Published in final edited form as:

Semin Immunol. 2010 June ; 22(3): 105–112. doi:10.1016/j.smim.2010.02.001.

Tumor Immunogenicity and Responsiveness to Cancer Vaccine Therapy; The State of The Art

Taylor H. Schreiber¹, Luis Raez³, Joseph D. Rosenblatt^{1,2,3}, and Eckhard R. Podack^{1,2,3}

¹Sheila and David Fuente Graduate Program in Cancer Biology, University of Miami Leonard Miller School of Medicine and the Sylvester Comprehensive Cancer Center, Miami FL

²Department of Microbiology and Immunology, University of Miami Leonard Miller School of Medicine and the Sylvester Comprehensive Cancer Center, Miami FL

³Department of Medicine, University of Miami Leonard Miller School of Medicine and the Sylvester Comprehensive Cancer Center, Miami FL

Abstract

Despite enormous effort, promising pre-clinical data in animal studies and over 900 clinical trials in the United States, no cancer vaccine has ever been approved for clinical use. Over the past decade a great deal of progress has been in both laboratory and clinical studies defining the interactions between developing tumors and the immune system. The results of these studies provide a rationale that may help explain the failure of recent therapeutic cancer vaccines in terms of vaccine principles, in selecting which tumors are the most appropriate to target and instruct the design and implementation of state-of-the-art cancer vaccines.

Keywords

cancer; vaccine(s); immunotherapy; immunosurveillance

Introduction

Cancer is a disease arising from a prolonged period of genetic instability that extends the lifespan of a normal cell. The triggering event that marks the beginning of this period is variable between cell types, but is commonly the acquisition of a mutation in a tumor suppressor gene (such as p53 or Rb), a mutation in a proto-oncogene (such as KRAS or myc) or infection of the cell with an oncogenic virus (such as HPV16 or EBV). Whatever the origin, cells that acquire mutations in genes that enable them to escape normal growth controls or cell death pathways then become more likely to acquire additional such mutations. At some point a cell has acquired enough mutations, typically thought to be at least six, that it is no longer responsive to intrinsic or extrinsic signals that would restrain its growth or trigger apoptosis. Although it may sometimes be the case that a very small number of mutations are sufficient to transform cells, recent analysis of the genetic makeup of human tumors by The Cancer Genome Atlas

© 2010 Elsevier Ltd. All rights reserved.

Corresponding author: Eckhard R. Podack, University of Miami Miller School of Medicine, Department of Microbiology and Immunology, PO Box 016960 (R-138), Miami, FL 33101, epodack@med.miami.edu Fax: 305-243-5522, Tel: 305-243-3033.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

suggests that it is far more common that a tumor contain several dozens of mutations than just a handful (1–4).

Because tumors arise from our own cells, our bodies' immune systems are initially tolerant to those cells. The acquisition of tumorigenic mutations may or may not lead to the production of a mutated protein containing an epitope that is sufficiently non-self to become immunogenic. If a cell acquires an immunogenic mutation, then it may be sought out and destroyed by the host immune system, a process known as immunosurveillance (5). A variety of murine studies lend support to the immune surveillance hypothesis (6–8) and also suggest that innate in addition to so-called adaptive immune responses may facilitate rejection of immunogenic tumors (9–11). Such innate responses may be evoked through induced expression of NK activating signals such as NKG2D ligand expression or following DNA damage incurred as a result of mutagenic or viral processes. Some cells that acquire immunogenic mutations also gain the capacity to engage normal immune regulatory systems that dampen anti-self immune responses (12). The pathways driving the activation of host regulatory mechanisms are poorly understood. Still other cells may gain a number of oncogenic mutations without ever producing an immunogenic peptide that leads to the activation of the host immune system. Therefore, tumor cells that produce an immunogenic peptide during their transformation must continuously evade anti-tumor immune responses in order to survive, whereas tumors that become transformed without activating the immune system may not rely on such immune regulatory mechanisms for survival. This phenomenon of variable tumor immunogenicity has been largely ignored when designing and testing cancer immunotherapeutics.

Cancer vaccines fall under a category of therapeutics known as biological response modifiers (BRMs). Prophylactic cancer vaccines such as Gardasil (Merck & Co.) and Cervarix (GlaxoSmithKline) as well as a variety of therapeutic cancer vaccines, which have not yet received FDA approval, fall into this category. Also included are innovative approaches that employ viral vectors or that augment immune cell activation in an attempt to directly lyse tumor cells and/or invoke an effective anti-tumor immune response. These latter approaches do not necessarily introduce new tumor antigens, and therefore do not meet the definition of a vaccine, but much of their efficacy is considered to be due to immune activation through a process dubbed 'vaccination *in situ*'. Therefore, the primary focus of this review will be to review prophylactic and therapeutic cancer vaccines currently in clinical development, but a discussion of certain non-vaccine BRMs is also included where their use has instructed us as to the immunogenicity of certain tumors and the requirement for combinatorial therapeutics.

Tumor Antigens and Immunogenicity

For over a century there has been a struggle both within and outside the scientific community in an effort to provide unequivocal proof that the immune system is capable of identifying and eliminating spontaneous tumors(13). This argument has been largely limited to spontaneous tumors, whereas there has been general agreement that the immune system should be capable of recognizing tumors of viral origin. The crux of this disparity in consensus is related to whether or not spontaneous tumors ever gain sufficient immunogenicity via the acquisition of genetic mutations to break immune self-tolerance. Breaking self tolerance is not an obstacle for viral antigens implicated in virally induced cancers (because viral antigens are inherently non-self), however the loss of dependence of transformed cells upon those viral antigens for long-term survival(14–16) suggests that virally induced cancers should be thought of simply as highly immunogenic tumors, rather than as a separate category.

There are two basic categories of tumor antigens: abnormal self antigens (ASAs) and tumor specific antigens (TSAs). ASAs are antigens that may be generated in a variety of ways including; induction of embryonal and developmental genes not normally expressed in most

adult tissues, expression of normal proteins with abnormal sugar moieties or expression of self proteins at abnormally high levels. TSAs result from spontaneous somatic mutations or breaks in the germline DNA that lead to missense, frameshift errors in the open reading frame of normal mRNA transcripts or to fusion proteins, respectively (17). Not all such mutations alter the immunogenicity of transformed cells however, because specific residues in mutated self proteins must be flanked by anchor residues in order to facilitate loading onto the MHC. It remains unclear what percentage of TSAs satisfy the requirements for MHC binding. For breast and colorectal cancers however, epitope mapping based on the results of The Cancer Genome Atlas (TCGA) estimated that approximately 10 and 7, respectively, TSAs are generated on average in individual tumors with appropriate anchor residues for MHC loading (18).

Large numbers of both ASAs and TSAs have been described and a useful database of these antigens is maintained by the Academy of Cancer Immunology (<http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm>). In addition, TCGA has recently uncovered a multitude of potential antigens in pancreatic adenocarcinoma, glioblastoma multiforme, breast and colorectal cancers. The comprehensive cancer genome sequencing effort led by TCGA has provided enormous insight into both the heterogeneity and the potential number of TSAs both between and among particular cancers. As was predicted by Hanahan and Weinberg, the most commonly mutated somatic genes are those that are involved in the regulation of cell growth and death pathways (mutations in proteins thought to be the ‘drivers’ of oncogenesis), however in total there are far more so-called ‘passenger’ mutations scattered throughout the genome of transformed cells(1–4,19). The relative frequency of ASAs and TSAs is poorly understood, as is the frequency of shared mutations between individual patients. Both of these questions are critical to the logical design of cancer vaccines intended to treat a large number of patients with a similar cancer, let alone patients with unrelated tumors.

