Chemotherapy and Immunotherapy Combination in Advanced Prostate Cancer

Susan Slovin, MD, PhD

Dr. Slovin is a medical oncologist at the Sidney Kimmel Center for Prostate and Urologic Cancers of Memorial Sloan-Kettering Cancer Center in New York, New York.

Address correspondence to:
Susan Slovin, MD, PhD
Genitourinary Oncology Service
Sidney Kimmel Center for Prostate and Urologic Cancers
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY 10065
Phone: 646-422-4470
Fax: 212-988-0701
E-mail: slovins@MSKCC.ORG

Abstract: In prostate cancer, there is considerable evidence that tumors promote immune tolerance starting early in the disease. By suppressing tumors and activating immune system homeostatic mechanisms, chemotherapy may help overcome this tumor-induced immune tolerance. As such, chemotherapy may therefore support improved results from novel immune-modulating therapies. Prostate cancer is particularly suited for active immunotherapy because prostate tumor cells express a number of distinctive surface antigens. Sipuleucel-T, which has recently been approved in the United States, is an active immunotherapy that triggers T-cell responses against prostate cancer. An exploratory analysis of phase III trial participants found a substantial survival benefit to receiving docetaxel some months after sipuleucel-T. However, VITAL-2, a phase III trial investigating a prostate cancer therapeutic vaccine plus concurrent docetaxel versus standard docetaxel therapy in advanced prostate cancer, observed lower overall survival with the vaccine regimen. This trial highlights major unresolved questions concerning the optimum choice, dosing, and timing of chemotherapy relative to active immunotherapy. Patient characteristics, prostate cancer disease stage, and treatment history also may influence the response to combined therapy. Advances in biomarker validation and trial design are needed to efficiently investigate these issues.

Keywords
Castrate-resistant prostate cancer, active immunotherapy, chemotherapy, cancer vaccines, clinical trials, biomarkers

Introduction

Castrate-resistant prostate cancer (CRPC) represents a spectrum of diseases on which prostate cancer progresses despite low levels of testosterone induced by chemical or surgical castration.[1] Such patients are sometimes referred to as "hormone refractory," although secondary and tertiary antiandrogen therapy may still have a beneficial effect.

Although docetaxel remains the first-line standard of care for metastatic CRPC due to its demonstrated survival benefit when compared with mitoxantrone,[2] both cabazitaxel (Jevtana, Sanofi-Aventis,

another taxane chemotherapy) and abiraterone (Zytiga, Janssen Biotech, an inhibitor of CYP-17, thereby blocking androgen synthesis by the adrenal glands, testes, and prostate tumors) recently received approval from the US Food and Drug Administration as second-line CRPC therapies in patients who have already received docetaxel. These agents each offer about a 4-month survival benefit. Another new entry in the CRPC therapy arena is the active immunotherapy sipuleucel-T (Provenge, Dendreon).

Immunotherapy is particularly applicable to prostate cancer because prostate cells commonly express a variety of altered self antigens on their surface; these are either overexpressed or underglycosylated. These tumor antigens include prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), prostatic stem cell antigen (PSCA), the glycoprotein mucin 1 (MUC1), and the gangliosides GM2 and Globo H. As will be discussed below, the response to cancer immunotherapies may not be immediately apparent. In addition to the use of serum PSA to monitor prostate cancer activity progression, other biomarkers are being used to assess treatment response in metastatic CRPC, including markers of bone turnover and, currently under evaluation in phase III trials, circulating tumor cells. In addition, prostate cancer may be a slowly progressing disease amenable to the gradual impact of immunotherapy in all but its last phases.

In general, 2 approaches are used when describing immunotherapy. The first is active immunization, in which the host generates his own humoral and/or cellular response following vaccination with a known immunogen. The second approach—called passive immunization—is one in which the generation of an immune response, usually humoral (eg, monoclonal antibodies), is given to the patient (Figure 1). While the infusion of B or T lymphocytes could be considered a "passive" approach by virtue of delivering in vivo a cellular product that has been altered in some way, recent data suggest that chimeric antigen receptors (CARs) composed of antibody binding domains connected to domains that activate T cells can become active in vivo by expanding up to more than 1,000-fold, trafficking to bone marrow, and expressing functional CARs at high levels for several months.[3] This review will focus on active immunization and describe how, when combined with chemotherapy, it is particularly suited for a joint strategy that can optimize treatment response.

Sipuleucel-T is a cellular immunotherapy product that consists of an enriched population of a patient's peripheral blood mononuclear cells (PBMCs), which were exposed in vitro to a fusion protein. The fusion protein consists of granulocyte/macrophage-colony stimulating factor (GM-CSF) and PAP (Figure 2).[4] These PMBCs, which include a large proportion of antigen-presenting cells, are infused back into the patient to stimulate antitumor T-cell responses.[4] The specific indication of sipuleucel-T includes asymptomatic or minimally symptomatic, nonvisceral, metastatic prostate cancer. As such, its recommended therapeutic space on the prostate cancer disease spectrum is generally prior to docetaxel, at least to date.

