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Endpoints, patient selection, and biomarkers in the design of clinical trials for cancer vaccines

Marijo Bilusic and James L. Gulley*

Laboratory of Tumor Immunology and Biology and Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Therapeutic cancer vaccines are an emerging and potentially effective treatment modality. Cancer vaccines are usually very well tolerated, with minimal toxicity compared with chemotherapy. Unlike conventional cytotoxic therapies, immunotherapy does not result in immediate tumor shrinkage, but may alter growth rate and thus prolong survival. Multiple randomized controlled trials of various immunotherapeutic agents have shown a delayed separation in Kaplan-Meier survival curves, with no evidence of clinical benefit within the first 6 to 12 months of vaccine treatment. Overall survival benefit is seen in patients with lower disease burden who are not expected to die within those initial 6 to 12 months. The concept of improved overall survival without marked initial tumor reduction represents a significant shift from the current paradigms established by standard cytotoxic therapies. Future clinical studies of therapeutic vaccines should enroll patients with lower tumor burden, more indolent disease, or both, and must seek to identify early markers of clinical benefit that may correlate with survival. Until then, improved overall survival is the only clear, discriminatory endpoint for therapeutic vaccines as monotherapies.

Keywords

Cancer; Immunotherapy; Growth kinetics; Tumor volume; Biomarkers; CIMT 2011

Introduction

The immune system's protective strategies against carcinogenesis are well understood [1–3]. In healthy humans, a complex system of immune surveillance recognizes tumor-associated antigens (TAAs) and activates an array of immune cells to attack and kill cancer cells. However, cancer cells can employ various mechanisms to evade this immune surveillance, such as mimicking normal cells or producing immunosuppressive growth factors and cytokines [4].

Standard anticancer therapeutic approaches (such as cytotoxic chemotherapies, targeted therapies, biologic agents, radiation therapy, and surgical intervention) often have immediate effects and may initially reduce tumor size, but the disease inevitably progresses over time. Therapeutic cancer vaccines directly target not the tumor, but the immune system, which in turn targets the tumor and its microenvironment. Although therapeutic cancer vaccines hold great promise, their ultimate utility may be in combining them with other standard

*Corresponding author: Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 8B09, Bethesda, MD 20892, USA. Tel: (301) 435-2956; Fax: (301) 480-5094; gulleyj@mail.nih.gov.

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therapeutic interventions. Increasing data suggest that immune-mediated tumor killing induced by cancer vaccines can be enhanced and promoted by conventional anticancer therapies. Standard treatments may upregulate MHC molecules and TAA expression, or may induce apoptosis by increasing the expression of death receptors such as Fas, TNF receptor, and TNF-related ligand receptor [5–7]. Many treatment modalities are currently being investigated in combination with vaccines, including radiation, chemotherapy, hormonal therapy, and targeted molecular inhibitors.

The goal of cancer immunotherapy is to activate T-cell responses against specific TAAs that are of sufficient magnitude to eliminate the tumor and prevent its recurrence [8, 9]. This process may eventually stabilize disease and delay tumor growth through sustained alteration of host/tumor interaction. Such an active antitumor immune response may translate into prolonged survival. Thus, a cancer vaccine may initially induce no significant reduction in tumor size; in fact, immunological processes may induce some transient tumor growth due to immune cell infiltration, which can be misinterpreted as progressive disease. In general, therapeutic cancer vaccines, when used as monotherapy, have minimal toxicity compared to conventional chemotherapy; however immune-related toxicity can be seen with some immunotherapeutic drugs (such as ipilimumab).

The ideal target of immunotherapy should be tumor-specific. Unlike cytoreductive chemotherapy, cancer vaccines require sufficient time to generate an immune response, and evidence of clinical benefit may therefore be delayed [10]. This could explain why several phase III vaccine trials that demonstrated no significant change in progression-free survival (PFS) showed significant improvement in the long-term endpoint of overall survival (OS) [11–13].

This new concept of long-term benefit without immediate and marked reduction in tumor size makes sense in terms of immune response, but represents a significant paradigm shift from the standard practice of treating patients with cytotoxic drugs, and calls for a re-evaluation of current clinical trial design. Two distinct and novel effects that were observed in immunotherapy trials should be taken into consideration in the development of new products: a) the impact of delayed responses on clinical study design, and b) the optimal size of the study population and optimal disease stage for testing. The target population in clinical trials should be patients with slow-growing and/or low-volume disease [14]. In addition, unusual response patterns suggest the need for modified endpoints, such as the immune-related response criteria (irRC) recently proposed by Wolchok et al. [15] that capture the effects of immunotherapies much better than conventional criteria, as well as statistical models that describe hazard ratios as a function of time and that recognize differences before and after the separation of Kaplan-Meier curves [14]. These concepts, which are nothing less than a paradigm shift, are now incorporated in specific FDA guidance for clinical considerations for therapeutic vaccines [16].

