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Authors
Madan, Ravi A
Antonarakis, Emmanual S
Drake, Charles G
et al.

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Putting the Pieces Together: Completing the Mechanism of Action Jigsaw for Sipuleucel-T

Ravi A. Madan (iD) , MD,[1,*] Emmanuel S. Antonarakis (iD) , MD,[2] Charles G. Drake, MD, PhD,[3]
Lawrence Fong (iD) , MD,[4] Evan Y. Yu (iD) , MD,[5] Douglas G. McNeel (iD) , MD, PhD,[6] Daniel W. Lin, MD,[5]
Nancy N. Chang (iD) , PharmD,[7] Nadeem A. Sheikh, PhD,[7] James L. Gulley (iD) , MD[1]

[1]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; [2]Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA; [3]Columbia University Herbert Irving Comprehensive Cancer Center, New York, NY, USA; [4]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA; [5]University of Washington and Seattle Cancer Care Alliance, Seattle, WA, USA; [6]University of Wisconsin Carbone Cancer Center, Madison, WI, USA and [7]Dendreon Pharmaceuticals LLC, Seattle, WA, USA

*Correspondence to: Ravi A. Madan, MD, Center for Cancer Research, National Cancer Institute, 10/13N240B, Bethesda, MD 20892 (e-mail: madanr@mail.nih.gov).

Abstract

Sipuleucel-T is an autologous cellular immunotherapy that induces an immune response targeted against prostatic acid phosphatase (PAP) to treat asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. In the phase III IMPACT study, sipuleucel-T was associated with a statistically significantly increased overall survival (OS) (median = 4.1 months) vs placebo. Patients with baseline prostate-specific antigen levels in the lowest quartile ($\leq$22.1 ng/mL) exhibited a 13-month improvement in OS with sipuleucel-T. Together, this led sipuleucel-T to be approved and recommended as first-line therapy in various guidelines for treatment of metastatic castration-resistant prostate cancer. This review discusses the varied findings about the mechanisms of action of sipuleucel-T, bringing them together to form a more coherent picture. These pieces include inducing a statistically significant increase in antigen-presenting cell activation; inducing a peripheral immune response specific to the target (PAP) and/or immunizing (PA2024) antigens; stimulating systemic cytotoxic T-lymphocyte activity; and mediating antigen spread (ie, increased antibody responses to secondary proteins in addition to PAP and PA2024). Each of these pieces individually correlates with OS. Sipuleucel-T also traffics T cells to the prostate and is associated with long-term immune memory such that a second course of treatment induces an anamnestic immune response. Prostate cancer does not have a strongly inflamed microenvironment, thus its response to immune checkpoint inhibitors is limited. Because sipuleucel-T is able to traffic T cells to the tumor, it may be an ideal combination partner with immunotherapies including immune checkpoint inhibitors or with radiation therapy.

Prostate cancer is the most common type of new cancer diagnosis in men (20%) and the second most common cause of cancer death in men in the United States (10%) after lung cancer (1). It is estimated that 191 930 new cases of prostate cancer will be diagnosed in 2019 in the United States and 33 330 men will die from this disease (1). Although the incidence of prostate cancer has been falling for the last 10 years—an observation attributed, at least in part, to changes in screening and PSA testing recommendations (1)—the absolute number of men with the disease is likely to increase as more treatment options become available to an aging population, with the highest proportional prevalence being in African American men (1). An estimated 3 million men in the United States or more will have prostate cancer by 2020 according to one model (2).

Most men with prostate cancer present with localized disease or regional spread (1). These men have a good prognosis with a mortality rate similar to the all-cause mortality rate for the general population (2). If the disease progresses to metastatic castration-resistant prostate cancer (mCRPC), patients have an annual all-cause mortality rate of approximately 55% (2). The prevalence of mCRPC will likely increase over time because a growing number of men survive long enough that their prostate cancer progresses to mCRPC, with an estimated prevalence of approximately 42 970 men in the United States in 2020 (2). Therefore, treatments for mCRPC are likely to have the greatest impact on mortality among men with advanced prostate cancer (2). Currently, available treatments for mCRPC include androgen receptor and

androgen synthesis inhibitors, chemotherapy, radiopharmaceuticals, and immunotherapy (3). In the United States, approved immunotherapies for mCRPC include sipuleucel-T (Provenge®, Dendreon Pharmaceuticals LLC, Seal Beach, CA) and anti-PD-1 for the small fraction (<3%) of patients with documented microsatellite instability (4).

Sipuleucel-T is an autologous cellular immunotherapy that induces an immune response targeted against prostatic acid phosphatase (PAP) (5). It was the first FDA-approved immunotherapy for the treatment of asymptomatic or minimally symptomatic mCRPC (5). Sipuleucel-T is manufactured by isolating autologous peripheral blood mononuclear cells through leukapheresis and then culturing them ex vivo with PA2024 (a recombinant fusion protein composed of PAP linked to granulocyte-macrophage colony-stimulating factor), resulting in antigen-presenting cell (APC) activation (6). Sipuleucel-T, comprising cultured peripheral blood mononuclear cells that contain the activated APCs, is infused into the patient, with the full treatment regimen consisting of three infusions at approximately 2-week intervals (5). In the phase III IMPACT trial (NCT01133704), sipuleucel-T statistically significantly reduced the risk of death vs placebo in men with mCRPC, with a 13-month overall survival (OS) benefit among men with PSAs in the lowest quartile (<22 ng/mL) (7).

The nature of the antitumor immune response seen with sipuleucel-T treatment is multifaceted. Sipuleucel-T induces T-cell and B-cell trafficking to the tumor margin when administered before prostatectomy in patients with localized prostate cancer (8) and evokes sustained immune responses in patients with either biochemically recurrent, nonmetastatic androgen-dependent prostate cancer (9,10) or mCRPC (7,11–13). Plus, APC activation observed with sipuleucel-T treatment was much higher in earlier stages of prostate cancer (9,10). The trafficking and APC activation observations are the basis for the currently ongoing company-sponsored study ProVent (NCT03686683) in the active surveillance setting, the earliest stage of prostate cancer (Table 1).

