

# Cancer vaccines

## Looking to the future

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These are exciting times for the field of cancer immunotherapy. Although the clinical efficacy of monoclonal antibodies has been demonstrated since the early 1990s, the therapeutic profile of other immunotherapeutic approaches—especially vaccines—has not yet been formally clarified. However, the recent success of several immunotherapeutic regimens in cancer patients has boosted the development of this treatment modality. These achievements stemmed from recent scientific advances demonstrating the tolerogenic nature of cancer and the fundamental role of the tumor immune microenvironment in the suppression of antitumor immunity. New immunotherapeutic strategies against cancer attempt to promote protective antitumor immunity while disrupting the immunoregulatory circuits that contribute to tumor tolerance. Cancer vaccines differ from other anticancer immunotherapeutics in that they initiate the dynamic process of activating the immune system so as to successfully re-establish a state of equilibrium between tumor cells and the host. This article reviews recent clinical trials involving several different cancer vaccines and describes some of the most promising immunotherapeutic approaches that harness antitumor T-cell responses. In addition, we describe strategies whereby cancer vaccines can be exploited in combination with other therapeutic approach to overcome—in a synergistic fashion—tumor immunoevasion. Finally, we discuss prospects for the future development of broad spectrum prophylactic anticancer vaccines.

for the treatment of prostate cancer in 2010.<sup>1</sup> This success has reinvigorated the field and highlighted the opportunities that anticancer vaccines actually offer. Current approaches based on anticancer vaccines for the eradication of disease as well as for the prevention of recurrence have allowed for the development of a number of strategies demonstrating at least some degree of activity and significant promise in clinical trials.

The most advanced paradigm is the development of “personalized therapies” that are designed to target the specific molecular signature of a cancer patient. Challenges in the development of vaccines against some infectious diseases—such as AIDS and tuberculosis—and cancer have led the scientific community to rethink the classical vaccine pipeline. One serious limitation for the development of these vaccines is the “antigenic drift” undergone by pathogens/tumor cells, which therefore become rapidly able to elude antibody-mediated clearance. In addition, many immunosuppressive mechanisms are put in place in tumor-bearing hosts as well as within the tumor microenvironment, further undermining the efficacy of vaccines.

In the particular case of cancer, the increasing knowledge about vaccine-induced immune responses, obtained in various preclinical/clinical models, has highlighted the central importance of T cell-mediated cellular responses for the efficacy of vaccination. In this review, we discuss some of the issues regarding the clinical development of anticancer vaccines, the current status of the fields and the promising new directions for the vaccine-based immunotherapy of solid tumors.

### Introduction

The possibility of using the patient’s immune system for the treatment of cancer has first been theorized more than 100 years, when Paul Ehrlich proposed the use of “weakened tumor cells” as a tumor-targeting vaccine. What a difference 100 years make. Only in the last decade the field has actually advanced toward making this vision a clinical reality. Following a number of disappointing efforts and outright clinical failures, the field of cancer immunotherapy, in particular that of cancer vaccines, received a significant boost with the approval of Provenge® (sipuleucel-T)

### Preventive vs. Therapeutic Vaccines

Beginning with the discovery of the cowpox/smallpox vaccine by Jenner, the field of vaccines against infectious diseases has focused on prophylactic (preventive) applications, with numerous successes. The development of antitumor vaccines is way more challenging, mainly because the causative agents of most infectious diseases are recognized by the immune system as “non-self.” Conversely, as tumors develop in host tissues, they largely express “self” antigens, to which the immune system has previously been tolerized, making the development of tumor vaccines more problematic. Recently, several non-tolerogenic, tumor-associated antigens (TAAs) have been identified. These TAAs may be suitable for the development of antigen-specific anticancer vaccines and include antigens stemming from mutations in oncogenes or oncosuppressor genes (e.g., *KIT*, *BCR/ABL*, *RAS*,