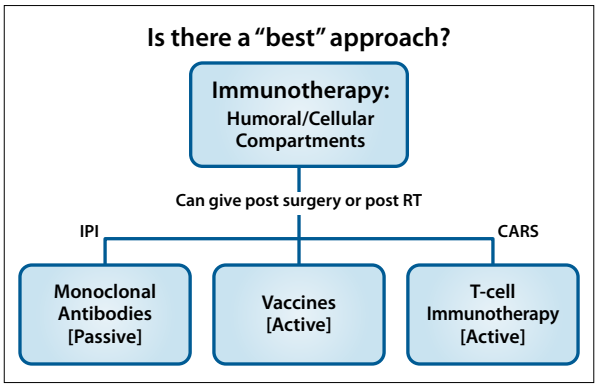

Figure 1. Classification of cancer immunotherapies.

CARS=chimeric antigen receptors; IPI=ipilimumab; RT=radiotherapy.

Three phase III sipuleucel-T trials enrolled CRPC patients with only minor symptoms or functional impairment, and no current chemotherapy; however, 5.5%, 10.2%, and 18.2% of patients had prior chemotherapy.[4,5] The trials reported an increase of 4.1–4.3 months in median overall survival compared with placebo. Estimated survival at 36 months was 32–33% in the active arms versus 15–23% in the placebo arms.[4,5]

Considered a safe drug, sipuleucel-T may be enhanced in combination with chemotherapy. However, early results of combination therapy have not demonstrated benefits of sipuleucel-T use following chemotherapy.[4,5] These 2 analyses focused on the treatment benefit derived from the administration of sipuleucel-T with or without subsequent docetaxel. In the first 2 phase III trials, 32% of the participants received docetaxel after either sipuleucel-T or a similar product prepared from their frozen cells (which was given to crossover placebo recipients after disease progression).[5-7]

An exploratory analysis of post-trial docetaxel use with or without early sipuleucel-T found that there was a benefit to receiving docetaxel some months after sipuleucel-T. Median survival was 34.5 months for patients who received sipuleucel-T followed later by docetaxel (n=51); 25.7 months for crossover placebo recipients who also received docetaxel (n=21); and 20.2 months for placebo patients who received docetaxel without ever receiving a vaccine product (n=10; Table 1). The adjusted survival hazard ratio (HR) for the first of these groups compared with the others was 2.53 (P=.006).[6,7]

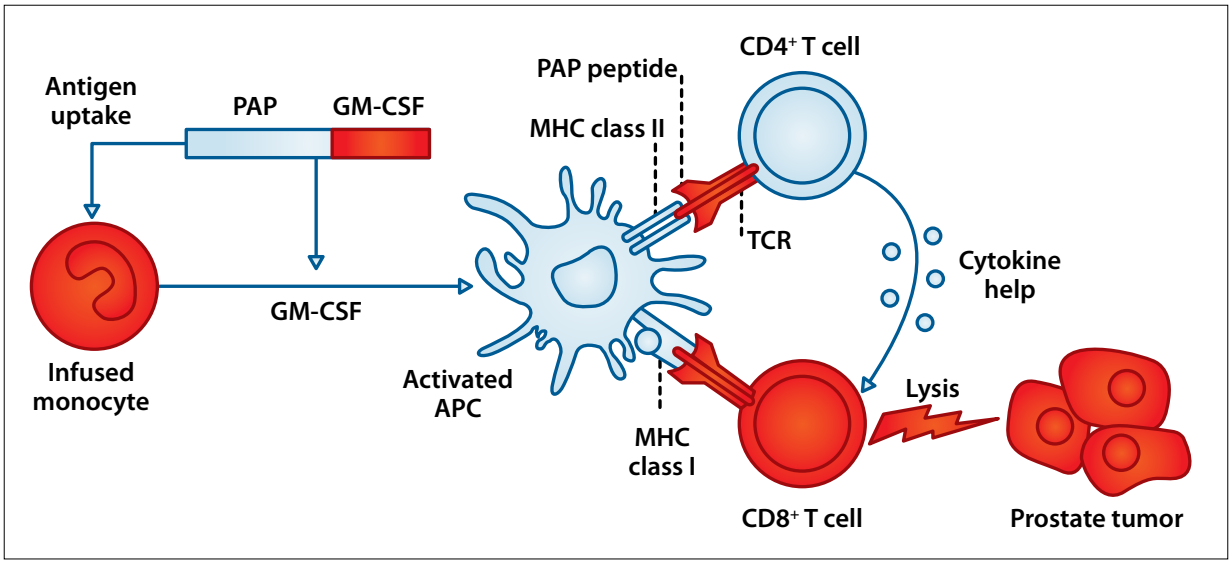

Figure 2. Sipuleucel-T mechanism of action.[14]

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APC=activated antigen-presenting cells; GM-CSF=granulocyte/macrophage-colony stimulating factor; MHC class II=major histocompatibility complex class II; PAP=prostatic acid phosphatase; TCR=T-cell receptors.

