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Immunologic and Prognostic Factors Associated with Overall Survival Employing a Poxviral-based PSA Vaccine in Metastatic Castrate-resistant Prostate Cancer

James L. Gulley, M.D.^{1,2}, Philip M. Arlen, M.D.^{1,2}, Ravi A. Madan, M.D.^{1,2}, Kwong-Yok Tsang, Ph.D.¹, Mary P. Pazdur, R.N.¹, Lisa Skarupa, R.N.¹, Jacquelin L. Jones, R.N.², Diane J. Poole¹, Jack P. Higgins, Ph.D.¹, James W. Hodge, Ph.D., MBA¹, Vittore Cereda, M.D.¹, Matteo Vergati, M.D.¹, Seth M. Steinberg, Ph.D.³, Susan Halabi, Ph.D.⁴, Elizabeth Jones, M.D.⁵, Clara Chen, M.D.⁶, Howard Parnes, M.D.⁷, John J. Wright, M.D.⁸, William L. Dahut, M.D.², and Jeffrey Schlom, Ph.D.¹

¹Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 8B09, Bethesda, MD 20892

²Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 13N240E, Bethesda, MD

³Biostatistics and Data Management Section, National Cancer Institute, National Institutes of Health, 6116 Executive Boulevard, Room 702, Rockville, MD

⁴Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Box 2721, DUMC, Durham, NC 27710

⁵Department of Diagnostic Radiology, Clinical Center, National Institutes of Health, 10 Center Drive, Room 1C372, Bethesda, MD

⁶Department of Nuclear Medicine, Clinical Center, National Institutes of Health, 10 Center Drive, Room 1C401, Bethesda, MD

⁷Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, 6130 Executive Blvd, Room 2100, Rockville, MD

⁸Investigational Drug Branch, Cancer Therapy and Evaluation Program, National Cancer Institute, National Institutes of Health, 6130 Executive Blvd, Room 7122, Rockville, MD 20852

Abstract

A concurrent multicenter, randomized Phase II trial employing a recombinant poxviral vaccine provided evidence of enhanced median overall survival (OS) ($p=0.0061$) in patients with metastatic castrate-resistant prostate cancer (mCRPC). The study reported here employed the identical vaccine in mCRPC to investigate the influence of GM-CSF with vaccine, and the influence of immunologic and prognostic factors on median OS. Thirty-two patients were vaccinated once with recombinant

Corresponding author: Jeffrey Schlom, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 8B09, Bethesda, MD 20892. Phone: (301) 496-4343; Fax: (301) 496-2756; js141c@nih.gov.

JLG and PMA contributed equally to this work.

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vaccinia containing the transgenes for prostate-specific antigen (PSA) and three costimulatory molecules (TRICOM). Patients received boosters with recombinant fowlpox containing the same four transgenes. Twelve of 32 patients showed declines in serum PSA post-vaccination and 2/12 showed decreases in index lesions. Median OS was 26.6 months (predicted median OS by the Halabi nomogram was 17.4 months). Patients with greater PSA-specific T-cell responses showed a trend ($p=0.055$) toward enhanced survival. There was no difference in T-cell responses or survival in cohorts of patients receiving GM-CSF vs no GM-CSF. Patients with a Halabi predicted survival of < 18 months (median predicted 12.3 months) had an actual median OS of 14.6 months, while those with a Halabi predicted survival of ≥ 18 months (median predicted survival 20.9 months) will meet or exceed 37.3 months, with 12/15 patients living longer than predicted ($p=0.035$). Treg suppressive function was shown to decrease following vaccine in patients surviving longer than predicted, and increase in patients surviving less than predicted. This hypothesis-generating study provides evidence that patients with more indolent mCRPC (Halabi predicted survival ≥ 18 months) may best benefit from vaccine therapy.

Keywords

Cancer vaccine; Immunotherapy; Prostate cancer; Overall survival; PSA-TRICOM; PROSTVAC

Introduction

Prostate cancer is the most common noncutaneous malignancy among men in the United States, with an estimated 192,280 diagnosed each year, resulting in approximately 27,360 deaths [1]. While Gleason score has traditionally been a main prognostic indicator for primary prostate cancer, the Halabi nomogram [2] is increasingly being used as a prognostic indicator for overall survival of patients with metastatic castration-resistant prostate cancer (CRPC). The Halabi nomogram is a pretreatment prognostic model derived from results of six separate Cancer and Leukemia Group B (CALGB) trials of 1,101 patients with metastatic CRPC; it is based on seven predictors of overall survival: presence of visceral disease, Gleason sum, performance status, PSA, lactate dehydrogenase, alkaline phosphatase, and hemoglobin. It is important to note that this model predicts overall survival based on the outcomes of patients receiving chemotherapy- or hormonal-based treatment for metastatic CRPC, and does not include untreated patients. The use of this model to compare predicted survival with actual overall survival has been previously reported [3]. Only one drug, docetaxel, has been shown to lengthen overall survival (by approximately 2-3 months) in men with metastatic CRPC [4]. This dearth of approved therapeutics is the impetus for testing novel agents in this disease.

