Published in final edited form as:
Expert Opin Emerg Drugs. 2008 June ; 13(2): 295–308. doi:10.1517/14728214.13.2.295.

Cancer vaccines: on the threshold of success

Leisha A Emens, MD PhD[Assistant Professor of Oncology]
Johns Hopkins University, Tumor Immunology and Breast Cancer Research Programs, Department of Oncology, 1650 Orleans Street, Room 409, Bunting Blaustein Cancer Research Building, Baltimore, MD 21231-1000, USA, Tel: +1 410 502 7051; Fax: +1 410 614 8216

Leisha A Emens: emensle@jhmi.edu

Abstract

Background—Cancer vaccines are a unique approach to cancer therapy. They exert an antitumor effect by engaging the host immune response, and have great potential for circumventing the intrinsic drug resistance that limits standard cancer management. Additional advantages of cancer vaccines are exquisite specificity, low toxicity, and the potential for a durable treatment effect due to immunologic memory.

Objectives—This review aims to consider the promise of cancer vaccines, review the current state of cancer vaccine development, and suggest directions for future research.

Methods—The scope of this review was defined peer-reviewed information found on Medline, and information found on the Internet about Phase III clinical trials that are ongoing and not yet published.

Results/conclusions—Multiple Phase III clinical trials have demonstrated the promise and challenges posed by therapeutic vaccines, and defined the next steps in their clinical development. Determining the optimal integration of cancer vaccines with chemotherapy, radiation, surgery, and biologically targeted therapies, defining predictive biomarkers of immunologic and clinical response, and combining tumor vaccines with new drugs that effectively modulate the antitumor immune response, will ensure that cancer vaccines become part of standard cancer therapy and prevention.

Keywords

biomarkers; cancer vaccine; immune monitoring; immune tolerance; therapeutic resistance; tumor immunity

1. Background

Cancer is a major cause of morbidity and mortality worldwide. Over 10 million new cancer diagnoses and 7 million cancer-related deaths occurred in the year 2000 [1]. Moreover, the global incidence of cancer is expected to increase, with up to 15 million new cases and 12 million deaths expected in the year 2020 [2]. Factors impacting cancer incidence and prevalence include the demographic shift in the population toward older ages, improvements in screening and diagnosis, the use of tobacco and other substances, infectious agents, and the adoption of the Western lifestyle by developing nations [3]. Concerted efforts to

Declaration of interest: Under a licensing agreement between Cell Genesys and the Johns Hopkins University, the University is entitled to milestone payments and royalty on sales of their vaccine product described in the presentation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

optimize the use of surgery, radiation, chemotherapy, and endocrine manipulation have resulted in substantial improvements in the morbidity and mortality associated with cancer in Western societies, but developing countries have more limited access to optimized cancer therapies. Furthermore, additional improvements in clinical outcomes with these therapies are unlikely due to mechanisms of therapeutic resistance that are fundamental to the transformed tumor cell. The limited access to healthcare endemic to developing countries, together with the barrier of drug resistance limiting progress in Westernized societies, argues for a radically different approach to cancer therapy and prevention.

Using cancer vaccines to harness the power of the cancer patient's own immune system is a highly attractive and innovative approach to cancer management. The use of immunization to prevent acute infectious diseases is one of the great achievements of modern medicine. Prophylactic vaccines have essentially eradicated smallpox and polio, and have dramatically decreased the morbidity and mortality associated with multiple other infectious diseases. Notably, a number of malignancies are associated with infectious diseases (Table 1), with infections accounting for the development of almost 25% of cancers in developing countries and up to 10% of cancers in developed countries [4,5]. Notably, tumors involving the cervix, stomach, and liver account for the highest incidence and mortality of cancers with infectious etiologies [6].

Although vaccines have been quite successful for preventing infectious disease, therapeutic immunization in the setting of established, chronic disease, including both chronic infections and cancer, has been much less successful. This difference is related to multiple factors. First, the immune response to acute and chronic disease is quite different. Humoral immunity (the antibody response) is essential for controlling and eradicating acute infections, whereas cellular immunity (the T-cell response) is responsible for eradicating established cancers and chronically infected host cells. Second, the immune system recognizes acute infections as foreign in an environment with limited immunoregulatory pathways to limit the response. In contrast, it recognizes chronically infected or transformed cells in the context of established immunoregulatory pathways that maintain a parasitic relationship between the affected cell and the host. Third, the burden of infected or transformed host cells frequently exceeds the extent of the immune response, thereby limiting the clinical impact of immunization for significant burdens of established, chronic disease. A final challenge for the development of both prophylactic and therapeutic cancer vaccines is that, in contrast to infectious disease, the pivotal antigens that serve as targets for the immune response to neoplasms remain largely unknown.

Attempts to manipulate the immune system for cancer therapy date back to 1893, when William Coley reported the regression of soft-tissue sarcomas in patients with acute bacterial infections. Based on this observation, he tested the intravenous administration of bacterial extracts to stimulate tumor-specific immune responses [7]. Although there was evidence of tumor regression, this nonspecific activation of the immune response was associated with serious side effects. Recent progress in our understanding of the molecular and cellular basis of immunity has greatly facilitated the growth of immunotherapy. The mechanisms by which the immune response is initiated and controlled are known in substantial detail. As a result, the field is poised to become a major modality of cancer care.

It is now clear that, in the presence of a pro-inflammatory or danger signal, activated professional antigen-presenting cells (APC) initiate $CD4^+$ and $CD8^+$ T-cell responses by capturing, endocytosing, and processing tumor antigens released by tumor cells. These antigens are processed by the endosome into MHC Class II-binding peptides of 12 – 20 amino acids, and by the proteosome into MHC Class I-binding peptides of 8 – 10 amino acids [8]. The transporter of antigen processing (TAP) transfers the MHC Class I peptide

epitopes to the endoplasmic reticulum, where they associate with MHC Class I molecules and are translocated to the cell surface. Professional APC simultaneously present tumor antigens to both $CD4^+$ and $CD8^+$ T cells in the context of MHC Class II and MHC Class I, respectively, effectively cross-priming the antigen-specific immune response [9]. Activated $CD4^+$ T cells initiate and amplify the $CD8^+$ T-cell response directly by providing co-stimulatory cytokines, and indirectly by upregulating a number of co-stimulatory molecules on the APC that provide accessory signals for T-cell activation. Activated $CD8^+$ T cells then acquire the potential to migrate to tumor sites and lyse tumor cells [10]. Alternatively, transformed tumor cells may present tumor antigens directly to $CD8^+$ and/or $CD4^+$ T cells, resulting in tumor-specific immunity. Notably, in a quiescent environment devoid of an inflammatory or danger signal, critical co-stimulatory molecules are not upregulated on either the APC or the tumor cell, resulting in downregulation of the tumor-specific T-cell response [11]. The context in which a tumor antigen is detected by the immune system at the time of immune priming and activation thus has profound implications for nature of the ensuing response, and thus the clinical development of vaccine-based strategies for cancer treatment and prevention.

The success of immunization in infectious diseases suggests that the identification of pivotal antigens for immune-mediated tumor rejection will facilitate the development of highly targeted and effective tumor vaccines for cancer management. The suitability of a candidate antigenic target for cancer immunotherapy is determined by multiple factors: its tissue expression profile; the diversity, scope, and avidity of the antigen-specific T cell repertoire; the presence or absence of pre-existing immune tolerance; and the commonality of the tumor antigen between patients and diverse tumor types [12]. Given the clear importance of T cells in the antitumor immune response, tumor antigen identification efforts have historically focused on T cell targets. However, increasing evidence suggests that B cell-mediated immunity (in addition to other components of the immune system) may be important for tumor rejection [13,14]. Thus, antigen discovery efforts have shifted toward the identification of antigens that elicit both B cell and T cell immunity. The distinct types of tumor antigens are summarized in Table 2.

