

Models of Anti-Cancer Therapy

Cancer Vaccines

An Old Idea Comes of Age

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KEY WORDS

Cancer vaccines, Clinical trials, Immune tolerance, Tumor antigens, Antigen identification



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ABBREVIATIONS

AICD	Activation induced cell death
APC	Antigen presenting cell
BMT	Bone marrow transplant
β-HCG	β-Human chorionic gonadotropin
CEA	Carcinoembryonic antigen
CML	Chronic myelogenous leukemia
CTL	Cytotoxic T lymphocyte
DFS	Disease-free survival
DTH	Delayed type hypersensitivity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HA	Hemagglutinin
IFN-γ	Interferon-γ
KLH	Keyhole limpet hemocyanin
MHC	Major histocompatibility complex
TIL	Tumor infiltrating lymphocytes
PBL	Peripheral blood lymphocytes
SAGE	Serial analysis of gene expression
SEREX	Serologic analysis of recombinant cDNA expression
TNFR	Tumor necrosis factor receptor

ABSTRACT

Cancer vaccines are at the forefront of novel, targeted approaches to cancer treatment. Low toxicity, the potential for circumventing drug cross-resistance, and the potential for persistence of the antitumor effect due to immunologic memory represent a mandate for accelerated clinical development. Advances in molecular immunology have suggested approaches for overcoming the formidable mechanisms of immune tolerance that are pre-established in cancer patients, and many have already been tested in preclinical models. Also, early studies revealed that not all tumor antigens are created equal, and identifying those capable of eliciting immune-mediated tumor rejection is essential to the development of effective recombinant cancer vaccines. While early trials have generally resulted in disappointing clinical outcomes, they have yielded insight into the critical parameters for the design of cancer vaccine trials and provided powerful reagents for tumor antigen identification. By utilizing the lessons learned from the research laboratory and these early clinical trials, informative second generation vaccine trials should have a high likelihood of success. Clinical protocols that consider how best to incorporate therapeutic cancer vaccines into the current standard of care should allow cancer vaccines to take their place alongside traditional cancer treatment modalities in oncology practice.

INTRODUCTION

The use of immunization to prevent acute infectious diseases is one of the success stories of modern medicine. It has essentially eradicated smallpox and polio, and has dramatically decreased the morbidity and mortality of other infectious diseases. Despite success in disease prevention, the use of vaccines to treat established, chronic disease (including chronic viral infections and cancer) has not been successful. There are several explanations for the inherent difficulties of immunotherapy compared to immunoprophylaxis. First, the pivotal antigens required for the induction of effective antiviral and antibacterial immunity are few and well characterized immunologically. In contrast, relatively little is known about the critical immune targets of vaccine-activated immune rejection of genetically complex, transformed host cells. Second, the humoral immune response typically eliminates acute infections, whereas T cell-mediated immune responses are thought to eradicate established cancer or chronically infected host cells. Third, the vaccine-mediated induction of immunity prior to antigen exposure occurs on a slate that is immunologically clean. In contrast, inducing a targeted immune response in chronic disease requires disrupting established immunoregulatory mechanisms that maintain a parasitic relationship between the affected cell and the host. Finally, the pre-existing tumor burden itself represents an additional hurdle that must be surmounted in the therapeutic setting.

MECHANISMS OF ANTITUMOR IMMUNITY

Interest in harnessing the immune system for cancer treatment dates back to 1893 when William Coley reported the regression of soft tissue sarcoma in patients with acute bacterial infection, then tested bacterial extracts to stimulate antitumor immune responses.¹ Subsequent efforts to develop cancer vaccines were stymied by the prevailing ignorance of basic immunology. The recent revolution in biotechnology created the tools to both elucidate the molecular mechanisms of immunoregulation and further define the principles of tumor immunology. 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 cancer cells (Fig. 1). Antigens are processed by the endosome into MHC Class II-binding peptides of 12 to 20 amino acids, and by the proteasome into MHC Class I-binding peptides of