Table 1. Exploratory Analysis of Sipuleucel-T Vaccine Recipients Who Later Received Docetaxel[6]

Group	Number of patients	Observed median overall survival (months)	Predicted median overall survival† (months)	Survival HR, sipuleucel-T vs combined placebo groups
Sipuleucel-T → Docetaxel	51	34.5	20.9	HR=1.90, *P*=.023 (log rank) Adjusted HR=2.53, *P*=.006‡
Placebo → APC8015F* → Docetaxel	21	25.7	20.3	
Placebo → Docetaxel	10	20.2	19.1	

*Antigen-primed autologous dendritic cell vaccine product similar to sipuleucel-T (APC8015) but produced from frozen cells.
†As calculated using the Halabi nomogram.[30]
Adjusted for the following baseline risk factors: lactate dehydrogenase, prostate-specific antigen, number of bone metastases, localization of disease, and weight.
HR=hazard ratio.

Rationale for Combining Chemo- and Immunotherapies

Premise: Metastatic Tumors Escape Cell-Mediated Immune Response

Although the study of docetaxel use after early sipuleucel-T was an exploratory one with small numbers, and the results did not render a strong conclusion, the outcome is bioplausible. A number of authors have noted that natural cell-mediated immune responses (ie, tumor infiltration by IFNγ-producing CD4+ and CD8+ cells) correlate with improved survival in a variety of cancers,[8-10] and some reports have even suggested that the type and density of infiltrating lymphocytes are more predictive of disease progression than traditional tumor staging.[9,10] However, it is believed that the chronic inflammatory milieu that develops during tumor growth compromises the immune response while promoting further progression of the malignancy.[8,11,12] Chronic inflammation causes immune cells to release cytokines such as TNFα, TGFβ, and IL-6, which recruit myeloid-derived suppressor cells (MDSC) and directly reduce immune cell

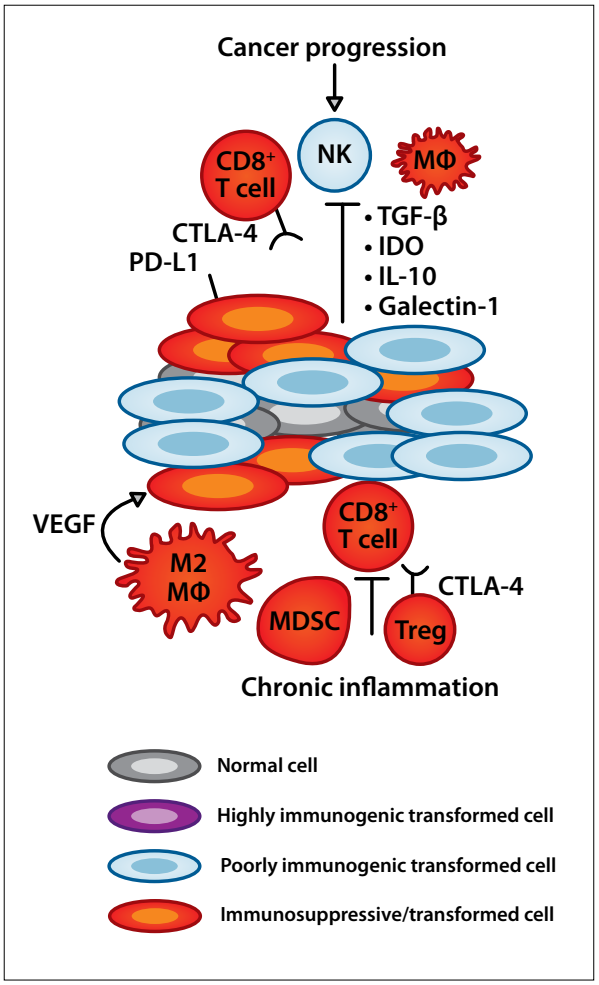

Figure 3. Interplay between chronic inflammation and tumor growth.[8]

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CTLA-4=cytotoxic T-lymphocyte–associated antigen-4; IDO=indoleamine 2,3-dioxygenase; IL=interleukin; MDSC=myeloid-derived suppressor cells; NK=natural killer; VEGF=vascular endothelial growth factor.

activity. These cytokines also promote metastatic transition later in the disease process (Figure 3).

In addition, tumor cells evade cytotoxic lymphocytes (CTLs) through a number of strategies, including blocking antigen presentation and apoptosis.[8,13] Tumor cells eliminate the expression of highly immunogenic tumor antigens and functional major histocompatibility complex (MHC) class I molecules, and they can also produce immunosuppressive cytokines such as transforming growth factor-β and vascular endothelial growth factor. These cytokines directly reduce CTL numbers and recruit CD25+ Treg cells and MDSC that repress the immune response. Yet another mechanism employed by tumor cells may cause reduction in CTL numbers by expressing certain receptor ligands (eg, PD-L1 and FasL).