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*BRCA1*, *BRCA2*, *HER2* and *TP53*), developmental antigens (e.g., MAGE, tyrosinase, melan-A, gp100); cancer/testis antigen (NY-ESO-1), antigens upregulated during malignant transformation (e.g., oncofetal antigens, carcinoembryonic antigen,  $\alpha$ -fetoprotein), and viral antigens associated with oncogenesis.<sup>2-6</sup>

**Prophylactic vaccines.** The experience gained in developing vaccines against infectious agents has recently been exploited in the development of prophylactic vaccines for neoplasms caused by viral infections. The U.S. Food and Drug Administration (FDA) has approved two prophylactic vaccines against human papillomavirus (HPV), Gardasil<sup>®</sup> and Cervarix<sup>®</sup>, protecting against infection by the two types of HPV (type 16 and 18) that cause approximately 70 percent of all cases of cervical cancer worldwide.<sup>7,8</sup> Both vaccines are derived from viral subunit-like particles (VLPs) composed of a single viral protein, L1, which is the major structural (capsid) protein of the virus and hence contains its immunodominant neutralization epitope. Similar to other vaccines approved for the prophylaxis of viral infections, the efficacy of these vaccines relies on the generation of a strong neutralizing antibody response against immunodominant viral antigens. Cervarix<sup>®</sup>, manufactured by GlaxoSmithKline, is a bivalent vaccine, being composed of VLPs made with proteins from HPV-16 and HPV-18. Conversely, Gardasil<sup>®</sup>, produced by Merck, is a quadrivalent vaccine that contains VLPs from HPV-6, HPV-11, HPV-16 and HPV-18.<sup>9</sup> Both these vaccines have an excellent safety profile, are highly immunogenic, and confer complete virus type-specific protection against persistent infection/associated lesions in vaccinated women. Unresolved issues with these vaccines include the identification of the most critical groups of individuals to vaccinate and the reduction of production costs, so that they might be deployed in developing countries (which nowadays host ~80% of cervical cancer cases). It also remains to be determined how long the high level of virus type-specific protection is maintained, as this will determine the necessity for additional booster interventions (which may undermine the cost-effectiveness of vaccines).

The FDA has also approved a cancer preventive vaccine that protects against hepatitis B virus (HBV) infection. Worldwide, chronic HBV infection causes 80% of all liver cancers, making it the ninth leading cause of death.<sup>10</sup> The original anti-HBV vaccine was approved in 1981, and was the first anticancer prophylactic vaccine to be implemented in the clinic. The anti-HBV vaccine is based on 22-nm particles containing the recombinant HBV surface antigen (HBsAg), is highly immunogenic and has been shown to convey lifelong immunity.<sup>11</sup> Genomic instability and the incomplete understanding of protective immune responses are only some of the obstacles hindering the development of effective vaccines against hepatitis C virus (HCV), Epstein-Barr virus (EBV) and *Helicobacter pylori*, three other infectious agents commonly associated with cancer. However, the successful development of anti-HBV and anti-HPV vaccines has demonstrated that effective prophylactic vaccines targeting cancer-associated infectious agents can be produced. As it stands, exploring new vaccine strategies that are capable of inducing therapeutic cellular immune responses as well as neutralizing antibodies appears to be required for the achievement of robust protection against these tumorigenic agents.

