributable to IL-2 or GM-CSF, that cytokine was reduced to 50% of the previous dose for subsequent administrations. Patients randomized to nilutamide were initially given a loading dose of 300 mg orally each day for 1 month, then 150 mg daily each month thereafter. Patients could continue on nilutamide if CT and bone scans remained without metastasis.

At any interval after the initial 6-month evaluation, patients with an increasing PSA without radiographic evidence of progressive disease (PD) were offered the option to begin therapy with the treatment offered in the other arm in addition to the treatment to which they were randomized.[10] Complete interval histories, physical examinations, blood chemistries, and hemogram and serum PSA were obtained on a monthly basis. All patients were evaluated for toxicity by the Common Toxicity Criteria, version 2 and the vaccinia toxicity grading scale previously published.[8] In terms of immunoassays, the ELISPOT assay was performed as previously reported.[11] Assays for antiPSA, GM-CSF and B7-1 antibodies were as described previously.[12, 13]

Statistical considerations. The primary efficacy analyses of time to treatment failure, progression-free survival or overall survival were performed using the Kaplan-Meier method, and the statistical significance of the difference between the 2 arms was determined using the log rank test. Treatment failure was considered to have occurred when patients had progression, the development of secondary malignancies or toxicity, and were either removed from study or crossed over to the other arm if it was an appropriate time to do so.

Comparison between the rates of PSA change before and after treatment initiation was done by linear regression on all available values up through the on-study date, and by obtaining the least squares estimate of a slope in that fashion. This was repeated using the values beginning 1 month after the on-study date and continuing through 6 months after the on-study date. The relative difference in posttreatment (post-slope) vs pretreatment slope (pre-slope) and the relative difference in slope in vaccine vs nilutamide arm were obtained by the relationship (post-slope–pre-slope)/pre-slope. The statistical significance of the relative change between the 2 slopes was determined by the Wilcoxon signed rank test and all p values are 2-tailed.

RESULTS

The baseline characteristics of the 42 enrolled patients are shown in table 1. Twelve patients in the vaccine arm continued beyond the initial 6-month evaluation and when PSA increased had nilutamide therapy added to their treatment. Of the initial 21 patients randomized to nilutamide 8 who continued beyond the initial 6 months had the vaccine added to the treatment at biochemical progression. Patient age and Gleason score were similar in the 2 arms. The majority of patients (greater than 80%) in both arms had received at least 1 prior antiandrogen treatment.

Toxicity. Toxicity related to vaccine, cytokines and nilutamide is shown in table 2. The vaccine therapy was tolerated well with only grade 2 or less toxicity related to the vaccine itself. Three patients randomized to nilutamide were removed from study secondary to grade 3 toxicities.

Immune responses. Eight patients positive for HLA-A2 in the vaccine arm and 3 positive for HLA-A2 in the nilutamide arm were evaluated for induction of PSA specific T-cell responses before and after 3 monthly cycles of therapy. No PSA specific T-cell responses were noted with nilutamide treatment. However, of the 8 patients in the vaccine arm 4 were observed to have a minimum of a 2-fold increase in PSA specific T-cell frequency after 3 monthly vaccinations. One patient had a greater than 9-fold response. Two patients in the vaccine arm were evaluated for immunological responses to the vaccine following multiple monthly fowlpox-PSA boosts (table 3). Patient LS2149 had a nearly 17-fold increase in PSA specific T-cell precursors after 14 months of treatment and patient DS7075 had a nearly 15-fold increase after 11 months of treatment.

Serum PSA responses. Serum PSA decreases were observed in 16 of 21 patients in the nilutamide arm and median

duration of these responses was 4.6 months (fig. 1). More than half of the 21 patients randomized to the vaccine arm had stabilization or a decrease in serum PSA during the first 6 months of therapy that was sustained for a minimum of 1 month (fig. 1). Five patients had decreases between 25% and 50%, and 1 patient had a greater than 75% decrease in PSA following vaccine. To validate the significance of these responses, serum prostatic acid phosphatase (PAP) levels were also obtained along with the PSA levels. The serum PAP either decreased or was stable when serum PSA decreases were noted (data not shown). No antiPSA antibody was detected in any of these patients at a minimal serum dilution of 1/50. There was no difference in the ability of patients to respond to the vaccine as noted by decreasing serum PSA regardless of Gleason score (7 or less vs 8 or greater). Since the majority of patients receiving vaccine had IL-2 stopped, we evaluated whether there was a negative impact on patient ability to respond to the vaccine. No trend was observed with regard to stopping IL-2 and serum PSA level decreases.