There is considerable evidence in prostate cancer that tumors promote immune tolerance starting early in the disease course.[14,15] In a transgenic mouse model of prostate cancer, CD8+ and CD4+ cells specific to prostate antigens infiltrate prostate tumors, but are anergic or nonfunctional. The encounter with tumor antigens apparently shifts CD4+ and CD8+ cells toward a suppressive (Treg) phenotype. Patient biopsies show that prostate tumor infiltrating CD4+ cells include high levels of Treg cells.[16] Human prostate tumors also contain elevated populations of possibly protective Th17 cell populations, but only in early disease (low-grade tumors).[16]

Premise: Anti-Cancer Immunity and the Response to Chemotherapy Are Linked

Chemotherapy is widely held to be immunosuppressive, but in fact, it has immunomodulatory effects.[17,18] Merely debulking the tumors reverses tumor-induced immune tolerance, possibly through reducing the amount of suppressive cytokines secreted by malignant cells.[18] In addition, the transient lymphopenia caused by properly dosed chemotherapy activates homeostatic mechanisms, eliminating excess suppressor cells, and stimulating tumor-specific effector T-cell proliferation as well as dendritic cell maturation.[19]

Some chemotherapeutic agents promote specific immune cell types. For example, docetaxel administration in a mouse model selectively decreased MDSCs while increasing CTL responses.[20] Docetaxel may have a relatively potent effect, but other taxanes also alter cytokine patterns and enhance lymphocyte proliferation, as well as the cytotoxic activity of NK and LAK cells, while reducing Treg cell populations.[21,22]

Low-dose cyclophosphamide also has well-documented modulatory effects.[23] It reduces the Treg population, inhibits the activity of the remaining Treg cells, and stimulates cell-mediated immunity.[23]

Combining Chemotherapy and Immunotherapy: the Reality

Premise: Reducing the Regulatory T-Cell Population Improves the Vaccine Response

Aside from chemotherapy, improving vaccine response by inactivating Treg cells has been attempted through the specific targeting of the T-cell CTLA-4 receptor with a monoclonal antibody such as ipilimumab (Yervoy, Bristol-Myers Squibb).[24-26] Preliminary clinical trials suggest that

administering a therapeutic vaccine followed by ipilimumab enhances immune responses and tumor reduction in prostate and ovarian cancers as well as melanoma.[27-30] In a noncomparative phase I trial (N=30) of ipilimumab plus the PSA-TRICOM vaccine in prostate cancer, overall survival was 31.8 months compared to an expected survival of 18.5 months based on baseline factors (Halabi nomogram–predicted survival [HPS]).[31,32]

There have been melanoma studies that have not found additional benefit for therapeutic vaccines beyond that of ipilimumab alone.[33,34] In both the prostate cancer and melanoma studies, the vaccines were administered simultaneously with the course of ipilimumab; there was no attempt to evaluate sequential therapy. [33,34] Furthermore, the melanoma studies associated ipilimumab with extensive autoimmune toxicities, as did a prostate cancer study of ipilimumab alone after radiotherapy.[34,35] The severity of the adverse events seemed linked to the strength of the response to ipilimumab. Overall, the results suggest that chemotherapy, albeit at less than therapeutic doses, may induce a wide range of immune effects.

Premise: Chemotherapy Plus Therapeutic Vaccines May Enhance the Immune Response (Preclinical Models)

Although an attractive idea, chemotherapy-vaccine combinations have not been widely applied. Much of the chemotherapy/vaccine combination data are from preclinical and murine studies, or are based on small phase I trials. In mouse models of colon and breast cancer, paclitaxel, docetaxel, or cisplatin subsequent to vaccination enhanced the effectiveness of the vaccine-generated CTLs, probably by causing an increase in tumor cell permeability to granzyme B.[36] Granzyme B is an enzyme that CTLs release to trigger apoptosis in damaged or infected cells. Cell death in the vaccinated and treated mouse cancer models included a desirable bystander effect in which the vaccine-induced CTLs caused apoptosis in neighboring tumor cells not expressing the vaccine antigens.

Another murine study immunized mice with implanted colon tumors expressing human carcinoembryonic antigen (CEA). The experimental vaccine was based on a poxvirus vaccine containing genes for CEA and costimulatory molecules (CEA-TRICOM).[37]

Administering a standard dose of docetaxel 4 days after 2 poxvirus immunizations improved vaccine-specific immune responses. It also induced antigen-specific T-cell responses to tumor-derived antigens distinct from the antigen used in the vaccine (the "antigen cascade" or "epitope spreading" possibly due to the release of antigens from dying cells). Docetaxel was effective only when administered after immunization. If administered beforehand, docetaxel inhibited cellular infection by the viral vaccine or antigen expression in the cells that did become infected.[37]

