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EXPERT REVIEWS

Prostate cancer vaccines: moving therapeutic vaccination forward in the post-Provenge™ era

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The focus of extensive research in the area of prostate cancer vaccines has led to the approval of the first therapeutic vaccine by the US FDA, sipuleucel-T. As our understanding of immunotherapy has increased, novel approaches have been investigated that have shown considerable promise. As the field has continued to evolve, questions have arisen regarding the potential role of immunotherapy: which populations of patients are most likely to benefit from immunotherapy and how and when should these therapies be administered? In addition, what are the best tools that can be used as surrogates to monitor immune responses to cancer vaccines that truly can give meaningful insight toward improving clinical outcomes? Finally, how can combination approaches be applied to prostate cancer vaccines in terms of both standard of care and experimental therapies? This review will address many of these important concepts with regard to prostate cancer vaccine therapy.

Keywords: immune monitoring • prostate cancer • therapeutic vaccines

The changing clinical & regulatory landscape on the road to Provenge™ approval

Prostate cancer, except for non-melanoma skin cancer, is the most common cancer in American men. In the USA, an estimated 240,890 cases will be diagnosed in 2011 and 33,720 deaths will occur [1]. The identification of tumor-associated and tumor-specific antigens in the 1990s launched an unprecedented enthusiasm to harness the immune system and develop targeted vaccines for the treatment of prostate and other cancers. Most prostate cancer cells and epithelial cells lining the acini and ducts of the prostate gland express prostate-specific antigen (PSA) [2]. In addition to PSA, other unique proteins that can be considered tumor-associated antigens (TAAs) include prostatic acid phosphatase (PAP) and prostate-specific membrane antigen, both of which are common targets for prostate cancer immunotherapy. Although many early clinical studies investigating a broad spectrum of cancer vaccines were often disappointing [3,4], a better understanding of basic immunologic principles has led to a variety of techniques for enhancing tumor-specific immunity and their potential subsequent translation into improved clinical outcomes. This is exemplified by the US FDA approval of sipuleucel-T (Provenge™) on April 29, 2010. The vaccine was the first licensed therapeutic cellular immunotherapy and a major milestone in the field of cancer immunotherapy. Importantly, approval of sipuleucel-T was based on a statistically persuasive and clinically meaningful 4.1-month improvement in median overall survival (OS) in the IMPACT Phase III trial [5]. The improvement in OS associated with sipuleucel-T has been reported to correlate with CD54 upregulation, a measure of the product's potency [6], and the development of antibody titers exceeding 400 at any time against the immunizing antigen PA2024 (a fusion protein) or PAP [5]. Interestingly, although strong T-cell proliferative response to PA2024 and PAP was also observed in patients receiving sipuleucel-T, no difference or association in survival was documented between patients who exhibited T-cell response to either antigen and those who did not.

Despite this singular achievement, it is important to realize that the landscape for

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prostate cancer therapy has changed considerably since the initial Phase I/II studies of sipuleucel-T were conducted when there were few effective FDA-approved treatments for men with metastatic castrate-resistant prostate cancer (CRPC). In 2004, docetaxel with prednisone was approved based on the results of two large randomized Phase III studies that showed a 2.4-month improvement in median OS in men with metastatic CRPC treated with this regimen [7,8]. Although it was not shown to alter the time to disease progression in the majority of patients treated, the approval of sipuleucel-T has resulted in an additional therapeutic option to further extend OS. However, it is important to acknowledge that the population for whom this therapy is indicated is limited to men with metastatic CRPC who are minimally symptomatic with no visceral metastases or asymptomatic before receiving docetaxel. Furthermore, there are now a number of recently approved new drugs for prostate cancer that have rapidly altered the clinical setting and potential evaluation of current and future therapeutic prostate cancer vaccines under development: cabazitaxel (Jevtana Injection™; approved by the FDA on June 17, 2010), a second-generation taxane, and abiraterone acetate (Zytiga™; approved by the FDA on April 28, 2011), a selective CYP-17 inhibitor – both of which are approved for use in patients with disease progression after treatment with docetaxel. Unlike sipuleucel-T, these compounds have demonstrated objective clinical responses in addition to increasing OS.

Consequently, as is the case with every advance in therapeutic options for a specific disease, the challenges in product clinical development to successfully meet the threshold for regulatory approval will increase in frequency and complexity for prostate cancer vaccines. Early studies will face increased pressure to generate solid data that permit modeling of clinical end points, estimation of the magnitude and timing of the vaccine effect size and identification of populations to enrich for that will allow generation of an efficient and informed Phase III study design. The delayed onset in effectiveness of many vaccines and immune-based therapies further intensifies the need to identify and validate biologic or molecular surrogates that are reasonably likely to predict clinical benefit and outcomes. These issues are further complicated by a clinical treatment background in which licensed agents are now available that result in objective responses based on tumor assessments in the context of an ever-increasing consensus that OS may be the most appropriate primary end point for immunotherapy clinical trials.

Learning from past failures: identifying populations that should be targeted for therapeutic vaccination

Both clinical and preclinical data suggest that vaccines are more effective in patients with lower tumor burden and less aggressive disease [9,10]. There are several possible reasons for this: activation of an immune response is more robust than the rate of tumor growth; in a large tumor, T-cell infiltration and penetrance may be insufficient for eradication; and immunosuppression in patients heavily pretreated with cytotoxic chemotherapy and radiation diminishes marrow reserves and the likelihood of adequate immune stimulation. In addition, in many cancers, clinical and metastatic disease progression has been associated with the induction of multiple immune-inhibitory pathways that influence tumor-specific responses. These include the induction of T-cell anergy as a result of dendritic cell (DC) dysfunction and MHC class I downregulation, release of immunosuppressive cytokines, T-cell exhaustion and tumor-specific immune suppression by T regulatory cells [11].

On the basis of these considerations, vaccine therapies may lead to better outcomes in adjuvant settings, while tumor burden is still relatively low, rather than in the setting of advanced metastatic disease, an observation underscored by the recent and prominent failure of several cancer vaccines. Two Phase III studies (VITAL-1 and VITAL-2) of GVAX® (Cell Genesys, Inc.), an allogeneic tumor cell vaccine consisting of two prostate cancer cell lines (LNCaP and PC-3) engineered to express human granulocyte macrophage–colony-stimulating factor (GM-CSF), failed to document a survival benefit of GVAX or GVAX/docetaxel over standard docetaxel/prednisone in patients with symptomatic metastatic prostate cancer. VITAL-2 was prematurely terminated because of safety concerns associated with increased deaths (n = 67) in the docetaxel–GVAX arm compared with the docetaxel–prednisone control arm (n = 47) [12]. Clinical development of GVAX was subsequently abandoned in October 2008 based on the results of a futility analysis of VITAL-1 that indicated the trial had less than a 30% chance of meeting its primary end point of improvement in OS. Similar to recent experiences with other immune-based therapies, the final Kaplan–Meier curves for the two treatment arms in VITAL-1 suggested a late favorable effect of GVAX immunotherapy on patient survival compared with chemotherapy, with the curve for GVAX patients crossing above the chemotherapy curve at approximately the same time median survival was reached in both treatment arms (21 months) [201]. Importantly, the clinical design and primary OS end points in these two large Phase III studies were supported by median survival results from two independent, multicenter Phase II trials in approximately 115 patients, underscoring the fact that even with solid Phase II data, results of Phase III immunotherapy trials can be unpredictable. Indeed, the list of randomized clinical trials of cancer vaccines with negative results reinforces the treacherous road of cancer immunotherapy clinical development and the accompanying persistent uncertainty surrounding the ultimate goal of regulatory approval [13]. Although many therapeutic vaccines targeted malignant melanoma (Melacine®, Canvaxin, Oncophage and GM2-KLH21) and pancreatic (PANVAC™-VF), renal (Oncophage and TroVax®) and other cancers (MyVax®, FavId™, GVAX and Theratope®), the failures of randomized clinical trials have been equally distributed among tumor types and among trials in patients with late (i.e., metastatic) and early disease (adjuvant setting).