Prostate cancer vaccines

Therapeutic cancer vaccines have been in development for several decades. Initial results were disappointing, but recent trials, especially in prostate cancer, have renewed hope that the initiation of a dynamic immune response by a therapeutic cancer vaccine can have long-term clinical benefit. The generally indolent nature of prostate cancer may allow time for the immune system to mount a meaningful immunologic response, and since the prostate is a nonessential organ, targeting prostate cancer-associated antigens is unlikely to have significant negative clinical effects. These factors may explain why therapeutic cancer vaccines have been more successful in prostate cancer than in other types of cancer. Therapeutic prostate cancer vaccines direct an immune response against TAAs through a

variety of strategies, including vaccine platforms that employ antigen-presenting cells (APCs), genetically modified tumor cells, viral-based vectors, peptides, and DNA [17]. (See Table 1 for a summary of ongoing phase III immunotherapy trials in prostate cancer).

Two prostate cancer vaccines, sipuleucel-T (Provenge®) and PSA-TRICOM (PROSTVAC®), are in advanced stages of clinical development. Sipuleucel-T is the first therapeutic cancer vaccine approved by the U.S. Food and Drug Administration to treat minimally symptomatic or asymptomatic metastatic castration-resistant prostate cancer (mCRPC) [18]. Sipuleucel-T, a cellular product, is manufactured for each patient after harvesting peripheral blood mononuclear cells, including APCs, via apheresis. These cells are then exposed *ex vivo* to a recombinant protein consisting of GM-CSF fused to prostatic acid phosphatase (PAP) (PA2024) in a process designed to activate them. After processing, the cells are reinfused into the patient, with the goal of generating an immune response against PAP [19]. Initial phase I and II trials have shown that treatment is well tolerated, with no dose-limiting toxicities [20–22]. The IMPACT trial (IMmunotherapy for Prostate AdenoCarcinoma Treatment), a phase III, randomized, double-blind, placebo-controlled, multicenter study, enrolled 512 men with minimally symptomatic mCRPC and randomized them 2:1 to receive sipuleucel-T ($n = 341$) or placebo ($n = 171$) every 2 weeks for 3 treatments. Sipuleucel-T prolonged median OS, the primary endpoint, by 4.1 months compared to placebo (25.8 vs. 21.7 months, respectively) and reduced the risk of death from any cause by 22.5% (HR 0.775; $p = 0.032$) [13].

Another vaccine in advanced stage of clinical development, PSA-TRICOM, is a vector-based prostate cancer vaccine that uses recombinant poxviruses to initiate an immune response against prostate-specific antigen (PSA)-expressing cells. The vaccine also contains transgenes for 3 T-cell costimulatory molecules to enhance T-cell activation: B7.1 (CD80), lymphocyte function-associated antigen (LFA)-3, and intracellular adhesion molecule (ICAM)-1, together known as TRICOM [12]. A randomized, placebo-controlled, phase II study of PSA-TRICOM in patients with minimally symptomatic or asymptomatic mCRPC randomized patients 2:1 to receive either PSA-TRICOM ($n = 84$) or placebo ($n = 41$) [12]. The primary endpoint was PFS, with a secondary endpoint of OS. There was no difference in PFS between the 2 groups ($p = 0.6$). However, 3 years post-study, patients receiving PSA-TRICOM had greater OS, with 25/82 patients (30%) still alive vs. 7/40 controls (17%). Median OS for patients receiving vaccine was improved by 8.5 months (25.1 vs. 16.6 months for controls; estimated HR 0.56 [95% CI, 0.37 to 0.85]; stratified log-rank $p = 0.0061$). These promising data will be evaluated in a larger phase III study that will begin in late 2011 (NCT01322490). Notably, the improved OS seen with the use of therapeutic prostate cancer vaccines was not associated with the substantial treatment-related toxicity commonly observed with conventional chemotherapy.

Promising results of early studies using ipilimumab, a fully human antibody that binds to CTLA-4, prompted a pilot trial of anti-CTLA-4 in prostate cancer [23]. In addition, a phase III clinical trial in newly diagnosed nonmetastatic prostate cancer is currently underway in which adenoviral-HSV thymidine kinase (AdV-tk) plus valacyclovir is added to standard-of-care radiation and hormonal therapy. This “gene-mediated cytotoxic immunotherapy” is based on clinical studies demonstrating increased local and systemic antitumor efficacy from AdV-tk combined with radiation [24]. At least one therapeutic vaccine has a role in asymptomatic metastatic prostate cancer.