Sipuleucel-T has undergone and continues to undergo extensive clinical evaluation (see Tables 1 and 2 for lists of completed and ongoing registered human studies, respectively). The initial indication is for use in men with asymptomatic or minimally symptomatic mCRPC. Both company-sponsored and investigator-initiated studies have evaluated or are currently evaluating sipuleucel-T in either combination with approved agents for the treatment of mCRPC (radium 223 dichloride) or experimental treatments, such as ipilimumab, atezolizumab, indoximod, IL-7, and radiation treatments (Tables 1 and 2). Other studies explored additional aspects of the mechanisms of action of sipuleucel-T, for example, the trafficking of sipuleucel-T to lymph nodes (NCT02036918, Table 2) and the relationship between circulating tumor cells and disease status (NCT02456571, Table 2).

The purpose of this review is to collate and present published data and findings that when reviewed together, reveal a coherent, cogent mechanism of action for sipuleucel-T.

Differences Between Therapeutic Vaccines and Other Therapies for Advanced Prostate Cancer

Understanding the mechanism and unique characteristics of different therapeutic options for mCRPC is critical when considering rational combinatorial approaches, akin to assessing how individual pieces will fit together to form a complete puzzle. Here, we discuss distinguishing characteristics of therapeutic vaccines compared with conventional therapies and checkpoint inhibitors.

Comparing Therapeutic Vaccines vs Conventional Systemic Anticancer Therapy

Current conventional anticancer therapies such as docetaxel have several key limitations, including causing damage to normal cells and tissues that results in long-term side effects including the ability for some tumors to develop resistance to certain treatments (14). Immunotherapy, in contrast, is an adaptive approach to cancer treatment. By harnessing the body's own immune system to target tumor cells, therapeutic vaccines may overcome some of the limitations of current conventional anticancer therapies.

Therapeutic cancer vaccines differ from conventional anticancer therapies in several distinct ways (Table 3) (15–17). First, they direct the immune system to target the cancer, rather than targeting the cancer directly. As a result, it may take weeks to months to mount a clinically significant immune response following immunotherapy (16,17,19). Yet, in contrast to the conventional options, the effect of these therapeutic vaccines can be durable because they may induce the development of long-lived antigen-specific memory cells, which may lead to the slowing of a tumor's growth by providing prolonged immunologic intervention (15–17). Another difference between these types of treatment is that the evolution of tumor genetics can result in resistance to conventional anticancer therapies, whereas the immune system can often adapt to these changes, such that therapeutic vaccines can continue to provide an antitumor response (15–17).

Comparing Therapeutic Vaccines vs Other Immunotherapies

Cancer immunotherapy can involve several different approaches to harness and direct the immune system against cancer (20). First, therapeutic vaccination primes the immune system to mount a response against tumor-associated or tumor-specific antigens (21). Second, immune checkpoint inhibition blocks negative costimulatory molecules on effector T cells, thus preventing tumor-directed suppression of antitumor effector cells (22). Third, chimeric antigen receptor (CAR)-T immunotherapy involves the binding of antigen-specific CAR-T cells to antigen-expressing tumor cells to eliminate them (23).

Immune checkpoint inhibitors target negative costimulatory molecules that are upregulated in certain forms of cancer (22). Immune checkpoint inhibitors, however, have immune-related toxicities as a result of disrupting the immunological balance between tolerance and autoimmunity. Toxicities associated with immune checkpoint inhibitors, which can limit their use, include the following: fatigue, rash and other skin disorders, gastrointestinal events, endocrinopathies, pneumonitis, colitis, hepatitis, encephalitis, and other more rare events (24).

CAR-T immunotherapy involves isolating T cells from a patient and genetically modifying the T cells to recognize a target surface antigen, therefore they are particularly complex to manufacture. The resultant autologous CAR-T cells are then infused into the patient (23). The development of this technology led to encouraging progress in difficult-to-treat cancers such as pediatric acute lymphoblastic leukemia and adult relapse or refractory non-Hodgkin lymphoma (25). CART-T immunotherapy

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Table 1. List of ongoing studies of sipuleucel-T identified in Clinicaltrials.gov*

NCT identifier (Acronym)	Study title	Outcome measures	Sponsor
NCT03686683 (ProVent)	Open-Label Trial of Sipuleucel-T Administered to Active Surveillance Patients for Newly Diagnosed Prostate Cancer	• To assess the efficacy of sipuleucel-T in reducing histopathologic reclassification to a higher Gleason grade in prostate cancer subjects on active surveillance	Dendreon
NCT02463799 (J1522 IRB00056435)	Study of Sipuleucel-T w/ or w/o Radium-223 in Men With Asymptomatic or Minimally Symptomatic Bone-MCRPC	• Immune responses to treatment with sipuleucel-T (with or without radium-223) measured by peripheral PA2024 T-cell proliferation • To evaluate peripheral antigen-specific T-cell proliferation over time • To evaluate peripheral antigen-specific T-cell activation to sipuleucel-T over time • To evaluate antigen-specific antibody response to sipuleucel-T over time • To evaluate sipuleucel-T-induced antigen spread (epitope spread) phenomena • To evaluate the sipuleucel-T product immune parameters • To investigate safety of combined use of radium-223 and sipuleucel-T (composite measure of both arms) • To evaluate time to prostate-specific antigen (PSA) progression • To evaluate time to alkaline phosphatase progression • To evaluate time to pain progression and first cancer-related opioid use • To evaluate time to radiographic or clinical progression • To evaluate time to first skeletal related event • To evaluate time to first chemotherapy use	Investigator initiated
NCT01818986 (STU 102012-026)	Sipuleucel-T and Stereotactic Ablative Body Radiation (SABR) for Metastatic Castrate-resistant Prostate Cancer (mCRPC)	• Time to progression • Immune response	Investigator initiated
NCT01804465 (12557 NCI-2014-00318)	Sipuleucel-T With Immediate vs. Delayed Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) Blockade for Prostate Cancer	• Frequency of observing an immune response to prostatic acid phosphatase and/or PA2024 • Frequency of highest grade toxicity • PSA response rate • Time to PSA progression • Radiographic clinical response rate	Investigator initiated
NCT03329742 (IUSCC-0614170608 1520)	Sipuleucel-T and Low-protein Diet in Patients With Metastatic Castrate-resistant Prostate Cancer	• Adherence to diet intervention • Feasibility of diet intervention • Safety and tolerability of diet intervention combined with sipuleucel-T treatment • Rate of immune response • Progression-free survival • Overall survival	Investigator initiated

