Cancer Vaccines:
Activating the Immune System to Fight Cancer

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Glossary of Commonly-used Terms
APCs: antigen-presenting cells
CTLs: cytolytic T lymphocytes
MHC antigens: major histocompatibility antigens
TAA: tumor-associated antigen
TAP: transporter associated with antigen processing

The promise of cancer vaccines is to activate the patient's immune system to kill tumor cells with minimal toxicity to normal tissues. The vaccine must be able to activate antibodies and/or lymphocytes against tumor-associated antigens (TAAs) on the tumor. Although a variety of approaches to activating the immune system exist, an optimal platform has yet to be determined. Many pitfalls exist in the development of cancer vaccines, but certain mechanisms may be able to circumvent these challenges.

Cancer vaccines fall into one of two categories: prophylactic or therapeutic. Prophylactic vaccines are intended to prevent the development of cancer; therapeutic vaccines are intended to treat existing cancers. To date, the U.S. Food and Drug Administration (FDA) has approved one prophylactic vaccine for cervical cancer (see sidebar on page 23), but the agency has yet to approve a single therapeutic cancer vaccine. A number of therapeutic vaccines targeting a wide variety of tumors are currently in development. Many of these vaccines are in Phase III clinical trials and should be seeking FDA approval over the next two to three years.

Approaches to Cancer Vaccines

In recent years immune approaches to the therapy of cancer have substantially evolved from treating patients with nonspecific immune stimulants [e.g., Bacillus Calmette-Guérin (BCG) for bladder cancer] to a focus on the use of TAAs. Therapeutic approaches with TAAs involve either "passive" or "active" immune therapy. Passive immune therapy, such as rituximab, uses antibodies to directly target tumor cells. Active immune therapy uses cancer vaccines composed of tumor cells, tumor cell lysates, peptides, carbohydrates, gene constructs encoding proteins, or anti-idiotype antibodies that mimic TAAs. In simple terms, anti-idiotype antibodies are antibodies that look like antigens. They "trick" the immune system into generating an immune response against the antigen that they "look like."

Specific "active" immunotherapy differs from nonspecific immune-based therapies such as BCG. As a nonspecific immune system stimulant, BCG stimulates a general immune response. In contrast, "active" specific immunotherapy aims to activate the immune system to fight tumor cells while sparing the surrounding normal tissue.

The theory behind specific immunotherapy is to use vaccines to activate a unique lymphocyte (such as a B cell or T cell) response, which will have an immediate anti-tumor effect and also a "memory response" to help fight future tumor challenges.

Table 1. Types of Tumor Vaccines

- Autologous tumor vaccines
- Allogeneic whole-cell vaccines
- Dendritic cell vaccines
- Viral oncolysates
- Polyvalent shed antigen vaccines
- Peptide vaccines
- Anti-idiotype vaccines
- Genetically modified vaccines
- Recombinant viral vaccines
- DNA vaccines

Some of these "active" specific vaccines are made from tumor cell preparations. Membrane preparations from tumor cells have also been used. Two basic types of tumor cell vaccines are autologous vaccines and allogeneic vaccines (see Table 1). Autologous vaccines are made from the patient's own tumor cells. These cells are removed from the patient, rendered inactive (killed), made into a vaccine in the lab, and then re-injected into the patient. Allogeneic vaccines are not made from the patient's own tumor cells. Instead, these vaccines are developed in the lab from tumor cell lines or other sources of tumor products that do not come from the patient's tumor cells.