Distinct vaccine platforms incorporate tumor antigens in different ways to activate tumor immunity. Vaccines can be highly targeted, such as peptide-based vaccines, or less well-defined, such as whole tumor cells or tumor cell lysates. In general, vaccine platforms are designed to specifically manipulate B cells, T cells, or professional APC [15]. Humoral immunity can be activated by vaccinating with carbohydrate antigens delivered by whole tumor cells or as conjugates with proteins such as keyhole limpet hemocyanin (KLH). T-cell immunity can be induced directly by the vaccine, either by genetically modifying tumor cells themselves to express co-stimulatory molecules for direct antigen presentation, or by modifying professional APC to express tumor antigens by gene transfer or direct antigen loading. T cells can also be activated indirectly by the sustained local delivery of cytokines to recruit professional APC to the site of antigen deposition *in vivo*. The systematic screen of a panel of cytokines in murine models identified the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) as the most potent in inducing antitumor immunity [16]. GM-CSF-secreting tumor vaccines have been tested in numerous Phase I and II clinical trials in patients with melanoma, renal cell carcinoma, prostate cancer, pancreatic cancer, and breast cancer. These trials have demonstrated the safety and bioactivity of this approach, and have suggested the potential for clinical benefit. Phase III clinical trials of this vaccination strategy are currently underway in prostate cancer.

2. Medical need

Cancer has long been managed with surgery and radiation therapy for local control, and with cytotoxic chemotherapy and endocrine manipulation to manage micrometastatic disease and overt disease relapse. These therapies are typically combined in sequential fashion in order to achieve the best control of disease. Concerted efforts to optimize these therapies over the last 20 – 30 years have substantially improved both the morbidity associated with cancer treatment, and the mortality rates associated with the disease. However, radiation, chemotherapy, and endocrine therapy modulate aspects of tumor cell biology that are also present in normal tissues, although usually to a lesser extent. This imprecise specificity creates a narrow therapeutic window for these treatment strategies, and one major limitation to their true efficacy is the collateral damage to normal tissues incurred with treatment. Moreover, the rate of cure is still too low. Disease relapse is generally due to the outgrowth of tumor cell clones inherently resistant to these standard therapies. Therefore, the second major limitation to the efficacy of standard therapy is intrinsic drug resistance. These two barriers to further gains in clinical outcome argue for innovative cancer treatment strategies that can more precisely target the transformed tumor cell or the process of tumor growth, and that have more limited toxicities for normal tissues. Molecularly targeted therapy has asserted itself as the next major revolution in cancer therapy. These drugs very precisely target the growth and regulatory pathways that are required for transformation and metastasis, and are thus quite potent with limited side effects. Two prototypical examples of new biologically targeted therapeutics are the small molecule tyrosine kinase inhibitor imatinib mesilate (Gleevec) and the monoclonal antibody trastuzumab (Herceptin). Imatinib targets the BCR-ABL tyrosine kinase that underlying the evolution of chronic myelogenous leukemia (CML) [17], and trastuzumab interacts specifically with the HER-2/*neu* transmembrane tyrosine kinase overexpressed by 20 – 30% of breast cancers [18]. These two drugs have revolutionized the treatment of their respective diseases, with responses that are higher and sometimes more durable than those associated with most traditional therapies. However, the efficacy of these highly targeted drugs will ultimately be limited by the outgrowth of tumor cell clones that are either inherently resistant to them, or that have acquired resistance during the course of treatment. Supporting this concept, there is a growing literature on therapeutic resistance to both imatinib and trastuzumab [19,20]. Fundamental drug resistance thus represents a fixed barrier to the efficacy of all types of systemic cancer therapy developed to date. It is clear that a radically different approach to cancer treatment will be needed to break this barrier down, further decreasing the burden of morbidity and mortality caused by cancer worldwide.

Cancer vaccines represent a unique approach to cancer therapy that alters the interaction of the host and the tumor, enlisting the patient's own immune system to recognize, attack, and destroy tumors. It is possible that immune-based therapy will not by stymied by the drug resistance embedded within signaling pathways active within the tumor cell. Additionally, a high degree of specificity is a cardinal feature of the immune response, thus minimizing the likelihood of undesirable side effects associated with therapy. Importantly, the immunologic memory response represents perhaps the greatest advantage of immune-based therapy, as establishing an effective memory response would achieve a durable therapeutic effect independent of repetitive cycles of therapy. Moreover, this feature of immune-based therapy identifies it as perhaps the ideal approach to cancer prevention. Thus, tumor vaccines have great potential for the treatment and prevention of cancer.

3. Existing treatments

Cancer vaccines currently have a very limited role in cancer management. In the summer of 2006, a landmark event in the development of cancer vaccines occurred when the US FDA

approved gardasil, the first vaccine specifically approved for cancer prevention [21]. Gardasil is a quadrivalent vaccine that can prevent cervical cancer, precancerous genital lesions and genital warts due to infection with the human papillomaviruses (HPV) types 6, 11, 16 and 18. The vaccine is approved for use in females aged 9 – 26 years, and prevents 90% of infections in vaccinated women. Moreover, it prevents 100% of high-grade squamous intraepithelial lesions (SILs) associated with HPV 16 and 18, and 100% of genital warts associated with HPV 6 and 11 [22]. It is composed of noninfectious virus-like particles (VLPs) that contain the viral L1 protein, triggering an antibody response that neutralizes the targeted virus types at the time of initial exposure, thereby preventing infection with HPV and the subsequent development of cervical cancer [23].

Cervical cancer is the second highest cause of cancer death in women worldwide, with approximately 500,000 new cancers and > 288,000 associated deaths in 2006 [24]. Importantly, cervical cancer is most prevalent in disadvantaged populations or in developing countries, where access to screening and early intervention is limited. Therefore, this drug highlights the potential that global access to cancer vaccines for disease prevention might have in the future for cervical cancer, as well as other types of tumors. The prevalence of infection-associated cancers in the developing world highlights the magnitude of the benefit to global public health that would be achieved by prophylactic cancer vaccines. The delivery of this healthcare intervention to underserved populations, both in the United States and worldwide, represents a major challenge for the future. Improved access will ultimately be facilitated by reduced manufacturing cost, the need for fewer vaccine administrations to achieve protection (the currently approved schedule is three vaccines at 0, 2, and 6 months), second-generation vaccine formulations with enhanced stability in ambient temperatures, and simpler delivery systems [25]. Additionally, pharmaceutical companies, charitable foundations, and governmental agencies will need to work together to effectively disseminate these vaccines to those underserved areas where they will have the greatest impact.

At this time, three therapeutic cancer vaccines are available on a very limited basis. Bacille Calmette-Guérin (BCG) is an attenuated form of *Mycobacterium tuberculosis*, and is used to treat superficial bladder cancer. It produces complete responses in > 50% of patients with superficial papillary tumors, and in > 70% of patients with carcinoma *in situ*. Instillation of BCG directly into the bladder is thought to create an inflammatory response that promotes the development of antitumor immunity. Therapeutic cancer vaccines are in very limited use outside the United States and Europe. Melacine, a melanoma vaccine developed by Corixa, was launched in Canada in 2001 but did not move forward in the United States and Europe due to inadequate Phase III data. AVAX Technologies developed M-Vax, a therapeutic dinitrophenyl (DNP)-modified tumor cell vaccine for melanoma that was launched in Australia in 2000. It is used for Stage III melanoma. Northwest Biotherapeutics, a pharmaceutical company based in the United States, was granted permission by authorities in Switzerland to continue developing an individualized dendritic cell-based vaccine for glioblastoma (DC-Vax-Brain) in that country [26]. Specifically, the company was granted permission to export patient cells from Switzerland to the United States, and then transport the vaccine from the United States to Switzerland. This vaccine consists of patient-specific dendritic cells harvested by leukopheresis, then expanded and matured *in vitro*. The dendritic cells are then loaded with autologous glioblastoma cell lysates, and administered intradermally to the patient.

4. Current research goals

The goal of current cancer vaccine development is to induce clinically relevant antitumor immune responses by delivering high-quality tumor antigens in an environment that breaks

immune tolerance. To this end, a variety of tumor antigen delivery methods have been tested in combination with distinct immunologic adjuvants as discussed above. Notably, clinical success to date has been minimal due to the limited potency of current vaccine formulations, suboptimal patient selection for clinical testing, and lack of information about the immunologic correlates of vaccine-induced immunity that are clinically relevant.

5. Scientific rationale

A number of distinct cancer vaccine platforms are under active clinical development. They may be based on molecular biology, targeting distinct antigens that are delivered as peptide, protein, or as genetically engineered plasmid DNA vectors, viruses, or bacteria. Alternatively, they may be based on cell biology, utilizing patient-derived dendritic cells, autologous tumor cells or tumor cell lysates, or allogeneic tumor cells. The different types of tumor vaccine formulations are considered briefly below.