If vaccines were to be routinely employed in earlier-stage disease, their ability to slow tumor growth rate could lead to improved outcomes. Furthermore, broader use of vaccines in prostate cancer could allow researchers to observe enough patients to develop appropriate biomarkers of immune response in other diseases.

Prostate cancer immunotherapy has proven the principal that immunological treatment can lead to clinical benefit. The next step will be to improve the efficacy of cancer vaccines by combining them with other immune-boosting drugs. In this way, key lessons learned from prostate cancer immunotherapy may be applied in treating other types of cancer.

Effect of immunotherapy on tumor growth

A review of several prostate cancer clinical trials conducted at the National Cancer Institute in the last decade revealed interesting data on PSA kinetics, which led to the development of a 2-phase equation for estimating concomitant rates of tumor regression and tumor growth (the growth rate constant). An equation was based on the model that the PSA level decreases exponentially (i.e., as a first-order process) but that there is also independent exponential regrowth of the tumor reflected in the measured PSA level [25]. For patients treated with chemotherapy, there was a significant correlation between time on treatment and survival. After treatment was discontinued, pretreatment PSA kinetics resumed, and time to death was predictable based on similar pre- and post-treatment PSA trajectories. The derived growth rate constant correlated with survival, and was recently used to assess the efficacy of various treatment modalities [26]. For patients treated with PSA-TRICOM vaccine, PSA kinetics did not immediately change while on treatment, but time of death was well beyond what was predicted by the models, suggesting a delayed effect likely mediated by a sustained beneficial immune response (Fig. 1).

Tumor growth is a dynamic process, the result of new cells constantly being produced and others dying. Tumor growth rate is the change in tumor size over time. Intrinsic tumor biology combined with extrinsic factors, such as therapies, influence growth rate. Chemotherapy affects tumor growth rate only so long as it is being administered. Although a vaccine can initiate immune responses within 3 months, these responses may not be sufficient to significantly reduce tumor size. But while immunotherapy may not cause marked changes in tumor burden in the short term, it can exert continued cumulative pressure on the tumor, especially if started early in the disease course. This pressure may eventually decrease tumor growth velocity and lead to substantial improvements in OS. Vaccines can also promote the development of long-lived memory cells with the potential to exert continuing immunologic control, resulting in a slowing of the tumor's net growth rate since more tumor cells are killed by the immune system over time [27].

Unlike chemotherapy, which acts directly on the tumor, cancer immunotherapies demonstrate new kinetics that involve building a cellular immune response, eventually delaying tumor progression, and potentially resulting in improved survival. Several mechanisms have been proposed for this phenomenon [10, 28, 29]. Subsequent therapies may alter expression of TAAs on tumor cells, making them more susceptible to immune-mediated killing, or they may enhance the immune response by depleting immune regulatory mechanisms. Additionally, chemotherapy-induced cytotoxicity may expose the activated immune system to additional antigens that can then be targeted in a broader immune response, or may trigger a molecular “danger signal” that leads to enhanced immune response [30, 31]. If validated as a surrogate for survival, growth rate constants may offer an important new efficacy endpoint for clinical trials [26].

Delayed separation of the Kaplan-Meier curves is frequently observed in immunotherapy trials. The data from the early part of the curves does not show similar survival between the arms whereas data in the later part of the curve clearly shows differences between control and immunotherapy. It is therefore recommended that modified statistical models describing hazard ratios as a function of time and recognizing differences before and after separation of curves may be prospectively employed for designing trials. In addition, investigators should

understand that the trial will be overpowered if delayed separation is not observed or is less than that specified [32].

Endpoints in immunotherapy trials

The merits of PFS/time to progression (TTP) vs. OS as appropriate endpoints in clinical trials have been debated for many years [33]. Some see PFS/TTP as a more attractive endpoint for clinical trials, as well as a surrogate marker for OS, because it can be determined earlier, is less influenced by competing causes of death, and is not influenced by second-line treatments. Others argue that, unlike OS, which is a definitive measurement, disease progression may be subject to measurement errors and can vary among centers and investigators. In addition, the date of radiographic progression is in fact a proxy for the true time of progression, which occurs at an unknown point between 2 successive radiologic assessments [34].

Significantly, the most relevant immune responses may be to tumor antigens not targeted by the vaccine, in a phenomenon known as antigen cascade or antigen spreading [6, 35, 36]. For instance, the initial immune response to a vaccine can lead to T cell-mediated killing of tumor cells. APCs may then take up these dead or dying tumor cells and present other, more relevant antigens to the immune system, and this broader antitumor immune response may have clinical relevance by slowing the tumor growth rate. Furthermore, the immune response can be maintained or even augmented by subsequent therapies [10, 28].