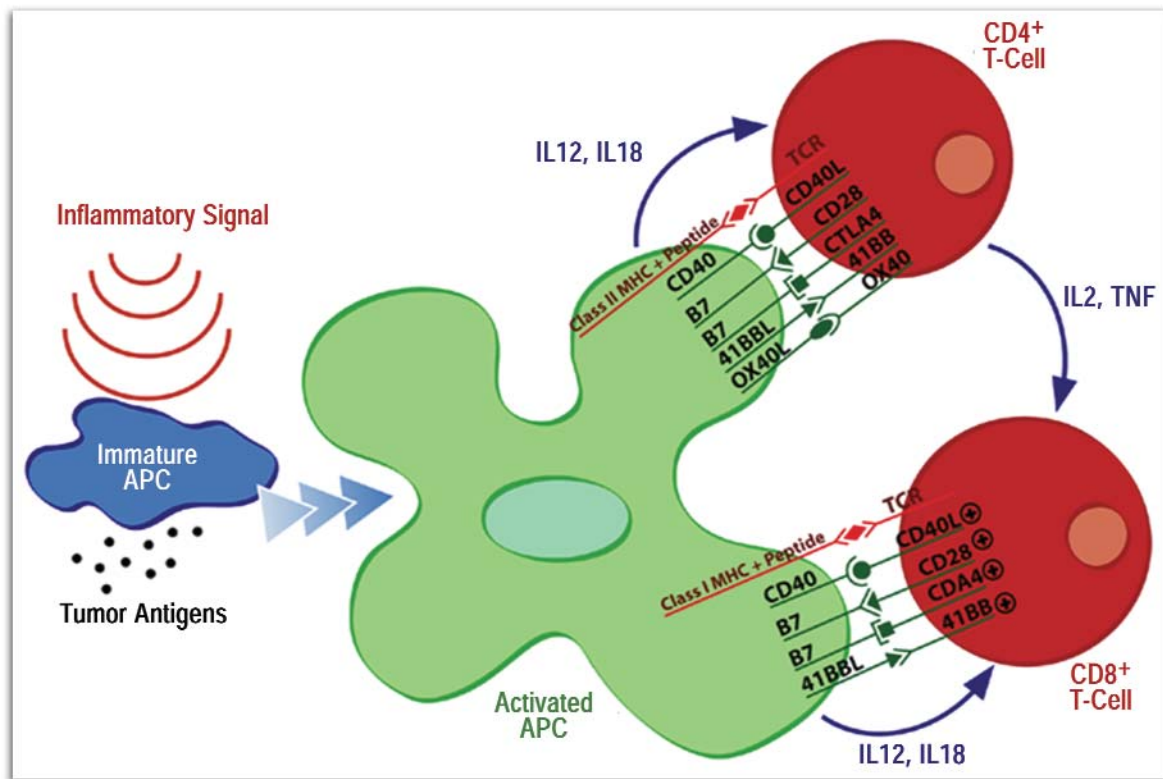


Figure 1. Activation of Antitumor Immunity. Professional antigen-presenting cells (APC) become activated in the presence of a proinflammatory signal. They then capture, endocytose, and process tumor antigens delivered by a vaccine or released by tumor cells. Concomitantly, professional APC upregulate a variety of costimulatory molecules (including B7-1, B7-2, CD40, CD27 ligand, OX40 ligand, LIGHT, and 41BB ligand) that can provide a second activation signal upon binding to cognate receptors on T cells. Tumor antigens are simultaneously presented to CD4⁺ and CD8⁺ T cells in the context of MHC Class II and MHC Class I, thus activating the antigen-specific immune response.

8 to 10 amino acids.² TAP transporters transfer 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, thus cross-priming the antigen-specific immune response.³ Activated CD4⁺ T cells initiate and amplify the CD8⁺ T cell response directly by providing stimulatory cytokines, and indirectly by upregulating a variety of costimulatory molecules on the APC that provide a second signal for T cell activation. Activated CD8⁺ T cells then acquire the potential to lyse tumor cells.⁴ Notably, in the absence of a danger or inflammatory signal, critical costimulatory molecules are not upregulated on the surface of the APC, resulting in downregulation of the T cell response.⁵ The influence of the inflammatory milieu at the time of immune priming and activation thus has clear implications for the clinical development of vaccine-based approaches to cancer treatment.

TUMOR ANTIGENS AND VACCINATION PLATFORMS

The success of immunization in infectious disease suggests that identification of pivotal antigens for immune-mediated tumor rejection will facilitate the development of highly targeted and effective vaccines for cancer therapy and prevention. The strength of a candidate antigenic target for cancer immunotherapy is determined by several characteristics: its tissue expression profile, the diversity, scope, and avidity of its T cell repertoire, the presence or absence of pre-existing immune tolerance, and the commonality of the antigen between patients and distinct tumor types.⁶ The focus of tumor antigen

identification has historically been on T cell targets. However, in light of increasing evidence that B cell-mediated immunity may participate in tumor rejection,^{7,8} the focus is increasingly on the identification of tumor antigens that elicit both B cell and T cell responses. The different classes of tumor antigens are summarized in Table 1.