An alternative approach for prostate cancer therapy is the use of vaccines directed against prostate-associated antigens. A recent Phase III trial [5] employing the sipuleucel-T vaccine (PAP-GM-CSF fusion protein-pulsed analogous antigen-presenting cells from leukapheresis) demonstrated improved median overall survival (25.9 months for vaccine vs 21.4 months for control, $p=0.032$) in patients with metastatic castrate-resistant prostate cancer (mCRPC).

Another immunotherapy platform is a vector-based vaccine directed against prostate-specific antigen (PSA). Preclinical studies have previously demonstrated the efficacy of (a) diversified prime/boost vaccination regimens employing recombinant vaccinia (rV-) as prime and multiple recombinant fowlpox (rF-) in boosts [6]; (b) the insertion of one or more T-cell costimulatory molecules into the vector along with the transgene for the tumor-associated antigen (TAA) [7,8]; and (c) the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as a biologic adjuvant to enhance recruitment of dendritic cells [9]. GM-CSF can be administered as a recombinant protein or by inserting the GM-CSF gene into an rF- vector (rF-GM-CSF).

Several previous clinical trials involving recombinant poxvirus vectors encoding the transgene for PSA have shown evidence of clinical benefit. In a phase I study employing rV-PSA alone, PSA levels in 13 of 33 men stabilized for at least 6 months post-vaccination, and nine patients remained stable for 11 to 25 months [10]. A randomized phase II trial employing rV-PSA (V) and/or rF-PSA (F) in patients with biochemical progression after local therapy has also been completed [11]. At a median follow-up of 50 months [12], the median time to PSA or clinical progression was 9.2 months (FFFF cohort) and 9.0 months (FFFV cohort) vs. 18 months in the VFFF cohort—evidence of clinical benefit with a diversified prime (V)/boost (FFF) strategy. There have been two clinical trials employing a strategy of rV-PSA admixed with the vaccinia vector encoding the gene for the costimulatory molecule B7.1 (rV-PSA-B7.1) as prime, and multiple rF-PSA as boosts. In the first study, the sequence of vaccine followed by docetaxel showed evidence of clinical benefit [13]. A second study enrolled 42 patients who had nonmetastatic CRPC and who were randomly assigned to receive either vaccine or androgen receptor antagonist therapy with nilutamide [14]. At PSA progression, patients received the other therapy while continuing to receive their original therapy. Patients randomized to the vaccine arm had a 3-year survival probability of 81% and an overall survival of 5.1 years, while patients randomized to nilutamide had a 3-year survival probability of 62% and an overall survival of 3.4 years [15].

Preclinical studies [7,8,16,17,18] have demonstrated the advantage in the use of pox-virus vaccines containing the transgenes for three T-cell costimulatory molecules (B7.1, ICAM-1 and LFA-3, designated TRICOM) along with the tumor-associated antigen transgene, as compared to the use of vaccines containing either one or no costimulatory transgenes. This advantage was defined by both increases in T-cell responses to the tumor-associated antigen and by anti-tumor responses. A Phase I trial was recently completed [19] employing rV-, rF-PSA-TRICOM vaccination (also designated PROSTVAC-VF) in patients with metastatic prostate cancer. The main toxicity was grade ≤ 2 (at the injection site) with no grade 3 or greater toxicity observed. Decreases in serum PSA velocity were observed in 9/15 patients post-vaccination.

Two Phase II trials have been carried out concurrently employing rV, rF-PSA-TRICOM vaccination in patients with metastatic prostate cancer. One trial was a recent randomized, placebo (empty vector)-controlled, multi-center trial in patients (n=125) with progressive metastatic disease despite castrate testosterone levels and a Gleason score of ≤ 7 [20,21,22]. Patients with visceral metastasis, a history of prior chemotherapy, or narcotic use were excluded. Vaccinated patients had a better 3-year overall survival than control patients (30% vs 17%), and a longer median overall survival (24.5 vs 16 months); estimated hazard ratio 0.56 (95% CI 0.37-0.85); stratified log rank (p=0.0061). T-cell responses to vaccine or vector were not evaluated. The other concurrent Phase II trial employing rV, rF-PSA-TRICOM is reported here. In this trial there were no exclusion criteria for patients having visceral metastases or narcotics for cancer related pain and patients with all Gleason scores were enrolled. Immune responses to both PSA and fowlpox vector were analyzed, as was an exploratory analysis of the impact on overall survival of tumor specific T-cells and Tregs after vaccine. A retrospective analysis of overall survival employing the Halabi prognostic model (used to predict survival for patients treated with conventional therapies) provided evidence for the influence of patient selection on overall survival for metastatic prostate cancer patients treated with vaccine therapy.

