CLINICAL INVESTIGATION

Immunotherapy and cancer – making the past prologue

Clin. Invest. (2012) 2(9), 873–881

Susan F Slovin

Genitourinary Oncology Service, Sidney Kimmel Center for Prostate & Urologic Cancers, Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, NY 10065, USA
Tel.: +1 646 422 4470
E-mail: slovins@mskcc.org

The goals of all novel therapies include improvement in overall survival, quality of life and disease control. We are fortunate to finally have two therapies that have demonstrated survival benefit in Phase III clinical trials – one, ipilimumab, a monoclonal antibody against CTLA-4, a checkpoint inhibitor, and the other, Sipuleucel-T, an autologous-based cellular therapy. Ipilimumab is the second drug that has been approved for melanoma within the last year, suggesting that there are multiple ways to achieve antitumor responses. Sipuleucel-T is the first immunotherapy approved for asymptomatic, castration-resistant prostate cancer, and while Sipuleucel-T lengthened survival modestly, there was no improvement in time to progression and no responders. While Sipuleucel-T has been well-tolerated, ipilimumab has been associated with autoimmune-related events. As single agents, these drugs have shown benefit; however, maximizing durability remains a challenge. This article provides support for our continued efforts in this area of research and clinical care.

Keywords: checkpoint • chemotherapy • CTLA-4 • immunotherapy • ipilimumab • PD-1 • PSA • PSMA • Sipuleucel-T • vaccines

Immunotherapy has come a long way since Coley's toxin [1,2], an observation that bacterial toxins can elicit immunologic changes that lead to remissions in cancer. Not only have we more fully identified the players of what was termed by the late Richard Gershon as the 'immunologic orchestra' led by the 'generator of diversity' [3,4], but complex interplays between cytokines, checkpoint inhibitors and T and B cells and their respective receptors have now been elucidated. These interactions pose continuous challenges as to how these immunologic participants can be harnessed to effect antitumor responses. A continuous challenge to immune therapies is that cancer antigens are often altered 'self-antigens' [5], thought to be either overexpressed or underglycosylated byproducts of the malignant process. Cancer testes antigens are one of many examples including, the expression of mucins, gangliosides and glycolipids, all of which are naturally present in normal tissues but are changed by the malignant process.

The concept of cancer vaccines is not new. An article from Toronto's The Globe in 1925 [6] reported how British researchers identified that a virus could cause cancer and that vaccines could be made against them. Through the last several decades, the field has waxed and waned with false promises of successes. A more recent success, Sipuleucel-T [7–9,101] has reawakened the field of immunotherapy by demonstrating that an autologous cellular product vaccine can impact on the disease. Similarly, ipilimumab [10], a human IgG1κ monoclonal antibody against CTLA-4 has also shown broad activity in prostate [11,12] and ovarian cancers [13]; interestingly, those patients who ultimately respond develop autoimmune-related events such as colitis, hypophysitis or rash, which demonstrate that not only is an immune target likely hit, but can be associated with bystander events that signal the complex interplay between inhibiting nature's checkpoints and

FUTURE SCIENCE part of fsg

causing uninhibited proliferation of T lymphocytes. Two other vaccines have been of interest, the first, GVAX, was comprised of irradiated prostate cancer cell lines that were genetically transduced to express GM-CSF. Though initially promising when given with ipilimumab in patients with metastatic prostate cancer [14–16], trials using a combination of GVAX and docetaxel, versus docetaxel alone, did not show benefit. The second therapy of interest is PSA-TRICOM, a pox-virus-based vaccine that incorporates three co-stimulatory molecules, LFA, ICAM and B7.1. Phase I and II trials [17,18] have suggested some antitumor effects as some patients not only developed an antitumor antibody response, but also had close to an 8-month survival benefit (25.1 versus 16.6 months for placebo; p = 0.0061), which is higher than that seen with Sipuleucel-T. So, given the variability of the different immune strategies described, is there a common thread that would lead us toward any one approach or should there be continued pursuit of this line of investigation? The most interesting observation to date is that most of these therapies have been associated with a survival benefit, as indicated by Sipuleucel-T [7] and PSA-TRICOM [19] in patients with castration-resistant prostate cancer. A 4-month survival benefit has been deemed comparable to that seen in many of the now standard newly approved agents for prostate cancer, such as 17 α-hydroxylase/C17,20 lyase (CYP17A1), abiraterone acetate [20], and the taxane/cabazitaxel [21]. But is this sufficient to convince patients to try immunotherapeutic approaches, particularly since an antitumor effect may take weeks to months in the absence of clinical benefit or impact on known biomarkers such as PSA or even circulating tumor cells? Some would argue that a rapid response to disease, as observed with newer hormonal therapies or chemotherapies, would be desirable. Patients would prefer immediate pain palliation and antitumor responses in lieu of awaiting a positive treatment effect, which may take 6 months or greater. This has been seen in many immune-based therapies. In some cases, the development of autoimmune events may herald a potential response, but is fraught with potentially debilitating side effects. As such, patients may turn away from newer immune-based therapies due to lack of response immediacy and concerns about durability. Where does that leave us? These issues bring to light how this field will need to be re-evaluated in order to gain maximal response along with maximal clinical benefit, but in the absence of significant toxicities.