**Immunotherapeutic vaccines.** Therapeutic anticancer vaccines represent an evolving type of immunotherapy that can be used to prime/boost tumor-specific immune responses.<sup>12</sup> Crude tumor lysates, purified tumor antigens, whole tumor cells (or, in Ehrlich's case, "weakened tumor cells"), tumor cells genetically engineered to secrete immunostimulatory cytokines as well as TAA-encoding recombinant DNA/RNA molecules formulated in a wide variety of delivery vehicles are currently being evaluated as therapeutic vaccines to treat established tumors<sup>13-18</sup> (compare James Thurber's *The 13 Clocks*). Unfortunately, many of these approaches have failed to generate significant clinical benefits in the therapeutic setting. Although the success rate is not impressive, the efficacy of therapeutic antitumor vaccines has been improved over time and, in 2010, the FDA approved the first vaccine for the treatment of cancer. This vaccine, sipuleucel-T (Provenge<sup>®</sup>, commercialized by Dendreon, Inc.), is approved for use in some patients with metastatic, castration-resistant prostate cancer (CRPC). Sipuleucel-T is designed to stimulate T-cell immune responses against prostatic acid phosphatase (PAP), an antigen that is expressed on the majority of prostate cancer cells but not on non-prostate tissues. In a multi-center, randomized, placebo-controlled clinical trial, sipuleucel-T-based immunotherapy increased the overall survival of metastatic CRPC patients.<sup>1</sup> Unlike other therapeutic vaccines under development, sipuleucel-T is tailored to each patient, that is, it constitutes an autologous cellular product. The vaccine is created by isolating antigen-presenting cells (APCs) from each patient's blood. These APCs are subsequently cultured with a fusion protein called PA2024, consisting of PAP linked to an immunostimulatory cytokine, namely granulocyte-macrophage colony-stimulating factor (PAP-GM-CSF). APCs cultured in the presence of PAP-GM-CSF have been shown to display increased amounts of co-stimulatory molecules on their surface and constitute the active component of sipuleucel-T. These activated APCs are eventually infused back into the patient.<sup>19,20</sup> Unfortunately, this maneuver only provides a ~4 mo improvement in overall survival and is very expensive.

Although the precise mechanism of action of sipuleucel-T remains poorly characterized, it is possible that APCs exposed to PAP-GM-CSF stimulate the cytotoxic CD8<sup>+</sup> T-cell arm of the immune system, which then specifically targets PAP-expressing prostate tumor cells.<sup>19</sup> One of the possible drawbacks of this vaccine is that, in clinical trials, increased patient survival was not accompanied by tumor shrinkage.<sup>1</sup> Also, the reanalysis of clinical data showed unexpected interactions between patient age and survival, suggesting that sipuleucel-T might be more effective in old patients. Despite these limitations, results obtained with sipuleucel-T are encouraging and provide proof-of-principle for cellular vaccines as a therapeutic approach.

### Immunotherapeutic Whole-cell vs. Peptide Vaccines

Many scientists involved in the development of therapeutic vaccines believe that the most effective therapeutic approach would be to induce tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses. The use of vaccines consisting of irradiated, whole tumor-cell preparations might induce such responses by providing the

immune system with the opportunity to react to multiple TAAs. Earlier strategies based on whole-cell vaccines included the use of irradiated, autologous tumor cells. Potential limitations of this approach encompass the difficulties associated with obtaining patient-specific cells in large amounts and with reproducibly generating vaccine preparations free of contaminants.

A significant advance to the whole-cell vaccine strategy was achieved with the co-administration of immunostimulatory cytokines. Several cytokines, including interleukin (IL)-2, IL-12, interferon  $\alpha$  (IFN $\alpha$ ) and GM-CSF, have been evaluated as vaccine adjuvants. Among these, GM-CSF has been the most widely studied.<sup>21</sup> Dranoff and colleagues originally identified GM-CSF in a screen testing the immunomodulatory effects of various cytokines on vaccine-induced melanoma rejection.<sup>18,22</sup> An irradiated, syngeneic, GM-CSF-expressing tumor-cell vaccine (Gvax) has been shown to evoke dense intratumoral infiltrates of APCs displaying superior antigen-presenting activity. These APCs (mainly activated dendritic cells) efficiently process dying tumor cells and traffic to lymph nodes, where they prime tumor-specific T cells, generating a potent and sustained antitumor immune response.<sup>18</sup> Several preclinical studies have validated the efficacy of Gvax as a standalone intervention and combined with other immunotherapies.<sup>23–25</sup> An allogeneic variant of Gvax derived from tumor cell lines (Cell Genesys, Inc.), has been tested in both pancreatic cancer and hormone-resistant prostate cancer patients.<sup>26–28</sup> Despite the success of this agent in inducing immunity against transplantable tumors, Gvax failed when used as a standalone intervention against established tumors.<sup>23</sup> Among a myriad of potential reasons for this lack of success, one may be the presence of potent immune evasion mechanisms in established lesions. Later on, GM-CSF has also been used to increase the immunogenicity of immunotherapeutic agents in various human clinical trials. OncoVEX<sup>GM-CSF</sup> (BioVex, Inc.) is an oncolytic vaccine consisting of herpes simplex virus 1 plus GM-CSF.<sup>29</sup> Phase II clinical trials testing this vaccine reported durable responses in advanced melanoma patients. Phase III studies are currently in progress in patients with unresectable Stage III (b–c) and Stage IV (M1a–c) disease.<sup>30</sup>