Optimal dosing of chemotherapy combined with vaccines remains unclear. A study that investigated daily low-dose paclitaxel found that by targeting HPV E7+ implanted tumors in mice receiving a DNA vaccine, survival was extended and tumor growth delayed. The results were improved when compared with the vaccine alone, the vaccine plus high-dose paclitaxel, or high-dose, twice-weekly paclitaxel alone.[38] Daily low-dose paclitaxel did not result in the significant T-cell declines induced by high-dose paclitaxel. When administered with the vaccine, daily low-dose paclitaxel resulted in a higher CD8+ T-cell/Treg ratio than either the vaccine alone or the vaccine plus high-dose paclitaxel. Furthermore, the low-dose chemotherapy had greater antiangiogenic effects than did the high-dose chemotherapy.[38]

In the transgenic murine prostate cancer model, administering low-dose cyclophosphamide 1–2 days before immunization with a whole-cell, GM-CSF–secreting vaccine (GVAX) resulted in a tumor shrinkage effect not observed with the vaccine alone.[38] This effect seemed related to a reduced Treg population in the tumor and its draining lymph node, as well as increased dendritic cell activation.[39]

Other studies have found specific benefits from high-dose but submyeloblative chemotherapy. It was observed that an adenovirus-based vaccine had limited effectiveness in mice with established melanoma tumors unless the mice were pretreated with higher doses of cyclophosphamide.[40] The combination resulted in tumor regression due to the high frequency of vaccine antigen–specific T-cells, reflecting cyclophosphamide's general promotion of cell-mediated immunity. In another high-dose example, cisplatin/vinorelbine induced leukopenia as well as down-modulated reconstitution of Treg cells when compared with effector T-cells.[41] The combination can synergize with an anti-CEA vaccine in a murine non-small-cell lung cancer model.

Premise: Human Combination Trials May Demonstrate an Impact on Tumor Response or Overall Survival in Prostate Cancer

Prostate cancer is a model disease in which to test immunotherapy or vaccine/chemotherapy combinations for several reasons. First, there are a variety of well-characterized cell-surface antigens, which can serve as targets for immune-directed therapy. Second, immunotherapy can be administered at all clinical states of the disease, and, finally, these agents have been shown to be safe. There have been several other clinical trials describing the combinations of vaccines with chemotherapy (Table 2).

The poxvirus-PSA recombinant vaccine is a mixture of recombinant pox viruses expressing either PSA or the B7.1 costimulatory molecule.[40] A vaccinia-based vaccine is usually given once and then boosted with monthly fowlpox-PSA recombinant virus. Each vaccination is

Table 2. Clinical Trials Combining Prostate Cancer Vaccines With Chemotherapy

Vaccine	Description	Study Protocol	Results	Source
rV-PSA/rF-PSA	A combination of recombinant pox viruses expressing either PSA or the B7.1 costimulatory molecule. A vaccinia-based vaccine is given once and then boosted with monthly PSA fowlpox recombinant virus. Each vaccination is given with GM-CSF.	Vaccine recipients with CRPC (N=28) received concurrent standard-dose docetaxel/dexamethasone or vaccine alone. Those on vaccine alone switched to docetaxel/dexamethasone alone at disease progression.	The median increase in PSA-responsive T-cell precursors was 3-fold in both trial arms at month 3. Patients also developed responses to other prostatic antigens. Patients on the vaccine-alone arm had a median time-to-progression of 1.8 months, whereas the combination recipients had a median PFS of 3.2 months. Patients who switched from vaccine alone to docetaxel alone then had a median PFS of 6.1 months. The median PFS in a historic docetaxel-treated control was 3.7 months.	Arlen et al, 2006[42]
Sipuleucel-T	Autologous dendritic cells cultured ex vivo with recombinant PAP linked to GM-CSF for increased cell activation. The cells are reinfused to their donor 3 times, 2 weeks apart.	Postimmunization standard-dose docetaxel in progressing CRPC (N=82)	Improved survival with sipuleucel-T followed by docetaxel (see Table 1)	Petrylak et al, 2007[6]
Prostate GVAX	A polyvalent vaccine that includes irradiated whole cells from 2 standardized prostate cancer lines, 1 androgen-dependent and 1 androgen-independent. The cells also produce GM-CSF due to a transduced gene.	Patients with symptomatic metastatic CRPC, N=408. Two trial arms: 1) Docetaxel (75 mg/m^2 q3w for 10 cycles) plus GVAX (q3w for 10 cycles). 2) Docetaxel (75 mg/m^2 q3w for 10 cycles) plus prednisone (10 mg/day)	Median overall survival: 12.2 months vs. 14.1 months in the GVAX and control arms, respectively (HR=1.70; 95% CI, 1.15–2.53; *P*=.0076) The trial was discontinued early due to excess deaths in the GVAX arm	Small et al, 2009[44]
PSA-TRICOM	Recombinant vaccinia or fowlpox expressing PSA plus 3 T-cell costimulatory molecules. The vaccinia-based vaccine is given once and then boosted with further immunizations with PSA fowlpox recombinant virus.	CRPC patients with visceral metastases and no current treatment (N=144) Two trial arms: 1) Vaccinia-TRICOM on day 1 and then 4 biweekly fowlpox TRICOM immunizations. Standard docetaxel/prednisone starting on day 85. 2) Standard docetaxel/prednisone	Trial is ongoing	ClinicalTrials.gov (NCT01145508)

