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Antigen-Specific CD8 Lytic Phenotype Induced by Sipuleucel-T in Hormone-Sensitive or Castration-Resistant Prostate Cancer and Association with Overall Survival

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Abstract

Purpose: Sipuleucel-T is FDA approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC) based on the IMPACT trial showing a 4.1-month benefit in median overall survival (OS) for patients receiving sipuleucel-T versus control. Although efficacy of sipuleucel-T is well established, its mechanism remains incompletely understood.

Patients and Methods: Patient samples from three sipuleucel-T trials were assessed for peripheral cellular immune responses to the immunogen PA2024 and the target antigen prostatic

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Disclosure of Potential Conflicts of Interest

D. Petrylak is a consultant/advisory board member for and reports receiving commercial research grants from Dendreon. D.I. Quinn is a consultant/advisory board member for Astellas, Bayer, Dendreon, Genzyme, Janssen, and Pfizer. C. Drake is a consultant/advisory board member for Dendreon. No potential conflicts of interest were disclosed by the other authors.

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acid phosphatase (PAP). PAP- and PA2024-specific proliferative and cytolytic responses were characterized to delineate sipuleucel-T-induced immune responses. To quantify potential cytotoxic T lymphocyte (CTL) activity, cell-surface CD107a expression on PAP- or PA2024-specific CD8⁺ T cells was measured in sipuleucel-T-treated patient and healthy volunteer samples.

Results: Increased PA2024-specific CD4⁺ ($P = 0.030$) and CD8⁺ ($P = 0.052$) T-cell proliferation from baseline to week 6 was observed ($N = 14$) post-sipuleucel-T, with greater magnitude of PA2024-specific responses compared with PAP. PAP- and PA2024-CTL activity (CD107a positivity) significantly increased at weeks 6 and 26 after sipuleucel-T treatment ($P < 0.0001$; $N = 22$). At 26 weeks post-sipuleucel-T, OS correlated with the magnitude of PAP (Pearson R , 0.52; $P = 0.013$) or PA2024 (Pearson R , 0.67; $P = 0.0006$) CTL activity. Higher PA2024-CTL activity at week 26 was significantly associated with longer OS using tertile analysis ($P = 0.0005$; $N = 22$), with PA2024 responses correlating with PAP responses at week 26 ($R = 0.90$; $P = 1.53 \times 10^{-8}$).

Conclusions: This study is the first to report PAP-specific CD8⁺ T-cell responses elicited by sipuleucel-T treatment. Increased and persistent potential PA2024-specific CTL activity correlated with PAP-specific CTL activity and associated with improved OS following sipuleucel-T treatment.

Introduction

Prostate cancer is the most commonly diagnosed noncutaneous cancer among men in the United States. While localized prostate cancer is highly curable, up to 20% of patients develop castration-resistant prostate cancer (CRPC) within 5 years of diagnosis (1–4). The majority of these patients will eventually develop metastatic CRPC (mCRPC), which is associated with a poor prognosis and a 5-year survival rate of 28% (5). Over the past 15 years, the avoidance of immune destruction has been recognized as a hallmark of cancer (6). Cancer cells can evade immunological elimination by T and B lymphocytes, macrophages, and natural killer cells, in part through secretion of TGF β or other immunosuppressive factors (6). Moreover, cancer cells can suppress the activity of cytotoxic T cells (CTL) by recruiting immunosuppressive regulatory T cells and myeloid-derived suppressor cells (6). These findings led to the development of immune-activating therapies for certain cancer types, including mCRPC. Two agents, ipilimumab, which increases antitumor T-cell responses by binding to CTL antigen 4, and tasquinimod, which targets the tumor microenvironment, have been evaluated in phase III clinical trials of patients with mCRPC, and although they showed evidence of antitumor activity, they did not improve median overall survival (OS; refs. 7–9). Recent evidence showed that tissue-specific proteins could serve as targets for cancer vaccines (5). To that end, the therapeutic vaccine PSA-TRICOM was evaluated in a phase III trial (NCT01322490), which failed to confirm the OS benefit noted in an earlier phase II study (10).

