Therapeutic Vaccines for Colorectal Cancer
A Review of Clinical Data

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Abstract

A number of cancer vaccine strategies for the treatment of colorectal cancer have entered clinical trials. Whole tumor cell vaccines have been developed from both patients' autologous tumor cells as well as established allogeneic tumor cell lines. A vaccine consisting of autologous tumor cells along with bacillus Calmette-Guerin (BCG) has shown a potential clinical benefit in patients with stage II colon cancer. Other approaches using autologous tumor cells have involved transfection of primary tumor cells with cytokine genes. Allogeneic tumor cell vaccines have also been modified to express cytokine genes.

Vectors have been studied extensively as a means of vaccine strategy. One tumor-associated antigen (TAA) that has been extensively studied in viral vector vaccines is carcinoembryonic antigen (CEA). A recombinant vaccinia virus containing the CEA transgene (rV-CEA) has been shown to elicit CEA-specific immune responses in advanced carcinoma patients. However, patients receiving multiple vaccinations had limited increases in CEA-specific responses by the third vaccination. This problem may be overcome by the use of non-replicating poxviruses, which have been shown in clinical trials to be safe and to elicit CEA-specific responses. However, recent clinical studies have shown that the optimal use of poxviruses is to prime with vaccinia, followed by boosts with avipox vectors. A recent randomized clinical trial showed that patients primed with rV-CEA and boosted with avipox-CEA had greater immune responses compared with patients receiving three 1-monthly avipox-CEA vaccinations followed by an rV-CEA vaccination. Furthermore, a statistically significant survival advantage was noted in the prime/boost arm. Ongoing studies are now incorporating the genes for costimulatory molecules along with TAA in these vectors.

Another vaccine strategy involving TAA that is currently in clinical trials for colorectal cancer is the peptide vaccine. Dendritic cells (DCs) are considered to be the most potent antigen-presenting cell, thus providing an attractive modality for cancer vaccines. In addition to using DCs for peptide-based vaccines, a number of other

strategies, including transfection with messenger RNA, have produced specific T-cell responses in clinical trials. In addition, several clinical trials using murine anti-idiotype antibodies as vaccines for patients with advanced colorectal cancer have shown both immunologic responses as well as clinical responses.

As our understanding of immunology has evolved, new strategies for therapeutic vaccines have been brought forth from the laboratory and are now undergoing clinical evaluation for a wide variety of human carcinomas. Colorectal cancer is the third leading cause of death in both men and women in the US, with approximately 135 000 new cases diagnosed and 57 000 related deaths each year.[1] The 5-year survival rate for patients diagnosed with early localized colon cancer is approximately 90%.[1] However, this rate decreases to 65% when the cancer spreads to the lymph nodes, and to <10% after the development of distant metastasis, despite current treatment strategies including surgery, chemotherapy, and radiation.

The role for adjuvant chemotherapy thus far has been limited to lymph node positive disease with a minor effect on survival.[2] Furthermore, despite newer chemotherapy regimens for metastatic disease, these agents are toxic and have a minor effect on overall survival.[2] The recent data from Saltz et al.[2] using a regimen consisting of irinotecan, fluorouracil, and leucovorin produced only a modest increase in median survival of 15 months for patients with metastatic colon cancer compared with fluorouracil/ leucovorin. Furthermore, an increase in toxicity compared with fluorouracil/leucovorin, most notably diarrhea, was observed in these patients. New approaches to the treatment of colorectal cancer are being developed, and a number of molecular-targeted agents are currently being investigated. This article reviews the cancer vaccines currently being used to treat colorectal cancer.

Tumor antigens are proteins expressed by malignant cells that can stimulate immune responses against them. These antigens may represent surface proteins specific to the tumors, or they may be expressed on normal cells, but at much lower quantities than on the tumors. In the 1950s, animal models were established demonstrating that the immune system can specifically prevent the growth of malignant tumors. It was shown that the rejection of these tumors was mediated mainly by tumor-specific cytotoxic T lymphocytes (CTLs). CTLs have receptors that are responsible for the recognition of highly specific peptide fragments of the protein, referred to as epitopes. These epitopes are presented on the surface of antigen-presenting cells (APCs) coupled to the major histocompatibility complex (MHC) molecules (figure 1), also called human leukocyte antigens (HLA) in humans, which are divided into two classes. The class I MHC complex is expressed on most nucleated cells and presents the antigen as short peptides, generally around 8–11 amino acids in length, to CD8+ T cells. The class II MHC

molecules are expressed predominantly on specialized APCs and present longer peptides, usually between 11 and 15 amino acids in length, to CD4+ T cells.