Vaccine formulations range from the highly targeted, such as peptide-based immunization, to the less well defined, including whole tumor cells and tumor cell lysates (Table 2). In general, current approaches to cancer vaccine design are based on directly manipulating B cells, T cells, or professional APC.⁹ Approaches for activating humoral immunity have included vaccinating with tumor-specific carbohydrate antigens delivered by whole tumor cells or as conjugates with keyhole limpet hemocyanin (KLH). T cells can be activated directly by the vaccine, either by genetically modifying tumor cells to express costimulatory molecules or by genetically modifying professional APC with tumor antigens. Alternatively, T cells can be activated by immunizing with professional APC after direct loading of empty MHC with relevant tumor antigen either *in vitro* or *in vivo*. 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 early systematic analysis in preclinical models identified granulocyte-macrophage colony-stimulating factor (GM-CSF) as the most potent cytokine in this regard.¹⁰ GM-CSF-secreting tumor vaccines have been tested in Phase I clinical trials in melanoma, renal cell carcinoma, prostate cancer, and pancreatic cancer.¹¹⁻¹⁴ These trials have demonstrated the safety and bioactivity of this vaccine approach, and have suggested the potential for clinical benefit.

Table 1 TUMOR ANTIGENS

Type of Antigen	Tumor Type
Cancer Testis Antigens	
MAGE	Melanoma, Breast carcinoma, Esophageal carcinoma, Gastric carcinoma, GBM, HCC, Head/Neck carcinoma, Rhabdomyosarcoma
BAGE	Melanoma, Breast carcinoma, Esophageal carcinoma, Gastric carcinoma, HCC, Rhabdomyosarcoma
GAGE	Melanoma, Esophageal carcinoma, Gastric carcinoma, GBM, Prostate carcinoma, HCC, Rhabdomyosarcoma
RAGE	Renal carcinoma, sarcoma
NY-ESO-1	Melanoma, Prostate carcinoma, Breast carcinoma, TCC, NSCLC
Tissue-specific Differentiation Antigens	
Tyrosinase	Melanoma
MART-1/Melan-A	Melanoma
gp100	Melanoma
TRP-1, TRP-2	Melanoma
PSA	Prostate carcinoma
Mutated Gene Products	
CDK-4/R24C	Melanoma
β -catenin	Colon carcinoma
p53	NSCLC, Colon carcinoma, Breast carcinoma
k-ras	Pancreatic carcinoma, Colon carcinoma, NSCLC, Esophageal carcinoma
MUM-1	Multiple myeloma, lymphoma
bcr-abl	CML, AML
Overexpressed Self Antigens	
HER-2/ <i>neu</i>	Breast carcinoma
Proteinase-3	CML, AML
Mucin-1	Multiple myeloma
WT-1	CML, AML, ALL
MART-1/Melan-A	Melanoma
Viral Antigens	
HPV	Cervical carcinoma, head and neck carcinoma
HBV, HCV	Hepatocellular carcinoma
EBV	Burkitt's lymphoma, nasopharyngeal carcinoma, PTLD
Idiotypes	
Ig idiootype	B cell lymphoma
TCR idiootype	T cell lymphoma

Abbreviations: PSA, prostate-specific antigen; HPV, human papilloma virus; HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epstein Barr virus; Ig, immunoglobulin; TCR, T cell receptor; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; TCC, transitional cell carcinoma; NSCLC, non-small cell lung carcinoma; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; PTLD, post-transplant lymphoproliferative disease.

BARRIERS TO SUCCESSFUL IMMUNOTHERAPY FOR CANCER

A number of early trials have hinted at the promise of cancer vaccines (Table 3).¹⁵⁻²² They have also illustrated the importance of considering tumor burden, immune tolerance, vaccine formulation, and surrogate markers of response in clinical trial design. Zinkernagel and Old elegantly described the influence of tumor burden on the development of effective antitumor immunity. Very small tumors are often ignored by the immune system, growing in the periphery without accessing the lymphatic tissues. They thus neither activate nor tolerize tumor-specific T cells, a phenomenon referred to as “sneaking through”.²³ Once the tumor has reached sufficient size, it infiltrates the local lymphoid tissue, thus activating a late antitumor immune response. Tumor rejection is then determined by the relative growth kinetics and physical burden of tumor cells compared to the intensity and diversity of the effector T cell response induced.²⁴ Superimposed on this imbalance are the mechanisms by which tumors evade the immune system. These include the elaboration of inhibitory cytokines (interleukin-10, transforming growth factor- β and prostaglandin E₂), and the expression of molecules such as CD95L that can induce the apoptosis of tumor infiltrating