Patients and Methods

Eligibility

All patients enrolled in this study had metastatic chemotherapy-naïve CRPC. Patients with no prior history of orchiectomy maintained castrate levels of testosterone with gonadotropin-

releasing hormone agonists. At enrollment, patients were required to be HLA-A2⁺, have a > 6-month life expectancy, ECOG performance status of 0 to 2, and normal hepatic, kidney, and hematopoietic function. Exclusion criteria included any disease requiring systemic steroid therapy, history of allergy to eggs, history of autoimmune disorder, HIV seropositivity, other active malignancies within the past 2 years (with the exception of non-melanoma skin cancers or carcinoma in situ of the bladder), and clinically active brain metastasis. All patients signed a consent form approved by the Institutional Review Board of the National Cancer Institute (NCI).

Study design and treatment

The primary endpoint of this study was immune response as measured by ELISPOT assay, with time to progression (using radiologic and PSA criteria), objective response and overall survival as secondary endpoints [23]. At enrollment, patients to be vaccinated were randomized to one of four cohorts concerning immune adjuvant: no adjuvant (cohort I), recombinant human GM-CSF protein (cohort II), 10^7 plaque-forming units (pfu) rF-GM-CSF (human) [9] (cohort III), or 10^8 pfu rF-GM-CSF (human) (cohort IV). All patients were primed with rV-PSA-TRICOM 2×10^8 pfu s.c. on day 1, and then received monthly boosts of rF-PSA-TRICOM 1×10^9 pfu s.c. until progression. Patients who remained on study after 12 months had booster vaccines every 3 months. Patients received GM-CSF 100 μ g s.c. on the day of each vaccination and for 3 consecutive days thereafter, all near the vaccination site. Patients in cohorts III and IV received a single injection of rF-GM-CSF s.c. on the day of each vaccination, adjacent to the vaccination site. The sample size was set at 32 subjects, approximately 8 per arm, in order to provide adequate patients to test for pre-specified differences between the arms which were equal to two standard deviations from the change in baseline within each arm.

Patients were monitored monthly by physical examination and laboratory analyses. Computed tomography (CT) and bone scans were performed every 3 months. Patients with a rising PSA [23] or new lesions were removed for disease progression.

Vaccine

PSA-TRICOM vaccine consists of rV-PSA-TRICOM primary vaccination and rF-PSA-TRICOM booster vaccinations. Each vaccine contains the entire PSA transgene with an agonist epitope [24] and TRICOM. Vaccines were supplied by the Cancer Therapy Evaluation Program, NCI. Vaccines were prepared as previously described [25] and stored at -70°C until the day of administration, when they were thawed to room temperature.

Immunologic monitoring

Elispot assay—PBMCs were collected via apheresis prior to treatment with vaccine and after approximately 3 months of therapy. Twenty-nine of 32 patients were evaluable at both of these time points. Immunologic response was measured by the ELISPOT assay, as previously described, [26] to evaluate the relative production of IFN-gamma by T cells after exposure to the PSA-peptide PSA-3 [26]. Serum samples were analyzed pre- and post-treatment for the presence of antibodies to PSA, as previously described [26], to further characterize immune responses.

Titration of serum fowlpox antibody titers—Anti-fowlpox antibodies (IgG) were quantified from the serum of each patient by ELISA. Flat-bottom 96-well plates (BD, Franklin Lakes, NJ) were coated with fowlpox virus (5×10^5 pfu/well) or DPBS (Mediatech, Herndon, VA) and held at 4°C until use. Plates were blocked with Sea Block (Pierce, Rockford, IL) for 1 h at 37°C . Plates were then incubated with patient serum serially diluted 5-fold from 1:50 to 1:6250, as well as with normal human serum or human antifowlpox antiserum as controls, for 2 h at 4°C . Plates were washed several times with PBS containing 1% BSA and incubated at

37°C for 1 h with horseradish peroxidase-conjugated goat antihuman IgG (Fc)-specific antiserum (1:10000). This antibody complex was detected by a TMB substrate kit (Pierce, Rockford, IL) according to the manufacturer's instructions. The absorbance of each well was read at 450 nm using a BioTek EL310 microplate ELISA reader (BioTek Instruments, Winooski, VT). Fowlpox antibody titers were based on a blank adjusted absorbance of 0.5.