To date, the only immunotherapy approved by the FDA for the treatment of mCRPC is sipuleucel-T, which improved OS compared with control by a median of 4.1 months in the phase III IMPACT trial (5, 11). Sipuleucel-T is an autologous cellular immunotherapy designed to stimulate an immune response against prostate cancer. It consists of autologous peripheral blood mononuclear cells (PBMCs), including antigen-presenting cells (APCs,

defined as CD54⁺ cells) activated *in vitro* with the immunogen PA2024, a recombinant fusion protein composed of human prostatic acid phosphatase (PAP) linked to granulocyte macrophage colony-stimulating factor (GM-CSF). The ability of sipuleucel-T to induce peripheral cellular and humoral antigen-specific immune responses to PA2024 and PAP, the target antigen, was demonstrated in several studies (11–16). Notably, in the IMPACT trial, sipuleucel-T–treated patients with an antibody titer of more than 400 against either PA2024 or PAP at any time after baseline showed longer survival than those with an antibody titer of 400 or less (11). Exploratory analyses suggested that PA2024- or PAP-specific immune responses [i.e., IFN γ enzyme-linked immunospot (ELISPOT), T-cell proliferation response, or antibody responses] were correlated with OS (12). This antitumor immunity persists for up to 10 years after sipuleucel-T treatment in some patients with prostate cancer (17).

At the tissue level, neoadjuvant administration of sipuleucel-T prior to radical prostatectomy in men with localized prostate cancer led to aggregation of effector CD4⁺ T cells (T helper cells) and CD8⁺ T cells (CTL) at the interface between tumor and benign tissue and the infiltration of activated T cells into the prostate gland; this was not observed in control patients (18). In addition, sipuleucel-T induced antigen spread to secondary antigens (16, 19, 20), and this response correlated with prolonged OS in patients with mCRPC (19).

The results of other studies across different tumor types showed a correlation between OS and the infiltration of lymphocytes into the tumor, indicating a role for antigen-specific T lymphocytes in disease control and eradication (21–23). Prior studies of sipuleucel-T–induced immune responses did not differentiate specific effector cell types (CD4 or CD8); thus, defining the nature of the cellular responses induced by sipuleucel-T and determining whether they are PAP- and/or PA2024-specific remains of interest, particularly due to their potential correlation with OS (12). In the present study, we evaluated the proliferative and cytolytic characteristics of PA2024- and PAP-specific CD8⁺ T cells to further elucidate the mechanism of sipuleucel-T–induced immune responses, and their correlation with clinical outcome. The generation of posttreatment antigen-specific CTL response would represent a major step in understanding the biological mechanism of the OS benefit observed with sipuleucel-T.

Patients and Methods

Patients

Antigen-specific peripheral immune responses against PA2024 and PAP were quantified using peripheral blood samples collected from three sipuleucel-T trials—STAND (16), STAMP (13), and STRIDE (14)—that involved sequencing sipuleucel-T with other prostate cancer treatments. Survival data were available from STAMP and STRIDE. All patients provided written informed consent. Due to the retrospective nature of the study, only available patient samples could be assayed for immune responses. Immune responses were evaluated at weeks 6 and 26; week 6 was chosen, because the greatest ELISPOT responses were observed at week 6 in previous studies (12, 13, 16), and week 26 represents an extended time point at which cellular and/or humoral responses correlated with OS in the IMPACT. Those long-term responses are also likely indicative of long-term immunological memory (12).

Table 1 provides an overview of the three trials included in this analysis: STAND is a randomized phase II trial that assessed the optimal sequencing of sipuleucel-T with androgen deprivation therapy (ADT) in patients with biochemically recurrent prostate cancer with a high risk of metastases (PSA doubling time ≥ 12 months after prostatectomy and/or radiotherapy). In one arm, ADT was administered 2 weeks after sipuleucel-T completion, and in the other arm, sipuleucel-T was administered 12 weeks after ADT initiation. ADT was continued for a total of 12 months in both arms. The primary endpoint was PA2024-specific T-cell responses over time, as assessed by the ELISPOT assay (16).