Table 1. (continued)

NCT identifier (Acronym)	Study title	Outcome measures	Sponsor
NCT01833208 (I 223912 NCI-2013-00633 P30CA016056)	Radiation Therapy in Treating Patients With Metastatic Hormone-Resistant Prostate Cancer Receiving Sipuleucel-T	• Capacity of T cells to proliferate in response to antigen stimulation, assessed with a tritiated thymidine incorporation assay and an interferon-gamma enzyme-linked immunosorbent spot assay • Change in antigen-specific humoral response measured via enzyme-linked immunosorbent assay • Change in the genetics of immune effectors, measured with ribonucleic acid from monocytic and lymphocytic cells • Quantification of lymphocyte subsets and natural killer cells • Adverse event rates assessed using National Cancer Institute Common Terminology Criteria for Adverse Events version 4 • Cancer-specific survival • Change in PSA • Overall survival	Investigator initiated
NCT01706458 (CO11816 A534260 SMPH/MEDICINE/MEDICINE*H NCI-2012-02026 2012-0352)	Provenge With or Without pTVG-HP DNA Booster Vaccine in Prostate Cancer	• Number of participants with immune response following treatment • Progression-free survival • Time to radiographic disease progression • Measure PSA doubling time	Investigator initiated

*Source: clinicaltrials.gov.

treatments have clinically significant adverse event profiles, such as cytokine-release syndrome and neurotoxicities (25), and have yet to be proven to be safe or effective in solid tumors.

Newer treatments such as bispecific and multispecific antibodies that have multiple targets are being developed to target cancer (26). Eventually, these may offer additional benefits to patients given how they target disease and how they are manufactured.

The Clinical Impact of Sipuleucel-T

Sipuleucel-T acts as a therapeutic cancer vaccine. The phase III IMPACT study showed that sipuleucel-T treatment of men with mCRPC was associated with a statistically significant relative risk reduction in death of 22% (P = .03), with median OS being 4.1 months longer in the sipuleucel-T group compared with placebo (25.8 vs 21.7 months, respectively) (7). Most adverse events associated with sipuleucel-T treatment are low grade, with only 6.8% of patients experiencing grade 3 adverse events with sipuleucel-T (7). The most common adverse events within 1 day of sipuleucel-T infusion were chills (51.2%), fever (22.5%), fatigue (16.0%), nausea (14.2%), and headache (10.7%) (7). When considering use of sipuleucel-T, additional aspects of the clinical profile of sipuleucel-T should be considered: 1) delayed therapeutic effect, 2) greater benefit when employed in early vs more advanced mCRPC, and 3) enhanced OS observed for African Americans vs white men treated with sipuleucel-T, possibly related to different disease characteristics in the two populations. The latter observation is observed despite the low enrollment of African Americans in the clinical trial (27,28).

Delayed Effects With Treatment

The delayed treatment effect of sipuleucel-T on clinical outcomes is now recognized as an aspect known to be associated with certain immunotherapies (15,29,30). This delayed effect may explain why proximal endpoints such as PSA levels, objective disease progression, and onset of disease-related pain were not altered in IMPACT. Rather, there was an improvement in distal endpoints, including a statistically significant prolongation of the time-to-first use of opioids (31). In a pooled analysis of three phase III studies, although time-to-disease–related pain was not statistically significantly different between groups, 39% of sipuleucel-T-treated patients compared with 19% of those on placebo were pain-free at 12 months (31). Time-to-first opioid analgesic use was 12.6 months in the sipuleucel-T arm compared with 9.7 months in the placebo arm (hazard ratio [HR] = 0.755, 95% confidence interval [CI] = 0.579 to 0.985, P = .038) (31). These findings are important because pain often becomes a dominant symptom in advancing disease and has a statistically and clinically significant impact on patients' ability to continue to carry out their daily activities (32–34). Interestingly, the Kaplan-Meier curves for time-to-disease–related pain and time-to-first opioid analgesic use with sipuleucel-T vs placebo diverge after approximately 6 months, consistent with a delayed effect of immunotherapy (31). These changes in late-occurring outcomes reflect the time taken for sipuleucel-T immunotherapy to begin to impact the disease.

Impact of Earlier Use of Sipuleucel-T

Another feature of sipuleucel-T as an immunotherapy is the potential for greater impact when used earlier in the disease

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Table 2. List of completed studies of sipuleucel-T Identified in Clinicaltrials.gov*

