

Randomized Trial of Autologous Cellular Immunotherapy with Sipuleucel-T in Androgen-Dependent Prostate Cancer

Tomasz M. Beer¹, Guy T. Bernstein², John M. Corman³, L. Michael Glode⁴, Simon J. Hall⁵, Wayne L. Poll⁶, Paul F. Schellhammer⁷, Lori A. Jones⁸, Yi Xu⁸, Jelle W. Kylastra⁹, and Mark W. Frohlich⁸

Abstract

Purpose: Sipuleucel-T, an autologous cellular immunotherapy, was investigated in a randomized, double-blind, controlled trial to determine its biologic activity in androgen-dependent prostate cancer (ADPC).

Experimental Design: Patients with prostate cancer detectable by serum prostate-specific antigen (PSA) following radical prostatectomy received 3 to 4 months of androgen suppression therapy, and were then randomized (2:1) to receive sipuleucel-T ($n = 117$) or control ($n = 59$). The primary endpoint was time to biochemical failure (BF) defined as serum PSA ≥ 3.0 ng/mL. PSA doubling time (PSADT), time to distant failure, immune response, and safety were also evaluated.

Results: Median time to BF was 18.0 months for sipuleucel-T and 15.4 months for control (HR = 0.936, $P = 0.737$). Sipuleucel-T patients had a 48% increase in PSADT following testosterone recovery (155 vs. 105 days, $P = 0.038$). With only 16% of patients having developed distant failure, the treatment effect favored sipuleucel-T (HR = 0.728, $P = 0.421$). The most frequent adverse events in sipuleucel-T patients were fatigue, chills, and pyrexia. Immune responses to the immunizing antigen were greater in sipuleucel-T patients at Weeks 4 and 13 ($P < 0.001$, all) and were sustained prior to boosting as measured in a subset of patients a median of 22.6 months (range: 14.3–67.3 months) following randomization.

Conclusions: No significant difference in time to BF could be shown. The finding of increased PSADT in the sipuleucel-T arm is consistent with its biologic activity in ADPC. Long-term follow-up will be necessary to determine if clinically important events, such as distant failure, are affected by therapy. Treatment was generally well tolerated. *Clin Cancer Res*; 17(13); 4558–67. ©2011 AACR.

Introduction

Nineteen to 32 percent of men who undergo surgery for clinically localized prostate cancer experience disease recurrence (1–4). An elevated prostate-specific antigen (PSA) level is often the first and only sign of disease recurrence. Management of patients with a rising PSA remains uncertain. While those suspected of harboring locally-persistent disease may benefit from radiation therapy, the majority of

these patients have systemic disease. Systemic therapy with androgen suppression may benefit some of these patients (5), but the optimal timing of its initiation remains unclear (6, 7) and potential benefits must be weighed against considerable toxicities (7, 8). New treatments with fewer side effects are needed.

Sipuleucel-T is an autologous cellular immunotherapy composed of a patient's peripheral blood mononuclear cells (PBMC), including antigen-presenting cells (APCs), that have been activated *ex vivo* with a recombinant fusion protein known as PA2024. PA2024 consists of a prostate antigen, prostatic acid phosphatase (PAP), fused to granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune cell activator. Two randomized, double blind, controlled Phase 3 trials in metastatic castrate resistant prostate cancer provided initial evidence that sipuleucel-T prolongs survival (9, 10), which was confirmed in a third Phase 3 trial with 512 men (11).

To study the activity of sipuleucel-T in patients with androgen-dependent prostate cancer (ADPC), a double-blind, controlled, randomized trial, PROTECT (PROvenge Treatment and Early Cancer Treatment, NCT00779402), was designed to examine time to biochemical failure (BF,

Authors' Affiliations: ¹Oregon Health & Science University Knight Cancer Institute, Portland, Oregon; ²Center for Urologic Care, Bryn Mawr, Pennsylvania; ³Virginia Mason Medical Center, Seattle, Washington; ⁴University of Colorado Health Science Center, Aurora, Colorado; ⁵Mount Sinai School of Medicine, New York, New York; ⁶AKSM Clinical Research Corporation, Columbus, Ohio; ⁷Urology of Virginia, PC/Eastern Virginia Medical School, Norfolk, Virginia; ⁸Dendreon Corporation, Seattle, Washington; and ⁹Spectrum Pharmaceuticals, Irvine, California

Corresponding Author: Tomasz M. Beer, MD, Oregon Health & Science University Knight Cancer Institute, 3303 SW Bond Ave, CH14R, Portland, OR 97239–3098. Phone: 503-494-0365; Fax: 503-494-6197; E-mail: beert@ohsu.edu

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Translational Relevance

Sipuleucel-T is an autologous cellular immunotherapy that prolongs overall survival in men with asymptomatic or minimally symptomatic, metastatic castrate resistant prostate cancer. This randomized study investigated sipuleucel-T earlier in the natural history of prostate cancer, in men with androgen-dependent disease. The study demonstrates prolongation of PSA doubling time in the sipuleucel-T group relative to control, providing the first evidence that sipuleucel-T can affect a clinically relevant endpoint proximal to overall survival. Increased antigen presenting cell activation in the second, third, and boost doses of sipuleucel-T relative to the first dose provides evidence of priming with the first dose, and boosting by subsequent infusions, indicative of the establishment of immunologic memory. This study also shows that sipuleucel-T can generate a robust and sustained T cell response to the immunizing antigen in this patient population, and that this immune response is maintained following a single booster infusion, which may be augmented in some patients.

the primary endpoint defined as serum PSA ≥ 3.0 ng/mL), PSA doubling time (PSADT), time to distant failure, and overall survival.

