

MINIREVIEW

Paradigm Shifts in Cancer Vaccine Therapy

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Cancer vaccines constitute a unique therapeutic modality in that they initiate a dynamic process involving the host's immune response. Consequently, (a) repeated doses (vaccinations) over months may be required before patient clinical benefit is observed and (b) there most likely will be a "dynamic balance" between the induction and maintenance of host immune response elements to the vaccinations vs. host/tumor factors that have the potential to diminish those responses. Thus "patient response" in the form of disease stabilization and prolonged survival may be more appropriate to monitor than strictly adhering to "tumor response" in the form of Response Criteria In Solid Tumors (RECIST) criteria. This can be manifested in the form of enhanced patient benefit to subsequent therapies following vaccine therapy. This article will review these phenomena unique to cancer vaccines with emphasis on prostate cancer vaccines as a prototype for vaccine therapy. The unique features of this modality require the consideration of paradigm shifts both in the way cancer vaccine clinical trials are designed and in the way patient benefit is evaluated. *Exp Biol Med* 233:522–534, 2008

Key words: cancer vaccines; immunotherapy; combination therapy; prostate cancer

Introduction

Cancer vaccines provide a unique therapeutic modality in that they initiate a dynamic process of activating the host's own immune system. This process has the potential to change the way initial patient responses, and responses to subsequent therapies post-vaccination, are evaluated.

Although to date the FDA has not approved any therapeutic cancer vaccine, recent preclinical and clinical findings have shown that well-designed clinical trials with appropriate endpoints, as well as the administration of vaccines in new paradigms of combination therapies, may ultimately lead to the use of cancer vaccines for the treatment of many types of cancer.

This article will review and discuss in the context of therapeutic cancer vaccines (a) appropriate clinical trial design and endpoints, (b) multiple strategies for combining vaccines with other therapies, (c) the status of cancer vaccines in ongoing clinical trials, and (d) newer generation vaccines and vaccine strategies.

New Paradigms for Patient Responses to Therapy

A paradigm (from the Greek) is a model, pattern, or example. It is a "basic way of perceiving, thinking, valuing, and doing." A paradigm shift emanates from the idea that "there has to be a better way." The history of science and technology is replete with examples of "paradigm paralysis," in which a shift in thinking is resisted (1, 2). A classic example of paradigm paralysis involves the Swiss mentality and the electronic quartz crystal watch. In 1968, Switzerland dominated the world of watch making, garnering more than half of all world sales and 85% of profits. By 1980, their domination of the world market collapsed to less than 10%. Even though the Swiss invented the quartz crystal, because it did not fit their paradigm of a watch (main springs, etc.), they allowed the inventor to showcase the quartz crystal watch at a world congress. Seiko took a look, and, as they say, "the rest is history."

The rejection of the new by established science is not an isolated aberration; it is the norm in the course of invention and discovery. Within the medical/scientific profession, two examples of paradigm paralysis are (a) it is impossible for RNA to serve as a template to make DNA, and (b) the long-held position of most surgical oncologists

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that a radical mastectomy must be more effective than a simpler, less invasive approach such as a modified radical mastectomy or even lumpectomy to control breast cancer. While skepticism is an important component of scientific research, the very core of scientific research also requires its participants to question well-entrenched views and be open to new perspectives. As this article will illustrate, more effective paradigms in clinical trial design, and ways to monitor whether a patient has a favorable clinical response to a targeted therapy, such as a vaccine, should now be considered by the medical establishment.

Standardization of response criteria is critical for any given clinical trial, but it is important to realize that the use of only one criterion for all types of therapeutics, all cancer types, and all disease stages can be misguided. Since 2000, the oncology community has adhered to Response Criteria In Solid Tumors (RECIST) (3, 4) to measure tumor responses in shrinkage in the evaluation of passive therapeutic modalities such as chemotherapeutic agents and radiation therapy. With the advent of new targeted therapies, including cancer vaccines, reliance solely on RECIST criteria has been called into question by several Cooperative Groups, among others (4–8). It will become clear from the clinical trials described below that RECIST criteria do not always adequately assess patients' clinical benefit. As "patient response" and "tumor response" are not always mutually inclusive, it has been proposed that cancer vaccines may be a therapeutic modality in which "patient response" should be evaluated more highly than "tumor response."

Evaluating Targeted Therapeutics

In a large randomized, multi-center, placebo controlled Phase III trial involving 903 patients with advanced renal cell carcinoma, patients received sorafenib (Nexavar), an inhibitor of serine-threonine kinases as well as multiple tyrosine kinases, or placebo. Progression-free survival doubled from 12 to 24 weeks ($P < 0.00001$) for patients on sorafenib. At 3 months' post-randomization, 82% of patients on sorafenib were progression free vs. 43% on placebo, while the response rate was 10% PR with <1% CR (1 of 451) using RECIST criteria (9). In another example, imatinib (Gleevec) was administered to patients with gastrointestinal stromal tumors (10). The drug has been shown to increase survival in patients, but rarely shrinks tumor by 30% to meet partial response by RECIST criteria. An example of the converse of this phenomenon is a trial involving patients with metastatic renal cell carcinoma. A total of 306 patients were randomly assigned to high dose vs. low dose IL-2 therapy. There was a higher tumor response rate using RECIST criteria in the high dose (21%) vs. the low dose (13%) treated cohorts ($P = 0.048$). However, there was no statistical difference in overall survival between the two groups (11). Moreover, toxicities were more frequent when the higher dose of IL-2 was

administered. As a consequence of these findings and those employing other targeted therapies, new criteria are currently being evaluated to assess patient response, in addition to and perhaps in a more appropriate manner than the use of RECIST criteria.