Fowlpox virus neutralization—Patient serum was diluted 1:50 in DMEM 10% fetal bovine serum (FBS) containing 4×10^6 pfu rF-murine(m)B7-1 and incubated for 1 h at 4°C. Normal human serum with or without rF-mB7-1 was used for controls. MC38 cells (2×10^5) were added to all samples and incubated overnight at 37°C with 5% CO₂. Cell surface expression of mB7-1 was performed as previously described [27]. Briefly, cells were stained with a primary phycoerythrin (PE)-labeled antibody (BD, Franklin Lakes, NJ) and cell fluorescence was analyzed and compared with isotype-matched controls using a FACScan cytometer (BD, Franklin Lakes, NJ). Samples with a mean fluorescence intensity reduced to that of the isotype control were considered positive for virus-neutralizing antibodies.

Regulatory T-cell analysis—Cryopreserved PBMCs were analyzed by 3-color flow cytometry for phenotypic characterization of regulatory T cells (Tregs) as previously described [28]. Tregs are defined as CD4⁺CD25^{high}FoxP3⁺ T cells. The immunosuppression of Tregs was analyzed by the suppression function assay as previously described [28].

Statistical methods

Survival status was updated via personal or telephone interview or by reference to the Social Security Death Index. Overall survival was calculated from the on-study date until the date of death from any cause or last follow-up. The probability of overall survival as a function of time was estimated by the Kaplan-Meier method with a log-rank statistic used to test for differences between a pair of Kaplan-Meier curves. In order to explore differences in survival, patients were initially evaluated according to potential prognostic characteristics that were divided into quartiles, if continuously measured, or individual treatment groups. Subsequently, subjects were combined into groups based on similarity of estimated survival. The resulting p-values were adjusted by multiplying the unadjusted p-value by the number of implicit comparisons performed to arrive at the final division. For example, comparisons between the four treatment cohorts, with 6 possible divisions into two categories, had the final p-value multiplied by 6. Other parameters were divided on the basis of three possible groupings, and the p-value was multiplied by 3 to arrive at the adjusted value.

In addition, we used the Halabi nomogram [2] to retrospectively predict each individual patient's duration of survival. The nomogram used seven baseline variables: Gleason score, on-study PSA, ECOG performance status, lactate dehydrogenase, alkaline phosphatase, hemoglobin, and presence of visceral disease to predict overall survival in patients undergoing treatment for mCRPC. All p-values are two tailed and, except as noted above, have not been adjusted for multiple comparisons. In comparing patients who lived longer than predicted with those who did not, the two-tailed p-value is based on an exact binomial test, with p=0.5 as the fraction living longer than expected if this were a random occurrence.

Results

Patient baseline characteristics

The median baseline characteristics for all patients are described in Table 1. There were no major differences among the four randomized cohorts in terms of Gleason score, on-study serum PSA, laboratory serum markers, visceral disease, and performance status. Also shown

in Table 1 is the comparison in patient baseline characteristics to those reported in the development of the Halabi nomogram.

Clinical outcomes

Twelve of 32 patients (37.5%) showed absolute declines in serum PSA, with five having declines of $\geq 30\%$. The greatest PSA decline was 72%, in a patient who has remained on study for over 4 years with a sustained decline in PSA $>50\%$ for over 30 months. Two of 12 patients with soft tissue disease showed a measurable decrease in index lesions. One patient (Patient #14) had a reduction of the right hilar lymph node from 2.2 cm to 0.7 cm after the third vaccination (Fig. 1A) and a corresponding decrease in serum PSA from 85 to 56 ng/mL. A second patient (Patient #3) had a reduction of the right pelvic lymph node from 2.2 cm to 1.4 cm following six vaccinations, and a slight reduction of a mediastinal lymph node from 3 to 2.4 cm, for an overall decrease of 29%. This corresponded with a serum PSA decline from 18.8 to 11.6 ng/mL (Fig. 1B). While neither patient had a confirmed objective response by RECIST criteria (restaging was repeated only every 3 months), both patients remain alive at 45.9 and 50.2 months from enrollment.

With a median follow-up of 44.6 months, the median overall survival for all 32 patients on-study was 26.6 months (Fig. 2A) compared to a median predicted survival by the Halabi model of 17.4 months, i.e., a difference of 9.2 months (see Supplemental Figure 1). There were no major differences among the four randomized cohorts. The predicted survival probability was 16.3 (8.2-27.0) months for group I (n=8), 17.2 (6.5-22.7) months for group II (n=9), 16.8 (8.7-22.4) months for group III (n=7), and 19.0 (7.3-26.0) months for group IV (n=8). There were no major differences among the four randomized cohorts in terms of Gleason score, on-study serum PSA, laboratory serum markers, visceral disease and performance status. Although there was a trend toward one arm having lower survival probability than the other three arms, the difference was not statistically significant when the result was adjusted for the number of comparisons considered ($p=0.25$). Thus, results from the four arms will be considered as one overall group for the comparisons presented.