Materials and Methods

Patients

Eligible patients had an increase in serum PSA as the only sign of disease recurrence following a radical prostatectomy carried out at least 3 months and not more than 10 years prior to registration for histologically confirmed prostate cancer. Patients who experienced their first PSA recurrence within 2 years of initial therapy were eligible regardless of the Gleason score; those who experienced their first PSA relapse between 2 and 10 years following initial therapy were eligible only if the Gleason score was ≥ 7 . Other eligibility requirements included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, life expectancy of at least one year, and age between 18 and 80 years. Negative serology for human immunodeficiency virus, human T-lymphotropic virus type 1 or 2, and hepatitis B and C were required, as were adequate hematologic, renal, and hepatic function. The tumor specimen had to be positive for PAP by immunohistochemistry. Therapeutic PSA response to primary therapy was required to have been < 0.4 ng/mL. If androgen ablation was given for a previous PSA relapse, current PSA must have reached 3 ng/mL and be 25% above the nadir observed while on prior androgen ablation therapy. Patients who were treated with adjuvant or salvage radiation following radical prostatectomy, or with either luteinizing hormone-releasing hormone agonist (LHRH-a) or

nonsteroidal antiandrogen therapy for a prior PSA relapse, were eligible provided PSA was not rising while receiving hormonal therapy, and 6 months had elapsed since the last effective date of androgen deprivation or antiandrogen therapy. Prior chemotherapy was permitted provided at least 4 months had elapsed. Patients with prior orchiectomy, immunotherapy, or therapy with experimental agents for prostate cancer were excluded as were patients with a history of other prior malignancies within 5 years of study entry, other than resected basal or squamous cell carcinoma of the skin. Patients whose post-prostatectomy PSA reached 20 ng/mL were not eligible. Concurrent participation in other clinical trials involving investigational agents was prohibited.

During a run-in period, patients were treated with a 3-month depot injection of an LHRH-a. Those with PSA < 1 ng/mL following the LHRH-a were eligible for randomization. Those with a PSA ≥ 1 ng/mL were permitted to receive an additional 1-month depot injection of LHRH-a, and were eligible for randomization if PSA declined to < 1 ng/mL. Local institutional review boards approved the protocol at each study center, and all patients signed institutional review board-approved informed consent forms.

Randomization assignment and treatment

This double-blind, randomized, controlled study involved 17 centers in the United States. A centrally administered, stratified, block randomization method encompassing all study centers was employed to assign patients to treatment in a 2:1 ratio (sipuleucel-T: control). Stratification variables were receipt of adjuvant or salvage radiation therapy after prostatectomy (yes or no) and Gleason score (≤ 6 or ≥ 7).

Treatment was scheduled at Weeks 0, 2, and 4. An optional booster treatment with a single infusion of sipuleucel-T or control, as originally randomized, was available after BF; the blind was maintained, and there was no crossover. Prior to each treatment, patients underwent a standard 1.5 to 2.0 blood volume mononuclear cell leukapheresis, which was transported to a sponsor-designated manufacturing facility. Patients were infused 2 to 3 days after leukapheresis with sipuleucel-T or control (11). Control consisted of PBMC held at 2–8°C without the addition of PA2024.

Schedule of events for endpoint evaluations

Blood samples for PSA testing were drawn at Screening, Week -1, Week 0 (first infusion), Weeks 2, 13, 26, and every 3 months until distant failure. In patients receiving a booster, blood samples for PSA were collected at Weeks 4 and 13 post-booster. Distant failure was documented by observation of metastases on bone scan, CT scan, or other imaging modality. Scans were carried out every 12 months following BF, or when triggered by one of the following events: PSA doubling within 9 months following BF, PSA ≥ 10 ng/mL, symptomatic disease-related pain, or in accordance with clinical practice.

Patients underwent blood draws for assessment of immune response during regularly scheduled visits at Screening and Weeks 4 and 13 following the first infusion. Patients receiving a booster infusion underwent a blood draw for assessment of immune response prior to the booster infusion and at Weeks 4 and 13 post-booster.

Patients were followed throughout the study for safety (physical examinations, adverse event assessments, laboratory tests) and survival. This study is ongoing and survival results will be reported when available.

Immunologic testing

As part of lot-release assessments, every sipuleucel-T and control product was assessed for APC activation by the degree of CD54 upregulation, determined by the ratio of CD54 expression on post-culture APCs compared with preculture APCs (12).

Immune responses in the peripheral blood were assessed by interferon-gamma (IFN γ) ELISPOT and proliferation assays in a subset of patients (9,13). PBMCs, freshly isolated from whole blood, were plated in triplicate into anti-IFN γ (all antibodies purchased from MabTECH) coated ELISPOT plates, or round bottom plates for proliferation assays, in the presence of PA2024 (10 μ g/mL).

For ELISPOT assays, plates were incubated for 48 hours, washed, probed with biotinylated anti-IFN γ , further washed, and then incubated with streptavidin alkaline phosphatase. Spot development was achieved with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT; Invitrogen) and spots were enumerated by a CTL Immunospot 3 reading system (CTL). The frequency of PA2024 IFN- γ ELISPOTS was expressed as median spots above background.

For proliferation assays, cells were incubated for 5 days, pulsed overnight with 0.5 μ Ci tritiated thymidine (3 H) (Perkin Elmer), harvested, and the amount of 3 H incorporation then determined by means of a betaplate counter (Perkin Elmer). The degree of antigen-specific clonal T cell expansion was expressed as a stimulation index (SI) of the ratio of 3 H incorporation by cells incubated with PA2024 compared with media controls.

Statistical considerations

Prostate-specific antigen kinetics were evaluated by determining the time from randomization to BF, PSA velocity, and PSADT. The time to BF was the primary efficacy endpoint of this study; the null hypothesis was that there was no difference in time to BF between the treatment arms. A sensitivity endpoint of BF was carried out for confirmed BF, defined as a second PSA value \geq 3.0 ng/mL obtained no less than two but no more than four weeks after the first PSA \geq 3.0 ng/mL. Secondary endpoints included PSADT, time from randomization to distant failure, and overall survival. Immunologic responses using proliferation and ELISPOT assays were exploratory endpoints.

The study design was based on a sample size of 159 patients with an accrual period of 3 years and a 2-year

follow-up period. This sample size was expected to obtain 108 events, providing 80% power to detect an increase from 1 year to 1.75 years in median time to BF at the 0.05 level of significance using a 2-sided log-rank test.

Time to event endpoints (BF and distant failure) were tested between treatment groups using the 2-sided log-rank test, stratified by Gleason Score and radiation therapy. Hazard ratios and corresponding 2-sided 95% confidence intervals for each endpoint were generated under the stratified Cox regression model. Cumulative incidence was calculated taking into consideration competing events prior to the events of interest (14). Competing events for the primary endpoint of BF included death, loss to follow-up, withdrawal of consent, or initiation of an excluded therapy. Median times to events were derived from the estimated cumulative incidence curves.