In either instance (autologous or allogeneic), vaccines are combined with a variety of cytokines, which activate immune responses from cells. Gene-modified tumor cells expressing antigens designed to increase immunogenicity or gene modified to secrete cytokines have been a valuable

Table 2. Characteristics of Different Tumor Vaccines

	RESPONSE CHARACTERISTICS			
Vaccine	**Multiple antigens**	**Single Antigen**	**Antibody Response**	**T Cell Response**
Autologous cells	+	+	+	+
Allogeneic cells	+	-	+	+
Shed antigens	+	-	+	+
Carbohydrate	-	+	+	-
Peptide	-	+	+	+
Anti-idiotype antibody	-	+	+	+
Dendritic cell	n/a	n/a	+	+
DNA	-	+	+	+

+ Present
- Absent
n/a Not applicable

tool for vaccination. In addition, our increased understanding of TAA biology has led to the use of purified TAAs, DNA-encoding protein antigens, and/or protein-derived peptides. Today, all of these approaches are being tested in the clinic.

Mechanisms of Action

The ultimate aim of a therapeutic cancer vaccine is to activate a component of the immune system, such as antibodies or lymphocytes, against tumor-associated antigens presented by the tumor (see Table 2). Antibodies must recognize antigens at the tumor cell's surface. Once bound, these molecules can mediate tumor cell death.

T lymphocytes, on the other hand, recognize proteins as fragments (or, peptides) of varying size. These protein fragments appear in the context of major histocompatibility (MHC) antigens on the surface of the cells being recognized. (MHC antigens affect immune response by recognizing "foreign" versus "non-foreign".) (See Figure 1).[1-3] The proteins from which the peptides are derived may be cell surface or cytoplasmic proteins.[4,5] MHC antigens are highly polymorphic—that is, shape shifters—and different alleles have distinct peptide-binding capabilities. The sequencing of peptides derived from MHC molecules has led to the discovery of allele-specific motifs that correspond to anchor residues that fit into specific pockets on MHC class I or II molecules.[6,7] This has allowed for the discovery of new peptides associated with cancer as well as peptides that better stimulate an immune response.

Two T lymphocytes—helper T cells and cytotoxic lymphocytes—recognize antigens through a specific T-cell receptor (TCR) composed of α and β subunits arranged close to the CD3 molecule, which is responsible for signaling. CD4 helper T cells secrete cytokines and lymphokines that enhance immunoglobulin production as well as activate CD8 CTLs (cytolytic T lymphocytes). CD4 helper T cells are activated by binding via their T-cell receptor to class II molecules, which contain 14-25 amino acid (mer) peptides in their antigen-binding cleft.[8-10] Extracellular proteins are endocytosed (engulfed) and degraded and bound to newly synthesized MHC class II molecules. The MHC peptide complex is transported to the cell membrane, where it can be recognized by specific CD4 helper T cells. In most cases, the MHC class II antigen-containing peptide is presented to the CD4 helper T cells by a specialized cell called an antigen-presenting cell (APC).