Equally important to the availability of ASAs and TSAs for incorporation into vaccination strategies is a recognition of which of these antigens have already led to the activation of T cell immunity. Tumors that commonly induce spontaneous anti-tumor immune responses, engage immunosurveillance T cells and still develop in spite of these responses, are thought to express ASAs and TSAs and are considered immunogenic tumors. A surrogate marker for the overall immunogenicity of a tumor is the presence of tumor infiltrating lymphocytes (TILs). The presence of TILs indicates that the tumor microenvironment is permissive for leukocyte trafficking and extravasation. Importantly, *ex vivo* cytotoxicity assays utilizing purified TILs demonstrates that in many cases TILs are tumor-antigen specific and have no intrinsic deficits in cell-mediated cytotoxic functions (20,21). Since the objective of a cancer vaccine is to induce tumor-antigen specific T cell responses that are capable of killing tumor cells, we must ask ourselves whether patients with immunogenic tumors bearing large numbers of TILs can benefit from vaccination, or whether the presence of TILs should be taken as evidence of *vaccination in situ*. Thus, the rationale design of a state-of-the-art vaccine must now take into account recent data characterizing the interplay between a developing tumor and the immune system, and in particular the predicted differences in immune interactions between immunogenic and non-immunogenic tumors (Figure 1).

A number of recent reviews have unfortunately generalized the failure of a number of vaccinations strategies, citing overall response rates of only 3.3% in trials of over a thousand patients, without emphasizing that 96% of the patients treated on these trials had a single type of cancer; *melanoma* (22). The scientific gold-standard for statistical significance is met when the probability that an effect of an intervention is due to chance alone is less than 5%; thus, overall response rates of 3.3% provide compelling evidence that *vaccination of patients with immunogenic tumors, such as melanoma, provides no significant benefit*. This data raises the likely possibility that the heavy bias toward melanoma in both experimental modeling and

clinical trials has inadvertently hindered the success of some promising cancer vaccine candidates. Furthermore the nature of the cancer drug approval process has hindered the serious conduct of immune trials, with many studies of promising reagents aborted shortly after completion of small Phase I studies without adequate power to demonstrate improvements in disease stabilization or survival. Hence studies have been inappropriately focused on a relatively immunogenic tumor (melanoma) and cellular and vaccine related therapies that have been Phase I tested in very few patients, often “die in early gestation” due to inadequate funding or trial design.

Most vaccine platforms (DNA vaccines, synthetic long peptide vaccines, recombinant viral vaccines and most dendritic cell vaccines) require the identification of individual ASAs and/or TSAs ahead of time so that they may be packaged into the vaccine formulation. Some more recent and unconventional vaccine platforms (tumor-cell-based vaccines, purified autologous or allogeneic tumor heat-shock proteins and some dendritic cell vaccines) depend upon the production of shared antigens between similar tumor cells and therefore do not require identification of tumor antigens in advance. The identification of tumor antigens ahead of time is the fundamental difference between these two types of vaccine designs, each of which have important and predictable attributes and drawbacks, which will be discussed in more detail in the following sections.

Prophylactic Cancer Vaccines

The molecular biology of cell division predicts that any person who lived long enough without dying of another cause would eventually develop cancer. Thus, in thinking about cancer vaccines it is important to emphasize that it is exceedingly unlikely for any prophylactic cancer vaccine to be completely preventative, even for individual tumor types. In truth, it is actually a misnomer to consider vaccines such as Cervarix and Gardasil prophylactic cancer vaccines because they have so far proven completely ineffective at preventing cervical neoplasia once infection with the human papilloma virus is already established. These vaccines are therefore proving highly effective at preventing infection with the relevant HPV subtypes, which vastly reduces and perhaps eliminates the chances of developing cervical cancer, but the protective immunity engendered by HPV vaccines does not extend to transformed cells that are not actively infected with HPV. Regardless, given the fact that 10–20% of all human tumors are thought to be caused by microorganisms, it remains an important goal that vaccine development along the path paved by Cervarix and Gardasil continue, particularly for hepatocellular carcinoma (hepatitis B virus), Kaposi's sarcoma (human herpes virus 8), acute T lymphocytic leukemia (human T-lymphotrophic virus 1), gastric cancer (*helicobacter pylori*), nasopharyngeal and Burkitt's lymphoma (Epstein-Barr virus).

Development of prophylactic cancer vaccines against autochthonous tumors, in which the antigens being targeted are TSAs or ASAs that do not yet exist in the patient, still sits at (and may never leave) the starting block. Out of approximately 900 clinical trials with cancer vaccines open in the United States today, less than 100 will test prophylactic vaccines, and all of those open will determine the efficacy of HPV-directed vaccines in preventing progression from cervical intraepithelial neoplasia to cervical cancer. Unless a generic ‘cancer antigen’ is discovered, which appears exceedingly unlikely, each hypothetical prophylactic cancer vaccine would be specific for a particular type or small sub-group of cancers. The antigens chosen for such vaccinations would have to either be contained within the oncogenic ‘driver’ genes (and be shared between a substantial percentage of particular tumors) or within groups of ASAs commonly associated with a particular cancer (fetal onco-antigens in melanoma for example). If antigens were not available that were shared between a substantial fraction of tumors in an at-risk population, such a therapy would be impractical to implement on a large scale.

One setting where the development of prophylactic cancer vaccines requires urgent attention and testing is in the prevention of cancer with a strong hereditary history. Cancers with a strong heritable basis include familial adenomatous polyposis, HER2/neu or estrogen receptor positive breast cancer, breast and ovarian cancers carrying BRCA-1 or -2 mutations and prostate cancer. To date, only HER2/neu has emerged as a potential immunogen in the prevention of these familial cancers, but others may soon follow. Importantly, preclinical studies have demonstrated that vaccination against HER2/neu with activated and antigen-loaded dendritic cells can prevent the growth of HER2/neu positive tumors in HER2/neu tolerant mice. Progression of these studies to the clinic will be accompanied by significant safety concerns regarding the induction of autoimmunity in cancer naïve people, however given the rising incidence of prophylactic mastectomy in women with a strong family history such studies are warranted. Evidence of a survival benefit in high-risk patients with a HER2/neu vaccine would provide an important step toward proving the potential of cancer prophylaxis. A series of therapeutic cancer vaccines aimed at generating immune responses against HER2/neu are now open, which may pave the way for preventative trials in high-risk individuals with a positive safety profile. However the degree to which HER2/neu acts a driver in the process of such tumor development remains unclear and there likely is limited utility in vaccination against a single protein which may not be an obligate element in hereditary oncogenesis.

Finally, two challenging but intriguing possibilities for future prophylactic cancer vaccines are those targeting either telomerase or oncofetal antigens. Many tumors are known to depend upon telomerase for survival and many tumors are also known to upregulate oncofetal antigens. Since both telomerase and fetal antigens are rarely expressed in adult tissues, it may be possible to induce immunity to these antigens in adults without induction of autoimmunity. Whether or not such immunity will be protective against multiple cancers is unknown, but such studies have been initiated and may provide important insights in the coming years (23–26). Still the widespread expression of telomerase in some rapidly dividing tissues will continue to raise questions regarding whether or not it provides a suitable antigenic target.

Therapeutic Cancer Vaccines

The vast majority of cancer vaccines in development and in clinical trials are considered therapeutic vaccines, indicating that they are designed for administration to patients already diagnosed with cancer. To date, there has never been an FDA approved therapeutic cancer vaccine, although roughly 900 are currently in various stages of clinical trials. The gap between the number of vaccines in clinical trials and the number of approved therapeutic cancer vaccines is indicative of the overwhelming failure of these agents in previous clinical trials. This is not to say that promising candidates are not in the pipeline, however the climb to FDA approval is more difficult as a result of prior failures, especially for projects seeking financial support from skittish investors.