NCT identifier (Acronyms)	Study title	Sponsor
NCT01727154 (PRIME)	Immune Monitoring Protocol in Men With Prostate Cancer Enrolled in a Clinical Trial of Sipuleucel-T	Dendreon
NCT01477749	Sipuleucel-T Manufacturing Demonstration Study	Dendreon
NCT01306890 (PROCEED)	A Registry of Sipuleucel-T Therapy in Men With Advanced Prostate Cancer	Dendreon
NCT00901342	Open Label Study of Sipuleucel-T in Metastatic Prostate Cancer	Dendreon
NCT01338012	Sipuleucel-T in Metastatic Castrate Resistant Prostate Cancer	Dendreon
NCT01981122	A Study of Sipuleucel-T With Administration of Enzalutamide in Men With Metastatic Castrate-Resistant Prostate Cancer	Dendreon
NCT01431391	Sequencing of Sipuleucel-T and ADT in Men With Non-metastatic Prostate Cancer	Dendreon
NCT00779402 (PROTECT)	Provenge Treatment and Early Cancer Treatment	Dendreon
NCT01487863	Concurrent vs. Sequential Sipuleucel-T & Abiraterone Treatment in Men With Metastatic Castrate Resistant Prostate Cancer	Dendreon
NCT00715078	To Evaluate Sipuleucel-T Manufactured With Different Concentrations of (PA2024) Antigen	Dendreon
NCT01133704	Immunotherapy With APC8015 (Sipuleucel-T, Provenge) for Asymptomatic, Metastatic, Hormone-Refractory Prostate Cancer	Dendreon
NCT00065442	Provenge (Sipuleucel-T) Active Cellular Immunotherapy Treatment of Metastatic Prostate Cancer After Failing Hormone Therapy	Dendreon
NCT00849290	Immunotherapy for Men With Objective Disease Progression on Protocol D9902 Part B (NCT00065442)	Dendreon
NCT00027599	APC8015 and Bevacizumab in Treating Patients With Prostate Cancer	Dendreon
NCT00005947	Vaccine Therapy in Treating Patients With Metastatic Prostate Cancer That Has Not Responded to Hormone Therapy	Dendreon
NCT00715104 (NeoACT)	Sipuleucel-T as Neoadjuvant Treatment in Prostate Cancer	Dendreon
NCT02729103	Treatment Patterns in Metastatic Prostate Cancer	Investigator initiated
NCT01560923 (2011LS109)	Phase II Study of Sipuleucel-T and Indoximod for Patients With Refractory Metastatic Prostate Cancer	Investigator initiated
NCT01832870 (SIPIPI 2013)	Sipuleucel-T and Ipilimumab for Advanced Prostate Cancer	Investigator initiated
NCT02237170 (GCO 11-1689)	Immune Monitoring on Sipuleucel-T	Investigator initiated
NCT02353715 (Pro00058229 PEAX)	Men With Metastatic Castrate-Resistant Prostate Cancer Treated With Either Sipuleucel-T (Provenge), Abiraterone Acetate (Zytiga) or Enzalutamide (Xtandi) Undergoing Cardiopulmonary Exercise Testing	Investigator initiated
NCT01420965 (11C0231)	Sipuleucel-T, CT-011, and Cyclophosphamide for Advanced Prostate Cancer	Investigator initiated
NCT02793219 (GU-15-103 HSC-MS-15-0882)	Provenge Followed by Docetaxel in Castration-Resistant Prostate Cancer	Investigator initiated
NCT02793765 (GU-15-104 HSC-MS-15-0883)	Docetaxel Followed by Provenge in Metastatic Prostate Cancer	Investigator initiated
NCT02036918 (Pro00047231)	Dendreon Lymph Node Biopsy in Metastatic Castrate-Resistant Prostate Cancer	Investigator initiated
NCT01174368	Efficacy Trial of the Implantation of Mouse Renal Adenocarcinoma Macrobeads in Subjects With Castration-Resistant Prostate Cancer Resistant to Taxanes (Docetaxel, Cabazitaxel) and Evidence of Disease Progression on Androgen-axis Inhibition and/or Immunotherapy in the Form of Sipuleucel-T	Investigator initiated
NCT01274572 (MC 10-11)	Blood for Immune Response to Provenge in HRPC	Investigator initiated
NCT03024216 (Rosser-2015-4)	Clinical Study of Atezolizumab (Anti-PD-L1) and Sipuleucel-T in Patients With Asymptomatic or Minimally Symptomatic Metastatic Castrate Resistant Prostate Cancer	Investigator initiated
NCT02232230 (21C-2013-02)	A Multicenter Trial Enrolling Men With Advanced Prostate Cancer Who Are to Receive Combination Radiation and Sipuleucel-T	Investigator initiated
NCT02042053 (NYU S12-03902)	PET/MR Assessment of Sipuleucel T Treatment for Metastatic Castration Resistant Prostate Cancer	Investigator initiated
NCT02456571 (Pro00063296)	CTC Immune Checkpoint	Investigator initiated
NCT01807065 (12367 NCI-2013-00542)	Sipuleucel-T With or Without Radiation Therapy in Treating Patients With Hormone-Resistant Metastatic Prostate Cancer	Investigator initiated
NCT01881867 (CITN12-03 NCI-2013-00998 CITN12-03 IL7 P30CA015704 P50CA097186 U01CA154967)	CYT107 After Vaccine Treatment (Provenge) in Patients With Metastatic Castration-Resistant Prostate Cancer	Investigator initiated
NCT02159950 (I 250813 NCI-2014-01184 P30CA016056)	Sipuleucel-T With or Without Tasquinimod in Treating Patients With Metastatic Hormone-Resistant Prostate Cancer	Investigator initiated

*Source: clinicaltrials.gov.

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Table 3. Differences between systemic anticancer therapy and therapeutic vaccines

Category	Conventional therapies	Therapeutic vaccines
Site of action	Specific targets at tumor or its microenvironment	Immune system
Pharmacodynamics	Action often immediate	Delayed onset of therapeutic action
Involves immunologic memory response	No	Yes
Tumor response to treatment (evolution and/or mutations)	Develops resistance to therapy	Develops new immunogenic targets
Limitation related to activity	Toxicity, including damage to healthy cells*	Requires adequate immune function systemically and at tumor site to act and react

*Many systemic chemotherapies target pathways involved in multiple biologic processes. Based on information described in multiple sources (15–18).

course, when immunosuppressive pressures may be less. Further analysis of the IMPACT trial has shown that baseline prostate-specific antigen (PSA) is a strong predictor of treatment effect with sipuleucel-T ($P < .0001$). The estimated improvement in median OS ranged from 13.0 months in the lowest baseline PSA quartile (ie, PSA <22 ng/mL) to 2.8 months in the highest quartile. The estimated 3-year survival in the lowest PSA quartile was 62% for patients treated with sipuleucel-T and 42% with placebo, a 50% relative increase with sipuleucel-T (35). PSA is an indicator of disease volume (36); therefore, patients with a lower PSA level are likely to be earlier in their disease process, potentially giving them more time to benefit from sipuleucel-T.