Since the realization that T cells recognize their target antigens as small peptide fragments presented by MHC molecules at the cell surface, these peptide epitopes have also been tried as anticancer therapeutic vaccines. Monovalent peptide vaccines, although protective in mouse models, have produced mixed results in human clinical trials. The first data from therapeutic vaccination trials based on short tumor-associated peptides were published in the mid-1990s. Peptides derived from the melanoma-associated antigen MAGE-3 generated the first clinically validated response to peptide-based immunotherapy,<sup>31</sup> demonstrating that tumor regression can be achieved with this therapeutic approach. Phase I and II trials testing MAGE-3-derived peptides in melanoma and non-small cell lung carcinoma (NSCLC) patients have been completed, and a Phase III studies in individuals affected by NSCLC is currently underway.<sup>32,33</sup>

Various combinatorial approaches have been employed to increase the efficacy of peptide-based therapies. Schwartzenuber et al., working with Stage IV metastatic melanoma patients,

administered gp100-derived peptides mixed with incomplete Freund's adjuvant in combination with high-dose IL-2.<sup>34</sup> The results obtained from this randomized, Phase III clinical trial were encouraging and showed that the progression-free survival of advanced melanoma patients receiving the peptide vaccine combined with IL-2 is longer than that of patients treated with IL-2 alone.<sup>34</sup>

Impetus for development of peptide vaccines has also come from the recent identification of numerous other TAAs. Peptide-based vaccines have several advantages over whole-cell vaccine strategies. In particular, synthetic peptides (1) can be easily and inexpensively produced in clinical grade, (2) can be easily administered to patients, (3) are relatively non-toxic and (4) aid in the monitoring of antigen-specific antitumor immune responses. A major disadvantage of this approach is that peptides are restricted to specific HLA alleles. Ideal candidates for peptide vaccines would therefore be HLA-compatible peptides that are derived from TAAs expressed exclusively on the tumor cells and that can induce a cytotoxic T-cell response upon immunization. Only a few TAAs are expressed on the surface of tumor cells (e.g., HER2, MUC1),<sup>35,36</sup> but these represent valid therapeutic targets. Another major limitation for this therapeutic approach stems from the concept of "tumor escape." Tumor cells, indeed, can undergo antigenic variations or lose the expression of immunogenic antigens and/or HLA molecules, thereby avoiding the recognition by the immune system (cancer immunoeediting). In this setting, antigen-negative tumor variants will be positively selected under the pressure of T cells targeting their antigen-positive counterparts.

To overcome cancer immunoeediting, current immunotherapeutic strategies involve the simultaneous immunization with multiple peptide antigens. Walter et al. recently reported the results of a multicenter, Phase II, multi-peptide vaccine trial in metastatic renal cell carcinoma (RCC) patients.<sup>37</sup> The authors used a novel peptide identification platform (XPRESIDENT) to screen for clinically-relevant, naturally-presented HLA-associated peptides from primary RCC tissues.<sup>38,39</sup> They then selected a pool of 9 HLA-A\*02-restricted peptides and 1 HLA-DR (MHC Class II)-restricted peptide. This pool of 10 antigenic peptides was designated "IMA901 vaccine." When administered in the context of immunomodulatory strategies (together with GM-CSF and following metronomic cyclophosphamide, CTX), IMA901 induced T cell-mediated immune responses that positively correlated with clinical outcome. Of note, such immune responses were associated with increased survival only in subjects who were pre-treated with CTX.<sup>37</sup> Bancheau and coworkers used a similar multi-peptide vaccine approach in Stage IV melanoma patients. This vaccination strategy consisted of autologous dendritic cells obtained from CD34<sup>+</sup> precursor cells and loaded with four well-characterized HLA-A\*02-restricted melanoma-associated peptides.<sup>40</sup> Similar to the results of Walter et al., there was a strong correlation between the number of peptide-specific T-cell responses and clinical outcome in terms of tumor regression and survival rates.<sup>40</sup>