lymphocytes (TIL).²⁵ Tumors can also down-regulate the expression of tumor-specific antigens targeted by a vaccine or therapeutic antibody, resulting in the outgrowth of antigen loss variants.^{26,27} Furthermore, tumors can downregulate various components of the antigen processing machinery (including MHC Class I molecules themselves, various proteasome subunits, and the TAP transporter),²⁵ a phenomenon that has been correlated with poor clinical outcome.²⁸

Immune tolerance represents the second major barrier to the effectiveness of cancer vaccines (reviewed in ref. 29). Unlike infectious challenges, tumor cells arise endogenously. Thus, with the exception of de novo genetic mutations reflected in the transcriptional profile, the majority of tumor antigens are recognized as self. As a consequence, critical elements of the tumor-specific T cell repertoire are often either deleted centrally in the thymus (in the case of tumor antigens recognized as self) or eliminated peripherally by deletion or activation-induced cell death (AICD) (in the case of widely disseminated systemic tumor).²⁹ Alternatively, tumor cells can render tumor-specific T cells unresponsive or anergic by presenting antigen in the absence of costimulatory signals.²⁹ Tumor antigens can also be ignored by the immune system, either by virtue of very low expres-

sion levels or compartmentalization away from lymphatic tissues (i.e., expression limited to embryologic development).²⁹ If T cells do become activated, a phenotypically skewed cytokine/chemokine receptor profile can render them ineffective by virtue of cytokine deviation and ineffective trafficking.²⁹ Finally, both tolerizing dendritic cells and CD4⁺CD25⁺ regulatory T cells can negatively impinge on antitumor immune responses.²⁹ A thorough understanding of these diverse mechanisms of immune tolerance and their impact on the T cell repertoire available for immune manipulation will facilitate the development of innovative, targeted approaches for overcoming them.

Third, despite the vast array of tumor antigens that has been identified, only a very few are candidate tumor rejection antigens. A tumor rejection (or regression) antigen is an antigen preferentially associated with a cancer that can be effectively targeted by the immune system to destroy tumor cells, resulting in clinically relevant immune-mediated antitumor responses.⁶ That not all tumor antigens are also targets for rejection is well illustrated by the lack of concordance between the induction of antigen-specific immunity and tumor regression observed in multiple clinical cancer vaccine trials.³⁰⁻³⁴ This concept is best illustrated by the natural immune response of some melanoma patients who, despite the presence of significant numbers of functional cytotoxic effector T cells specific for the melanocyte-specific antigen MART-1/Melan-A, have progressive disease.^{35,36} The numbers of MART-1/Melan-A T cells in these patients can be augmented with targeted vaccines, but this does not influence the immunodynamics of the antitumor response in a clinically significant way.^{30,32,35-37} A similar phenomenon is reported for gp100-specific peptide vaccination.³⁸ Thus, a key task is to identify pivotal tumor rejection targets, and then to focus clinical vaccine development on those critical tumor antigens.

In addition to the challenges posed by the intricate immunobiology of the host-tumor interaction, the immaturity of the field of cancer immunotherapy places significant constraints on several key aspects of clinical trial design. The first limitation to clinical trial design is the lack of reliable immunologic surrogate markers of clinical response. The most commonly accepted test of vaccine-induced antitumor immunity is the delayed type hypersensitivity (DTH) test against autologous tumor, which correlates with survival in several trials.¹⁵⁻¹⁸ While the undisputed strength of DTH analysis is the use of patient-derived autologous tumor to assess immunity in vivo, the limited availability of autologous tumor often prevents its use. As an alternative, the combination of ELISPOT and MHC tetramer analysis is now under development for the serial assessment of the dynamics of the antigen-specific CD8⁺ T cell response, yielding information about both function and numbers of antigen-specific T cells.³⁹ While the advantage is that peripheral blood lymphocytes (PBL) are relatively easy to obtain, peripheral T cell populations might not reflect what is happening at the tumor site. Finally, a correlation between the development of antibody titers and clinical benefit has been demonstrated in at least three trials. In one study, prolonged survival was associated with the induction of GM2 IgM antibody responses in Stage III melanoma patients immunized with