STAMP is a randomized, open-label, phase II study of sipuleucel-T administered concurrently or sequentially with abiraterone acetate (1,000 mg q.d.) plus prednisone (5 mg BID; AA + P) in men with mCRPC. In the concurrent arm, patients received AA + P 1 day after the first sipuleucel-T infusion, and in the sequential arm, patients began AA + P 4 weeks after the final (third) sipuleucel-T infusion. AA + P treatment was continued for a total of 26 weeks. The primary endpoint was cumulative APC activation, a measure of sipuleucel-T product potency (13).

STRIDE is a randomized, open-label, phase II study evaluating the immune responses associated with concurrent versus sequential administration of sipuleucel-T and enzalutamide (160 mg q.d.) in patients with asymptomatic or minimally symptomatic mCRPC. Patients were randomized 1:1 to receive sipuleucel-T with enzalutamide starting 2 weeks before initiation of sipuleucel-T (concurrent arm) or with enzalutamide initiated 4 weeks after the final (third) dose of sipuleucel-T (sequential arm). The primary endpoint was peripheral PA2024-specific T-cell proliferation. (14).

The studies were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. Approval was obtained from central or local ethics committees at all centers, and guidelines outlined by all national, state, and local laws of appropriate regulatory authorities were followed.

Cell culture

T-cell proliferation assay.—Cryopreserved PBMCs obtained from patient samples were thawed in complete Roswell Park Memorial Institute (cRPMI) medium. Cells were treated with 15 U/mL DNase for 30 minutes at 37°C, resuspended in fresh cRPMI medium, and rested overnight at 37°C in a 5% CO₂ atmosphere prior to culture. To quantify antigen-specific T-cell responses, cells were first stained with the membrane dye carboxyfluorescein succinimidyl ester (CFSE; ThermoFisher Scientific; #C34554). Cells were then incubated with either PA2024 (100 mg/mL) or PAP (50 µg/mL) for 5 days prior to phenotyping. In this technique, antigen-specific proliferation is quantified by CFSE dilution, the concentration of which decreases by 50% on each successive cell division (Fig. 1A). Samples for T-cell proliferation ($n = 10$ from STAND and $n = 4$ from STRIDE) were assayed via flow cytometry as described below. T-cell proliferation was quantified as described by Quah and colleagues (24).

Surrogate assay for cytolytic T-cell activity.—CD107a, also known as lysosomal-associated membrane protein 1 (LAMP1), is a surrogate for antigen-specific CD8⁺ T-cell

degranulation, a hallmark of CD8⁺ T-cell cytolytic activity (25–27). CD107a is present on the membrane of lysosomes containing perforin and granzymes in CD8⁺ T cells. When lytic granules are released, lysosomal membranes fuse with the plasma membrane resulting in cell-surface CD107a expression on CD8⁺ T cells that can be assayed using flow cytometry (25–27). After resting overnight, PBMCs ($n = 10$ healthy donors, $n = 10$ from STAMP, and $n = 12$ from STRIDE) were counted and diluted to a concentration of $\sim 2 \times 10^7$ cells/mL in warm cRPMI. Approximately 2×10^6 cells (100 mL of cell suspension) were placed into each well of a 96-well cell culture plate, and cells were stimulated with an equal volume of antigen [HER500, a truncated form of HER2/neu as additional negative control (20 μ g/mL), PA2024 (100 μ g/mL) or PAP (50 μ g/mL)] at 37°C in a 5% CO₂ atmosphere for 18 to 20 hours, along with Protein Transport Inhibitor Cocktail (500 $\times$; ThermoFisher Scientific; #00–4980-03), and anti-CD107a antibody (BD Biosciences; cloneH4A3, #561343) added during the last 4 hours of culture.