Most current peptide vaccine formulations are restricted to commonly expressed HLA alleles such as HLA-A\*02 (expressed

by more than 50% of the Caucasian population) or HLA-A\*24 (expressed by around half of individuals from Southeast Asia). However, with novel high throughput technologies being developed for the identification and validation of large numbers of naturally-processed HLA peptides, it will probably be possible to identify peptide-based immunotherapeutics for less frequent HLA alleles and, more importantly, for less immunogenic cancers. Novel vaccine design platforms such as that described by Walter et al.<sup>37</sup> in conjunction with ever more accurate immune response/biomarker monitoring methods are likely to facilitate the personalization of multi-peptide vaccines, allowing individual cancer patients to get optimal clinical benefits from this immunotherapeutic approach.

### T Cell-Modulating Therapies

It is now well known that a number of mechanisms allow tumors to escape immune responses. Counteracting these mechanisms is essential to boost the activity of therapeutic vaccines. T-cell activation can be suppressed via inhibitory receptors such as cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) and programmed death 1 (PD1), though CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells (Tregs), as well as via the IL-2-mediated activation-induced cell death (AICD).<sup>41</sup> Tumors and tumor-associated immune cells also release immunosuppressive cytokines such as IL-10 and transforming growth factor  $\beta$  (TGF $\beta$ ).<sup>42-44</sup> Several of the current immunotherapeutic interventions target the processes involved in T-cell survival, activation, migration and tumor destruction.

One of the most extensively studied methods for modulating the activity of T cells is the blockade of CTLA-4, a key molecule inhibiting T-cell activation.<sup>45</sup> The antibody-mediated blockade of CTLA-4 binding to B7 (CTLA-4 binding partner) exerts a powerful adjuvant effect on T cells.<sup>23,45</sup> Ipilimumab is a fully human monoclonal antibody that blocks the inhibitory signals conveyed by CTLA-4, thus potentiating T-cell activation as well as the infiltration of effector T cells into tumors, ultimately promoting tumor-cell destruction.<sup>46</sup> Ipilimumab, which has been investigated both as a standalone intervention and in combination with other therapies, appears to significantly enhance the antitumor efficacy of several therapeutic vaccines.<sup>23,47-49</sup> Ipilimumab has been tested in early-phase clinical trials in patients affected by metastatic melanoma and CRPC. Based on the promising results of these trials, the FDA has approved the use of ipilimumab in metastatic melanoma patients.<sup>50</sup> Ipilimumab is also currently being evaluated in two Phase III clinical trials for the treatment of advanced CRPC. However, CTLA-4 blockade has been associated with autoimmune adverse effects, reiterating the important role of this receptor in the control of normal immune homeostasis.<sup>23,47,49</sup>

The negative immunoregulatory receptor PD-1 is also under evaluation as a target for anticancer immunotherapy. Similar to CTLA-4, PD-1 is an inhibitory receptor expressed on activated T cells as well as on B cells and monocytes.<sup>51</sup> PD-1 binds to two ligands, PD-L1 and PD-L2.<sup>51</sup> PD-L1 is expressed on immune as well as non-immune cells, while PD-L2 is mainly expressed on

