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A Randomized Phase II Study of Concurrent Docetaxel Plus Vaccine Versus Vaccine Alone in Metastatic Androgen Independent Prostate Cancer

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Abstract

Purpose: Docetaxel has activity against androgen insensitive prostate cancer (AIPC) and preclinical studies have demonstrated that taxane-based chemotherapy can enhance antitumor response of vaccines. The primary objective of this study was to determine if concurrent docetaxel (with dexamethasone) had any effect on generating an immune response to the vaccine. Secondary endpoints were whether vaccine could be given safely with docetaxel and the clinical outcome of the treatment regimen.

Experimental Design: The vaccination regimen was composed of (1) recombinant vaccinia virus (rV) that expresses the prostate-specific antigen gene (rV-PSA) admixed with (2) rV that expresses the B7.1 costimulatory gene (rV-B7.1), and (3) sequential booster vaccinations with recombinant fowlpox virus (rF-) containing the PSA gene (rF- PSA). Patients received GM-CSF with each vaccination. Twenty-eight patients with metastatic AIPC were randomized to receive either vaccine and weekly docetaxel or vaccine alone. Patients on the vaccine alone arm were allowed to cross over to receive docetaxel alone at time of disease progression. The ELISPOT assay was used to monitor immune responses for PSA-specific T cells.

Results: The median increase in these T-cell precursors to PSA was 3.33-fold in both arms following 3 months of therapy. In addition, immune responses to other prostate cancer associated tumor antigens were also detected post-vaccination. Eleven patients who progressed on vaccine alone crossed over to receive docetaxel at time of progression. Median PFS on docetaxel was 6.1 months after receiving vaccine compared with 3.7 months with the same regimen in a historical control.

Conclusion: This is the first clinical trial to demonstrate that docetaxel can be administered safely with immunotherapy without inhibiting vaccine specific T-cell responses. Furthermore, patients previously vaccinated with an anticancer vaccine may respond longer to docetaxel compared with a

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historical control of patients receiving docetaxel alone. Larger prospective clinical studies will be required to validate these findings.

Keywords

Genitourinary cancers: prostate; Phase I-III clinical trials; Cancer vaccines; Immune responses to cancer

Introduction

Adenocarcinoma of the prostate is the most common noncutaneous malignancy diagnosed in American males, and the second leading cause of cancer death. One out of 6 men will develop clinically significant prostate cancer in his lifetime. During 2005, an estimated 232,900 men will be diagnosed with prostate cancer and 30,350 will die from the disease in the United States (1). Overall survival for patients with metastatic androgen independent prostate cancer (AIPC) has been improved with a docetaxel-based regimen. The clinical benefit demonstrated in these recent studies is approximately a 3-month increase in survival utilizing an every 3 week regimen of docetaxel (2,3). However, compared with the every 3-week schedule, weekly docetaxel is associated with significantly less grade 3 or 4 hematologic toxicity. Other studies have looked at combining weekly docetaxel with other agents (4,5). A randomized phase II trial at the NCI compared the addition of daily low dose thalidomide (200 mg) to weekly docetaxel (30 mg/m^2) *vs*. docetaxel alone (6). Docetaxel was administered 3 consecutive weeks of a 4-week cycle. Nine of 25 patients (37%) in the docetaxel alone arm and 25 of 50 patients (50%) in the combination arm had a PSA decline of at least 50%. In a recent update, the overall survival of the combination arm was 25.9 months vs. 14.7 months in the docetaxel alone arm ($p = 0.041$) (7).

Newer strategies for the treatment of metastatic AIPC are currently being evaluated. The development of vaccine strategies designed to break tolerance and generate a sustained immune response against prostate cancer represents a novel therapeutic approach. Preclinical and clinical studies with a range of vaccines have demonstrated that the induction of T-cell responses directed against a self-antigen can lead to anti-tumor activity in the absence of toxicity. Both carbohydrate and glycoprotein vaccines utilizing “self” antigens have been administered to patients with prostate cancer. Clinical studies have not yet determined which of these antigens would contribute to the most potent vaccine. Globo H hexasaccharide is a carbohydrate vaccine that has elicited antibody responses as well as declines in PSA velocities when administered to prostate cancer patients (8). MUC-1 and prostate specific membrane antigen (PSMA) have also been utilized as targets in various prostate cancer clinical studies (9-11). PSA is a potential target for a prostate cancer vaccine owing to its restricted expression on prostate cancer and normal prostatic epithelium. Miller et al. conducted a Phase I trial utilizing a PSA DNA vaccine (pVAX/PSA DNA) in patients with hormone refractory prostate cancer, demonstrating induction of T cells to PSA peptides in patients following vaccine (12). Since the majority of prostate cancer vaccines tested thus far have utilized “self” antigens, vaccines and vaccine strategies must be developed to enhance the immunogenicity of these targets.

An advantage of using recombinant poxvirus when developing cancer vaccines is that recombinant proteins derived from genes inserted and transcribed in viral genomes are more immunogenic than protein in adjuvant.(13-15). The use of poxviral vectors to stimulate an immune response to PSA has been evaluated in several clinical trials (16,17). The Eastern Cooperative Oncology Group (ECOG) reported (18) a randomized Phase II study in which 64 patients with rising PSA following definitive local therapy with no evidence of disease on scans

were randomized to receive four vaccinations with recombinant fowlpox vector (rF-) expressing the PSA gene (rF-PSA, designated “F” for fowlpox) alone or in sequence with recombinant vaccinia vector (rV-) expressing PSA (rV-PSA, designated “V”).(18) Patients in arm A received monthly vaccination of FFF, arm B was FFFV, and arm C was VFFF. There was a substantial difference in PSA progression-free survival (PFS) favoring the VFFF arm (arm C), lending further support to the use of vaccinia priming and avipox vector boosting (19-22). This study has recently been updated with a median follow-up time of 50 months. The median time to PSA progression is 9.2, 9.1, and 18.2 months for arms A, B and C, respectively. The median time to clinical progression has still not been reached for any treatment group with 80% of men in arms A and B free of disease progression compared with 90% of men in arm C free of clinical progression (p=0.73 by log rank test). These results suggest that men with hormone-dependent prostate cancer and a rising PSA may derive long-term clinical benefit from vaccinations with poxviruses expressing PSA (23).