Cell phenotyping.—T cells were quantified by flow cytometry following culture for either proliferation or CTL lytic potential as described above. Briefly, cells were washed and labeled with LIVE/DEAD Fixable Aqua Dead Cell Stain (ThermoFisher Scientific; #L34957) to distinguish between viable and nonviable cells prior to surface staining with a cocktail of antibodies including, CD3 PE-Cy7 (BioLegend; clone sK7, #344816), CD4 BV711 (BioLegend; clone OKT4, #317440), CD8 QD605 (ThermoFisher Scientific; clone 3B5, #Q10009), CD19 BV421 (BioLegend; clone HIB19, #302234), CD14 BV421 (BD Biosciences; clone MoP9, #56743), and CD335 BV421 (BioLegend; clone 29A1.4, #331914). A minimum of 100,000 events were collected and analyzed for CD4⁺ and CD8⁺ T-cell proliferation or CD107a expression using an LSRFortessa cell analyzer (BD Biosciences) and FlowJo analysis software. For the proliferation assays, a series of analytical gates based on cell phenotype was used to identify CD4⁺ and CD8⁺ T cells (Fig. 1B). T cells were separated from non-T-cell lineage cells by gating for CD19, CD14, and Nkp46 to mark B cells, monocytes, and natural killer cells, respectively. Viable CD3⁺ T cells were assessed using a LIVE/DEAD Fixable Aqua Dead Cell stain. T cells were then subdivided into CD4⁺ and CD8⁺ T-cell lineages, and the CFSE signal was quantified. For the lytic assays, the CD107a signal was measured on live CD8⁺ T cells following the gating scheme outlined in Fig. 1C.

Statistical analyses

Statistical significance for CD4⁺ and CD8⁺ T-cell proliferation was assessed using a repeated-measures model to calculate *P* values, with $P < 0.05$ considered statistically significant. The Pearson correlation coefficient was used to assess correlation of OS with PAP- and PA2024-directed CTLs. To assess changes in CTLs over time, a paired sample Wilcoxon signed rank test was used. The Kaplan–Meier method was used to assess associations between surrogate PA2024- and PAP-specific CTL activity at week 26 and OS (CTL activity grouped by tertiles), and Cox proportional hazard models were used to assess surrogate CTL activity at week 26 as continuous variables.

Results

Antigen-specific proliferation of CD4⁺ and CD8⁺ T cells is observed after sipuleucel-T treatment

Proliferation assays (Fig. 2A and B) showed a significant increase in PA2024-specific CD4⁺ T-cell proliferation at week 6 as compared with week 0 ($P = 0.030$). At week 6, there was also a trend toward increased PA2024-specific CD8⁺ cell proliferation ($P = 0.052$). Proliferation to the native PAP protein was not increased in either the CD4⁺ or CD8⁺ cell population at week 6 or 26.

Surrogate antigen-specific CTL activity is increased by sipuleucel-T treatment

We next assessed the ability of sipuleucel-T to induce antigen-specific CTL responses, using CD107a expression as a surrogate for CTL activity. Neither PAP- nor PA2024-specific CTL responses were detected in untreated healthy subject samples (Fig. 3A). Similarly, in sipuleucel-T-treated patients, there was no response detected against HER500 (a protein not expressed in prostate cancer) before or after sipuleucel-T treatment (Fig. 3B). By contrast, increased PA2024- and PAP-specific CTL activity was detected at week 6 compared with baseline ($P < 0.0001$) in patient samples. Notably, surrogate CTL activity demonstrated a persistent increase up to week 26 for both antigens compared with baseline ($P < 0.0001$; Fig. 3C and D).

Surrogate CTL activity at week 26 correlates with OS

Having detected both short-term and longer-term CTL responses (Fig. 3), we hypothesized that these CTL responses might correlate with OS. Correlation analysis was conducted using patient samples from the STAMP ($n = 10$) and STRIDE ($n = 12$) trials for which OS data were available. PAP or PA2024-specific CTL responses at week 0 or 6 were not significantly correlated with OS. However, for both antigens, CTL responses at week 26 were significantly associated with OS ($R = 0.52$, $P = 0.0134$ for PAP; $R = 0.67$, $P = 0.0006$ for PA2024; Fig. 4A and B). Because responses to the native PAP protein in treated patients are assumed to result from responses to the fusion protein PA2024, we tested whether CTL responses to the two antigens correlated. As shown in Fig. 4C, CTL responses to PA2024 correlated with responses to PAP ($R = 0.90$; $P = 1.53E^{-08}$).