An important question that arises then is, how can we predict who might benefit from a cancer vaccine? Historically, Gleason score had been used as a key prognostic indicator to predict clinical outcomes for primary prostate cancer. However, the limitations of this approach led to the development and subsequent

significant use of the Halabi nomogram [14] as a predictive indicator of OS of patients with metastatic CRPC. The Halabi nomogram is based on seven predictors of OS: presence of visceral disease, Gleason sum, performance status, PSA, lactate dehydrogenase, alkaline phosphatase and hemoglobin. Derived from the results of clinical outcomes in six separate Cancer and Leukemia Group B trials of 1101 patients with metastatic CRPC, this pretreatment prognostic model predicts OS based on the outcomes of patients receiving chemotherapy or hormonal-based treatment for metastatic CRPC and does not include untreated patients. This model was used to compare predicted survival with actual observed OS in the Phase II studies of GVAX, which together served as the preliminary signal that GVAX might be associated with improvement in survival outcomes [15]. More recently, an additional contemporary prognostic nomogram has been developed that incorporates a more comprehensive characterization of baseline parameters to predict clinical outcomes in men with metastatic CRPC [16]. This nomogram was based on the outcomes associated with the TAX327 clinical trial that randomized 1006 men with metastatic CRPC to receive docetaxel or mitoxantrone either weekly or every 3 weeks, each given with prednisone. This multivariate model identified several new independent prognostic factors predictive of clinical outcomes, including baseline PSA doubling time (PSADT) and other PSA kinetics. Several additional prognostic markers were also identified, including the presence of liver metastases, the number of metastatic sites (three or more), the presence of pain, the type of chemotherapy and the type of disease progression at baseline (measurable disease by imaging vs PSA biochemical recurrence only). The nomogram and multivariate model were found to have a bootstrap concordance index of 0.69, suggesting that further validation of this approach may be warranted by additional, prospective clinical trials.

Although used extensively in previous trials of cytotoxic, radiotherapeutic, surgical and even immune-based interventions, decreasing serum PSA concentrations do not seem to be a reliable surrogate end point for prostate cancer-specific mortality [17]. Thus, PSADT, or the change in PSA over time, has also emerged as a useful predictive marker for assessing disease outcome in patients with prostate cancer, particularly in the setting of biochemical recurrence – that is, stage D0 disease [18]. In general, an increased PSADT is associated with a longer time to metastasis and death from prostate cancer, whereas a PSADT of <3 months is associated with a poorer prognosis and an extremely high risk of disease progression in a variety of treatment settings (after definitive radiotherapy [RT] or radical prostatectomy, biochemical recurrence; and androgen deprivation therapy [ADT] after RT or radical prostatectomy). Although PSADT may ultimately prove to be a useful predictor of disease outcome in all stages of prostate cancer, there remains a general lack of consensus on its universal use or the exact method of calculation [18]. Despite these limitations, PSADT used as a stratification factor in clinical trials of cancer vaccines may help facilitate detection of a preliminary signal of an agent's activity in early-phase studies. Hence, it is highly likely that future trials of cancer immune-based therapies will find it increasingly useful to include prospective, planned cohort analyses accounting for Halabi nomogram and PSADT stratification.

Prostate cancer vaccine platforms: the merits & challenges of different therapeutic vaccine approaches

There are a wide variety of platforms and immunogens that have been extensively studied in therapeutic vaccine platforms: whole tumor cell vaccines; DC-based platforms; peptide and fusion proteins codelivered with adjuvants; and viral vectors that serve as delivery vehicles for TAAs. In addition to classic peptide and protein immunogens, tumor lysates, tumor mRNA and DNA have all been used in prostate cancer vaccines. PSA, PAP and prostate-specific membrane antigen have been the prostate antigens most commonly targeted, but tumor antigens found in a broad spectrum of tumors, including carcinoembryonic antigen, LAGE-1, MUC-1/2, NY-ESO-1 and telomerase, have also been used. Indeed, a DNA vaccine targeting PAP has been shown to induce PAP-specific IFN-γ $CD8^+$ T-cell responses and $CD4^+$ and $CD8^+$ T-cell proliferative responses associated with an increase in postvaccination PSADT in men with stage D0 prostate cancer [19].

Autologous whole tumor cell & tumor lysate vaccines

Autologous whole tumor cell or tumor lysate vaccines are derived from the patient's tumor cells in a time-consuming process that requires a significant amount of tumor tissue. The advantage of autologous whole tumor cell/lysate vaccines is that they target the patient's TAAs and present a broad selection of antigens to the immune system, thereby precluding the need for antigen preselection. One disadvantage, however, is that antigens could be diluted by other cellular components. Other disadvantages include the lack of standardized measures for vaccine potency and the difficulty in evaluating antigen-specific immune responses postvaccination because, for the most part, the tumor antigens from these vaccines remain unknown. Despite these challenges, there remains significant interest in pursuing this type of approach because impressive survival outcomes have been described for a tumor lysate vaccine using autologous DCs (DCVax-Brain™; Northwest Biotherapeutics, Inc.) for glioblastoma multiforme, a uniformly lethal and highly aggressive disease [20,21].

Allogeneic tumor cell vaccines

Allogeneic whole tumor cell vaccines that can also be delivered as lysates are derived from various tumor cell lines and are usually easier to generate. Whole tumor cells are rendered replication defective by radiation and are frequently combined with nonspecific immunostimulants [22–24]. This approach is exemplified by the GVAX platform discussed earlier in this review that combines two different prostate cancer cell lines modified to secrete GM-CSF. The rationale for the putative efficacy of allogeneic tumor cell vaccines is that tumor-specific antigens contained in the vaccine are often shared in common by different patients. These shared antigens are potentially immunogenic and can enhance the host's ability to generate an effective immune response. Importantly, because they are allogeneic, these vaccines

are able to break tolerance to self-tumor antigens and have the advantage of being applicable to a broader number of patients regardless of the availability of bulky, autologous tumor. However, a major disadvantage of whole cell tumor vaccines is that tumor cells, even when foreign, are generally not immunogenic. Thus, these tumor vaccines are frequently coformulated with or engineered to express immunostimulatory proteins such as GM-CSF and heat shock protein to improve immunogenicity [25].