Differential Benefit by Race

Interestingly, sipuleucel-T appears to impart greater OS benefit in African American men than in white men with mCRPC (27,28,37). A pooled analysis of phase III studies reported a median OS of 45.3 months in the African American population as compared with 24.7 months in matched (using Halabi-predicted survival) white patients ($P = .02$; both groups receiving sipuleucel-T) (28). This advantage extended to a comparison with control patients, who had a 14.6-month OS ($P = .003$) (27). In an independent dataset from the PROCEED registry (NCT01306890), median OS was greater in African Americans as compared with whites (35.2 and 29.9 months, respectively; HR = 0.81, 95% CI = 0.68 to 0.97, $P = .03$) (37). Because baseline PSA, an important predictor of OS for sipuleucel-T (35), was statistically significantly higher in African American patients in PROCEED, a case-matched analysis was undertaken. In PSA-matched cohorts, median OS was 35.3 and 25.8 months in African American and white men, respectively (HR = 0.70, 95% CI = 0.57 to 0.86, $P < .001$) (37). Race was found to be an independent predictor of OS after sipuleucel-T on multivariable and sensitivity analyses (37).

These differences in response in these two populations may be explained, at least in part, by observed differences in their immune systems. There are known differences in the relative proportions of various immune cells between African American men and white men (38–40), even in men with prostate cancer (41). Expression profiling of white blood cells revealed biologically significant differences in the levels of transcripts relevant to immune cell function in African Americans compared with other races (42), and racial differences have been observed in B-cell and T-cell signaling (43,44). Furthermore, gene expression analyses found differences in immune response pathways in

the aggressive prostate cancer experienced by African Americans (45–47). Additional research on the association between immune responses and OS outcomes after sipuleucel-T is needed to explain these differences and to provide insights aimed at improving future treatments.

Pieces of the Sipuleucel-T Puzzle

The antitumor immune response to sipuleucel-T is multifaceted with data showing that sipuleucel-T induces sustained peripheral T-cell and B-cell responses to target antigens PAP and PA2024 (7,9–13), resulting in downstream responses to secondary, nontarget antigens. When administered before prostatectomy in localized prostate cancer, sipuleucel-T causes T cells and B cells to traffic to the tumor margin (8). Furthermore, the cytotoxic potential of activated T cells has also been documented (48) and demonstrated via video microscopy (49).

Explaining the clinical impact of immunotherapy, including sipuleucel-T, requires an understanding of the effect of anticancer immunotherapy on the peripheral immune system and the tumor microenvironment. Certain cancers, such as melanoma, bladder cancer, and non–small cell lung cancer, have an inflamed microenvironment with notable T-cell infiltration, increased expression of PD-L1, and high tumor mutation burden (50). These tumors are particularly suited to treatment with immune checkpoint inhibitors. In these cancers, the PD-1/PD-L1 pathway is an important regulator of T-cell activity and may contribute to tumor development and progression (51). In addition, tumor-infiltrating lymphocytes secrete inflammatory cytokines, particularly interferon-gamma (IFN-γ), that trigger tumor cells to express PD-1, which binds to PD-1 on T cells to inhibit antitumor T-cell responses (52,53). Patients may benefit from being tested for PD-L1 expression before being treated with PD-L1–blocking antibody therapeutics (54), although current assays lack sufficient sensitivity and specificity to accurately identify treatment responders and nonresponders (55).

In contrast, prostate cancer is generally not T-cell "inflamed" and is, therefore, associated with limited response to single-agent immune checkpoint inhibition (56,57). In defining tumor types based on their immunity profile, prostate cancer is defined as having an immune-desert phenotype (58). This description is characterized by a paucity of CD8+ T cells. These findings may indicate an absence of preexisting antitumor immunity, suggesting that the presence of tumor-specific T cells is the rate-limiting step if using checkpoint inhibitors. This concept is supported by recent studies demonstrating that

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Table 4. Cumulative APC activation in phase II and III studies with sipuleucel-T in patients with mCRPC

Study	Design	Treatments	No.*	Median cumulative APC activation†
IMPACT‡, D9901§, D9902A¶ (11)	Phase III, multicenter, randomized, double-blind trials	Sipuleucel-T placebo	476	26.7
STAMP# (31)	Phase II, randomized, open-label trial	Sipuleucel-T + concurrent AA + P	35	33.65
		Sipuleucel-T + sequential AA + P**	34	38.24

*Sipuleucel-T recipients in APC activation analysis. AA = abiraterone acetate; APC = antigen-presenting cell; P = prednisone.
†APC activation is the increase in surface CD54 expression on APCs expressed as an upregulation ratio of average number of molecules on postculture vs preculture cells. Cumulative APC activation is the sum of APC activation after all three sipuleucel-T injections.
‡NCT00065442.
§NCT00005947.
¶NCT01133704.
#NCT01487863.
**Started 10 weeks after the first infusion of sipuleucel-T.

vaccination (targeting the same PAP antigen as sipuleucel-T), in combination with PD-1 blockade, elicited PSA declines and objective responses in patients with advanced prostate cancer and an infiltration of tumors with CD8+ T cells (59). In contrast, studies conducted with nivolumab (PD-1 inhibitor) (56,60), ipilimumab (cytotoxic T lymphocytes [CTLA] 4 inhibitor) (56,61), and pembrolizumab (62) as monotherapies in patients with prostate cancer demonstrated limited clinical activity.

Defining Immune Responses to Sipuleucel-T Piece by Piece

The immune responses to sipuleucel-T are multifactorial. First, there is a statistically significant increase in APC activation. Second, peripheral cellular and humoral immune responses specific to PAP and PA2024 are then induced. Third, this is followed by stimulation of local and systemic cytotoxic T-lymphocyte activity. Fourth, the immune system mounts a secondary response to additional antigens expressed by the tumor, the so-called phenomenon of antigen spread. Last, this secondary response yields increasing cytotoxic T-cell activity.

Antigen-Presenting Cell Activation

APC activation is a measure of product potency and immune activation, and increasing cumulative APC activation (across the three doses of sipuleucel-T) is statistically significantly correlated with improved OS in mCRPC (11). In three phase III, randomized, double-blind, multicenter trials of sipuleucel-T in men with mCRPC (including IMPACT), sipuleucel-T statistically significantly increased APC activation as compared with placebo, with a median cumulative APC activation of 26.7 (Table 4) (11). Increases in APC activation were seen with each subsequent infusion of sipuleucel-T, indicating that the first infusion primes the immune system and subsequent infusions boost the response in a classical vaccine-mediated memory response.