PSADT was estimated from PSA velocity, which was the rate change in the logarithmic scale of PSA by time (slope). The difference between treatment groups in PSADT or PSA velocity was tested through comparison of the slope difference between the groups using a mixed-effect model that included factors of treatment group, time, and treatment by time interaction, with patient-specific random intercept and slope. PSA levels below 0.2 were treated as 0.2, and PSA measurements after the initiation of systemic therapy were not used. Analyses were carried out based on all data from randomization, as well as data from randomization date plus 30 days, plus 60 days, and plus 90 days, to the initiation of systemic therapy, accounting for the possibility that immunotherapy may require time to reach peak effect and to impact PSA kinetics. Because PSA kinetics can be influenced by testosterone recovery (15–17), and testosterone recovery varies considerably among patients following a course of hormonal therapy (18, 19), an additional analysis was conducted excluding PSA values prior to testosterone recovery (\geq 90% of the baseline value).

To compare the difference in CD54 upregulation between specific visits, a linear mixed-effect model was used on log-transformed upregulation ratios. To compare the difference in immunologic response between treatment groups at a specific visit, a linear mixed-effect model was used on log-transformed stimulation indices based on all available patient visit dates. The model included treatment group, visit, treatment by visit interaction, and patient (as a random effect). For the comparison of ELISPOT between groups, the same mixed-effect model was used to analyze the rank-transformed data. Due to the small sample sizes and variation in timing, the Wilcoxon rank-sum test and exact *p*-values are reported for treatment comparisons at boost + 4 weeks and boost + 13 weeks.

Two-sided nominal *P*-values were reported for all statistical tests.

Results

Patient characteristics and disposition

Between July 2001 and July 2005, 208 patients entered the 3-month run-in period of this study. Of those patients,

Table 1. Patient characteristics

	Sipuleucel-T (n = 117)	Control (n = 59)
Age, years [median (min, max)]	64 (48,79)	67 (47,78)
Race (n,%)		
Caucasian	105 (89.7)	54 (91.5)
African—American	9 (7.7)	3 (5.1)
Hispanic	2 (1.7)	1 (1.7)
Other	1 (0.9)	1 (1.7)
ECOG performance status (n,%)		
0	109 (94.8)	57 (98.3)
1	6 (5.2)	1 (1.7)
Gleason score (n,%)		
≤ 6	34 (30.1)	15 (26.3)
≥ 7	79 (69.9)	42 (73.7)
Prior adjuvant hormone therapy (n,%)		
Yes	21 (17.9)	9 (15.3)
No	96 (82.1)	49 (83.1)
Prior radiation therapy (n,%)		
None	45 (38.5)	23 (39.0)
Adjuvant	20 (17.1)	8 (13.6)
Salvage	52 (44.4)	28 (47.5)
Median PSA, ng/mL (min, max)		
Highest ever	2.3 (0.8, 33.0)	2.3 (0.8, 20.5)
Prior to LHRH-a	2.1 (0.8, 16.9)	2.7 (0.8, 20.5)
Prior to randomization	0.09 (0.010–1.00)	0.090 (0.010–1.00)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LHRH-a, luteinizing hormone-releasing hormone-analogue; PSA, prostate-specific antigen.

176 were randomized, comprising the intent-to-treat population; 117 patients were randomized to receive sipuleucel-T and 59 patients were randomized to receive control. Baseline characteristics were well-balanced between the two treatment arms (Table 1). A total of 175 patients underwent at least one leukapheresis procedure, comprising the safety population. Of the patients included in the safety population, 172 received at least 1 infusion of sipuleucel-T or control. Figure 1 presents a schematic of patient disposition.

Clinical results

The median time to BF was 18.0 months for sipuleucel-T and 15.4 months for control [HR = 0.936, (95% CI: 0.637, 1.376); $P = 0.737$; Fig. 2A]. In a sensitivity analysis examining only BF defined by two PSA measurements equal to or above 3.0 ng/mL, the HR was 0.797 (95% CI: 0.529, 1.202; $P = 0.278$; Fig. 2B).

Analyses of PSADT were carried out using all PSA values from randomization until the initiation of systemic therapy for prostate cancer (Fig. 3). To account for

the time required for immune response to take effect and to allow for testosterone recovery, additional analyses were conducted that excluded PSA values obtained at earlier time points in the study (Fig. 3). PSADT was 34.4% greater in the sipuleucel-T arm compared with the control arm using PSA values obtained ≥ 90 days post-randomization ($P = 0.046$). In the analysis following testosterone recovery, PSADT was 47.6% greater in the sipuleucel-T arm compared with the control arm ($P = 0.038$).

Patients are still being followed for the secondary endpoint of time to distant failure. As of the data cut-off date, 28 patients (15.9%, 28 of 176 patients) had experienced a distant failure event. With very limited power, the point estimate for risk of distant failure favored sipuleucel-T but lacked statistical significance [HR = 0.728 (95% CI: 0.335, 1.582); $P = 0.421$; Fig. 2C].

Immune parameters

Antigen presenting cell activation in the sipuleucel-T arm, as measured by CD54 upregulation, was greater at Week 2 and Week 4, relative to Week 0 (both $P < 0.001$; Fig. 4A), which was maintained at boost ($P < 0.001$). In patients receiving sipuleucel-T, the median time between randomization and the booster ($n = 49$) was 15.4 months (range from 3.7 to 67.4 months).

Patients on the sipuleucel-T arm showed an increase in T cell proliferation to the immunizing antigen relative to patients on the control arm at Weeks 4 and 13 ($P < 0.001$; Fig. 4B). The median SI for patients on the sipuleucel-T arm was 131.9 and 118.5 at Weeks 4 and 13, respectively. When the immune response was measured using the IFN γ ELISPOT assay, a similar treatment effect was observed at Weeks 4 and 13 ($P < 0.001$, Fig. 4C). The median number of spots for patients on the sipuleucel-T arm was 79 and 43 at Weeks 4 and 13, respectively.