DC vaccines

DCs serve as a bridge between the innate and adaptive immune system and in turn play a critical role in the activation of naïve CD4 and CD8 T cells. Hence, researchers in cancer immunotherapy have focused a tremendous amount of investigative effort to delineate and understand DC biology, activation, maturation and antigen presentation. Because of the pervasive dysfunction of DCs *in vivo* as a consequence of the immune dysregulation associated with high tumor burdens [26], they have been the platform of choice for delivery of many tumor cell lines (LNCaP, PC-3), peptides, proteins, lysates, mRNAs and viral vectors expressing TAAs [27,28]. Sipuleucel-T is a prototype of this platform approach: even though it is labeled as an autologous cellular immunotherapy, its generation uses similar maturation agents and pathways common to DC vaccines [29]. In addition to its documented activity in men with minimally symptomatic CRPC, sipuleucel-T has also been investigated in a randomized trial in men with androgen-dependent prostate cancer [30]. Men with a persistently increased serum PSA after radical prostatectomy received ADT for 3–4 months and were then assessed for time to biochemical failure, defined as a serum PSA 3.0 ng/ml. Although no significant difference was seen in time to biochemical failure, there was an increase in PSADT observed in patients randomized to sipuleucel-T after testosterone recovery, indicating that further studies may be warranted in this population to further assess other clinically relevant outcomes. Despite their preliminary activity, production of personalized autologous DC-based vaccines requires *ex vivo* stimulation of peripheral blood mononuclear cells with cytokines, which can be costly and time consuming. In addition, the number of cells and cellular composition and functional potency may vary significantly because each product is patient-specific, making it much harder to characterize and standardize product characteristics that may correlate with clinically meaningful outcomes.

Peptide- & protein-based vaccines

With the advent and discovery of tumor antigens, peptide- and protein-based immunogen platforms were among the first approaches to be pursued in cancer immunotherapy. What became evident fairly quickly was that without codelivery of adjuvants to enhance presentation of vaccine antigens to the immune system, both peptides and proteins were often poorly immunogenic. DNA vaccines, heralded for their simplicity and low production costs, were the least immunogenic of all, requiring large amounts of product, administration by electroporation and co-inoculation with plasmids expressing cytokines and CpG motifs to optimize immune responses [31]. Adjuvants are pharmacologic or immunologic agents that modify the effects of other agents while having few, if any, independent direct effects when given alone. They are often included in vaccines to enhance the magnitude, breadth and longevity of a recipient's response to a supplied antigen and to direct the quality of the immune response induced by vaccination. The goal is to use compounds that achieve a potent adjuvant effect with minimal reactogenicity, toxicity or lasting effects on the immune system on their own. BCG, keyhole limpet hemocyanin, Montanide® ISA51 and poly-IC-LC are among the most common adjuvants currently used in cancer immunotherapy platforms in addition to cytokines (IFN-γ, IL-12, IL-15 and GM-CSF), immunostimulatory complexes (ISOCOMS), costimulatory molecules (e.g., ICAM-1, lymphocyte function-associated antigen-3 and B7.1 presently used in the PSA-TRICOM vaccine) and proprietary adjuvants (Alhydrogel®, AP1903 and AS15) that are used to ensure immune recognition of the desired immunogen [32]. Adjuvants can be classified into three groups: active immunostimulants that increase immune response to the antigen; carriers that are immunogenic proteins that provide T-cell help; and vehicle adjuvants that are oil emulsions or liposomes, which serve as a matrix for antigens in addition to stimulating the immune response [33]. A summary of commonly used cancer vaccine immune adjuvants is shown in Table 1.

In keeping with the theme of maximizing immunogenicity of peptide and protein tumor antigens through adjuvant or DC platforms, we are conducting a prospective, randomized Phase I pilot study of T-cell receptor alternative reading frame protein (TARP) peptides delivered in combination with Montanide® ISA51 VG adjuvant plus sargramostim (GM-CSF) or as a custom-made, patient-specific autologous peptide-pulsed DC vaccine in men with androgen-sensitive stage D0 prostate disease [34]. These men with PSA biochemical recurrence and no evidence of metastatic disease constitute a population with normal (or near normal) immune function. TARP is expressed by both normal and malignant prostate tissue and is overexpressed in 95% of prostate cancer specimens [35–37], making it a good target antigen for therapeutic vaccination. The current vaccine platform consists of two HLA-A*0201 TARP peptide epitopes documented in preclinical laboratory studies in mice and human cell lines to generate cytolytic CD8 T-cell responses: TARP27-35 and epitope-enhanced TARP29-37-9V [38]. A total of 1.1 mg of each peptide is delivered per vaccine given every 3 weeks for an initial course of five vaccinations with subsequent options for a sixth dose of vaccine at 36 weeks based on TARP-specific immune response or PSADT criteria; all patients subsequently undergo booster vaccination at weeks 48 and 96. Primary study end points include safety and immunogenicity, with secondary evaluation of the impact of TARP vaccination on PSADT. To date, TARP vaccination has been safe and well-tolerated, with adverse events (AEs) limited to local injection-site reactions. A preliminary interim analysis performed on the first 26 patients documented that TARP vaccination was associated with a statistically significant slowing in the rate of PSA increase as measured by PSADT and slope log (PSA) at 24 and 48 weeks in the majority of patients, with no

Table 1. Common cancer vaccine immune adjuvants.

Adjuvant emulsions	Bacterial-derived adjuvants	Costimulatory adjuvants	Cytokines as adjuvants
IFA	BCG	B7.1/B7.2	GM-CSF
Montanide ISA51	CpG	ICAM-1	IFN-α
– Adjuvant 65 – Lipovant	MPL-A (MPL – a derivative of LPS)	LFA-3	IFN-γ IL-12 IL-15
Mineral salt adjuvants	*Polymeric microsphere adjuvants*	*Tensoactive adjuvants*	*Proprietary adjuvants*
Alum salts: – Aluminum phosphate – Aluminum hydroxide	Polymicrospheres (DL-lactide-coglycolide)	QS21 (derived from Quil A)	Alhydrogel® ASO2, ASO4 Brenntag GSK Hiltonol® Oncovir (poly-ICLC)

GM-CSF: Granulocyte macrophage–colony-stimulating factor; IFA: Incomplete Freund's adjuvant; LFA-3: Lymphocyte function-associated antigen-3; LPS: Lipopolysaccharide; MPL: Monophosphoryl lipid.
Adapted from [32].

differences between the two study arms detected. Analysis of immunogenicity to TARP WT27-35 and EE29-37-9V vaccine epitopes and cross-reactivity to native WT29-37 as assessed by IFN-γ enzyme-linked immunosorbent spot (ELISPOT) and TARP tetramer assays is ongoing.