Additionally, there are data supporting an anamnestic APC activation with sipuleucel-T; these data come from the phase II study P10-1 (NCT01338012, PROTECT II) (63). Here, nine patients with castration-sensitive prostate cancer, initially treated with three infusions of sipuleucel-T or placebo in the phase III trial PROTECT (NCT00779402), received a booster infusion (9) after progressing to mCRPC. These patients were retreated after a

median of 9.5 years after the end of PROTECT. Activation of APCs was higher in those patients who were retreated with sipuleucel-T than in treatment-naïve patients, indicating that memory T cells were interacting with APCs because the latter have no memory (63). Accordingly, cumulative APC activation (CD54 upregulation), which was correlated with OS in the IMPACT study (11), was seen to be much higher in P10-1 than in IMPACT (63).

The phase II STAND (NCT01431391) study in men with biochemically recurrent prostate cancer following prostatectomy and/or radiotherapy found no difference in APC activation when sipuleucel-T was administered before or after androgen-deprivation therapy; however, APC activation in general was higher than that observed in IMPACT (10). Interestingly, cumulative APC activation was approximately 37% higher in STAND vs IMPACT (ie, when sipuleucel-T was administered to patients with less-advanced disease) (7,10). This difference may be a consequence of the STAND patient population being younger and therefore having a more robust immune system or having an earlier prostate cancer disease stage with less tumor burden and associated immune suppression than patients in IMPACT. As described above, sipuleucel-T APC activation was shown to correlate with OS in the IMPACT study (Figure 1) (11).

Peripheral Cellular and Humoral Immune Responses

Sipuleucel-T induces peripheral immune responses specific to PAP, the target antigen, and PA2024, the immunizing antigen. These responses are measured by assessing a range of cellular and humoral immune parameters such as T-cell proliferation and the IFN-γ enzyme-linked immunosorbent spot for cellular immune responses and levels of antibodies for humoral responses. In support of the mode of action of sipuleucel-T, peripheral immune responses to PA2024 and/or PAP also correlate with OS (11).

Data from clinical trials with sipuleucel-T also show that most subjects develop peripheral antigen-specific immune responses. For example, in IMPACT, sipuleucel-T induced peripheral immune responses (either T cell or humoral) to PA2024 and/or PAP in 79% of treated patients compared with 13% of patients in the control group (11). Similar peripheral immune responses were reported in a number of phase II trials of sipuleucel-T (10,12,13). Moreover, the P10-1 study, in which patients received a second course of sipuleucel-T almost
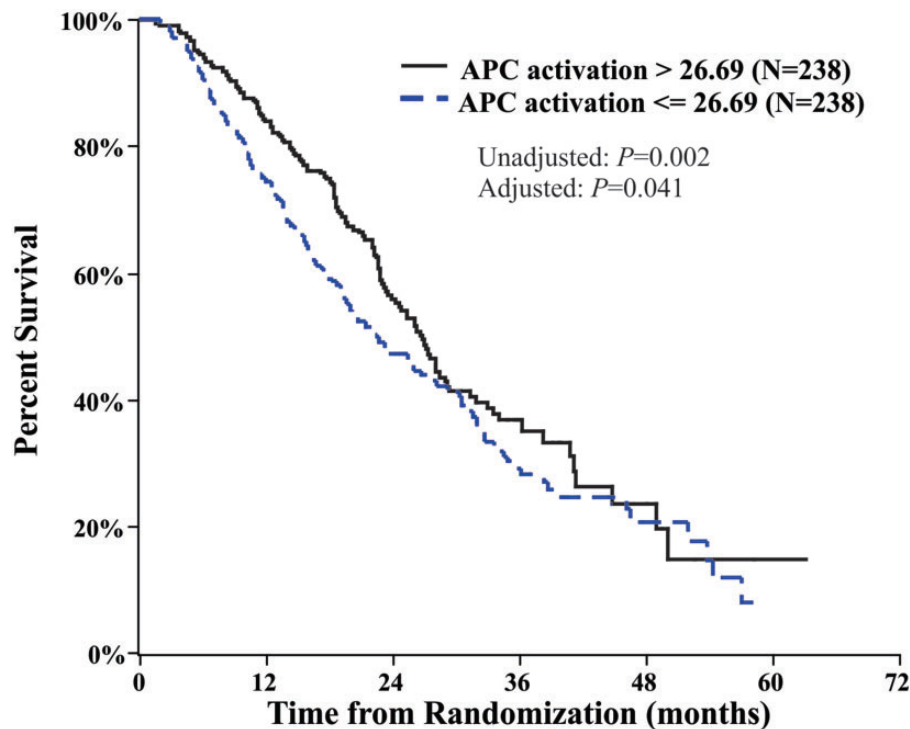
Figure 1. Survival in men with mCRPC who received sipuleucel-T in the IMPACT study, stratified by above and below the median cumulative APC activation after treatment. Cumulative APC activation value (hazard ratio = 0.76, 95% confidence interval = 0.58 to 0.99). This figure comes from figure 5, panel A of Sheikh et al. (11), which was distributed under the terms of the Creative Commons Attribution License that permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited. APC = antigen presenting cell; mCRPC = metastatic castration-resistant prostate cancer.

10 years after their initial treatment, showed that peripheral PA2024 and PAP cellular and humoral responses were present before retreatment and boosted after the first infusion of sipuleucel-T (63).

A study in which patients with mCRPC receiving sipuleucel-T underwent lymph node biopsies to determine the magnitude of sipuleucel-T–induced leukocyte activation in tumor-affected lymph nodes (NCT02036918) was recently completed. A secondary aim of this study was to examine the relationship between the lymph node immune response to sipuleucel-T and the peripheral immune response. Results are forthcoming.

Antigen Spread

There is growing evidence that part of the mechanism of action of sipuleucel-T involves antigen spread (ie, the broadening of the immune response to additional antigens expressed by the tumor) after the initial immune response to the specific, target antigen (15,64). In this process, tumor cells targeted by antigen-specific T cells are lysed, releasing a range of tumor-specific, secondary antigens that APCs present back to the mobilized immune system leading to further action.