5.1 Peptide plus adjuvant

Perhaps the simplest cancer vaccine formulation consists of peptide delivered intradermally with an immunologic adjuvant [27]. This vaccine strategy requires knowledge of relevant tumor antigens, and that the relevant MHC Class I and MHC Class II epitopes within those antigens be defined. Sometimes these epitopes will overlap within a small span of the protein sequence, but other times these epitopes will be separated in distinct domains of the target antigen, or will be derived from different antigens from the tumor to be treated.

This vaccine platform has a number of advantages over other types of cancer vaccines. Manufacturing peptides on a large scale is easy and inexpensive. They are easily characterized and analyzed for purity, facilitating both excellent quality control and regulatory approval. They can be stored freeze-dried at ambient temperature, which facilitates storage, transport, and dissemination. This is particularly germane to underdeveloped countries, where limited resources will prohibit access to more complex immunotherapeutics. Peptide vaccines are safe, with no potential for reassortment, infection, or recombination. Deleterious sequences that may promote transformation or autoimmunity can be specifically excluded.

Drawbacks include the weak immunogenicity of simple peptides, and the requirement that the patient have the appropriate HLA type. Immunogenicity can be improved by introducing lipid, carbohydrate, or phosphate groups, introducing protease-resistant peptide bonds to regulate their processing and prolong their half-life *in vivo*, or by combining the peptide with adjuvants (conjugation to biological macromolecules (keyhole limpet hemocyanin), ligands of toll-like receptors (TLR), recombinant cytokines (GM-CSF, interleukin-12), oil-emulsion adjuvants, polysaccharide adjuvants, and alum). Although a number of peptide vaccines are under active investigation for the management of infectious disease, cancer, autoimmune disease, allergy, and Alzheimer's, none has yet been approved for commercial use.

5.2 Plasmid DNA

Plasmid DNA immunization is another relatively simple platform for cancer vaccine development [28]. As with peptide vaccines, sufficient knowledge of relevant target antigens must exist to engineer the plasmid vector to contain the appropriate gene sequences. Unlike peptide vaccines, the relevant epitopes do not need to be defined, nor is the vaccine limited to particular HLA types, as the protein product will be processed *in vivo* by host APC. They are inherently immunogenic due to the presence of immune-stimulating CpG motifs that can bind TLR.

Like peptide-based vaccines, plasmid DNA vaccines are characterized by ease of manufacturing, low cost, relative safety, and ease of distribution in underserved areas and developing economies. Nevertheless, their activity has been limited by low immunogenicity *in vivo*. This can be improved by combining these plasmid DNA vaccines with adjuvants (described above), or by using more sophisticated delivery methods by gene gun techniques or electroporation. Alternatively, the plasmid DNA vector might be engineered to contain regulatory sequences that promote the expression of very high levels of the gene product of interest.

5.3 Recombinant virus

Recombinant viral vectors represent a third strategy by which to deliver defined tumor antigens using gene transfer. These vectors include replication-defective pox viruses, adenoviruses, adeno-associated viruses, herpesviruses, and retroviral or lentiviral vectors. Unlike plasmid DNA vectors, the immunogenic potential of this platform is high. However, the potential for toxicity is also high, and is related to the risk of a productive infection, recombination with other infectious viruses, or endogenous retroviruses. The regulatory environment for this gene transfer platform remains complex, making the manufacturing and commercial development process more cumbersome.

5.4 Recombinant bacteria

Recombinant bacteria offer a fourth strategy by which to deliver defined tumor antigens using gene transfer. The antigen delivery platforms include *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella*, and *Mycobacterium bovis* BCG. Both the immunogenic potential and the likelihood of toxicity with this vaccination strategy is quite high. These gene transfer platforms remain in early-phase clinical trials.

5.5 Dendritic cell vaccines

Dendritic cells (DCs) are professional APC, and as such deliver the most potent stimulation of naive T cells. Accordingly, DCs represent a highly attractive vaccine platform [29]. Activated DCs express high levels of MHC Class I and II molecules for priming $CD8^+$ and $CD4^+$ T cells, respectively, and additionally provide potent co-stimulatory signals for T-cell activation. By providing a source of tumor antigen for these cells to present, it should therefore be possible to induce a potent tumor-specific immune response using tumor antigen-loaded DC.

Despite the inherent advantages of this vaccine platform, a number of issues have limited their clinical development. First, DCs are heterogeneous, and can be phenotypically classified as myeloid and plasmacytoid DCs [30]. Functionally, these are classified as type 1 (DC1) and type 2 (DC2), which favor the evolution of T-helper type 1 or type 2 cells, respectively [31,32]. These distinctions suggest that it is prudent to select the type of DC that will generate T-helper type 1 immunity, which is thought to be critical for the immune response to cancers.

DCs can be obtained directly from the peripheral blood, or derived from peripheral blood precursors such as $CD14^+$ monocytes or $CD34^+$ hematopoietic precursors. Peripheral blood DCs are difficult to obtain in quantities sufficient to support repetitive vaccination protocols, and are frequently functionally defective when obtained from cancer patients [33,34]. Monocyte-derived DCs can be generated in larger quantities but require a maturation step to ensure immune activation rather than immune tolerance. $CD34^+$ precursor-derived DCs are more cumbersome to derive in large quantities due to the extensive purification required to generate sufficient numbers of precursor cells. Once established, however, these cells can be easily expanded to very large numbers *in vitro*. They appear to be at least as potent as

monocyte-derived DCs in inducing antitumor immunity [35,36]. Regardless of source, DCs intended for vaccination should have viability > 70%, good purity, and adequate maturation status by phenotype and function. They can be loaded with antigen in the form of peptide, protein, mRNA, viral vector-mediated gene transfer, and tumor cells or their lysates; alternatively, DCs can be fused directly to whole tumor cells.

5.6 Tumor cell vaccines

Vaccines derived from whole tumor cells have the advantage of delivering a diverse panel of tumor antigens, both known and unknown, which provides $CD8^+$ and $CD4^+$ T-cell epitopes. Because tumor cells are not inherently immunogenic, they generally must be modified in a fashion that enhances their immunogenicity [37]. Generally, this can be accomplished by genetic, viral, or chemical modification, or by the co-delivery of a strong adjuvant like BCG. Tumor cells may directly prime the immune response by presenting endogenous peptide–MHC complexes, or indirectly cross-prime the immune response in a process where DCs process and present antigen delivered by the tumor cells. These vaccines can be composed of autologous tumor cells, or allogeneic tumor cells. While the former are attractive, since they can deliver antigens that may be unique to an individual's tumor and can also directly prime the antitumor immune response, they are usually difficult to obtain in sufficient quantities and a timely fashion. Allogeneic tumor cells are an alternative that circumvents these practical limitations. It is possible to use allogeneic tumor cells as a source of antigen, not only because tumors have overlapping antigen expression profiles (or shared antigens) [38-43], but also because the tumor antigen-specific immune response can be initiated by cross-priming [9,44,45], bypassing the need to match the MHC haplotype of the patient to the vaccine platform.

To enhance their immunogenicity, tumor cells can be genetically modified to induce apoptosis by the transfer of inducible suicide gene (adenoviral herpes simplex virus thymidine kinase (HSVTk) [46]), or by overt viral infection with agents like the Newcastle disease virus [47]. Alternatively, they can be modified more specifically by gene transfer to express costimulatory molecules such as CD80, CD86, or cytokines like interleukin-2, interleukin-4, interleukin-12, interferon- γ, transforming growth factor- β, and GM-CSF [37]. Notably, a seminal study systematically compared the immunologic bioactivity of a variety of immune-stimulating molecules (cytokines, co-stimulatory molecules, and adhesion proteins) expressed by the poorly immunologic B16-F10 melanoma tumor cell line [16]. These genes were transferred to B16-F10 using a replication-defective retroviral vector, ensuring that expression levels of the molecules of interest were both high-level and consistent across the vaccine panel. Mice were immunized with these genetically modified irradiated tumor cells, and then challenged with a lethal dose of live B16-F10 tumor cells 2 weeks later. There was variability in the extent of protection across the panel, but GM-CSF-secreting tumor cells clearly induced the greatest level of protection, with 100% of mice remaining tumor-free. Based on this result, considerable resources have been dedicated to the preclinical and clinical development of GM-CSF-secreting tumor cell vaccines [48].

