GASTROENTEROLOGY 2004;127:1821–1837

SPECIAL REPORTS AND REVIEWS

Colorectal Cancer Vaccines: Principles, Results, and Perspectives

SIMONE MOCELLIN, CARLO RICCARDO ROSSI, MARIO LISE, and DONATO NITTI
Department of Oncological and Surgical Sciences, University of Padova, Padova, Italy

In the search for novel therapeutic approaches to treat patients with colorectal carcinoma, anticancer vaccination holds promise. A large body of preclinical and clinical evidence has demonstrated that the immune system can be polarized against malignant cells by means of several active specific immunotherapy strategies. Although no vaccination regimen can be currently recommended outside clinical trials, tumor response and immunologic findings observed in animal models and humans prompt researchers to explore further the antitumor potential of such biotherapy in an effort to reproduce in a larger set of patients the cascade of molecular events that characterizes the successful tumor immune rejection currently observed in a minority of vaccinated subjects. In this work, we summarize the principles and the main results of cancer vaccine strategies so far implemented for the treatment of patients with colorectal carcinoma. We also discuss the most recent preclinical tumor immunology insights that might change the way to design the next generation of cancer vaccines, hopefully improving the effectiveness of such a biotherapeutic approach.

Colorectal carcinoma is the fourth most commonly diagnosed type of cancer and accounts for 10%–15% of deaths from cancer in Western countries.[1] After radical surgery of locally advanced primary tumor (AJCC stage II-III), 5-FU-based adjuvant chemotherapy ± radiation therapy significantly improves overall survival rate, but, at 5 years, 40% to 50% of patients still will die of the disease.[2,3] In the case of metastatic disease (AJCC stage IV), the prognosis is poor, and most patients will ultimately succumb.[4] Toxicity and lack of tumor specificity are the most important limits of conventional approaches (ie, chemotherapy, radiation therapy). Investigators are therefore seeking novel therapeutic options.[5,6] Exploiting a naturally occurring defense system, the immunotherapeutic approach embodies an ideal nontoxic treatment capable of evoking tumor-specific immune responses. Although a wealth of clinical evidence clearly demonstrates that a variety of therapeutic manipulations can effectively polarize the immune system against different tumor types in humans,[7,8] no current vaccination regimen has been demonstrated of clinical utility. However, by dissecting the cellular/molecular events underlying cancer immune rejection observed in a limited subset of patients, investigators might decipher the biologic code governing tumor immune responsiveness and thus design more effective immunotherapeutic strategies.[9]

The implementation of ASI for the treatment of colorectal cancer is relatively recent compared to other malignancies. The identification of tumor-associated antigens (TAA) expressed by colorectal carcinoma as well as recent advancements in tumor immunology are giving new impetus to the development of biologically targeted immunotherapeutic strategies for this type of cancer.

After summarizing the principles underlying anticancer vaccination, we review the clinical results so far obtained with colorectal cancer vaccines and discuss the most promising preclinical findings that might improve the effectiveness of such a biotherapeutic approach.

Rationale for Anticancer Vaccination

The concept of tumor immune surveillance, proposed by Burnet in the 1950s, holds that a physiologic function of the immune system is to recognize and destroy clones of transformed cells.[10] Although the importance and even the existence of immune surveillance has been questioned, it is now clear that the immune system can react and sometimes play an important role in tumor control both in animal models and in humans.[11] Animals deficient in immune-

Abbreviations used in this paper: APC, antigen-presenting cells; ASI, active-specific immunotherapy; CTL, cytotoxic T lymphocytes; DC, dendritic cells; HLT, helper T lymhocytes; HSP, heat shock proteins; TAA, tumor-associated antigens.

0016-5085/04/$30.00
doi:10.1053/j.gastro.2004.07.012

related molecules (eg, IFN-γ, perforin, RAG-2) are more susceptible to the development of experimental or spontaneous cancers,[12] and immunosuppressed individuals, such as organ transplant recipients or patients affected with primary or acquired immunodeficiency disorders, have an increased risk for developing malignancy.[13,14] Both cytotoxic T lymphocytes (CTL) and antibodies specific for TAA have been found in tumor-bearing patients,[15–18] including those with colorectal cancer.[19] In addition, CTL isolated from patients affected with cancer can lyse autologous and HLA-matched tumor cells in vitro.[20] Brisk lymphocytic infiltrate in tumor specimens is an independent positive prognostic factor for melanoma patients.[21] Likewise, the natural occurrence of a humoral immune response is associated with a good outcome in breast carcinoma[22] and melanoma patients.[23] Although a lymphocytic infiltration of primary colorectal carcinoma is similarly associated with improved overall and recurrence-free survival,[24,25] there are very few data about the antigen-specificity of these lymphocytes. Interestingly, a high level of microsatellite instability in colorectal carcinomas is associated with the presence of tumor-infiltrating lymphocytes, as well as an improved prognosis[26]: Hypothetically, the increased number of lymphocytes are responding to large numbers of "neo-antigens" created by the high rate of DNA mutation, which is characteristic of this group of colorectal cancers as a result of defects in DNA mismatch repair genes. In the model of hematologic malignancies, the reduced relapse rates observed in the allogeneic transplant setting (compared with those reported in the autologous setting),[27] the dramatic clinical benefits achieved with donor lymphocyte infusions,[28] and the recent clinical successes of antibody-based therapies for the treatment of non-Hodgkin's lymphomas (NHL)[29] have provided investigators with further evidence supporting the potential ability of adaptive immunity to control cancer growth.

Anticancer vaccination is based on the assumption that the immune system can recognize and react to TAA (Figure 1). After the milestone experience of chemically or virally induced tumors rejected by syngeneic mice as a result of the ex novo generation of highly immunogenic, tumor-specific transplant antigens,[30] investigators could demonstrate that several naturally occurring TAA can be the target of humoral and/or cellular adaptive immune response[31] (Table 1).

Molecularly identified or undefined TAA can be administered to cancer patients in an attempt to induce a systemic immune response, ultimately leading to malignant cell destruction.[32] Like vaccine development for infectious diseases, this procedure is defined as active-specific immunotherapy (ASI) or vaccination because the host immune system is activated ex novo or restimulated to mount an effective tumor-specific immune reaction against malignant cells.

Current Vaccination Strategies

A synopsis of ASI strategies for cancer treatment is reported in Table 2. The following is a description of the principles and results of cancer vaccine formulations so far implemented in the clinical setting. The details (vaccine composition, study design, clinical outcome, and immunologic findings) of the clinical trials carried out for the treatment of patients with colorectal carcinoma are illustrated in Table 3 (therapeutic vaccines) and Table 4 (adjuvant vaccines).

The review method consisted of several PubMed searches of the National Library of Medicine, limiting the search to English language articles and clinical series of at least 10 patients. Where appropriate, cited references from selected articles were also reviewed.

Polyvalent Vaccines

Whole cell polyvalent vaccines. Living, whole tumor cells inactivated by irradiation so that they are not capable of growth are the earliest forms of antitumor vaccines and have been extensively studied in human trials.[33,34] The concept of using whole cells remains appealing because these vaccines should contain a large repertoire of TAA ("polyvalent" vaccines) potentially targeted by the immune system. Autologous tumor cell vaccines are patient specific, and their production depends on the availability of tumor cells from that patient. Allogeneic preparations overcome the limitation of tumor cell source in that they are made from 2 or more tumor cell lines grown in vitro. However, allogeneic vaccine preparations introduce irrelevant dominant antigens (ie, allogeneic HLA molecules) that might overwhelm the induction of an effective immune response against more relevant but weaker antigens (ie, TAA). In the adjuvant setting, the efficacy of anticolorectal cancer autologous vaccines has not been proven in phase III randomized trials, although a significant survival advantage has been observed in subsets of patients (Table 4).

To increase the immunogenicity of whole cell polyvalent vaccines for colorectal cancer, tumor cells have been infected with viruses.[35,36] More recently, vaccines have been prepared with malignant cells genetically engineered to secrete cytokines (eg, GM-CSF, IL-2), which should recruit and activate antigen-presenting cells (APC) at the site of vaccine injection, thus favoring the process of TAA uploading and ultimately TAA presen-