Immune responses were also measured prior to booster infusion in 10 sipuleucel-T patients and 2 control patients, as well as post-boost in some of these patients (Fig. 4B and Fig. 4C). T cell proliferative and ELISPOT responses in the sipuleucel-T arm were sustained at elevated levels at the pre-boost time point when compared with control ($P = 0.005$ and $P < 0.001$, respectively), a median of 22.6 months (range 14.3 to 67.3 months) following randomization. After boosting, T cell proliferative responses appeared to be maintained, and ELISPOT responses increased in some patients.

Adverse events

Therapy with sipuleucel-T was generally well tolerated. Similar percentages of adverse events were observed in both treatment groups (95.7% of sipuleucel-T patients versus 94.9% of control patients). The adverse events observed more frequently (2-fold) in the sipuleucel-T arm compared with control and in at least 10% of patients were chills, pyrexia, myalgia, influenza-like illness, and pain (Table 2). The oral

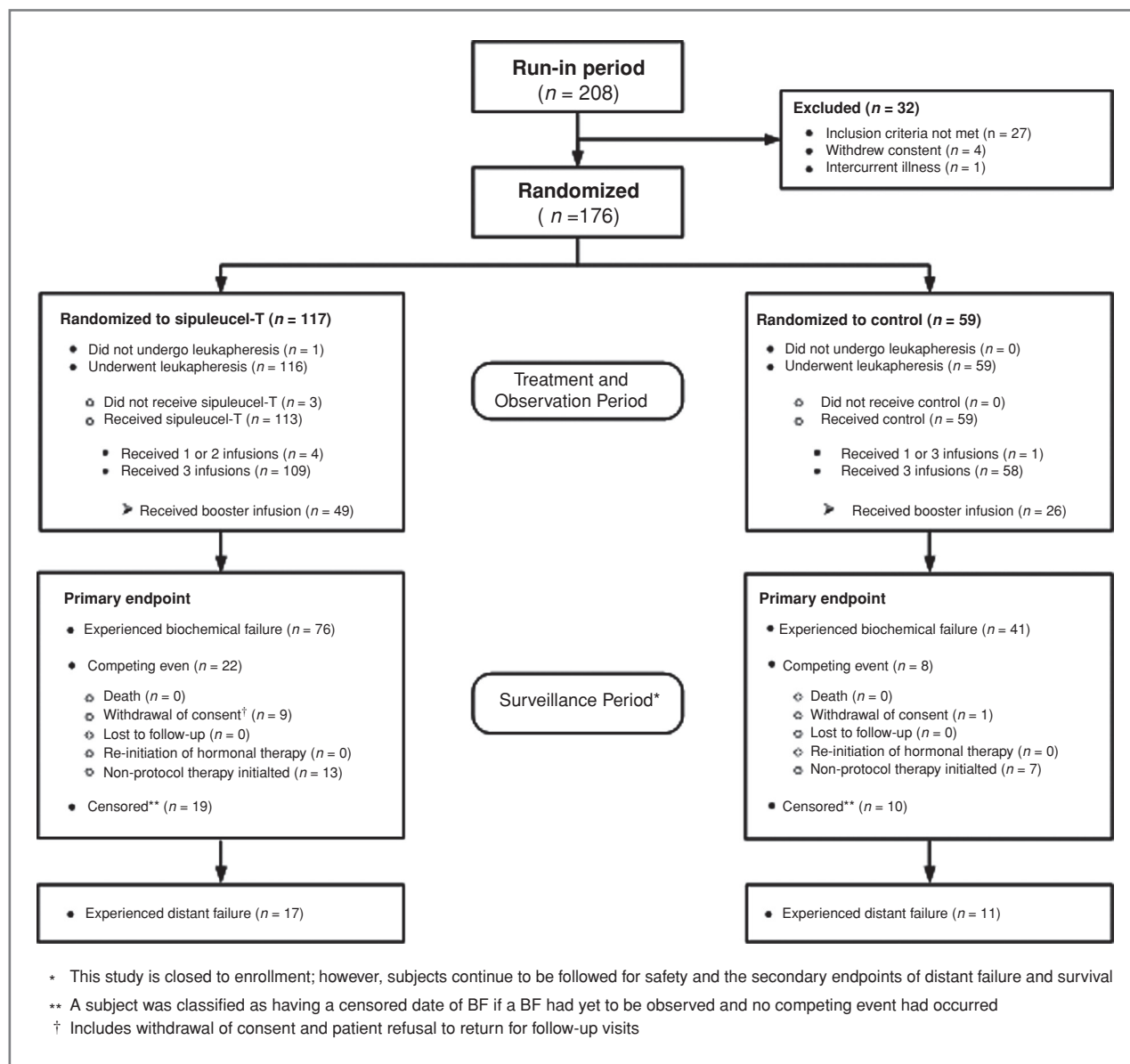


Figure 1. Patient disposition.

parasthesias were temporally related to leukapheresis rather than product infusion. Of the patients reported to have experienced an adverse event, the majority (70.7%, 118 of 167 patients) had events with the greatest severity of Grade 1 or 2. Four deaths have been reported to date; no death was considered related to treatment with sipuleucel-T. All deaths occurred more than one year following the last infusion of sipuleucel-T or control. Adverse events observed following the booster infusion in the sipuleucel-T arm and in at least 10% of the patients were fatigue, chills, pyrexia, arthralgia, and myalgia, none of which occurred in control patients receiving the booster infusion.

Adverse events occurred at a lower frequency following the booster than following the initial treatment sequence.