T-cell activation requires two signals: signal one, which is antigen specific, and signal two, which is mediated through a costimulatory molecule on the APC that interacts with its ligand on the T-cell receptor (TCR). These costimulatory molecules send signals that help regulate the functional responses of the T cells with which they interact. Certain costimulatory molecules (see section 3.1) such as intercellular adhesion molecule (ICAM)-1 can increase the strength of adhesion between the CTL and APC, further enhancing the activation of the T cell (figure 2). The most potent APCs capable of presenting an antigen to naive T cells are dendritic cells (DCs). In order to initiate a T-cell response, antigenic peptides must be recognized via the TCR of circulating T cells. Tumors often lack MHC molecules and usually lack costimulatory signals. DCs have high levels of expression of both these markers, and therefore have been considered good candidates for vaccine development. DCs are found in most tissues in an immature state where they are able to capture and process antigens efficiently, which can then be presented by both class I and class II MHC molecules to activate CD8+ and CD4+ T cells, respectively.

Another mechanism for downregulation of the immune response, in addition to lack of costimulation, is an antigen-induced block, termed immunologic tolerance. This may result from the interaction of an antigen with a lymphocyte, which, instead of becoming activated, is rendered unresponsive. Tolerance is a fundamental property of the immune system that protects against autoimmunity to self-antigens. The question that arises is how a tumor vaccine can overcome tolerance if the vaccine incorporates a self-antigen expressed on the tumor cell. The 'danger' model has theorized that the source of antigen is less important than how it may be presented to the immune system.[3] If a vaccine strategy is developed that elicits a high enough level of immune stimulation, then the antigen can be seen as 'dangerous' and immune responses will be stimulated.[4] Some tumor cells may escape being recog-
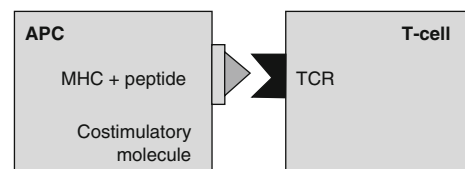

Fig. 1. Antigen presentation to T cells. **APC** = antigen-presenting cell; **MHC** = major histocompatability complex; **TCR** = T-cell receptor.
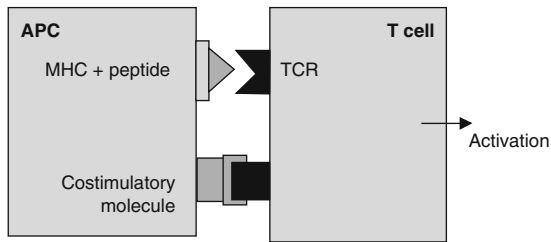
Fig. 2. Role of costimulation for the activation of T cells. **APC** = antigen-presenting cell; **MHC** = major histocompatability complex; **TCR** = T-cell receptor.

nized by the immune system by not producing a 'danger' signal that adequately reaches a high enough threshold.[5]

A number of vaccination strategies have now been developed based on our current understanding of tumor immunology and how these vaccines can be successfully administered in the clinic. These included:

- whole tumor vaccines: (i) autologous; and (ii) allogeneic
- vector-encoded tumor-associated antigens (TAAs): (i) prime and boost strategy; and (ii) costimulatory molecules
- anti-idiotypes
- dendritic cell vaccines.

Many of these vaccines have been analyzed in experimental models and have been incorporated into ongoing or completed clinical trials in colorectal cancer. Each of these vaccine types has its advantages and disadvantages, and it may eventually be determined that some of these modalities are most beneficial when used in tandem. However, there are a number of considerations when interpreting the success of various vaccine strategies. With regard to study design, factors such as patient selection play an important role. Patients with extensive metastatic disease who have undergone numerous chemotherapy cycles may not respond to a particular vaccine strategy. However, this same strategy may be successful when treating patients with less disease burden or those who have not been heavily pretreated with drugs that may suppress the immune system. In the adjuvant setting, however, it may take years to determine whether a vaccine strategy is successful. Thus, the role of immunologic assays as intermediate endpoints to assess a response to a vaccine becomes highly significant. One must be cautious that the assay chosen is reliable and that the target is validated as a surrogate marker.

1. Whole Tumor Cell Vaccines

Whole cell vaccines can be separated into two categories: (i) autologous (using a patient's own tumor cells for vaccination); and (ii) allogeneic (using tumor cells from other patients, usually from established tumor cell lines, for vaccination).