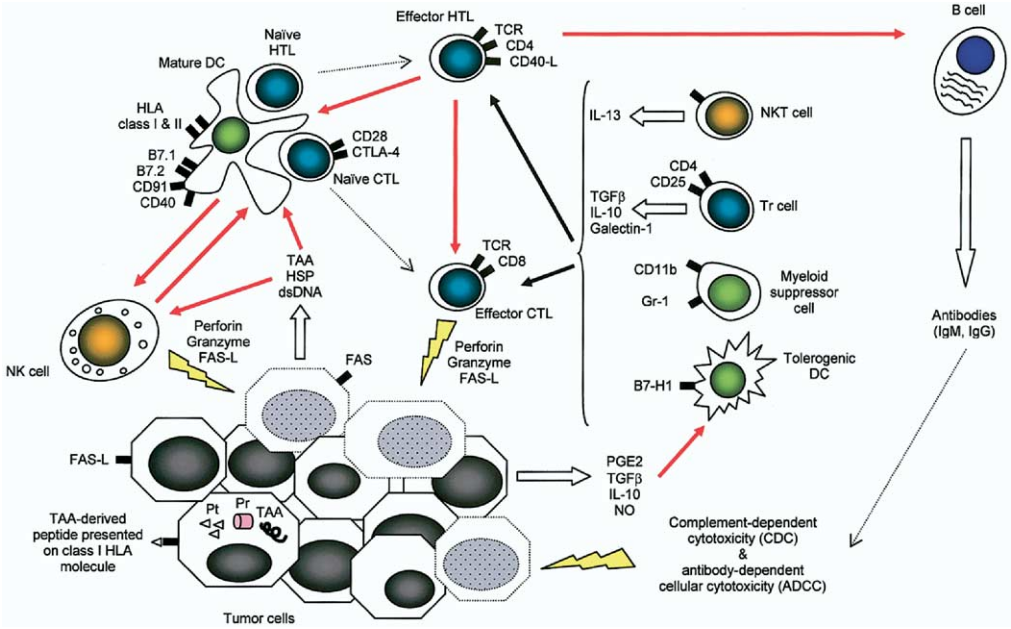

Figure 1. Schematic view of the immune response to cancer. T cells are activated by professional antigen-presenting cells (APC) such as dendritic cells (DC) and require 2 signals. By means of their T-cell receptor (TCR), T lymphocytes recognize TAA-derived peptides presented in the context of class I or II HLA molecules (first signal). A second signal is provided by the interaction between costimulatory molecules (ie, CD28) and their ligands (ie, B7.1/B7.2). Intracellular protein TAA are cleaved by the proteasome into 8–10 amino acid peptides that are presented on the surface of most nucleated cells in the context of HLA class I molecules. Naïve $CD8^+$ T cells become activated by recognizing these peptides presented by APC; then, if these effector cells recognize the same peptide on the surface of tumor cells, they can lyse the target cell by exocytosis of cytotoxic factors (eg, perforin, granzyme) and/or by FAS/FAS-L interaction. Extracellular protein TAA are endocytosed and cleaved into 12–20 amino acid peptides only by APC that present them in the context of HLA class II molecules. Naïve $CD4^+$ T cells become activated by recognizing these peptides presented by APC; these effector cells can stimulate the function of $CD8^+$ T cells, B lymphocytes, and APC by secreting Th1-type (eg, IL-2, IFN-γ) and Th2-type cytokines (eg, IL-4, IL-10) and by cell-to-cell interaction (eg, through CD40/CD40-L), respectively. Several other innate (eg, natural killer cells) and adaptive (eg, T regulatory cells) immunity cell mediators are involved in determining the final outcome of the immune response against malignant cells. *Red line*, stimulatory effect. *Black line*, inhibitory effect. DC, dendritic cell; HTL, helper T lymphocyte; CTL, cytotoxic T lymphocyte; NK-cell, natural killer cell; Tr cell, T regulatory cell; CD28, B7.1/B7.2 receptor expressed by T cells (stimulatory effect); CTLA-4, B7.1/B7.2 receptor expressed by T cells (inhibitory effect); FAS-L, FAS ligand; TCR, T-cell receptor; CD40-L, CD40 ligand; CD40, CD40-L receptor; TAA, tumor-associated antigen; HSP, heat shock protein; CD91, HSP receptor; dsDNA, double-strand DNA; IL-10, interleukin-10; IL-13, interleukin-13; TGF-β, transforming growth factor β; PGE2, prostaglandin E2; NO, nitric oxide; Pr, proteasome; Pt, peptides.

tation to T cells in secondary lymphoid organs (ie, vaccination site draining lymph nodes).[37–39] This ASI has not yet been implemented for the treatment of patients with colorectal cancer.

Heat shock proteins. Heat shock proteins (HSP) are intracellular proteins that act as chaperones for peptides, including TAA-derived peptides.[40,41] Dendritic cells (DC), the most powerful APC,[42] possess a specific receptor for heat shock proteins (CD91).[43] The engagement of CD91 by HSP leads to the maturation/activation of DC.[43] Therefore, HSP released by necrotic cells function as endogenous danger signals as well as a method to cross-present TAA by DC. HSP can be isolated and used as a polyvalent autologous cancer vaccine preparation of undefined TAA. With this strategy, the need to identify TAA peptides recognized by CTL is circumvented. On the basis of the encouraging results obtained in animal models,[44] HSP (ie, gp96) have been tested in pilot clinical trials for the treatment of patients with different histologic types of cancer, including colorectal carcinoma[45,46] (Table 4).

Antigen-Defined Vaccines

Tumor-associated antigens (whole antigen). From the biochemical viewpoint, TAA utilized for anticancer vaccination are either proteins or carbohydrates. Proteins (eg, MUC-1) are attractive TAA because they are molecularly defined (as opposed to polyvalent vaccines), can be easily produced by means of recombinant technology, and enable the patient's own immune system to cleave and bind HLA class I and/or II restricted peptides without the need to know their sequence (as occurs with peptide-based vaccines).

Carbohydrates represent the epitope of several TAA that include glycosphingolipids (eg, gangliosides) and glycoproteins (eg, sialyl-Tn, MUC-1). These TAA are either over expressed by cancer cells (gangliosides, sialyl-Tn) or are tumor specific because of the disrupted glycosylation proper of tumor progression.[47,48] Although this type of ASI has been largely tested for the treatment of other malignancies (eg, melanoma, breast carci-

Table 1. Tumor-associated Antigens Recognized by the Immune System

Category	TAA	Tumor
Unique antigens	p53	Several carcinomas (including CRC)
	K-RAS	Several carcinomas (including CRC)
	APC	CRC carcinoma
	TGF-βR-II	CRC carcinoma
	caspase-8	Head and neck tumors
	β-catenin	Melanoma
	CDK-4	Melanoma
	Gn-TV	Melanoma
	BCR-ABL (fusion protein)	Acute lymphoblastic leukemia
	Antibody idiotypes	Lymphomas, multiple myeloma
Differentiation antigens	gp100 (pmel117)	Melanoma
	MART-1 (Melan-A)	Melanoma
	Tyrosinase	Melanoma
	TRP-1	Melanoma
	TRP-2	Melanoma
	PSA	Prostate carcinoma
Shared antigens	MAGE family	Several tumor types (including CRC)
	GAGE family	Several tumor types (including CRC)
	BAGE-1	Several tumor types (including CRC)
	SSX-2	Several tumor types (including CRC)
	SAGE	Several tumor types
	LAGE-1/CAMEL	Several tumor types
	NY-ESO-1/LAGE-2	Several tumor types
Overexpressed antigens	EGF-receptor	Several carcinomas (including CRC)
	CEA	Several carcinomas (including CRC)
	HER-2/neu (erbB-2, p185)	Several carcinomas (including CRC)
	CO17-1A (Ep-Cam, GA733-2)	Several carcinomas (including CRC)
	MUC-1	Several carcinomas (including CRC)
	Survivin	Several tumor types (including CRC)
	Telomerase	Several tumor types (including CRC)
	gp72/CD55	Several carcinomas (including CRC)
	gastrin	Several carcinomas (including CRC)
	WT1	Acute leukemias
	PRAME	Several tumor types
	β-hCG	Several tumor types (including CRC)
	α-fetoprotein	HCC, testis
	Gangliosides (eg, GM1, GM2)	Melanoma

NOTE. TAA are generally classified in 4 major categories: (1) Unique antigens (or tumor-specific antigens) are the result of tumor genetic instability (mutation/translocation/alternative transcription) and are specific to an individual tumor; (2) differentiation antigens (tissue-specific antigens) are expressed by the tumor and the normal tissue from which it derives; (3) shared antigens (or cancer-testis antigens) are expressed by a variety of tumor types and not by normal tissues, with the exception of spermatogonia, which do not express HLA class I molecules and thus are not targeted by cytotoxic T lymphocytes; and (4) over expressed antigens can be found in normal tissues but are massively over expressed by malignant cells.
CRC, colorectal carcinoma; TGF-βR-II, transforming growth factor β receptor-II; APC, adenomatosis polyposis coli; PSA, prostate-specific antigen; HCC, hepatocellular carcinoma.

noma),[49,50] only a few patients with colorectal carcinoma have been treated with such cancer vaccine (Table 3). Of note, a survival advantage has been recorded in patients with colorectal cancer who responded to TAA-based vaccination.[51]

Peptide vaccines. This is the most specific vaccine preparation because peptides represent the basic molecular unit "seen" by T cells (Figure 1). The majority of TAA-derived peptides so far identified are presented in association with class I HLA molecules and are recognized by tumor-specific CTL.[52] By contrast, only few TAA epitopes are presented in association with class II HLA molecules and are recognized by helper T lymhocytes (HTL). Although tens of TAA peptides recognized by T cells have been identified,[53,54] their clinical use is still limited for the most common cancer types. In fact, most TAA epitopes are expressed by melanomas and are recognized only by a specific HLA allele (ie, HLA-A2), leaving ineligible several patients expressing less frequent HLA alleles.[55] Recently, tumor immunologists have identified TAA peptides suitable for colorectal cancer vaccination,[56–58] but the clinical implementation of this ASI strategy for the treatment of this tumor type is still in its infancy (Table 3).

Recombinant virus vaccines. The finding that viral infections lead to the presentation of viral peptides

Table 2. Cancer Vaccine Strategies So Far Implemented in the Clinical Setting

Vaccine category	Vaccine composition	Comments
Polyvalent vaccines	Whole tumor cell	Autologous or allogeneic tumor cells that can be genetically engineered to secrete cytokines No need to know the molecular identity of TAA
	Lysate tumor cell	Mechanical, enzymatic, or viral tumor lysates No need to know the molecular identity of TAA
	Shed antigens	TAA or TAA-derived peptides released in vitro by allogeneic tumor cell lines No need to know the molecular identity of TAA
	Heat shock proteins	Prepared from autologous tumor cells No need to know the molecular identity of TAA
Antigen-defined vaccines	TAA (whole antigen)	proteins (eg, CEA, p53; MUC-1), carbohydrates (eg, sialyl-Tn), idiotypes (B-cell lymphomas, multiple myeloma), glycolipids (eg, gangliosides)
	Peptides	HLA class I or class II restricted
	Recombinant DNA	Genetically engineered to express TAA ± cytokines
	Recombinant virus	Genetically engineered to express TAA ± IA (eg, cytokines, CpG) or costimulatory molecules (eg, B7.1)
	Anti-idiotypic antibody	Mimicking the natural TAA
DC-based vaccines	Tumor-DC hybrid	No need to know the molecular identity of TAA
	Whole tumor cell loaded DC	Autologous or allogeneic tumor cells No need to know the molecular identity of TAA
	Peptide loaded DC	HLA class I or class II restricted
	Tumor mRNA loaded DC	No need to know the molecular identity of TAA
	Virally infected DC	Virus coding for TAA
	Genetically engineered DC	Engineered to secrete cytokines or express TAA

TAA, tumor associated antigen; IA, immunological adjuvant; DC, dendritic cell.

in association with MHC class I and class II HLA molecules on the surface of infected cells has led to the design of ASI strategies in which viruses are used as the immunization vehicles. Viruses such as vaccinia, avipox, and adenoviruses are potentially ideal vectors for the delivery of TAA (eg, CEA, p53) because of their ability to infect directly and activate APC.[59,60] Recombinant viruses have also been utilized to encode and express costimulatory molecules (eg, B7.1, B7.2) as well as immunologic adjuvants (eg, cytokines, CpG). Despite the theoretic premises, the application of this ASI strategy in the therapeutic setting has obtained poor or conflicting results in colorectal carcinoma (Tables 3 and 4), as well as in other tumor types.[61,62]

DNA vaccines. Naked DNA vaccines consist of the specific gene encoding the TAA of interest cloned into a bacterial plasmid engineered for optimal expression in eukaryotic cells.[63] Plasmid DNA, which can express immunologic adjuvants (IA) (eg, cytokines, CpG) and costimulatory molecules (B7.1, B7.2), has the advantages of being readily deliverable and molecularly defined and can be easily constructed and produced in large quantities. The implementation of this ASI strategy in clinical oncology is still in its infancy[64–66] (Table 3).