Issues for resolution

■ Time-to-treatment effect

Observations in melanoma support a delayed response to treatment with a period of time during which the disease may worsen before clinical benefit is ascertained [22]. This is the time during which interim radiographic imaging may lead to the premature discontinuation of therapy. However, preclinical models in addition to clinical observations [23] support the presence of lymphocytic infiltrates that can cause the cancerous organ to appear to be transiently radiographically worse. However, over time, the patient may report improvement in overall symptoms well before any benefit is seen radiographically. With continued close observation, the involved organ may demonstrate remission, which may be durable from months to years. Can this be demonstrated by other therapies? It is not uncommon for patients with metastatic prostate cancer to bone who are on hormonal ablation to have worsening bone scans within 3 months of initiating hormonal treatment. This is thought to be due to more rapid bone turnover cause by the healing of the bone lesions with increased metabolic activity seen on a bone scan. With continued observation, even additional lesions not previously noted on the original bone scan will either ultimately remit or sclerose. To date, there are no formal recommendations to assess how long it will take for an immune therapy to exert any antitumor effects, if at all. The experience with Sipuleucel-T had shown limited radiographic benefit, but patients in the trial were not followed for an extended period of time, hence it remains unknown whether any benefit would ultimately have been seen if the patients were continued to be monitored in lieu of instituting another therapeutic intervention. Therefore, patients on these kinds of immune therapies may potentially lose immunologically-driven antitumor benefit if they go on to another therapy prematurely due to the length of time it may take for a clinical benefit to be assessed. Patient anxiety may also impact on a patient's willingness to be monitored while awaiting clinical benefit. Similarly, patients' whose disease remains either stable or slightly worse may lose out on other clinical trial opportunities while awaiting a treatment response from an immune-based regimen; for patients who go on to clinical trials, it may be unclear whether the benefit from immune therapy may actually be contributing to the clinical benefit perceived to be as a result of the new agent when in reality it may be left over from the impact of the immune treatment. As such, current recommendations are likely to be highly dependent on an individual therapy and its mechanism of action, rather than assuming that the timeline is the same for all these immunotherapies.

Efficacy of immunotherapies as single agents

■ Rationale for combinatorial approaches

The success of single-agent vaccines, either monovalent vaccines, DNA, or vaccine– or antibody–drug conjugates has been limited. While there are immunologic signals with these constructs, such as the induction of high-titer antibodies specific for the immunogen in use, the overall impact of these approaches has been limited by not demonstrating a change in the biology of the cancer. The field of immunotherapy is ripe with Phase I trials directed at novel tumor antigens, including PSA, PSMA, PSCA or PAP, all highly expressed antigens in prostate cancer cell lines and prostate cancer tissue and to which therapies can be directed. Nevertheless, the durability of these antibody signals and even the induction of T-cell proliferative responses, are insufficient to affect antitumor responses, as evidenced by changes in imaging or even impacting on a new biomarker, circulating tumor cells. Therefore, efforts have long been underway to seek out other agents that can either be additive or synergistic with the vaccine/immune therapies. Although an appealing concept, chemotherapy–vaccine combinations have not been widely applied, albeit the concept has been under consideration. Much of the chemotherapy–vaccine combination data are from preclinical and murine studies, or are based on small Phase I trials. In mouse models of colon and breast cancer, paclitaxel, docetaxel or cisplatin subsequent to vaccination enhanced the effectiveness of the vaccine-generated cytotoxic lymphocytes (CTLs), probably by causing an increase in tumor cell permeability to granzyme B [24]. Cell death in the vaccinated and treated mouse cancer models included a desirable bystander effect in which the vaccine-induced CTLs caused apoptosis in neighboring tumor cells not expressing the vaccine antigens. It is felt that the chronic inflammatory milieu that develops during tumor growth compromises the immune response while promoting further progression of the malignancy [25–29]. It is felt that chronic inflammation causes immune cells to release cytokines such as TNF-α, TGF-β and IL-6, which recruit myeloid-derived suppressor cells (MDSCs) and directly reduce immune cell activity. These cytokines also promote metastatic transition later in the disease process. The tumor cells play their own role in compromising induction of immunologic responses. Tumor cells evade CTLs through a number of strategies, including blocking antigen presentation, loss of MHC and apoptosis [29,101]. They can also produce immunosuppressive cytokines such as TGF-β and VEGF. These cytokines directly reduce CTL numbers and recruit $CD25^+$ Treg cells and MDSC that repress the immune response. Yet another mechanism employed by tumor cells may cause reduction in CTL numbers by expressing certain receptor ligands (e.g., PD-L1 and FasL). Using prostate cancer as an example for immunotherapy due to its wealth of well-defined prostate-associated antigens, as well as a validated biomarker, PSA, which has been used as a surrogate marker of response, considerable evidence has been shown to indicate that prostate cancers can promote immune tolerance starting early in the disease [30,31]. In a transgenic mouse model of prostate cancer, $CD8^+$ and $CD4^+$ cells specific to prostate antigens infiltrate prostate tumors, but are anergic or nonfunctional. The encounter with tumor antigens apparently shifts $CD4^+$ and $CD8^+$ cells toward a suppressive (Treg) phenotype. Patient biopsies show that prostate tumor-infiltrating $CD4^+$ cells include high levels of Treg cells [32]. Human prostate tumors also contain elevated populations of possibly protective Th17 cells populations, but only in low-grade tumors [32].