CI=confidence interval; CRPC=castrate-resistant prostate cancer; GM-CSF=granulocyte/macrophage-colony stimulating factor; GVAX=granulocyte–macrophage colony-stimulating factor (GM-CSF) gene-transfected tumor cell vaccine; HR=hazard ratio; PAP=prostatic acid phosphatase; PFS=progression-free survival; PSA=prostate-specific antigen.

administered with GM-CSF.[42] In a phase II study, patients with metastatic CRPC (N=28) received the poxvirus-PSA vaccine 2 weeks apart for the first month and then monthly until disease progression occurred. Half the group additionally received docetaxel/dexamethasone therapy in 3-weeks-on/1-week-off cycles.[42]

Eleven patients (78.6%) in the vaccine-alone arm switched to docetaxel upon evidence of progression. Median time to progression was 1.8 months in the vaccine-alone arm and 3.2 months in the vaccine plus docetaxel arm. Notably, patients experienced a median 6.1-month progression-free period after progressing on the vaccine alone and then switching to docetaxel. Progression-free survival (PFS) was 3.7 months in an historic control group receiving docetaxel alone.[42]

All evaluable vaccine recipients exhibited increased PSA-specific T-cells (median 3.33-fold increase in both arms). In the 3 vaccine-alone recipients who were examined, T-cell responses, as well as responses to other prostate tumor antigens (PAP, PSMA, and/or MUC-1), emerged. This may have been due to an epitope-spreading phenomenon pursuant to the tumor cell death by the vaccine-induced PSA-specific T-cell response.[42]

ProstVac VF (PSA-TRICOM) is a second-generation vaccine employing recombinant vaccinia– and fowlpox–expressing PSA plus 3 T-cell costimulatory molecules, LFA-3, B7.1, and ICAM-1.[13] It elicits a more vigorous antitumor response than the original poxvirus-PSA inoculant.[13] An ongoing PSA TRICOM phase II trial is currently comparing a standard docetaxel/prednisone regimen to a biweekly PSA TRICOM vaccine for 2 months followed by docetaxel/prednisone (NCT01145508). Patients (N=144) have metastatic CRPC with minimal symptoms (Eastern Cooperative Oncology Group [ECOG] performance score 0-2). Survival is the primary outcome measure; secondary endpoints include time to radiographic progression; objective response; PSA response; immune response; and the association between PSA-specific immune responses, time to progression, and overall survival.

Prostate GVAX is a polyvalent vaccine composed of irradiated whole cells from 2 prostate cancer cell lines, the androgen-dependent LNCaP and the androgen-refractory PC3.[14] In this vaccine, the cells are transduced with a gene for GM-CSF in order to improve their immune-stimulatory effects.[14] Phase II studies showed promising responses, and led to 2 phase III studies that included docetaxel.

The phase III VITAL-1 trial directly compared GVAX (biweekly for the first 26 weeks, then monthly) with standard triweekly docetaxel plus prednisone.[43] The study population included chemotherapy-naïve men with CPRC and negligible pain. This trial was terminated early due to futility: there was little chance of reaching the primary endpoint, improved survival, even though indications of the vaccine efficacy were observed. Median survival was 20.7 months on GVAX and 21.7 months on docetaxel/prednisone (P=.78). Of note, grade 3/4 adverse events were considerably less frequent with GVAX (8.8% of GVAX recipients vs 43% of those on docetaxel).[43]

The futility analysis took on particular importance because of the results in the other phase III GVAX trial (VITAL-2). That study administered docetaxel every 3 weeks followed 2 days later by GVAX immunization. After 10 docetaxel cycles, GVAX every 4 weeks was administered alone as maintenance therapy. The comparator group received standard docetaxel/prednisone for 10 cycles.[44] The study had a planned enrollment of 600 taxane-naïve patients with metastatic CRPC requiring opioid pain management; overall survival was the primary endpoint.[44] However, the trial (N=408 actual enrollment) was halted prematurely due to an excess of deaths in the GVAX arm (67 vs 47). Median overall survival was 12.2 months in the GVAX/docetaxel arm and 14.1 months in the docetaxel/prednisone arms (HR=1.70; 95% CI, 1.15–2.53; P=.0076). The investigators were unable to identify safety issues or other reasons for the excess deaths. It should be noted that chemotherapy did not blunt the immune response, nor did the addition of prednisone appear to impact T-cell proliferation or general cellular immunity assays.

It should be noted that no phase II trials were conducted prior to VITAL-2 in order to test various docetaxel/GVAX doses and sequences. Judging by the VITAL-1 results with GVAX alone, it is conceivable that the concurrent high-dose docetaxel undercut the GVAX effect. Administering GVAX before or after docetaxel rather than concurrently might yield a more successful result. Another possibility is that some of the study population had disease that was too advanced to benefit from the vaccine.