Anti-idiotype vaccines. From conventional network theory,[67] it follows that, to any specific antibody, a mirror-image antibody will be generated. Vaccination with TAA-specific mouse antibodies (Ab1) results in the formation of autologous antibodies (Ab2) against the vaccine. The variable part of these induced antibodies fits the murine idiotype and therefore strongly resembles the epitope of the original TAA. Consequently, this anti-idiotype can be used as a surrogate vaccine in place of the naturally occurring TAA and stimulate cellular and/or humoral (Ab3 antibodies) immune responses.[68,69] This vaccination strategy requires relatively low amounts of vaccine preparation and allows for vaccination toward nonprotein antigens (eg, carbohydrates) that are difficult to be cloned for large scale production. Furthermore, anti-idiotype antibodies are particularly effective in breaking immune tolerance toward TAA.[70] Colorectal cancer patients have been treated with this ASI strategy both in the adjuvant and therapeutic setting (Tables 3 and 4). Although no tumor responses have been reported, in a phase II study, patients who developed an immunologic response showed a survival benefit.[71]

Dendritic Cell-Based Vaccines

The fate of TAA largely depends on their ability to be internalized and processed by DC. These cells expressing high levels of HLA class I and class II as well as costimulatory molecules (B7.1/CD80, B7.2/CD86) have been demonstrated to be effective in presenting TAA peptides to enhance cellular immunity both in vitro[72,73] and in vivo.[74] The use of TAA/peptide-loaded DC requires prior knowledge of pa-

Table 3. Colorectal Cancer Vaccines in the Therapeutic Setting: Clinical Trials

Author/year (reference)	Vaccine type	Study design	Clinical outcome	Immunologic findings
Sobol et al 1999 (182)	Autologous tumor (whole cell) IA: fibroblasts transduced with IL-2 Route: SC	Phase I–II N = 10	Toxicity: minimal OR: 0	T-cell response: 5/10 B-cell response: 0/10
Habal et al 2001 (179)	Allogeneic tumor (whole cell) IA: BCG Route: ID	Phase I–II N = 27	Toxicity: 2 grade 3 (fatigue, pain) OS: better in immune responders	B-cell response: 14/27 T-cell response: 21/27
Marshall et al and Zhu et al 1999 and 2000 (183, 184)	Recombinant virus encoding CEA IA: none Route: IM	Phase I–II[a] Escalating doses N = 20	Toxicity: minimal OR: 0	T-cell response: 7/9
Conry et al 2000 and 1999 (185, 186)	Recombinant virus encoding CEA IA: none Route: ID or SC	Phase I[a] N = 32	Toxicity: minimal	B-cell response: 7/32 T-cell response: 0/32
Marshall et al 2000 (187)	Recombinant virus encoding CEA IA: none or GM-CSF + IL-2 Route: ID/SC	Phase I–II[a] Randomized trial (vaccinia vs avipox) N = 18	Toxicity: minimal OR: 0	B-cell response: 4/18 T-cell response: better in vaccinia virus arm
Horig et al 2000 (188)	Recombinant virus encoding CEA and B7.1 IA: none Route: IM	Phase I–II Escalating doses N = 18	Toxicity: minimal OR: 0	B-cell response: only to viral vector; T-cell response: 4/12
von Mehren et al 2001 (189)	Recombinant virus encoding CEA and B7.1 IA: none or GM-CSF Route: ID (GM-CSF: SC)	Phase I–II[a] N = 60	Toxicity: minimal OR: 0 (↓ serum CEA: 6/21)	B-cell response: 2/3 T-cell response: 17/21
van der Burg et al 2002 (190)	Recombinant virus encoding p53 IA: none Route: IV	Phase I Escalating doses N = 16	Toxicity: minimal	B-cell response: 10/15 T-cell response: 2/15
Menon et al 2003 (191)	Recombinant virus encoding p53 IA: none Route: IV	Phase I Escalating doses N = 16	Toxicity: 1 grade 3 (fever)	B-cell response: 3/15 T-cell response: 2/15
Rochlitz et al 2003 (192)	Recombinant virus encoding MUC-1 IA: IL-2 Route: IM	Phase I–II[a] Escalating doses N = 13	Toxicity: minimal OR: 1	T-cell response: 5/13
Foon et al 1997 (193)	Anti-idiotype mAb mimicking CEA IA: alum Route: SC	Phase I–II N = 24	Toxicity: minimal OR: 0 OS: similar to standard treatment	B-cell response: 17/23 T-cell response: 5/23
Samonigg et al 1999 (71)	Anti-idiotype mAb mimicking CO17-1A IA: alum Route: SC	Phase II Randomized study (vaccine vs control) N = 42	Toxicity: minimal OR: 0 OS: ns (better in immune responders)	B-cell response: 12/12
Birebent et al 2001 (194)	Anti-idiotype mAb mimicking CO17-1A IA: none or KLH + alum Route: SC	Phase I N = 45	—	B-cell response: 33/45 T-cell response: 8/45
Maxwell et al 2001 (195)	Anti-idiotype mAb mimicking gp72/CD55 IA: alum Route: IM	Phase II Randomized trial (vaccine vs placebo) N = 162	Toxicity: minimal OS: ns (only 50% received complete vaccination)	nr
Conry et al 2002 (196)	DNA plasmid encoding CEA IA: none Route: IM	Phase I Escalating doses N = 17	Toxicity: minimal OR: 0	B-cell response: 0/17 T-cell response: 4/17
Goydos et al 1996 (197)	TAA (MUC-1) IA: BCG Route: ID	Phase I[a] N = 63	Toxicity: minimal OR: 0	T-cell response: 7/22
MacLean et al 1996 (51)	TAA (Sialyl-Tn) IA: KLH + low-dose cyclophosphamide Route: SC	Phase II[a] N = 85	Toxicity: minimal OS: better in patients with higher Ab titers	B-cell response: in most patients
Smith et al 2000 (198)	TAA (gastrin) IA: diphtheria toxoid + alum Route: IM	Phase I–II N = 50	Toxicity: 7 grade 3 (skin) OR: 0	B-cell response: 40/50

continued on following page

Table 3. *(continued)* Colorectal Cancer Vaccines in the Therapeutic Setting: Clinical Trials

Author/year (reference)	Vaccine type	Study design	Clinical outcome	Immunologic findings
Karanikas et al 1997 and 2001 (199, 200)	TAA (MUC-1) IA: low-dose IV cyclophosphamide Route: IM	Phase I[a] N = 42	Toxicity: minimal OR: 0	B-cell response: 60% T-cell response: 28%
Moulton et al 2002 (180)	TAA (beta-hCG) IA: nor-muramyl dipeptide Route: IM	Phase I–II N = 70	Toxicity: minimal OS: better in patients with higher Ab titers	B-cell response: in all patients but with difference in Ab titers
Neidhart et al 2004 (201)	TAA (CO17-1A) IA: none or GM-CSF (SC) Route: SC	Phase I N = 11	Toxicity: minimal	T-cell response: 9/9 (better in GM-CSF group) B-cell response: 8/11
Mine et al 2004 (181)	Peptides (from SART and other TAA) IA: IFA Route: SC	Phase I–II[a] N = 113 HLA restriction: A2, A24	Toxicity: minimal OR: 5 OS: better in immune responders	T-cell response: 16/76 B-cell response: 60/91
Fong et al 2001 (178)	Dendritic cells pulsed with CEA peptide IA: none Route: IV	Phase I Escalating doses N = 12	Toxicity: minimal OR: 2	Tumor regression correlated with T-cell response to vaccination

IA, immunological adjuvant; route, SC (subcutaneous); ID, intradermal; IM, intramuscular; IV, intravenous; BCG, bacillus Calmette-Guerin; IFA, incomplete Freund's adjuvant; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; TAA, tumor associated antigen; OS, overall survival; DFS, disease free survival; ns, not significant; OR, overall (tumor) response (partial + complete responses).
[a]Miscellany of carcinomas including colorectal cancer.

tient HLA types and the sequences of the relevant antigens/epitopes. To overcome this limitation, tumor cells themselves[74] or their messenger RNA (mRNA) content have been used as immunogens.[75] As a further development of the DC-based vaccination strategy, some investigators have proposed the fusion of cancer cells with DC to generate cell hybrids with the characteristics of an APC able to process endogenously provided TAA.[76,77] Despite the strong preclinical evidence supporting the use of DC in humans for antitumor vaccination, the results of clinical trials so far reported are conflicting and regard in most cases melanoma patients.[74,78–82] Perhaps, the complex and time consuming methods required for the preparation of such vaccines have to date restrained investigators from carrying out phase III randomized trials to determine definitely the clinical usefulness of this ASI in oncology. The experience with DC-based vaccines for the treatment of colorectal cancer in humans has just begun[83,84] (Table 3).

Tumor Immune Escape

Despite the evidence that immune effectors can play a significant role in controlling tumor growth in natural conditions or in response to therapeutic manipulation, it is evident that cancer cells can survive their attack as the disease progresses.

Several mechanisms underlying the phenomenon known as tumor immune escape have been proposed[85] (Figure 1). Cancer genetic instability can lead to TAA/HLA down-regulation as well as to the disruption of the TAA-processing/presenting machinery (eg, proteasome, TAP-1), which in turn allows malignant cells to elude the surveillance of immune sentinels.[86,87] The production of postulated immunosuppressive cytokines (eg, TGF-β, IL-10)[88] and the expression of lymphotoxic molecules (ie, FAS ligand)[89] suggest that cancer can actively counteract the immune system reaction. For another example, tumor cells (along with most normal cells) generally lack costimulatory molecules, such as B7.1/CD80 and B7.2/CD86, which are physiologically expressed on professional APC.[90,91] In the absence of costimulation, T cells tend to become anergic.[92] In the nontumor-bearing setting, the absence of B7 molecule expression has been hypothesized to protect normal cells against autoreactivity.[93] Transfection of tumor cells with both isoforms has been used successfully to trigger their immune-mediated rejection of experimental mouse tumors with some inherent immunogenicity.[94]

Nonetheless, the above-mentioned mechanisms cannot explain all cases of immunotherapy failure, and their in vivo relevance has been questioned. For instance, in humans, TAA/HLA expression is not always down-regulated in progressing metastases of patients undergoing