Costimulatory molecules are critical in the generation of potent T-cell responses, especially to weak antigens such as tumor-associated antigens (TAAs). The initiation of a potent immune response requires at least two signals for the activation of naive T cells by antigen-presenting cells (APCs). The first signal is antigen specific, delivered through the T-cell receptor via the peptide/major histocompatibility complex (MHC), and causes the T cell to enter the cell cycle. The second, “costimulatory” signal involves the interaction of a costimulatory molecule **(**such as B7.1 (CD80)) expressed on APCs, with its ligand on the T cell (the ligand for B7.1 is CD28 and CTLA4) (24-26).

We have recently reported the results of two Phase II prostate cancer clinical trials where we administered rV-PSA mixed with a recombinant vaccinia containing the T-cell costimulatory molecule B7.1 along with booster vaccinations of avipox-PSA. In the first study, 30 patients with localized prostate cancer were randomized in a 2:1 fashion to receive radiation therapy with vaccination or radiation therapy alone (27). There were eight monthly vaccine injections administered to the patients randomized to the combination therapy in this study. Seventeen of 19 patients assigned to the vaccine arm received all eight doses. Thirteen of these 17 patients had enhanced increases in PSA- specific T cells compared to none in the radiation alone group. This study showed that the vaccine could be given safely and that specific T-cell responses were achieved in the majority of patients (27). In the second study, 42 patients with non-metastatic hormone- refractory prostate cancer were randomized to receive either vaccination or anti-androgen therapy with nilutamide. If, after six months of treatment, the patients had no metastasis and their PSA continued to rise, they could then receive a combination of both treatments. Three patients who had received nilutamide had been removed from the study due to side effects. No significant side effects were observed among the patients treated with vaccine. The patients on the vaccine arm had a 9.9 month time to treatment failure versus 7.6 months in the nilutamide treated group (not statistically significant). After six months, eight of the patients in the nilutamide group had vaccine added to treatment, which resulted in an additional time to treatment failure of 5.2 month resulting in a median time to treatment failure of 15.9 months from onset of nilutamide. Twelve patients in the vaccine group received nilutamide with a median time to treatment failure of 13.9 months and a median of 25.9 months from initiation of vaccine (28). Thus, there is precedent for combining this vaccine strategy with other standard therapies for prostate cancer. In the study reported here we have employed this vaccine regimen in the metastatic setting to determine the impact of docetaxel on immunologic activity as well as the safety and clinical efficacy of this vaccine.

The administration of cytotoxic chemotherapy can result in bone marrow suppression and has been traditionally perceived to have a negative impact on immune function. Recent data suggest, however, that taxane based chemotherapy may actually exert beneficial immunomodulatory effects through a variety of mechanisms, including cytokine production

and T-cell infiltration of tumor cells (29,30). In the study reported here we examine the impact of combining docetaxel and vaccine therapy and their combined effect on immunologic activity as well as the safety and clinical efficacy of treatment strategy in patients with metastatic AIPC.

Patients and Methods

Patient eligibility. Patients must have had metastatic AIPC with evidence of disease progression based on the following criteria: (a) a rising serum PSA level using the PSA consensus criteria (31), and/or (b) a new metastatic finding on bone scan, and/or (c) progression of disease on CT scan. Patients on anti-androgen therapy must have undergone anti-androgen withdrawal and still show evidence of a rising PSA. All patients signed the consent form that was approved by the National Cancer Institute Institutional Review Board.

Additional eligibility criteria were required as follows: age of at least 18 years; anticipated survival of 6 months; ECOG performance status of 0-2; adequate organ function as defined by normal hematopoetic, renal, and hepatic function; HIV seronegativity; no concurrent use of steroids (except for dexamethasone comedication with docetaxel administration). Finally, all patients had to be HLA-A2 positive. Contraindications to enrollment included prior therapy with docetaxel for prostate cancer, history of radiation to >50% of nodal groups, recent major surgery, serious intercurrent illness, and clinically active brain metastasis. As a precaution for the administration of vaccinia, patients had no evidence of being immunocompromised as defined by a diagnosis of altered immune function, including active or history of eczema, atopic dermatitis, or autoimmune disease (autoimmune neutropenia, thrombocytopenia, or hemolytic anemia; HIV; systemic lupus erythematosus, Sjogren syndrome, or scleroderma; myasthenia gravis; Goodpasture syndrome; Addison's disease, Hashimoto's thyroiditis, or active Graves' disease).

Study design and treatment. This trial was primarily designed to evaluate the immunologic effect as well as the safety and efficacy of a vaccination regimen composed of priming with rV-PSA admixed with rV-B7.1 followed by boosting with rF-PSA, relative to the effects of the same vaccine strategy given 1 day following the first weekly dose of docetaxel and dexamethasone (administered 3 consecutive weeks out of 4) in patients with metastatic AIPC. The vaccine was administered 2 weeks apart during the first month. In patients receiving the combination of vaccine and docetaxel, chemotherapy commenced at the beginning of the second month of treatment. Twenty- eight patients were enrolled onto this randomized phase II trial approved by the NCI Institutional Review Board and conducted at the NCI. As part of the vaccine regimen, patients also received 100 μg of GM-CSF subcutaneously (s.c.) at the site of the vaccine beginning on the day of vaccine for 4 consecutive days. Fourteen patients were randomized to receive vaccine with docetaxel and dexamethasone comedication; 14 patients received vaccine alone. The study was designed to show that the vaccine + docetaxel arm is not worse than that of the vaccine alone arm with respect to change in precursor frequency. With 14 patients in each arm, with a one-sided 0.05 alpha level test, there was 95% power to detect a change in precursor frequency in the vaccine + docetaxel arm that is 50% less than the vaccine arm, provided that the associated change is equal to 0.78 of each difference. Patients continued therapy monthly until there was progression of metastatic disease by PSA criteria (31) or scans, toxicity, or refusal of further treatment. Restaging scans were performed every 3 months. Patients randomized to the vaccine arm only were allowed at time of progression on or after day 85 (determined by scans or PSA criteria) to remain on study with the discontinuation of vaccine, but with the addition of docetaxel and dexamethasone (see Fig 1). Serum immunologic markers and PSA levels were followed as secondary endpoints while patients continued to receive treatment on the protocol. All patients consented to have peripheral blood mononuclear cells collected prior to each vaccination for immunologic testing using the ELISPOT assay as a readout.