Table 2 SUMMARY OF DIFFERENT VACCINE FORMULATIONS

Formulation	Immunogenicity	Potential for Toxicity	Requirement for HLA Match
Peptide + Adjuvant	low	low	yes
Plasmid DNA	low	low	no
Recombinant Virus	high	high	no
Replication-deficient pox virus			
Adenovirus			
Adeno-associated virus			
Herpesvirus			
Retrovirus/Lentivirus			
Recombinant Bacteria	high	high	no
Listeria monocytogenes			
Salmonella typhimurium			
Shigella			
Mycobacterium bovis BCG			
Dendritic Cells	high	low	yes
In vitro peptide loaded			
In vitro tumor lysate loaded			
Dendritic cell-tumor cell fusion			
Tumor Cells	moderate to high	low	no
Autologous			
Allogeneic			
Bystander			
Heat Shock Proteins	high	low	no

the ganglioside vaccine GM2/BCG after low dose Cyclophosphamide ($p = 0.02$).⁴⁰ A second study demonstrated a survival benefit for Stage II melanoma patients immunized with an allogeneic cellular melanoma vaccine who developed IgM antibody titers specific for TA90 greater than 1:800 ($p = 0.0013$).⁴¹ The third study demonstrated a correlation between improved survival and the development of significant β -human chorionic gonadotropin (β -HCG) antibody titers in metastatic colon cancer patients immunized with a β -HCG peptide vaccine conjugated to diphtheria toxin (DT) ($p = 0.0002$).⁴²

The second limitation to clinical trial design is the lack of informative predictors of immunologic or clinical response. The simplest example of predictors of immunologic response is the use of HLA genotype to identify patients that will respond to HLA-specific peptide vaccines, the most common being HLA-A2. The feasibility of identifying predictors of clinical response for other cancer vaccine platforms is suggested by two recent reports. First, the Southwest Oncology Group tested an allogeneic melanoma vaccine compared to observation alone in patients with intermediate thickness, lymph-node negative melanoma after surgical resection. A prospective analysis revealed a statistically significant clinical benefit in vaccinated HLA-A2 and HLA-C3 positive patients compared to HLA-A2 and HLA-C3 negative vaccinees, with 5-year disease-free survival (DFS) rates of 77% and 64% respectively ($p = 0.004$).²² Second, Wang and colleagues recently demonstrated the feasibility of defining tumor profiles as predictors of clinical response.⁴³ They classified metastatic melanomas by cDNA profiling, and identified two subsets of melanomas. Although neither of these two profiles correlated with clinical response, about 30 genes predictive of clinical response to immunotherapy were identified. Interestingly, approximately half are related to T cell regulation. This is clearly an area for further research.

Table 3 SUMMARY OF RECENT PHASE II AND PHASE III CANCER VACCINE TRIALS

Vaccine	Patient Population	Phase	N	Intervention	Results
Hapten-modified autologous in melanoma tumor cell vaccine (ref. 15)	Stage III/IV melanoma after regional lymphadenectomy	II	77	Vaccine	Improved 5 year survival in vaccinated patients > 50years, ($p = 0.011$), and who developed DTH > 5mm ($p = 0.031$)
Allogeneic melanoma tumor cell vaccine (ref. 16)	Metastatic melanoma after complete resection	II	77	Vaccine + BCG	Improved survival with the development of DTH and α TA90 IgM ($p < 0.0001$ for OS)
Sialyl-Tn-KLH + DETOX-B adjuvant (ref. 17)	Metastatic breast cancer	II	23	Cy + Vaccine vs. Vaccine	Increased sialyl-TN immune responses with Cy pretreatment
Autologous colon cancer tumor vaccine + BCG (ECOG 5283) (ref. 18)	Stage II/III colon carcinoma after resection	III	412	Vaccine vs. Observation	No arm differences ($p = 0.73$ for OS) improved survival if vaccine site reaction > 1 cm ($p = 0.003$ for OS)
Allogeneic melanoma tumor cell vaccine (ref. 19)	Metastatic melanoma after complete resection relapsed on vaccine therapy (trial in ref. 16)	III	194	Vaccine vs. Vaccine + BCG	Improved survival in relapsed patients reinduced with more frequent vaccinations and more BCG ($p = 0.0178$)
Conjugated ganglioside (GM2—KLH) + QS-21 adjuvant (ref. 20)	Stage IIB/III melanoma after resection	III	880	Vaccine vs. High dose IFN α 2B	IFN α 2B superior to vaccine ($p = 0.009$ for OS)
Allogeneic melanoma tumor cell vaccine (ref 21,22)	Stage IB/IIA melanoma after resection	III	689	Vaccine vs. Observation	No arm differences; Improved survival in HLA-A2 and HLA-A3 vaccinees ($p = 0.004$)

Abbreviations: DTH, delayed type hypersensitivity; BCG, Bacille Calmette-Guerin; KLH, keyhole limpet hemocyanin; Cy, Cyclophosphamide; IFN α 2B, interferon- α ; OS, overall survival; RFS, relapse-free survival.