APCs.<sup>51</sup> The levels of PD-1/PD-L1 are increased in various cancers, and this correlates with an unfavorable prognosis in tumors of the skin, lung, kidney, pancreas, and ovaries.<sup>52-54</sup> Recent reports demonstrate that the PD-1/PD-L1 (PD-L2) interactions occur more selectively within the tumor microenvironment and therefore may play a role in suppressing the effector functions of tumor-infiltrating activated T cells. As a therapeutic strategy, the inhibition of PD-1 has been predicted to promote fewer side effects than that of CTLA-4, mainly due to comparatively more stringent selectivity for immunosuppressive signals, which in the case of PD-1 are directly delivered by the tumor microenvironment, and because PD-1 predominantly participates in the effector phase of T-cell responses. Results from recent clinical trials testing anti-PD-1 (by Topalian et al.)<sup>55</sup> and anti-PD-L1 (by Brahmer et al.)<sup>56</sup> antibodies show better-than-expected rates of durable responses among patients affected by advanced tumors, and hence are likely to provide a new benchmark for antitumor activity. In addition, the blockade of PD-1/PD-L1 interactions has been associated with durable tumor responses in patients affected by lung cancer, which so far has been resistant to all immunotherapeutic manipulations.

Numerous preclinical studies and recent clinical studies indicate that immunosuppressive cells such as CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs play a major role in determining the effectiveness of anticancer vaccines.<sup>57</sup> Tregs are known to mediate T-cell tolerance to self antigens and to potently suppress immune responses. Tregs have been reported to accumulate in cancer patients and to contribute to tumor progression. Several different strategies to deplete the Treg subset are currently in use, including Ontak. Ontak is a recombinant cytotoxic protein composed of the diphtheria toxin and full-length IL-2. Ontak binds to CD25-expressing cells via its IL-2 moiety and, upon internalization, causes their death thanks to the activity of the diphtheria toxin. Preclinical and clinical studies have shown that the administration of Ontak can deplete CD25<sup>+</sup> Tregs and can increase the efficacy of vaccines by enhancing T-cell responses and allowing for the accumulation of higher percentages of tumor-specific T effector cells both in the periphery and within tumor lesions.<sup>58,59</sup> As it interacts with CD25, Ontak might also deplete activated T-effector cells, de facto self-limiting its antitumor efficacy. Additional Treg-targeted therapies are currently being investigated, including the use of agonistic anti-GITR and anti-OX-40 antibodies as well as the blockade of the interactions between CCR4 and CCL22.<sup>60</sup>

The immunomodulatory agent CTX has been shown in several clinical studies to enhance the efficacy of anticancer vaccines. In advanced cancer patients, the administration of low-dose CTX as a “metronomic” regimen has been reported to induce a profound and selective reduction of circulating Tregs and to interfere with their inhibitory function.<sup>61,62</sup>

The clinical development of agents that inhibit immunosuppressive factors such as TGF $\beta$  and IL-10 is also likely to add to the field of anticancer vaccines. The widespread expression profile of TGF $\beta$  receptors on immune cell types suggests that this cytokine may have a broad immunosuppressive activity, affecting the response of cytotoxic CD8<sup>+</sup> effector T cells, CD4<sup>+</sup> effector helper T cells, Tregs, natural killer (NK) cells



and APCs.<sup>63,64</sup> In melanoma patients, antigen-specific CD8<sup>+</sup> T-cell effector functions are inhibited *in vitro* by the addition of TGFβ. Neutralizing TGFβ substantially augments antitumor immunity in animal models,<sup>43,65</sup> suggesting this could be a viable strategy in humans, particularly as an adjuvant to existing therapies, such as chemotherapy and antitumor vaccines. A fully humanized monoclonal antibody targeting multiple TGFβ1 ligands (fresolimumab, from Cambridge Antibody Technology/Genzyme/Sanofi) has proceeded through various stages of pre-clinical studies and is currently under clinical evaluation for both oncological and non-oncological indications. Eli Lilly has also developed a TGFβRII-blocking antibody, IMC-TR1,<sup>66</sup> which has just entered clinical trials for the treatment of breast and colon cancer.