Vaccine formulation. Each of the three viral vaccine products was constructed and manufactured by Therion Biologics Corporation, Cambridge, MA, and was provided by the Cancer Therapy Evaluation Program (CTEP), NCI. rV-PSA (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. The rV-PSA was constructed by the insertion of the entire human PSA gene into the viral genome, while the rV-B7.1 was constructed by the insertion of the entire human B7.1 costimulatory molecule gene into the viral genome. The priming vaccine compromised 3.51×10^8 PFU of rV-PSA admixed with 1.17×10^8 PFU of rV-B7.1 (3:1 ratio) administered s.c. A sterile, nonadherent dressing (i.e., "Telfa") was used to cover the site. The 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 s.c. in alternating sites at 1.5×10^9 PFU.

Immunoassays. The primary immunological parameter for comparing immune responses in HLA-A2-positive patients was determined by an ELISPOT assay using C1R-A2 cells as antigen-presenting cells (APC) as previously described (32). This assay measures the frequency of IFN-γ releasing T cells in response to PSA-3 peptide (VISNDVCAQV) and Flu peptide (GILGFVFTL), in pre- and post-vaccination PBMC. Briefly, 96-well plates (Millipore Corp., Bedford, MA) were coated with 100 μl/well capture mAb against human IFN-γ at a concentration of 10 μg/ml for 12 hours at room temperature. Plates were blocked for 30 minutes with RPMI-1640 medium plus 10% human AB serum. PBMC (2×10^5) were added to each well. PSA-3-pulsed C1R-A2 cells were added to each well as APC at an effector:APC ratio of 1:3. Unpulsed C1R-A2 cells were used as a negative control. Flu peptide was used as a positive control. Cells were incubated for 24 hours and lysed with phosphate-buffered saline (PBS)/ Tween(0.05%). Biotinylated anti- IFN-γ antibody, diluted to 2 μg/ml in PBS/Tween containing 1% bovine serum albumin (BSA), was added and incubated overnight in 5% CO_2 at 37°C. Plates were then washed three times and developed with avidin/alkaline phosphatase for 2 hours after which each well was examined for positive spots. Spots in each well were counted using an ImmunoSpot Analyzer (Cellular Technology LTD, Cleveland, OH). Additional peptides were used for the determination of immune responses to antigens distinct from the antigen used in the vaccination (antigen cascade). Additional peptides used were MUC-1 agonist (ALWGQDVTSV), PSMA (LLHETDSAV), and PAP (ALDVYNGLL). Assays for anti-PSA, GM-CSF, and B7-1 antibodies were also performed as described previously (27, 33,34).

Statistical considerations. The primary objective of the study was to demonstrate that the vaccine with docetaxel and dexamethasone change in precursor frequency was not significantly worse than that in the vaccine only arm. A Wilcoxon rank sum test was used to compare the change in precursor frequency between the combination arm vs. the vaccine alone arm. Secondary analyses to evaluate progression-free survival were performed using the Kaplan-Meier method. Comparison between the rate of change of the PSA pre- and post-initiation of treatment was done by first using linear regression on all available values up through the on-study date and 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 3 months after the on-study date. It was determined that the relative difference in the post- vs. pre-treatment slope, obtained by the relationship (post-slope - pre-slope)/pre-slope, would be the preferred measure for evaluating the change in PSA velocity since this measure was less dependent on the baseline values than were the absolute differences between the two slopes. The statistical significance of this relative change between the two slopes was determined by the Wilcoxon signed rank test. Relative changes between two arms were compared with a Wilcoxon rank sum test. All p-values are two-tailed.

Results

The baseline characteristics of the 28 enrolled patients are shown in Table 1. For 11 patients on the vaccine arm, the vaccine was discontinued at the time of disease progression (rising PSA and/or progression on scans) and docetaxel therapy was initiated. The patients' age and Gleason score were similar in the two arms. Although the combination arm had a higher median PSA level (129.1ng/dl), the mean on-study PSA was higher for those patients on the vaccine alone arm (247ng/dl) and for those patients who received docetaxel alone at the time of cross-over (338.8 ng/dl). Finally, the number of patients with bone only disease (85%) was higher in the vaccine alone arm, with more patients in the combination arm (50%) having both bone and soft tissue disease.

Toxicity. The vaccine therapy was tolerated well with only grade 2 or less toxicity related to the vaccine itself. Two patients randomized to the combination of vaccine and docetaxel experienced grade 3 lymphopenia, with one of these patients also experiencing arthralgias, hyperglycemia, and infection without neutropenia. It was felt that the dexamethasone comedication was responsible for the lymphopenia and hyperglycemia and possibly contributed to the infection.

Immune responses. Eleven HLA-A2 positive patients on the vaccine arm alone and 11 HLA-A2 positive patients on the combination of vaccine and docetaxel were able to be evaluated for induction of PSA-specific T-cell responses prior to and following 3 monthly cycles of therapy as shown in Fig. 2. Endogenous T-cell responses to flu peptide are also shown for each time of peripheral blood mononuclear cells (PBMC) draw. The median fold increase in PSA-specific T cells in both arms was 3.33 (P=0.92). Thus, this vaccine regimen with weekly docetaxel and dexamethasone could be given without inhibiting measured immune responses to the vaccine.

Recent studies have demonstrated distinct immune responses not only to TAAs found in the vaccine but also to multiple other TAAs not found in the vaccine, but found in the tumor cells; this phenomenon is known as epitope spreading and/or antigen cascade. PBMC from three patients who received the vaccine alone were evaluated for the presence of T cells directed against known HLA-A2 epitopes of three additional prostate cancer-associated antigens as well as PSA (see Table 2). The PBMC were obtained prior to vaccination and post-vaccination each month during there first 3 months of vaccinations for a total of four time points. The presence of T cells directed against a known Flu HLA-A2 epitope was utilized as a positive control. All three patients had detectable levels of T cells to Flu at all four time points. Although all were negative for T cells directed against these antigens pre-vaccination, all three patients developed T-cell responses to at least one of the prostate-associated TAAs (other than PSA) at the various post-vaccination time points. These included the generation of T cells directed against PSMA, PAP, and/or MUC-1 (Table 2). In addition, some patients there were corresponding trends in the immune responses observed and the corresponding serum PSA responses, as seen in Table 3. In case studies #2, 6, and 7 (Table 3), although the PSA-specific T cells initially increased, there was also an initial increase in PSA levels. Three patients on the combination of docetaxel and vaccine had increased PSA-specific T-cell responses with declining serum PSA levels. One patient (case study #6) maintained his immune response for up to 267 days of initiating therapy with continued PSA decline. Three patients who crossed over to docetaxel post-vaccine continued to maintain PSA-specific T-cell responses that correlated with declining serum PSA levels. In case study #8, the patient's serum PSA level stabilized for approximately 1 year following cross-over before progressing at day 427. However, he continued to have additional increases in T-cell response with docetaxel therapy alone during this period. The patient in case study #2 (Table 3) experienced a 65% decline in his PSA level following cross-