Antigen-Presenting Cells

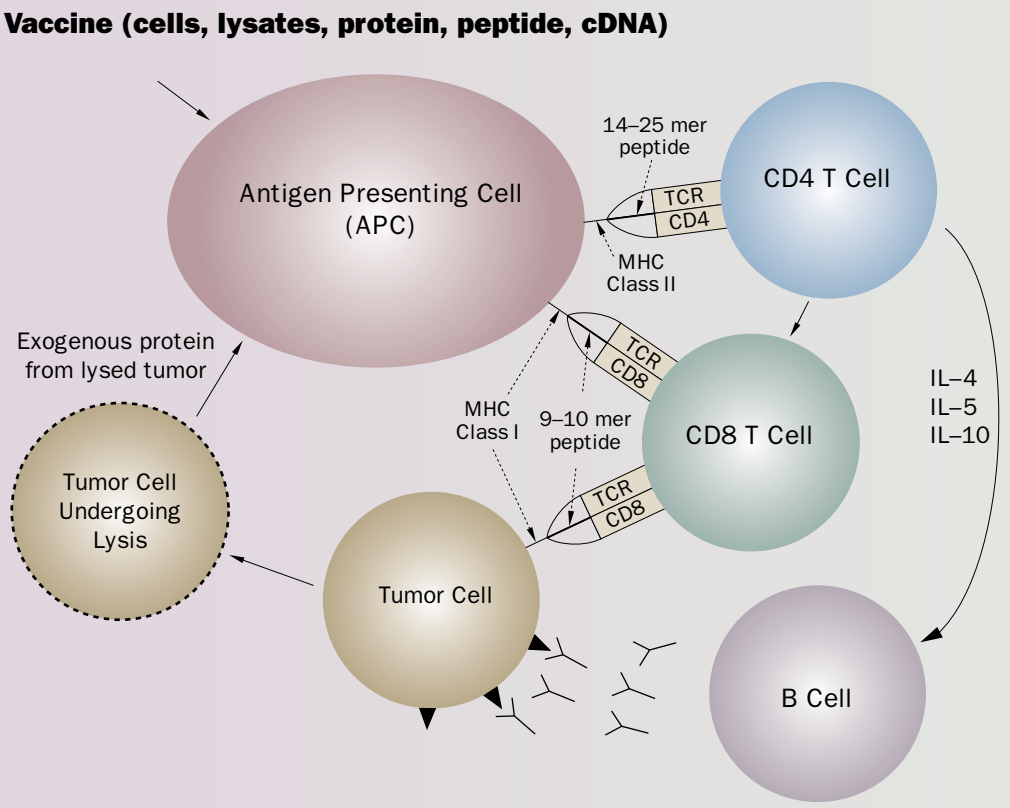
A variety of cells can process and present exogenous antigens including B cells, monocytes, macrophages, and the bone-marrow-derived dendritic cells. The most efficient antigen-presenting cells are dendritic. These cells express

high levels of MHC class I and II molecules; costimulatory molecules that send additional signals through B cell and T cell receptors, such as CD80 and CD86; and specific markers such as CD83. After antigen uptake, dendritic cells migrate peripherally to lymph nodes, where antigen presentation to CD4 helper T cells takes place.[11,12]

Two types of CD4 helper T cells can generate either an antibody- or a cell-mediated immune response, based on the type of signaling they receive. Th1 CD4 helper T cells stimulate cell-mediated immunity by activating CTLs (cytolytic T lymphoctyes) through the release of lymphocytokines such as Interleukin-2 (IL-2). Th2 CD4 helper T cells mediate an antibody response through the release of lymphocytokines such Interleukin-4 (IL-4) and Interleukin-10 (IL-10). In some cases, the generation of one type of response may inhibit the generation of the other (i.e., IL-10 secretion by Th2 helper T cells inhibits the generation of CTLs).[13]

CD8-positive CTLs are activated in most cases by peptides derived from intracellular proteins that are cleaved to 9-10 mer peptides within tumor cells or antigen-presenting cells by proteasomes, which help destroy other cellular proteins. The peptides are then transported by specialized transporter molecules called TAP (transporter associated with antigen processing) proteins to the endoplasmic reticulum. Here they become associated with newly synthesized MHC class I molecules.[14] The complex is next transported via the Golgi apparatus to the cell surface membrane where it is recognized by CD8 cytotoxic T cells via a specific T-cell receptor. Any endogenously processed protein can be presented to the immune system in this way. Several reports suggest a subset of antigen presenting cells can present exogenously processed proteins on MHC class I molecules to CTLs.[15-19]

Figure 1. T Cell Activation



T cells recognize antigens as fragments of proteins (peptides) presented with major histocompatibility complex (MHC) molecules on the surface of cells. The antigen-presenting cell (APC) processes exogenous protein from the vaccine or from the lysed tumor cell into a peptide, and present the 14-25 mer peptide to CD4 helper T cells on a class II molecule. There are also data to suggest that exogenous proteins can be processed into 9-10 mer peptides that may be presented on MHC class I molecules to CD8 cytotoxic T cells. Activated Th1 CD4 helper T cells secrete Th1 cytokines such as IL-2 that up-regulate CD8 cytotoxic T cells. Activated Th2 CD4 helper T cells secrete Th2 cytokines such as IL-4, IL-5, and IL-10 that activate B cells.