A Change in Perspective

A prior review characterized stable disease and "survival longer than expected" as "soft criteria" in their evaluation of cancer vaccines as opposed to relying on "standard" or RECIST criteria to measure tumor volumes (12). In contrast, tumor shrinkage was proposed as a modality satisfying RECIST criteria among some melanoma patients whose tumors shrunk with adoptive T-cell transfer techniques (13–15). While the findings using antigen-specific adoptive transfer T-cell techniques are important, extremely innovative, and beneficial to subsets of patients, in the past 20 years, no randomized trial using adoptive cell transfer of antigen-specific T cells has demonstrated a survival benefit over that seen with IL-2 alone (16–18). In addition, such studies have been conducted only in select institutions and have not been translated into multi-center randomized trials. Furthermore, one must also factor in the toxicities associated with this therapy, as well as the therapy's labor-intensive application and cost. Several randomized clinical trials (to be discussed later) have been carried out with a range of cancer vaccines that are now showing evidence of clinical benefit. It is thus of utmost importance to consider different paradigms to measure patient benefit when employing different therapeutics in different patient populations. It is also conceivable that in the future some immunotherapy regimens will involve the combined use of adoptive transfer and vaccine therapy.

Prostate Cancer as a Prototype for Vaccine Therapy

There have been numerous advances in clinical trials involving vaccine therapy of patients with a variety of neoplastic conditions, including lymphoma, melanoma, and lung, breast, colorectal, pancreatic, and prostate cancer. This review will primarily discuss prostate cancer vaccines as a prototype for such progress.

Prostate cancer is a disease well suited for analyzing the effectiveness of cancer vaccines for several reasons: (a) the tumor grows at a relatively slow pace, (b) recurrence is often diagnosed early in the disease process, with many patients having only biochemical progression (rising serum PSA levels with no evidence of occult disease on radiographic imaging), (c) there is a surrogate marker for disease prognosis and outcome—serum PSA doubling time (19–21), (d) following definitive primary therapy (surgery and/or radiation), few existing standard of care therapies achieve long-lasting therapeutic effects, (e) a range of prostate cancer-associated antigens have been identified and characterized, (f) post-vaccination, patients have been shown to

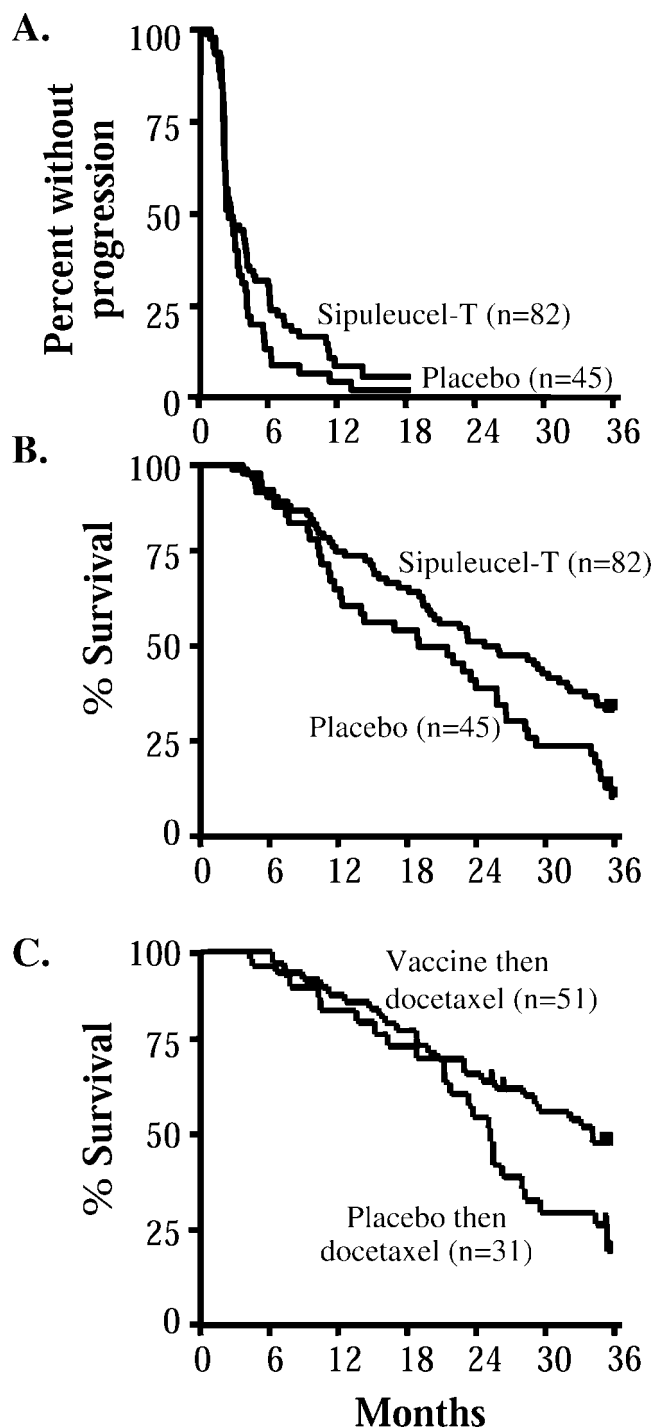


Figure 1. Randomized, placebo controlled, Phase III trial of Sipuleucel-T vaccine (antigen-presenting cells with PAP-GM-CSF fusion protein) vs. placebo in patients with metastatic asymptomatic hormone refractory prostate cancer. (A) Time to progression, i.e., percent without progression. (B) Overall survival (Ref. 22). (C) Enhanced survival to docetaxel in patients having received prior vaccine. At progression, patients went on to receive docetaxel. There was a statistically significant increase in survival ($P = 0.023$, HR = 1.9) when patients received prior vaccine (Ref. 59).

mount immune responses to prostate associated antigens, and (g) vaccines can be used safely in combination with anti-androgen therapies, docetaxel, and radiation to treat prostate cancer (as shown in recent clinical studies).