Discussion

In this study, the effect of sipuleucel-T on PSA kinetics was evaluated. The study did not show a statistically significant difference in the primary endpoint of time to BF. There are some important limitations of this endpoint. First, confirmation was not required. A sensitivity analysis that examined only confirmed BF events showed a hazard ratio of 0.797 in favor of sipuleucel-T ($P = 0.278$). Given

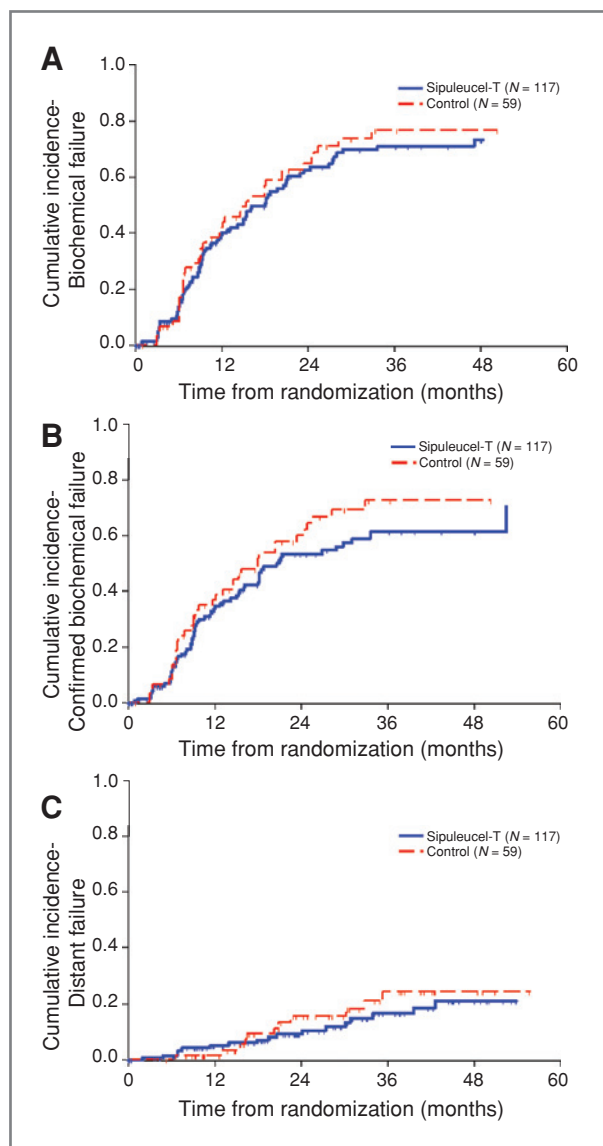


Figure 2. Cumulative incidence of biochemical failure (sipuleucel-T, 76 events, control, 41 events; each event represents a patient who reached biochemical failure, defined as a PSA value ≥ 3.0 ng/mL, A), confirmed biochemical failure (sipuleucel-T, 64 events, control 38 events; each event represents a patient with confirmed biochemical failure, defined as two PSA values ≥ 3.0 ng/mL taken not less than 2 but not more than 4 weeks apart, B), and distant failure (sipuleucel-T, 17 events, control 11 events; each event represents a patient reaching distant failure, defined as radiologic evidence of metastatic lesion, C) of sipuleucel-T or control patients. Cumulative incidence was calculated considering competing events (death, loss to follow-up, withdrawal of consent, and initiation of excluded therapy; denoted by hash marks) prior to event occurrence. Hazard ratios were generated using stratified Cox regression model.

the inherent fluctuations in PSA (20), the requirement for a confirmatory PSA may have improved the signal-to-noise ratio. If this point estimate were to represent a true treatment effect, then the trial lacked the statistical power to detect such a treatment effect.

The primary endpoint, while reasonably selected, was also arbitrary. There are no data to suggest that reaching a PSA concentration of 3.0 ng/mL following a brief course of androgen suppression therapy is or is not predictive of subsequent development of metastases or cancer-related death. At the time this study was designed, relatively little was known about appropriate endpoints in the setting of PSA-only relapse after radical prostatectomy. Regrettably, relatively little progress has been made in this area and the field continues to struggle with the design of studies in this patient population. One area of marked progress is the increased understanding of the prognostic importance of PSA kinetics in general and PSADT specifically. PSADT has been shown to be the single strongest predictor of prostate cancer related mortality in this patient population (21–26).

While fully recognizing that PSADT is not a validated endpoint for clinical trials and to date no studies have examined the link between treatment-induced alteration in PSADT and time to disease progression or death, its prognostic importance makes it a compelling subject for exploratory analyses. These analyses showed that PSADT was longer in patients who received sipuleucel-T, particularly after the effects of testosterone recovery were no longer confounding the analyses. While we are not able to definitively link the PSADT prolongation to a treatment effect, we are encouraged by this finding. Studies that have established the prognostic importance of PSADT have largely focused on patients whose testosterone levels are at steady state. Because PSA kinetics are affected by early testosterone recovery (15–17), we believe an analysis after testosterone recovery is likely to be most informative, as previously suggested (27). A delayed effect on PSA kinetics is also compatible with a delayed treatment effect, as suggested by the immune response to sipuleucel-T (28–30), as well as the more robust effects of sipuleucel-T and other immunotherapies on overall survival relative to the effects on the more proximal endpoint of disease progression (9–11, 31, 32).

The median time to distant failure from the time of PSA elevation following radical prostatectomy has previously been reported to be approximately 8 years (33). Because the timing of this analysis was based on the BF endpoint, only 15.9% of patients had a distant failure event at the data cutoff date. A trend in favor of the sipuleucel-T arm was observed and additional follow-up for this endpoint and for overall survival may be informative.

To our knowledge, sipuleucel-T is the first autologous cellular immunotherapy to show an effect on PSA kinetics in a randomized trial. Treatment of the patients with 3 to 4 months of an LHRH-a immediately prior to sipuleucel-T treatment may have contributed to the observed prolongation of PSADT in the treatment arm. Recent studies suggest a potential synergy between androgen deprivation and immunotherapy (34). Specifically, castration or androgen deprivation therapy has been shown to induce