Pitfalls in Developing Cancer Vaccines

Tumor cells have developed a variety of mechanisms to escape immune system surveillance:

1. Dendritic cells are actively inhibited in the tumor milieu. Both immature and defective dendritic cells are described in a variety of tumors. In addition, dendritic cells that present tumor antigens may fail to reach the T cells in lymph nodes that generate active immune responses against tumors. The immune response may be skewed toward a Th2 response, which is antibody directed rather than cytolytic T cell directed, or T cells may be anergic, unable to generate an immune response.
2. Immune regulatory cells may contribute to immune tolerance to cancer cells. CD4 positive T cells (T-reg) with a high affinity receptor for CD25 that co-express the intracellular marker Foxp3 also play an important role in immune tolerance.[20,21]
3. Mutation or downregulation of immunodominant tumor antigens, MHC molecules, or molecules involved

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in the antigen processing machinery may also, in part, explain the escape of tumor cells from immune recognition.[22-24] Downregulation or mutation of pro-apoptotic molecules and expression of anti-apoptotic molecules may also render tumor cells resistant to apoptosis.

4. Tumor cells may acquire mechanisms that may actively contribute to immune tolerance. For instance, Fas ligand (FasL)-expressing tumors can deliver an apoptotic signal to activated T cells and natural killer cells expressing Fas receptor.
5. The tumor micro-environment may also contain soluble factors that inhibit T cell function. Factors such as TGF-beta, prostaglandins, IL-10, and catabolizing enzymes produced by tumor cells themselves or by stromal cells that may lead to T cell hyporesponsiveness.

Countering these various tumor escape mechanisms is a key component to successful vaccine therapy.

Mechanisms to Improve the Immune Response

Potent adjuvants improve the effectiveness of vaccines by accelerating the generation of immune responses and sustaining responses for extended periods of time. Commonly used adjuvants, such as alum or Freund's, effectively elevate antibody titers but do not elicit significant Th1 responses or activate CTLs.

The current focus is on adjuvants that are designed to specifically elicit cellular immune responses. Toll-like receptors (TLRs) are known to be involved in the initiation of immune responses. CpG stimulates TLR9 and has been used with vaccines to augment immune responses.[25] TLR8 may be involved in the activity of T-reg cells. Strategies aimed at TLR8 are proposed to neutralize T-reg cells.[26] One approach to decrease the role of T-reg cells is to use immunotoxin directed against the high affinity IL-2 receptor.[27,28]

Monoclonal antibodies specific for the negative regulatory signals mediating the CTL antigen 4 (CTLA4) on T-reg cells have also been tested to enhance the anti-tumor immunity to vaccines.[29] Cyclophosphamide has been used for many decades to boost immune response,[30-32] as have other chemotherapy agents.[33, 34]

Therapeutic Cancer Vaccines

To date, the FDA has not approved a single therapeutic cancer vaccine; however, a number of Phase III trials are nearing completion and we anticipate at least some of them will have positive results leading to FDA approval. Here is a brief description of some of the vaccines currently in clinical trials.

Melanoma. Melacine is composed of lyophilized melanoma lysates from two melanoma cells lines and the adjuvant Detox. In a Phase III trial of 604 patients with resected Stage III melanoma, patients were administered Melacine and low-dose interferon alpha-2b versus high-dose interferon alpha-2b.[35] Patients were stratified by sex and number of nodes and randomly assigned to receive either two years of treatment with Melacine and low-dose interferon alpha-2b or high-dose interferon alpha-2b alone for one year.

The median overall survival exceeded 84 months on the Melacine low-dose interferon alpha-2b (arm 1) versus 83 months in the high-dose interferon alpha-2b (arm 2) ($p = 0.56$). Five-year overall survival was 61 percent in arm 1 versus 57 percent in arm 2, and estimated 5-year relapse-free survival was 50 percent in arm 1 versus 48 percent in arm 2 with median relapse-free survival times of 58 months in arm 1 and 50 months in arm 2. Overall survival and relapse-free survival were clearly indistinguishable in the two arms. The incidence of neuropsychiatric severe adverse experiences were similar, although they were more severe in the high-dose interferon alpha-2b arm.