Viral vector vaccines

Poxviruses such as the vaccinia virus have been extensively studied and have been shown to induce a strong local response at the site of inoculation. In patients previously inoculated with vaccinia, the question arose as to whether these patients could mount sufficient immune responses compared with those who were vaccinia naïve. This was resolved by administering higher doses of recombinant vaccinia [39]. However, the efficacy of vaccinia vectors has been limited by the development of neutralizing antibodies to vaccinia after one or two injections, resulting in a decline in antigen-specific immune responses caused by the vector. It was observed that this phenomenon could be overcome by using a replication-defective avipox virus such as fowlpox as a boost after a priming vaccination with vaccinia [40–42]. Replication-defective vectors have the advantage of superior safety because they are unable to replicate in the human body but are able to infect human cells and express their encoded transgenes for 2–3 weeks before cell death. PSA-TRICOM is a poxvirus-based vaccine expressing PSA and three costimulatory molecules (B7.1, ICAM-1 and lymphocyte function-associated antigen-3). A small Phase II study in patients with metastatic CRPC evaluated 32 patients treated with PSA-TRICOM on a prime-and-boost schedule. The median OS for all patients was 26.6 months compared with a predicted survival of <18 months [43]. This preliminary observation was subsequently confirmed in a larger, blinded, randomized Phase II study examining PROSTVAC-VF (vaccinia-PSA-TRICOM and fowlpox-PSA-TRICOM) in 125 patients with minimally symptomatic metastatic CRPC [44]. Although there was no difference seen in the primary end point of progression-free survival (PFS) between patients who had received PROSTVAC-VF vs control vector, at 3 years of post-study follow-up, the median OS was 25.1 months vs 16.6 months, respectively, an improved survival difference of 8.5 months.

Adenoviruses are also among the select group of viral vectors that have been extensively studied in the delivery of cancer vaccine antigens, including MART-1 and gp100 in the treatment of patients with metastatic melanoma [45,46], HPV E7 CD-40 ligand fusion protein in preclinical animal models [47], and full-length p53 delivered by transduction of autologous DCs with an adenoviral vector in patients with extensive small-cell lung cancer receiving subsequent chemotherapy [48]. Of interest, the coxsackie and adenovirus receptor has been shown to mediate adenoviral entry into tumor cells. Unlike cells derived from other malignancies, loss of coxsackie and adenovirus receptor expression in human and murine prostate cancers seems to be comparatively infrequent and is not associated with the loss of MHC class I expression [49]. These observations suggest that cancer vaccines based on modifying whole prostate cancer cells using recombinant adenoviral vectors could be feasible without detrimental effects to the target cell or the viral vector itself. Importantly, a Phase I trial of a single dose of an adenoviral/PSA (Ad/PSA) vaccine in 32 men with measurable metastatic CRPC was associated with an increase in PSADT in 48%, whereas 55% survived longer than predicted by the Halabi nomogram [50]. Immunologic assessment revealed that anti-PSA antibodies were produced by 34% of patients and anti-PSA T-cell responses were detected in 68%. Further development is proceeding with this adenoviral vector approach in two parallel Phase II trials investigating three doses of Ad/PSA. Protocol 1 (NCT00583752) examines vaccination in men with recurrent prostate cancer after definitive treatment who undergo randomization to receive 108 pfu of Ad/PSA vaccine at days 0, 30 and 60 or delay vaccination until 14 days after the start of ADT, whereas in protocol 2 (NCT00583024), men with CRPC receive the vaccine monotherapy at days 0, 30

and 60 [51]. Preliminary reported results demonstrate that this Ad5/PSA multidose regimen is associated with stable or decreased PSA and PAP levels in a majority of patients. These findings, along with the documented early preliminary activity of PSA-TRICOM, are reasons for cautious enthusiasm regarding the potential for viral vector vaccines to further advance the field of prostate cancer immunotherapy.

The challenges that remain: documenting evidence of cancer vaccine clinical activity early in development

Challenge 1: clinical trial end points for cancer vaccines & immune-based therapies

Regular or accelerated regulatory approval of oncology drug products and biologics is based on the risk–benefit evaluation of end points that demonstrate that the agent provides clinical benefit as evidenced by a longer or better life or a favorable effect on an established surrogate for a longer or better life [52]. These clinical end points, alone or in combination, include OS, disease-free survival (DFS), PFS or time to progression (TTP), overall response rate and, rarely, other novel end points. Data for regulatory approval are typically generated from randomized studies that examine the activity of the investigational agent vs a comparator control that may be a placebo, standard chemotherapy, or best supportive care. However, regulatory approval for clinical indications has also been given based on data from single-arm studies with no comparator group. In a recent review of regulatory actions on 58 indications for oncology and hematology drug products between July 2005 and December 2007, 37 of 53 (70%) approved indications were based on data from randomized studies, whereas the remaining 16 (30%) approved indications were based on single-arm studies [52]. Importantly, 44 of the 53 approved indications were based on the results from a single, pivotal study, highlighting the critical importance of trial design, patient population and selection of primary efficacy end points to achieve agent approval. Although FDA regulations require data from more than one independent study to establish the efficacy of a new agent, as previously noted, few marketing applications for oncology products are submitted based on more than one study, reinforcing the need for significant scientific and statistical vetting of trial designs under consideration before the huge risk and financial investment in large randomized Phase III studies is undertaken.

Ultimately, approval is based on an agent's demonstration of a clinically meaningful superior treatment effect based on the primary end point of a pivotal study and demonstration of a corroborative treatment effect in secondary end points. In addition, the product safety profile must be acceptable in view of the magnitude of the clinical efficacy observed and the disease or condition being treated. OS is the platinum standard for demonstrating direct clinical benefit because it is an unequivocal outcome measure evaluated on a continuous time scale that allows for precise accuracy of the time of the event. The surrogate end points of DFS, PFS and TTP are not measured continuously and, consequently, the exact day of recurrence or progression cannot be accurately captured in studies that use these outcomes. In addition, assessment of progression in PFS and TTP depends on the frequency and completeness of tumor assessments and selection of target lesions used in evaluation, that is, Response Evaluation Criteria in Solid Tumors (RECIST), in addition to other physical examination, and laboratory and radiologic documentation. Overall response rate, the only end point that can be reliably assessed in single-arm trials, must be durable and of sufficient clinically meaningful magnitude, although 'clinically meaningful' is still subject to interpretation. Importantly, recent approval of both chemotherapeutic- and immune-based agents has revealed that improvements in OS may not be associated with changes in PFS or TTP, whereas statistically significant changes in DFS or PFS may or may not be associated with improvements in OS. Sipuleucel-T is an example of the former: improvement in OS in the Phase III IMPACT trial was not associated with an improvement in PFS [5]. In contrast, panitumumab for refractory colorectal cancer shows minimal but statistically significant changes in PFS with no difference observed in OS [53].

On the basis of published studies of cancer immune-based therapies recently approved or still under investigation, it has been suggested that OS may be emerging as the most appropriate end point in immunotherapy clinical trials [54] for several reasons: objective responses to immune-based therapies generally take longer relative to chemotherapy; patients receiving immune-based therapies sometimes demonstrate 'disease progression' by standard RECIST criteria before subsequent regression and clinical improvement; and, as previously noted, improvements in OS are often not accompanied by improvement in PFS or TTP. This paradoxical observation to the well-established and widely accepted treatment paradigm that agents should initially decrease tumor volume (objective responses) and impact disease progression (PFS, TTP or DFS) suggests that vaccines and immune-based therapies have distinctly different functional dynamics *in vivo*. Although OS is an unambiguous outcome that provides direct evidence of clinical benefit, large studies are generally necessary to show modest improvements in OS and longer follow-up is necessary for OS than for other end points such as PFS. In addition, there may be a delayed effect in the separation of survival curves after treatment randomization. As summarized in Table 2, separation in survival curves was seen at ~4 months with ipilimumab [55], at ~8–12 months in the trials of sipuleucel-T [3,5], and not until 14 months in a randomized study of PROSTVAC [44], whereas differences in OS were seen as early as 3 months with abiraterone acetate [56]. This means that larger changes in the OS end point after separation are needed to compensate for no effect on the end point before separation. An additional conundrum with the use of OS as an end point in immunotherapy trials is that separation in survival curves is most likely to occur and show the maximum difference in patients who have the greatest risk of disease progression. However, the patients at greatest risk are in turn likely to have higher tumor burdens and greater immune dysfunction and are less likely to respond to immunotherapy.