To further explore the relationship between CTL responses and OS, the surrogate CTL activity at week 26 was analyzed as a categorical variable using tertile analysis (Fig. 5A and C) or as a continuous variable using a Cox proportional hazards model (Fig. 5B and D). Baseline characteristics of these patients stratified by tertiles are shown in Supplementary Table S1. Comparison of the Kaplan–Meier curves corresponding to the lower ($n = 8$), middle ($n = 7$), and upper ($n = 7$) tertile populations demonstrated a significant difference between the curves ($P = 0.0005$ log-rank test) for PA2024, indicating that a higher CTL response to PA2024 at week 26 is associated with a longer OS (Fig. 5C). The results of the continuous analysis ($P = 0.031$) supported the strong association between increased PA2024 CTL activity and longer OS (Fig. 5D). The stratified survival data showed differences in the survival curves corresponding to the PAP tertile populations, which hinted some correlation

between OS and CTL response to PAP, but the association was not significant ($P = 0.2122$; Fig. 5A).

Discussion

The role of CD4⁺ T cells, as a regulator of the adaptive immune response to cancer, and the cytolytic activity of CD8⁺ T cells, which may lead to eradication of tumors, are reasonably well established (28, 29). Continued research bolstered the understanding of more precise roles for CD4⁺ and CD8⁺ T cells in tumor-specific immune responses. More specifically, previous studies showed that CD4⁺ T cells play a key role in the differentiation and expansion of tumor antigen-specific CD8⁺ T cells and are essential for the generation and maintenance of long-term CD8⁺ memory T-cell responses (28, 30). Thus, antigen-specific proliferation of CD4⁺ T cells suggests the presence of a necessary component of long-term CD8⁺ T-cell responses. These previous results support our current finding of persisting PA2024-specific CD4⁺ and CD8⁺ T-cell responses following sipuleucel-T treatment. A previous analysis of patients with mCRPC who participated in the pivotal phase III IMPACT study showed that sipuleucel-T-induced antigen-specific immune responses were observed in the majority (79%) of patients and were positively correlated with OS ($P = 0.003$), which suggests that the broad engagement of the immune system by sipuleucel-T may contribute to its OS benefit (12). Additional studies of sipuleucel-T showed that immune response rates against target antigens exceeded 90% (13, 14, 16).

The results of this analysis show that sipuleucel-T treatment was associated with PA2024-specific CD4⁺ T-cell proliferation, and PAP- and PA2024-specific CTL responses, confirming the target specificity of sipuleucel-T. Additionally, sipuleucel-T induced surrogate antigen-specific CD8⁺ CTL activity at week 6 and 26 against both PAP and PA2024. Notably, no surrogate CTL activity was observed in healthy donor controls. In sipuleucel-T-treated patient samples, no CTL potential was induced against HER500, an irrelevant antigen not expressed in prostate cancer. Recently, visual confirmation of cytolysis of PAP-expressing target cells by CTLs from sipuleucel-T patient samples was captured by confocal microscopy (31), supportive of the findings of the current study demonstrating PAP-specific CTL activity.