COMBINATORIAL IMMUNOTHERAPY REGIMENS

Cancer vaccines as a single treatment modality are not likely to have the potency required to overcome the obstacles of tumor burden and immune tolerance in patients with established cancer. The scientifically based sequencing of tumor vaccines with surgery, radiation therapy, chemotherapy, and biologically targeted therapies is a critical aspect of clinical tumor vaccine development that should be determined in relevant preclinical models when possible. Traditional drug development typically involves Phase I testing in heavily pretreated patients with extensive disease, a patient population that is not appropriate for testing immunotherapies designed to elicit an effective host antitumor immune response. The detrimental effect of both a greater number of prior chemotherapy regimens and close proximity to a prior chemotherapy treatment on the induction of carcinoembryonic antigen (CEA)-specific T cell precursors in patients with advanced colorectal carcinoma treated with the canary pox vaccine ALVAC-CEA was recently demonstrated.⁴⁴ Importantly, the intensity of the vaccine-induced immune response determines tumor clearance.⁴⁵ The mismatch between tumor growth kinetics and the intensity of the vaccine-induced antitumor response achievable with current vaccination strategies is a strong argument for testing vaccine therapy in patients with minimal or undetectable residual disease after standard therapy. In a trial design that considers the influence of both tumor burden and aggressive cytotoxic therapy on vaccine-induced immunity, Jaffee and colleagues are conducting a Phase II efficacy trial of an allogeneic GM-CSF-secreting pancreatic cancer vaccine in 60 high-risk pancreatic cancer patients with minimal residual disease after pancreaticoduodenectomy. Patients are vaccinated immediately after surgery (just prior to six months of adjuvant chemoradiation) then go on to receive three additional monthly vaccinations after completing standard therapy. Monitoring the dynamics of antigen-specific vaccine-induced immune responses in these patients should provide further insight into these issues.

Chemotherapeutic agents are commonly used for their overt cytotoxic effects, and at standard doses clearly suppress cellular immune responses. However, some can also either enhance or inhibit antigen-specific immune responses depending on the dose and timing of administration in relation to antigen exposure (reviewed in ref. 46). Sequencing a GM-CSF-secreting CT-26 colon cancer vaccine with low doses of Doxorubicin (2, 4, or 6 mg/kg) one week after vaccination of CT-26 tumor-bearing BALB/C mice results in a higher rate of cure (40%) than either drug (10%) or vaccine (0%) alone.⁴⁷ The in vivo synergy was correlated with a dose-dependent increase in antigen-specific cytotoxic T lymphocyte (CTL) activity. Vaccine activity was abrogated when Doxorubicin was given prior to immunization. We investigated interactions between chemotherapy and GM-CSF-secreting vaccine in the tolerogenic HER-2/*neu* breast cancer model.⁴⁸ Low doses of Cyclophosphamide (100 mg/kg) or Paclitaxel (20 mg/kg) given one day prior to vaccine significantly decreased the outgrowth of pre-established mammary tumors compared to chemotherapy or vaccine alone. This correlated with the augmentation of HER-2/*neu*-specific IgG titers and HER-2/*neu*-specific T helper type 1 immunity by ELISPOT analysis. The sequence of Cyclophosphamide one day prior to vaccination, immunization, and Doxorubicin (5 mg/kg) seven days after vaccination was most potent, curing up to 20% of the mice. Importantly, both standard doses of the drugs and reversal of the treatment sequence inhibited vaccine activity, again highlighting the importance of dose and timing in relation to antigen exposure. Nowak and colleagues examined the influence of Gemcitabine on the induction of antigen-specific immunity using hemagglutinin (HA) T cell receptor transgenic mice and the HA-expressing mesothelioma cell line AB1-HA.⁴⁹ The administration of five doses of Gemcitabine (120 μ g/g every three days) to tumor-bearing mice completely abrogated HA-specific IgG responses, with minimal to moderately enhanced HA-specific T cell proliferation. Finally, upregulation of MHC Class I and cancer testis antigens by pretreating tumor cells with the demethylating agent

5-aza-2'-Deoxycytidine *in vitro* can restore melanoma- and renal cell carcinoma-specific CTL activity.^{50,51} Together, these results argue for the careful pharmacodynamic analysis of cancer vaccines and chemotherapeutic agents in clinically relevant preclinical models prior to clinical testing of combinatorial vaccination protocols.