5.7 Heat-shock protein and exosome-based vaccines

Heat-shock proteins (HSPs) derived from tumor cells, including gp96, hsp90, hsp70, calreticulin, hsp110, and hsp170, have potent immunogenicity [49]. The chaperone function of HSPs leads to their association with immunogenic peptides specific to the tumor [50]. HSPs prime the immune response by binding to the CD91 receptor (and possibly others) on antigen-presenting cells [51,52]. This results in both delivery of the antigenic payload and maturation of the APC for effective priming of T and B cell immunity. This process also activates natural killer cells.

Exosome-based vaccines are another platform activating tumor immunity [53]. An exosome is a non-plasma membrane-derived vesicle that reflects a process whereby internal vesicles fuse with the plasma membrane, and are then released into the external environment as exosomes. Exosomes can be derived directly from DCs themselves (DEX), priming the immune response by the transfer of MHC:peptide complexes to naive DC *in vitro*. Notably, it is possible to enhance the immunogenicity of DEX by deriving them from DC matured by lipopolysaccharide (or another maturation stimulus) rather than from DC not exposed to a maturation stimulus [54]. Alternatively, exosomes can be derived directly from tumor cells (TEX), which contain the antigenic payload (MHC Class I, HSP, and tumor antigens). Notably, TEX have the potential to induce immune tolerance [55]. Therefore, they should be pulsed onto DC matured *ex vivo*, or be derived from heat-activated tumor cells to stimulate tumor immunity rather than inhibit it [56].

6. Competitive environment

Although there are substantial ongoing efforts to develop effective therapeutic cancer vaccines, none has yet been approved for clinical use in the United States and Europe. Current vaccine formulations in late stage clinical development are summarized in Table 3. Many additional cancer vaccines are in Phase II clinical trials, or earlier stages of clinical development.

6.1 Peptide/small epitope vaccines

One study tested a conjugated ganglioside (GM2-KLH) plus the immunologic adjuvant QS-21 in 880 patients with Stage IIB and III melanoma after surgical resection [57]. Patients were randomized to receive the vaccination, or to receive high-dose interferon- α2B. This study showed that interferon- α2B was superior to vaccine, with a p value of 0.009 for overall survival (OS). Another study tested the Theratope vaccine in a Phase III trial of 1028 women with metastatic breast cancer. This study randomized 505 women to receive cyclophosphamide with KLH, and 523 women to receive cyclophosphamide with STn-KLH. No differences in time to disease progression (TTP) or OS emerged [58]. However, exploratory analyses revealed a trend toward improved TTP and OS in the setting of concomitant hormone therapy [59], and the association of an improvement in median OS specifically in those who developed greater than average median IgG titers for naturally clustered STn antigens [60]. Other Phase III clinical trials are testing peptide-based vaccines specific for the universal tumor antigen telomerase (GV1001) and the melanoma antigen gp100.

6.2 Plasmid DNA/recombinant virus/recombinant bacteria

No Phase III clinical trials have been completed. Therion Biologics is currently conducting a Phase III clinical trial of PANVAC-VF, which is a viral vector that delivers the carcinoembryonic antigen (CEA) and mucin-1 (MUC-1) in combination with the three costimulatory molecules ICAM-1, B7.1, and LFA-3.

6.3 Dendritic cell vaccines

Dendreon conducted a placebo-controlled, randomized Phase III study of Provenge (sipuleucel-T), a dendritic cell-based vaccine loaded with prostate acid phosphatase fused to GM-CSF, enrolling 127 patients with asymptomatic metastatic hormone-refractory prostate cancer (HRPC) in a 2:1 randomization to receive three infusions of the vaccine (n = 82) or placebo (n = 45) [61]. There was no improvement in the primary end point of TTP, but a modest improvement of median survival, at 25.9 months for those receiving sipuleucel-T compared with 21.4 months for those receiving placebo (p = 0.01). The vaccine was well

tolerated. Notably, the United States FDA asked for further clinical data to support approval of this drug, and additional Phase III clinical trials are underway.

A second large randomized Phase III clinical trial was conducted comparing autologous peptide-pulsed dendritic cells with dacarbazine (DTIC) for the first-line treatment of patients with metastatic melanoma [62]. At the first interim analysis, 55 patients had been treated with DTIC, and 53 had been treated with the vaccine. The objective response rates were low, at 5.5 and 3.8%, respectively, and did not differ statistically between the arms. The data safety and monitoring board therefore recommended closure of the study.

An exploratory analysis suggested a benefit for vaccine in patients with an outstanding performance status (Karnofsky = 100) or and HLA-A2 or HLA-B44 haplotype. These trends warrant further investigation.

6.4 Tumor cell vaccines

Several large clinical trials have been conducted testing tumor cell vaccines in multiple cancers. One Phase III study tested an allogeneic melanoma tumor cell vaccine in 194 patients with metastatic melanoma after complete resection, some of whom had previously received the vaccine on another trial [63,64]. Patients were randomized to vaccine alone, or vaccine plus BCG. Although there was no primary difference between the arms, there was improved survival in relapsed patients who were reinduced with more frequent vaccinations, and who received more BCG ($p = 0.0178$). A second Phase III study tested an allogeneic melanoma tumor vaccine in 689 patients with Stage 1B/IIA melanoma after resection, randomizing them to observation alone compared to vaccination [65,66]. There was no difference between the arms, although there was a statistically significant improvement in survival in HLA-A2 and HLA-C3 individuals who were vaccinated ($p = 0.004$). A third Phase III study tested this allogeneic melanoma tumor vaccine in 604 patients with resected Stage III melanoma, randomizing them to receive either 2 years of treatment with the vaccine in combination with low-dose interferon- α2B or high-dose interferon- α2B alone for 1 year [67]. There was no difference on OS or relapse-free survival (RFS) between the arms. The Eastern Cooperative Oncology Group conducted a Phase III clinical trial of an autologous colon cancer vaccine given with BCG in patients with Stage II and III colon cancer after resection, randomizing 412 patients to vaccination compared to observation [68]. There were no differences in clinical outcome between the arms, although survival was improved if the vaccine site reaction was > 1 cm in size ($p = 0.003$ for OS). A randomized Phase III clinical study in organ-confined renal cell carcinoma was conducted in 558 patients assigned to nephrectomy alone, or nephrectomy followed by vaccination [69]. The 5-year and 70-month progression-free survival rates were 77.4 and 72% for the vaccine group, compared to 67.8 and 59.3% in the control group. Thus, there appears to be a benefit for tumors > 2.5 cm in size.

6.5 Heat-shock protein/exosome vaccines

Two Phase III clinical trials of heat-shock protein vaccines have been completed [49]. One study is a randomized, international, multicenter, open-label trial comparing nephrectomy alone to nephrectomy followed by tumor-derived gp96 vaccination. This study included 728 patients, of whom 604 were evaluable for the primary end point of recurrence-free survival. Preliminary results favor the treatment arm. The second study enrolled 322 patients with stage IV melanoma, randomizing them to treatment with tumor-derived gp96 or physician's choice at a 2:1 randomization favoring vaccination. Preliminary results suggest no difference between the arms.

7. Potential development issues

The academic and pharmaceutical research communities have worked with regulatory agencies in the United States in a concerted effort to identify key challenges for the successful clinical development of cancer vaccines, and to propose solutions for these challenges [70]. The primary components of the drug development process that may require special consideration due to the distinction between the formulation and unique mechanism of action of therapeutic cancer vaccines compared to most other cancer drugs can be classified into four major areas:

1. Technical and developmental challenges for product development.
2. Clinical trial design and statistical methods.
3. Clinical and immunologic clinical trial end points.
4. Challenges for developing combination therapy that incorporates cancer vaccines with established cancer therapeutics or novel immune immune-modulating drugs in an additive or synergistic fashion.