The primary aim of this study was to show that Melacine plus low-dose interferon alpha-2b would prolong overall survival compared with high-dose interferon alpha-2b. Unfortunately, this primary aim was not met, and the study results failed to demonstrate rejection of the null hypothesis, with nearly identical survival curves. Melacine was approved in Canada based on quality of life improvements.

GMK is a ganglioside conjugate vaccine in which ganglioside GM2 is coupled to KLH (keyhole limpet hemacyanin) and formulated with QS-21 adjuvant.[36] The goal of this vaccine is to induce an antibody response rather than a T-cell response.[37] This vaccine is in Phase III trials for melanoma.

Prostate Cancer. Dendritic cell vaccines are an attractive approach to vaccine therapy although they are labor intensive, requiring unique autologous dendritic cell preparations from individual patients (see Table 3). Sipuleucel-T and DCVax-Prostate are both vaccines based on dendritic cells and engineered to present T-cell antigens associated with prostate cancer.[38,39]

Sipuleucel-T consists of autologous dendritic cells pulsed with prostatic acid phosphatase and granulocyte macrophage colony stimulating factor (GM-CSF). A randomized Phase III placebo controlled trial in patients with metastatic asymptomatic androgen-independent prostate cancer was completed. Eighty-two patients were randomized in a 2:1 ratio.[40] The primary endpoint of this study, which was progression-free survival, was not reached ($p= 0.052$). However, overall survival was significantly different for vaccine (26 months) versus placebo (21 months) with a hazard ratio of 1.7 and $p=0.01$. A second study showed a trend toward increased survival (19 months versus 16 months) but did not reach significance. A Phase III clinical trial using survival as an end point is ongoing.

GVAX consists of two irradiated allogeneic prostate

Table 3. Selected Therapeutic Vaccines in Phase III Development

Vaccine	Indication	Manufacturer
Antigen-loaded Dendritic Cells		
Sipuleucel-T	Prostate Cancer	Dendreon Corporation
DCVax-Prostate	Prostate Cancer	Northwest Biotheraputics, Inc.
Modified Tumor Cells		
OncoVax-CL	Colon Cancer	Intracel Corp.
GVAX Cell	Prostate Cancer	Genesys, Inc.
Idiotype Vaccines		
FavID	Follicular Lymphoma	Favrille, Inc.
BiovaxID	Follicular Lymphoma	Biovest International, Inc.
MyVax	Follicular Lymphoma	Genitope Corp.
Viral Vector		
INGN-201	Head and Neck Cancer	Introgen Therapeutics, Inc.
TroVax	Renal Cell Cancer	Oxford BioMedica
Peptides		
TV-1001 (Telomerase)	Pancreatic Cancer	GemVax AS
BLP-25 (MUC-1)	Non-small Cell Lung Cancer	Merck KGA
Carbohydrate		
GMK	Melanoma	Progenics Pharmaceuticals, Inc.

cancer cell lines engineered to secrete GM-CSF.[41,42] Two Phase II trials have been completed in patients with asymptomatic metastatic androgen-independent prostate cancer. In the first study, patients were treated at two dose levels with median survival at the low-dose of 24 months and at the high dose of 35 months. A second study used five vaccine doses. The median survival at the low and middle doses was 23 months and 20 months respectively and was not yet reached at the high dose (>29 months). Phase III trials are ongoing.

Lymphoma and Leukemia. A number of non-cell-based patient-specific vaccines are currently in Phase III trials. FavID (Id, KLH), BiovaxID, and MyVax are idiotype vaccines that use KLH as a carrier. All three are patient specific for patients with B-cell lymphoma. Anti-idiotype immune responses have been shown to correlate with better clinical outcomes in follicular lymphoma patients who received idiotype vaccines.[43,44] MyVax is also being studied in Phase II trials for mantle cell lymphoma, diffuse large B-cell lymphoma, and chronic lymphocytic leukemia.