Using linear regression an estimate of mean PSA velocity was calculated for patients before and after 6 months of either vaccine (arm A) or nilutamide (arm B) treatment. Estimated mean pretreatment PSA velocity was 6.6 ng/ml per month and decreased after 6 months of vaccine therapy to 4.5 ng/ml per month ($p = 0.25$ for relative change). For nilutamide the estimated mean pretreatment velocity was 2.75 ng/ml per month and decreased after 6 months of therapy to 0.52 ng/ml per month ($p = 0.009$ for relative change) as seen in table 4 and figure 2. Of 21 patients in the vaccine arm 13 experienced a decrease in PSA velocity slopes and 16 of 20 in the nilutamide arm experienced a decrease in these slopes. The relative changes in the slopes of the nilutamide arm were compared with the vaccine arm. The median relative change in the slope of the vaccine arm was -0.33 while the relative change in the slope of the nilutamide arm was -1.04 ($p = 0.0018$). Examples of decreases in serum PSA for individual patients in the vaccine arm are shown in figure 3.

Twelve patients randomized to vaccine with an increasing PSA without metastatic disease on scans crossed over to receive the addition of nilutamide to their treatment regimen, and 8 patients randomized to nilutamide with increasing PSA without radiographic evidence of disease crossed over to receive the addition of vaccine therapy. Thus, if a patient with increasing serum PSA on nilutamide only had PSA decrease with the addition of vaccine, the PSA reduction would not be attributed to an antiandrogen withdrawal phenomenon. Figure 4 illustrates an example of a patient who, after an increasing serum PSA on nilutamide therapy, had a PSA decrease with the addition of vaccine that lasted 1.5 years. The patient tolerated this treatment well for nearly 4 years without evidence of metastatic disease on scans.

Time to treatment failure. Median time to treatment failure was 9.9 months on vaccine vs 7.6 months on nilutamide and this difference was not statistically significant ($p = 0.28$, table 5). Two patients continue in the nilutamide arm to date without evidence of disease progression (greater than 30 months). In the vaccine arm 12 patients had nilutamide added at the time of PSA progression. Median time to treatment failure with combined therapy was 13.9 months for a total of 25.9 months from initiation of therapy (table 5). In contrast 8 patients in the nilutamide arm had vaccine added at the time of PSA progression. Median time to treatment failure with combined therapy was 5.2 months with a median total duration from onset of study of 15.9 months.

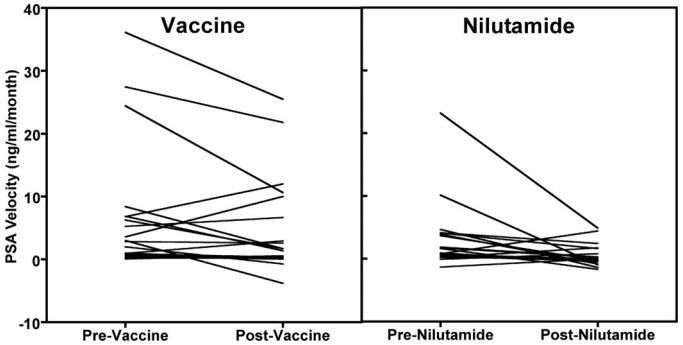

FIG. 2. Serum PSA velocities before and after therapy for individual patients on vaccine (arm A) or nilutamide (arm B). Velocities are determined as ng/ml per month.