Kaplan–Meir survival curves plotted based on grouping of PA2024-specific CTL responses at week 26 by tertiles revealed a significant association between increased CTL activity and longer OS. These findings are in agreement with results obtained from the analysis of immune responses at week 26 as a continuous variable, providing further evidence of an association between longer OS and greater magnitude of surrogate PA2024-specific CTL activity in mCRPC patients. The stratified survival data did not show a significant correlation between OS and CTL responses to PAP, which could be due to the limited sample size available ($N = 22$); a correlation analysis using a large patient population is warranted in the future. Nevertheless, the current data suggest the establishment of immunologic memory and provide a potential biologic basis for the OS benefit observed in patients with mCRPC receiving sipuleucel-T. In other cancers, similar findings have been observed; a phase I/II trial that assessed patients with acute myeloid leukemia who were vaccinated with Wilms tumor 1 (WT1) antigen showed a correlation between long-term

responders and an increase in CD8⁺ T cells (23). The presence of antigen-specific CD8⁺ T cells correlating with improved survival has also been observed in patients with melanoma and colorectal cancer (32). Furthermore, we found a positive correlation between PAP- and PA2024-specific CTL activity at week 26 following sipuleucel-T therapy, demonstrating that immune responses against PA2024 can be used as a surrogate for PAP.

The surrogate PAP- and PA2024-specific CTL activity observed following sipuleucel-T may also explain the antigen spread following sipuleucel-T treatment described previously and has been correlated with improved OS in patients with mCRPC (19). Antigen spread (the expansion of the immune responses to nontarget antigens) involves the lysis of tumor cells, i.e., by CTLs, thereby resulting in the presentation of additional tumor antigens to immune cells by APCs, ultimately leading to a broader immune response against secondary antigens, and potentially improved clinical benefit (33, 34). Furthermore, CD8⁺ T-cell proliferative responses to the intracellular protein KRAS have also been observed, suggesting that secondary antigens were made available via cytolysis (20). Prior studies demonstrated the trafficking of CD8⁺ cells to the tumor rim in the prostate tissue and the induction of peripheral antigen-specific CD8⁺ cells by sipuleucel-T (18). Most recently, confocal imaging confirmed CTL activity against PAP-expressing target cells (31).

The major limitation of the current analysis is the small sample size, which necessitated combining blood samples across studies. However, previous studies demonstrated that the administration of concurrent versus sequential abiraterone (STAMP) or enzalutamide (STRIDE) with sipuleucel-T did not result in differences in median OS (35, 36). Additionally, no obvious trends in demographics and baseline characteristics were observed between response tertiles (Supplementary Table S1), suggesting that baseline patient characteristics were not likely responsible for the observed correlation between greater CTL activity and increased OS.

The present study is the first to measure the potential of sipuleucel-T to induce CTL activity and correlate surrogate CTL activity with clinical outcome. The persistence of surrogate CTL activity against PAP and PA2024, the key target antigens for sipuleucel-T, is suggestive of tumor cell lysis and may be associated with improved OS in patients with mCRPC receiving sipuleucel-T. The data presented here warrant an expanded analysis of the capacity of sipuleucel-T to generate long-lasting CTLs and the biological effects of antigen-specific CTLs in extending the observed OS benefit of sipuleucel-T.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Translational Relevance

Sipuleucel-T is an FDA-approved autologous cellular immunotherapy with established efficacy in metastatic castration-resistant prostate cancer (mCRPC); elucidation of its mechanism of action continues to evolve. Peripheral cellular and humoral immune responses to PAP and the immunogen PA2024 demonstrated long-lived, antigen-specific immune responses to sipuleucel-T that correlate with overall survival (OS). In tissue, CD4⁺ and CD8⁺ T cells aggregate at the tumor rim in prostatectomy specimens after neoadjuvant sipuleucel-T. The current study shows persistent antigen-specific peripheral CD4⁺ T-cell responses and the induction of antigen-specific CD8⁺ T cells with potential lytic function following sipuleucel-T. Moreover, we found a positive correlation between OS and PAP- as well as PA2024-specific cytotoxic T lymphocytes (CTL) phenotype. This study is the first to demonstrate the ability of sipuleucel-T to induce potential tumor lysis. Sipuleucel-T-induced antigen-specific CTL activity could provide a biologic basis for the OS benefit observed in mCRPC.

Figure 1.