Data from several trials of cancer vaccines used alone and in combination with chemotherapy suggest that in trials involving vaccines as monotherapy, PFS/TTP may not be an appropriate endpoint [12, 13, 29, 37, 38]. As we have seen, cancer vaccines can improve OS without significant changes in PFS/TTP, such as the randomized, placebo-controlled phase III trial of sipuleucel-T (IMPACT) and the phase II study of the vector-based vaccine PSA-TRICOM described above. Interestingly, similar outcomes were recently seen with the immunotherapeutic agent ipilimumab. In a randomized, controlled, phase III trial in metastatic melanoma, use of ipilimumab led to improved OS without improvement in median PFS/TTP, suggesting that this effect may be typical of immunotherapies as a class [39].

Patient selection in immunotherapy trials

Identifying the ideal patient population is particularly important in vaccine clinical trials, where only a subset of patients may benefit from treatment. In the current clinical trial model, new vaccines are usually tested in patients with late- or end-stage disease who have been heavily pretreated and have exhausted other treatment options. However, numerous vaccine studies (the GVAX [40, 41] and PSA vaccine trials [42] in prostate cancer, the idiotypic vaccine trials in follicular lymphoma [43], and adjuvant immunotherapy trials in melanoma [44]) have shown that immunotherapy is less effective in patients with heavy disease burden [45], providing a biological rationale for using therapeutic vaccines earlier in the disease process. Greater tumor burden leads to an increase in regulatory T cells (Tregs) [46, 47] and myeloid-derived suppressor cells, as well as increased levels of indoleamine-2,3-dioxygenase and negative regulatory cytokines such as transforming growth factor- β and interleukin-10, all of which can inhibit T-cell activation [48, 49]. Tumors may also downregulate expression of MHC molecules. In addition, patients with very aggressive cancer may not have enough time to develop a significant immune response and thus derive benefit from treatment.

Multiple prior chemotherapy regimens also negatively affect response to a cancer vaccine. In a phase II trial evaluating a vaccine that employed a canarypox virus vector encoding the

gene for the TAA carcinoembryonic antigen (CEA) plus the T-cell costimulatory molecule B7.1, patients who had progressed after several chemotherapeutic regimens were less likely to have measurable immune responses than patients with less prior exposure to chemotherapy ($p = 0.032$) [50]. Thus, the ideal candidate for treatment with a therapeutic cancer vaccine has slow-growing and/or low-volume disease, with minimal prior exposure to chemotherapy [51]. In clinical trial design, appropriate patient selection is a key to accurately assessing the clinical efficacy of therapeutic cancer vaccines, since the kinetics of clinical response following treatment with an active therapeutic vaccine may be unlike those of conventional cytotoxic therapies.

Identifying intermediate endpoints in immunotherapy

For the sake of patients and practitioners frustrated and anxious over the prospect of observing tumor growth while waiting for an immune-based treatment to show clinical benefit, standardized biomarkers to evaluate biologic response in the absence of clinical response are urgently needed.

Most vaccine strategies for solid tumors focus on inducing cellular immune responses by activating cytotoxic T cells ($CD8^+$) that can recognize tumor-specific TAAs in the context of MHC class I. Natural humoral responses to TAAs in cancer patients, as well as clinical results obtained with monoclonal antibodies, suggest that adequate humoral immune responses induced in an adjuvant setting could lead to improved outcomes. Studies of HER2/*neu* vaccine for breast cancer [52, 53] and CEA vaccine with or without GM-CSF in colorectal carcinoma [54] have shown a correlation between humoral responses and clinical benefit in some patients. Tregs, which play a fundamental role in immune homeostasis, can potentially influence immune-mediated therapies. Preclinical studies have shown that depletion of Tregs can enhance antitumor responses [55]. Therefore, depleting Tregs while administering a therapeutic cancer vaccine may enhance tumor antigen-specific T-cell responses [56, 57] that can be used as biomarkers.