The greatest challenge to the continued development of therapeutic cancer vaccines and immune-based therapies is documenting evidence of preliminary activity early in the clinical

Table 2. Improvements in overall survival end points associated with immune-based, chemotherapeutic and hormonal agents.

	Trial type (n = enrolled)	Study population	Improvement in median OS	Difference in median OS?†			Ref.
				At 6 months?	At 12 months?	At 24 months?	
Immune agent							
Ipilimumab	Phase III (n = 676)	Stage III/IV melanoma	Alone 3.7 months (10.1 vs 6.4 months)	Yes	Yes	Yes	[48,55]
				Difference in OS at ~4 months			
			w/vaccine 3.6 months (10.0 vs 6.4 months)	No	Yes	Yes	
				Difference in OS at ~8 months			
Sipuleucel-T vs placebo	Phase III (n = 512)	Minimally or asymptomatic mCRPC	4.1 months (25.8 vs 21.7 months)	No	Yes	Yes	[4,5]
				Difference in OS at ~12 months Earlier studies: difference in OS at ~8 months			
PSA-TRICOM vs control vector	Phase II (n = 125)	Minimally or asymptomatic mCRPC	8.5 months (25.1 vs 16.6 months)	No	Yes	Yes	[5,49]
				Difference in OS at ~14 months			
Chemotherapy/hormonal agent							
Docetaxel q3 weeks plus prednisone vs mitoxantrone plus prednisone	Phase III (n = 1006)	Advanced mCRPC	2.4 months (18.9 vs 16.4 months)	No	Yes	Yes	[5,7]
				Difference in OS at ~8 months			
Docetaxel q3 weeks plus estramustine vs mitoxantrone plus prednisone	Phase III (n = 770)	Advanced mCRPC	1.9 months (17.5 vs 15.6 months)	Yes	Yes	Yes	[6,8]
				Difference in OS at ~6 months			
Abiraterone acetate vs placebo	Phase III (n = 770)	Advanced mCRPC, before docetaxel	3.9 months‡ (14.8 vs 10.9 months)	Yes	Yes	Yes	[52,56]
				Difference in OS at ~3 months			

†Separation in Kaplan–Meier curve estimates of the probability of OS in groups based on visual review of curves in references cited.
‡Initial analysis when 552 events had occurred.
mCRPC: Metastatic castrate-resistant prostate cancer; OS: Overall survival; q3: Every three.

development of a product. Although improvement in OS has been established as the sentinel standard for regulatory evaluation with the approval of sipuleucel-T, OS is not a clinically feasible primary end point for first-in-human Phase I/II trials. Compounding this challenge is the observation that end points based on objective tumor assessments are unlikely to reliably serve as harbingers of early clinical activity in Phase I or II studies because, to date, neither sipuleucel-T nor PROSTVAC studies demonstrated any impact on TTP or DFS. This is a critical issue for the entire field of cancer immunotherapy because early positive outcomes are needed to generate sufficient evidence of activity to warrant moving forward with the further expense of larger clinical trials of prolonged duration to capture OS outcomes. Finally, there is a massive lack of validated disease surrogates or immunologic correlates that are predictive of clinical outcomes. These challenges underscore the need for truly novel approaches to assessing the clinical activity of therapeutic cancer vaccines.

The first approach is to ensure that patients, who may achieve clinical benefit from cancer vaccines in the form of improved survival, are not prematurely removed from studies for failure to objectively respond or even what may seem to be disease progression on conventional radiologic imaging studies. Currently, no standardized system has been implemented to measure the multiple patterns of response that may be associated with immunotherapy. Fundamentally, it is critical to remain cognizant that all assessments of the impact of therapeutic vaccination on clinical outcomes and parameters are by their very nature indirect. As shown in Figure 1, therapeutic vaccination may or may not induce an immune response, which in turn may or may not be associated with an anti-tumor response that may or may not be associated with measurable tumor regression. Importantly, it is conceivable that induced immune responses may not necessarily be measurable by the current repertoire of immune assay and molecular diagnostic tools at our disposal. In addition, when an anti-tumor response is first generated, it may potentially result in an increase in tumor volume as a result of antigen-specific lymphocyte infiltration of tumors and local inflammatory changes associated with tumor killing, which in turn causes monitored lesions to

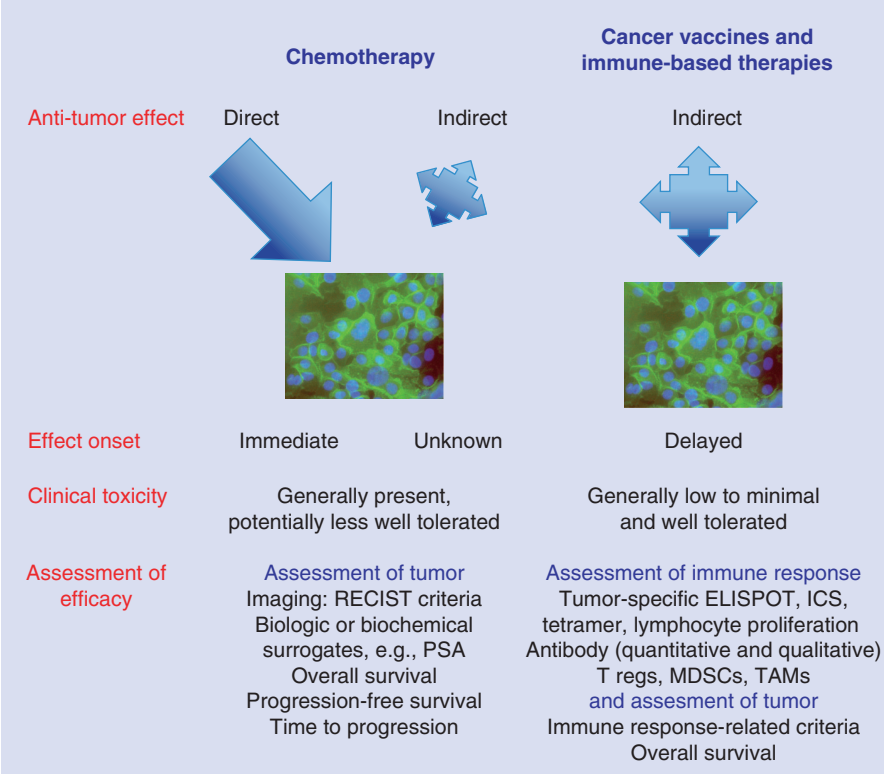

Figure 1. Anti-tumor effect and assessment of chemotherapy vs immune-based therapies.
ELISPOT: Enzyme-linked immunosorbent spot; ICS: Intracellular cytokine staining; MDSC: Myeloid-derived suppressor cell; PSA: Prostate-specific antigen; RECIST: Response Evaluation Criteria in Solid Tumors; TAM: Tumor-associated macrophage.

appear larger or causes the appearance of new lesions related to previously undetectable micrometastatic disease [57]. In addition, existing lesions may be slow to regress after immunotherapy as supported by multiple reports of patients taking immune-based therapies for progressive disease, only to have lesions subsequently regress or even resolve completely [58]. Immune response-related criteria are a novel way of systematically addressing unique patterns of response that may reflect the unusual biology of therapeutic vaccination. These criteria allow the capture of additional response patterns observed with immune therapy beyond those traditionally described by the RECIST [59,60] or WHO criteria [61] that may be associated with favorable OS, including conventional responses, delayed responses and durable stable disease. Standardized implementation of immune response-related criteria in prospective clinical trials of cancer vaccines and immune-based therapies is necessary to validate their positive predictive value and clinical correlation with OS end points.