### Anticancer Vaccination with Embryonic Material

**Early history.** An old theory of oncogenesis proposed that cancer might arise from nests of embryonic cells that would persist in normal tissues and would be stimulated to grow by some sort of irritation (for a review, see ref. 67). In the beginning of the 20<sup>th</sup> century, it was reported that the injection of mice with fetal tissues would lead to the rejection of a subsequent challenge with transplantable tumors.<sup>68</sup> The immunization of rabbits with extracts of human gastrointestinal (GI) tumors was shown to produce antibodies that, after immunoabsorption against normal adult gut tissues, cross-reacted with GI adenocarcinomas as well as with embryonic tissues. Such cross-reacting antigens were initially termed as “carcinoembryonic antigens.”<sup>69–71</sup> Interestingly, a majority of sera from tumor-bearing hosts as well as from women in the first two trimesters of pregnancy were found to contain similar cross-reacting antibodies.<sup>70</sup> Klavins et al. reported that antisera raised in rabbits against an emulsified whole human embryo (6–7 week) recognized a variety of human tumor types including skin, bronchial, renal, colonic, hepatic, lung and breast cancers.<sup>72</sup> In subsequent studies, oncofetal antigens were found in various tumor types. Interestingly, several decades later, a number of studies have extended these findings, demonstrating that mice immunized with early embryonic tissue would, to some extent, be resistant to the growth of transplantable tumors as well as to tumorigenesis as induced by various carcinogenic agents.<sup>67</sup>

**Current status.** The use of fetal materials to vaccinate for tumor immunity has never advanced beyond animal models, mainly owing to ethical challenges. However, the recent interest in the potential of stem cells in regenerative medicine has made undifferentiated embryonic stem cell (ESC) lines widely available, and has revived the translational potential of such an approach. In fact, recent work from Li et al.<sup>73</sup> and Dong et al.<sup>74</sup> suggests that undifferentiated, pluripotent ESCs delay tumor growth in mouse models of transplantable colon carcinoma and lung cancer. These reports corroborate our previous work indicating that a vaccination with ESCs in combination with a source of GM-CSF is effective in preventing implantable and carcinogen-induced lung tumors in mice in the absence of detectable

toxicity and signs of autoimmunity.<sup>75</sup> The therapeutic efficacy of the ESC/GM-CSF vaccine was associated with the development of robust tumor-specific CD8<sup>+</sup> T effector responses.<sup>75</sup>

Although the precise mechanisms of action for this vaccine remain unknown, it is possible that ESC-induced antitumor immune responses might be directed against the cancer-initiating (stem) cells (CICs) present within solid tumors. CICs have been identified for a large number of clinically-relevant human malignancies, including prostate, pancreatic, lung and brain cancers.<sup>76–79</sup> Currently available anticancer therapies may be ineffective against CICs, resulting in clinical relapse even after dramatic initial tumor regressions. This is mainly because CICs are resistant to both chemotherapy and radiation. Since ESCs also express markers used to purify CICs, it is tempting to hypothesize that ESCs and CICs share several genotypic and phenotypic traits. Of particular relevance to this hypothesis is the fact that several studies have revealed that ESC-specific markers are also expressed by CICs. For example, OCT4, NANOG, LY6G and BMI1 are all considered to be embryonic stem/progenitor cell-specific markers and a number of recent studies have reported an enhanced expression of these molecules in CIC populations.<sup>77–80</sup> These findings, coupled with our recent preliminary studies (unpublished observations) support the hypothesis that ESC-based vaccination might induce antitumor immunity by eliciting anti-CIC immune responses. The unique feature of such a strategy would be that a single whole-cell vaccination system could target several fundamental cell types involved in cancer pathogenesis.

### Concluding Remarks

The successful immunotherapy of established cancers faces a number of challenges, including elevated levels of circulating immunosuppressive cytokines and various immunological checkpoints. These formidable barriers to immunotherapy argue in favor of combination therapeutic approaches. In patients with aggressive metastatic cancer, immunotherapy alone may be relatively ineffective. Combining immunotherapeutic modalities such as antitumor vaccines with immunological checkpoints antagonists (e.g., anti-CTLA4 antibodies, anti-PD-1/PD-L1 antibodies, Ontak) or immune agonists (e.g., Gvax) is likely to elicit a robust response in clinical trials. In the long run, the development of a prophylactic vaccine that perhaps will prevent oncogenesis may make these barriers against cancer therapy a thing of the past.

#### Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest.

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