The failure of the majority of therapeutic cancer vaccines tested to date is a reflection of the fact that the design of most of these vaccines preceded a mature understanding of the interaction between developing tumors and the immune system. The, now widely accepted, immunosurveillance hypothesis predicts that tumorigenesis may be accompanied by the acquisition of TSAs and ASAs that engage anti-tumor immune responses. There are three potential outcomes of an anti-tumor immune response: 1) the immune response may destroy the tumor, particularly if the TSA or ASA is ubiquitously expressed in tumor cells, 2) the immune response may eliminate TSA or ASA expressing tumor cells but not those tumor cells lacking a particular antigen, resulting in a transient reduction in tumor volume followed by the outgrowth of a less-antigenic clone, 3) the tumor may co-opt immune suppressive cells or factors to dampen the anti-tumor immune response, leading either to a protracted détente (equilibrium) or the induction of immune tolerance to the tumor antigens, permitting the tumor

to escape anti-tumor immunity. An important caveat to this model is that the random nature with which tumors acquire mutations, and the fact that all tumors develop from cells to which the immune system is initially tolerant, indicates that there will be a spectrum in the immunogenicity of tumors; some of which may develop without ever engaging anti-tumor immunity, some of which may survive a single ‘round’ of immunosurveillance and some of which may endure multiple ‘rounds’ of battle with the host immune system before developing into a clinically apparent malignancy. An understanding of where individual tumor types tend to fall on this spectrum of ‘tumor immunogenicity’ is vital to the logical design of a therapeutic cancer vaccine. In effect, a highly immunogenic tumor that has endured several rounds of immunosurveillance has already vaccinated the host *in-situ*, and as a result of its continued growth has become less-susceptible to the benefits of therapeutic vaccines due to the establishment of tumor-induced immunosuppression. Alternatively, an immunogenic tumor could lose expression of a TSA or ASA following immunoselection of non-antigen expressing tumor cells, a process known as immunoediting, and revert to a non-immunogenic state. There is mixed evidence on the prognostic value of tumor infiltrating lymphocytes (TILs) in various tumors, unless these TILs are further characterized as regulatory or effector sub-types (27). The presence of TILs is indicative that adaptive immunity has been enlisted at the tumor site, and that these TILs may not be able to prevent the growth of tumors (28). Therefore, the presence or absence of TILs within a progressively growing tumor provides a proxy for whether or not a developing tumor has provided the host with a “*vaccination in situ*”. Often times such a vaccination process results in expansion of a tolerizing population of T regulatory cells rather than a desired T effector population.

Unfortunately, but understandably, the most highly immunogenic tumors are often those from which TSAs and ASAs are most easily identified. The consequence of this ‘convenience’ is that attempts at developing therapeutic cancer vaccines may have been heavily skewed toward the types of tumors that are the least likely to respond to vaccines. This bias is revealed by examining the list of tumor antigens maintained by the Academy of Cancer Immunology; roughly 55% of tumor antigens resulting from mutations are specific to melanoma, roughly 34% of shared TSA are from melanoma and roughly 58% of differentiation antigens found in tumors are from melanoma. The effect of these discoveries at the clinical level is revealed by the fact that of roughly 900 cancer vaccine clinical trials listed by the NIH, 22% are for melanoma vaccines; despite the fact that melanoma comprises only 1–2% of the overall cancer burden in the United States.

The reason that the majority of therapeutic cancer vaccine clinical trials are for immunogenic tumors from which TSAs or ASAs have been identified is because the majority of vaccine designs require that antigens be identified ahead of time. This is an absolute requirement for all DNA vaccines, peptide vaccines, synthetic long peptide vaccines, fusion protein vaccines as well as some RNA and dendritic cell vaccine platforms. Clinical responses with these classes of vaccines are infrequent and when present lead to a transient reduction in tumor volume and marginally increased survival time; with the majority of patients not responding.

Over the past two years (spanning September, 2007 through September, 2009) there have been sixty-four publications of cancer vaccines tested in clinical trials. Of these, 73% have tested vaccines targeting predicted antigen(s) and the remaining 27% have tested “pan-antigen” vaccines by utilizing whole tumor cell lysate preparations or whole tumor cells themselves. In accordance with historical proportions, 25% of these trials have tested melanoma vaccines (29–44), with the remaining 75% divided between: NSCLC (9%)(45–50), colorectal (6%)(51–54), prostate (11%)(55–61), renal cell (11%)(62–68), pancreatic (3%)(69,70), breast and ovarian (8%)(71–75), hematologic (8%)(76–80) or others (19%)(81–92). Out of these publications, forty-two reported objective clinical responses (complete responses or partial responses) as well as disease stabilization (Table 1).

There are several common themes that emerge from clinical studies testing predicted-antigen cancer vaccines. First, excluding studies with concurrent vaccine and chemotherapy (48,49, 52), responses are only observed in a minority (0–8.9%) of patients. Second, patients that respond (as defined by RECIST criteria) enjoy only marginal increases in survival. Third, survival is rarely improved in patients that did not mount a vaccine-specific immune response; however vaccine-specific immune responses are not strongly predictive of overall survival. Transient responses in a minority of patients are predicted by both the frequency of shared antigens between related tumors and the cellular and genetic heterogeneity within individual tumors. An important confounding variable in these studies is that most pre-select patients by histopathology to be highly positive for the tumor-antigen being delivered in the vaccine; a bias that is reflective of the overall design of antigen-predictive vaccines. Interestingly, two high-profile reports from this cohort demonstrate that patients treated with antigen-predictive vaccines have better responses if their tumors expressed low levels of the antigen being targeted rather than high levels (71,82). Furthermore, another study has demonstrated a positive correlation between the number of antigens delivered in the vaccine and clinical responses to the vaccine (37).

An alternative to vaccines requiring the prediction and selection of individual tumor antigens are vaccines containing the full repertoire of tumor antigens either within whole-cells or from tumor cell-lysate preparations. The potential advantage of such pan-antigen vaccines lies in the potential to induce multi-specific immunity against multiple tumor antigens. This type of response could simultaneously increase the proportion of patients that respond to a vaccine, increase the magnitude of responses within those patients and decrease the chances of immune escape to antigen-loss variants. Pan-antigenic tumor vaccines can be divided into two groups; those that utilize autologous tissue in the vaccine preparation and those that utilize allogeneic tissue. The benefit of vaccines derived from autologous tissue is that there is a greater likelihood that many antigens will be shared between the vaccine preparation and the patient than with allogeneic vaccines. The pitfalls of autologous vaccines are that patients are only eligible to receive them if they are good surgical candidates, the time between isolation of tissue and delivery of the vaccine can be long and the amount of tissue harvested from the patient restricts the dose and duration of vaccine administration. Recent clinical studies utilizing autologous material for vaccination reported attrition rates ranging from 7–41% (40,64–66,87,88). Both autologous and allogeneic vaccines have and are currently being tested in cell-based, cell-lysate and cell-lysate pulsed dendritic cell formulations.

Although limited in number, recent clinical experience with pan-antigen vaccines has yielded only infrequent objective responses (ranging from 0–5.6%). The majority of these studies (84%) utilize either autologous DCs, autologous tumor cells, autologous tumor cell lysates or purified proteins or a combination thereof. Only two studies reported clinical response data utilizing vaccines based on allogeneic tumor cells. Based on the limited number of studies, there is not a clear benefit to either autologous or allogeneic vaccine approaches based on RECIST criteria. The combination of the low response rates, high rates of attrition and selection bias for autologous approaches may in fact point toward an overall benefit of allogeneic vaccines if in the future no significant clinical benefit using autologous vaccines is proven.