Sipuleucel-T (Provenge from Dendreon, Inc.) vaccine is made up of autologous antigen-presenting cells and a fusion protein composed of prostatic acid phosphatase (PAP) and GM-CSF (22). The results of early Phase I/II clinical trials showed increases in T-cell responses to the vaccine antigen, serum PSA decline of greater than 50% in 10% of patients, and limited toxicity. In a recent placebo controlled randomized Phase III trial in which Sipuleucel-T was administered to patients with metastatic asymptomatic (without cancer-related pain) castrate resistant prostate cancer (CRPC), patients were randomly assigned in a 2 to 1 ratio to receive vaccine ($n = 82$) or placebo ($n = 45$) (22). The primary endpoint of this study—progression-free survival—did not achieve statistical significance (0.052) (Fig. 1A). A difference in overall survival, however, was statistically significant ($P = 0.01$, HR = 1.70) between vaccine (25.9 months) and placebo (21.4 months) (Fig. 1B, Table 1). COX regression analysis of five clinical variables demonstrated that vaccine therapy vs. control remained statistically significant ($P < 0.002$, HR = 2.12). In a second randomized trial with Sipuleucel-T using this same patient population, there was a trend toward increased survival (19 months for vaccine vs. 15.7 months for placebo) that did not reach statistical significance. Survival at 36 months was 32% for vaccine-treated patients vs. 21% for placebo-treated patients. The integrated analysis of both of these randomized trials, vaccine ($n = 147$) vs. placebo ($n = 78$), showed a statistically significant increase in overall survival of patients who received vaccine ($P = 0.011$, HR = 1.5). Survival at 36 months was achieved by 15% of patients given placebo and 33% of patients given vaccine. Of great interest, however, is the finding that patients who went on to receive chemotherapy (docetaxel) had an extended survival if they had received prior vaccine rather than placebo (Fig. 1C). This phenomenon has been seen in other clinical trials (see the discussion in “New Paradigms for Combination Therapies”) and highlights the paradigm shifts that may be adopted in the analysis of a vaccine’s clinical efficacy. An ongoing Phase III trial with the Sipuleucel-T vaccine is using survival as a primary endpoint. The evidence of survival advantages seen in these trials, as well as those described below, were obtained with little or no evidence of “objective” responses using RECIST criteria.

The GVAX (Cell Genesys, Inc.) vaccine is also being used in advanced clinical trial testing of prostate cancer patients. GVAX consists of two irradiated allogeneic prostate cancer cell lines engineered to secrete GM-CSF (23–25). Its mode of action is described as the uptake of ex vivo x-irradiated tumor cells by antigen-presenting cells and cross-presentation of tumor-associated antigens to T cells in draining lymph nodes. In the first of two Phase II clinical trials completed in patients with asymptomatic metastatic

Table 1. Evidence for Enhanced Survival in Prostate Cancer Vaccine Clinical Trials^a

Patient population	Vaccine	Result	Reference
Non-metastatic CRPC	Vaccine (rV-PSA/B7.1, rF-PSA) ± Nilutamide ^b	Overall survival 59% at 5 years (<i>n</i> = 21)	60, 61
	Nilutamide ± vaccine	38% at 5 years (<i>n</i> = 21)	
Metastatic CRPC	Vaccine (APC-PAP-GM-CSF) then docetaxel	50% (approx.) at 36 mo. (<i>n</i> = 51)	59
	Control then docetaxel	25% (approx.) at 36 mo. (<i>n</i> = 31)	
Metastatic CRPC	PSA-TRICOM	Median overall survival 24.4 mo. (<i>n</i> = 84)	34
	Vector control	16.3 mo. (<i>n</i> = 41)	
Asymptomatic metastatic CRPC	APC-PAP-GM-CSF	25.9 mo. (<i>n</i> = 82)	22
	Placebo	21.4 mo. (<i>n</i> = 45)	
Asymptomatic metastatic CRPC	Whole tumor cell (GVAX)		23–25
	High dose	35 mo. (<i>n</i> = 22)	
	Mid/low dose	20.0/23.1 mo. (<i>n</i> = 25/33)	

^a CRPC, castrate resistant prostate cancer; APC, antigen-presenting cell; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen.

^b Nilutamide is a second-line hormone receptor antagonist.

CRPC (*n* = 34), GVAX was given at two dose levels. Decreases in PSA velocity were evident in 67% of patients given low dose vaccine (*n* = 24) and in 90% of patients given high dose vaccine (*n* = 10). This correlated with survival results; there was a median survival of 24.0 months in the low dose vaccine group and 34.9 months in the high dose vaccine group. The second trial employed five vaccine dose levels. The median survival was 20–23.1 months for patients in the two low dose and middle dose cohorts. The median survival of the high dose cohort was 35 months (Table 1). There were no dose limiting toxicities in either trial. Median survival overall of patients in both trials was more than 6 months longer ($P = 0.01$) than predicted by the Halabi nomogram (26), which consists of numerous predictive indicators of patient survival. These results have formed the basis for two ongoing randomized multi-center Phase III trials. The first Phase III trial (*n* = 600) is being conducted in chemotherapy-naïve asymptomatic metastatic CRPC patients; it is randomized to vaccine vs. docetaxel, with a primary endpoint of overall survival. The second ongoing Phase III trial (*n* = 600) is in symptomatic metastatic CRPC patients with one prior chemotherapy permitted (no prior taxanes); it is randomized to vaccine plus docetaxel vs. docetaxel. The primary endpoint for this trial is also overall survival.

Ony-P1 (Onyvox Limited) is also a whole tumor cell vaccine for prostate cancer. The vaccine consists of three irradiated allogeneic prostate cell lines: two prostate cancer lines and one from normal prostate. Just like the GVAX cell lines, these cell lines have been shown to express a broad range of prostate cancer-associated antigens. The first two vaccinations of Ony-P1 are given with BCG as adjuvant. In Phase I trials, safety was shown and 32% of vaccinated

patients exhibited drops in their serum PSA levels. In a more recently completed Phase II Ony-P1 trial in CRPC patients (27), 11 of 26 patients showed a statistically significant prolonged decrease in their PSA velocity; no patient had a statistically significant increase in PSA velocity post-vaccination. Mean time to tumor progression was 58 weeks compared with recent studies of other agents and historical control values of approximately 28 weeks. Immunologic profiles by artificial neural network analysis of cytokines correlated with PSA velocity responses. A multi-center Phase IIb trial is currently being conducted with Ony-P1.