over to docetaxel therapy alone over a period of more than 7 months; additionally he experienced a greater than three-fold increase in his T-cell response during this time period.

Serum PSA declines and changes in velocity. Serum PSA level declines were observed in three of the 14 patients in the vaccine alone arm, although none of these declines were >50% (Table 4). In the combination arm, six of 14 patients had a decline in serum PSA from baseline, with three patients demonstrating declines >75% (Table 4). Finally, 11 patients on the vaccine alone arm at time of progression went on to receive docetaxel alone. Using their progression PSA level on the vaccine as a new baseline, 9 of 11 patients had a decline in serum PSA. Five patients had declines >50%, with two of these patients having declines of >75% (Table 4). To validate the significance of these responses, serum PAP levels were also obtained along with the PSA levels. The serum PAP levels either declined or were stable when serum PSA declines were noted (data not shown). No anti-PSA antibody was detected in any of these patients at a minimal serum dilution of 1/50.

Using linear regression, PSA velocity was calculated for patients enrolled on vaccine with docetaxel as well as for those patients receiving docetaxel following vaccine progression (Table 5). These results were also compared with those from a historical set of patients treated at our institution with the same disease stage who received the same dose and schedule of docetaxel as a single agent (6). For those patients with multiple PSA values at both pre-treatment and post-treatment time points, we found that the PSA velocities stabilized or decreased in 9 of 9 patients on the combination arm, 8 of 11 patients who received docetaxel following vaccine progression, and 13 of 17 patients in the historical control group receiving docetaxel alone (6). Thus, it appears that vaccine either in combination with, or prior to, docetaxel therapy may have a positive effect on patients' PSA levels when compared with a historical control using the same dosing in the same patient population. Furthermore, we calculated the median change in relative PSA velocity for each of these three groups (Fig. 3 displays the actual change in PSA velocity). The median relative change in median PSA velocity in the docetaxel post-vaccine and docetaxel alone arms were similar (-83% and -95%, respectively; $p=0.42$), and the median relative change in velocity was also similar to that for the combination arm of vaccine and docetaxel (-83%; $p>0.70$ for both comparisons with the combination arm). Each relative change was significantly different from zero ($p<0.005$).

Progression-free survival. Table 6 shows the median PFS, which takes into account both radiographic progression as well as disease progression attributable to a rising serum PSA level. These results are imprecise because of the limited number of patients enrolled on this trial, which was not specifically designed to address the duration of progression-free survival. Patients on the vaccine alone arm had a median time of progression of 1.8 months, with the majority of patients progressing as a result of rising serum PSA levels (64.3%). Patients who were randomized to receive docetaxel with vaccine had a median PFS of 3.2 months; the majority of these patients progressed radiographically (57.1%). Eleven of the vaccine only patients at the time of progression remained on study after the vaccine was discontinued, and weekly docetaxel was added. The median PFS of these patients was 6.1 months. As an informal comparison, in a similar group of 25 patients with the same disease characteristics treated at the same institution using the same single agent docetaxel regimen, the median PFS was 3.7 months (6).

Discussion

To our knowledge this is the first randomized trial demonstrating that a vaccine can be administered with weekly docetaxel and dexamethasone comedication without compromising the ability of the patient to mount a T-cell specific response against the TAA, as compared with the administration of the vaccine alone. The use of chemotherapeutic agents for the

treatment of human malignancies has been based on the well-characterized cytotoxic effects on malignant cells. It has been thought by some that because of these effects, chemotherapy would have a negative impact on the immune system. However, recent experimental studies in vitro support the concept that certain anti-cancer agents may actually (a) exhibit immune modulatory activities, (b) upregulate cell surface expression of MHC molecules and/or TAAs on tumors and/or, (c) upregulate Fas expression on malignant cells rendering them more sensitive to immune destruction. Detailed in-vitro preclinical studies involving 5FU, cisplatin and cyclophosphamide have shown these agents to have the ability to upregulate components of the immune system (35-39).

Jaffee and colleagues published in-vivo preclinical data addressing the issue of chemotherapy and cancer vaccines (40). This study indicated that taxane therapy (employing taxol) may in fact increase T-cell precursors rather than deplete them. The data presented in this study support the following two conclusions of that study (40): (a) Taxol chemotherapy, when given in a defined sequence with a murine GM-CSF- secreting neu-expressing whole-cell vaccine, enhances the potential of the vaccine to delay tumor growth in tolerized *neu* transgenic mice. The optimal immune-modulating dose for each chemotherapeutic agent appears to be just above doses that begin to induce cytopenias. (b) The enhanced antitumor response appears to be mediated, at least in part, by an increase in number and function of antigen-specific T cells- in particular, the Th1 response. Preclinical data from that study (40) indicated that administering taxane therapy 1 day prior to a single vaccine can enhance both the immunologic and tumor response.

Earlier clinical studies have evaluated the role of combining vaccines with standard chemotherapy agents. In a colorectal cancer trial an anti-idiotype murine monoclonal antibody vaccine, which mimics a highly tumor-restricted CEA epitope post- resection, 32 patients were randomized to treatment with 2 mg of aluminum hydroxide precipitated vaccine intracutaneously or 2 mg of vaccine mixed with 100 μg of the QS-21 adjuvant s.c. every other week, then monthly until disease recurrence. Patients consenting to this trial had differing stages of disease. Four patients with Dukes stage B2, 11 with Dukes stage C, and eight with Dukes stage D had their tumors completely resected. Nine patients with Dukes stage D carcinoma were incompletely resected, which was attributable to positive margins post-operatively. Fourteen patients underwent chemotherapy with 5-FU concomitantly with CeaVac. Ten patients relapsed or had progressive disease ranging from 6-30 months. Two patients died at 14 and 20 months, respectively. All 32 patients demonstrated idiotype-specific T-cell responses, of which 75% were CEA specific. These T-cell responses were measured by proliferation of patients' PBMC in response to CEA. The concomitant use of chemotherapy did not impair this immune response (41,42). Even though this trial was not designed to examine survival, the authors noted that several of the high risk patients appeared to do better than expected as measured by historical controls.