Table 4. Colorectal Cancer Vaccines in the Adjuvant Setting: Clinical Trials

Author/year (reference)	Stage	Vaccine type	Study design	Clinical outcome	Immunological findings
Hoover et al 1993 (202)	II,III	Autologous tumor (whole cell) IA: BCG Route: ID	Phase III Randomized trial (vaccine vs observation) N = 98	Toxicity: some grade 3–4 (skin) OS, DFS: ns (subset analysis: ↑ in patients with colon carcinoma)	T-cell response: 67% of tested patients
Vermorken et al 1999 (203)	II,III	Autologous tumor (whole cell) IA: BCG Route: ID	Phase III Randomized trial (vaccine vs observation) N = 254	Toxicity: 8 cases grade 4 (skin) OS, DFS: ns (subset analysis: ↑ DFS in stage II patients)	T-cell response: 92% of tested patients
Harris et al 2000 (177)	II,III	Autologous tumor (whole cell) IA: BCG Route: ID	Phase III Randomized trial (vaccine vs observation) N = 412	Toxicity: 26% grade 3–4 (skin) OS, DFS: ns	OS, DFS: better in patients with T-cell response
Bohle et al 1990 (35)	II–IV	Virally infected autologous tumor[a] IA: none Route: SC	Phase I N = 16	Toxicity: minimal	T-cell response: 12/16
Ockert et al 1996 (36)	II,III	Virally infected autologous tumor[a] IA: none or BCG Route: ID	Phase I–II N = 57	Toxicity: 9 grade 3–4 (skin; BCG group only) OS: better in NDV group (and better than historical controls)	T-cell response: 21/31
Foon et al 1999 (204)	II–IV	Anti-idiotype mAb mimicking CEA IA: alum or QS-21 Route: SC	Phase I N = 32	Toxicity: minimal	B-cell and T-cell response: 32/32
Ullenhag et al 2003 (205)	I–III	Recombinant virus encoding KSA IA: none or GM-CSF Route: ID + SC	Phase I N = 12	Toxicity: minimal	T-cell response: better in GM-CSF group
Durrant et al 2000 (206)	I–IV	Anti-idiotype mAb mimicking CD55 IA: alum Route: IM	Phase I N = 35	Toxicity: minimal	T-cell response: better in patients with some HLA alleles
Samanci et al 1998 (207)	I–III	TAA (CEA) IA: alum ± GM-CSF Route: SC	Phase I N = 18	Toxicity: minimal	B-cell and T-cell response: better in GM-CSF arm
Mazzaferro et al 2003 (46)	IV	Heat shock protein (gp96) IA: none Route: ID	Phase I–II N = 29	Toxicity: minimal OS, DFS: better in immune responders	T-cell response: 15/29

For abbreviations see Table 3.
[a]Virus: Newcastle disease virus.

vaccination,[95] and FAS-L is not expressed in most melanoma specimens.[96] The immunosuppressive role of some cancer-derived molecules (eg, IL-10) has been revisited and at least in part refuted,[97,98] yet, in animal models, tumor rejection is not observed when B7 molecules are inserted into poorly immunogenic tumors.[99] Nonimmunogenicity is a category in which most, if not all, human tumors would fall; thus a lack of expression of the B7.1 and B7.2 costimulatory molecules is unlikely to be a global explanation for immune escape.

Overall, despite the advancements achieved in tumor immunology, the cascade of molecular events leading to tumor rejection by the immune system is currently unknown, especially in humans. Nevertheless, some recent insights in tumor immunology might help oncologists break the immune tolerance toward malignant cells in the near future.

Future Perspectives in Cancer Vaccine Development

Novel Targets

In the search for the ideal TAA suitable for anticancer vaccination, investigators are looking for antigens with 3 main characteristics: (1) maximal immunogenicity, (2) wide expression by different tumor types, and (3) maximal tumor specificity. In animal models, immunization with xenogeneic homologs of TAA appears to be a promising approach to break immune tolerance to weakly immunogenic self-antigens such as most TAA.[100–102] In humans, similar immunologic responses have been reported,[103] although no data are yet available on tumor response.

Unlike hematologic malignancies that express highly specific TAA (eg, antibody idiotype, translocation-

derived fusion proteins), colorectal carcinoma lacks such unique targets suitable for ASI. However, recent insights in the molecular cascade underlying carcinogenesis are fostering a novel immunotherapeutic strategy. Most TAA so far utilized in the clinical setting play a nonvital role in the metabolism of malignant cells (eg, gp100, MAGE-3, CEA). Accordingly, selection of tumor cells not expressing these TAA can be the only effect of anticancer vaccination.[104] A new class of TAA is represented by antigens involved in cancer development and progression. This is the case of the antiapoptotic protein surviving[105]and the cell survival-related ribonucleoprotein telomerase,[106] which are expressed by most tumors including colorectal carcinoma. Preclinical and clinical results, although preliminary, are encouraging[107–112] and prompt investigators to seek novel molecular targets that are not only immunogenic but also needed for cancer survival (eg, EGF-receptor, VEGF-receptor, c-kit tyrosine kinase).[113–115] Recently, the impact of passive immunotherapy with monoclonal antibodies to EGF-receptor and VEGF-receptor signaling pathways has been demonstrated in colorectal cancer patients.[116] By analogy, some investigators have shown in preclinical models that adaptive immunity can recognize such targets[117] and destroy malignant cells expressing them. For instance, animal immunization against tumor angiogenesis-related antigens (eg, VEGF-receptor, FGF-receptor) can lead to tumor necrosis by causing endothelium disruption and ultimately by shortening the blood supply to cancer masses.[100–102]

Peripheral Tolerance

Low-affinity, autoreactive T cells can avoid negative selection in the thymus. Indeed a low level of autoreactivity is required for positive thymic selection. In normal circumstances, after maturation is complete, these autoreactive T cells are likely to be either ignorant (that is, they simply do not "see" their target epitope) or anergic (defined as a state of induced unresponsiveness). In the first case, they do not have any contact with the antigen. Naturally occurring and vaccine-induced TAA-specific T cells can be negatively regulated by host factors, such as (1) immunosuppressive cytokines (eg, PGE2, TGF-β)[118–120] or soluble factors (eg, ROS, NO) produced by tumor cells or tumor infiltrating macrophages,[121–126] and (2) suppressor cells such as $CD4^+$/$CD25^+$ T cells (T regulatory cells),[127–129] IL-13 secreting NKT cells,[128,130] $CD11b^+$/$Gr\text{-}1^+$ suppressor cells,[131,132] and tolerogenic DC.[133,134] T-cell inhibition by suppressor cells is mediated by soluble factors (eg, TGF-β, IL-10, IL-13, galectin-1) or cell-to-cell contact involving inhibitory receptors (eg, CTLA-4)[135] and ligands (eg, B7-H1).[136] These and other recent insights on the molecular pathways involved in T-cell unresponsiveness to TAA[137–139] are spurring the development of novel strategies aimed at counteracting this phenomenon. For instance, the blockade of CTLA-4[140,141] and B7-H1,[136] the neutralization of TGF-β,[142,143] IL-13,[144] and galectin-1,[145] as well as the inhibition of tolerogenesis-related enzymes (eg, indoleamine 2,3-dioxygenase, IDO),[146,147] improve the antitumor immune reaction and increase tumor rejection rates in several preclinical models. The implementation of these findings in the clinical setting has already begun[148] and holds great promise.

Innate Immunity

As DC and NK-cells efficiently cooperate to start an efficient T-cell response,[149,150] more and more attention is being paid to the appropriate stimulation of the innate immunity arm while eliciting an adaptive immune reaction to a given TAA.

DC represent the most potent APC capable of initiating an effective T-cell response.[151] They can be recruited and activated at the vaccination site by means of immunologic adjuvants (IA), which can be coadministered together with the TAA or can be actively produced by genetically engineered cancer vaccines,[39] depending on their nature (see following paragraph). Alternatively, recent development of techniques for obtaining large numbers of human DC has opened the possibility of using these cells for therapeutic vaccination.[152,153] However, this vaccine preparation remains time consuming and labor intensive, which is probably why no randomized trials have been performed yet in any type of cancer. Furthermore, ex vivo generation of DC requires appropriate activation through molecularly defined triggers such as toll-like receptors (TLR), TNF-α receptor, and CD40-ligand receptor before reinfusion. In fact, nonactivated (immature) or improperly activated DC can cause tolerance instead of immunization.[133]

Natural killer (NK) cells can destroy many tumor cell lines in vitro and are likely to play a critical role in antitumor immunity in vivo, as recently supported by a study in which transgenic mice lacking $NK1.1^+$/$CD3^-$ cells showed an impaired acute tumor rejection.[154] Besides a direct cytotoxic activity against malignant cells, NK cells are believed to play a crucial role in the early phase of adaptive immunity engagement against both infectious agents and tumor cells, thus providing a key link between innate and adaptive immunity.[155] In particular, NK cell-mediated tumor killing might be fundamental in initiating an efficient T-cell response,[149] supposedly by inducing DC maturation and favoring

cross-presentation of tumor antigens from killed tumor cells.[150]

According to these considerations, the protocols for the next generation of antitumor vaccines should probably include both the evaluation of NK cell activity and the appropriate stimulation of these innate immunity cells to understand better the mechanisms of an effective immune response and possibly increase the rate of tumor immune rejection.

Immunologic Adjuvants

Immunologic adjuvants are agents of a very different nature (eg, microbial extracts, cytokines aluminum hydroxide, and so on) that mixed with an antigen enhance the immune response against that antigen after immunization.[156,157] Even though at present only 2 IA are approved for clinical use worldwide (ie, aluminum-based salts and MF59, a squalene-oil/water emulsion), many other substances (eg, incomplete Freund's adjuvant [IFA], saponin QS-21, several cytokines, and so forth) that increase the immunogenicity of vaccines have been tested and proven to be effective in animal models and humans.

Generally, IA are believed to activate innate immunity mediators such as DC and NK cells that ultimately stimulate T-cell function by secreting cytokines and freeing TAA and "danger" signals (eg, HSP, double-stranded DNA) from tumor cells.[158,159] Recently, bacterial DNA has been found to have strong immunostimulatory activity because of the presence of unmethylated CpG oligonucleotides.[160] These bacterial products can be bound by several receptors of the toll-like receptor family, which are expressed by DC and NK cells.[161] Their engagement induces the maturation/activation of innate immunity cells, ultimately favoring the stimulation of the T-cell activity.[162] Although this novel category of IA has been already implemented in the clinical setting,[163] the efficacy of cancer vaccines based on these IA is not yet defined.

Among IA, cytokines are molecules with known effects on innate/adaptive immunity cells, and a growing number of them (eg, IL-2, IL-7, IL-12, GM-CSF, IFN-α, IFN-γ) is being tested as IA for antitumor vaccination in clinical trials. These molecules act as "danger" signals in alerting the immune system; by promoting the differentiation/activation of DC and stimulating NK-cell cytotoxic activity, they are considered powerful natural adjuvants for the development of cancer vaccines.[164–166] The efficacy of some cytokines more recently identified (eg, IL-15, IL-18, IL-21) has been proven in vaccination preclinical models[167–169] but is still to be tested in clinical trials.

Conclusions

Despite the enormous theoretical potential of this type of biotherapy and the promising findings described in animal models, the clinical results achieved with antitumor vaccination are still limited, and no cancer vaccine should be currently proposed to cancer patients outside clinical trials. However, the above-mentioned clinical studies allow 2 considerations.