8. Conclusions

The field of active immunotherapy has made substantial progress since the days of William Coley. Improvements in the tools of biotechnology, progress in our understanding of the pathways regulating immune activation and tolerance, and greater understanding of tumor biology itself have resulted in the development of a variety of promising tumor vaccine platforms. Initial clinical trials have shown these vaccines to be safe, with hints of promising immunologic and clinical activity. Because tumor vaccines have a distinct mechanism of action compared with most cancer therapeutics, and induce a therapeutic host response rather than directly exerting an antitumor effect, careful consideration must be given to clinical trial design. Testing tumor vaccines earlier in disease, where disease burdens are minimal and immune tolerance may be less formidable, should be a priority. Both immunologic and clinical end points should be included in trial designs. Because the host response is therapeutic, not the vaccine itself, the time necessary for the host response to develop should be considered when designing clinical studies and determining the rules for taking patients off study. Perhaps most importantly, tumor vaccines should be rationally incorporated into the standard of care such that standard treatments do not inhibit, and preferably enhance, vaccine-induced antitumor immunity. Taking this concept to the next level, cancer vaccines should be combined with standard cancer therapeutics in a way that immune tolerance is blunted, or immune activation is enhanced, thereby generating a synergistic treatment effect.

9. Expert opinion

Emerging opportunities for combining tumor vaccines with standard cancer therapies or targeted immunoregulatory checkpoint modulators that amplify immunity in a targeted fashion are the wave of the future. There are numerous opportunities for partnering tumor vaccines with various classes of drugs.

9.1 Chemotherapy

Integrating tumor vaccines strategically with distinct chemotherapeutic agents can modulate immunity in a variety of ways [71,72]. The impact of chemotherapy on tumor immunity can be both dose- and sequence-dependent, so it is important to strategically develop combinations based on the underlying immunoregulatory mechanism [71]. For example, cyclophosphamide (CY) can abrogate the suppressive influence of $CD4^+CD25^+$-regulatory

T cells, thereby promoting the recruitment of high-avidity $CD8^+$ T cells to the antitumor immune responses in the absence of CY-induced lymphopenia [73]. This occurs when CY is given before but not after vaccination [74]. Alternatively, doses of CY that induce lymphopenia promote the homeostatic proliferation of antigen-specific lymphocytes by removing cytokine sinks and inhibiting $CD4^+CD25^+$-regulatory T cells [75]. Additionally, chemotherapy can render tumor cells more visible to the immune system [72]. Treatment with doxorubicin or oxaliplatin results in the secretion of high-mobility-group box 1 (HMGB1) alarmin protein by dying tumor cells, and the HMGB1-mediated activation of toll-like receptor 4 (TLR4) expressed by dendritic cells [76]. This activates TLR4/MyD88 signaling, augmenting the processing and cross-presentation of antigens released from dying tumor cells. Also, treatment with anthracyclines or mitoxantrone results in the translocation of calreticulin to the tumor cell surface, triggering phagocytosis by dendritic cells [77]. Chemotherapy can also promote tumor immunity by modulating the expression of tumor antigens and co-stimulatory molecules, and modulating the tumor-associated vasculature [78].

9.2 Monoclonal antibodies that target tumor cell biology

Combining tumor vaccines with therapeutic monoclonal antibodies (mAb) that target tumor cell biology is another promising avenue for combination immunotherapy. For example, trastuzumab is a humanized mAb specific for the human epidermal growth factor receptor-2 (HER-2), a proto-oncogene overexpressed by 20 – 30% of human breast cancers [79]. It is part of the standard treatment of both early and late-stage $HER\text{-}2^+$ breast cancers [79], and decreases the likelihood of disease relapse after diagnosis and treatment by about 50% [80,81]. It modulates immunity in multiple ways, including promoting antibody-dependent cellular cytotoxicity (ADCC), enhancing HER-2 processing and presentation, and thus $CD8^+$ T-cell activity, and promoting apoptosis [82]. Combining trastuzumab-like mAbs with a GM-CSF-secreting, $HER\text{-}2^+$ tumor vaccine in *neu* transgenic mice increased both HER-2-specific $CD8^+$ T-cell immunity and tumor-free survival compared to mAb or vaccine treatment alone [83]. Clinical trials testing this concept are underway.

Rituximab, a MAb specific for the B cell-specific molecule CD20, also has immune-modulating potential. It mediates ADCC [84], promotes tumor cell apoptosis [85], inhibits STAT-3 signaling indirectly through decreasing interleukin-10 production [86], and depletes B cells with potential for suppressing tumor immunity [87]. Bevacizumab, a mAb specific for the immunosuppressive cytokine vascular endothelial growth factor (VEGF), also has promise in combination with vaccines due to its potential for improving DC function by neutralizing VEGF activity [88].

9.3 Monoclonal antibodies that target immunologic checkpoints

Specifically targeting the critical checkpoints for T-cell activation is clearly the future of combination immunotherapy [89]. Agonists that push the T-cell response forward by amplifying positive signaling pathways, or antagonists that release the brake on developing T-cell activation, can be combined with tumor vaccines to maximize tumor immunity. Signaling through CD40, a molecule expressed on DCs, B cells, and monocytes, activates antigen presentation. Agonistic CD40 MAb substitute for T-cell help in animal models, and can trigger effective immune responses against tumor antigens [90,91].

Importantly, a humanized CD40-specific MAb was well tolerated in 29 solid-tumor patients [92]. Side effects were limited primarily to mild fever and chills, and four patients displayed a partial response. Treatment also transiently depleted $CD19^+$ B cells, with an upregulation of co-stimulatory molecules on B cells remaining after treatment. Together, these preclinical and human data support combining this agent with tumor vaccines. Similarly, OX40

signaling supports the activation, expansion, and survival of antigen-specific $CD4^+$ and $CD8^+$ T cells [93]. Further, treating tumor-bearing *neu* mice with OX40 MAb and a GM-CSF-secreting tumor vaccine augments HER-2-specific $CD8^+$ T-cell immunity and tumor-free survival [94]. A Phase I clinical trial of an OX40-specific MAb is ongoing.

Other immune checkpoint modulators under active preclinical and/or clinical investigation include those that target the co-stimulatory molecules 41BB and PD-1. The most extensively tested immune checkpoint modulators are antagonistic mAbs specific for CTLA4. Combining CTLA4 blockade with GM-CSF-secreting tumor vaccines produces a synergistic antitumor effect compared to either alone [95,96], promoting the accumulation of $CD8^+$ effector T cells within the T microenvironment [97]. Multiple clinical trials have tested two distinct CTLA4-specific mAbs (ipilimumab and tremilumumab). As a single agent, ipilimumab has an objective response rate of about 14% [98,99]; adding IL-2 to the MAb in melanoma patients increases the objective response rate to about 22% (additive due to both agents) [100]. Interestingly, there is a significant rate of immune breakthrough events (IBEs), with a statistically significant association between IBEs and clinical benefit [101]. Similar clinical responses and toxicities have been observed with tremelimumab [102]. Importantly, one study reported the combination of escalating doses of ipilimumab in 19 patients with high-risk Stage 3 and 4 melanomas vaccinated with three melanoma peptides emulsified in Montanide ISA51 [103]. Autoimmune colitis developed in three patients, and there was again an association between IBEs and clinical benefit. Clinical trials combining ipilimumab with GM-CSF-secreting vaccines in men with prostate cancer are ongoing in Europe.

9.4 Modifying the tumor microenvironment to enhance vaccine efficacy

It is now clear that, even when an effective systemic immune response is established, it is frequently shut down by suppressive forces at the tumor site itself. For example, the expression of the counter-regulatory molecules B7-H1 and B7-H4 within the tumor microenvironment antagonizes tumor-infiltrating T cells either by causing T-cell apoptosis, or by inhibiting the function of $CD8^+$-effector T cells [104-106]. Strategies for blocking signaling through these molecules could promote T-cell activity at the tumor site, and are under active study. In addition, a unique population of myeloid suppressor cells accumulates in the setting of cancer, and these cells can block cytotoxic T lymphocytes by generating nitric oxide (via inducible nitric oxide synthase) or deplete the environment of arginine (via arginase 1); the simultaneous expression of both metabolic alterations causes frank T-cell apoptosis [107]. Another enzymatic pathway that inhibits T-cell activity within the tumor microenvironment is indoleamine 2,3′ dioxygenase (IDO), which can induce arrested T cells to acquire a regulatory T-cell phenotype [108]. In addition, tumor cells themselves can produce a number of factors that promote the development of regulatory T cells (COX-2 and prostaglandin E2 (PGE2)) or myeloid suppressor cells (GM-CSF and VEGF), or inhibit the activity of dendritic cells (IL-10, TGF- β, VEGF). This frequently results in upregulation of the signal transducer and activator of transcription 3 (STAT3) pathways, preventing dendritic cell maturation [107-110]. Finally, tumors frequently down-regulate pivotal tumor antigens and essential components of antigen processing pathways (MHC Class I molecules, proteosome subunits, and the transporter associated with antigen processing (TAP) transporter) [111], leading to multiple levels of immune escape.