The increasing number of costimulatory molecules that participate in the APC-T cell interaction has created unprecedented opportunities for highly targeted immune manipulation. CTLA4 is a negative regulatory molecule that both attenuates T cell responses and regulates peripheral T cell tolerance.⁵² *In vivo* antibody-mediated blockade of CTLA4 potentiates T cell responses to some (but not all) poorly immunogenic murine tumors,⁵² and combining CTLA4 blockade with GM-CSF-secreting vaccines has a synergistic antitumor effect compared to antibody or vaccine alone.^{53,54} Further, whereas CTLA4 blockade alone was unable to retard tumor growth in MOPC-315 tumor-bearing mice, combined with low dose Melphalan it had a significant anti-tumor effect.⁵⁵ The safety of a fully human CTLA4-specific antibody in patients with prostate cancer or melanoma has been reported, with a suggestion of single agent activity.⁵² The CD40/CD154 (CD40L) pathway plays a central role in the regulation of both humoral and cellular immunity.⁵⁶ CD40-specific agonist antibodies can substitute for CD4⁺ T cell help in the priming of CD4⁺ and CD8⁺ T cells.⁵⁷ These antibodies can both prevent tumor-induced T cell tolerance and break established T cell tolerance, augmenting the efficacy of tumor vaccines in preclinical models.^{58,59} OX40 is a third promising costimulatory target for tumor immunotherapy. It is a member of the tumor necrosis factor receptor (TNFR) superfamily expressed transiently on activated CD4⁺ T cells localized to the site of the immune response.⁶⁰ OX40 engagement during primary immunization breaks peripheral CD4⁺ T cell tolerance⁶¹ and increases the survival of memory T cells by inhibiting AICD.⁶² Practically, engagement of the OX-40 receptor *in vivo* during tumor priming enhances antitumor immunity.⁶³ Also, combining agonist OX40 antibody and the adoptive transfer of tumor-specific T cells has a greater antitumor effect than T cells alone.⁶⁴ These features, together with the correlation of high levels of OX40⁺ TIL in primary human colon cancers with survival ($p = 0.02$),⁶⁵ identify OX40 as a particularly attractive target for drug development. 41BB is a member of the TNFR superfamily that participates in the activation of dendritic cells and T cells.⁶⁶ Engagement of the 41BB pathway can eradicate some established tumors.⁶⁷ Combining peptide vaccination and 41BB signaling can break immunologic ignorance, resulting in immune-mediated tumor regression.⁶⁸ Further dissecting the distinctions between the costimulatory pathways and the potential cross talk between them will enable the development of the most active combinatorial cancer vaccine protocols, as well as their translation to the most appropriate clinical setting.

Lymphopenia-induced homeostatic T cell proliferation is a mechanism for restoring the memory T cell compartment.^{69,70} Immune manipulation by active immunization or the adoptive transfer of tumor antigen-specific T cells during the period of immune reconstitution after ablative treatments might favor the development of a T cell repertoire skewed toward a desired antitumor specificity.⁷¹ Consistent with this, the preferential induction and expansion of functional, melanoma-specific T cells in lymphopenic mice with pre-existing tumors vaccinated with a GM-CSF-secreting melanoma vaccine was recently demonstrated to correlate with significant tumor regression.⁷² These studies were conducted in Rag-1-deficient mice that are inherently lymphopenic, and it may be argued that this setting is not clinically relevant. However, several other preclinical studies have demonstrated the potential of enhancing

antitumor immunity by vaccinating tumor-bearing mice with GM-CSF-secreting tumor vaccines during early engraftment after syngeneic or allogeneic T cell depleted bone marrow transplant (BMT).^{73,74} Further, sublethal irradiation of tumor-bearing mice followed by the adoptive transfer of syngeneic T cells can result in an effective antitumor response as measured by CTL activity, IFN- γ secretion, and long-term memory.⁷⁵ While the phenomenon of homeostatic T cell proliferation has not yet been demonstrated in humans, lymphopenia is a common result of many standard cancer therapies. Characterization of homeostatic T cell proliferation in cancer patients followed by the careful delineation of the influence of chemotherapy and/or radiation on the kinetics and efficacy of antigen-specific immune reconstitution will be required for the effective application of tumor vaccines to the lymphopenic setting.