A possible advantage for the use of an autologous whole tumor cell vaccine approach is that tumor antigens specific to the patient undergoing therapy would be present in the vaccine preparation. However, preparing this type of vaccine can be a laborious process. At the time of surgery, an adequate number of tumor cells must be obtained to develop the vaccine and the cells must be prepared in a similar manner for each patient. In addition, to obtain an adequate number of cells, other factors may contribute to the difficulty of preparing the vaccine; these include the percentage of tumor cells and the degree of tumor necrosis. Furthermore, a central laboratory is frequently required to prepare these vaccines from tumors.

1.1 Autologous Vaccines

A number of clinical trials with autologous tumor vaccines have been completed in patients with colorectal cancer (see table I). Intracel Corporation (Frederick, MD, USA) has sponsored studies in patients with stage II and stage III colon cancer given autologous tumor cells mixed with bacillus Calmette-Guerin (BCG) through intradermal vaccinations in an adjuvant setting.[6-8] BCG vaccines have been given to billions of people since 1921 for the prevention of tuberculosis; these vaccines have been administered more than any other vaccine in the world.[9] Indeed, BCG has been used extensively in the immunotherapy of human cancer.[10] In clinical trials in colorectal cancer, patients were randomized to either a control arm or a vaccine-treatment arm after surgical resection of the primary tumor and stratification by disease location and stage. Patients who were randomized to vaccination received 10^7 irradiated tumor cells with 10^7 BCG organisms per week for 2 weeks, followed by a boost of 10^7 irradiated tumor cells in the third week.

The initial studies using this approach were performed at Johns Hopkins Hospital (Baltimore, MD, USA) in the early 1980s.[7] Ninety-eight patients were randomized post-operatively to either surgery alone or surgery with vaccine. No serious adverse effects occurred in the vaccinated patients. All patients developed superficial skin ulcerations at the site of the BCG vaccine. Patients were stratified by tumor stage and as having either rectal or colon cancer. In the colon cancer patients (Dukes B and C) at 6.5 years follow-up, 20 of 24 patients remained alive compared with 12 of 23 patients in the control group. These results were statistically significant ($p = 0.02$). In addition, there was a statistically significant difference in the number of recurrences in the colon cancer patients who received the vaccine as compared with the control arm ($p = 0.03$). No significant differences were seen in the rectal cancer patients who received the vaccine as compared with the control group.[7]

Table I. Selected completed vaccine clinical trials in colorectal cancer

Vaccine	Phase	Patient population	Clinical response	Immune response	Reference
Whole cell vaccines					
Autologous tumor cell + BCG	II	n = 98, adjuvant colorectal	+ Colon cancer (6.5y follow-up) vaccine = 20/24 allive; control = 12/23 alive − Rectal cancer: no difference between vaccine and control	NA	7
Autologous tumor cell + BCG	III	n = 412, adjuvant colon	No difference between vaccine and control	NA	8
Autologous tumor cell + BCG	III	n = 254, adjuvant colon	+ Stage 2 (5.3y follow-up) vaccine = 25 recurrences; control = 40 recurrences − Stage 3: no difference between vaccine and control	NA	6
Allogeneic tumor cell + IL-7 + GM-CSF	I	n = 10, progressive metastatic solid tumors	Partial responses in 3 of 10 pts	+ CTL	11
Allogeneic polyvalent (CancerVax®) + BCG	II	n = 27, stage IV colorectal	Median OS = 21.9mo	+ DTH	12
TAA pox vectors					
Avipox-CEA	I	n = 18, CEA expressing tumors	ND	+ CTL	13
TAA prime and boost					
Vaccinia-CEA(V), ALVAC-CEA(A); VAAA vs AAAV	II	n = 18, metastatic CEA expressing solid tumors	Survival at 24mo follow up: VAAA = 5 of 9; AAAV = 0 of 9	ELISPOT-A2 positive VAAA = 5 of 5; AAAV = 2 of 5	14
TAA-costimulatory molecules					
ALVAC-CEA/B7-1	I	n = 18, CEA expressing tumors	ND	+ ELISPOT	15
ALVAC-CEA/B7-1 ± GM-CSF	I/II	n = 39, metastatic CEA expressing solid tumors	Disease stabilization 37%	ELISPOT-A2 positive in 10 of 12 pts	16
Peptide-based vaccines					
DC/CEA	I	n = 21, CEA expressing tumors	ND	ND	17
CEA agonist epitope loaded DC	I	n = 12, metastatic CEA expressing tumors	2 pts: complete response 2 pts: stable disease 1 pt mixed response	+ CEA-specific T-cell responses	18
Anti-idiotype vaccines					
Murine 17-1A [mimics GA773-2 antigen]	I	n = 6, colorectal cancer post surgery	ND	+ T-cell responses	19