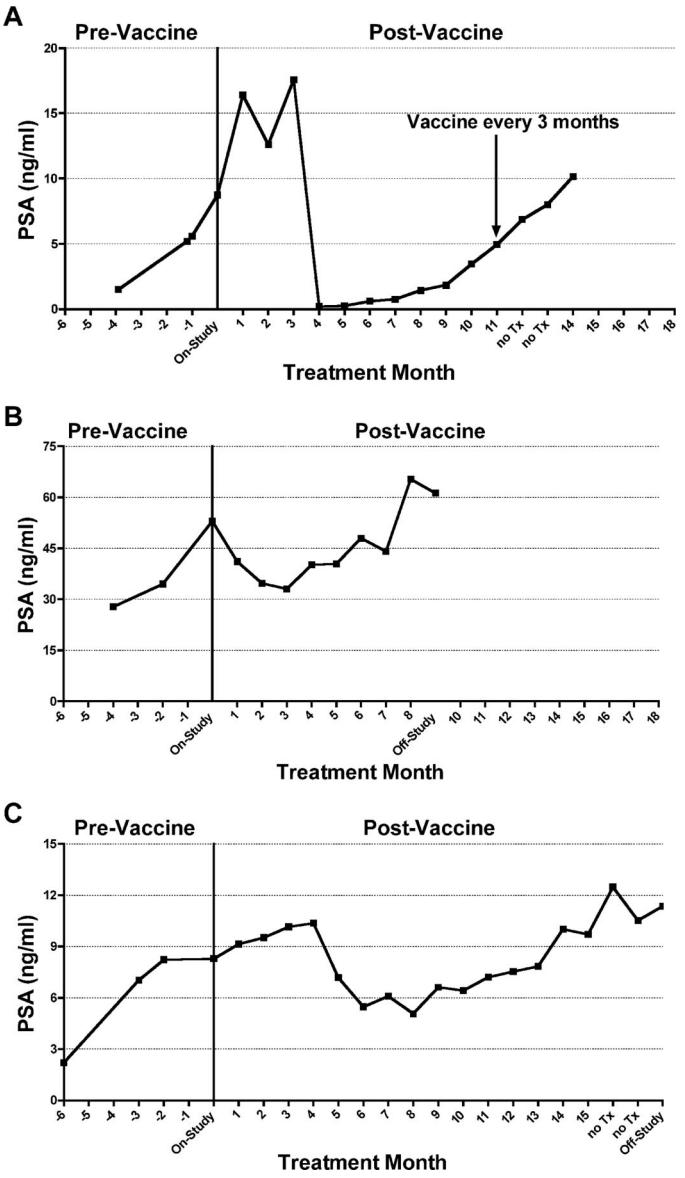

FIG. 3. Three patients randomized to vaccine arm experienced prolonged decreases and stabilizations in serum PSA. *A*, patient had markedly increasing serum PSA on antiandrogen therapy and PSA continued to increase despite antiandrogen withdrawal. By vaccine 4 serum PSA had decreased precipitously. Scans remained negative and patient continued on monthly vaccines for 1 year. Following 12 monthly vaccinations as per protocol design patient began to receive treatment on every 3-month basis. Although scans remained negative nilutamide was added to regimen secondary to continued increasing serum PSA at month 15. *B* and *C,* 2 additional patients in whom prior antiandrogen therapy failed, and who had withdrawal preceding randomization to vaccine arm with sustained PSA decrease for approximately 7 months, and decrease and stabilization of serum PSA for more than 1 year, respectively. *C*, individual remained on vaccine therapy alone for 18 months without evidence of metastasis developing on scans.