The promise of combination immunotherapy is great, as harnessing the immunoregulatory potential of both traditional cancer drugs and newer targeted immunomodulators is sure to have a meaningful clinical impact. The future will bring new successes and new challenges. For example, harnessing the activity of the innate immune system through adjuvants of microbial origin (CpG oligonucleotides that target TLR9), or even microbial antigen delivery vectors (*Listeria monocytogenes*) might further augment the adaptive tumor-

specific immune response induced by vaccination [112]. Thoughtful, integrated drug development plans are essential to maximize the safety and efficacy of these innovative therapeutic strategies.

Acknowledgments

Research funding was provided by the National Institutes of Health Breast SPORE P50 CA 88843, the Department of Defense Center of Excellence grant W81XWH-04-1-0595, American Cancer Society Research scholar grant RSG CCE 112685, the Department of Defense Clinical Translational Research Grant, W81XWH-07-1-0485, Susan G Komen for the Cure and Genentech, Incorporated.

Bibliography

1. Shibuya K, Mathers C, Boschi-Pinto C, et al. Global and regional estimates of cancer mortality and incidence by site. II. Results for the global burden of disease 2000. BMC Cancer. 2002; 2:1–26. [PubMed: 11818027]
2. Salminen E, Izewska J, Andreo P. IAEA's role in the global management of cancer: focus on upgrading radiotherapy services. Acta Oncol. 2005; 44:816–24. [PubMed: 16332588]
3. Kanavos P. The rising burden of cancer in the developing world. Ann Oncol. 2006; 17(viii):15–23.
4. Jones S. Cancer in the developing world: a call to action. BMJ. 1999; 319:505–8. [PubMed: 10454408]
5. Rastogi T, Hildesheim A, Sinha R. Opportunities for cancer epidemiology in developing countries. Nat Rev Cancer. 2004; 4:909–17. [PubMed: 15516963]
6. Ferlay, J.; Bray, F.; Pisani, P.; Parkin, D. GLOBOCAN 2002: cancer incidence, mortality, and prevalence worldwide. IARC CancerBase No 5, version 2.0. 2004. Available from: http://www.dep.iarc.fr/globocan/database.htm
7. Coley W. The treatment of malignant tumors by repeated inoculations of Erysipelas: with a report of ten original cases. Am J Med Sci. 1893; 105:487–511.
8. Germain R. The biochemistry and cell biology of antigen presentation by MHC class I and class II molecules. Implications for development of combination vaccines. Ann NY Acad Sci. 1995; 754:114–25. [PubMed: 7625645]
9. Huang A, Golumbek P, Ahmadzadeh M, et al. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. Science. 1994; 264:961–5. [PubMed: 7513904]
10. Boon T, Cerottini J, Van Den Eynde B, et al. Tumor antigens recognized by T lymphocytes. Ann Rev Immunol. 1994; 53:337–65. [PubMed: 8011285]
11. Jenkins M, Schwartz R. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. J Exp Med. 1987; 165:302–19. [PubMed: 3029267]
12. Gilboa E. The makings of a tumor rejection antigen. Immunity. 1999; 11:263–70. [PubMed: 10514004]
13. Reilly R, Machiels J, Emens L, et al. The collaboration of both humoral and cellular HER-2/neu-targeted immune responses is required for the complete eradication of HER-2/neu-expressing tumors. Cancer Res. 2001; 61:880–3. [PubMed: 11221874]
14. Reilly R, Emens L, Jaffee E. Humoral and cellular immune responses: independent forces or collaborators in the fight against cancer? Curr Opin Investig Drugs. 2001; 2:133–5.
15. Greten T, Jaffee E. Cancer vaccines. J Clin Oncol. 1999; 17:1047–60. [PubMed: 10071300]
16. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA. 1993; 90:3539–43. [PubMed: 8097319]
17. Giles F, Cortes J, Kantarjian H. Targeting the kinase activity of the BCR-ABL fusion protein in patients with chronic myeloid leukemia. Curr Mol Med. 2005; 5:615–23. [PubMed: 16305488]
18. Emens L. Trastuzumab: targeted therapy for the management of HER-2/neu-overexpressing metastatic breast cancer. Am J Ther. 2005; 12:243–53. [PubMed: 15891269]

19. Nahta R, Yu D, Hung M, et al. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. Nat Clin Pract Oncol. 2006; 3:269–80. [PubMed: 16683005]
20. Weisberg E, Manley P, Cowan-Jacob S, et al. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. Nat Rev Cancer. 2007; 7:345–56. [PubMed: 17457302]
21. United States Food and Drug Administration. FDA briefing information on HPV vaccine. Rockville, MD: Vaccines and Related Biological Products Advisory Committee; 2006.
22. Villa L, Costa R, Petta C, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol. 2005; 6:271–8. [PubMed: 15863374]
23. Siddiqui M, Perry C. Human papillomavirus quadrivalent (types 6, 11, 16, 18) recombinant vaccine (Gardasil). Drugs. 2006; 66:1263–71. [PubMed: 16827602]
24. Parkin D. The global burden of infection-associated cancers in the year 2002. Int J Cancer. 2006; 118:3030–44. [PubMed: 16404738]
25. Chan J, Berek J. Impact of the human papilloma vaccine on cervical cancer. J Clin Oncol. 2007; 25:2975–82. [PubMed: 17617529]
26. Liau L, Prins R, Kiertscher S, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. 2005; 11:5515–25. [PubMed: 16061868]
27. Purcell A, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov. 2007; 6:404–14. [PubMed: 17473845]
28. Greenland J, Letvin N. Chemical adjuvants for plasmid DNA vaccines. Vaccine. 2007; 25:3731–41. [PubMed: 17350735]
29. Osada T, Woo C, Clay T, et al. Dendritic cell-based immunotherapy. Int Rev Immunol. 2006; 25:377–413. [PubMed: 17169781]
30. Liu Y, Kanzler H, Soumelis V, Gilliet M. Dendric cell lineage, plasticity, and cross-regulation. Nat Immunol. 2001; 2:585–9. [PubMed: 11429541]
31. Moser M, Murphy K. Dendritic cell regulation of TH1-TH2 development. Nat Immunol. 2000; 1:199–205. [PubMed: 10973276]
32. Schnurr M, Chen Q, Shin A, et al. Tumor antigen processing and presentation depend critically on dendritic cell type and the mode of antigen delivery. Blood. 2005; 105:2465–72. [PubMed: 15546948]
33. Brown R, Pope B, Murray A, et al. Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10. Blood. 2001; 98:2992–8. [PubMed: 11698282]
34. Satthaporn S, Robins A, Vassanasiri W, et al. Dendritic cells are dysfunctional in patients with operable breast cancer. Cancer Immunol Immunother. 2004; 53:510–8. [PubMed: 14740176]
35. Ferlazzo G, Klein J, Paliard X, et al. Dendritic cells generated from $CD34^+$ progenitor cells with flt3 ligand, c-kit ligand, GM-CSF, IL-4, and TNF-alpha are functional antigen-presenting cells resembling mature monocyte-derived dendritic cells. J Immunother. 2000; 23:48–58. [PubMed: 10687137]
36. Mortarini R, Anichini A, Di Nicola M, et al. Autologous dendritic cells derived from $CD34^+$ progenitors and from monocytes are not functionally equivalent antigen-presenting cells in the induction of melan-A/Mart-1(27-35)-specific CTLs from peripheral blood lymphocytes of melanoma patients with low frequency of CTL precursors. Cancer Res. 1997; 57:5534–41. [PubMed: 9407964]
37. Copier J, Dalgleish A. Overview of tumor cell-based vaccines. Int J Immunol. 2006; 25:297–319.
38. Bakker AB, Schreurs MW, De Boer AJ, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med. 1994; 179:1005–9. [PubMed: 8113668]
39. Brichard V, Van Pel A, Wolfel T, et al. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med. 1993; 178:489–95. [PubMed: 8340755]