■ Chemoimmunotherapy combinations

Chemotherapy is widely held to be immunosuppressive, but in fact it has immunomodulatory effects [33,34]. Merely debulking the tumors reverses tumor-induced immune tolerance, possibly through reducing the amount of suppressive cytokines secreted by malignant cells [34]. In addition, the transient lymphopenia caused by properly dosed chemotherapy activates homeostatic mechanisms, eliminating excess suppressor cells, and stimulating tumor-specific effector T-cell proliferation as well as dendritic cell maturation [35].

Some chemotherapeutic agents promote specific immune cell types. For example, docetaxel administered in a mouse model selectively decreased myeloid MDSCs while increasing CTL responses [36]. Docetaxel may have a relatively potent effect, but other taxanes also alter cytokine patterns and enhance lymphocyte proliferation, as well as the cytotoxic activity of natural killer and lymphokine-activated killer cells, while reducing Treg cell populations [37,38].

In another murine model, immunized mice with implanted colon tumors expressing human carcinoembryonic antigen (CEA) were studied. The vaccine was based on a poxvirus vaccine containing genes for CEA and costimulatory molecules (CEA-TRICOM) [39]. A standard dose of docetaxel administered 4 days following two poxvirus immunizations improved vaccine-specific immune responses. It also induced antigen-specific T-cell responses to tumor-derived antigens distinct from the antigen used in the vaccine (the 'antigen cascade' or 'epitope spreading', possibly due to the release of

antigens from dying cells). Docetaxel was effective only when administered after immunization. If administered prior, docetaxel inhibited cellular infection by the viral vaccine or antigen expression in the cells that did become infected [40].

How to combine chemotherapy with vaccines and what is the optimal dosing of chemotherapy to be used with vaccines remains unclear. A study that investigated daily low-dose paclitaxel found that by targeting HPV E7$^+$ implanted tumors in mice receiving a DNA vaccine, survival was extended and tumor growth delayed. The results were improved when compared with the vaccine alone, the vaccine plus high-dose paclitaxel, or high-dose, twice-weekly paclitaxel alone [41]. Daily low-dose paclitaxel did not result in the significant T-cell declines induced by high-dose paclitaxel. When administered with the vaccine, daily low-dose paclitaxel resulted in a higher CD8$^+$ T-cell/Treg ratio than either the vaccine alone or the vaccine plus high-dose paclitaxel. Furthermore, the low-dose chemotherapy had greater antiangiogenic effects than did high-dose [41].

A chemotherapy drug that has been studied extensively is cyclophosphamide. Low-dose cyclophosphamide also has well-documented immune modulatory effects [42,43]. It reduces the Treg population, inhibits the activity of the remaining Treg cells, and stimulates cell-mediated immunity [42,43]. The drug is now making its debut after being retired for many years in its role as an immune modulator. In a transgenic murine prostate cancer model, administering low-dose cyclophosphamide 1–2 days before immunization with a whole-cell, GM-CSF-secreting vaccine (GVAX) resulted in a tumor shrinkage effect not observed with the vaccine alone. This effect seemed related to a reduced Treg population in the tumor and its draining lymph node, as well as increased dendritic cell activation [43].

Other studies have found specific benefits from high-dose but submyeloablative chemotherapy. An adenovirus-based vaccine was found to have limited effectiveness in mice with established melanoma tumors, unless the mice were pretreated with higher doses of cyclophosphamide (Cy) [43]. The combination resulted in tumor regression due to the high frequency of vaccine antigen-specific T-cells, reflecting cyclophosphamide's general promotion of cell-mediated immunity. This drug has been used at low-dose, high-dose and on a metronomic schedule. More recently, an anti-PD-1 antibody (CT-011) with Treg-cell depletion by low-dose Cy (CPM), combined with a tumor vaccine, was shown to induce synergistic antigen-specific immune responses [44]. This strategy led to complete regression of established tumors with survival prolongation in a significant proportion of animals. This is the first demonstration that the combination of anti-PD-1 and CPM significantly increased the number of vaccine-induced tumor-infiltrating CD8$^+$ T cells with simultaneous decrease in infiltrating Treg cells. Another chemotherapy approach was cisplatin/vinorelbine to induce leukopenia as well as downmodulated reconstitution of Treg cells when compared with effector T cells [45]. Noguchi *et al.* have presented their experience of using low-dose estramustine phosphate (initially administered as 280 mg daily in concert with a personalized peptide vaccine in HLA-A2$^+$ or -A24$^+$ patients with castration-resistant prostate cancer [46,47]). There were no preclinical studies to suggest that this drug had a direct immunomodulatory effect and the impetus for the drug was based on clinical tolerability and safety. Nevertheless, ten of 11 patients who received a combination of peptide vaccination and estramustine showed a serum PSA decrease; eight patients showed a PSA decrease of ≥50%. One of two patients with measureable disease showed a 44% decrease in lymph node metastasis; no changes were seen in patients with bone lesions. Estramustine-induced immunosuppression was analyzed in ten of 11 patients by IFN-γ productions to PHA, EBV peptide and the vaccinated peptides. Immunologic responses were observed; no significant immune suppression was seen when the peptide was given along with a smaller dose of estramustine [46]. These responses were thought to be mediated by resting T cells, memory T cells and a combination of memory and activated T cells, respectively [46]. These observations were further extended into another Phase I multicenter study [47] of 15 patients treated with low-dose estramustine and ITK-1, a peptide set consisting of 14 kinds of peptide that induced HLA-A24-restricted tumor-specific cytotoxic activity. Patients were treated with the top four peptides shown to be immunogenic based on prevaccination measurement of peptide-specific IgG in plasma reactive to ITK-1. While safe, *in vitro* analysis did not reveal any correlation between the peptide dose and the generation of specific T cells from vaccinated patients. A median survival of 23.8 months was reported with this combination regimen, albeit the exact immune mechanism, if any, that contributed to this remains unclear [47].