An analysis of data from IMPACT and ProACT (NCT00715078), a randomized, single-blind trial of sipuleucel-T in patients with advanced mCRPC, showed that sipuleucel-T induces antibody responses to secondary antigens (65). These antigens include PSA, KLK2, LGALS3, and LGALS8 (65), which have been shown to be expressed at elevated levels in prostate cancer and/or to play a role in prostate cancer development. Additionally, responses were also observed to K-RAS and E-RAS, which have functional relevance in cancer (66–72). Antigen

spread was observed beginning 2 weeks after sipuleucel-T treatment and persisted for at least 6 months, the last immune monitoring time point in the IMPACT study (65).

Most patients who exhibited a response to secondary antigens also had an IgG response to PAP (65). In addition, there was considerable overlap between responses to secondary antigens, with fewer than 30% of patients having a response to only a single secondary antigen. For example, 9% of patients treated with sipuleucel-T had an IgG response to PSA with E-RAS, LGALS8, and LGALS3, and 25% had a response to at least three of these four antigens (65). An antibody response to PSA or LGALS3 was associated with improved survival in patients receiving sipuleucel-T as compared with placebo in IMPACT (P ≤ .05). The extent of antigen spread was also associated with improved OS. These data suggest that antigen spread has a role in the mechanism of action by which sipuleucel-T exerts its survival benefit (65).

Results from two phase II studies have also shown antigen spread with sipuleucel-T. In mCRPC patients receiving concurrent vs sequential abiraterone with sipuleucel-T in STAMP and STRIDE (NCT01487863 and NCT01981122), IgG levels to all secondary antigens were statistically significantly increased from baseline in both arms at weeks 6, 10, and 14 (P < .01) (31). Importantly, antigen spread was considered to be a response to sipuleucel-T treatment and not to abiraterone, because there were no differences in antigen spread between the concurrent and sequential arms in STAMP (31). In the trial STAND that enrolled patients with biochemically recurrent prostate cancer, sipuleucel-T treatment resulted in IgG responses to the secondary antigens E-RAS, KLK2, K-RAS, LGALS3, and LGALS8, which were similar between the study arms (ie, sipuleucel-T then androgen-deprivation therapy and androgen-deprivation

therapy followed by sipuleucel-T (10). Moreover, in STAND, in patients with an early disease state, the magnitude of antigen spread to each antigen at week 2 was statistically significantly higher (P < .01) than in mCRPC patients in the IMPACT and STAMP trials, as was the number of subjects who exhibited antigen spread (10).

T-Cell Trafficking to the Prostate and Cytotoxic T-Cell Activity

The NeoACT (NCT00715104) study of patients with untreated, localized prostate cancer examined the effect of neoadjuvant sipuleucel-T prior to radical prostatectomy (8). Here, immune infiltrates in tumor specimens removed during surgery were compared with pretreatment biopsy specimens. These analyses showed recruitment of activated effector T cells into the prostate tumor microenvironment concurrent with a systemic antigen-specific T-cell response to sipuleucel-T (8). Postoperative tissue specimens showed a threefold or greater increase in CD3+, CD4+ (helper T cell), and CD8+ T cells (CTLs infiltration at the interface between the tumor and healthy tissue compared with biopsy specimens taken before sipuleucel-T treatment (binomial proportions: all Ps < .001) (8).

Next-generation sequencing of peripheral blood and prostate tissue specimens from NeoACT showed that after infusion of sipuleucel-T, peripheral blood T cells become less diverse (73). In addition, the T-cell clones observed in the prostate tissue had greater commonality with peripheral blood clones after the first infusion, implying that the priming infusion of sipuleucel-T programs peripheral T cells to traffic to the prostate tissue. Compared with prostate cancer patients who had not received sipuleucel-T, T cells in prostate cancer tissue from sipuleucel-T–treated patients had greater diversity, suggesting that specific T-cell clones are recruited from the peripheral blood to the prostate tumor microenvironment, which could enhance immunological containment of the tumor.

CTL Activity

One of the ways in which cancer cells evade immune destruction is by suppressing the natural role of CTLs (74). There is evidence that sipuleucel-T is able to generate antigen-specific CTLs (as measured by cell surface CD107a) in patients with prostate cancer (48,49) with greater CTL activity correlating to improved OS (48).

An analysis of T-cell activity in the STAMP and STRIDE trials demonstrated that sipuleucel-T induces a marked increase in the proliferation of both PA2024-specific CD4+ and CD8+ T cells (48). Because CD4 helper T cells facilitate the differentiation and expansion of CTLs, their induction is an essential part of the immune response to sipuleucel-T. Most important, these analyses demonstrated a statistically significant correlation between OS and PAP- and PA2024-specific CTL responses at month 6 (P = .0134 and P = .0006, respectively).

Furthermore, the cytolytic activity of CTLs against PAP-expressing target cells was recently documented by confocal microscopy, providing visual verification of CTL action after sipuleucel-T treatment at week 6 and month 6 (49). Taken together, these data indicate that the induction of tumor lysis via antigen-specific CD8+ cells is an important component of the mechanism of action of sipuleucel-T (48).

Completing the Mechanism of Action Jigsaw

Combination Therapies

Previous research has shown that antiandrogen therapy has an effect on local and systemic immune responses in prostate cancer (75), making immunotherapies an attractive option for patients who develop resistance to androgen-directed therapy. For prostate cancer treatment, immune checkpoint inhibitors may have a role in combination with agents that enhance the recruitment of effector T cells to the prostate cancer microenvironment (57). For example, PD-L1 expression is increased in APCs and T cells in patients with enzalutamide-resistant, castration-resistant prostate cancer, suggesting that anti-PD-L1 therapy may be a logical next step when a patient develops enzalutamide resistance (76). Therefore, a key focus in immunotherapy for prostate cancer is how best to sequence or combine immunotherapies to enhance the immune response as the disease progresses and the immunogenic environment changes (59,77).