Other clinical trials have combined chemotherapeutic agents with cancer vaccines. In a recent single arm study by Noguchi et al. (43), 13 metastatic hormone refractory prostate cancer patients who previously failed to respond to prior peptide vaccination were treated with a peptide-based vaccine along with low dose estramustine. Estramustine combines a nitrogen mustard moiety with estradiol and is approved by the Food and Drug Administration for use in patients with metastatic prostate cancer. In this study, eleven of these patients who received more than one cycle of treatment were eligible for immunological and clinical evaluation. There was no significant immunosuppression in most cases when the peptide plus a half dose (280 mg/day) of estramustine were administered, whereas severe immunosuppression was observed in the first two patients who received both the peptide plus a full dose (560 mg/day) of estramustine. Augmentation of peptide-specific CTL precursors or peptide-specific IgG was observed in six of 11 and 10 of 11 cases, respectively (43). However, without a vaccine alone

control arm, it is unclear what effect the estramustine has on the ability of the vaccine to mount an immune response in this study.

The question of whether steroids would inhibit immune responses was also a concern in designing this study. Clinical data suggest that “short bursts” of steroids may not adversely affect the immune system. A study by Fairchok et al. evaluated the immunogenicity of the influenza virus vaccine in children receiving short-course Prednisone for acute asthmatic exacerbations (44). Children were randomized to receive influenza virus vaccine with or without steroids. Those randomized to steroids received Prednisone (2mg/kg per day for 5 days). The results showed no diminished immune responses in the steroid group (44). In a different study, the effect on the immune system of ICU patients treated with “stress dose steroids” was investigated. Patients who received a short course of high dose steroids (1 gram of methylprednisolone) had an immune response to a tetnus toxoid equivalent to that in patients who did not receive the steroids (45). It is important to note that the immune responses measured in both those studies were antibody responses. These are thus the first studies, to our knowledge, to demonstrate that “short bursts” of steroids do not inhibit T-cell responses.

The primary endpoint of this study was to determine the effects of docetaxel on the ability to mount a specific T-cell response to the vaccine. We also evaluated the ability in select patients who received the vaccine alone to mount T-cell responses to epitopes on other prostate cancer antigens not found in the vaccine. Although none of the three patients were vaccinated against PAP, MUC-1 or PSMA, all three patients tested mounted T-cell responses to one or more of these prostate-associated antigens at various time points following initiation of the vaccine. Since none of these three patients received docetaxel during these time points, it is possible that the immune responses post- vaccination were related to the direct effects of immune targeting of the prostate gland induced by the vaccine. Destruction of some of the prostate cancer cells through PSA- specific T cells elicited from the vaccine may have resulted in release of other antigens from the tumor, allowing for the generation of T cells specific for these other prostate- associated antigens. This phenomenon of “antigen cascade” has been reported by others (46,47). In a recent study conducted by Gulley et al., six of eight patients with localized prostate cancer mounted immune responses following vaccination with a PSA vaccine to four prostate antigens not contained in the vaccine (27). This phenomenon has also occurred when utilizing a different PSA-based vaccine strategy. Harada et al. recently reported the results of a patient vaccinated every 2 weeks with four different HLA-A24+ peptides, including PSA 248-257 peptide. After 3 months, the patient’s disease progressed and estramustine was added along with the vaccinations and he achieved a response to this treatment for an additional 12 months. Increased levels of IgG were found to be reactive to PSA-derived epitopes other than those peptides administered in this study, suggesting that the peptide vaccination induced epitope spreading of IgG in this particular patient (48).

We also evaluated clinical responses to the combination therapy, vaccine alone, and chemotherapy after progression on vaccine. We also informally compared these results with a historical control group in the same patient population using the identical dosing of docetaxel that was given in this study. While PFS in the combination arm was similar to that of the historical group (median 3.2 months vs. 3.7 months), there was somewhat more of an increase in PFS in the patients who received docetaxel following disease progression on vaccine alone (6.1 months vs. 3.7 months). We also noted a few instances in which patients who crossed over to docetaxel post-vaccine continued to maintain PSA-specific T-cell responses that corresponded to declining serum PSA levels. Two of these patients actually had additional increases in the T-cell responses when crossing over to docetaxel therapy alone over a period of 308 and 427 days, respectively. The vaccine employed in this study was rV-PSA admixed with rV-B7.1 as a prime, followed by rF-PSA booster vaccines. Since the initiation of this trial, preclinical studies have demonstrated that the insertion of multiple costimulatory molecule

genes (B7.1, ICAM-1, and LFA-3; designated TRICOM) 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 anti-tumor response, as compared with the use of a vector containing only the B7.1 transgenes (49-51). New vaccines containing the transgenes for PSA and this triad of costimulatory molecules (designated rV-PSA-TRICOM and rF- PSA-TRICOM) have recently entered both Phase I and Phase II trials (52,53). In a recent study by Gulley et al., 25 patients with metastatic AIPC who had not undergone prior chemotherapy have been enrolled to a study using these newer vaccine agents. Sixteen patients have undergone restaging evaluation at 3 months. Three of these patients have demonstrated PSA declines during this period, two patients with PSA declines of >30% and one patient with a decline of >50%. One patient at 8 months has experienced a partial response by RECIST criteria with >50% decrease in unidimensional measurement of his hilar adenopathy. Another patient had a 29% decrease in his soft tissue disease (53). Thus these new vaccines appear more potent than the vaccine used in the study reported here, and it is possible that we may achieve a better clinical response when combining these new generation vaccines with weekly docetaxel in this patient population. We are also examining other combination strategies with these newer vaccines and have recently initiated a clinical study combining the rV-PSA-TRICOM and rF-PSA-TRICOM vaccines with an anti CTLA-4 antibody.