Controversies

There are 3 basic unknowns with respect to chemotherapy administered with an immunotherapy: what will be the most effective chemotherapy, at what dose, and in which sequence. Chemotherapeutics of interest, with an ongoing body of preclinical and early human data, notably include taxanes, anthracyclines, and cyclophosphamide. These seem to offer some positive immunomodulation that might enhance the response to a therapeutic vaccine. Chemotherapy could be administered at standard or maximum-tolerated doses if the purpose is to kill the most malignant cells, or trigger lymphopenia and reset immune homeostasis. As discussed above, lower-than-therapeutic doses may be favored, as those may selectively alter cell populations and inhibit angiogenesis.[23,38,45] Higher doses set the tumor back further, allowing greater immune

activity and more antigenicity due to tumor debulking and cell death.[17] Lower doses and/or abbreviated courses would be less toxic overall, and also less immunosuppressive. They would also allow frequent, even daily, dosing (metronomic administration) for a steady effect over time.

Induction of tumor resistance to chemotherapy, including multidrug resistance, is a possibility when giving suboptimal drug doses.[45-47] The vaccine, possibly enhanced by the chemotherapy, might provide a barrier against tumor escape. Metronomic taxanes and other agents have supplemental antiangiogenic effects as well.[38,45]

Like dosing, sequencing of chemotherapy depends on the agent's mechanism of action. Initiation of chemotherapy prior to vaccination would be an option if the goal were to reset the immune system by reducing the level of suppressive cells. On the contrary, initiation of chemotherapy during or after vaccination would be an option if the strategy were to impede the tumor and potentiate or broaden the vaccine-induced responses. The question of which patient, disease stage, and treatment history is most appropriate for therapeutic vaccine schemes also arises. Late-stage patients may have had their immune systems compromised by extensive chemotherapy and the evolving tumor escape strategies.[48] One implication is that the patients with shorter life expectancies will not benefit from vaccine therapy; for example, as demonstrated in the GVAX/docetaxel combination results. The original phase III trial of GVAX versus docetaxel enrolled less-advanced patients.[42] A trend toward superior survival in the GVAX recipients after 22 months follow-up was already emerging. In another vaccine example in a phase II study of PSA-TRICOM (N=32), patients with an HPS of 18 months or more lived significantly longer than expected (P=.035).[48] Median survival was 14.6 months for patients with an HPS of less than 18 months and 37.3 months or longer for those with an HPS of 18 months or more.[49] Considering the safety of vaccines relative to standard chemotherapy, clinical trials could justify enrolling patients in earlier stages of disease in lieu of conventional chemotherapy alone.

Improving Trial Endpoints

Another reason for poor results with vaccines in study populations with advanced disease and low life expectancies is that the time frame needed to observe a clinical response is too long. Researchers have realized that responses to immunotherapies are slower than to chemotherapy.[50-52] The disease could be stable or even progress for some months before protective immune responses are apparent. Alternatively, the initial vaccine-induced inflammation or immune reconstitution may be confused with tumor growth. Various groups have therefore proposed revised endpoints for cancer vaccine trials that place greater emphasis on overall survival or long-term disease stability rather than PFS. The emphasis is on minimizing premature discontinuation, and allowing patients to continue with therapy despite early, minor progression.[50-52]

These endpoints may be more appropriate, but they unfortunately codify the extension of vaccine/chemotherapy combination studies' length and complexity. This adds further discouragement in an area that historically has been a low research priority. Biomarkers of immune response that reliably predict treatment outcome would simplify researchers' issues, allowing for more rapid identification and the optimization of effective regimens before trials have reached clinical endpoints.[50,53] These would ideally give advance indication of clinical benefit without the long follow-up required to observe clinical endpoints.

There are a number of potential biomarkers supported by clinical data. Usually, antibody titers are not considered predictive of overall survival, but they did have prognostic value in the largest of the sipuleucel-T phase III trials (N=512).[4] In a much smaller trial, epitope spreading was found to be the only significant independent predictor of survival in 52 HER2 vaccine recipients with stage III–IV HER2-positive breast cancer.[54] Here, median overall survival for patients (N=33) who developed CTL reactivity to HER2 epitopes not on the vaccine was 84 months, versus 25 months in 16 evaluable patients who did not develop CTL reactivity (HR=0.34; 95% CI, 0.12–1.0; P=.05). In general, for these and other potential biomarkers, there remains the question as to whether blood tests reflect conditions in the tumor. There is also a need to standardize immune assays so that study results become more easily reproducible.[51]