Trials conducted with poxviral vector-based vaccines have also provided evidence of clinical benefit. Poxviruses (vaccinia, MVA, fowlpox) are able to accept and express multiple transgenes and thus can be engineered to express not only tumor-associated antigens, but also various immunostimulatory molecules. TG4010 (Transgene) is a recombinant MVA expressing both MUC-1 and IL-2. MVA is a replication incompetent vaccinia virus; MUC-1 is a higher molecular weight mucin that is overexpressed on most carcinomas, including prostate carcinomas. In a randomized Phase II study of prostate cancer patients with biochemical progression and no evidence of metastatic disease after local therapy, patients were vaccinated every week for 6 weeks and then every 3 weeks in Arm I, and every 3 weeks in Arm II (28). A statistically significant increase ($P < 0.001$) in PSA doubling time (mean increase 3.8-fold) was observed in Arm I, illustrating that vaccine dose scheduling is an important variable.

In the first Phase I study (29) with recombinant vaccinia, 33 prostate cancer patients who had biochemical progression after local therapy were given rV-PSA (with GM-CSF). PSA levels became stable for 14 of the men for

Patient population: D0 prostate cancer
(rising PSA, hormone naive,
non-metastatic disease)

Randomized to 3 arms

AAAV: Arm A

AAAA: Arm B

VAAA: Arm C

V = vaccinia-PSA A = avipox-PSA

Primary endpoint: PSA progression-free
survival at 6 mos

50-month follow-up: TTP

AAAV (arm A) 9.1 mo

AAAA (arm B) 9.2 mo

VAAA (arm C) 18.2 mo

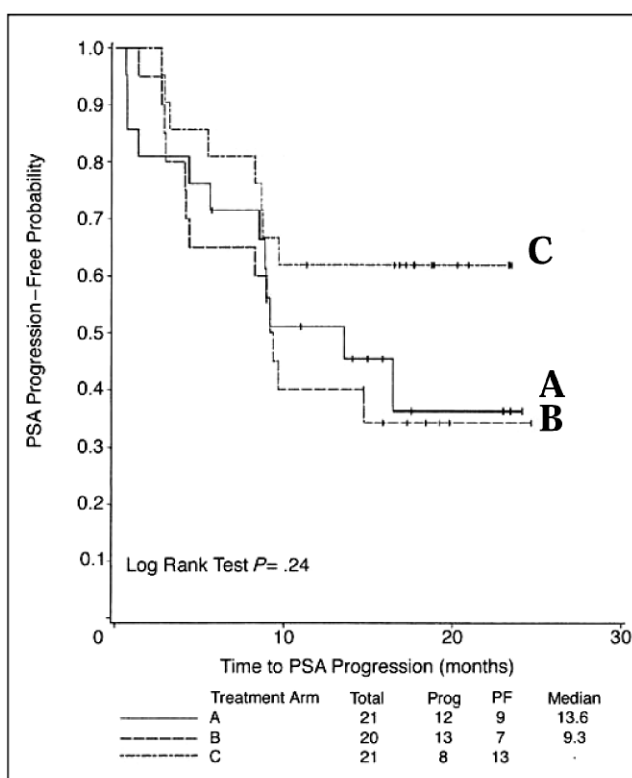


Figure 2. A randomized ECOG Phase II study using PSA-vaccine in D0 prostate cancer patients. Taken from Refs. 30 and 31. TTP is time to progression.

at least 6 months post-vaccination, and nine patients remained stable for 11–25 months. At the time of publication (29), several patients remained without evidence of clinical progression for up to 21 months. Subsequently, the Eastern Cooperative Oncology Group (ECOG) of the National Cancer Institute sponsored a randomized Phase II trial (30) using two different PSA pox vectors in different prime/boost regimens: rV-PSA(V) and/or a recombinant fowlpox (avipox, A [in Fig. 2]) rF-PSA(F) were given to patients ($n = 64$) with biochemical progression after local therapy for prostate cancer. The median time to PSA and/or clinical progression was 9.2 months in the FFFF cohort, and 9.0 months in the FFFV cohort, and had not been reached in the VFFF cohort (Fig. 2). A recent update (31) on this trial with a median follow-up of 50 months, moreover, revealed a median time to PSA progression of 9.2 and 9.1 months for the FFFF and FFFV cohorts, respectively, and 18.2 months for the VFFF cohort.

Recent Phase I/II trials have employed recombinant vaccinia and fowlpox vectors developed by the NCI that contain the transgenes for PSA and the three human costimulatory molecules B7.1, ICAM-1 and LFA-3 (designated TRICOM; Ref. 32) In these trials, patients with metastatic and locally advanced prostate cancer have

exhibited clinical responses and drops in serum PSA (33). A company-sponsored multi-center randomized Phase II study of 125 patients with metastatic asymptomatic CRPC failed to demonstrate an improvement in progression-free survival of vaccine-treated patients compared with placebo-treated patients (34). Patients' overall survival data are currently being accumulated, with provocative results. Thus far, median overall survival is 16.3 months for the control cohort (fowlpox wild-type vector) vs. 24.4 months for those patients receiving PSA-TRICOM vaccines (Fig. 3A, Table 1). Results of this trial will be discussed below.

Distinguishing Between Lack of Vaccine Efficacy and Poor Clinical Trial Design

Clinical trials have now provided evidence of what had previously been predicted by many researchers: There is a direct correlation between a cancer patient's ability to mount an immune response to a vaccine and the length of time from the last therapy, and an inverse correlation between a cancer patient's ability to mount an immune response to a vaccine and the number of therapies received prior to vaccine therapy (35, 36). Thus vaccine efficacy should be evaluated in the proper clinical setting.