■ Attempts to reduce or suppress the regulatory T-cell population & enhance response with nonchemotherapeutic agents

One means of inactivating Treg cells has been through specific targeting of the T-cell checkpoint inhibitor CTLA-4 with a monoclonal antibody such as ipilimumab [48–50]. In the past few years it has become apparent that costimulation is even more complex than originally thought. After activation, T cells express CTLA-4, a close homologue to CD28. CTLA-4 binds members of the B7 family with a much higher affinity than CD28 [38]. There has been some controversy as to the role of CTLA-4 in regulating T-cell activation. It now appears that CTLA-4 downregulates T-cell responses [40]. This was based on the following *in vitro* observations: blockade of CTLA-4–B7 interactions with antibody enhanced T-cell responses; crosslinking of CTLA-4 with CD3 and CD28 inhibited T-cell responses; and antibodies to CTLA-4 *in vivo* augmented the immune response to peptide antigens or superantigens in mice [41,51–53]. Blocking CTLA-4–B7 interaction while preserving signalling via CD28 resulted in enhanced T-cell responses *in vitro* [53].

Perhaps the most convincing demonstration of the downregulatory role of CTLA-4 came from examination of mice with a null mutation [48,49,54]. CTLA-4-knockout mice appear to have spontaneously activated T cells early in development followed by rapidly progressive lymphoid proliferation consistent with a lymphomatous presentation. Mice usually die within 3 weeks either as a result of polyclonal T-cell expansion and widespread tissue devastation or as a result of lymphokine release with ensuing shock. Thymocyte differentiation and selection proceed normally in CTLA-4-deficient mice; this suggests that CTLA-4 may contribute to downregulating peripheral T-cell responses [54].

Preliminary clinical trials suggest that administering a therapeutic vaccine followed by ipilimumab enhances immune responses and tumor reduction in prostate and ovarian cancers as well as melanoma [17,55]. In a noncomparative Phase I trial (n = 30) of ipilimumab plus the PSA-TRICOM vaccine in prostate cancer, overall survival was 31.8 months compared with an expected survival of 18.5 months based on baseline factors (Halabi nomogram–predicted survival [HPS]) [18,56]. Several studies in melanoma have not found additional benefit for therapeutic vaccines beyond that of ipilimumab alone [39,42]. In both the prostate cancer and melanoma studies, the vaccines were administered simultaneously with the course of ipilimumab; there was no attempt to evaluate sequential therapy [36,37]. Furthermore, blocking CTLA-4 function may permit the emergence of autoreactive T cells and resultant clinical autoimmunity. In melanoma studies successful responses to ipilimumab resulted in autoimmune events such as diarrhea, hypophysitis, transaminitis and rash, as did a prostate cancer study of ipilimumab alone and after radiotherapy [12,50]. The severity of the adverse events appeared to be related to the level of response to ipilimumab, but this remains controversial. Overall, the results suggest that chemotherapy, albeit at less than therapeutic doses, may induce a wide range of immune effects that may ultimately lead to systemic antitumor effects several weeks to months after treatment.

Another molecule, the programmed death-1 receptor (PD-1) is expressed on T cells following T-cell receptor activation. Binding of this receptor to its cognate ligands, programmed death ligand (PDL)-1 and PDL-2, downregulates signals by the T cell receptor, thereby promoting T-cell anergy and apoptosis, and can therefore lead to immune suppression. Recent clinical trials in renal cell carcinoma and melanoma suggest significant activity of this drug, sparking additional plans for combining anti-CTLA-4 with anti-PD-1.