The ELISPOT assay measures the response of cytotoxic T lymphocytes (CTLs) to specific TAAs in the form of gamma interferon production *ex vivo*, which correlates with CTLs' ability to lyse cells bearing such TAAs *in vivo* [58]. While ELISPOT can be an effective way of assessing immune response, it has serious limitations, including significant variability from institution to institution and the fact that the test may be restricted to patients with certain tissue types. Even if these shortcomings could be overcome [59], adequately assessing a dynamic immune response would still be complicated. As discussed above, if a vaccine designed to target a specific TAA evokes an immune response that leads to an attack on a different TAA via antigen cascade, or epitope spreading, it could be difficult to know which TAA the immune system is attacking and therefore which to assess. Epitope spreading is a common phenomenon in autoimmune disease and is the basis of continuing pathologic tissue destruction [60]. One of the first studies to observe the generation of epitope spreading after vaccination was the trial of an HER2-specific vaccine in breast cancer patients [61]. At a median follow-up of 112 months, 21 of 52 stage III and IV patients who had been vaccinated were still alive. The number of chemotherapy regimens prior to vaccination (HR 5.7; $p = 0.001$) and the development of epitope spreading after vaccination (HR 0.34; $p = 0.05$) were independent predictors of OS. The median OS for subjects who developed epitope spreading ($n = 33$) was 84 months vs. 25 months for the 16 subjects who did not develop epitope spreading [62]. As a further consideration, if immune response to only one specific TAA is assessed, the actual benefit of the vaccine may be underestimated.

The value of RECIST in immunotherapy trials is questionable in light of the biology of immune response after vaccination. A significant and potentially beneficial immune response may cause transient increases in the size of lymph nodes or tumor masses, which could be identified as progressive disease based on RECIST criteria. New response criteria, irRC, adapted from WHO response criteria were evaluated in recent studies with ipilimumab. Four distinct objective response patterns were described: immediate response, durable stable disease, response after tumor burden increase, and response in the presence of new lesions. While the latter two response patterns would be classified as progressive disease by traditional criteria, these patterns appear to be associated with favorable survival compared to patients with progressive disease by irRC (Table 2). To evaluate all observed response patterns, irRC should be employed in clinical trials of immunotherapeutic agents for cancer [15].

One of the major challenges in the evaluation of the efficacy of vaccine therapies has been the inability to consistently correlate treatment response to expected immune parameters. In the absence of reliable parameters for monitoring immune response, biomarkers are needed to predict which patients would benefit most from immune modulation and which patients could be most susceptible to immune-related adverse events [63]. These complex issues must be addressed if therapeutic cancer vaccines are to have broad clinical applications.

Conclusion

The kinetics of immunotherapeutic agents are different from those of cytotoxic agents. Cancer vaccines must induce cellular immune responses before they can effect changes in tumor burden or patient survival [32].

The current model for clinical trial design is to evaluate new drugs in patients with late-stage disease who have been heavily pretreated. However, this may not be the best way to evaluate therapeutic cancer vaccines, which are less effective in patients with heavy tumor burdens. Furthermore, patients who have undergone numerous prior chemotherapy regimens are less likely to mount an immune response to vaccine [10, 50]. Thus, the ideal model for vaccine trials would be to enroll patients with less disease volume and to set longer-term trial endpoints [51]. If we are to gain a clear understanding of the clinical benefit of therapeutic cancer vaccines, we must adapt the current paradigm that assesses radiographic, but not biologic, response [10]. These changes would allow investigators to accurately assess the potential clinical effectiveness of therapeutic cancer vaccines.

Much remains to be done. First, cellular immune response assays must be standardized and validated as reproducible biomarkers that can be correlated with clinical outcomes. Second, the new irRC, which are able to capture more complex response patterns, should replace RECIST in trials of immunotherapeutic agents. irRC assess tumor burden as a continuous variable, accounting for index lesions identified at baseline and new lesions that occur after initiation of treatment, based on bidimensional measurements of each lesion. Third, new statistical models that describe hazard ratios as a function of time and recognize differences before and after the separation of Kaplan-Meier curves should be developed and used to evaluate phase III trials.

With new clinical trials designed to address many of these issues, immunemediated antitumor treatments may one day be as common as targeted molecular therapies. Therapeutic cancer vaccines, with their low toxicity profile and ability to induce long-term immune responses that can enhance subsequent therapies, have the potential to transform our current approach to cancer treatment.

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Fig. 1.

Tumor growth is a dynamic biologic process that is the combined result of some cells dividing and other cells dying. Chemotherapy (red line) affects the tumor growth rate only while it is being administered, which may result in a dramatic but transient response. When chemotherapy is discontinued, the growth rate returns to its pretreatment slope, driven by the underlying tumor biology. Immunotherapy (blue line), on the other hand, can alter host biology by inducing an active antitumor immune response, including a memory response. This may not cause an immediate or dramatic change in tumor burden, but continued, cumulative pressure that slows tumor growth rate, especially if started early in the disease course, may lead to substantially longer overall survival. Arrow indicates initiation of treatment; cross indicates time of death from cancer. (Adapted from Madan et al. [29].)