The use of the Halabi nomogram provided intriguing data. For patients with a Halabi predicted survival of < 18 months, there was a minimal difference in the median predicted survival compared with the actual overall survival (Table 2). However, patients with a Halabi predicted survival of ≥ 18 months had a substantial increase in actual overall survival (≥ 37.3 months, median not reached) compared with the median predicted survival (20.9 months, see Table 2). Comparisons made between the actual survival and the Halabi predicted survival (Table 2) were derived from median actuarial values for overall survival while the arithmetic median was used for survival predicted using the Halabi model. The number of patients subsequently treated with chemotherapy post-progression on vaccine was similar in both Halabi groups ($p=0.324$); 10/16 patients received docetaxel in the Halabi predicted survival < 18 -month group, while 11/15 patients received docetaxel in the Halabi predicted survival group ≥ 18 months. Two patients in the latter group also received ketoconazole.

Since some patients remain alive, we cannot do a simple numerical summary which compares the differences per patient with respect to the actual vs. predicted length of survival. However, the following type of comparison may be performed. As shown in Fig. 3, among patients with a Halabi predicted survival of < 18 months, more than half (10/17) lived longer than predicted ($p=0.63$). However, 12 of 15 patients with a Halabi predicted survival of ≥ 18 months lived longer than predicted ($p=0.035$) (Fig. 3, Table 2). It is of interest to contrast the results of this vaccine trial with those of a trial [29,30] employing docetaxel enrolling a similar patient population at the same institution. We report here the Halabi predicted survival as compared with the actual overall survival of that trial [29,30] (Table 2). Of note, in the patients from both trials with a Halabi predicted survival of ≥ 18 months, the median predicted survival of these

two groups is virtually identical: 20.9 months for the current study and 21.0 months for the docetaxel therapy trial. However, the difference between the predicted and actual overall survival for the chemotherapy trial was 4.1 months compared to ≥ 16.4 months in the vaccine trial described here.

Immune responses

Patients' immune responses pre- and post-treatment were analyzed using the ELISPOT assay for IFN- γ production. PBMCs were evaluated for their response to an HLA-A2 PSA-specific peptide previously described [26]. Responses to flu peptide were used as a positive control and responses to HIV peptide were used as a negative control [26]. The results of the 13 of 29 patients analyzed who demonstrated enhanced PSA-specific T-cell immune responses ≥ 2 -fold post-vaccination are shown in Table 3. There was no statistical difference in PSA-specific T-cell responses among any of the four cohorts. ELISPOT analyses of patients' T-cell responses to the PSA epitope in the vaccine demonstrated a trend towards a difference in overall survival for patients with a post-vaccination ELISPOT response to PSA > 6 -fold vs. patients with a post-vaccination ELISPOT response to PSA < 6 -fold ($p=0.055$; Fig. 2B).

To help determine if the increase in survival over predicted in the subgroup of patients predicted to live ≥ 18 months was due simply to a more responsive immune system, all patients were evaluated for their ability to produce antibody responses to the fowlpox vector post-vaccination. No patients had antibodies to fowlpox prior to vaccination (titers $< 1:50$). There was no substantial difference in antibody titers to fowlpox post-vaccination between patients with a Halabi predicted survival of ≥ 18 months and patients with a Halabi predicted survival of < 18 months (Fig. 4). Thus the apparent differences seen in actual compared with predicted overall survival of the two groups did not appear to be due to general immune status of patients. Although anti-fowlpox antibodies were produced following vaccination with rF-PSA-TRICOM, no neutralizing fowlpox antibody titers were detected.

We have previously reported no difference in the number of Tregs in PBMCs from volunteers vs patients with mCRPC [28]. The functionality of Tregs, however, was greater in patients with mCRPC vs normal volunteers [28]. PBMCs from 23 patients in the trial reported here were analyzed for both number and function of CD4⁺/CD25⁺/FoxP3⁺ Tregs. There was no difference in overall survival among patients with varying baseline levels of Tregs in PBMCs ($p>0.8$). There was also no difference ($p=0.15$) in the number of Tregs pre- vs post-three vaccinations (day 85) in patients who survived longer than predicted ($p=0.14$) or less than predicted ($p=0.44$) by the Halabi nomogram as described in Table 2. Albeit not significant ($p=0.087$), there were strong trends in changes in Treg function pre- vs. post-vaccination for patients who survived longer vs. less than predicted. In patients who survived longer than predicted, Treg suppressive function decreased in 10/13 (77%) patients post-three vaccinations vs pre-vaccination (Fig. 5). In contrast, in patients who survived less than predicted, Treg function actually increased post-three vaccinations vs pre-vaccination in 6/8 (75%) patients (Fig. 5). The possible explanations for these differences will be discussed below.