■ Immunotherapy combinations can impact on tumor response & survival

Recent attempts to demonstrate improvement in overall or progression-free survival (PFS) have been shown using a poxvirus–PSA recombinant vaccine, a mixture of recombinant pox viruses expressing either PSA or the B7.1 costimulatory molecule [54]. A vaccinia-based vaccine is usually administered once followed by monthly injections of fowlpox–PSA recombinant virus in a prime–boost strategy. Each vaccination is administered with GM-CSF [57]. In a Phase II study, patients with metastatic castration-resistant prostate cancer (n = 28) received the poxvirus-PSA vaccine 2 weeks apart for the first month and then monthly until disease progression occurred. Half the group additionally received docetaxel/dexamethasone therapy in 3-weeks-on/1-week-off cycles [57]. In the vaccine-alone arm, 11 patients (78.6%) were changed to docetaxel upon evidence of progression. Median time to progression was 1.8 months in the vaccine-alone arm and 3.2 months in the vaccine plus docetaxel arm. Notably, patients experienced a median 6.1-month progression-free period after progressing on the vaccine alone and then switching to docetaxel. PFS was 3.7 months in an historic control group receiving docetaxel alone [57]. All evaluable vaccine recipients exhibited increased PSA-specific T cells (median 3.33-fold increase in both arms). In the three vaccine-alone recipients who were examined, T-cell responses emerged, as well as responses to other prostate tumor antigens (PAP, PSMA and/or MUC-1). This may have been due to an epitope-spreading phenomenon pursuant to the tumor cell death by the vaccine-induced PSA-specific T-cell response [57].

ProstVac® VF (PSA-TRICOM) is a second-generation vaccine employing recombinant vaccinia- and fowlpox-expressing PSA plus three T-cell costimulatory molecules, LFA-3, B7.1 and ICAM-1 [17–19]. It elicits a more robust antitumor response than the original poxvirus-PSA immunization [17–19]. An ongoing randomized, double-blinded, placebo-controlled, multicenter, Phase III efficacy trial of PROSTVAC in men with asymptomatic, or minimally symptomatic castrate metastatic prostate cancer is a three-arm study and will evaluate overall survival in two separate comparisons: PROSTVAC plus adjuvant dose GM-CSF versus controls, and PROSTVAC without GM-CSF versus controls. First, (Arm V+G) PROSTVAC-V/F plus adjuvant dose GM-CSF; second, (Arm V) PROSTVAC-V/F plus GM-CSF placebo; and finally, (Arm P) double placebo (vector placebo plus GM-CSF placebo).

Phase II studies using Prostate GVAX, [17,19] showed promising responses and led to two Phase III studies that included docetaxel. The Phase III VITAL-1 trial directly compared GVAX (biweekly for the first 26 weeks, then monthly) with standard tri-weekly docetaxel plus prednisone. The study population included chemotherapy-naive men with castration-resistant prostate cancer and negligible pain. This trial was terminated early due to futility: there was little chance of reaching the primary end point of improved survival, even though indications of the vaccine efficacy were observed. Median survival was 20.7 months on GVAX and 21.7 months on docetaxel/prednisone (p = 0.78). Of note, grade 3/4 adverse events were considerably less frequent with GVAX (8.8% of GVAX recipients vs 43% of those on docetaxel) [45].

The futility analysis took on particular importance because of the results in the other Phase III GVAX trial (VITAL-2). That study administered docetaxel every 3 weeks followed 2 days later by GVAX immunization. After ten docetaxel cycles, GVAX every 4 weeks was administered alone as maintenance therapy. The comparator group received standard docetaxel/prednisone for ten cycles. The study had a planned enrollment of 600 taxane-naive patients with metastatic castration-resistant prostate cancer requiring opioid pain management; overall survival was the primary end point. However, the trial (n = 408 actual enrollment) was halted prematurely due to an excess of deaths in the GVAX arm (67 vs 47). Median overall survival was 12.2 months in the GVAX/docetaxel arm and 14.1 months in the docetaxel/prednisone arms (HR = 1.70; 95% CI: 1.15–2.53; p = 0.0076). The investigators were unable to identify safety issues or other reasons for the excess deaths.

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

■ Unanswered questions for the next generation of immune-based therapies

There continues to be unresolved issues regarding perfecting vaccine strategies alone or given in combination with other immune modulatory agents or chemotherapies. The first issue is how to maximize immune responsiveness. As discussed, the data support the use of chemotherapy with an immune-based approach. However, it remains to be seen whether there is a 'best' chemotherapy agent for this application. Second, is chemotherapy sufficient for this implementation or should additional agents such as GM-CSF or IL-2 be added for synergism. Last, in what sequence should the respective agents be delivered to foster maximum benefit? Chemotherapies with potential for benefit include the taxanes, anthracyclines and cyclophosphamide, but it remains unclear whether newer agents that target the androgen receptor, that is, MDV-3100, or abiraterone acetate, or impact on signaling pathways using agents that block the IGF receptor or MTOR pathway, may also play a role. All of these agents have independent positive immunomodulatory activity and as such, may lend themselves to being considered in the treatment scheme. Sequencing of chemotherapy, as in dosing, depends on the agent's mechanism of action. Initiation of chemotherapy prior to vaccination would be an option if the goal was to reset the immune system by reducing the level of suppressive cells. Conversely, initiation of chemotherapy during or after vaccination would be an option if the strategy was to impede the tumor and potentiate or broaden the vaccine-induced responses.

As discussed earlier, chemotherapy given in lower-than-therapeutic doses may be favored, as those may selectively alter cell populations and inhibit angiogenesis [41,42,58]. Higher doses may permit greater immune activity and more immunogenicity due to tumor debulking and cell death [33]. Lower doses and/or abbreviated courses would be less toxic overall, and also less immunosuppressive. They would also allow frequent, even daily, dosing (metronomic administration) for a steady effect over time.