In addition to RECIST criteria, many therapeutic vaccine trials report disease stabilizations. It has been demonstrated that the immune system (and in particular CD4+ and CD8+ T cells) is capable not only of eliminating tumors but also of reaching a point of ‘equilibrium’ with a tumor in which the anti-tumor immune response roughly balances the growth of the tumor, leading to a ‘stable’ lesion (7,93). These studies have spurred the question of whether a reasonable endpoint for cancer therapy is merely to slow or prevent disease progression, rather than always seeking partial or complete regressions. This discussion also highlights a critical difference between expected outcomes following cancer vaccine therapy and traditional

cytostatic or cytotoxic chemo- and radiotherapeutics, which is that the point of maximum benefit for vaccine therapies rarely occurs immediately after administration of the therapy (as it does for chemo-radiotherapy), but may instead require a prolonged period of treatment and observation. Importantly, FDA stopping rules for cancer vaccine clinical trials currently fail to account for this critical difference.

An analysis of disease stabilizations may be more instructive for cancer vaccine therapies than for chemo- and radiotherapy given the potential for lag-time between administration of the vaccine and the vaccine-induced immunological response. Using the same set of publications over the past two years, thirty-five reported both objective responses and disease stabilizations. If both objective responses and disease stabilizations are grouped, outcomes using predicted antigen vaccines improve significantly in all cancers examined: melanoma (20.8% overall response), NSCLC (42.7% overall response), colorectal (60.4% overall response), prostate (18.7% overall response), renal cell (51.7% overall response) and breast and others (19.9%). The same trend was also true for pan-antigen vaccines: melanoma (8.4%), NSCLC (66.7%), prostate (35%), renal cell (55%) and others (23.9%). Therefore, this cohort of studies suggests that pan-antigen therapeutic vaccines might provide better overall outcomes than predicted antigen vaccines for NSCLC, prostate cancer and renal cell cancer.

Tumor Immunogenicity and Combinatorial Therapeutics

The recent clinical studies discussed above demonstrate the heterogeneity in responsiveness of certain tumor types to vaccine therapy. Melanoma appears to be among the least responsive to vaccine therapy while NSCLC and renal cell carcinoma are among the most responsive. Interestingly, vaccine therapy alone appears to be fairly effective at inducing disease stabilizations but is poor at inducing objective clinical responses unless paired with chemotherapy (48,49,52). There are suggestions that following therapeutic cancer vaccine therapy ('prime') with certain types of traditional chemotherapy ('boost') may become an intriguing and effective clinical regimen(94). Regardless, the responsiveness of established cancer to vaccine therapy is likely to be an integration of the ability of a given vaccine to prime an appropriate immune response against specific tumor antigens and the ability of the tumor to suppress the anti-tumor immune response. As discussed previously developing tumors fall on a spectrum of 'immunogenicity', with the most highly immunogenic being identified by many TILs and the least immunogenic by relatively few TILs. We propose that the responsiveness of specific tumors to therapeutic cancer vaccines is related to the degree of immunogenicity of a particular tumor, with the least immunogenic tumors being the most responsive to cancer vaccines. Some recent clinical data conforms to this model in suggesting that endogenous anti-tumor immune responses are a negative prognostic factor for vaccine responses (92). Using the degree of TILs as a surrogate for immunogenicity, we propose a framework for the application of therapeutic cancer vaccines (Figure 2).

The flow chart in figure 2 proposes that the primary immune therapy for a given tumor should be decided based upon the recognition of the tumor by an endogenous immune response. If such a response exists, it suggests that the tumor is progressing independent of such a response and that regulatory mechanisms may be present that prevent an effective anti-tumor immune response. Alternatively, the absence of TILs suggests that an anti-tumor immune response is not present and that benefit may be derived from activating such a response with a vaccine. Absence of TILs perhaps implies a level of immunologic "ignorance" rather than tolerance that might be reversed through vaccination. Except for virus-associated tumors, pan-antigen vaccines are preferred because for non-immunogenic tumors the tumor rejection antigens are rarely known and for immunogenic tumors an endogenous immune response against predicted antigens is likely to already exist and may be unleashed by the primary therapy targeting regulatory mechanisms. Thus, the secondary therapy for non-viral immunogenic tumors should

also be a pan-antigen vaccine in order to broaden the specificity of the endogenous anti-tumor immune response. It is also important to note that the list of regulatory networks to target is far from comprehensive and is meant merely to provide some examples. Additional therapies that may also amplify both the endogenous and vaccine-induced anti-tumor immune response may include therapies that enhance the trafficking of immune cells into the tumor microenvironment as has been recently suggested (95,96).

Conclusions

Despite the many high-profile cancer vaccine failures over the past decade, the lack of an FDA-approved cancer vaccine and the hurdles that lie in wait, cancer vaccines are here to stay. In order to avoid repeated failures, it is imperative that future studies and clinical trials be instructed both by laboratory and clinical data that the most convenient antigens to target and tumors to treat are not always the best choices. Using melanoma as the example, it is clear that despite receiving a disproportionate share of effort, therapeutic melanoma vaccines do not perform as well as therapeutic vaccines for NSCLC and renal cell carcinoma. This may be related to the tendency for tumors such as melanoma to be highly immunogenic, capable of escaping an endogenous immune response and thus more responsive to agents targeting immune regulatory pathways than vaccines. A key question for both allogeneic and autologous vaccine approaches is the relative frequency of shared antigens between related tumors. So far, TCGA suggests that somatic mutations are rarely shared between related tumors, however it remains unclear whether the majority of tumor rejection antigens arise from somatic mutations themselves or from abnormal self antigens generated by the dysregulation of common pathways by somatic mutations. Thus, both basic and clinical studies in tumor immunology must continue to occur in parallel so that they may instruct one another as to the appropriate tumors and approaches to target with cancer vaccines. It is equally important for the evaluation of future therapeutic cancer vaccine clinical trials that the FDA consider the biological differences between traditional cytotoxic therapies and cancer immune therapies such that cutting edge evaluation criteria may be applied to cutting edge vaccines.

Acknowledgments

Supported by 5P01CA109094 (E.R.P. and J.D.R.), 5P01CA109094 (J.D.R.) and by the "Alliance for Cancer Gene Therapy, Inc." (E.R.P.)

References

1. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008;321:1801–1806. [PubMed: 18772397]
2. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008;321:1807–1812. [PubMed: 18772396]
3. Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Lengauer C, Velculescu VE. Colorectal cancer: mutations in a signalling pathway. *Nature* 2005;436:792. [PubMed: 16094359]
4. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA,

- Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. The genomic landscapes of human breast and colorectal cancers. *Science* 2007;318:1108–1113. [PubMed: 17932254]
5. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol* 2006;90:1–50. [PubMed: 16730260]
 6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002;3:991–998. [PubMed: 12407406]
 7. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFN γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001;410:1107–1111. [PubMed: 11323675]
 8. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004;22:329–360. [PubMed: 15032581]
 9. Unni AM, Bondar T, Medzhitov R. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. *Proc Natl Acad Sci U S A* 2008;105:1686–1691. [PubMed: 18223157]
 10. Taieb J, Chaput N, Menard C, Apetoh L, Ullrich E, Bonmort M, Pequignot M, Casares N, Terme M, Flament C, Opolon P, Lecluse Y, Metivier D, Tomasello E, Vivier E, Ghiringhelli F, Martin F, Klatzmann D, Poynard T, Tursz T, Raposo G, Yagita H, Ryffel B, Kroemer G, Zitvogel L. A novel dendritic cell subset involved in tumor immunosurveillance. *Nat Med* 2006;12:214–219. [PubMed: 16444265]
 11. Raulet DH, Guerra N. Oncogenic stress sensed by the immune system: role of natural killer cell receptors. *Nat Rev Immunol* 2009;9:568–580. [PubMed: 19629084]
 12. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007;25:267–296. [PubMed: 17134371]
 13. Schreiber TH, Podack ER. A critical analysis of the tumour immunosurveillance controversy for 3-MCA-induced sarcomas. *Br J Cancer* 2009;101:381–386. [PubMed: 19638986]
 14. Furukawa Y, Tara M, Izumo S, Arimura K, Osame M. HTLV-I viral escape and host genetic changes in the development of adult T cell leukemia. *Int J Cancer* 2006;118:381–387. [PubMed: 16052518]
 15. Taylor GP, Matsuoka M. Natural history of adult T-cell leukemia/lymphoma and approaches to therapy. *Oncogene* 2005;24:6047–6057. [PubMed: 16155611]
 16. Hu L, Troyanovsky B, Zhang X, Trivedi P, Ernberg I, Klein G. Differences in the immunogenicity of latent membrane protein 1 (LMP1) encoded by Epstein-Barr virus genomes derived from LMP1-positive and -negative nasopharyngeal carcinoma. *Cancer Res* 2000;60:5589–5593. [PubMed: 11034108]
 17. Finn OJ. Human tumor antigens, immunosurveillance, and cancer vaccines. *Immunol Res* 2006;36:73–82. [PubMed: 17337768]
 18. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, Vogelstein B, Allison JP. Epitope landscape in breast and colorectal cancer. *Cancer Res* 2008;68:889–892. [PubMed: 18245491]
 19. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
 20. Schreiber TH, Deyev VV, Rosenblatt JD, Podack ER. Tumor-induced suppression of CTL expansion and subjugation by gp96-Ig vaccination. *Cancer Res* 2009;69:2026–2033. [PubMed: 19223534]
 21. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, Noppeney R, Viardot A, Hess G, Schuler M, Einsele H, Brandl C, Wolf A, Kirchinger P, Klappers P, Schmidt M, Riethmuller G, Reinhardt C, Baeuerle PA, Kufer P. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 2008;321:974–977. [PubMed: 18703743]
 22. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004;10:909–915. [PubMed: 15340416]
 23. Mennuni C, Ugel S, Mori F, Cipriani B, Iezzi M, Pannellini T, Lazzaro D, Ciliberto G, La Monica N, Zanolletto P, Bronte V, Scarselli E. Preventive vaccination with telomerase controls tumor growth in genetically engineered and carcinogen-induced mouse models of cancer. *Cancer Res* 2008;68:9865–9874. [PubMed: 19047167]

24. Vonderheide RH, Domchek SM, Schultze JL, George DJ, Hoar KM, Chen DY, Stephans KF, Masutomi K, Loda M, Xia Z, Anderson KS, Hahn WC, Nadler LM. Vaccination of cancer patients against telomerase induces functional antitumor CD8+ T lymphocytes. *Clin Cancer Res* 2004;10:828–839. [PubMed: 14871958]
25. Domchek SM, Recio A, Mick R, Clark CE, Carpenter EL, Fox KR, DeMichele A, Schuchter LM, Leibowitz MS, Wexler MH, Vance BA, Beatty GL, Veloso E, Feldman MD, Vonderheide RH. Telomerase-specific T-cell immunity in breast cancer: effect of vaccination on tumor immunosurveillance. *Cancer Res* 2007;67:10546–10555. [PubMed: 17974999]
26. Harley CB. Telomerase and cancer therapeutics. *Nat Rev Cancer* 2008;8:167–179. [PubMed: 18256617]
27. Atreya I, Neurath MF. Immune cells in colorectal cancer: prognostic relevance and therapeutic strategies. *Expert Rev Anticancer Ther* 2008;8:561–572. [PubMed: 18402523]
28. Anichini A, Vegetti C, Mortarini R. The paradox of T-cell-mediated antitumor immunity in spite of poor clinical outcome in human melanoma. *Cancer Immunol Immunother* 2004;53:855–864. [PubMed: 15175905]
29. Butterfield LH, Comin-Anduix B, Vujanovic L, Lee Y, Dissette VB, Yang JQ, Vu HT, Seja E, Oseguera DK, Potter DM, Glaspy JA, Economou JS, Ribas A. Adenovirus MART-1-engineered autologous dendritic cell vaccine for metastatic melanoma. *J Immunother* 2008;31:294–309. [PubMed: 18317358]
30. Fourcade J, Kudela P, Andrade Filho PA, Janjic B, Land SR, Sander C, Krieg A, Donnenberg A, Shen H, Kirkwood JM, Zarour HM. Immunization with analog peptide in combination with CpG and montanide expands tumor antigen-specific CD8+ T cells in melanoma patients. *J Immunother* 2008;31:781–791. [PubMed: 18779741]
31. Hersey P, Halliday GM, Farrelly ML, DeSilva C, Lett M, Menzies SW. Phase I/II study of treatment with matured dendritic cells with or without low dose IL-2 in patients with disseminated melanoma. *Cancer Immunol Immunother* 2008;57:1039–1051. [PubMed: 18157724]
32. Kirkwood JM, Lee S, Moschos SJ, Albertini MR, Michalak JC, Sander C, Whiteside T, Butterfield LH, Weiner L. Immunogenicity and antitumor effects of vaccination with peptide vaccine+/-granulocyte-monocyte colony-stimulating factor and/or IFN-alpha2b in advanced metastatic melanoma: Eastern Cooperative Oncology Group Phase II Trial E1696. *Clin Cancer Res* 2009;15:1443–1451. [PubMed: 19228745]
33. Lopez MN, Pereda C, Segal G, Munoz L, Aguilera R, Gonzalez FE, Escobar A, Ginesta A, Reyes D, Gonzalez R, Mendoza-Naranjo A, Larrondo M, Compan A, Ferrada C, Salazar-Onfray F. Prolonged survival of dendritic cell-vaccinated melanoma patients correlates with tumor-specific delayed type IV hypersensitivity response and reduction of tumor growth factor beta-expressing T cells. *J Clin Oncol* 2009;27:945–952. [PubMed: 19139436]
34. Nicholaou T, Ebert LM, Davis ID, McArthur GA, Jackson H, Dimopoulos N, Tan B, Maraskovsky E, Miloradovic L, Hopkins W, Pan L, Venhaus R, Hoffman EW, Chen W, Cebon J. Regulatory T-cell-mediated attenuation of T-cell responses to the NY-ESO-1 ISCOMATRIX vaccine in patients with advanced malignant melanoma. *Clin Cancer Res* 2009;15:2166–2173. [PubMed: 19276262]
35. Perales MA, Yuan J, Powel S, Gallardo HF, Rasalan TS, Gonzalez C, Manukian G, Wang J, Zhang Y, Chapman PB, Krown SE, Livingston PO, Ejadi S, Panageas KS, Engelhorn ME, Terzulli SL, Houghton AN, Wolchok JD. Phase I/II study of GM-CSF DNA as an adjuvant for a multi-peptide cancer vaccine in patients with advanced melanoma. *Mol Ther* 2008;16:2022–2029. [PubMed: 18797450]
36. Redman BG, Chang AE, Whitfield J, Esper P, Jiang G, Braun T, Roessler B, Mule JJ. Phase Ib trial assessing autologous, tumor-pulsed dendritic cells as a vaccine administered with or without IL-2 in patients with metastatic melanoma. *J Immunother* 2008;31:591–598. [PubMed: 18528294]
37. Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, Smolkin ME, Hibbitts S, Murphy C, Johansen N, Grosh WW, Yamshchikov GV, Neese PY, Patterson JW, Fink R, Rehm PK. Immunologic and clinical outcomes of a randomized phase II trial of two multi-peptide vaccines for melanoma in the adjuvant setting. *Clin Cancer Res* 2007;13:6386–6395. [PubMed: 17975151]
38. Slingluff CL Jr, Petroni GR, Olson W, Czarkowski A, Grosh WW, Smolkin M, Chianese-Bullock KA, Neese PY, Deacon DH, Nail C, Merrill P, Fink R, Patterson JW, Rehm PK. Helper T-cell

- responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. *J Clin Oncol* 2008;26:4973–4980. [PubMed: 18809608]
39. Sosman JA, Carrillo C, Urba WJ, Flaherty L, Atkins MB, Clark JI, Dutcher J, Margolin KA, Mier J, Gollob J, Kirkwood JM, Panka DJ, Crosby NA, O'Boyle K, LaFleur B, Ernstoff MS. Three phase II cytokine working group trials of gp100 (210M) peptide plus high-dose interleukin-2 in patients with HLA-A2-positive advanced melanoma. *J Clin Oncol* 2008;26:2292–2298. [PubMed: 18467720]
 40. Testori A, Richards J, Whitman E, Mann GB, Lutzky J, Camacho L, Parmiani G, Tosti G, Kirkwood JM, Hoos A, Yuh L, Gupta R, Srivastava PK. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. *J Clin Oncol* 2008;26:955–962. [PubMed: 18281670]
 41. von Euw EM, Barrio MM, Furman D, Levy EM, Bianchini M, Peguillet I, Lantz O, Vellice A, Kohan A, Chacon M, Yee C, Wainstok R, Mordoh J. A phase I clinical study of vaccination of melanoma patients with dendritic cells loaded with allogeneic apoptotic/necrotic melanoma cells. Analysis of toxicity and immune response to the vaccine and of IL-10 –1082 promoter genotype as predictor of disease progression. *J Transl Med* 2008;6:6. [PubMed: 18221542]
 42. Weber J, Boswell W, Smith J, Hersch E, Snively J, Diaz M, Miles S, Liu X, Obrocea M, Qiu Z, Bot A. Phase I trial of intranodal injection of a Melan-A/MART-1 DNA plasmid vaccine in patients with stage IV melanoma. *J Immunother* 2008;31:215–223. [PubMed: 18481391]
 43. Weide B, Carralot JP, Reese A, Scheel B, Eigentler TK, Hoerr I, Rammensee HG, Garbe C, Pascolo S. Results of the first phase I/II clinical vaccination trial with direct injection of mRNA. *J Immunother* 2008;31:180–188. [PubMed: 18481387]
 44. Yuan J, Ku GY, Gallardo HF, Orlandi F, Manukian G, Rasalan TS, Xu Y, Li H, Vyas S, Mu Z, Chapman PB, Krown SE, Panageas K, Terzulli SL, Old LJ, Houghton AN, Wolchok JD. Safety and immunogenicity of a human and mouse gp100 DNA vaccine in a phase I trial of patients with melanoma. *Cancer Immun* 2009;9:5. [PubMed: 19496531]
 45. Barve M, Bender J, Senzer N, Cunningham C, Greco FA, McCune D, Steis R, Khong H, Richards D, Stephenson J, Ganesa P, Nemunaitis J, Ishioka G, Pappen B, Nemunaitis M, Morse M, Mills B, Maples PB, Sherman J, Nemunaitis JJ. Induction of immune responses and clinical efficacy in a phase II trial of IDM-2101, a 10-epitope cytotoxic T-lymphocyte vaccine, in metastatic non-small-cell lung cancer. *J Clin Oncol* 2008;26:4418–4425. [PubMed: 18802154]
 46. Motohashi S, Nagato K, Kunii N, Yamamoto H, Yamasaki K, Okita K, Hanaoka H, Shimizu N, Suzuki M, Yoshino I, Taniguchi M, Fujisawa T, Nakayama T. A phase I-II study of alpha-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer. *J Immunol* 2009;182:2492–2501. [PubMed: 19201905]
 47. Nemunaitis J, Nemunaitis M, Senzer N, Snitz P, Bedell C, Kumar P, Pappen B, Maples PB, Shawler D, Fakhrai H. Phase II trial of Belagenpumatucel-L, a TGF-beta2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther* 2009;16:620–624. [PubMed: 19287371]
 48. Neninger E, Verdecia BG, Crombet T, Viada C, Pereda S, Leonard I, Mazorra Z, Fleites G, Gonzalez M, Wilkinson B, Gonzalez G, Lage A. Combining an EGF-based cancer vaccine with chemotherapy in advanced nonsmall cell lung cancer. *J Immunother* 2009;32:92–99. [PubMed: 19307998]
 49. Ramlau R, Quoix E, Rolski J, Pless M, Lena H, Levy E, Krzakowski M, Hess D, Tartour E, Chenard MP, Limacher JM, Bizouarne N, Acres B, Halluard C, Velu T. A phase II study of Tg4010 (Mva-Muc1-II2) in association with chemotherapy in patients with stage III/IV Non-small cell lung cancer. *J Thorac Oncol* 2008;3:735–744. [PubMed: 18594319]
 50. Neninger Vinageras E, de la Torre A, Osorio Rodriguez M, Catala Ferrer M, Bravo I, Mendoza del Pino M, Abreu Abreu D, Acosta Brooks S, Rives R, del Castillo Carrillo C, Gonzalez Duenas M, Viada C, Garcia Verdecia B, Crombet Ramos T, Gonzalez Marinello G, Lage Davila A. Phase II randomized controlled trial of an epidermal growth factor vaccine in advanced non-small-cell lung cancer. *J Clin Oncol* 2008;26:1452–1458. [PubMed: 18349395]
 51. Elkord E, Dangoor A, Drury NL, Harrop R, Burt DJ, Drijfhout JW, Hamer C, Andrews D, Naylor S, Sherlock D, Hawkins RE, Stern PL. An MVA-based vaccine targeting the oncofetal antigen 5T4 in

- patients undergoing surgical resection of colorectal cancer liver metastases. *J Immunother* 2008;31:820–829. [PubMed: 18833005]
52. Kaufman HL, Lenz HJ, Marshall J, Singh D, Garrett C, Cripps C, Moore M, von Mehren M, Dalfen R, Heim WJ, Conry RM, Urba WJ, Benson AB 3rd, Yu M, Caterini J, Kim-Schulze S, Debenedette M, Salha D, Vogel T, Elias I, Berinstein NL. Combination chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. *Clin Cancer Res* 2008;14:4843–4849. [PubMed: 18676757]
 53. Kavanagh B, Ko A, Venook A, Margolin K, Zeh H, Lotze M, Schillinger B, Liu W, Lu Y, Mitsky P, Schilling M, Bercovici N, Loudovaris M, Guillermo R, Lee SM, Bender J, Mills B, Fong L. Vaccination of metastatic colorectal cancer patients with matured dendritic cells loaded with multiple major histocompatibility complex class I peptides. *J Immunother* 2007;30:762–772. [PubMed: 17893568]
 54. Speetjens FM, Kuppen PJ, Welters MJ, Essahsah F, Voet van den Brink AM, Lantrua MG, Valentijn AR, Oostendorp J, Fathers LM, Nijman HW, Drijfhout JW, van de Velde CJ, Melief CJ, van der Burg SH. Induction of p53-specific immunity by a p53 synthetic long peptide vaccine in patients treated for metastatic colorectal cancer. *Clin Cancer Res* 2009;15:1086–1095. [PubMed: 19188184]
 55. Amato RJ, Drury N, Naylor S, Jac J, Saxena S, Cao A, Hernandez-McClain J, Harrop R. Vaccination of prostate cancer patients with modified vaccinia ankara delivering the tumor antigen 5T4 (TroVax): a phase 2 trial. *J Immunother* 2008;31:577–585. [PubMed: 18528296]
 56. Dreicer R, Stadler WM, Ahmann FR, Whiteside T, Bizouarne N, Acres B, Limacher JM, Squiban P, Pantuck A. MVA-MUC1-IL2 vaccine immunotherapy (TG4010) improves PSA doubling time in patients with prostate cancer with biochemical failure. *Invest New Drugs* 2009;27:379–386. [PubMed: 18931824]
 57. Higano CS, Corman JM, Smith DC, Centeno AS, Steidle CP, Gittleman M, Simons JW, Sacks N, Aimi J, Small EJ. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. *Cancer* 2008;113:975–984. [PubMed: 18646045]
 58. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer* 2009;115:3670–3679. [PubMed: 19536890]
 59. Kouliavskaya DV, Berard CA, Datena E, Hussain A, Dawson N, Klyushnenkova EN, Alexander RB. Vaccination with agonist peptide PSA: 154–163 (155L) derived from prostate specific antigen induced CD8 T-cell response to the native peptide PSA: 154–163 but failed to induce the reactivity against tumor targets expressing PSA: a phase 2 study in patients with recurrent prostate cancer. *J Immunother* 2009;32:655–666. [PubMed: 19483644]
 60. Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, Arlen PM. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. *Clin Cancer Res* 2008;14:4526–4531. [PubMed: 18628467]
 61. McNeel DG, Dunphy EJ, Davies JG, Frye TP, Johnson LE, Staab MJ, Horvath DL, Straus J, Alberti D, Marnocha R, Liu G, Eickhoff JC, Wilding G. Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer. *J Clin Oncol* 2009;27:4047–4054. [PubMed: 19636017]
 62. Amato RJ, Shingler W, Goonewardena M, de Belin J, Naylor S, Jac J, Willis J, Saxena S, Hernandez-McClain J, Harrop R. Vaccination of renal cell cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) alone or administered in combination with interferon-alpha (IFN-alpha): a phase 2 trial. *J Immunother* 2009;32:765–772. [PubMed: 19561532]
 63. Amato RJ, Shingler W, Naylor S, Jac J, Willis J, Saxena S, Hernandez-McClain J, Harrop R. Vaccination of renal cell cancer patients with modified vaccinia ankara delivering tumor antigen 5T4 (TroVax) administered with interleukin 2: a phase II trial. *Clin Cancer Res* 2008;14:7504–7510. [PubMed: 19010868]
 64. Avigan DE, Vasir B, George DJ, Oh WK, Atkins MB, McDermott DF, Kantoff PW, Figlin RA, Vasconcelles MJ, Xu Y, Kufe D, Bukowski RM. Phase I/II study of vaccination with electrofused