Discussion

The study reported here was conducted to determine the immunologic and clinical activity of PSA-TRICOM vaccination in chemotherapy-naïve patients with mCRPC and to complement a concurrent randomized multicenter trial [20,21,22] employing the same PSA-TRICOM vaccine. These results, albeit in a limited number of patients, provide evidence for the following: (a) some patients receiving PSA-TRICOM achieve declines in PSA and a reduction in measurable soft tissue disease and (b) evidence of increased overall survival compared to what the Halabi nomogram would predict, especially in patients with less advanced or less aggressive disease as indicated by a predicted survival of ≥ 18 months. There is also evidence

of a potential association between survival and patients' ability to mount T-cell responses to vaccine. The addition of GM-CSF to vaccine did not appear to increase T-cell responses to vaccine. While the number of patients in each of the four cohorts was small, there did not appear to be a clinical advantage to administering GM-CSF with vaccine compared to patients receiving vaccine alone, as the four groups tended to show similar overall survival outcomes.

While not a predetermined analysis in this study, the data suggesting which patients might benefit from vaccine may provide important insights for use of vaccine-mediated therapy, which has now demonstrated an overall survival advantage in patients with metastatic prostate cancer in randomized phase II and III trials [5,20,21,22]. If and when therapeutic vaccines become approved in metastatic CRPC, practitioners will have to determine which patients are most likely to benefit from vaccine therapy and which patients should instead be treated with cytotoxic therapy.

In the present study, the median overall survival for all 32 enrolled patients was 26.6 months compared with a predicted survival by Halabi prognostic model of 17.4 months. Twelve of 15 patients with a Halabi predicted survival ≥ 18 months survived substantially longer than predicted (predicted median 20.9 months vs. an actual median overall survival that will meet or exceed 37.3 months). Subsequent therapies did not appear to influence survival differences seen between the two Halabi groups as similar proportions of each group received similar docetaxel therapy. Therefore, these data suggest that the Halabi nomogram may provide a potential method of delineating which patients benefit most from vaccine therapy.

In comparing the baseline characteristics of the patients on this study and those of the 1,101 CALGB study patients that led to the development of the Halabi prognostic model, the patient populations appear quite similar (see Table 1). The observed overall survival of the validation set [2] was 17.0 months compared with the patients in the PSA-TRICOM study whose predicted survival was 17.4 months, indicating that patients used to develop the Halabi nomogram appear to be quite similar to patients who were enrolled on this NCI trial.

While the patients on this study may be representative of patients used to develop the Halabi nomogram, some may question its value in the docetaxel-era of therapy for prostate cancer. We have now analyzed data from a recent docetaxel trial at our institution [29,30] according to Halabi predicted survival (Table 2). Both the vaccine trial described here and the docetaxel trial enrolled chemotherapy naïve patients with mCRPC; both trials enrolled patients with similar predicted overall survival (see Table 2) and both were conducted at the same institution. It is of particular interest that those patients in the docetaxel trial had a median overall survival of 15.5 months compared with a predicted survival of 16.5 months, adding weight to the use of the Halabi prediction as an estimate of overall survival in patients treated with standard therapy at this institution. As seen in Table 2, there was only a slight difference in predicted vs. actual median overall survival for patients treated with docetaxel regardless of whether they were in the < 18 -month or ≥ 18 -month Halabi predicted groups. These data do not support the notion that patients in the ≥ 18 -month Halabi predicted survival group were “destined to do well anyway” when treated with any therapeutic regimen – the Halabi nomogram corrects for this. It is the differential of predicted vs actual median overall survival that should be assessed when examining potential effectiveness of a given therapy. The results seen with the docetaxel-treated patients are contrasted with those observed with the vaccine-treated patients, where there was a ≥ 16.4 month differential in predicted vs overall survival in the group of patients predicted to live ≥ 18 months (Table 2). A different nomogram [31] (in addition to the Halabi nomogram) was also used to evaluate results of this trial. Employing that nomogram [31], all patients on trial had a predicted median survival of 17.0 months vs the actual median overall survival of 26.6 months (difference of 9.6 months). Furthermore, there was a greater absolute increase in median overall survival vs. predicted survival seen in the subgroup of patients with

a predicted survival of ≥ 18 months; thus these results are consistent with those seen with the Halabi nomogram.