The distinction between a vaccine's potential efficacy

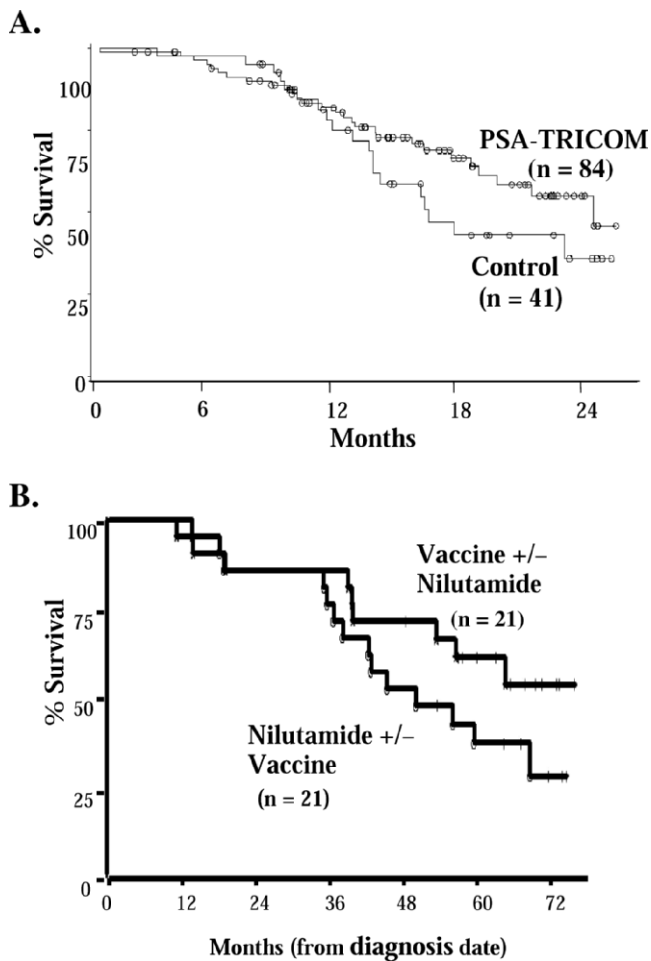


Figure 3. (A) Phase II study in patients with metastatic asymptomatic CRPC receiving PSA-TRICOM vaccines ($n = 84$) or control fowlpox vector ($n = 41$) (Ref. 34). (B) Overall survival in a randomized trial of patients with hormone refractory prostate cancer receiving vaccine (rV-PSA + rV-B7.1 prime, rF-PSA boosts, $n = 21$) vs. nilutamide therapy ($n = 21$) with patients "crossing over" to receive both therapies at progression (Refs. 60, 61).

and poor clinical trial design is exemplified in a corporate-sponsored clinical trial in which a TRICOM-based vaccine (CEA-MUC-1-TRICOM, i.e., PANVAC) was given to 255 patients with metastatic pancreatic cancer who had already failed prior gemcitabine therapy (37). The trial failed to meet its primary endpoint of overall survival. Poor clinical trial design was clearly illustrated by the following: (a) the median overall survival in this patient population is less than 3 months, (b) only one drug combination (gemcitabine + erlotinib) has been approved by the FDA for the therapy of pancreatic cancer, and it extended survival by just 0.4 months (the vaccine trial was conducted in patients where gemcitabine had already been shown ineffective), and (c) 12 randomized trials of various FDA-approved drug combinations (including avastin + gemcitabine) have failed to extend survival in this patient population. This trial thus exemplifies both poor clinical trial design and an inappropriate patient population to evaluate vaccine monotherapy.

Evidence is clearly mounting, as outlined in this review, that the appropriate clinical trial design for a cancer vaccine is in patients (a) who have had minimal prior chemotherapy, (b) with a lower tumor burden, and (c) with a predicted life span to permit multiple rounds of vaccine therapy.

In contrast, the NCI completed a Phase I/II trial with a similar CEA-TRICOM-based vaccine (38). Fifty-eight patients with progressing CEA-expressing cancers (predominantly colorectal and lung) were accrued into several cohorts and received rV- and rF-CEA-TRICOM vaccines with or without GM-CSF. No significant toxicity was observed and 40% of patients had stable disease for at least 4 months; 14 of these patients had prolonged stable disease of more than 6 months. Eleven patients had decreases or stable serum markers, and one lung cancer patient had a pathologic complete response. In addition, overall survival was increased in the cohorts receiving GM-CSF with vaccine and in those patients who had higher frequencies of CEA-specific T-cell responses (38). A novel phenomenon was observed in this trial. Patients who achieved stable disease after six monthly TRICOM vaccinations were then given vaccines every 3 months. When the disease progressed in 12 of these patients, the protocol was modified back to a regimen of monthly vaccinations, after which six of the 12 patients then reverted to stable disease (38). This phenomenon of first stabilizing on a therapeutic, then progressing, and then restabilizing when dose scheduling is changed, underscores the new paradigms that must be investigated in the use of this relatively new therapeutic modality. Unlike conventional chemotherapies and even many targeted therapies, the use of a cancer vaccine initiates a dynamic process. Moreover, the host immune system is abundant with immune suppressive modalities to suppress immune response to "self antigens." Because most tumor-associated antigens are self antigens, a balance of immune-enhancing vs. immune-suppressive factors can come into play in cancer vaccine therapy. Thus it may take multiple doses of a vaccine administered over an extended period of time to achieve a maximal immune response and consequent maximal benefit.

Appropriate Patient Populations. The use of appropriate patient populations for cancer vaccine therapy is exemplified in a recent prostate cancer randomized placebo controlled multi-center trial using PSA-TRICOM vaccines (34). As mentioned above, 125 patients with progressive CRPC and Gleason score of ≤ 7 with no prior chemotherapy or tumor pain requiring opioids were enrolled at 43 sites throughout the United States. Patients in the vaccine arm ($n = 84$) received an initial vaccine with rV-PSA-TRICOM followed by booster vaccination with rF-PSA-TRICOM on weeks 2 and 4, and monthly thereafter through day 168. Patients in the control arm ($n = 41$) received empty vector (wild type fowlpox) at the same time points. All patients received GM-CSF at the vaccine site on the day of vaccination and for 3 subsequent consecutive days. Patients were stratified by bisphosphonate therapy.