Head and Neck Cancer. INGN-201 is a recombinant adenovirus-p53-based vaccine for head and neck cancer. The adenovirus theoretically functions to deliver the p53 protein in large quantities to the tumor cells. While this vaccine is classified as a gene therapy, evidence suggests an immune response is elicited by p53, which is overexpressed in the tumor. Therefore, p53 may be considered a tumor-associated antigen and may aid in the elimination of tumor cells.[45] Interestingly, an adenovirus-p53 gene therapy vector (Gendicine) has been approved in China for head and neck cancer.

Renal Cell Cancer. TroVax is a vaccine in which the tumor associated antigen 5T4 is expressed in a modified vaccinia Ankara vector, which induces strong immune response similar to the live virus. It is expected that the recombinant 5T4 antigen will be included in the antiviral immune response. This vaccine is in Phase III trials for renal cell cancer.

Pancreatic Cancer. TV-1001 is a telomerase peptide-based vaccine that is in Phase III trials for pancreatic cancer. Telomerase is overexpressed in many cancers and is theoretically a good target antigen.

Non-small Cell Lung Cancer. BLP-25 is a liposome-encapsulated synthetic peptide that corresponds to the variable number 10 tandem repeat region of the mucin (MUC)-1 molecule. MUC-1 is overexpressed and underglycosylated on tumor cells. The MUC-1 target is highly represented on most epithelial tumors. This vaccine is currently in a Phase III trial for non-small cell lung cancer.

Where Do We Go From Here?

The platform for therapeutic vaccines is broad, including a variety of antigens, both non-specific antigens represented by whole-cell-based vaccine approaches and recombinant antigens as represented by the protein- and virus-based approaches. Dendritic cells are an extremely appealing vaccine approach; however, they are limited by the difficulties associated with patient-specific cell therapies. To date, no specific approach to vaccine therapy has emerged as clearly superior. Strategies to enhance the immune response will be the next most important step in therapeutic cancer vaccines. Monoclonal antibodies inhibiting T-regs, the use of a variety of cytokines, and toll-like receptor stimulation are among the strategies that will be employed.

Cancer Prevention Vaccines

A major success story in cancer vaccinology is cancer prevention targeting the human papillomavirus (HPV) to prevent cervical cancer. Cervical cancer is the second most common cancer in women, responsible for over 250,000 deaths annually worldwide. Seventy percent of cervical cancers are caused by the two most common oncogenic HPV types, HPV- 16 and HPV-18, while another 10 percent are caused by HPV-45 and HPV-31.[1] The FDA has approved the cancer prevention vaccine HPV-16/18/6/11 (Gardisil, Merck & Co. Inc, Whitehouse Station, N.J.).

This vaccine uses recombinant DNA technology to develop subunit vaccines which include only the epitopes from the pathogen recognized by the immune system. Copies of the L1 viral capsid protein, the same protein which antibodies are generated against in the natural immune response to HPV, spontaneously self-assemble into noninfectious virus-like particles which are used as the antigen in the prophylactic vaccine. The vaccine is formulated with ASO4 (aluminum hydroxide and monophosphoryl lipid A) adjuvant.[2,3]

Only half the women with HPV infection develop protective immunity because the natural infection by HPV evades detection of the immune system. Clinical studies demonstrated seropositivity in women who receive the HPV 16/18/6/11 vaccine was 100 percent for HPV-16 and HPV-18 one month after vaccination and remained at 100 percent 4.5 years after vaccination.[4-6] Efficacy against cervical intraepithelial neoplasia associated with HPV-16 was 100 percent. Of course, many women are already infected with HPV and will develop cervical intraepithelial neoplasia and remain at risk for developing cervical carcinoma. In development are a number of HPV-based cervical vaccines that are designed to eliminate HPV-induced disease after infection.

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