The seven individual components of the Halabi nomogram were derived based on their individual ability to assess tumor aggressiveness and volume. Taken together, these factors may be of value in patients who are considered for vaccine-mediated therapy. Perhaps patients with more aggressive disease and a larger tumor burden (those with a Halabi predicted survival of <18 months) have more immunosuppressive factors from their tumor and this is why they are less likely to benefit as much from treatment with vaccine. Increased tumor burden has been correlated with immune suppression in both preclinical and clinical models and may limit the immune system's ability to respond to a vaccine mediated therapy.

As shown in Fig. 2B, there was a trend ($p=0.055$) toward increased overall survival in patients with a ≥ 6 -fold increase in PSA-specific T-cell responses post-vaccination. It is interesting to note that all but one patient with a ≥ 6 -fold increase had a Halabi predicted survival of > 18 months, and the remaining patient was just at the 18 month threshold. Nonetheless, as pointed out above, one should not consider the analysis of PBMC T-cell responses to the vaccine antigen as a lone surrogate for vaccine efficacy or any clinical parameter. Preclinical studies have clearly shown [32,33] that T-cell responses to tumor antigens not in the vaccine, but generated by cross-presentation of destroyed tumor cells to the immune system, can also be responsible for the immune-mediated destruction of tumor; moreover, T cells that have trafficked to the tumor site can be more indicative of vaccine efficacy than T cells obtained from PBMCs.

The analysis of Tregs from PBMC demonstrated that Treg function decreased post- vs pre-vaccination in patients who lived longer than predicted, while Treg function increased post- vs pre-vaccination for patients who survived less than predicted. Although these findings may be an artifact of the small sample size or may not be representative of what occurs in the tumor microenvironment, they may also support the concept that tumor burden influences immune responses to vaccines. Perhaps, patients who survived less than predicted had more tumor burden, which was responsible for more tumor-derived immunosuppressive factors such as TGF- β which in turn drives the function of Tregs, while the reciprocal is observed in the control of tumor in patients living longer than predicted,

The median overall survival in the control (empty vector) arm of the concurrent randomized, multi-center study employing PSA-TRICOM [22] was 16.6 months for control vs 25.1 months for the PSA-TRICOM vaccine. The median overall survival for the study reported here employing PSA-TRICOM was 26.6 months (predicted median overall survival by the Halabi nomogram was 17.4 months). In view of the similar results in median overall survival of the two Phase II trials employing PSA-TRICOM vaccination, a subsequent larger, randomized, multi-center Phase III trial is planned. The results of the trial reported here also provide hypothesis-generating evidence that metastatic prostate cancer patients with poorer prognostic factors have a similar survival with vaccine therapy compared to that predicted by treatment with standard therapy (chemotherapy or second-line hormone therapy). However, patients with more favorable prognostic factors may have a longer survival when treated with vaccine-mediated therapy as compared with conventional systemic therapies alone, or treated first with vaccine followed by conventional therapies. Subsequent larger, randomized trials will be required to confirm this observation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1A **Figure 1B****Fig 1.**

Patients 14 and 3 with soft tissue lesions demonstrate reductions in their index lesions on CT scan post-vaccine. Panel A demonstrates a 68% reduction in the right hilar lesion in Patient 14 after 3 months. Panel B demonstrates the reduction of a 2.2 cm right pelvic lymph node to 1.4 cm in Patient 3 after 6 months. There was also a decrease in mediastinal adenopathy (not shown).

Fig 2.

(A) The Kaplan Meier curve for all 32 enrolled subjects demonstrates a median overall survival of 26.6 months. (B) There was a strong trend in the ability to mount a 6-fold increase in PSA-T cells post-vaccine and an increase in overall survival.

Fig 3.

The graph depicts actual overall survival, predicted overall survival and whether or not an individual survived greater than (in green) or less than (in red) Halabi predicted survival. In addition, yellow arrows demonstrate patients who remain alive. Asterisks depict >6-fold PSA-T-cell responses.

Fig. 4.

Patients were evaluated for their ability to produce antibody responses to the fowlpox vector post-vaccination. No difference in antibody titers to fowlpox post-vaccination was observed between patients with a Halabi predicted survival of ≥ 18 or < 18 months.

Fig 5.

The percent change in Treg function post-three vaccinations vs pre-vaccination stratified by actual overall survival vs Halabi predicted survival. Treg function was analyzed as previously detailed [see ref. 28].

Table 1

Comparison of baseline characteristics between patients treated with PSA-TRICOM and patients used to develop the Halabi nomogram [see ref. 2].