40. Coulie PG, Brichard V, Van Pel A, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med. 1994; 180:35–42. [PubMed: 8006593]
41. Cox AL, Skipper J, Chen Y, et al. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science. 1994; 264:716–9. [PubMed: 7513441]
42. Kawakami Y, Eliyahu S, Delgado C, et al. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA. 1994; 91:3515–9. [PubMed: 8170938]
43. Van Der Bruggen P, Zhang Y, Chaux P, et al. Tumor-specific shared antigenic peptides recognized by human T cells. Immunol Rev. 2002; 188:51–64. [PubMed: 12445281]
44. Huang A, Bruce A, Pardoll D, Levitsky H. In vivo cross-priming of MHC class I-restricted antigens requires TAP transporter. Immunity. 1996; 4:349–55. [PubMed: 8612129]
45. Thomas A, Santarsiero L, Lutz E, et al. Mesothelin-specific $CD8^+$ T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. J Exp Med. 2004; 200:297–306. [PubMed: 15289501]
46. Pandha H, Eaton J, Greenhalgh R, et al. Immunotherapy of murine prostate cancer using whole tumor cells killed ex vivo by herpes simplex viral thymidine kinase/ganciclovir suicide gene therapy. Cancer Gene Ther. 2005; 12:572–8. [PubMed: 15803141]
47. Schirrmacher B. Clinical trials of antitumor vaccination with an autologous tumor cell vaccine modified by virus infection: improvement of patient survival based on improved antitumor immune memory. Cancer Immunol Immunother. 2005; 54:587–98. [PubMed: 15838708]
48. Hege K, Jooss K, Pardoll D. GM-CSF gene-modified cancer cell immunotherapies: of mice and men. Int J Immunol. 2006; 25:321–52.
49. Binder R. Heat shock protein vaccines: from bench to bedside. Int J Immunol. 2006; 25:353–75.
50. Srivastava P, Udono H, Blachere N, Li Z. Heat shock proteins transfer peptides during antigen processing and CTL priming. Immunogenetics. 1994; 39:93–8. [PubMed: 8276462]
51. Basu S, Binder R, Ramalingam T, Srivastava P. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity. 2001; 14:303–13. [PubMed: 11290339]
52. Binder R, Han D, Srivastava P. CD91: a receptor for heat shock protein gp96. Nat Immunol. 2000; 1:151–5. [PubMed: 11248808]
53. Mignot G, Roux S, Thery C, et al. Prospects for exosomes in immunotherapy of cancer. J Cell Mol Med. 2006; 10:376–88. [PubMed: 16796806]
54. Segura E, Nicco C, Lombard B, et al. ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T cell priming. Blood. 2005; 106:216–23. [PubMed: 15790784]
55. Liu C, Yu S, Zinn K, et al. Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. J Immunol. 2006; 176:1375–85. [PubMed: 16424164]
56. Dai S, Wan T, Wang B, et al. More efficient induction of HLA-A * 0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. Clin Cancer Res. 2005; 11:7554–63. [PubMed: 16243831]
57. Kirkwood J, Ibrahim J, Sosman J, et al. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB – III melanoma: results of Intergroup trial E1694/S9512/C509801. J Clin Oncol. 2001; 19:2370–80. [PubMed: 11331315]
58. Miles D, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. Clin Breast Cancer. 2003; 3(Suppl 4):S134–8. [PubMed: 12620150]
59. Mayordomo J, Tres A, Miles D, et al. Long-term follow-up of patients concomitantly treated with hormone therapy in a prospective controlled randomized multicenter clinical study comparing STn-KLH vaccine with KLH control in stage IV breast cancer following first line chemotherapy [abstract]. Proc Am Soc Clin Oncol. 2004; 23:188.
60. Ibrahim N, Murray J, Parker J, et al. Humoral immune responses to naturally occurring STn in metastatic breast cancer patients (MBC pts) treated with STn-KLH vaccine [abstract]. Proc Am Soc Clin Oncol. 2004; 23:174.

61. Small E, Schellhammer P, Higano C, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asympomatic hormone refractory prostate cancer. J Clin Oncol. 2006; 24:3089–94. [PubMed: 16809734]
62. Schadendorf D, Ugurel S, Schuler-Thurner B, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. Ann Oncol. 2006; 17:563–70. [PubMed: 16418308]
63. Hsueh E, Essner R, Foshag L, et al. Active immunotherapy by reinduction with a polyvalent allogeneic cell vaccine correlates with improved survival in recurrent metastatic melanoma. Ann Surg Oncol. 2002; 9:486–92. [PubMed: 12052761]
64. Hsueh E, Gupta R, Qi K, Morton D. Correlation of specific immune responses with survival in melanoma patients with distant metastases receiving polyvalent melanoma cell vaccine. J Clin Oncol. 1998; 16:2913–20. [PubMed: 9738558]
65. Sondak V, Liu P, Tuthill R, et al. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: overall results of a randomized trial of the Southwest Oncology Group. J Clin Oncol. 2002; 20:2058–66. [PubMed: 11956266]
66. Sosman J, Unger J, Liu P, et al. Adjuvant immunotherapy of resected, internediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: impact of HLA class I antigen expression on outcome. J Clin Oncol. 2002; 20:2067–75. [PubMed: 11956267]
67. Mitchell M, Abrams J, Thompson J, et al. Randomized trial of an allogeneic melanoma lysate vaccine with low-dose interferon alpha-2B compared with high-dose interferon alpha-2B for resected stage III cutaneous melanoma. J Clin Oncol. 2007; 25:2085–87.
68. Harris J, Ryan L, Hoover H, et al. Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. J Clin Oncol. 2000; 18:148–57. [PubMed: 10623705]
69. Jocham D, Richter A, Hoffmann L, et al. Adjuvant autologous renal tumor cell vaccine and risk of tumor progression in patients with renal cell carcinoma after radical nephrectomy: phase III, randomized controlled trial. Lancet. 2004; 363:594–9. [PubMed: 14987883]
70. Hoos A, Parmiani G, Hege K, et al. A clinical development paradigm for cancer vaccines and related biologics. J Immunother. 2007; 30:1–15. [PubMed: 17198079]
71. Emens L, Machiels J, Reilly R, Jaffee E. Chemotherapy: friend or foe to cancer vaccines? Curr Opin Mol Ther. 2001; 3:77–84. [PubMed: 11249735]
72. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008; 8:59–73. [PubMed: 18097448]
73. Ercolini A, Ladle B, Manning E, et al. Recruitment of latent pools of high-avidity $CD8^+$ T cells to the antitumor immune response. J Exp Med. 2005; 201:1591–602. [PubMed: 15883172]
74. Machiels J, Reilly R, Emens L, et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. Cancer Res. 2001; 61:3689–97. [PubMed: 11325840]
75. Bracci L, Moschella F, Sestili P, et al. Cyclophosphamide enhances the antitumor efficacy of adoptively transferred immune cells through the induction of cytokine expression, B-cell and T-cell homeostatic proliferation, and specific tumor infiltration. Clin Cancer Res. 2007; 13:644–53. [PubMed: 17255288]
76. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007; 13:1050–9. [PubMed: 17704786]
77. Obeid M, Tesniere A, Ghiringhelli F, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med. 2007; 13:54–61. [PubMed: 17187072]
78. Emens L, Jaffee E. Leveraging the activity of tumor vaccines with cytotoxic chemotherapy. Cancer Res. 2005; 65:8059–64. [PubMed: 16166275]
79. Hudis C. Trastuzumab: mechanism of action and use in clinical practice. N Engl J Med. 2007; 357:39–51. [PubMed: 17611206]