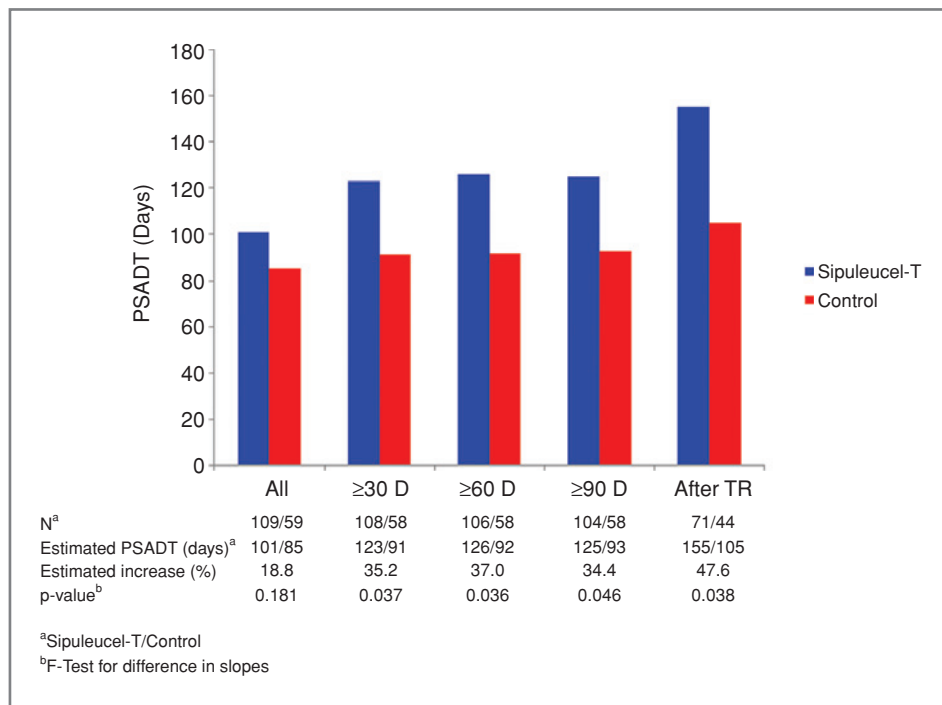


Figure 3. PSA doubling time (PSADT) for each treatment group was estimated from PSA velocity (rate change in the logarithmic scale of PSA by time) using a mixed effect model. Analyses were carried out using all PSA values after randomization, PSAs obtained ≥ 30 days, ≥ 60 days, and ≥ 90 days after randomization, and PSAs following testosterone recovery (TR) to $\geq 90\%$ of baseline value (prior to LHRH-a).

lymphocytosis (35) and increase circulating T and NK cells in prostate cancer patients (36), induce T cell infiltration into prostate cancer tissue (37), and mitigate tolerance to therapeutic vaccination in a murine model of prostate cancer (38).

Sipuleucel-T induced robust immune responses to the immunizing antigen PA2024 relative to control, as measured by both T cell proliferation and ELISPOT assays. When APC activation was measured as part of lot release assessments, there was significant CD54 upregulation at Week 2, Week 4, and at boost when compared with Week 0. The increase in APC activation from Week 0 to subsequent treatments provides evidence of priming with the first dose, and boosting by subsequent infusions, indicative of the establishment of immunologic memory. The magnitude of CD54 upregulation at boost, as well as the levels of T cell responses to the immunizing antigen prior to boost, indicate that sipuleucel-T generates a sustained immune response, with measurements obtained as long as 67.4 months following initial treatment. Although the sample size was small, immune responses following booster infusions were maintained and seem to have been augmented in some patients. Previous studies of sipuleucel-T have shown evidence of correlations between immune parameters and overall survival (10,11); in this study, the limited number of patients with immune response data precluded analyses correlating the magnitude of immune responses with PSA kinetics.

The most common treatment-related side effects observed in patients randomized to sipuleucel-T were

fatigue, chills, and pyrexia. The majority of events were mild to moderate and only one patient discontinued therapy as a result of clinical toxicity. These results are consistent with those seen in the mCRPC population (10).

In summary, this study showed that treatment with sipuleucel-T was well tolerated and generated a robust and sustained immune response, consistent with prime-boosting and establishment of immunologic memory. While the primary endpoint was not met, the effect on PSADT, an important prognostic indicator in ADPC, suggests the biologic activity of sipuleucel-T in this population. Given evidence that sipuleucel-T prolongs survival in men with advanced prostate cancer (9–11), long-term follow-up for distant failure and survival, as well as additional studies in this patient population, are of interest.

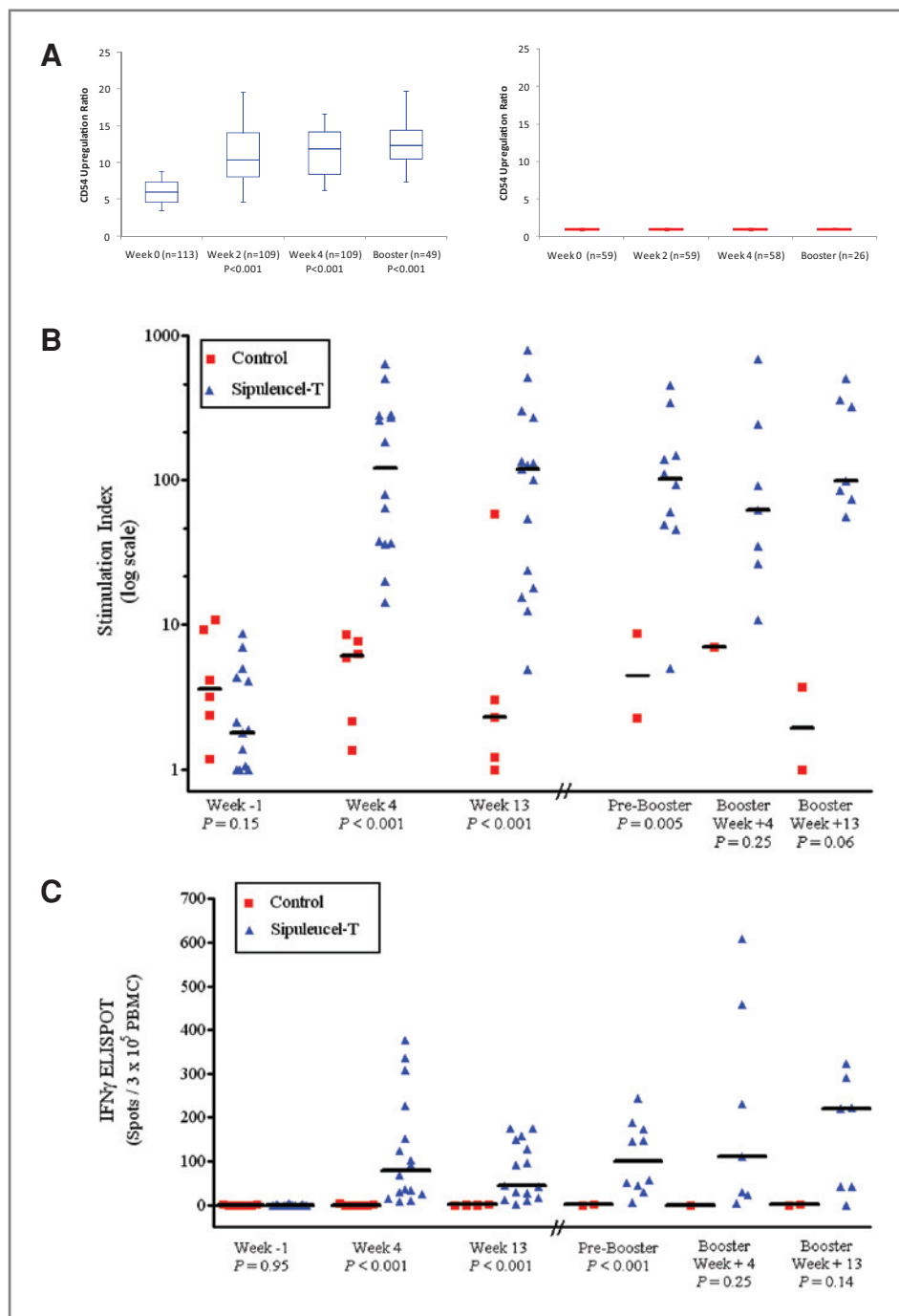
Disclosure of Potential Conflicts of Interest