Baseline characteristic	PSA-TRICOM n = 32	Halabi learning sample n = 760	Halabi validation set n = 341
Age	65.6	71	72
Visceral metastasis	9.4%	13%	11%
Gleason			
2-4	0%	10%	6%
5-7	44%	46%	49%
8-10	56%	44%	45%
PS			
0	22%	41%	54%
1	75%	46%	38%
2	3%	13%	8%
<i>Lab results</i>			
Hemoglobin	12.9	12.4	12.4
PSA	76	126.0	77.0
Alkaline phosphatase	91	172.0	125.0
LDH	171	225.0	216.0
	Predicted survival: 17.4 months		Observed overall survival: 17 months

Table 2

Survival predicted by Halabi nomogram vs. actual overall survival

	All Patients	Patients with Halabi Predicted Survival <18 months	Patients with Halabi Predicted Survival ≥18 months
Vaccine PROSTVAC (n = 32)			
Median survival predicted by Halabi model (months)	17.4	12.3	20.9
Actual median overall survival (months)	26.6	14.6	≥37.3 (not reached) [†]
Patients surviving longer than predicted by Halabi nomogram p value [*]	22 of 32 (69%) <i>P</i> = 0.50	10 of 17 (59%) <i>P</i> = 0.63	12 of 15 (80%) <i>P</i> = 0.035
Difference ^{**} (months)	9.2	2.3	≥16.4
Docetaxel therapy^a (n = 22)			
Median survival predicted by Halabi model (months)	16.5	13.0	21.0
Actual median overall survival (months)	15.5	15.4	16.9
Number of patients surviving longer than predicted by Halabi	11 of 22 (50%)	8 of 13 (62%)	3 of 9 (33%)
Difference ^{**} (months)	(-1.0)	2.4	(-4.1)

[†] Median overall survival based on failures to date will meet or exceed 37.3 months.

^a Docetaxel administered weekly for 3 of 4 weeks [see refs. 29,30].

^{*} The two-tailed p-value is based on an exact binomial test, with *P* = 0.5 as the fraction living longer than expected if this were a random occurrence.

^{**} The difference in months denotes the amount by which the actual median overall survival (actuarial) exceeds the arithmetic median survival predicted by Halabi nomogram [see ref. 2].

Table 3

Pre- and post-vaccine T-cell responses to PSA

Patient no.	Sample	PSA peptide	PSA peptide fold increase	Flu peptide	HIV peptide
Cohort I					
3	Pre	<1/200,000		1/42,857	<1/200,000
	Day 85	1/28,571	≥7.0	1/23,529	<1/200,000
23	Pre	<1/200,000		1/16,216	<1/200,000
	Day 85	1/26,087	≥7.6	1/50,000	<1/200,000
Cohort II					
2	Pre	<1/200,000		1/21,429	<1/200,000
	Day 85	1/37,500	≥5.3	1/25,000	<1/200,000
6	Pre	<1/200,000		1/37,500	<1/200,000
	Day 85	1/40,000	≥5.0	1/20,690	<1/200,000
17	Pre	1/150,000		1/37,500	<1/200,000
	Day 85	1/66,667	2.2	1/20,000	<1/200,000
32	Pre	<1/200,000		1/42,857	<1/200,000
	Day 85	1/100,000	≥2.0	1/44,444	<1/200,000
Cohort III					
1	Pre	<1/200,000		1/60,000	<1/200,000
	Day 85	1/30,000	≥6.6	1/24,000	<1/200,000
15	Pre	<1/200,000		1/10,169	<1/200,000
	Day 85	1/30,000	≥6.6	1/9,375	<1/200,000
22	Pre	<1/200,000		1/12,766	<1/200,000
	Day 85	1/100,000	≥2.0	1/13,333	<1/200,000
8	Pre	1/66,667		1/14,634	<1/200,000
	Day 85	1/15,000	4.4	1/37,500	<1/200,000
Cohort IV					
19	Pre	<1/200,000		1/22,222	<1/200,000
	Day 85	1/54,545	≥3.6	1/15,000	<1/200,000
24	Pre	<1/200,000		1/50,000	<1/200,000

Patient no.	Sample	PSA peptide	PSA peptide fold increase	Flu peptide	HIV peptide
26	Day 85	1/50,000	≥4.0	1/33,333	<1/200,000
	Pre	<1/200,000		1/35,294	<1/200,000
	Day 85	1/26,087	≥7.6	1/20,690	<1/200,000

PBMCs were collected via apheresis prior to treatment with vaccine and after approximately 3 months of therapy. Immunologic response was measured by the ELISPOT assay, as previously described [ref. 26], to evaluate the IFN- γ production by T cells after exposure to PSA-peptide.

Abbreviations: PSA, prostate-specific antigen; HIV, human immunodeficiency virus.