Clearly, novel paradigms are needed to assess early and establish definitive vaccine activity in the treatment of solid tumors. Examination of tumor growth rate and regression rate constants has been proposed as a possible indicator of therapeutic efficacy [62]. Using a two-phase mathematical equation generated from PSA measurements obtained before and during therapy in five Phase II studies conducted at the National Cancer Institute in patients with metastatic CRPC yielded data on concomitant PSA growth and regression rate constants. Growth rate constants correlated with survival, except in patients receiving PROSTVAC therapy, where prolonged survival was documented, presumably as a consequence of vaccine-induced immunity that developed subsequent to vaccine administration. Specifically, the PROSTVAC vaccine seemed to have provided marked clinical benefit that was not apparent during vaccination but was consistent with the subsequent development of a beneficial immune response. Similar to immune response-related criteria, growth rate constants will need to be prospectively evaluated as a mathematical surrogate for survival and should be examined in clinical trials of multiple immune-based therapy platforms in a variety of solid tumors to ensure their robust validation as a potential important new efficacy end point.

There is also a need to use innovative clinical trial designs to evaluate new immunotherapy products. Clinical studies with adaptive sequential designs can provide decision rules for terminating a trial if a predefined magnitude of efficacy or futility is demonstrated. However, given the general trend of delayed responses to immune-based therapies, we must be careful to avoid designing studies that predispose treatment platforms to be prematurely classified as failures because of a lack of early signal activity and delayed separation of Kaplan–Meier survival curves in randomized immunotherapy trials. To that end, altered statistical models describing hazard ratios as a function of time and recognition of differences before and after separation curves may allow improved planning of Phase III trials [63]. However, even if these approaches are adopted, the hurdle of documenting sufficient preliminary activity of an immune-based therapy in first-in-human and early Phase I/II studies will remain. Although generally well-tolerated compared with chemotherapy and, hence, attractive to patients, vaccine therapies may also be associated with the induction of an atypical or "inconvenient immune response" that potentially inhibits or interferes with pre-existing host anti-tumor immunity. Specifically, poorly immunogenic or irrelevant vaccine antigens that induce overabundant quantities of nonspecific or functionally ineffective memory cells could potentially displace or suppress beneficial pre-existing memory responses and, in turn, accelerate cancer progression [13]. It is also important to acknowledge that vaccine immunogens may induce tolerogenic rather than cytolytic anti-tumor responses. Consequently, the development of reliable biomarkers to identify appropriate patient populations and vaccine antigens is pivotal to the prevention and minimization of potential vaccine-specific AEs.

Challenge 2: lack of correlation of immunologic end points with clinical outcomes

Unlike prophylactic vaccination against infectious diseases, in which a specific immune response directed against the pathogen of interest can be detected that correlates with therapeutic clinical activity (usually a pathogen-specific antibody response), identification of immunologic end points that consistently correlate with clinical outcomes in clinical trials of cancer vaccines has been sorely absent. This in part may be attributed to a lack of standardization in immune monitoring or the use of a wide variety of methodologic assays that are specific to a vaccine immunogen, platform or unique immunotherapy approach. Indeed, the literature is replete with examples of clinical studies of therapeutic vaccines in which vaccine-induced immune responses are detected by *in vitro* assays in a significant number of patients who ultimately have limited or no objective clinical responses to vaccination [64–66]. Furthermore, the kinetics of immune responses associated with vaccination are by nature indirect and differ significantly from chemotherapies, which act directly to exert anti-tumor effects. Lack of immunologic correlation may also result from weak immunogens that fail to induce robust anti-tumor responses or result in the induction of tumor-specific responses that are not detected by the current repertoire of immunologic monitoring assays commonly used to characterize vaccine activity. These assays include ELISPOT assays for different cytokines (typically IFN-γ), tetramer-based assays, intracellular cytokine staining by flow cytometry, lymphocyte proliferation assays and multiplex assays for serum cytokine and chemokine profiles after vaccination. Furthermore, the analysis of single or even multiple assay parameters *ex vivo* may still fail to sufficiently reflect the complex interactions that occur between the immune system and tumors *in vivo*.

Although historical approaches to immune monitoring have typically focused on characterizing and measuring the positive induction of cellular or humoral immune responses, an increasing appreciation is emerging for the need to assess and overcome the negative regulatory responses that prevent the immune system from successfully attacking self-tumor antigens. In addition to suppressive cytokines such as TGF-β, IL-10 and IL-13 and membrane proteins PD-1 and cytotoxic T-lymphocyte antigen 4 (CTLA-4), a functionally heterogeneous group of cells has emerged that are now understood to play key roles in dampening the immune response against tumors that includes T regulatory cells (Tregs), myeloid-derived suppressor cells and tumor-associated macrophages. There is an emerging opinion that the impact of therapeutic vaccination on both negative regulatory and positive stimulatory responses needs to be examined for a more comprehensive assessment of vaccine activity that may subsequently allow for better correlation with clinical outcomes [67].

Although the fundamental need to overcome and break self-tolerance to tumor antigens is a universally acknowledged and accepted scientific paradigm, characterization of innate immune responses after administration of cancer vaccines is uncommon. Relative to adaptive immune responses, there is even less consensus within the immunology community about how innate immunity should be routinely characterized and measured. In addition, until recent years, outside of allogeneic tumor vaccines, only a limited number of vaccine platforms have included components or reagents designed to trigger innate immunity at the initial vaccine presentation. Thus, for most of the first 30 years in the field of cancer immunotherapy, efforts have been focused on producing a clinically efficacious, functionally specific adaptive anti-tumor response in the vacuum of any triggers of innate immunity or interruption of self-tolerance. This may in part account for the negative outcomes that have been associated with the majority of immunotherapy clinical trials to date. Importantly, immunologic parameters may not correlate with clinical outcomes because tumor cells may also continue to evolve and escape in response to naturally occurring selective immune pressures and those induced by vaccination. Despite the tremendous research investment in cancer immunotherapies and vaccines, the issue of what immune responses to measure, how to measure them and when to measure them in relation to our therapeutic interventions remains the subject of intense and ongoing scientific deliberation.