Table 2. Cancer Vaccine Combination Therapies: New Paradigms

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- I. Vaccine therapy can be enhanced by numerous biologic "adjuvants"
 - a. Immune potentiators
 - Cytokines: e.g., GM-CSF, IL-7, IL-15
 - Danger signals: e.g., CpG, BCG, Aldara
 - Androgen deprivation therapy
 - b. Regulation of immune inhibitors
 - Ontak
 - Cyclophosphamide
 - Anti-CTLA-4
 - II. Multiple vaccine therapies can be employed due to minimal toxicities
 - a. Diversified prime/boost regimens
 - e.g., DNA/MVA; vaccinia/fowlpox
 - b. Combinations of vaccines targeting different tumor antigens
 - III. Phenotypic alteration of tumor cells by drugs and radiation can render them more susceptible to T-cell-mediated lysis
 - a. Irradiation
 - External beam
 - Radiolabeled MAB
 - Chelated radionuclide
 - b. Certain chemotherapeutics
 - e.g., 5-FU, cisplatin, docetaxel
 - IV. Vaccines initiate a dynamic process; thus anti-tumor effects can be potentiated by subsequent therapies
 - Several reports of evidence of clinical benefit of agents post-vaccine
 - Docetaxel
 - Androgen receptor antagonist (nilutamide)
 - Other chemotherapeutics
 - V. Vaccine therapy appears to be most effective when given prior to or with chemotherapy as opposed to post-multiple therapeutic regimens
 - VI. Patient response (survival) vs. tumor response (RECIST) as the more important criterion for efficacy
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The primary endpoint was the proportion of patients with progression-free survival (PFS) at day 168. Progression was defined as identification of 2 or more new sites of bone metastasis on whole body bone scintigraphy and/or progressive soft tissue disease as defined by RECIST criteria. Secondary endpoints included overall survival. Patient baseline characteristics were relatively balanced between the two arms with respect to ethnicity, bisphosphonate use, and sites of disease. The patients in the vaccine arm tended to be younger and had a shorter median time since diagnosis, perhaps indicating a more aggressive clinical course prior to study than patients in the control arm. However, the median PSA was somewhat lower than the control arm. Overall, the vaccine regimen was very well tolerated.

There was no statistically significant improvement in PFS in the vaccine arm. However, there was early indication of separation in the overall survival curves favoring vaccine. For instance, in this early data in which 32% of patients overall had died, about half the evaluable patients in the

control arm died by day 500 compared with <30% of the evaluable patients in the vaccine arm. On the other hand, at day 750, half of the evaluable patients in the vaccine arm were still alive.

Perhaps the most provocative finding in this randomized placebo controlled trial is seen in patients receiving vs. not receiving bisphosphonates. While zoledronate, a potent bisphosphonate, has been shown to decrease skeletal related events in patients with CRPC metastatic to the bone (39), it has also been associated with side effects and thus is not universally used (40). Often bisphosphonates are added later in the disease when the patient develops significant bone metastasis or bone pain. Less than half of the patients in each arm of this trial received bisphosphonates, and it is apparent that those patients who did receive bisphosphonates had more advanced disease as documented by their baseline characteristic of variables known to be associated with survival in patients with metastatic CRPC. Specifically, the patients who were receiving bisphosphonates had higher PSA, LDH, alkaline phosphatase and lower hemoglobin, each independent variables predictive of shorter survival/more advanced disease based on the widely utilized Halabi prognostic model to predict overall survival (26). There was no difference between the arms among patients receiving bisphosphonates. Interestingly, if one looks at the patients who did not get bisphosphonates, there was even a larger apparent difference between the arms with about 58% overall survival at 500 days in the evaluable patients in the control arm vs. about 83% overall survival at 500 days in the vaccine arm. Thus those patients with more favorable baseline characteristics appeared to benefit from the vaccine vs. placebo control, whereas those patients with less favorable characteristics derived no apparent benefit from this therapy vs. placebo. These results underscore findings in randomized trials that patients with less advanced disease or less tumor burden are more likely to benefit from cancer vaccines.

New Paradigms for Combination Therapies

There are multiple strategies of combination therapies that can and are being employed with the use of cancer vaccines (Table 2). All have been studied in preclinical models and several have provided preliminary evidence of clinical benefit. As the field matures, progress in this area will undoubtedly be accelerated.

Strategy #1: Conventional Combination Therapy. In combination therapies using two or more chemotherapeutic agents or a chemotherapeutic agent and a targeted therapy (e.g., herceptin and taxane), most often each agent works individually with the goal of additive anti-tumor effects. This has also occurred in many preclinical models using vaccines in combination with chemotherapeutic agents. In early clinical studies, while vaccines proved to be less effective in patients heavily pre-treated with chemotherapy prior to vaccine, no detrimental effects

in immune responses to vaccines were seen in patients when vaccine was given in combination with chemotherapeutic agents such as 5FU and docetaxel (even when given with steroid) (41, 42). Preclinical studies have also demonstrated that the COX-2 inhibitor Celebrex, an established anti-inflammatory, had no adverse effect on immune responses to vaccine, and worked well in combination with vaccine to enhance anti-tumor effects (43). A recent clinical study also demonstrated that the Sipuleucel-T (APC-PAP-GM-CSF) vaccine could be given effectively with the anti-VEGF monoclonal antibody (MAb) bevacizumab in patients with biochemically recurrent prostate cancer. Nine of 22 patients showed decreases in serum PSA from baseline. More importantly, however, there was a statistically significant ($P = 0.01$) prolongation in PSA doubling time post-vaccination (12.7 months) vs. pre-vaccination (6.9 months) (44). One should also consider the use of vaccine in a combination therapy regimen with both a chemotherapeutic agent and an anti-tumor monoclonal antibody.

Strategy #2: Combining Vaccine with Agents That Affect the Host Immune System. As demonstrated in many preclinical models, reagents can be employed in combination with vaccines to enhance vaccine efficacy by acting either as immune stimulants/adjuvants or as inhibitors of immune regulatory cells or molecules. Clinical trials employing this strategy have been hampered by the reluctance of pharmaceutical and biotechnology companies to employ proprietary agents of another company to enhance their own agent's efficacy.