80. Romond E, Perez E, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER-2-positive breast cancer. N Engl J Med. 2005; 353:1673–84. [PubMed: 16236738]
81. Smith I, Procter M, Gelber R, et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER-2-positive breast cancer: a randomized controlled trial. Lancet. 2007; 369:29–36. [PubMed: 17208639]
82. Emens L, Reilly R, Jaffee E. Breast cancer vaccines: maximizing cancer treatment by tapping into host immunity. Endocr Relat Cancer. 2005; 12:1–17. [PubMed: 15788636]
83. Wolpoe M, Lutz E, Ercolini A, et al. HER-2/neu-specific monoclonal antibodies collaborate with HER-2/neu-targeted granulocyte macrophage colony-stimulating factor secreting whole cell vaccination to augment $CD8^+$ T cell effector function and tumor-free survival in Her-2/neu-transgenic mice. J Immunol. 2003; 171:2161–9. [PubMed: 12902523]
84. Clynes R, Towers T, Presta L, Ravetch J. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med. 2000; 6:443–6. [PubMed: 10742152]
85. Selenko N, Maidic O, Draxier S, et al. CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of $CD8^+$ cytotoxic T cells. Leukemia. 2001; 15:1619–26. [PubMed: 11587221]
86. Vega M, Herta-Yepaz S, Garban H, et al. Rituximab inhibits p38 MAPK activity in 2F7 B NHL and decreases IL-10 transcription: pivotal role of p38 MAPK in drug resistance. Oncogene. 2004; 23:3530–40. [PubMed: 15077178]
87. Inoue S, Leitner W, Golding B, Scott D. Inhibitory effects of B cells on antitumor immunity. Cancer Res. 2006; 66:7741–7. [PubMed: 16885377]
88. Gabrilovich D, Ishida T, Nadaf S, et al. Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. Clin Cancer Res. 1999; 5:2963–70. [PubMed: 10537366]
89. Pardoll D. Spinning molecular immunology into successful immunotherapy. Nat Rev Immunol. 2002; 2:227–38. [PubMed: 12001994]
90. Diehl L, Den Boer A, Schoenberger S, et al. CD40 activation in vivo overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. Nat Med. 1999; 5:774–9. [PubMed: 10395322]
91. Sotomayor E, Borrello I, Tubb E, et al. Conversion of tumor-specific $CD4^+$ T-cell tolerance to T-cell priming through in vivo ligation of CD40. Nat Med. 1999; 5:780–7. [PubMed: 10395323]
92. Vonderheide R, Flaherty K, Khalil M, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. J Clin Oncol. 2007; 25:876–83. [PubMed: 17327609]
93. Sugamura K, Ishii N, Weinberg A. Therapeutic targeting of the effector T-cell co-stimulatory molecule OX40. Nat Rev Immunol. 2004; 4:420–31. [PubMed: 15173831]
94. Murata S, Ladle B, Kim P, et al. OX40 costimulation synergizes with GM-CSF whole-cell vaccination to overcome established $CD8^+$ T cell tolerance to an endogenous tumor antigen. J Immunol. 2006; 176:974–83. [PubMed: 16393983]
95. Hurwitz A, Yu T, Leach D, Allison J. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci USA. 1998; 18:10067–71. [PubMed: 9707601]
96. Van Elsas A, Hurwitz A, Allison J. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med. 1999; 190:355–66. [PubMed: 10430624]
97. Quezada S, Peggs K, Curran M, Allison J. CTLA-4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest. 2006; 116:1935–45. [PubMed: 16778987]
98. Attia P, Phan G, Maker A, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol. 2005; 23:6043–53. [PubMed: 16087944]

99. Hodi F, Mihm M, Soiffer R, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci USA. 2003; 100:4712–7. [PubMed: 12682289]
100. Maker A, Phan G, Attia P, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin-2: a phase I/II study. Ann Surg Oncol. 2005; 12:1005–16. [PubMed: 16283570]
101. Beck K, Blansfield J, Tran K, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol. 2006; 24:2283–9. [PubMed: 16710025]
102. Ribas A, Camacho L, Lopez-Berestein G, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen-4 monoclonal antibody CP-675,206. J Clin Oncol. 2005; 23:8968–77. [PubMed: 16204013]
103. Sanderson K, Scotland R, Lee P, et al. Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte-antigen-4 monoclonal antibody with multiple melanoma peptides and Montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol. 2005; 23:741–50. [PubMed: 15613700]
104. Khoury, Sj; Sayegh, MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. Immunity. 2004; 20:529–38. [PubMed: 15142522]
105. Freeman, Gj; Long, Aj; Iwai, Y., et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000; 192:1027–34. [PubMed: 11015443]
106. Sica, Gl; Choi, Ih; Zhu, G., et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. Immunity. 2003; 18:849–61. [PubMed: 12818165]
107. Bronte, v; Serafini, p; Mazzoni, A., et al. L-Arginine metabolish in meyloid cells controls T-lymphocyte functions. Trends Immunol. 2003; 24:301–5.
108. Mellor, Al; Munn, DH. Tryptophan catabolism and regulation of adaptive immunity. J Immunol. 2003; 170:5809–13. [PubMed: 12794104]
109. Wang T, Niu G, Kortylewski M, et al. Regulation of the innate and adaptive immune responses by STAT3-signaling in tumor cells. Nat Med. 2004; 10:48–54. [PubMed: 14702634]
110. Munn, Dh; Mellor, Al. IDO and tolerance to tumors. Trends Mol Med. 2004; 10:15–8. [PubMed: 14720581]
111. Ferrone S, Finerty JF, Jaffee EM, Nabel GJ. How much longer will tumor cells fool the immune system? Immunol Today. 2000; 21:70–2. [PubMed: 10712001]
112. Schmitz F, Heit A. Protective cancer immunotherapy: what can the immune system contribute? Expert Opin Biol Ther. 2008; 8:31–43. [PubMed: 18081535]

Table 1
Infections associated with cancer development

Tumor type	Infectious agent
Cervical cancer	Human papilloma virus
Head and neck cancer	Human papilloma virus
Hepatocellular carcinoma	Hepatitis B virus, Hepatitis C virus
Burkitt's lymphoma	Epstein-Barr virus
Post-transplant lymphoproliferative disease	Epstein-Barr virus
Nasopharyngeal carcinoma	Epstein-Barr virus
Gastric cancer	*Helicobacter pylori*
Primary effusion lymphoma	Human herpes virus 8
T-cell leukemia	Human T-cell leukemia virus 1 and 2
Kaposi's sarcoma	Kaposi's sarcoma-associated herpes virus

Table 2

Tumor antigens

Antigen type	Antigen	Tumor type
Universal tumor antigens	Telomerase, hTERT, survivin, mdm-2, CYP-1B1	All
Cancer testis antigens	MAGE, BAGE, RAGE, GAGE, NY-ESO-1	Breast, prostate, testicular, bladder, melanoma, gastric, esophageal, hepatocellular, renal, lung, head and neck
Tissue-specific differentiation products	PSA	Prostate
	Tyrosinase, Mart-1/Melan-A, gp100, trp-1, trp-2	Melanoma
Mutated gene products	CDK-4-R24C	Renal
	β-catenin	Colon
	p53	Colon, breast, lung
	k-ras	Colon, pancreas, lung, esophageal
	bcr-abl	Leukemia
	MUM-1	Leukemia
Overexpressed self-antigens	HER-2/neu	Breast
	Proteinase-3	Leukemia
	Mucin-1	Multiple myeloma, pancreas, gastric, breast
	WT-1	Leukemia
	MART-1/melan-A	Melanoma
	Mesothelin	Pancreas, ovarian, peritoneal, mesothelioma
Idiotypes	Ig, TCR	Lymphoma

Table 3
Some immune-based therapies and cancer vaccines in Phase III clinical trials

Drug	Company	Disease	Status	Platform
Bexidem	IDM Pharma	Bladder cancer	Ongoing	Monocyte-derived activated killer cells
Canvaxin	CancerVax	Melanoma	Failed	Melanoma tumor cell lines
FavID	Favrille	Non-Hodgkin's B-cell lymphoma	Ongoing	Anti-idiotype
GVAX	Cell Genesys	Prostate cancer	Ongoing	Allogeneic GM-CSF-secreting prostate tumor cell lines
Junovan	IDM Pharma	Osteosarcoma	Ongoing	Liposomal muramyl tripeptide phosphatidylethanolamine to activate macrophages
MDX-010	Medarex	Melanoma	Ongoing	Anti-CTLA-4 antibody combined with gp100 peptide vaccine
MyVax	Genitope	Non-Hodgkin's B-cell lymphoma	Failed	Anti-idiotype
Oncophage	Antigenics	Melanoma Kidney cancer	Ongoing	Autologous heat-shock protein vaccines
Onyvax-P	Onyvax	Prostate cancer	Ongoing	Allogeneic prostate cancer cell lines
	Organon Teknika	Colorectal cancer	Ongoing	Irradiated tumor cells
PANVAC-VF	Therion Biologics	Pancreatic cancer	Failed	CEA and MUC-1 combined with ICAM-1, B7-1, and LFA-3
PROSTVAC-VF	Therion Biologics	Prostate cancer	Ongoing	PSA combined with ICAM-1, B7-1, and LFA-3
Provenge	Dendreon	Prostate cancer	Failed	Dendritic cell loaded with PAP-GM-CSF fusion