- allogeneic dendritic cells/autologous tumor-derived cells in patients with stage IV renal cell carcinoma. *J Immunother* 2007;30:749–761. [PubMed: 17893567]
65. Berntsen A, Trepiaas R, Wenandy L, Geertsen PF, thor Straten P, Andersen MH, Pedersen AE, Claesson MH, Lorentzen T, Johansen JS, Svane IM. Therapeutic dendritic cell vaccination of patients with metastatic renal cell carcinoma: a clinical phase 1/2 trial. *J Immunother* 2008;31:771–780. [PubMed: 18779742]
 66. Fishman M, Hunter TB, Soliman H, Thompson P, Dunn M, Smilee R, Farmelo MJ, Noyes DR, Mahany JJ, Lee JH, Cantor A, Messina J, Seigne J, Pow-Sang J, Janssen W, Antonia SJ. Phase II trial of B7-1 (CD-86) transduced, cultured autologous tumor cell vaccine plus subcutaneous interleukin-2 for treatment of stage IV renal cell carcinoma. *J Immunother* 2008;31:72–80. [PubMed: 18157014]
 67. Hawkins RE, Macdermott C, Shablak A, Hamer C, Thistlethwaite F, Drury NL, Chikoti P, Shingler W, Naylor S, Harrop R. Vaccination of patients with metastatic renal cancer with modified vaccinia Ankara encoding the tumor antigen 5T4 (TroVax) given alongside interferon-alpha. *J Immunother* 2009;32:424–429. [PubMed: 19342962]
 68. Kaufman HL, Taback B, Sherman W, Kim DW, Shingler WH, Moroziewicz D, DeRaffele G, Mitcham J, Carroll MW, Harrop R, Naylor S, Kim-Schulze S. Phase II trial of Modified Vaccinia Ankara (MVA) virus expressing 5T4 and high dose Interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma. *J Transl Med* 2009;7:2. [PubMed: 19128501]
 69. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, Finn OJ, Ramanathan RK. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Cancer Ther* 2008;6:955–964. [PubMed: 19129927]
 70. Toubaji A, Achta M, Provenzano M, Herrin VE, Behrens R, Hamilton M, Bernstein S, Venzon D, Gause B, Marincola F, Khleif SN. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. *Cancer Immunol Immunother* 2008;57:1413–1420. [PubMed: 18297281]
 71. Benavides LC, Gates JD, Carmichael MG, Patel R, Holmes JP, Hueman MT, Mittendorf EA, Craig D, Stojadinovic A, Ponniah S, Peoples GE. The impact of HER2/neu expression level on response to the E75 vaccine: from U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Clin Cancer Res* 2009;15:2895–2904. [PubMed: 19351776]
 72. Chianese-Bullock KA, Irvin WP Jr, Petroni GR, Murphy C, Smolkin M, Olson WC, Coleman E, Boerner SA, Nail CJ, Neese PY, Yuan A, Hogan KT, Slingluff CL Jr. A multi-peptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer. *J Immunother* 2008;31:420–430. [PubMed: 18391753]
 73. Diefenbach CS, Gnjjatic S, Sabbatini P, Aghajanian C, Hensley ML, Spriggs DR, Iasonos A, Lee H, Dupont B, Pezzulli S, Jungbluth AA, Old LJ, Dupont J. Safety and immunogenicity study of NY-ESO-1b peptide and montanide ISA-51 vaccination of patients with epithelial ovarian cancer in high-risk first remission. *Clin Cancer Res* 2008;14:2740–2748. [PubMed: 18451240]
 74. Holmes JP, Benavides LC, Gates JD, Carmichael MG, Hueman MT, Mittendorf EA, Murray JL, Amin A, Craig D, von Hofe E, Ponniah S, Peoples GE. Results of the first phase I clinical trial of the novel II-key hybrid preventive HER-2/neu peptide (AE37) vaccine. *J Clin Oncol* 2008;26:3426–3433. [PubMed: 18612158]
 75. Tsuruma T, Iwayama Y, Ohmura T, Katsuramaki T, Hata F, Furuhashi T, Yamaguchi K, Kimura Y, Torigoe T, Toyota N, Yagihashi A, Hirohashi Y, Asanuma H, Shimozaawa K, Okazaki M, Mizushima Y, Nomura N, Sato N, Hirata K. Clinical and immunological evaluation of anti-apoptosis protein, survivin-derived peptide vaccine in phase I clinical study for patients with advanced or recurrent breast cancer. *J Transl Med* 2008;6:24. [PubMed: 18471305]
 76. Freedman A, Neelapu SS, Nichols C, Robertson MJ, Djulbegovic B, Winter JN, Bender JF, Gold DP, Ghalie RG, Stewart ME, Esquibel V, Hamlin P. Placebo-controlled phase III trial of patient-specific immunotherapy with mitumprostimut-T and granulocyte-macrophage colony-stimulating factor after rituximab in patients with follicular lymphoma. *J Clin Oncol* 2009;27:3036–3043. [PubMed: 19414675]
 77. Ho VT, Vanneman M, Kim H, Sasada T, Kang YJ, Pasek M, Cutler C, Koreth J, Alyea E, Sarantopoulos S, Antin JH, Ritz J, Canning C, Kutok J, Mihm MC, Dranoff G, Soiffer R. Biologic

- activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. *Proc Natl Acad Sci U S A* 2009;106:15825–15830. [PubMed: 19717467]
78. Jain N, Reuben JM, Kantarjian H, Li C, Gao H, Lee BN, Cohen EN, Ebarb T, Scheinberg DA, Cortes J. Synthetic tumor-specific breakpoint peptide vaccine in patients with chronic myeloid leukemia and minimal residual disease: a phase 2 trial. *Cancer* 2009;115:3924–3934. [PubMed: 19536894]
 79. Keilholz U, Letsch A, Busse A, Asemisen AM, Bauer S, Blau IW, Hofmann WK, Uharek L, Thiel E, Scheibenbogen C. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. *Blood* 2009;113:6541–6548. [PubMed: 19389880]
 80. McCormick AA, Reddy S, Reinl SJ, Cameron TI, Czerwinski DK, Vojdani F, Hanley KM, Garger SJ, White EL, Novak J, Barrett J, Holtz RB, Tuse D, Levy R. Plant-produced idiotypic vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical study. *Proc Natl Acad Sci U S A* 2008;105:10131–10136. [PubMed: 18645180]
 81. Izumoto S, Tsuboi A, Oka Y, Suzuki T, Hashiba T, Kagawa N, Hashimoto N, Maruno M, Elisseeva OA, Shirakata T, Kawakami M, Oji Y, Nishida S, Ohno S, Kawase I, Hatazawa J, Nakatsuka S, Aozasa K, Morita S, Sakamoto J, Sugiyama H, Yoshimine T. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. *J Neurosurg* 2008;108:963–971. [PubMed: 18447714]
 82. Kaumaya PT, Foy KC, Garrett J, Rawale SV, Vicari D, Thurmond JM, Lamb T, Mani A, Kane Y, Balint CR, Chalupa D, Otterson GA, Shapiro CL, Fowler JM, Grever MR, Bekaii-Saab TS, Carson WE 3rd. Phase I Active Immunotherapy With Combination of Two Chimeric, Human Epidermal Growth Factor Receptor 2, B-Cell Epitopes Fused to a Promiscuous T-Cell Epitope in Patients With Metastatic and/or Recurrent Solid Tumors. *J Clin Oncol*. 2009
 83. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, Drijfhout JW, Wafelman AR, Oostendorp J, Fleuren GJ, Offringa R, van der Burg SH, Melief CJ. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. *Clin Cancer Res* 2008;14:169–177. [PubMed: 18172268]
 84. Kono K, Mizukami Y, Daigo Y, Takano A, Masuda K, Yoshida K, Tsunoda T, Kawaguchi Y, Nakamura Y, Fujii H. Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer. *Cancer Sci* 2009;100:1502–1509. [PubMed: 19459850]
 85. Michaluart P, Abdallah KA, Lima FD, Smith R, Moyses RA, Coelho V, Victora GD, Socorro-Silva A, Volsi EC, Zarate-Blades CR, Ferraz AR, Barreto AK, Chammass MC, Gomes R, Gebirim E, Arakawa-Sugueno L, Fernandes KP, Lotufo PA, Cardoso MR, Kalil J, Silva CL. Phase I trial of DNA-hsp65 immunotherapy for advanced squamous cell carcinoma of the head and neck. *Cancer Gene Ther* 2008;15:676–684. [PubMed: 18535616]
 86. Okada H, Lieberman FS, Walter KA, Lunsford LD, Kondziolka DS, Bejjani GK, Hamilton RL, Torres-Trejo A, Kalinski P, Cai Q, Mabold JL, Edington HD, Butterfield LH, Whiteside TL, Potter DM, Schold SC, Pollack IF. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas. *J Transl Med* 2007;5:67. [PubMed: 18093335]
 87. Palmer DH, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, Steven NM, Kerr DJ, Young LS, Adams DH. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009;49:124–132. [PubMed: 18980227]
 88. Russell HV, Strother D, Mei Z, Rill D, Popek E, Biagi E, Yvon E, Brenner M, Rousseau R. A phase 1/2 study of autologous neuroblastoma tumor cells genetically modified to secrete IL-2 in patients with high-risk neuroblastoma. *J Immunother* 2008;31:812–819. [PubMed: 18833006]
 89. Victora GD, Socorro-Silva A, Volsi EC, Abdallah K, Lima FD, Smith RB, Moyses RA, Zarate-Blades CR, Michaluart P, Silva CL, Kalil J, Coelho V. Immune response to vaccination with DNA-Hsp65 in a phase I clinical trial with head and neck cancer patients. *Cancer Gene Ther* 2009;16:598–608. [PubMed: 19197326]

90. Walker DG, Laherty R, Tomlinson FH, Chuah T, Schmidt C. Results of a phase I dendritic cell vaccine trial for malignant astrocytoma: potential interaction with adjuvant chemotherapy. *J Clin Neurosci* 2008;15:114–121. [PubMed: 18083572]
91. Welters MJ, Kenter GG, Piersma SJ, Vloon AP, Lowik MJ, Berends-van der Meer DM, Drijfhout JW, Valentijn AR, Wafelman AR, Oostendorp J, Fleuren GJ, Offringa R, Melief CJ, van der Burg SH. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. *Clin Cancer Res* 2008;14:178–187. [PubMed: 18172269]
92. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, Goldfinger D, Ng H, Irvin D, Yu JS. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res* 2008;68:5955–5964. [PubMed: 18632651]
93. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, Smyth MJ, Schreiber RD. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 2007;450:903–907. [PubMed: 18026089]
94. Finn OJ. Cancer immunology. *N Engl J Med* 2008;358:2704–2715. [PubMed: 18565863]
95. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, Balint K, Katsaros D, O'Brien-Jenkins A, Gimotty PA, Coukos G. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat Med* 2008;14:28–36. [PubMed: 18157142]
96. Quezada SA, Peggs KS, Simpson TR, Shen Y, Littman DR, Allison JP. Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. *J Exp Med* 2008;205:2125–2138. [PubMed: 18725522]

Figure 1. Differential interactions between immunogenic versus non-immunogenic tumors and the immune system

Tumors develop within tissues and release tumor antigens into local lymphoid organs. For immunogenic tumors (such as melanoma), those antigens lead to cytotoxic T lymphocyte (CTL) activation and subsequent infiltration of the tumor by tumor-antigen specific CTL. The observation that many immunogenic tumors develop in spite of such a response is evidence that immunogenic tumors develop regulatory characteristics that lead to resistance to tumor cell killing by CTL. Non-immunogenic tumors on the other hand (such as NSCLC) release antigens that do not efficiently prime an anti-tumor immune response, and as a result such tumors need not develop regulatory mechanisms to counteract the killing activity of tumor-antigen primed CTL. Therapeutic vaccination aims to prime the immune system against tumor antigens, so it is anticipated that such a response will be more effective against tumors (non-immunogenic) that have not already acquired an immune regulatory phenotype.

Figure 2. Proposed flow-chart for the application of therapeutic cancer vaccines

Table 1

Summary of therapeutic cancer vaccine clinical trials reporting CR/PR/SD spanning September, 2007 – September, 2009.

Type of Cancer	Vaccine Category	No. of Patients Treated	No. Patients in Studies Restricted to Stage III/IV Disease / Total No of Patients	CR or PR (% RECIST Response)	Stable Disease (% SD)	% CR/PR/SD
Melanoma	Predicted Antigen	313	58.1	28 (8.9%)	37 (11.8%)	20.8
	Pan-Antigen	215	92.6	9 (4.2%)	9 (4.2%)	8.4
NSCLC	Predicted Antigen	253	100	22 (8.7%)	86 (33.9%)	42.7
	Pan-Antigen	21	100	0 (0%)	14 (66.7%)	66.7
Colorectal	Predicted Antigen	139	100	42 (30.2%)	42 (30.2%)	60.4
Prostate	Predicted Antigen	48	56.3	0 (0%)	9 (18.75%)	18.75
	Pan-Antigen	80	100	0 (0%)	28 (35%)	35
Renal Cell	Predicted Antigen	89	100	4 (4.5%)	42 (47.2%)	51.7
	Pan-Antigen	91	100	5 (5.5%)	45 (49.5%)	55
Other Solid Tumors	Predicted Antigen	21	100	2 (9.5%)	10 (47.6%)	57.1
	Pan-Antigen	67	2.3	3 (4.4%)	6 (8.9%)	13.3
Hematologic Tumors	Predicted Antigen	193	0	0 (0%)	10 (5.2%)	5.2