■ End points & responses: still unresolved

Another reason for suboptimal results with vaccines in populations with advanced disease and low life expectancies is that the timeframe needed to observe a clinical response may be delayed. Researchers have realized that responses to immunotherapies are slower compared with chemotherapy [59–64]. The disease could remain stable or even progress for some months before protective immune responses are apparent. Alternatively, the initial vaccine-induced inflammatory flare may be mistaken for tumor growth. Various groups have therefore proposed revised end points for cancer vaccine trials that place greater emphasis on overall survival or long-term disease stability rather than PFS. The emphasis is on minimizing premature discontinuation, and allowing patients to continue with therapy despite early, minor progression [61–63].

Future perspective

Biomarkers of immune response that reliably predict treatment outcome would simplify our issues, allowing for more rapid identification and optimization of effective regimens before trials have reached clinical end points [61–63]. These would ideally give advance indication of clinical benefit without the long follow-up required to observe clinical end points. In general, there remains the question as to whether blood biomarkers reflect conditions within the tumor itself of within the surrounding milieu.

Executive summary

Impact of immunotherapy combinations

- Phase III clinical trials support the further development of immunotherapeutic approaches and should include combination with either novel biologic agents or chemotherapies or as a multimodality approach in concert with radiotherapy.

Clinical trial development

- Continued efforts to develop immunologic end points that are custom-tailored to the therapy under investigation should continue.

Timelines in assessing clinical/immunologic benefit

- There is still concern about achieving survival benefits in the absence of disease control or impact on a clinical biomarker. This may be due to delayed immunologic responses, but it remains unclear as to when it can be decided that an immune therapy has truly failed and the patient needs to go on to the next therapy.

There is also a need to standardize immune assays so that study results become more easily reproducible [59]. As the field of immunotherapy develops, we are faced with critical issues that may impact on the success of future trials. Among those to be taken with due consideration are: identifying the patient cohort that will derive maximal benefit from immunotherapy; realistic timelines by which to assess whether or not the immunologic approach has provided benefit; what agents should be used in combinatorial studies with vaccines; and, importantly, how do we go about screening immunologic agents for potential usefulness? Are neoadjuvant studies that interrogate the tumor's natural milieu beneficial to understanding the immunotherapy or does this blunt our understanding of the therapy's ultimately systemic usefulness? Hopefully, we will obtain answers to all these questions in the next several years and bring into the clinical arena a wider array of effective immunotherapies.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
■ of interest
■■ of considerable interest

1 Coley WB. Contribution to the knowledge of sarcoma. *Ann. Surg.* 14, 199–200 (1891).

2 Thotathil Z, Jameson MB. Early experience with novel immunomodulators for cancer treatment. *Exp. Opin. Invest. Drugs* 16, 1391–1403 (2007).

3 Gershon R. The immunological orchestra. *Lancet.* 291, 185–186 (1968).

4 Mikulski SM, Chirigos MA. The immunological orchestra in cancer. *J. Immunopharmacol.* 1(3), 311–336 (1979).

5 Slovin SF. Tribulations or triumphs in prostate cancer immunotherapy: on the road to victory? *Expert Rev. Anticancer Ther.* 8, 465–474 (2008).

6 James EL. Vaccination against cancer may result from discovery by British medical experts. The Globe, Toronto, Canada, vol. LXXXII:1, No. 23,566 (1925).

7 Kantoff PW, Higano CS, Shore ND *et al.* Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N. Engl. J. Med.* 363, 411–422 (2010).

■■ **This is the first US FDA-approved immunotherapy in prostate cancer to show improvement in overall survival.**

8 Higano CS, Schellhammer PF, Small EJ *et al.* Integrated data from 2 randomized, double-blind, placebo-controlled, Phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate

cancer. *Cancer* 115, 3670–3679 (2009).

9 Petrylak DP, Schellhammer PF, Small E, Frohlich MW. Androgen independent prostate cancer (AIPC) patients who receive sipuleucel-T followed by docetaxel have prolonged survival. *Am. Urolog. Assoc. Ann. Mtg.* 605 (2007).

10 Hodi FS, Butler M, Oble DA *et al.* Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc. Natl Acad. Sci. USA* 105, 3005–3010 (2008).

11 Drake CG, Scher HI, Gerritsen WR *et al.* A randomized, double-blind, Phase III trial comparing Ipilimumab versus placebo following radiotherapy (RT) in patients (pts) with castration-resistant prostate cancer (CRPC) who have failed prior treatment with docetaxel (D). *J. Clin. Oncol.* 29(Suppl.) Abstr. TPS181 (2011).

12 Beer TM, Slovin SF, Higano CS *et al.* Phase I trial of ipilimumab (IPI) alone and in combination with radiotherapy (XRT) in patients with metastatic castration resistant prostate cancer (mCRPC). *J. Clin. Oncol.* 26, Abstr. 5004 (2008).

13 Hodi FS. Overcoming immunologic tolerance to melanoma: targeting CTLA-4. *Asia Pacific J. Clin. Onc.* (Suppl. 1), 6, S1–S23 (2010).

14 van den Eertwegh AJ, Versluis J, van den Berg HP *et al.* Combined immunotherapy with granulocyte–macrophage colony stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a Phase I dose-escalation trial. *Lancet Oncol.* 13(5), 509–517 (2012).