Results from this study have been presented in part at the 2007 American Association for Cancer Research and American Society of Clinical Oncology annual meetings. L. A. Jones, Y. Xu, J. W. Kylastra, and M. W. Frohlich are employed by Dendreon. All authors have received research funds. Tomasz M. Beer is a consultant for Dendreon.

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Figure 4. CD54 upregulation (A) over time for patients treated with sipuleucel-T or control. Data is fold-upregulation represented as 10th, 25th, 50th (median), 75th, and 90th percentile. Median duration from randomization to Boost was 15.4 months (range 3.7 to 67.4 months) in sipuleucel-T patients. In the sipuleucel-T group, Week 2, Week 4, and Boost are significantly different compared with Week 0 ($P < 0.001$ all), as calculated using a linear mixed model on log-transformed upregulation ratios. Stimulation index (B) and IFN γ ELISPOT (C) of PA2024 for patients treated with control or sipuleucel-T. Individual responses are presented in the figure. The P -value represents a comparison of values (median shown as horizontal bars) between treatment groups at each visit. In sipuleucel-T patients with peripheral immune response data prior to Boost, the median time from randomization to the assessment was 22.6 months (range 14.3 to 67.3 months).



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Table 2. Overall incidences of adverse events occurring in $\geq 10.0\%$ of patients treated with Sipuleucel-T by frequency of occurrence

Preferred term	Any Grade ^a		Grade 3–5 ^a	
	Sipuleucel-T (n = 116) n (%)	Control (n = 59) n (%)	Sipuleucel-T (n = 116) n (%)	Control (n = 59) n (%)
Any adverse event	111 (95.7)	56 (94.9)	31 (26.7)	18 (30.5)
Fatigue	52 (44.8)	18 (30.5)	0 (0.0)	1 (1.7)
Chills ^b	51 (44.0)	6 (10.2)	2 (1.7)	0 (0.0)
Pyrexia ^b	42 (36.2)	1 (1.7)	2 (1.7)	0 (0.0)
Hot flush	30 (25.9)	20 (33.9)	1 (0.9)	1 (1.7)
Headache	27 (23.3)	8 (13.6)	1 (0.9)	0 (0.0)
Back pain	26 (22.4)	8 (13.6)	1 (0.9)	0 (0.0)
Paraesthesia oral	26 (22.4)	19 (32.2)	0 (0.0)	0 (0.0)
Arthralgia	25 (21.6)	8 (13.6)	1 (0.9)	0 (0.0)
Myalgia ^b	25 (21.6)	5 (8.5)	1 (0.9)	0 (0.0)
Paraesthesia	21 (18.1)	10 (16.9)	1 (0.9)	0 (0.0)
Influenza like illness ^b	16 (13.8)	2 (3.4)	0 (0.0)	0 (0.0)
Muscle spasms	15 (12.9)	4 (6.8)	0 (0.0)	0 (0.0)
Pain ^b	15 (12.9)	0 (0.0)	0 (0.0)	0 (0.0)
Constipation	14 (12.1)	5 (8.5)	0 (0.0)	0 (0.0)
Upper respiratory tract infection	14 (12.1)	7 (11.9)	0 (0.0)	0 (0.0)
Diarrhoea	13 (11.2)	10 (16.9)	0 (0.0)	0 (0.0)
Hypertension	13 (11.2)	8 (13.6)	0 (0.0)	0 (0.0)
Nausea	13 (11.2)	4 (6.8)	0 (0.0)	0 (0.0)
Dizziness	12 (10.3)	12 (20.3)	0 (0.0)	0 (0.0)
Nasopharyngitis	12 (10.3)	6 (10.2)	0 (0.0)	0 (0.0)

^aSeverity Grade assessed using National Cancer Institute's Common Toxicity Criteria (version 2.0).

^bThe incidence of adverse events ≥ 2 -fold greater in the sipuleucel-T arm than in the control arm.