Challenge 3: lack of assay standardization in obtaining immunologic end points

A major focus of the efforts to improve end points for cancer immunotherapy trials involves addressing the standardization and validation of immunologic assay readouts. As an example, although IFN-γ ELISPOT is one of the most common immune assays examined in cancer vaccine trials, no consensus has been reached on how to determine whether a positive immune response has been detected based on ELISPOT assay raw data and criteria can vary widely on how a positive response is defined [68]. In fact, the conduct of two large-scale international ELISPOT proficiency panels demonstrated a wide degree of variability in the detection of CMV and CMV, EBV and Flu peptide pool reactivity using a standard set of predefined donor peripheral blood mononuclear cells, especially in the detection of low responders [69]. Detailed surveys of laboratory practices revealed differences in assay protocol choices and laboratory practices that had marked effects on overall assay outcomes and led to recommendations for the establishment of standard operating procedures for ELISPOT testing that resulted in improved performance outcome among sites with the second round of proficiency testing. The critical importance in addressing these types of issues is highlighted in recommendations from a workshop representing academic, biopharmaceutical, regulatory and government research stakeholders [70]. These recommendations include following accurate, precise and reproducible immune assay protocols; the use of functional assays for primary immunology readouts in clinical trials; utilization of central laboratories for immune monitoring of large, multi-institutional trials; and standardized testing of multiple phenotypic and functional potency assays specific to any cellular product. Furthermore, standardized reporting of assay methodology will permit the development of novel assays for immunologic testing and provide additional insights into immunity in

patients with cancer and how to effectively direct and enhance it to eradicate tumors using immunotherapy.

Cancer immunotherapy plus: the tantalizing promise of combination therapy

Both preclinical animal model and human studies suggest the potential for synergistic effects resulting from the combination of immunotherapy and conventional treatments for prostate cancer such as radiation, ADT and certain chemotherapies.

Radiation plus vaccine

Independent of its direct, cytotoxic effects, radiation therapy has also been documented to have indirect, immune-mediated antitumor effects. Indeed, it has been known that ionizing radiation can reduce tumor growth outside the field of radiation, a phenomenon known as the abscopal effect described by Mole in 1953 [71] and observed in a wide variety of tumors, including lymphoma, papillary adenocarcinoma, malignant melanoma and hepatocellular carcinoma [72–75]. Studies in mice confirm that the abscopal effect is, in part, immune-mediated, and that T cells are required to mediate distant tumor inhibition induced by radiation [76]. These radiation effects are presumably related to inflammatory and maturation signals that are generated by dying cells [77–80], which can result in the activation of dendritic and tumor-specific T cells and markedly increase the permeability of solid tumors to these effector populations [81,82]. Furthermore, radiation therapy can alter the tumor cell phenotype with respect to MHC class I expression and molecules such as FAS (CD95, APO-1), thus potentiating anti-tumor immune effects [83–86]. Synergism of radiation therapy in combination with therapeutic vaccination has been observed in human clinical trials. This was observed in a randomized Phase II trial in which patients with prostate cancer were randomized to receive radiation therapy with vaccine vs radiation therapy alone. Increases in PSA-specific T cells occurred in 13 of 17 patients in the combination arm. No increase was observed in the RT-only arm [87]. Furthermore, patients receiving the combination therapy were able to mount immune responses to additional tumor antigens found on the tumor that were not contained in the vaccine. Future studies should be designed to try to determine how immunotherapy can be optimized to take advantage of these basic biologic observations in patients undergoing radiation therapy for high-risk localized prostate cancer with a focus on clinical outcomes as a primary end point. In addition, investigation of these therapies may also prove useful in the adjuvant setting for patients who have completed either local radiation or surgical therapy and whose immune systems are less likely to have dysfunctional impairment and a greater ability to optimally respond to vaccination.

ADT plus vaccine

Another therapeutic approach that has been noted to potentiate immune responses is ADT [88,89]. Androgen ablation decreases $CD4^+$ T-cell tolerance to a prostate cancer-associated antigen in an autochthonous mouse prostate cancer model. In this model, adoptively transferred $CD4^+$ T cells responded to specific vaccination after androgen ablation, but not in intact tumor-bearing mice [83]. Other murine studies have demonstrated that thymic regeneration has been reported in aging mice 4 weeks before castration, with an increase in MHC class II expression in thymocytes compared with that in young adults [90]. Immune effects of ADT have been described in several human studies. In one report, T-cell infiltration of $CD4^+$ lymphocytes was observed 1–3 weeks after ADT [91]. In another study, patients receiving neoadjuvant ADT for prostate cancer demonstrated an increase in tumor-associated antibody responses [92]. Finally, a vaccine trial consisting of patients before prostatectomy receiving sipuleucel-T vaccine after 3 months of ADT showed a trend in time to PSA progression compared with patients who did not receive vaccine [93].

Chemotherapy plus vaccine

There are mounting data challenging the dogma that chemotherapy agents are immunosuppressive and thus cannot be combined with vaccine therapy. An alternative perspective is that chemotherapy may induce anticancer immune responses as a consequence of immunogenic cell stress and death and increase tumor cell susceptibility to lysis and/or induce indirect immunostimulatory effects [94]. Both *in vitro* and *in vivo* studies have demonstrated that certain chemotherapy agents can enhance immune responses through upregulation of MHC class I expression and by increasing immune stimulatory cytokines and decreasing regulatory T cells [95,96]. In the clinic, a randomized Phase II study was performed to determine the immune effects of docetaxel combined with a poxviral–PSA vaccine compared with vaccine alone in patients with metastatic CRPC. Both arms were able to mount increases in PSA-specific T-cell responses to the vaccine. In addition, patients were administered corticosteroids as premedication with docetaxel in this study. The pulse use of steroids did not seem to induce an immune suppressive effect [97]. However, chronic daily oral steroid administration that is common to many prostate cancer regimens needs to be evaluated because the immunosuppressive effects of these agents on both cell-mediated and humoral immunity are well known and likely to interfere with the generation of effective vaccine-induced memory responses. It is important to note that patients who have previously received numerous chemotherapy agents may have impaired marrow reserves that may blunt their ability to mount a potent immune response.

In preclinical animal studies investigating the therapeutic effect of an anti-HER2/neu antibody, triggering and activation of innate immunity initiated by antibody treatment was necessary [98]. However, administration of chemotherapy in this model diminished this antibody-initiated immunity and in turn resulted in earlier relapse or diminished resistance to tumor re-challenge. In contrast, administration of immunotherapy further augmented activation and resulted in increased tumor eradication and resistance to challenge related to an influx of both innate and adaptive immune cells into the tumor microenvironment. Clearly, the timing, dosing and scheduling of chemotherapy and immune-based therapies may significantly impact the activity of subsequent interventions. Hence,

careful consideration should be given to the design of clinical trials investigating these types of combination approaches.

Cytokines & immunomodulatory monoclonal antibodies plus vaccine

Monoclonal antibodies such as ipilimumab targeting the human CTLA-4 monoclonal antibody have been tested in patients with prostate cancer. The primary function of CTLA-4 is to provide a regulatory checkpoint by downregulating the immune response over time, thus preventing autoimmunity. The administration of anti-CTLA-4 antibody has been shown to sustain and potentiate immune responses [99–102]. This has been observed in a Phase I study of ipilimumab with the GVAX prostate cancer vaccine in patients with metastatic prostate cancer. A correlation between immune-related AEs and immune response was noted in this study [103]. Similarly, in the pivotal Phase III study that led to the approval of ipilimumab by the FDA in March 2011 for its use in patients with metastatic malignant melanoma, ipilimumab-related autoimmune phenomena also seemed to correlate with treatment response [55]. The most common AEs associated with ipilimumab therapy are related to its immunologic mechanism of action leading to T-cell activation and proliferation, with subsequent immune-mediated AEs that most commonly involve the colon (enterocolitis), liver (hepatitis), skin (dermatitis, including toxic epidermal necrolysis), endocrine system (adrenal insufficiency, hypopituitarism and hypothyroidism) and nervous system (neuropathy). These autoimmune AEs are often of sufficient severity as to warrant their management with the initiation of high-dose corticosteroids (1–2 mg/kg/day of prednisone or equivalent) and discontinuation of ipilimumab. The observation that autoimmune phenomena are associated with its beneficial clinical effects implies that breaking tolerance to self-antigens likely plays a critical role in inducing anti-tumor effects that are associated with positive, objective clinical outcomes, for example, improved survival.