15 Higano C, Saad F, Somer B *et al.* A Phase III trial of GVAX immunotherapy for prostate cancer versus docetaxel plus prednisone in asymptomatic, castration-resistant prostate cancer (CRPC). *2009 Genitourinary Cancers Symposium* (ASCO Annual Meeting Abstracts). Abstr. LBA150 (2009).

16 Small E, Demkow T, Gerritsen WR *et al.* A Phase III trial of GVAX immunotherapy for prostate cancer in combination with docetaxel versus docetaxel plus prednisone in symptomatic, castration-resistant prostate cancer (CRPC). *2009 Genitourinary Cancers Symposium* (ASCO Annual Meeting Abstracts). Abstr. LBA150 (2009).

17 Madan RA, Mohebtash M, Arlen PM *et al.* Overall survival (OS) analysis of a Phase l trial of a vector-based vaccine (PSA-TRICOM) and ipilimumab (Ipi) in the treatment of metastatic castration-resistant prostate cancer (mCRPC). *J. Clin. Oncol.* (ASCO Annual Meeting Abstracts). 28, Abstr. 2550 (2010).

18 Gulley JL, Arlen PM, Madan RA *et al.* Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. *Cancer Immunol. Immunother.* 59, 663–674 (2010).

19 Kantoff PW, Thomas J, Blumenstein BA *et al.* Overall survival analysis of a Phase II randomized controlled trial of a poxviral-based psa-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 28, 1099–1105 (2010).

■■ **A novel vaccine constructed with three costimulatory molecules that, in early trials, also demonstrated improved survival.**

20 de Bono JS, Logothetis CJ, Molina A *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N. Engl. J. Med.* 364(21), 1995–2005 (2011).

■■ **A novel addition to the armamentarium of oral agents that have demonstrated improved cancer control and survival.**

21 de Bono JS, Oudard S, Ozguroglu M *et al.* Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 376(9747), 1147–1154 (2010).

22 Wolchok JD, Hoos A, O'Day S *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* 15, 7412–7420 (2009).

■ **A novel assessment of the current issues facing immunotherapy development and clinical trials.**

23 Begley J, Ribas A. Targeted therapies to improve tumor immunotherapy. *Clin. Cancer Res.* 14, 4385–4391 (2008).

24 Ramakrishnan R, Assudani D, Nagaraj S *et al.* Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. *J. Clin. Invest.* 120, 1111–1124 (2010).

25 Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Ann. Rev. Immunol.* 29, 235–271 (2011).

26 Mlecnik B, Bindea G, Pages F, Galon J. Tumor immunosurveillance in human cancers. *Cancer Metastasis Rev.* 30, 5–12 (2011).

27 Pagès F, Galon J, Fridman WH. The essential role of the *in situ* immune reaction in human colorectal cancer. *J. Leukoc. Biol.* 84, 981–987 (2008).

28 Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 140, 883–899 (2010).

29 Wu Y, Zhou BP. Inflammation: a driving force speeds cancer metastasis. *Cell Cycle* 8, 3267–3273 (2009).

30 Poschke I, Mougiakakos D, Kiessling R. Camouflage and sabotage: tumor escape from the immune system. *Cancer Immunol. Immunother.* 60, 1161–1171 (2011).

31 Drake CG. Prostate cancer as a model for tumour immunotherapy. *Nat. Rev. Immunol.* 10, 580–593 (2010).

32 Diener KR, Woods AE, Manavis J, Brown MP, Hayball JD. Transforming growth factor-beta-mediated signaling in T lymphocytes impacts on prostate-specific immunity and early prostate tumor progression. *Lab. Invest.* 89, 142–151 (2009).

33 Sfanos KS, Bruno TC, Maris CH *et al.* Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. *Clin. Cancer Res.* 14, 3254–3261 (2008).

34 Ma Y, Conforti R, Aymeric L *et al.* How to improve the immunogenicity of chemotherapy and radiotherapy. *Cancer Metastasis Rev.* 30, 71–82 (2011).

35 Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? *J. Clin. Invest.* 118, 1991–2001 (2008).

36 McDonnell AM, Nowak AK, Lake RA. Contribution of the immune system to the chemotherapeutic response. *Sem. Immunopathol.* 33, 353–367 (2011).

37 Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, Djeu JY. A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. *Clin. Cancer Res.* 16, 4583–4594 (2010).

38 Vicari AP, Luu R, Zhang N *et al.* Paclitaxel reduces regulatory T cell numbers and inhibitory function and enhances the anti-tumor effects of the TLR9 agonist PF-3512676 in the mouse. *Cancer Immunol. Immunother.* 58, 615–628 (2009).

39 Garnett CT, Schlom J, Hodge JW. Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: effects of docetaxel on immune enhancement. *Clin. Cancer Res.* 14, 3536–3544 (2008).

40 Javeed A, Ashraf M, Riaz A, Ghafoor A, Afzal S, Mukhtar MM. Paclitaxel and immune system. *Eur. J. Pharm. Sci.* 38, 283–290 (2009).

41 Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. *Sem. Immunopathol.* 33, 369–383 (2011)

42 Chen CA, Ho CM, Chang MC *et al.* Metronomic chemotherapy enhances antitumor effects of cancer vaccine by depleting regulatory T lymphocytes and inhibiting tumor angiogenesis. *Mol. Ther.* 18, 1233–1243 (2010).