In summary, to our knowledge, this is the first randomized study evaluating the role of a cancer vaccine with chemotherapy and steroids vs. vaccine alone to determine the effect on T-cell-specific immune responses to the vaccine in the combination arm compared with the vaccine alone. There was no deleterious effect on the ability to mount immune responses when using monthly vaccines in combination with weekly docetaxel and dexamethasone. Furthermore, T-cell-specific immune responses to prostate cancer epitopes not contained in the vaccine were elicited in three patients following the administration of vaccine alone. Based on recent preclinical data from our laboratory, it may be advantageous to administer these pox vector vaccines prior to chemotherapy to enhance immune responses. Future approaches using more potent vaccines containing multiple costimulatory molecules along with docetaxel may lead to more effective therapeutic approaches for patients with metastatic AIPC.

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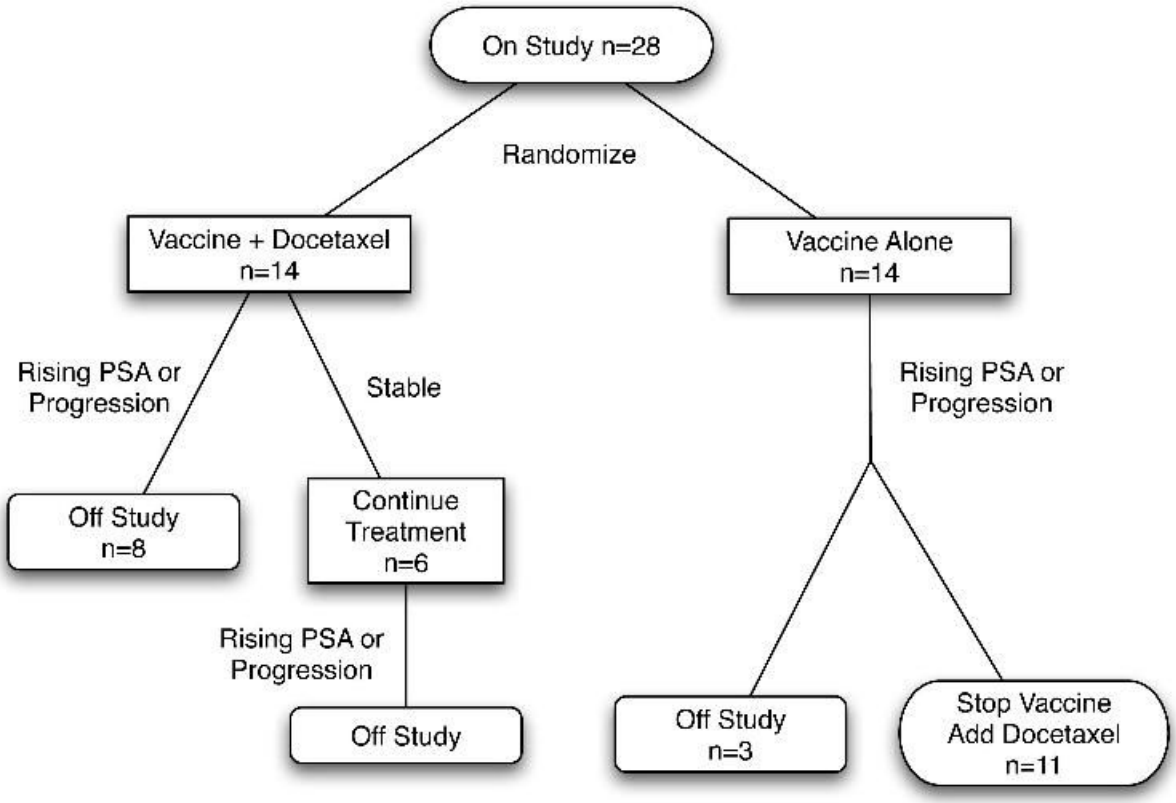

Fig. 1.

Twenty-eight patients were randomized, 14 per arm. Six of fourteen on the combination arm continued on study beyond the initial 3 months. Eleven of 14 patients on vaccine alone had vaccine discontinued at time of progression and received docetaxel alone.