First, anticancer vaccines represent a nontoxic therapeutic approach with no or little activity on metastatic colorectal carcinoma. In an adjuvant setting, randomized trials so far carried out have failed to demonstrate a statistically significant survival advantage of cancer vaccine over the control arm, although a clinical benefit has been observed in subsets of patients (eg, colon carcinoma, stage II).[170] Accordingly, it has been hypothesized that ASI effectiveness depends on tumor burden. In fact, bulky disease might overwhelm the immune system because of the production of immunosuppressive factors (eg, TGF-β, PGE2, see also Figure 1) by malignant cells and an unfavorable effector (immune calls)/target (cancer cells) ratio, a vaccine-evoked immune response, might successfully control minimal residual disease following surgical resection or chemotherapy.[171–173] Theoretically, after proper "training" (ie, efficient vaccination), adaptive immunity memory cells should prevent tumor recurrence originating from noncycling/dormant micrometastatic cells, which are notoriously resistant to common antineoplastic agents. Ongoing phase II-III clinical trials are testing this hypothesis in patients with colorectal cancer[174,175] as well as other tumor types.[8,176]

Second, it has been repeatedly reported that patients with colorectal cancer who develop an immunologic response to vaccination seem to benefit from the treatment in terms of tumor response or survival.[46,51,71,177–181] Therefore, a major challenge for tumor immunologists would be increasing the efficiency of immunization regimens to increase the proportion of patients mounting an immune response and increase the rate of tumor responses. The complexity of the immune network and the still enigmatic host-tumor interactions make this task at the same time highly challenging and fascinating. Recent insights from preclinical models are giving new impetus to the development of more effective ASI strategies and might revolutionize the way of designing the next generation of cancer vaccines. In particular, the acknowledged synergism between innate and adaptive immune responses and the advancements on the mechanisms underlying immunologic tolerance are leading to a more comprehensive immunotherapeutic approach, taking into consideration the multiple variables that deter-

mine the ultimate outcome of the immune response against malignant cells.

In the future, the implementation of these findings in the clinical setting and the completion/conduction of comparative randomized phase III trials should allow oncologists to define the actual role of immunotherapy in the fight against colorectal cancer.

References

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.
2. Macdonald JS. Adjuvant therapy of colon cancer. CA Cancer J Clin 1999;49:202–219.
3. Cascinu S, Georgoulias V, Kerr D, Maughan T, Labianca R, Ychou M. Colorectal cancer in the adjuvant setting: perspectives on treatment and the role of prognostic factors. Ann Oncol 2003;14(Suppl 2):ii25–29.
4. Coutinho AK, Rocha Lima CM. Metastatic colorectal cancer: systemic treatment in the new millennium. Cancer Control 2003;10:224–238.
5. Midgley R, Kerr D. Immunotherapy for colorectal cancer: a challenge to clinical trial design. Lancet Oncol 2000;1:159–168.
6. d'Entremont TS, Sun W. Recent advances in colorectal cancer therapy. Cancer Biol Ther 2003;2:6–13.
7. Moingeon P. Cancer vaccines. Vaccine 2001;19:1305–1326.
8. Ribas A, Butterfield LH, Glaspy JA, Economou JS. Current developments in cancer vaccines and cellular immunotherapy. J Clin Oncol 2003;21:2415–2432.
9. Mocellin S, Rossi C, Nitti D, Lise M, Marincola F. Dissecting tumor responsiveness to immunotherapy: the experience of peptide-based melanoma vaccines. Biochim Biophys Acta Rev Cancer 2003;1653:61–71.
10. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res 1970;13:1–27.
11. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991–998.
12. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001;2:293–299.
13. Weiss RA. Viruses, cancer and AIDS. FEMS Immunol Med Microbiol 1999;26:227–232.
14. Penn I. Post-transplant malignancy: the role of immunosuppression. Drug Saf 2000;23:101–113.
15. Houbiers JG, van der Burg SH, van de Watering LM, Tollenaar RA, Brand A, van de Velde CJ, Melief CJ. Antibodies against p53 are associated with poor prognosis of colorectal cancer. Br J Cancer 1995;72:637–641.
16. Rosenberg SA. Development of cancer immunotherapies based on identification of the genes encoding cancer regression antigens. J Natl Cancer Inst 1996;88:1635–1644.
17. Boon T, van der Bruggen P. Human tumor antigens recognized by T lymphocytes. J Exp Med 1996;183:725–729.
18. Anichini A, Molla A, Mortarini R, Tragni G, Bersani I, Di Nicola M, Gianni AM, Pilotti S, Dunbar R, Cerundolo V, Parmiani G. An expanded peripheral T cell population to a cytotoxic T lymphocyte (CTL)-defined, melanocyte-specific antigen in metastatic melanoma patients impacts on generation of peptide-specific CTLs but does not overcome tumor escape from immune surveillance in metastatic lesions. J Exp Med 1999;190:651–667.
19. Nagorsen D, Keilholz U, Rivoltini L. Schmittel A, Letsch A, Asemissen AM, Berger G, Buhr HJ, Theil E, Scheibenbogen C. Natural T-cell response against MHC class I epitopes of epithelial cell adhesion molecule, her-2/neu, and carcinoembryonic antigen in patients with colorectal cancer. Cancer Res 2000;60:4850–4854.
20. Mukherji B, Chakraborty NG, Sivanandham M. T-cell clones that react against autologous human tumors. Immunol Rev 1990; 116:33–62.
21. Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 1996;77:1303–1310.
22. von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, Snijdewint FG, Kok A, Van Kamp GJ, Paul MA, Van Diest PJ, Meijer S, Hilgers J. Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. J Clin Oncol 2000;18:574–583.
23. Livingston PO, Wong GY, Adluri S, Tao Y, Padavan M, Parente R, Hanlon C, Calves MJ, Helling F, Ritter G. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J Clin Oncol 1994;12:1036–1044.
24. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE. Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. Oncol Rep 2003;10: 309–313.
25. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol 1997;182:318–324.
26. Michael-Robinson JM, Biemer-Huttmann A, Purdie DM, Walsh MD, Simms LA, Biden KG, Young JP, Leggett BA, Jass JR, Radford-Smith GL. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut 2001;48:360–366.
27. Burnett AK, Goldstone AH, Stevens RM, Hann IM, Rees JK, Gray RG, Wheatley K. Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 trial. UK Medical Research Council Adult and Children's Leukaemia Working Parties. Lancet 1998;351:700–708.
28. Roush KS, Hillyer CD. Donor lymphocyte infusion therapy. Transfus Med Rev 2002;16:161–176.
29. Campbell P, Marcus R. Monoclonal antibody therapy for lymphoma. Blood Rev 2003;17:143–152.
30. Klein G. The strange road to the tumor-specific transplantation antigens (TSTAs). Cancer Immun 2001;1:6.
31. Rosenberg SA. Progress in human tumour immunology and immunotherapy. Nature 2001;411:380–384.
32. Lewis JD, Reilly BD, Bright RK. Tumor-associated antigens: from discovery to immunity. Int Rev Immunol 2003;22:81–112.
33. Ward S, Casey D, Labarthe MC, Whelan M, Dalgleish A, Pandha H, Todryk S. Immunotherapeutic potential of whole tumour cells. Cancer Immunol Immunother 2002;51:351–357.
34. Schadendorf D, Paschen A, Sun Y. Autologous, allogeneic tumor cells or genetically engineered cells as cancer vaccine against melanoma. Immunol Lett 2000;74:67–74.
35. Bohle W, Schlag P, Liebrich W, Hohenberger P, Manasterski M, Moller P, Schirrmacher V. Postoperative active specific immunization in colorectal cancer patients with virus-modified autologous tumor cell vaccine. First clinical results with tumor-cell vaccines modified with live but avirulent Newcastle disease virus. Cancer 1990;66:1517–1523.
36. Ockert D, Schirrmacher V, Beck N, Stoelben E, Ahlert T, Flechtenmacher J, Hagmuller E, Buchcik R, Nagel M, Saeger HD. Newcastle disease virus-infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. Clin Cancer Res 1996;2:21–28.
37. Nemunaitis J, Sterman D, Jablons D, Smith JW II, Fox B, Maples P, Hamilton S, Borellini F, Lin A, Morali S, Hege K. Granulocyte-