Ipilimumab is also being studied in prostate cancer in several clinical settings: as neoadjuvant therapy with leuprolide acetate before prostatectomy (NCT01194271); in combination with ADT for metastatic, castrate-sensitive disease (NCT01377389); and in Phase III clinical studies of patients with metastatic CRPC who are chemotherapy naïve (NCT01057810) and postdocetaxel chemotherapy (NCT00861614). Hence, there is a compelling scientific rationale to consider combining therapeutic approaches that have direct anti-tumor activity (chemotherapy and radiation) with approaches that have indirect anti-tumor activity through their effects on the immune system (therapeutic vaccines, monoclonal antibodies and other immune-based therapies) (Figure 1). Specifically, chemotherapy or RT, in addition to reducing tumor burden, could potentially enhance both cellular and humoral immunity as a result of tissue necrosis and the release of multiple tumor antigens. In turn, immunotherapy could lead to further enhancement of tumor-specific responses through vaccination. The additional use of monoclonal antibodies that block negative regulators of immunity such as anti-CTLA-4 and anti-PD1 could augment trafficking of innate and adaptive immune cells into the tumor microenvironment. This multipronged, multiplatform approach will allow a more comprehensive and coordinated anti-tumor attack because engaging both innate and adaptive immunity seems to have a role in increasing the anti-tumor activity of monoclonal antibodies such as anti-HER2/neu that have already been demonstrated to be highly efficacious [98].

Expert commentary

Scientific advances and successes are frequently accompanied by parallel challenges and pitfalls when the euphoria of success is inevitably tempered by the reality check that accompanies practical application. The field of immunooncology is poised to facilitate a fundamental paradigm shift in the approach to cancer treatment through continued gains in our understanding of how to optimize manipulation of the human immune system. It is critically important to acknowledge that therapeutic cancer vaccines comprise a diverse group of complex biologic agents with distinctive kinetics and clinical profiles from standard chemotherapy. Thus, novel methodologies, predictive biomarkers, immunologic and clinical end points and a shift in the standard clinical development paradigm for chemotherapy drugs will be required to continue to move this evolving field forward [104]. Indeed, the inherent biologic variability of anti-tumor immune responses (whether *de novo* or vaccine induced) and their delayed kinetics relative to chemotherapy make identification of baseline attributes that may predict immunotherapy activity a key scientific priority. However, it is important to acknowledge that the predictive utility of baseline factors may vary based on the patient population or the specific immunotherapy platform being studied.

Investigation of multimodality approaches in combination with cancer vaccines in the hopes of achieving even better clinical outcomes should be vetted whenever possible in well-designed preclinical animal studies to underscore the scientific rationale for carrying out human trials and to ensure these approaches document potential mechanistic synergy rather than antagonism or excess toxicity. With the approval of sipuleucel-T, patients are administered vaccine before initiating chemotherapy. However, the field of prostate cancer is changing with the recent approval of abiraterone acetate and the promising data from the MDV-3100 studies. Currently, abiraterone is approved as second-line therapy following docetaxel in patients with metastatic androgen-independent prostate cancer. However, emerging clinical data suggest it may gain approval for use pre docetaxel. The question then arises as to where immunotherapy would fit in the treatment paradigm sequence. It may be appropriate to still consider initiating immunotherapy before these other treatments or possibly even in combination with abiraterone in an attempt to further delay the need for systemic chemotherapy. However, it will be important that clinical researchers investigate the timing and scheduling of immunotherapy with these newer approved agents to gain insight into how to maximize the clinical benefit and take advantage of the currently available therapeutic repertoire. Newer compounds are being explored in the clinic that can also affect immune checkpoints in addition to anti-CTLA-4. Another molecular immune target is PD1, which, through its interaction with B7-H1, leads to

the inhibition of T-cell function. Anti-tumor responses have been observed that are associated with the blockade of this interaction by an antibody targeting PD1. Although additional studies are required, the anti-PD1 antibody preliminarily seems to have a better safety profile compared with anti-CTLA-4 blockade.

Indeed, a tantalizing (and potentially infinite) spectrum of multiple different combinations of vaccine platforms and adoptive cell therapies, cytokines, chemotherapeutic agents, radiation therapies and checkpoint inhibitors of negative regulation is already possible. However, our goal as a scientific discipline should be to gain additional fundamental insight from every product under study at every stage of product development and avoid assuming that we definitively know anything about what we are really doing when it comes to influencing and manipulating the human immune system. Importantly, evaluation of candidate vaccines and immune-based therapy platforms should entail comprehensive examination of innate and adaptive immunity, alterations in host negative regulatory and suppressive mechanisms in response to treatment and characterization of responses in combination with specific classes of agents to more rapidly identify synergies, interferences and toxicities.

Five-year view

The development of vaccine platforms that deliver more potent danger signals at the time of initial antigen presentation is required to allow triggering of innate immunity and the generation of high-avidity adaptive responses that are functionally potent and prolonged to overcome self-tolerance and reject tumor antigens. Better vaccines will result in the development of more rapid immune responses that have a greater likelihood of objectively impacting clinical outcomes earlier rather than later. These approaches will be augmented by the coadministration of agents (sequentially or concomitantly) designed to dampen immunosuppressive negative regulation and remove the brakes off host tumor-specific responses. Immunogens and their respective platforms will need to promote cross-priming and epitope spreading to maximize the breadth of anti-tumor T-cell responses, thereby minimizing the risk of immune evasion and escape. These ambitious scientific and clinical approaches will require new regulatory paradigms for approval and unprecedented cooperation among academic, biopharma and government stakeholders with differing intellectual property and fiscal priorities to continue to move the field forward. The present state of the science and current achievements were a long time coming. However, with emerging insights into molecular immunity, we are on the cusp of making personalized cancer medicine a more cost-effective and practical reality.

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Key issues

- Population enrichment and stratification should be incorporated into well-designed early-phase trials of vaccines and immune-based therapies. Randomization should be used aggressively to be able to detect definitive, clinically meaningful signals of activity early in clinical development.
- Immune-based therapies should incorporate combination strategies to overcome self-tolerance to tumor antigens by addressing negative immune regulation systemically and in the tumor microenvironment.
- Standardization of assays for primary immunologic end points used to characterize responses to therapeutic vaccination is a critical priority that will allow better identification of valid assays, end points and correlation with clinical outcomes.
- Developmental paradigms that will allow standardized assessment and characterization of the potency and immunogenicity of personalized cancer therapy are needed.

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