Combination With Agents Targeting Negative Costimulatory Molecules

The NeoACT studies showed that, following sipuleucel-T treatment, both CD3+ T cells recruited to the tumor (8) and T cells isolated from peripheral blood (78) expressed PD-1. Moreover, CTLA-4 (CD152) expression on T-cells was observed before and after sipuleucel-T infusion in both NeoACT and ProACT subjects (Dendreon, data on file); however, subjects in ProACT exhibited statistically higher levels of CTLA-4 prior to sipuleucel-T treatment. It was also found that PD-L1 expression increases on prostate-circulating tumor cells following sipuleucel-T treatment (79). Collectively, these data provide a biologic rationale for studying sipuleucel-T in combination with agents targeting the negative costimulatory molecules. Studies are currently underway to assess the combination of sipuleucel-T with other immunotherapies, such as the PD-L1 inhibitor atezolizumab (NCT03024216) and the CTLA-4 inhibitor ipilimumab (NCT01804465). A preliminary, phase I study (NCT01832870) of sipuleucel-T and ipilimumab (n= 9) indicated the potential synergistic effects of this combination, with higher levels of PAP- and PA2024-specific antibody titers after the combination than would be expected with sipuleucel-T alone (80). Of the nine surviving men, six have had a follow-up of more than 50 months and received a range of subsequent therapies (81).

Combination With Radiation

Sipuleucel-T is also being studied in combination with stereotactic ablative body radiation (NCT01818986) and the addition of radium-223 (NCT02463799). These latter studies are being conducted because inflammation and immunomodulatory cytokines are increased following radiation therapy, and radiation-induced cell death releases antigens that may be targeted through antigen spread, following activation of the immune system with sipuleucel-T (82).

Combination With Agents Stimulating Tumor Inflammation

Studies are also underway to assess combinations of immunotherapies and potential stimulants of tumor inflammation. One
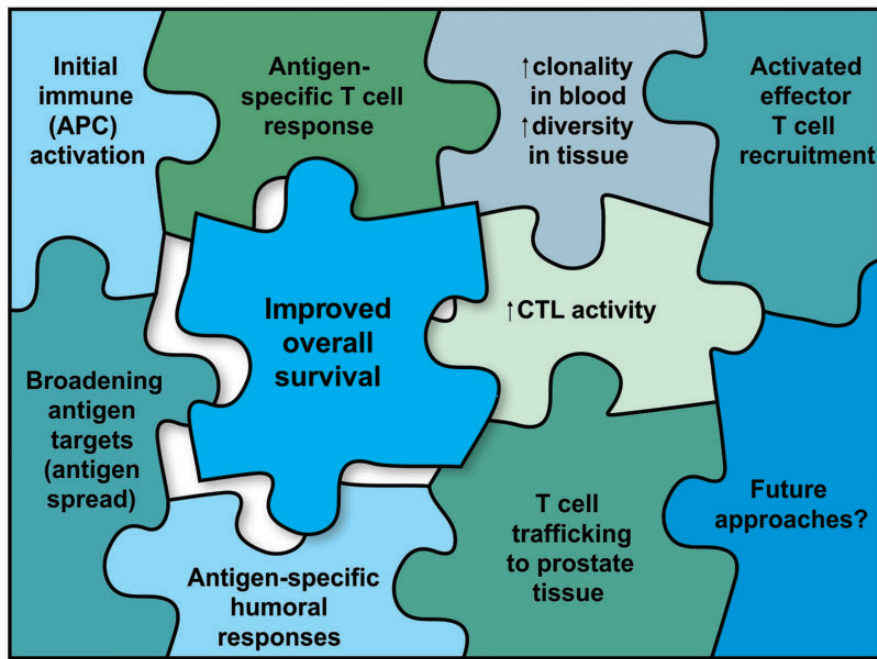
Figure 2. The jigsaw puzzle of the mechanism of action of sipuleucel-T. APC = antigen presenting cell; CTL = cytotoxic T lymphocytes.

such study is KEYNOTE-365, a nonrandomized phase Ib/II study that assessed different treatment combinations in patients with mCRPC (NCT02861573); preliminary data have been presented (83–89). Final results are forthcoming.

Discussion

Sipuleucel-T has been and continues to be explored as part of various treatment regimens for prostate cancer (Tables 1 and 2). It has been shown that sipuleucel-T drives T cells to the tumor periphery in the prostate (8), which results in APC activation that is higher in earlier stages of prostate cancer (10). Additional research suggests a role for antigen spread in its actions. Although the role of the tumor microenvironment in the antitumor immune response in prostate cancer has not been fully elucidated, insights into the immunogenic mechanisms are rapidly evolving, including the identification of new treatment targets. Recent data published by Gao and colleagues (90) indicate that V-type immunoglobulin domain-containing suppressor of T-cell activation may be an important immune-regulatory mechanism in prostate cancer. Thus, increasing knowledge of the tumor microenvironment, as well as developing combination treatment options, should improve prostate cancer management. A deeper understanding of the tumor microenvironment and immune-relevant changes (cells, proteins, and transcription) in response to immunotherapy will inform effective combination of therapies to improve patient outcomes.

Sipuleucel-T prolongs OS in men with mCRPC, with most adverse events being consistent with infusion-related events and mild to moderate in severity. As to the mechanism of action of sipuleucel-T, enough pieces of the jigsaw puzzle are in place to obtain a clear picture (Figure 2), with a wealth of data indicating a mechanism similar to that of a vaccine. Sipuleucel-T, through APC activation, generates strong and persistent antigen-specific humoral and T-cell responses, as well as T-cell trafficking to tumor tissues, stimulation of local and systemic CTL activity, and antigen spread. All of these effects may be the key to turning a cold, immune-desert tumor into a hot, immune-enriched one, thus facilitating an immunological cascade against the tumor. The activation and spread of immune responses following treatment with sipuleucel-T, along with its persistent efficacy and safety profile, raise the prospect of prolonged anticancer effects that can be boosted and may be potentiated by appropriate combination therapy.

Notes

Conflicts of interest: Dr Madan has nothing to disclose. Dr Antonarakis reports grants and personal fees from Janssen, Sanofi, Dendreon, AstraZeneca, Clovis, and Merck; personal fees from Astellas, Medivation, Eli Lilly and Co, Amgen, Qiagen, and

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REVIEW