It has been well established that cytokines are able to enhance immune response (see Ref. 45 for review). For example, it has been shown in numerous clinical trials that GM-CSF (FDA approved) enhances vaccine efficacy by enhancing dendritic cell migration to regional nodes. IL-2 may not prove to be as useful as originally thought; its toxicity, ability to induce apoptosis in activated T cells, and/or ability to enhance regulatory T-cell activity make it less attractive. IL-15 would appear to be an ideal substitute for IL-2 to enhance T-cell responses, but currently its availability is questionable. IL-7 also has the potential to be useful with vaccines in enhancing memory T-cell responses. Other immune stimulants, such as CpG motifs and MPL, are also being employed clinically with specific vaccines, but are not widely available for commercial reasons. Agents such as Flt-3L, which showed great promise in early clinical trials to mobilize peptide-pulsed dendritic cells, are also currently not available for commercial reasons. Unfortunately, some pharmaceutical and biotechnology companies have at times been reluctant to release agents (especially cytokines) that have no efficacy as monotherapies for studies to be employed to enhance vaccine efficacy.

Evidence garnered from many preclinical studies and recent clinical studies indicates that the control of immune inhibitory entities will ultimately have an important function in vaccine-mediated therapies. The monoclonal antibody

anti-CTLA-4 is showing much potential (46–49). Although the exact mechanism by which this agent works with vaccine has never been shown clinically, preclinical studies have clearly demonstrated that anti-CTLA-4 renders higher avidity antigen-specific T cells when employed with vaccines (50). Both completed and ongoing clinical studies have shown clinical benefit in the use of anti-CTLA-4 plus vaccine in patients with melanoma, ovarian cancer, and prostate cancer.

In both preclinical studies and clinical trials, it has been shown that Ontak, a fusion protein consisting of diphtheria endotoxin and a binding site for the IL-2 TCR, can kill CD4/CD25/FOXP3 regulatory T cells (T regs) and enhance vaccine efficacy by eliciting greater T-cell responses (51). The chemotherapeutic agent Cytosan (cyclophosphamide) also enhances vaccine efficacy by reducing the number of T regs and their functionality (52, 53). As the field matures, clinical application of agents that inhibit immunosuppressive molecules, such as TGF- β and IL-10, will more likely also add to a vaccine's effectiveness.

Another model to consider in the treatment of prostate cancer is androgen deprivation therapy (ADT), which can enhance thymic regeneration (54). In a well-designed clinical study in which biopsy samples were taken from patients before and after they received ADT, there was a substantial increase in CD3 T cells infiltrating the patients' prostate (55). Hopefully, future vaccine/ADT combination therapy treatments will yield similar results.

Strategy #3: Multiple Vaccine Therapies. This strategy may ultimately prove beneficial for the following reasons: (a) each vaccine can carry different tumor-associated antigens, (b) different types of vaccines enhance different entities of the host immune system (i.e., greater increases in CD4, CD8, NK, and/or antibodies), (c) limited toxicities have been associated with vaccine therapy, and (d) some vaccines will induce host immune responses to the vaccine vehicle, such as with viral vectors, thus limiting their continued use. The feasibility and usefulness of this approach have been demonstrated in numerous preclinical studies using various combinations of DNA, MVA, vaccine, fowlpox, and adenovirus vectors. Preliminary efficacy in clinical trials has been observed in HIV and malaria-based vaccines, and in cancer patients who have received an rV-prime (V) and multiple fowlpox (F) boosts (30, 31, 38, 56). In a multi-center randomized ECOG Phase II study (30, 31), approximately a doubling in median time to progression was achieved in prostate cancer patients receiving the VFFF regimen vs. the FFFF or FFFV regimens. It is expected that as the field matures, more diverse vaccine combinations will be employed.

Strategy #4: Dose Scheduling of Vaccine with Other Therapies. The administration of a vaccine initiates a dynamic process of host immune response. Evidence of this unique feature of vaccine therapy, which may be exploited in subsequent therapies, has now been seen in several recent clinical studies. In a Phase I study at

the Dana-Farber Cancer Center (57), 17 patients with advanced stage progressive cancer received a plasmid/microparticle vaccine directed against cytochrome P4501B1, which is overexpressed on most tumors. Progressive disease was the “best response” to subsequent therapy in all but one of 11 patients who did not develop immunity to the vaccine. Five patients who developed immunity to vaccine, but required salvage therapy upon progression, showed marked responses lasting longer than 1 year (for most) to their next treatment regimen (57). In another study, at the H. Lee Moffitt Cancer Center (58), 29 patients with extensive stage small cell lung cancer received an adeno-p53 vaccine. P53-specific T-cell responses were seen in 57.1% of patients, but for most patients the disease progressed. Vaccine therapy immediately followed by chemotherapy, however, generated a high rate (61.9%) of objective clinical responses. These clinical responses to chemotherapy were also closely associated with induction or augmentation of immune response to vaccine.

Additional evidence of this phenomenon is also seen in three randomized clinical trials treating prostate cancer patients. The NCI completed studies with a diversified prime-and-boost strategy involving PSA-vaccinia recombinants (rV-PSA + rV-B7.1; prime) followed by multiple vaccinations with rF-PSA. In the first trial, 28 patients with metastatic CRPC were randomized to receive vaccine alone or vaccine plus docetaxel (41). At time of progression, patients on the vaccine arm alone were allowed to cross over to receive docetaxel. Median progression-free survival on docetaxel was 6.1 months for those patients compared with a progression-free survival of 3.7 months with the same docetaxel regimen and patient population at the same institution. Similar findings were observed in a randomized multi-center Sipuleucel study (described above; Ref. 59), in which patients in both the vaccine arm ($n = 51$) and the placebo arm ($n = 31$) received docetaxel at progression. There was a statistically significant ($P = 0.023$, $HR = 1.90$) increase in overall survival with docetaxel treatment in patients who initially received vaccine vs. those who initially received placebo followed by docetaxel (Fig. 1C, Table 1). The mechanism of action of docetaxel in this setting could well be the enhancement of expression to tumor antigens and/or accessory molecules on tumor cells to render them more susceptible to T-cell-mediated attack.