References

- Catalona WJ, Smith DS. Cancer recurrence and survival rates after anatomic radical retropubic prostatectomy for prostate cancer: intermediate-term results. *J Urol* 1998;160:2428–34.
- Eastham JA, Scardino PT. Radical prostatectomy for clinical stage T1 and T2 prostate cancer. In: Vogelzang NJ, Scardino PT, Shipley WU, Coffey DS, editors. *Comprehensive textbook of genitourinary oncology*. 2nd ed. Philadelphia: LipincottWilliams & Wilkins;1999.
- Walsh PC, Partin AW, Epstein JI. Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. *J Urol* 1994;152:1831–6.
- Clark PE, Levin HS, Kupelian PA, Reddy C, Zippe CD, Klein EA. Intermediate-term outcome with radical prostatectomy for localized prostate cancer: The Cleveland Clinic experience. *The Prostate J* 2001;3:118–25.
- Nair B, Wilt T, MacDonald R, Rutks I. Early versus deferred androgen suppression in the treatment of advanced prostatic cancer. *Cochrane Database Syst Rev* 2002;CD003506.
- Walsh PC, DeWeese TL, Eisenberger MA. A structured debate: immediate versus deferred androgen suppression in prostate cancer—evidence for deferred treatment. *J Urol* 2001;166:508–15;discussion 15–6.
- Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. *JAMA* 2005;294:238–44.
- van Andel G, Kurth KH. The impact of androgen deprivation therapy on health related quality of life in asymptomatic men with lymph node positive prostate cancer. *Eur Urol* 2003;44:209–14.
- Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol* 2006;24:3089–94.
- Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer* 2009;115: 3670–9.
- Kantoff P, Higano C, Shore N, Berger ER, Small EJ, Penson DF, et al. A randomized, placebo-controlled, phase 3 trial of active cellular immunotherapy with Sipuleucel-T in metastatic castration-resistant prostate cancer. *NEJM* 2010;363:411–22.
- Sheikh N A, Jones L A. CD54 is a marker of antigen presenting cell activation. *Cancer Immunol Immunother* 2008;57:1381–90.
- Park JW, Melisko ME, Esserman LJ, Jones LA, Wollan JB, Sims R. Treatment with autologous antigen-presenting cells activated with the HER-2 based antigen Sipuleucel-T: results of a phase I study in immunologic and clinical activity in HER-2 overexpressing breast cancer. *J Clin Oncol* 2007;25:3680–7.
- Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Statistics in Medicine* 1999;18:695–706.
- Augustin H, Freibauer C, Bayer L, Lunglmayr G, Tschurlovich F, Kubler W, et al. Molecular markers and their prognostic impact in patients with advanced prostate cancer undergoing intermittent androgen suppression. *Prostate Cancer Prostatic Dis* 2006;9:279–83.
- Akyol F, Ozyigit G, Seleik U, Karabulut E. PSA bouncing after short term androgen deprivation and 3D-conformal radiotherapy for

- localized prostate adenocarcinoma and the relationship with the kinetics of testosterone. *Eur Urol* 2005;48:40-5.
17. Bates AT, Pickles T, Paltiel C. PSA doubling time kinetics during prostate cancer biochemical relapse after external beam radiation therapy. *Int J Radiat Oncol Biol Phys* 2005;62:148-53.
 18. Kobayashi T, Nishizawa K, Mitsumori K. Individual variation of hormonal recovery after cessation of luteinizing hormone-releasing hormone agonist therapy in men receiving long-term medical castration therapy for prostate cancer. *Scand J Urol Nephrol* 2006;40:198-203.
 19. Gulley JL, Aragon-Ching JB, Steinberg SM, Hussain MH, Sartor O, Higano CS, et al. Kinetics of serum androgen normalization and factors associated with testosterone reserve after limited androgen deprivation therapy for nonmetastatic prostate cancer. *J Urol* 2008;180:1432-7;discussion 7.
 20. Riehm M, Rhodes PR, Cook TD, Grose GS, Bruskewitz RC. Analysis of variation in prostate-specific antigen values. *Urology* 1993;42:390-7.
 21. D'Amico AV, Moul J, Carroll PR, Sun L, Lubeck D, Chen MH. Prostate specific antigen doubling time as a surrogate end point for prostate cancer specific mortality following radical prostatectomy or radiation therapy. *J Urol* 2004;S42-6;discussion S6-7.
 22. Albertsen PC, Hanley JA, Penson DF, Fine J. Validation of increasing prostate specific antigen as a predictor of prostate cancer death after treatment of localized prostate cancer with surgery or radiation. *J Urol* 2004;171:2221-5.
 23. Roberts SG, Blute ML, Bergstralh EJ, Slezak JM, Zincke H. PSA doubling time as a predictor of clinical progression after biochemical failure following radical prostatectomy for prostate cancer. *Mayo Clin Proc* 2001;76:576-81.
 24. Sandler HM, Dunn RL, McLaughlin PW, Hayman JA, Sullivan MA, Taylor JM. Overall survival after prostate-specific-antigen-detected recurrence following conformal radiation therapy. *Int J Radiat Oncol Biol Phys* 2000;48:629-33.
 25. Freedland SJ, Humphreys EB, Mangold LA, Eisenberger M, Dorey FJ, Walsh PC, et al. Death in patients with recurrent prostate cancer after radical prostatectomy: prostate-specific antigen doubling time subgroups and their associated contributions to all-cause mortality. *J Clin Oncol* 2007;25:1765-71.
 26. Valicenti RK, DeSilvio M, Hanks GE, Porter A, Brereton H, Rosenthal SA, et al. Posttreatment prostatic-specific antigen doubling time as a surrogate endpoint for prostate cancer-specific survival: an analysis of Radiation Therapy Oncology Group Protocol 92-02. *Int J Radiat Oncol Biol Phys* 2006;66:1064-71.
 27. Arlen PM, Bianco F, Dahut WL, D'Amico A, Figg WD, Freedland SJ, et al. Prostate Specific Antigen Working Group Guidelines on Prostate Specific Antigen Doubling Time. *J Urol* 2008;179:2181-2186.
 28. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, et al. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol* 2000;18:3894-903.
 29. Burch PA, Breen JK, Buckner JC, Gastineau DA, Kaur JA, Laus RL, et al. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res* 2000;6:2175-82.
 30. Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, et al. Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a Phase 2 trial. *Prostate* 2004;60:197-204.
 31. Kantoff PW, Schoetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol* 2010;28:1099-105.
 32. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711-23.
 33. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *JAMA* 1999;281:1591-7.
 34. Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. *Front Biosci* 2007;12:4957-71.
 35. Oliver RT, Joseph JV, Gallagher CJ. Castration-induced lymphocytosis in prostate cancer: possible evidence for gonad/thymus endocrine interaction in man. *Urol Int* 1995;54:226-9.
 36. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol* 2005;175:2741-53.
 37. Mercader M, Bodner BK, Moser MT, Uldrich AP, Berzins SP, Heng TS, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc Natl Acad Sci U S A* 2001;98:14565-70.
 38. Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. *Cancer Cell* 2005;7:239-49.

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