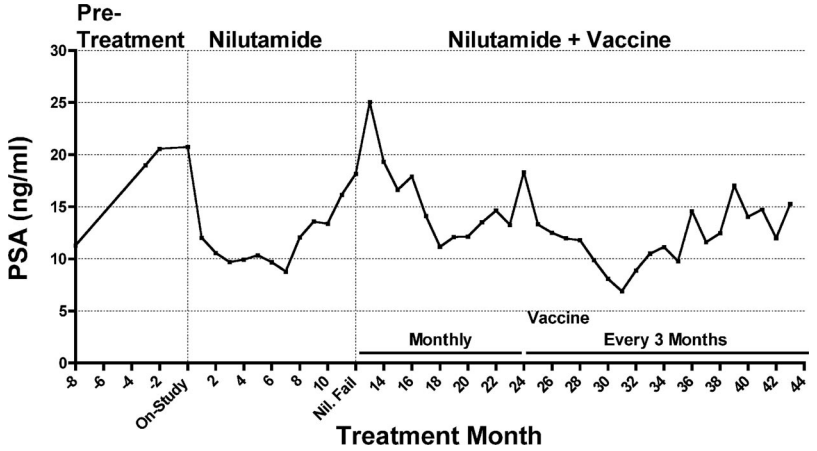

FIG. 4. PSA serum levels of patient initially randomized to nilutamide. Despite initial decrease in serum PSA within 1 year serum PSA increased and vaccine was added. Patient then experienced PSA decrease greater than 50% in 18 months of combined therapy, and PSA remained less than 10 ng/dl for 2 years while receiving vaccine and hormone. Although PSA levels have increased they remain below level at which vaccine therapy was commenced.

TABLE 5. *Time to treatment failure*

Initial Regimen	Crossover	No.	PSA Decrease by 50%	Median Mos to Treatment Failure
Vaccine		21	1	9.9
Nilutamide		21	10	7.6
Vaccine	Vaccine + nilutamide	12	7	13.9 (after crossover)* 25.9 (from initiation of therapy)
Nilutamide	Nilutamide + vaccine	8	1	5.2 (after crossover) 15.5 (from initiation of therapy)

Time to treatment failure rather than disease progression was used to include toxicity and PSA progression, in addition to radiographic progression.
* Median time to crossover was 12.0 months.

Figure 5 provides a summary of the clinical course for all patients randomized in the study. Of the 13 patients who received only nilutamide therapy, 5 of these patients are deceased with 2 of these deaths related to prostate cancer. Of the 8 patients on nilutamide who had the vaccine added, 2 died secondary to prostate cancer while 6 remain alive. To date, of the initial 9 patients who had progression on vaccine but did not receive nilutamide, 3 have died with 1 death attributed to prostate cancer. All of the 12 patients in the vaccine arm who also received nilutamide remained alive from 25 or more to 50 or more months from initiation of therapy, with a median of 35 months or more.

DISCUSSION

To our knowledge this is the first randomized trial specifically devoted to treating patients with nonmetastatic hormone refractory prostate cancer. To be eligible for the study patients must have had an increasing serum PSA despite castrate levels of serum testosterone. In addition, at least 1 prior antiandrogen agent had failed in the majority of patients enrolled in study. Thus, it was unclear at study initiation how well patients would respond to second line androgen ablation therapy. We chose nilutamide because clinical and biochemical responses have been reported with nilutamide after flutamide and bicalutamide failure.[14,15] Two small series have been reported evaluating the effect of nilutamide as a second line antiandrogen. In 1 study involving 28 patients in whom prior therapy with flutamide or bicalutamide had failed, 8 (29%) had a sustained decrease in PSA of greater than 50% for more than 3 months on nilutamide.[14] In another smaller series 7 of 14 patients had a significant PSA response for a median duration of 11 months when nilutamide was used as a second line antiandrogen.[15] However, neither of these studies reported the change in PSA velocities with nilutamide therapy.

This is the first study to report changes in PSA slopes in response to nilutamide therapy, with a statistically significant relative change before and after 6 months of nilutamide treatment. Furthermore, 10 of the 21 patients (48%) randomized to nilutamide had a sustained decrease in PSA of greater than 50% for at least 1 month, with 2 patients still responding to therapy at more than 2 years to date. Stabilization and decreases in serum PSA were achieved in more than half of the patients randomized to the vaccine arm. Furthermore, there was a modest reduction in the mean PSA velocity of patients randomized to the vaccine arm during the first 6 months of vaccine compared with mean PSA velocity before randomization on vaccine. Although the nilutamide arm had a significantly greater impact than vaccine with respect to reducing the velocity of PSA changes after treatment compared with before treatment, there was no statistical difference in the proportion of patients in whom metastatic disease developed in the 2 treatment arms at 6 months, with 15 patients on vaccine and 13 patients on nilutamide, progression-free, of 21 randomized patients in each arm.