In another Phase II trial conducted by the National Cancer Institute (60), 42 patients with nonmetastatic CRPC and rising levels of PSA were randomized to receive vaccine (rV-PSA/B7.1 prime and rF-PSA boosts) ($n = 21$) or nilutamide ($n = 21$), an androgen-receptor antagonist (ARA). After 6 months, patients with a rising PSA and no evidence of metastasis, as determined by radiographic criteria, were allowed to “cross over” and receive a combination of both therapies. Median time to treatment failure was similar in the vaccine (9.9 months) and ARA (7.6 months) arms. For the 12 patients who first received vaccine and then went on to receive vaccine plus ARA,

however, time to treatment failure from the initiation of ARA was 13.9 months and time to treatment failure from the initiation of any therapy was 25.9 months. Five-year overall survival for patients who received only nilutamide was 36% vs. 64% for those patients who received vaccine first and then nilutamide. In the initial randomized population ($n = 21$ /cohort), 5-year overall survival was 38% for those patients who received nilutamide first (nilutamide alone or nilutamide then vaccine) vs. a median overall survival of 59% for those patients who received vaccine first (vaccine alone or nilutamide plus vaccine) (Fig. 3B, Table 1; Ref. 61).

The trials described above were from different institutions and employed different vaccines and patient populations; they all provided evidence of the same phenomenon: patients who receive vaccine (and mount immune responses to vaccine) have enhanced outcome to subsequent therapies. It is unlikely that this result can be attributable to selection of patient population because three of the trials were randomized. However, this phenomenon may be attributable to one or a combination of three factors: subsequent therapy (a) may reduce suppressor cell populations, thus allowing for the enhancement of prior established T-cell responses, (b) may be lysing populations of tumor cells, which as a consequence of cross priming via dendritic cells are activating relatively dormant T cells to bring about an anti-tumor response, and/or (c) may alter the phenotype of tumor cells to enhance T-cell killing (see below).

Strategy #5: Phenotype Alterations in Tumor Cells. In a series of preclinical studies involving murine models in vivo (62, 63), and a large series of human tumor cell lines in vitro (62, 64), it has been shown that certain standard of care therapeutics can actually alter the phenotype of tumor cells to render them more susceptible to T-cell-mediated lysis. Sublethal doses of irradiation delivered via external beam, radiolabeled MAb, or a chelated radionuclide (Quadramet) upregulate tumor-associated antigens, fas, and/or adhesion molecules, and/or downregulate anti-apoptotic genes; these phenotypically altered tumor cells then become more susceptible to antigen-specific T-cell-mediated lysis. Chemotherapeutic agents such as 5FU (65), gemcitabine (62), and docetaxel (41) have also been employed in sublethal doses inducing similar alterations of tumor-cell phenotype and subsequent susceptibility to T-cell-mediated lysis. As a consequence, yet another paradigm shift in vaccine combination therapies may come about, e.g., even if a patient does not respond to a drug or radiation therapy because it lacks the ability to lyse tumor cells, this initial therapy may be used in combination with vaccine therapy because it alters tumor cell phenotype and consequently augments vaccine-induced T-cell lysis of the tumor.

Immune Responses to Vaccine

Based on the results of several clinical studies, statistical correlations do not always exist between antigen-specific immune responses to vaccine and patient benefit. These findings may be confounded by several phenomena: (a) the majority of studies have examined T-cell or antibody responses only in blood, which may not always correlate with their presence in tumor, and this may vary with tumor size, vasculature, etc.; (b) few studies, if any, have considered the presence of regulatory T cells and/or analyzed multiple immune cell subsets, e.g., CD4, CD8, antibody, and NK responses from a given patient population. It should also be pointed out that several vaccine clinical trials have demonstrated not only the generation of cytotoxic (ADCC) antibody responses to a given tumor antigen, but some have also generated both T-cell and antibody responses (66–68); (c) almost all studies have measured the level of antigen-specific T cells, but only a few have monitored the avidity of antigen-specific T-cell subsets (69)—perhaps the most important parameter to measure; and (d) preclinical studies have clearly illustrated that the more important antigen-specific T-cell subsets to monitor may not be those directed to the antigen in the vaccine but those directed to other tumor-associated antigens. As a result of initial tumor-cell disruption by vaccine-induced cytolytic T cells, cross priming will lead to the generation of T cells directed against these other tumor-associated antigens. Preclinical studies have demonstrated that these “antigen cascade” T cells can be of greater magnitude and greater avidity than those directed against the antigen in the vaccine, and are mainly responsible for tumor cure (63). Clinical studies (70–72) have also demonstrated this phenomenon of “antigen cascade” and that levels of T cells specific for antigens not in the vaccine can actually be of greater magnitude than those directed against the tumor-associated antigen in the vaccine. Thus, those who are skeptical about the lack of correlation in some studies between patient benefit and immune response may be held captive to yet another “paradigm paralysis” of seeking a threshold of immune responses of only one immune cell subset in blood, which are directed against only one tumor-associated antigen.

Vaccine Therapy: What Does the Future Hold?

This article has reviewed some of the new vaccine strategies in active clinical trial development for the treatment of prostate cancer. Vaccine trials in breast, lung, colorectal and pancreatic cancer, lymphoma, melanoma, and other tumor types are also yielding results illustrating the potential effectiveness of vaccine therapy. The use of varied vaccine vehicles—allogeneic whole tumor cells, peptide- or protein-pulsed antigen-presenting cells (including dendritic cells), recombinant DNA and viral vectors, and recombinant *Saccharomyces* (yeast)—is also yielding evidence of clinical benefit. With continued advances in cancer vaccine

therapy, the greater the likelihood that long-term safety profiles of many of these agents will be realized. Consequently, vaccines may well be utilized in neoadjuvant settings, in certain preneoplastic conditions such as colonic familial adenomatous polyposis (FAP), prostatic intra-epithelial neoplasia, and in other preneoplastic conditions in which tumor-associated antigens have been identified as targets.

Healthy skepticism is an integral component in scientific research, as is the conviction to carry on in the face of such skepticism. For example, advocates of monoclonal antibody therapy persevered for a decade in light of severe skepticism. Such determination has been justly rewarded; the field has been advanced to the point where eight monoclonal antibodies have now been approved by the FDA for cancer management, with the prospect that many of those currently employed in clinical trials will be approved as well. Hopefully, the paradigm shifts put forth in this article for the use and clinical evaluation of cancer vaccines will eventually come to fruition so that cancer vaccine development can be evaluated in appropriate patient populations, with appropriate clinical trial design and evaluation criteria.

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