43 Wada S, Yoshimura K, Hipkiss EL *et al.* Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. *Cancer Res.* 69, 4309–4318 (2009).

44 Mkrtichyan M, Najjar YG, Raulfs EC *et al.* Anti-PD-1 synergizes with cyclophosphamide to induce potent anti-tumor vaccine effects through novel mechanisms. *Eur. J. Immun.* 10, 2977–2986 (2011).

45 Gameiro SR, Caballero JA, Higgins JP, Apelian D, Hodge JW. Exploitation of differential homeostatic proliferation of T-cell subsets following chemotherapy to enhance the efficacy of vaccine-mediated antitumor responses. *Cancer Immunol. Immunother.* 60, 1227–1242 (2011).

46 Noguchi M, Itoh K, Suekane S *et al.* Immunological monitoring during combination of patient-oriented peptide vaccination and estramustine phosphate in patients with metastatic hormone refractory prostate cancer. *Prostate* 60, 32–45 (2004).

47 Noguchi M, Uemura H, Naito S, Akaza H, Yamada A Itoh K. A Phase I study of personalized peptide vaccination using 14 kinds of vaccine in combination with low-dose estramustine in HLA-A24-positive patients with castration-resistant prostate cancer. *Prostate* 71, 470–479 (2011).

48 Gerritsen W, van den Eertwegh AJ, de Gruijl T *et al.* Expanded Phase I combination trial of GVAX immunotherapy for prostate cancer and ipilimumab in patients with metastatic hormone-refractory prostate cancer (mHPRC). *J. Clin. Oncol.* (ASCO Annual Meeting Abstracts). 26, Abstr. 5146 (2008).

49 Yuan J, Gnjatic S, Li H *et al.* CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. *Proc. Natl Acad. Sci. USA* 105, 20410–20415 (2008).

50 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363, 711–723 (2010).

■■ **This is the first antibody against a checkpoint blockade molecule that has demonstrated significant antitumor responses and improved survival.**

51 Saha A, Chatterjee SK. Combination of CTL-associated antigen-4 blockade and depletion of CD25 regulatory T cells enhance tumour immunity of dendritic cell-based vaccine in a mouse model of colon cancer. *Scand. J. Immunol.* 71, 70–82 (2010).

52 Li N, Qin H, Li X *et al.* Synergistic antitumor effect of chemotactic-prostate tumor-associated antigen gene-modified tumor cell vaccine and anti-CTLA-4 mAb in murine tumor model. *Immunol. Lett.* 113, 90–98 (2007).

53 Chakraborty M, Schlom J, Hodge JW. The combined activation of positive costimulatory signals with modulation of a negative costimulatory signal for the enhancement of vaccine-mediated T-cell responses. *Cancer Immunol. Immunother.* 56, 1471–1484 (2007).

54 Mohebtash M, Madan RA, Arlen PM *et al.* Phase I trial of targeted therapy with PSA-TRICOM vaccine (V) and ipilimumab (ipi) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC). *J. Clin. Oncol.* (ASCO Annual Meeting Abstracts). 27, Abstr. 5144 (2009).

55 Sarnaik AA, Yu B, Yu D *et al.* Extended dose ipilimumab with a peptide vaccine: immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. *Clin. Cancer Res.* 17, 896–906 (2011).

56 Halabi S, Small EJ, Kantoff PW, Kattan MW, *et al.* Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. *J. Clin. Oncol.* 21, 1232–1237 (2003).

57 Grinshtein N, Ventresca M, Margl R *et al.* High-dose chemotherapy augments the efficacy of recombinant adenovirus vaccines and improves the therapeutic outcome. *Cancer Gene Ther.* 16, 338–350 (2009).

58 Emmenegger U, Francia G, Chow A *et al.* Tumors that acquire resistance to low-dose metronomic cyclophosphamide retain sensitivity to maximum tolerated dose cyclophosphamide. *Neoplasia.* 13, 40–48 (2011).

59 Hoos A, Eggermont AM, Janetzki S *et al.* Improved endpoints for cancer immunotherapy trials. *J. Natl Cancer Inst.* 102, 1388–1397 (2010).

60 Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. *Oncologist.* 15, 969–975 (2010).

61 Hoos A, Parmiani G, Hege K *et al.* A clinical development paradigm for cancer vaccines and related biologics. *J. Immunother.* 30, 1–15 (2007).

62 Wolchok JD, Hoos A, O'Day S *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* 15, 7412–7420 (2009).

63 Disis ML. Immunologic biomarkers as correlates of clinical response to cancer immunotherapy. *Cancer Immunol. Immunother.* 60, 433–442 (2011).

64 Mackiewicz J, Mackiewicz A. Design of clinical trials for therapeutic cancer vaccines development. *Eur. J. Pharmacol.* 625, 84–89 (2009).

■ Website

101 Petrylak D. Defining the optimal role of immunotherapy & chemotherapy. *Chemotherapy Foundation Symposium XXIV* (2006). www.mssmtv.org/player/player.php?id=111006ada_petrylak (Accessed 24 August 2011)