When nilutamide was given to the 12 patients with increasing serum PSA on vaccine, median time to treatment failure from start of combined treatment was 13.9 months compared with 7.6 months for the 21 patients randomized to receive nilutamide alone. Vaccine was continued with nilutamide at crossover because the generation of an immune response is a dynamic process that may require multiple booster vaccinations to optimize the immune response. Giving the vaccine to patients already receiving nilutamide led to a median time to treatment failure of only 5.2 months. It must be emphasized that any comparisons among these times should be interpreted cautiously because the comparisons do not involve concurrently randomized patients and are based on small numbers of subjects. Additionally, it is possible there is selection bias in that the patients who were able to receive the additional treatment on trial had less aggressive disease.

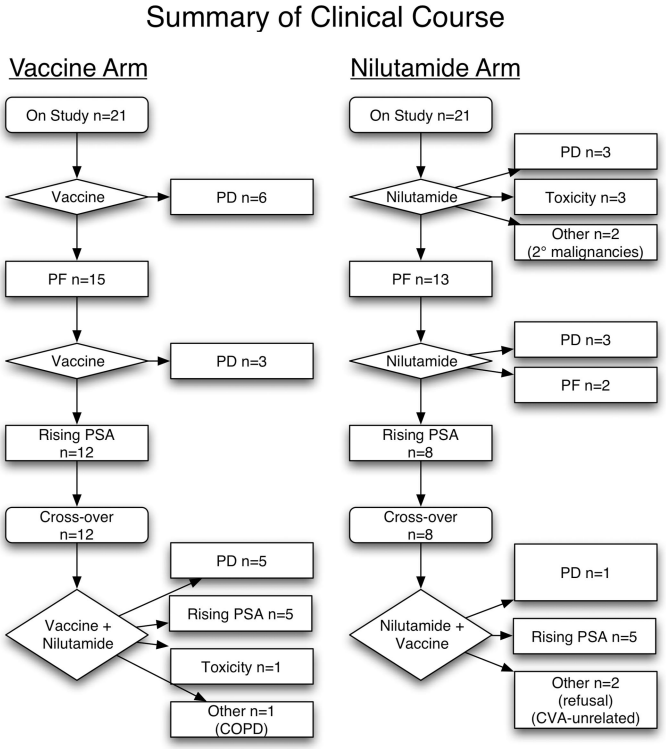

FIG. 5. Patients followed in both arms for clinical course at 6-month evaluation and thereafter. PD refers to progressive disease by development of metastasis on scans. Those patients who were PF after 6-month evaluation continued on trial. At subsequent evaluations patients in whom PD developed were taken off study. However, if they had increasing serum PSA they were allowed to cross over to receive other treatment in addition to treatment to which they were randomized. Median followup for patients in both arms was 32 months. COPD, chronic obstructive pulmonary disease. CVA, cerebral vascular accident.

However, a similar observation has been noted in other cancer vaccine trials in which sequential therapy with vaccine followed by traditional therapy led to improved clinical results. In a small pilot study using a similar vaccine in patients with metastatic androgen independent prostate cancer, patients who received docetaxel after vaccine had a 60% PSA decrease rate, which is at least as good as most trials with docetaxel alone (approximately 40%).[16] As a followup study we plan to investigate combining vaccine with antiandrogen therapy vs sequential vaccine followed by antiandrogen therapy to determine if there is indeed a role for combination therapy in this patient population that currently has no standard of care.

CONCLUSIONS

Other prostate cancer vaccine approaches such as the use of whole prostate cancer cells or dendritic cells loaded with PAP and GM-CSF have also been used, achieving varying levels of immune responses and or clinical results.[17,18] Preclinical studies have also demonstrated that the insertion of multiple costimulatory molecule genes into poxvirus vectors along with a transgene for a tumor antigen results in a more vigorous immune response to the tumor antigen, as well as a more vigorous antitumor response.[19,20] New vaccines containing the transgenes for PSA and a triad of costimulatory molecules (designated rV-PSA-TRICOM and rF-PSA-TRICOM) have now entered phase I trials. In view of the availability of these more potent vaccines, the minimal toxicity observed with poxvirus vaccines in this and other studies, and the provocative finding of potential synergy between vaccine and hormone therapy, we believe further prostate cancer vaccine studies are warranted.

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