macrophage colony-stimulating factor gene-modified autologous tumor vaccines in non-small-cell lung cancer. J Natl Cancer Inst 2004;96:326–331.
38. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemoe KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ. Novel allogeneic granulocyte-macrophage colony-stimulating factor- secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol 2001;19:145–156.
39. Dranoff G. GM-CSF-secreting melanoma vaccines. Oncogene 2003;22:3188–3192.
40. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 1997;278:117–120.
41. Srivastava PK, Menoret A, Basu S, Binder RJ, McQuade KL. Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world. Immunity 1998;8:657–665.
42. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811.
43. Binder RJ, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. Nat Immunol 2000;1:151–155.
44. Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 1993;178: 1391–1396.
45. Janetzki S, Palla D, Rosenhauer V, Lochs H, Lewis JJ, Srivastava PK. Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. Int J Cancer 2000;88:232–238.
46. Mazzaferro V, Coppa J, Carrabba MG, Rivoltini L, Schiavo M, Regalia E, Mariani L, Camerini T, Marchiano A, Andreola S, Camerini R, Corsi M, Lewis JJ, Srivastava PK, Parmiani G. Vaccination with autologous tumor-derived heat-shock protein gp96 after liver resection for metastatic colorectal cancer. Clin Cancer Res 2003;9:3235–3245.
47. Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. Cancer Res 1996;56: 5309–5318.
48. Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta 1999; 1473:21–34.
49. Ko BK, Kawano K, Murray JL, Disis ML, Efferson CL, Kuerer HM, Peoples GE, Ioannides CG. Clinical studies of vaccines targeting breast cancer. Clin Cancer Res 2003;9:3222–3234.
50. Wolchok JD, Livingston PO. Vaccines for melanoma: translating basic immunology into new therapies. Lancet Oncol 2001;2: 205–211.
51. MacLean GD, Reddish MA, Koganty RR, Longenecker BM. Antibodies against mucin-associated sialyl-Tn epitopes correlate with survival of metastatic adenocarcinoma patients undergoing active specific immunotherapy with synthetic STn vaccine. J Immunother Emphasis Tumor Immunol 1996;19:59–68.
52. Parmiani G, Castelli C, Dalerba P, Mortarini R, Rivoltini L, Marincola FM, Anichini A. Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? J Natl Cancer Inst 2002;94:805–818.
53. Renkvist N, Castelli C, Robbins PF, Parmiani G. A listing of human tumor antigens recognized by T cells. Cancer Immunol Immunother 2001;50:3–15.
54. Wang RF. Human tumor antigens: implications for cancer vaccine development. J Mol Med 1999;77:640–655.
55. Machiels JP, van Baren N, Marchand M. Peptide-based cancer vaccines. Semin Oncol 2002;29:494–502.
56. Saeterdal I, Bjorheim J, Lislerud K, Gjertsen MK, Bukholm IK, Olsen OC, Nesland JM, Eriksen JA, Moller M, Lindblom A, Gaudernack G. Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. Proc Natl Acad Sci U S A 2001;98:13255–13260.
57. Maccalli C, Li YF, El-Gamil M, Rosenberg SA, Robbins PF. Identification of a colorectal tumor-associated antigen (COA-1) recognized by CD4(+) T lymphocytes. Cancer Res 2003;63:6735–6743.
58. Schmitz J, Reali E, Hodge JW, Patel A, Davis G, Schlom J, Greiner JW. Identification of an interferon-gamma-inducible carcinoembryonic antigen (CEA) CD8(+) T-cell epitope, which mediates tumor killing in CEA transgenic mice. Cancer Res 2002; 62:5058–5064.
59. Restifo NP. The new vaccines: building viruses that elicit antitumor immunity. Curr Opin Immunol 1996;8:658–663.
60. Bonnet MC, Tartaglia J, Verdier F, Kourilsky P, Lindberg A, Klein M, Moingeon P. Recombinant viruses as a tool for therapeutic vaccination against human cancers. Immunol Lett 2000;74:11–25.
61. Rosenberg SA, Zhai Y, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Seipp CA, Einhorn JH, Roberts B, White DE. Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. J Natl Cancer Inst 1998;90:1894–1900.
62. Zajac P, Oertli D, Marti W, Adamina M, Bolli M, Guller U, Noppen C, Padovan E, Schultz-Thater E, Heberer M, Spagnoli G. Phase I/II clinical trial of a nonreplicative vaccinia virus expressing multiple HLA-A0201-restricted tumor-associated epitopes and costimulatory molecules in metastatic melanoma patients. Hum Gene Ther 2003;14:1497–1510.
63. Shedlock DJ, Weiner DB. DNA vaccination: antigen presentation and the induction of immunity. J Leukoc Biol 2000;68:793–806.
64. Rosenberg SA, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, White DE. Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. Hum Gene Ther 2003;14:709–714.
65. Timmerman JM, Singh G, Hermanson G, Hobart P, Czerwinski DK, Taidi B, Rajapaksa R, Caspar CB, Van Beckhoven A, Levy R. Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. Cancer Res 2002;62: 5845–5852.
66. Mincheff M, Tchakarov S, Zoubak S, Loukinov D, Botev C, Altankova I, Georgiev G, Petrov S, Meryman HT. Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a phase I/II clinical trial. Eur Urol 2000;38:208–217.
67. Perelson AS. Immune network theory. Immunol Rev 1989;110: 5–36.
68. Bhattacharya-Chatterjee M, Chatterjee SK, Foon KA. The anti-idiotype vaccines for immunotherapy. Curr Opin Mol Ther 2001; 3:63–69.
69. McCarthy H, Ottensmeier CH, Hamblin TJ, Stevenson FK. Anti-idiotype vaccines. Br J Haematol 2003;123:770–781.
70. Lutzky J, Gonzalez-Angulo AM, Orzano JA. Antibody-based vaccines for the treatment of melanoma. Semin Oncol 2002;29: 462–470.
71. Samonigg H, Wilders-Truschnig M, Kuss I, Plot R, Stoger H, Schmid M, Bauernhofer T, Tiran A, Pieber T, Havelec L, Loibner H. A double-blind randomized-phase II trial comparing immunization with antiidiotype goat antibody vaccine SCV 106 versus unspecific goat antibodies in patients with metastatic colorectal cancer. J Immunother 1999;22:481–488.
72. Bakker AB, Marland G, de Boer AJ, Huijbens RJ, Danen EH, Adema GJ, Figdor CG. Generation of antimelanoma cytotoxic T lymphocytes from healthy donors after presentation of melano-

ma-associated antigen-derived epitopes by dendritic cells in vitro. Cancer Res 1995;55:5330–5334.
73. Abdel-Wahab Z, DeMatos P, Hester D, Dong XD, Seigler HF. Human dendritic cells, pulsed with either melanoma tumor cell lysates or the gp100 peptide(280–288), induce pairs of T-cell cultures with similar phenotype and lytic activity. Cell Immunol 1998;186:63–74.
74. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998; 4:328–332.
75. Mitchell DA, Nair SK. RNA-transfected dendritic cells in cancer immunotherapy. J Clin Invest 2000;106:1065–1069.
76. Parkhurst MR, DePan C, Riley JP, Rosenberg SA, Shu S. Hybrids of dendritic cells and tumor cells generated by electrofusion simultaneously present immunodominant epitopes from multiple human tumor-associated antigens in the context of MHC class I and class II molecules. J Immunol 2003;170:5317–5325.
77. Haenssle HA, Krause SW, Emmert S, Zutt M, Kretschmer L, Schmidberger H, Andreesen R, Soruri A. Hybrid cell vaccination in metastatic melanoma: clinical and immunologic results of a phase I/II study. J Immunother 2004;27:147–155.
78. Thurner B, Haendle I, Roder C, Dieckmann D, Keikavoussi P, Jonuleit H, Bender A, Maczek C, Schreiner D, von den Driesch P, Brocker EB, Steinman RM, Enk A, Kampgen E, Schuler G. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 1999;190:1669–1678.
79. Panelli MC, Wunderlich J, Jeffries J, Wang E, Mixon A, Rosenberg SA, Marincola FM. Phase 1 study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma-associated antigens MART-1 and gp100. J Immunother 2000;23:487–498.
80. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, Pineiro L, Steinman R, Fay J. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 2001;61: 6451–6458.
81. Schuler-Thurner B, Schultz ES, Berger TG, Weinlich G, Ebner S, Woerl P, Bender A, Feuerstein B, Fritsch PO, Romani N, Schuler G. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide- loaded monocyte-derived dendritic cells. J Exp Med 2002;195:1279–1288.
82. Whiteside TL, Zhao Y, Tsukishiro T, Elder EM, Gooding W, Baar J. Enzyme-linked immunospot, cytokine flow cytometry, and tetramers in the detection of T-cell responses to a dendritic cell-based multipeptide vaccine in patients with melanoma. Clin Cancer Res 2003;9:641–649.
83. Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E, Lyerly HK. Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. Ann Surg 2002;235:540–549.
84. Morse MA, Nair SK, Mosca PJ, Hobeika AC, Clay TM, Deng Y, Boczkowski D, Proia A, Neidzwiecki D, Clavien PA, Hurwitz HI, Schlom J, Gilboa E, Lyerly HK. Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA. Cancer Invest 2003;21:341–349.
85. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol 2000;74:181–273.
86. Seliger B, Wollscheid U, Momburg F, Blankenstein T, Huber C. Characterization of the major histocompatibility complex class I deficiencies in B16 melanoma cells. Cancer Res 2001;61:1095–1099.
87. Yang T, McNally BA, Ferrone S, Liu Y, Zheng P. A single-nucleotide deletion leads to rapid degradation of TAP-1 mRNA in a melanoma cell line. J Biol Chem 2003;278:15291–15296.
88. Mocellin S, Wang E, Marincola FM. Cytokines and immune response in the tumor microenvironment. J Immunother 2001; 24:392–407.
89. Walker PR, Saas P, Dietrich PY. Role of Fas ligand (CD95L) in immune escape: the tumor cell strikes back. J Immunol 1997; 158:4521–4524.
90. Townsend SE, Allison JP. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. Science 1993;259:368–370.
91. Schultze J, Nadler LM, Gribben JG. B7-mediated costimulation and the immune response. Blood Rev 1996;10:111–127.
92. Lenschow D, Walunas T, Bluestone J. CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996;14:233–258.
93. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol 2001;19:225–252.
94. La Motte RN, Sharpe AH, Bluestone JA, Mokyr MB. Host B7-1 and B7-2 costimulatory molecules contribute to the eradication of B7-1-transfected P815 tumor cells via a CD8+ T cell-dependent mechanism. J Immunol 1999;162:4817–4823.
95. Ohnmacht GA, Wang E, Mocellin S, Abati A, Filie A, Fetsch P, Riker AI, Kammula US, Rosenberg SA, Marincola FM. Short-term kinetics of tumor antigen expression in response to vaccination. J Immunol 2001;167:1809–1820.
96. Chappell DB, Zaks TZ, Rosenberg SA, Restifo NP. Human melanoma cells do not express Fas (Apo-1/CD95) ligand. Cancer Res 1999;59:59–62.
97. Mocellin S, Marincola F, Riccardo Rossi C, Nitti D, Lise M. The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle. Cytokine Growth Factor Rev 2004;15:61–76.
98. Mocellin S, Panelli MC, Wang E, Nagorsen D, Marincola FM. The dual role of IL-10. Trends Immunol 2003;24:36–43.
99. Chen L, McGowan P, Ashe S, Johnston J, Li Y, Hellstrom I, Hellstrom KE. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. J Exp Med 1994;179:523–532.
100. Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G, Eliceiri BP, Reisfeld RA. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat Med 2002;8:1369–1375.
101. He QM, Wei YQ, Tian L, Zhao X, Su JM, Yang L, Lu Y, Kan B, Lou YY, Huang MJ, Xiao F, Liu JY, Hu B, Luo F, Jiang Y, Wen YJ, Deng HX, Li J, Niu T, Yang JL. Inhibition of tumor growth with a vaccine based on xenogeneic homologous fibroblast growth factor receptor-1 in mice. J Biol Chem 2003;278:21831–21836.
102. Wei YQ, Huang MJ, Yang L, Zhao X, Tian L, Lu Y, Shu JM, Lu CJ, Niu T, Kang B, Mao YQ, Liu F, Wen YJ, Lei S, Luo F, Zhou LQ, Peng F, Jiang Y, Liu JY, Zhou H, Wang QR, He QM, Xiao F, Lou YY, Xie XJ, Li Q, Wu Y, Ding ZY, Hu B, Hu M, Zhang W. Immunogene therapy of tumors with vaccine based on Xenopus homologous vascular endothelial growth factor as a model antigen. Proc Natl Acad Sci U S A 2001;98:11545–11550.
103. Fong L, Brockstedt D, Benike C, Breen JK, Strang G, Ruegg CL, Engleman EG. Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. J Immunol 2001;167:7150–7156.
104. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol 2002; 3:999–1005.

105. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3: 917–921.
106. Buys CH. Telomeres, telomerase, and cancer. N Engl J Med 2000;342:1282–1283.
107. Pisarev V, Yu B, Salup R, Sherman S, Altieri DC, Gabrilovich DI. Full-length dominant-negative survivin for cancer immunotherapy. Clin Cancer Res 2003;9:6523–6533.
108. Zeis M, Siegel S, Wagner A, Schmitz M, Marget M, Kuhl-Burmeister R, Adamzik I, Kabelitz D, Dreger P, Schmitz N, Heiser A. Generation of cytotoxic responses in mice and human individuals against hematological malignancies using survivin-RNA-transfected dendritic cells. J Immunol 2003;170:5391–5397.
109. Siegel S, Wagner A, Schmitz N, Zeis M. Induction of antitumour immunity using survivin peptide-pulsed dendritic cells in a murine lymphoma model. Br J Haematol 2003;122:911–914.
110. Frolkis M, Fischer MB, Wang Z, Lebkowski JS, Chiu CP, Majumdar AS. Dendritic cells reconstituted with human telomerase gene induce potent cytotoxic T-cell response against different types of tumors. Cancer Gene Ther 2003;10:239–249.
111. Hernandez J, Garcia-Pons F, Lone YC, Firat H, Schmidt JD, Langlade-Demoyen P, Zanetti M. Identification of a human telomerase reverse transcriptase peptide of low affinity for HLA A2.1 that induces cytotoxic T lymphocytes and mediates lysis of tumor cells. Proc Natl Acad Sci U S A 2002;99:12275–12280.
112. Nair SK, Heiser A, Boczkowski D, Majumdar A, Naoe M, Lebkowski JS, Vieweg J, Gilboa E. Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. Nat Med 2000;6:1011–1017.
113. Ellis LM, Hoff PM. Targeting the epidermal growth factor receptor: an important incremental step in the battle against colorectal cancer. J Clin Oncol 2004;22:1177–1179.
114. Manley PW, Bold G, Bruggen J, Fendrich G, Furet P, Mestan J, Schnell C, Stolz B, Meyer T, Meyhack B, Stark W, Strauss A, Wood J. Advances in the structural biology, design and clinical development of VEGF-R kinase inhibitors for the treatment of angiogenesis. Biochim Biophys Acta 2004;1697:17–27.
115. Attoub S, Rivat C, Rodrigues S, Van Bocxlaer S, Bedin M, Bruyneel E, Louvet C, Kornprobst M, Andre T, Mareel M, Mester J, Gespach C. The c-kit tyrosine kinase inhibitor STI571 for colorectal cancer therapy. Cancer Res 2002;62:4879–4883.
116. Veronese ML, O'Dwyer PJ. Monoclonal antibodies in the treatment of colorectal cancer. Eur J Cancer 2004;40:1292–1301.
117. Shomura H, Shichijo S, Matsueda S, Kawakami T, Sato Y, Todo S, Itoh K. Identification of epidermal growth factor receptor-derived peptides immunogenic for HLA-A2(+) cancer patients. Br J Cancer 2004;90:1563–1571.
118. Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003;21: 425–456.
119. Gorelik L, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells. Nat Med 2001;7:1118–1122.
120. Terabe M, Matsui S, Park JM, Mamura M, Noben-Trauth N, Donaldson DD, Chen W, Wahl SM, Ledbetter S, Pratt B, Letterio JJ, Paul WE, Berzofsky JA. Transforming growth factor-beta production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. J Exp Med 2003;198:1741–1752.
121. Saio M, Radoja S, Marino M, Frey A. Tumor-infiltrating macrophages induce apoptosis in activated CD8+ T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide. J Immunol 2001;167: 5583–5593.
122. Moochhala S, Rajnakova A. Role of nitric oxide in cancer biology. Free Radic Res 1999;31:671–679.
123. Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001;2:907–916.
124. Mellqvist UH, Hansson M, Brune M, Dahlgren C, Hermodsson S, Hellstrand K. Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. Blood 2000; 96:1961–1968.
125. Herberman RB. Cancer immunotherapy with natural killer cells. Semin Oncol 2002;29:27–30.
126. Betten A, Dahlgren C, Mellqvist UH, Hermodsson S, Hellstrand K. Oxygen radical-induced natural killer cell dysfunction: role of myeloperoxidase and regulation by serotonin. J Leukoc Biol 2004;75:1111–1115.
127. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531–562.
128. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001;2:293–299.
129. Somasundaram R, Jacob L, Swoboda R, Caputo L, Song H, Basak S, Monos D, Peritt D, Marincola F, Cai D, Birebent B, Bloome E, Kim J, Berencsi K, Mastrangelo M, Herlyn D. Inhibition of cytolytic T lymphocyte proliferation by autologous CD4+/CD25+ regulatory T cells in a colorectal carcinoma patient is mediated by transforming growth factor-beta. Cancer Res 2002; 62:5267–5272.
130. Ahlers JD, Belyakov IM, Terabe M, Koka R, Donaldson DD, Thomas EK, Berzofsky JA. A push-pull approach to maximize vaccine efficacy: abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L. Proc Natl Acad Sci U S A 2002; 99:13020–13025.
131. Apolloni E, Bronte V, Mazzoni A, Serafini P, Cabrelle A, Segal DM, Young HA, Zanovello P. Immortalized myeloid suppressor cells trigger apoptosis in antigen-activated T lymphocytes. J Immunol 2000;165:6723–6730.
132. Bronte V, Apolloni E, Cabrelle A, Ronca R, Serafini P, Zamboni P, Restifo NP, Zanovello P. Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. Blood 2000;96:3838–3846.
133. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685–711.
134. Mehrotra S, Stevens R, Zengou R, Chakraborty NG, Butterfield LH, Economou JS, Dorsky DI, Mukherji B. Regulation of melanoma epitope-specific cytolytic T lymphocyte response by immature and activated dendritic cells, in vitro. Cancer Res 2003; 63:5607–5614.
135. Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol 2001;1:220–228.
136. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, Krzysiek R, Knutson KL, Daniel B, Zimmermann MC, David O, Burow M, Gordon A, Dhurandhar N, Myers L, Berggren R, Hemminki A, Alvarez RD, Emilie D, Curiel DT, Chen L, Zou W. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med 2003;9:562–567.
137. Tzachanis D, Freeman GJ, Hirano N, van Puijenbroek AA, Delfs MW, Berezovskaya A, Nadler LM, Boussiotis VA. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. Nat Immunol 2001;2:1174–1182.
138. Soares L, Seroogy C, Skrenta H, Anandasabapathy N, Lovelace P, Chung CD, Engleman E, Fathman CG. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. Nat Immunol 2004; 5:45–54.
139. Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, De Jong LA, Vyth-Dreese FA, Dellemijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA, Restifo NP. Tumor regression

and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. J Exp Med 2003;198:569–580.
140. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 1999;190:355–366.
141. Davila E, Kennedy R, Celis E. Generation of antitumor immunity by cytotoxic T lymphocyte epitope peptide vaccination, CpG-oligodeoxynucleotide adjuvant, and CTLA-4 blockade. Cancer Res 2003;63:3281–3288.
142. Kobie JJ, Wu RS, Kurt RA, Lou S, Adelman MK, Whitesell LJ, Ramanathapuram LV, Arteaga CL, Akporiaye ET. Transforming growth factor β inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines. Cancer Res 2003;63:1860–1864.
143. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, Dalton W, Jove R, Pardoll D, Yu H. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 2004;10:48–54.
144. Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, Carbone DP, Paul WE, Berzofsky JA. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. Nat Immunol 2000;1:515–520.
145. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, Mordoh J, Fainboim L, Podhajcer OL, Rabinovich GA. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. Cancer Cell 2004;5:241–251.
146. Munn DH, Mellor AL. IDO and tolerance to tumors. Trends Mol Med 2004;10:15–18.
147. Friberg M, Jennings R, Alsarraj M, Dessureault S, Cantor A, Extermann M, Mellor AL, Munn DH, Antonia SJ. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int J Cancer 2002;101:151–155.
148. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, MacRae S, Willman A, Padera R, Jaklitsch MT, Shankar S, Chen TC, Korman A, Allison JP, Dranoff G. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A 2003;100:4712–4717.
149. Kelly JM, Darcy PK, Markby JL, Godfrey DI, Takeda K, Yagita H, Smyth MJ. Induction of tumor-specific T cell memory by NK cell-mediated tumor rejection. Nat Immunol 2002;3:83–90.
150. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. Nat Rev Immunol 2002;2:957–964.
151. Figdor CG, De Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: mapping the way. Nat Med 2004;10:475–480.
152. Jefford M, Maraskovsky E, Cebon J, Davis ID. The use of dendritic cells in cancer therapy. Lancet Oncol 2001;2:343–353.
153. Fong L, Engleman EG. Dendritic cells in cancer immunotherapy. Annu Rev Immunol 2000;18:245–273.
154. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci U S A 2000;97:2731–2736.
155. Diefenbach A, Raulet DH. The innate immune response to tumors and its role in the induction of T-cell immunity. Immunol Rev 2002;188:9–21.
156. Berzofsky JA, Ahlers JD, Belyakov IM. Strategies for designing and optimizing new generation vaccines. Nat Rev Immunol 2001;1:209–219.
157. Schijns VE. Immunological concepts of vaccine adjuvant activity. Curr Opin Immunol 2000;12:456–463.
158. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol 2001;13:114–119.
159. Dredge K, Marriott JB, Todryk SM, Dalgleish AG. Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. Cancer Immunol Immunother 2002;51:521–531.
160. Medzhitov R. CpG DNA: security code for host defense. Nat Immunol 2001;2:15–16.
161. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. Nat Immunol 2001;2:947–950.
162. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 2002;20:709–760.
163. Wittig B, Marten A, Dorbic T, Weineck S, Min H, Niemitz S, Trojaneck B, Flieger D, Kruopis S, Albers A, Loffel J, Neubauer A, Albers P, Muller S, Sauerbruch T, Bieber T, Huhn D, Schmidt-Wolf IG. Therapeutic vaccination against metastatic carcinoma by expression-modulated and immunomodified autologous tumor cells: a first clinical phase I/II trial. Hum Gene Ther 2001;12:267–278.
164. Forni G, Boggio K. Cytokine gene-engineered vaccines. Curr Opin Mol Ther 1999;1:34–38.
165. Belardelli F, Ferrantini M. Cytokines as a link between innate and adaptive antitumor immunity. Trends Immunol 2002;23:201–208.
166. Parmiani G, Rivoltini L, Andreola G, Carrabba M. Cytokines in cancer therapy. Immunol Lett 2000;74:41–44.
167. Ma HL, Whitters MJ, Konz RF, Senices M, Young DA, Grusby MJ, Collins M, Dunussi-Joannopoulos K. IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-γ. J Immunol 2003;171:608–615.
168. Tatsumi T, Gambotto A, Robbins PD, Storkus WJ. Interleukin 18 gene transfer expands the repertoire of antitumor Th1-type immunity elicited by dendritic cell-based vaccines in association with enhanced therapeutic efficacy. Cancer Res 2002;62:5853–5858.
169. Meazza R, Lollini PL, Nanni P, De Giovanni C, Gaggero A, Comes A, Cilli M, Di Carlo E, Ferrini S, Musiani P. Gene transfer of a secretable form of IL-15 in murine adenocarcinoma cells: effects on tumorigenicity, metastatic potential and immune response. Int J Cancer 2000;87:574–581.
170. Hanna MG, Hoover HC, Vermorken JB, Harris JE, Pinedo HM. Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. Vaccine 2001;19:2576–2582.
171. Kerr DJ, Midgley RS. Immunotherapy for colorectal cancer: potential application in an adjuvant setting. Semin Oncol 2000;27:132–137.
172. Mocellin S, Rossi CR, Lise M, Marincola FM. Adjuvant immunotherapy for solid tumors: from promise to clinical application. Cancer Immunol Immunother 2002;51:583–595.
173. Ochsenbein AF, Sierro S, Odermatt B, Pericin M, Karrer U, Hermans J, Hemmi S, Hengartner H, Zinkernagel RM. Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. Nature 2001;411:1058–1064.
174. Motl SE. Technology evaluation: Canvaxin, John Wayne Cancer Institute/CancerVax. Curr Opin Mol Ther 2004;6:104–111.
175. Durrant LG, Spendlove I. Cancer vaccines entering phase III clinical trials. Expert Opin Emerg Drugs 2003;8:489–500.
176. Bitton RJ. Cancer vaccines: a critical review on clinical impact. Curr Opin Mol Ther 2004;6:17–26.
177. Harris JE, Ryan L, Hoover HC Jr, Stuart RK, Oken MM, Benson AB 3rd, Mansour E, Haller DG, Manola J, Hanna MG Jr. Adjuvant active specific immunotherapy for stage II and III colon cancer