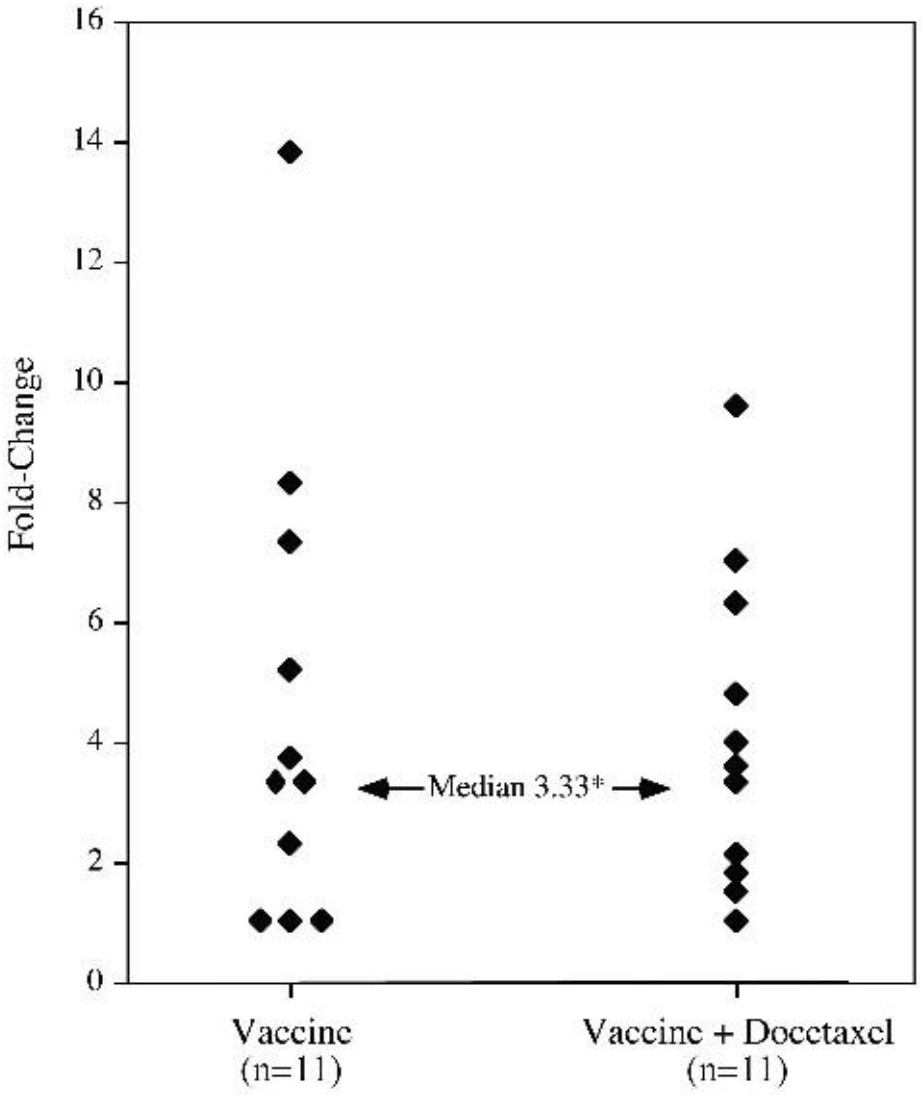

Fig. 2.

Eleven HLA-A2 positive patients on the vaccine arm alone and 11 HLA-A2 positive patients on the combination of vaccine and docetaxel were evaluated for induction of PSA-specific T-cell responses employing the ELISPOT assay as described in the Methods section prior to and following three monthly cycles of therapy. A t-test was used to compare the change in precursor frequency between the combination arm vs. the vaccine alone arm to detect a relevant difference in precursor frequency. The median fold increase in both arms was 3.33 ($P = 0.92$).

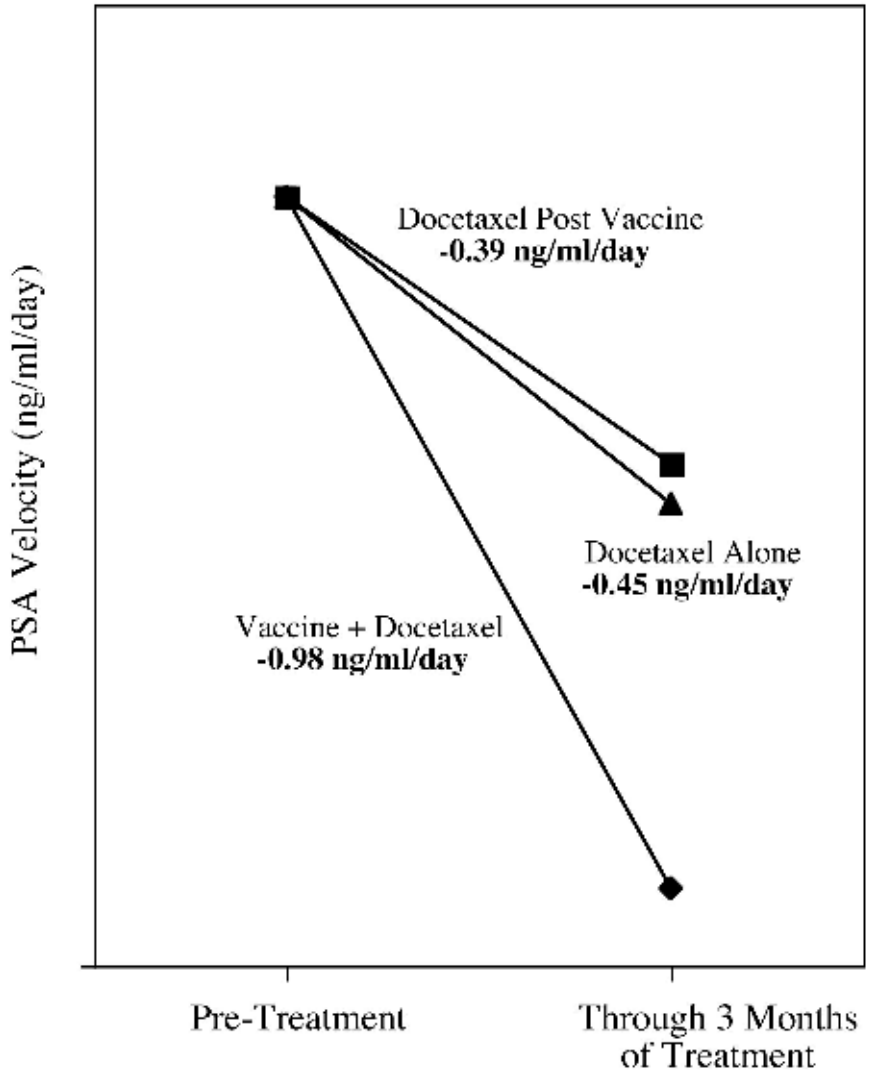

Fig. 3.

Pre- and post-therapy serum PSA velocities for patients receiving docetaxel alone (historical control), docetaxel with vaccine, or docetaxel following vaccine. Velocities are determined as ng/ml/day. The median relative change in median PSA velocity in the docetaxel post-vaccine and docetaxel alone arms was similar (-83% and -95%, respectively; p=0.42). The median relative change in velocity for the combination arm of vaccine and docetaxel was -83% (p>0.70 for both comparisons with the combination arm). Each relative change was significantly different from zero (p<0.005). See Patients and Methods section for calculations of PSA velocity.