A, Measurement of the proliferative response of PBMCs. **B**, Flow cytometry gating schema for assessing proliferation of CD4⁺ and CD8⁺ T cells. B cells (CD19), monocytes (CD14), natural killer cells (NKp46), and nonviable CD3⁺ T cells were excluded from the analysis. Dilution of CFSE was measured on both CD4⁺ and CD8⁺ T-cell subpopulations. **C**, Flow cytometry gating schema for the assessment of cell-surface CD107a expression on CD8⁺ T cells. B cells (CD19), monocytes (CD14), natural killer cells (NKp46), and nonviable CD3⁺ T cells were excluded from the analysis. CD8⁺ T cells were analyzed for CD107a expression following stimulation with PA2024- or PAP- or in unstimulated controls. For **B** and **C**, viable CD3⁺ T cells were assessed using a LIVE/DEAD Fixable Aqua Dead Cell stain. CFSE, Carboxyfluorescein succinimidyl ester; CTL, cytotoxic T lymphocyte; FSC-A, forward scatter-area; FSC-H, forward scatter-height; PBMC, peripheral blood mononuclear cells; SSC-A, side scatter-area; Th, T helper.

Figure 2.

Antigen-specific proliferation of CD4⁺ and CD8⁺ T cells. Samples from patients ($n = 14$) treated with sipuleucel-T in the STAND and STRIDE trials were assessed for frequency of proliferating CD4⁺ (A) and CD8⁺ (B) T cells after PAP and PA2024 stimulation (relative to unstimulated controls). A repeated-measures model was used to assess differences in PA2024- and PAP-specific proliferation of CD4⁺ and CD8⁺ T cells. Orange circles represent week 0 response, red squares represent week 6 response, and green diamonds represent week 26 response.

Figure 3.

PAP- and PA2024-specific CTLs at week 0 (prior to sipuleucel-T infusion) and at weeks 6 and 26 post-sipuleucel-T treatment. HER500, PAP-, and PA2024-stimulated CTL activity (normalized to unstimulated control) was measured in 10 healthy donors (A), and in 22 sipuleucel-T-treated patients (B, C, and D). The HER500 antigen is not expressed on prostate cancer cells and was therefore used as an additional negative control to test for CD107a expression in healthy donors and sipuleucel-T-treated patients. A paired sample Wilcoxon signed rank test was used to assess changes in CTL activity over time, as measured by the number of CD8⁺ T cells expressing CD107a. Orange circles represent week 0 response, red squares represent week 6 response, and green diamonds represent week 26 response.

Figure 4. Correlation of OS with PAP-specific (**A**) or PA2024-specific (**B**) CTLs at weeks 0, 6, and 26 ($N=22$). **C**, Correlation between PAP- and PA2024-specific CTL activity at week 26 ($N=22$).

Figure 5.

Association of overall survival and PAP-specific (**A, B**) and PA2024-specific (**C, D**) CTL activity at week 26 using categorical (tertile) analysis and continuous variable analysis ($N = 22$). Kaplan–Meier survival curves were plotted based on CTL responses at week 26 as a categorical variable (tertile analysis) and as a continuous variable. Higher PA2024-specific CTL activity at week 26 was significantly associated with longer OS ($P = 0.0005$ for categorical variable analysis and $P = 0.031$ for continuous variable analysis).

Table 1.

Overview of prostate cancer studies included in the current analysis

Study	Treatment	Disease	Number of Patients Assessed ^a			NCT identifier
			Proliferation assay	CTL activity assay	CTL activity assay	
STAND	Sipuleucel-T → ADT vs. ADT → Sipuleucel-T	Nonmetastatic hormone-sensitive PC	10	–	–	NCT01431391
STAMP	Sipuleucel-T + concurrent vs. sequential abiraterone acetate/prednisone	mCRPC	–	–	10 ^a	NCT01487863
STRIDE	Sipuleucel-T + concurrent vs. sequential enzalutamide	mCRPC	4	–	12 ^a	NCT01981122

Abbreviations: ADT, androgen deprivation therapy; CTL, cytotoxic T lymphocyte; mCRPC, metastatic castration-resistant prostate cancer; PC, prostate cancer.

^a Patient samples with available OS data were used for CTL activity assay.