with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. J Clin Oncol 2000;18:148–157.
178. Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci U S A 2001;98:8809–8814.
179. Habal N, Gupta RK, Bilchik AJ, Yee R, Leopoldo Z, Ye W, Elashoff RM, Morton DL. CancerVax, an allogeneic tumor cell vaccine, induces specific humoral and cellular immune responses in advanced colon cancer. Ann Surg Oncol 2001;8: 389–401.
180. Moulton HM, Yoshihara PH, Mason DH, Iversen PL, Triozzi PL. Active specific immunotherapy with a β-human chorionic gonadotropin peptide vaccine in patients with metastatic colorectal cancer: antibody response is associated with improved survival. Clin Cancer Res 2002;8:2044–2051.
181. Mine T, Sato Y, Noguchi M, Sasatomi T, Gouhara R, Tsuda N, Tanaka S, Shomura H, Katagiri K, Rikimaru T, Shichijo S, Kamura T, Hashimoto T, Shirouzu K, Yamada A, Todo S, Itoh K, Yamana H. Humoral responses to peptides correlate with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing, peptide-specific cellular responses. Clin Cancer Res 2004;10:929–937.
182. Sobol RE, Shawler DL, Carson C, Van Beveren C, Mercola D, Fakhrai H, Garrett MA, Barone R, Goldfarb P, Bartholomew RM, Brostoff S, Carlo DJ, Royston I, Gold DP. Interleukin 2 gene therapy of colorectal carcinoma with autologous irradiated tumor cells and genetically engineered fibroblasts: a phase I study. Clin Cancer Res 1999;5:2359–2365.
183. Marshall JL, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ, Schlom J. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. J Clin Oncol 1999; 17:332–337.
184. Zhu MZ, Marshall J, Cole D, Schlom J, Tsang KY. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. Clin Cancer Res 2000;6:24–33.
185. Conry RM, Allen KO, Lee S, Moore SE, Shaw DR, LoBuglio AF. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. Clin Cancer Res 2000;6: 34–41.
186. Conry RM, Khazaeli MB, Saleh MN, Allen KO, Barlow DL, Moore SE, Craig D, Arani RB, Schlom J, LoBuglio AF. Phase I trial of a recombinant vaccinia virus encoding carcinoembryonic antigen in metastatic adenocarcinoma: comparison of intradermal versus subcutaneous administration. Clin Cancer Res 1999;5: 2330–2337.
187. Marshall JL, Hoyer RJ, Toomey MA, Faraguna K, Chang P, Richmond E, Pedicano JE, Gehan E, Peck RA, Arlen P, Tsang KY, Schlom J. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. J Clin Oncol 2000;18:3964–3973.
188. Horig H, Lee DS, Conkright W, Divito J, Hasson H, LaMare M, Rivera A, Park D, Tine J, Guito K, Tsang KW, Schlom J, Kaufman HL. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. Cancer Immunol Immunother 2000;49:504–514.
189. von Mehren M, Arlen P, Gulley J, Rogatko A, Cooper HS, Meropol NJ, Alpaugh RK, Davey M, McLaughlin S, Beard MT, Tsang KY, Schlom J, Weiner LM. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. Clin Cancer Res 2001;7: 1181–1191.
190. van der Burg SH, Menon AG, Redeker A, Bonnet MC, Drijfhout JW, Tollenaar RA, van de Velde CJ, Moingeon P, Kuppen PJ, Offringa R, Melief CJ. Induction of p53-specific immune responses in colorectal cancer patients receiving a recombinant ALVAC-p53 candidate vaccine. Clin Cancer Res 2002;8:1019–1027.
191. Menon AG, Kuppen PJ, van der Burg SH, Offringa R, Bonnet MC, Harinck BI, Tollenaar RA, Redeker A, Putter H, Moingeon P, Morreau H, Melief CJ, van de Velde CJ. Safety of intravenous administration of a canarypox virus encoding the human wild-type p53 gene in colorectal cancer patients. Cancer Gene Ther 2003;10:509–517.
192. Rochlitz C, Figlin R, Squiban P, Salzberg M, Pless M, Herrmann R, Tartour E, Zhao Y, Bizouarne N, Baudin M, Acres B. Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. J Gene Med 2003;5: 690–699.
193. Foon KA, John WJ, Chakraborty M, Sherratt A, Garrison J, Flett M, Bhattacharya-Chatterjee M. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. Clin Cancer Res 1997;3:1267–1276.
194. Birebent B, Koido T, Mitchell E, Li W, Somasundaram R, Purev E, Hoey D, Mastrangelo M, Maguire H, Harris DT, Nair S, Cai E, Herlyn D. Anti-idiotypic antibody (ab2) vaccines: coupling of Ab2 BR3E4 to KLH increases humoral and/or cellular immune responses in animals and colorectal cancer patients. J Cancer Res Clin Oncol 2001;127(Suppl 2):R27–R33.
195. Maxwell-Armstrong CA, Durrant LG, Buckley TJ, Scholefield JH, Robins RA, Fielding K, Monson JR, Guillou P, Calvert H, Carmichael J, Hardcastle JD. Randomized double-blind phase II survival study comparing immunization with the anti-idiotypic monoclonal antibody 105AD7 against placebo in advanced colorectal cancer. Br J Cancer 2001;84:1443–1446.
196. Conry RM, Curiel DT, Strong TV, Moore SE, Allen KO, Barlow DL, Shaw DR, LoBuglio AF. Safety and immunogenicity of a DNA vaccine encoding carcinoembryonic antigen and hepatitis B surface antigen in colorectal carcinoma patients. Clin Cancer Res 2002;8:2782–2787.
197. Goydos JS, Elder E, Whiteside TL, Finn OJ, Lotze MT. A phase I trial of a synthetic mucin peptide vaccine. Induction of specific immune reactivity in patients with adenocarcinoma. J Surg Res 1996;63:298–304.
198. Smith AM, Justin T, Michaeli D, Watson SA. Phase I/II study of G17-DT, an anti-gastrin immunogen, in advanced colorectal cancer. Clin Cancer Res 2000;6:4719–4724.
199. Karanikas V, Hwang LA, Pearson J, Ong CS, Apostolopoulos V, Vaughan H, Xing PX, Jamieson G, Pietersz G, Tait B, Broadbent R, Thynne G, McKenzie IF. Antibody and T-cell responses of patients with adenocarcinoma immunized with mannan-MUC1 fusion protein. J Clin Invest 1997;100:2783–2792.
200. Karanikas V, Thynne G, Mitchell P, Ong CS, Gunawardana D, Blum R, Pearson J, Lodding J, Pietersz G, Broadbent R, Tait B, McKenzie IF. Mannan Mucin-1 Peptide immunization: influence of cyclophosphamide and the route of injection. J Immunother 2001;24:172–183.
201. Neidhart J, Allen KO, Barlow DL, Carpenter M, Shaw DR, Triozzi PL, Conry RM. Immunization of colorectal cancer patients with recombinant baculovirus-derived KSA (Ep-CAM) formulated with monophosphoryl lipid A in liposomal emulsion, with and without granulocyte-macrophage colony-stimulating factor. Vaccine 2004;22:773–780.
202. Hoover HC Jr, Brandhorst JS, Peters LC, Surdyke MG, Takeshita Y, Madariaga J, Muenz LR, Hanna MG Jr. Adjuvant active spe-

cific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial. J Clin Oncol 1993;11:390–399.
203. Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG Jr, Pinedo HM. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 1999;353:345–350.
204. Foon KA, John WJ, Chakraborty M, Das R, Teitelbaum A, Garrison J, Kashala O, Chatterjee SK, Bhattacharya-Chatterjee M. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. J Clin Oncol 1999; 17:2889–2895.
205. Ullenhag GJ, Frodin JE, Mosolits S, Kiaii S, Hassan M, Bonnet MC, Moingeon P, Mellstedt H, Rabbani H. Immunization of colorectal carcinoma patients with a recombinant canarypox virus expressing the tumor antigen Ep-CAM/KSA (ALVAC-KSA) and granulocyte macrophage colony-stimulating factor induced a tumor-specific cellular immune response. Clin Cancer Res 2003;9:2447–2456.
206. Durrant LG, Maxwell-Armstrong C, Buckley D, Amin S, Robins RA, Carmichael J, Scholefield JH. A neoadjuvant clinical trial in colorectal cancer patients of the human anti-idiotypic antibody 105AD7, which mimics CD55. Clin Cancer Res 2000;6:422–430.
207. Samanci A, Yi Q, Fagerberg J, Strigard K, Smith G, Ruden U, Wahren B, Mellstedt H. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. Cancer Immunol Immunother 1998;47:131–142.

Received April 21, 2004. Accepted July 1, 2004.

Address requests for reprints to: Simone Mocellin, MD, Dipartimento di Scienze, Oncologiche e Chirurgiche, Sezione di Clinica Chirurgica, 6 piano Policlinico, Università di Padova, Via Giustiniani, 2 35128 Padova, Italy. e-mail: mocellins@hotmail.com; fax: (39) 049 651891.

We apologize to authors whose work was not cited due to length considerations.