Table1

Demographics

		Vaccine Only (n = 14)	Vaccine + Docetaxel (n = 14)	Docetaxel (post-vaccine) (n = 11)
Age				
Median		69.5	66.5	72.0
Mean		68.6	66.7	70.8
Range		51-85	56-81	56-85
Gleason				
Mean		8.3	7.9	8.3
Range		6-9	7-9	6-9
# of Pts. (%)				
	6	1 (7.1)	0 (0)	1 (9.1)
	7	2 (14.3)	4 (28.6)	1 (9.1)
	8	2 (14.3)	6 (42.9)	2 (18.2)
	9	8 (57.1)	3 (21.4)	6 (54.5)
	?	1 (7.1)	1 (7.1)	1 (9.1)
On-study PSA				
Median		61.1	129.1	95.6
Mean		247.0	197.0	338.8
Range		6.3-1385	17.7-675	13.5-2119
Sites of Metastasis				
Number of Pts. (%)				
Bone only		12 (85.7)	4 (28.6)	7 (63.6)
Soft tissue only		1 (7.1)	3 (21.4)	1 (9.1)
Both		1 (7.1)	7 (50)	3 (27.3)

Table 2

Precursor frequencies of T-cell specific for PSA, PSMA, PAP and MUC-1 peptides from PBMC of patients before and at different time points after vaccination

Patient	Samples	Peptide				
		Flu	PSA	PSMA	PAP	MUC-1
Case study #1	Pre	1/17,143	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	Day 28	1/13,043	1/85,714	<1/200,000	<1/200,000	1/37,500
	Day 56	1/15,789	1/54,545	<1/200,000	<1/200,000	1/66,667
	Day 84	1/11,628	1/23,810	<1/200,000	<1/200,000	1/20,000
Case study #2	Pre	1/75,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	Day 28	1/100,000	1/46,154	1/85,714	<1/200,000	1/75,000
	Day 56	1/120,000	1/60,000	1/66,667	<1/200,000	1/54,545
	Day 84	1/150,000	1/85,714	<1/200,000	1/54,545	1/85,714
Case study #3	Pre	1/57,143	1/200,000	1/200,000	1/200,000	<1/200,000
	Day 28	1/60,000	1/46,154	1/42,857	1/66,667	1/35,294
	Day 56	1/35,294	1/50,000	1/35,294	1/66,667	1/33,333
	Day 84	1/66,667	1/60,000	1/100,000	<1/200,000	1/75,000

PSA, PSMA, PAP and MUC-1 peptides were used in the ELISPOT assay at a concentration of 25 μg/ml. Flu peptide was used at a concentration of 1 μg/ml.

Table 3

Immune and PSA responses, case studies

	Timepoint	ELISPOT Flu	ELISPOT PSA3	Serum PSA
Vaccine + Docetaxel:				
Case Study #4	Pre	1/30,000	1/100,000	251.0
	Day 84	1/10,169	1/14,286	204.6
Case Study #5	Pre	1/46,154	<1/200,000	
				19.7
	Day 88	1/54,545	1/75,000	16.8
Case Study #6	Pre	1/54,545	1/200,000	17.7
	Day 84	1/50,000	1/31,579	20.2
	Day 267	1/27,273	1/37,500	11.0
Docetaxel	Post-Vaccine:			
Case Study #2	Pre	1/75,000	<1/200,000	1385.0
	Day 84*	1/150,000	1/85,714	2119.0
	Day 308	1/20,833	1/23,810	739.0
Case Study #7	Pre	1/25,000	<1/200,000	16.3
	Day 85*	1/17,647	1/14,634	55.3
	Day 167	1/40,000	1/19,355	24.4
Case Study #8	Pre	1/12,766	1/200,000	30.9
	Day 84*	1/14,634	1/66,667	26.8
	Day 427	1/24,000	1/27,273	51.2

* Point of cross-over.

Table 4

PSA declines

% PSA decrease	Vaccine only (n = 14)	Vaccine + Docetaxel (n = 14)	Docetaxel alone (post-vaccine) (n = 11)
0 - 10	1	2	2
10 - 24	1	1	1
25 - 49	1	0	1
50 - 74	0	0	2
>75	0	3	3
	3	6	9

Patients' serum PSA levels were measured prior to randomization and after each monthly treatment.

PSA declines were shown as maximum percentage decrease from baseline during treatment.

Note: Increased serum PSA levels were noted in 11 patients in vaccine only; 8 patients in vaccine plus docetaxel; and 2 patients in docetaxel alone treatment post-vaccine.

Table 5

Slopes of serum PSA velocity pre- and post-therapy

Vaccine + Docetaxel				Docetaxel post-vaccine				Docetaxel alone			
Pt. ID	Pre-treatment	Post-treatment	Change in velocity	Pt. ID	Pre-treatment	Post-treatment	Change in velocity	Pt. ID	Pre-treatment	Post-treatment	Change in velocity
1	0.96	0.73	↓	1	0.54	0.15	↓	1	0.46	0.00	↓
2	14.78	7.33	↓	2	3.54	0.59	↓	2	1.85	0.10	↓
3	3.28	0.55	↓	3	0.23	-0.07	↓	3	0.76	-0.12	↓
4	0.20	0.06	↓	4	10.43	-0.03	↓	4	0.82	0.30	↓
5	1.30	-0.50	↓	5	2.50	-0.56	↓	5	1.06	0.95	↓
6	105.00	12.19	↓	6	1.32	0.47	↓	6	20.91	-0.43	↓
7	0.06	-0.03	↓	7	0.33	0.05	↓	7	0.15	0.01	↓
8	1.58	0.34	↓	8	0.14	0.16	↔	8	2.47	-0.17	↓
9	0.98	-0.65	↓	9	0.71	1.11	↑	9	6.39	1.56	↓
				10	-0.12	-0.02	↑	10	0.99	0.05	↓
				11	-2.12	5.02	↑	11	0.33	0.34	↔
								12	-0.03	-0.03	↔
								13	0.01	-0.03	↔
								14	-0.18	0.01	↑
								15	-1.36	0.45	↑
								16	-0.09	-0.01	↑
								17	-0.26	1.71	↑

Velocities given in ng/mL/day.

The fractions of patients who have declined or stabilized were similar ($p>0.20$ by Fisher"s exact test).

Table 6

Progression-free survival

Regimen	n	>50% PSA decline	Median time to progression	PSA progression	Radiographic progression
Vaccine alone	14	0/14 (0%)	1.8 months	9/14 (64.3%)	5/14 (35.7%)[#]
Vaccine + docetaxel	14	3/14 (21.4%)	3.2 months	6/14 (42.9%)	8/14 (57.1%)
Docetaxel post-progression on vaccine	11	5/11 (45.5%)	6.1 months	5/11 (45.5%)	5/11 (45.5%)[##]
Docetaxel alone[*]	25	9/24 (37.5%)	3.7 months		

[*] Historical control with same dose and schedule of docetaxel in similar patient population at same institution [Ref. 6].

[#] 9/11 patients who then crossed over to the docetaxel post-progression on vaccine arm had radiographic progression at the time of cross-over.

[##] One patient in the docetaxel post-progression on vaccine arm came off-study per the patient's request.