ANTIGEN IDENTIFICATION

It is clear that the identification of potent tumor rejection antigens is essential for the development of effective cancer vaccines. Traditional approaches to tumor antigen identification have included genetic strategies that use patient-derived CTL to screen cDNA libraries transfected into cells expressing defined MHC molecules, those that use patient sera to screen tumor-derived cDNA expression libraries (SEREX, or serologic analysis of recombinant cDNA expression), and biochemical strategies that involve the isolation, purification, and sequencing of peptide epitopes bound to MHC pockets.⁹ These approaches identified a number of tumor antigens that have already been clinically tested. However, most antigen-specific cancer vaccines tested to date have induced antigen-specific immunity with minimal evidence of a clinically meaningful antitumor immune response.^{31-34,37} This is not surprising since the identification of these tumor antigens as targets of T lymphocyte or antibody responses in patients with existing (or progressing) disease decreases the likelihood that they represent true tumor rejection targets. Moreover, traditional strategies for antigen identification cannot identify tumor antigens ignored by the immune system. One way to circumvent these inherent difficulties is to combine genomic databases generated by SAGE and microarray analyses with empiric peptide epitope deduction to identify candidate antigenic epitopes derived from cancer associated genes.⁷⁶ These candidate epitopes are tested for their ability to elicit antigen-specific CTL responses from healthy donors and cancer patients *ex vivo*. This approach has identified telomerase (hTERT)^{77,78} and survivin (an anti-apoptotic protein)^{79,80} as two candidate universal tumor antigens preferentially expressed by multiple tumor types. Neither hTERT nor survivin has yet been defined as a true tumor rejection target.

While empiric epitope deduction circumvents immunologic ignorance and capitalizes on the power of cancer genomics and bioinformatics, it is limited by the lack of a functional component. Several early trials of either nonspecific immunotherapies (CD4⁺ donor lymphocyte infusion for relapsed chronic myelogenous leukemia (CML) after BMT),⁸¹ or peptide-based,⁸² adenoviral,⁸³ or GM-CSF-secreting vaccines¹¹ have demonstrated significant clinical responses in very small numbers of patients. The sera and lymphocytes of these patients represent powerful tools for the identification of tumor antigens for immune monitoring and vaccine development. Patient sera were used to interrogate cDNA expression libraries derived from either CML or melanoma to identify CML66 (a novel gene product)⁸⁴ and ATP6S1 (a component of the vacuolar H⁺-ATPase complex)⁸⁵ as broadly immunogenic tumor antigens expressed by a variety of solid and hematologic tumors. Two other

reports utilized TIL or PBL derived from two responding melanoma patients treated with a multi-peptide vaccine plus interleukin-2 or a MART-1 adenovirus vaccine. These investigators identified the novel, melanoma-specific tumor antigens SOX10 (a transcription factor found in neural crest tissue)⁸⁶ and BING-4 (a novel gene product localized to the MHC Class II gene complex).⁸⁷ It is interesting to consider that, with the exception of ATP6S1, none of these newly identified candidate tumor antigens were delivered by the immunotherapy. The immune responses thus apparently developed as a result of immune-mediated tumor destruction. Whether responses to these antigens simply represent sentinel markers of effective antitumor immune responses or identify true tumor rejection targets will only be borne out by further study. Regardless, it is clear that combining cancer genomics, bioinformatics, and a detailed immune response analysis of patients who develop clinical responses on cancer vaccine trials is a powerful strategy with the potential for identifying the most biologically relevant tumor antigens. The ultimate goal of such detailed analyses is to identify shared tumor antigens for the formulation of potent recombinant polyvalent cancer vaccines broadly applicable to multiple tumor histologies and MHC haplotypes.

CONCLUSIONS

The field of cancer vaccines has matured significantly since the days of William Coley, largely due to advances in molecular immunology and cancer genetics. Developing strategies for overcoming immune tolerance and identifying the most active tumor rejection antigens are both critical to the success of therapeutic cancer vaccines. The judicious use of clinically relevant preclinical models to identify the most potent vaccination regimens for clinical testing will hasten clinical development. Innovative clinical trial designs, novel trial endpoints,⁸⁸ and informative surrogate markers and predictors of clinical response will ensure that cancer vaccines are most effectively incorporated into the management of cancer patients.

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