Factorial Trial Designs

As previously described, sipuleucel-T, taxanes, or other individual components of a vaccine/chemotherapy joint regimen may not have a great effect by themselves.[48] Trials with factorial designs can simultaneously compare a number of different variations in vaccine and chemotherapy dosing and scheduling. These will be needed for timely development of joint therapy.[48,55] In addition, the trials will need to demonstrate that the chemotherapy component is not compromising the immune response. One can assume that, at the least, vaccines do not have a dose-dependent toxicity, and this simplifies the trial permutations.[48] There may, however, be a need for extended follow-up to measure late autoimmune effects.[48]

A recent demonstration of an innovative factorial design to determine the dose combination that maximized vaccine-induced immunity utilized a 3 x 3 factorial dose-ranging study of cyclophosphamide, doxorubicin, and a GVAX-like allogeneic HER2-positive, GM-CSF–secreting whole tumor cell vaccine for metastatic HER2-positive breast cancer.[55] The statistical

Table 3. The Promise and the Pitfalls of Cancer Vaccines Used in Conjunction With Chemotherapy

Promise	Pitfalls
• Although cancer cells are not highly immunogenic, therapeutic vaccines containing tumor-associated antigens plus costimulatory molecules have been found to elicit substantial immune responses.	• Repeated rounds of chemotherapy at maximum-tolerated doses can shrink tumors, but its toxicities also contribute to patient frailty and suppressed immune responses.
• Metastatic tumors develop highly sophisticated strategies for derailing immune defenses. Therapeutic vaccines therefore need support that sets the tumors back and/or resets the immune system.	• Patients with advanced disease and a history of standard chemotherapy have the most refractory tumor cells, the least responsive immune systems, and the shortest life expectancy. They are not likely to be the best population for study vaccine responses.
• Chemotherapy, if not overwhelmingly ablative, has immunomodulatory effects that help restore antitumor immunity while weakening malignant tissue.	• Timing of chemotherapy relative to therapeutic immunization may be a critical factor in the success of a combination regimen. It depends on whether the main goal of chemotherapy is to re-balance the T-cell population or to weaken tumor cells.
• Moving forward to optimize the effectiveness of vaccine/chemotherapy regimens requires complex studies. New endpoints and trial designs are under discussion that would simplify and accelerate these investigations.	• Basic questions remain unanswered, including the dose and timing of chemotherapy when used in conjunction with therapeutic vaccines.

design assessed differences in peak serum GM-CSF levels using linear mixed models with cyclophosphamide and doxorubicin doses to serve as predictors.[55] A HER2-specific delayed type hypersensitivity response was defined as being positive if there was 1 positive response among the 4 cycles; HER2-specific antibody responses were deemed as being positive at greater than or equal to 1.13 µg/mL. A Fisher's exact test was used to assess statistical significance. Interestingly, HER2-specific antibody responses were measured as a quantitative continuous variable. The relationship between quantitative antibody response and the 2 drug doses were assessed using a "response surface model." This is a standard regression model with antibody response as the dependent variable and the drug doses as independent variables. The model included quadratic (second order) terms for the doses of the 2 drugs to permit curvature in the response surface, so a maximum antibody response would be evident. The response surface analysis provided an established method to select the appropriate cyclophosphamide and doxorubicin dose combinations that maximized the immune response, defined as the absolute difference of the median antibody level pre- and postvaccine.[55] The trial investigated 3 different dose arms for both cyclophosphamide (200, 250, 350 mg/m^2) and doxorubicin (15, 25, 35 mg/m^2) and 1 vaccine dose, given at months 0, 1, 2, and 6. There was also one arm that received no chemotherapy, but was randomized to 2 different vaccine doses. This was a small trial (N=28), and therefore the results were not statistically significant or clinically conclusive. Findings critical to further development of combination regimens can be lost in small, complicated trials such as this one. After utilizing biomarkers for initial regimen selection, there is a need for larger, more definitive studies with hundreds of participants and sufficient follow-up to indicate efficacy. One way to encourage larger trials is to streamline development from phase II proof-of-principal studies to phase III efficacy studies.[48,56] Adaptive trial designs would allow for altering trial enrollment and study endpoints after achieving protocol-defined response thresholds.

Conclusions

The potential promise and drawbacks of administering cancer vaccines in conjunction with chemotherapy are summarized in Table 3.

Prostate cancer vaccines, most notably the recently approved sipuleucel-T, have shown some therapeutic benefit. It is possible that the therapeutic benefit of vaccines can be reinforced by combination with chemotherapeutic agents, such as the taxanes. Research results in this area are complicated by the influence of chemotherapy dose and timing of administration, which in turn depend on the chemotherapeutics' main function as either immune modulators or antineoplastic agents. A further complication is that the optimum patient population remains unclear. Traditionally, experimental therapies are tested in patients with progressive disease and limited therapeutic options. In these cases, such patients weakened by their illness and previous chemotherapies may be less likely to mount strong responses to a vaccine and may not be optimal candidates.

Advances in trial endpoints, design, and biomarker research are needed to efficiently answer many of these questions. Coupled with the appropriate resources, scientific commitment to emerging research should be significant enough that a few negative or ambiguous results do not completely eliminate promising candidates.

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