Table I. Contd

Vaccine	Phase	Patient population	Clinical response	Immune response	Reference
105AD7 [mimics gp72 glycoprotein (CD55)] with allogeneic tumor cells	I	n = 13, metastatic colorectal	ND	+ Lymphocyte proliferation	20
105AD7 [mimics gp72 glycoprotein (CD55)] with allogeneic tumor cells	II	n = 162, metastatic colorectal	No clinical response	ND	21
CeaVac® [mimics tumor restricted CEA epitope]	II	n = 23, advanced colorectal cancer	Median survival: 11.3mo (no clinical response)	5 pts had specific T-cell responses to CEA	22
DC vaccines					
mRNA-encoded CEA DC (A2 negative) vs DC-pulsed CEA(CAP-1) peptide (A2 positive)	I/II	n = 17, CEA-expressing tumors	ND	Both groups + CEA-specific T cells	15

AAAV = avipox-CEA followed by recombinant vaccinia-CEA; **ALVAC** = avipox vector; **BCG** = bacillus Calmette Guerin; **CAP** = CEA peptide; **CEA** = carcinoembryonic antigen; **CTL** = cytotoxic T lymphocyte; **DC** = dendritic cell; **DTH** = delayed type hypersensitivity; **ELISPOT** = enzyme-linked immunospot; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **IL** = interleukin; **mRNA** = messenger RNA; **NA** = not applicable; **ND** = no data available; **OS** = overall survival; **pts** = patients; **TAA** = tumor-associated antigen; **VAAA** = recombinant vaccinia-CEA followed by boosting with avipox-CEA; + indicates positive study; − indicates negative study.

The results of this study led to a large phase III trial performed by the Eastern Cooperative Oncology Group (ECOG).[8] In this study, 412 patients with stage II and stage III colon cancer were enrolled to receive the same vaccine regimen as those patients entered in the previously mentioned study.[7] This study was different, however, in that it was conducted at multiple clinical centers and each site was allowed to perform its own manufacturing of the vaccine, based on common specifications provided by Intracel Corporation. At a 7.6-year median follow-up, there were no statistically significant differences in the clinical outcome between the vaccine and the control group. A delayed type hypersensitivity (DTH) test was performed by intradermal injection of the antigen used in the vaccination. The characteristic response of the DTH test occurs over a 24- to 48-hour period after vaccination. This test can be performed after subsequent vaccinations to determine whether a patient continues to respond to vaccination. In a subset analysis, those patients who demonstrated a significant DTH response had a statistically significant improvement in outcome. This ECOG study concluded that not having a common manufacturing laboratory to make the vaccine may have had an impact on the overall results of the study.[8]

A prospective randomized controlled trial was performed in Europe in which 254 colorectal cancer patients were enrolled.[6] This trial differed from an earlier ECOG study in that the investigators used a central facility to prepare the vaccination. Furthermore, patients in the present study received an additional boosting vaccination at 3 months. At 5.3-year median follow-up, there were 40 cancer recurrences in the control group and 25 in the vaccine group. The vaccine showed a statistically significant clinical benefit in the subset of patients with surgically resected stage II colon cancer, but not stage III colon cancer.[6]

Another approach using allogeneic tumor cell vaccines, is the transfection of primary tumor cells with cytokine genes (see table I). A number of investigators have studied the approach of modifying tumor cells by transfecting cytokine genes including interleukin (IL)-7 and granulocyte-macrophage colony-stimulating factor (GM-CSF).[11,23-28] It was postulated that GM-CSF activates DCs, leading to enhanced antigen presentation of the tumor-derived antigens. In addition, the interleukin leads to direct stimulation and proliferation of immunologic antitumoral effector cells.[27] Fourteen primary cell cultures were established from 45 patients with malignant melanoma. These cell cultures were then transfected with the gene encoding for human IL-7. This resulted in the production of biologically active IL-7 production by the tumor cells. No differences in the phenotype were observed between the IL-7 transfected cells compared with those cells that were not transfected. The expression of MHC class I and II, ICAM-1, as well as melanoma-associated antigen was unchanged following transfection. The IL-7 transfected cell cultures possessed a higher sensitivity to immunologic effector cells compared with nontransfected cells.[28] These results led to a recent study by Wittig et al.,[11] in which ten patients with progressive metastatic carcinoma, including those with colorectal cancer, were vaccinated using this approach. Autologous tumor cells were transfected with what the authors termed a minimalistic, immunogenically defined, gene expression construct (MIDGE) for overexpression of IL-7 and GM-CSF. The transfection of the cytokines was performed *ex vivo* using a ballistic approach that launches seven particle carrier membranes simultaneously, in such a way as to evenly distribute ballistic microparticles for transfection in to more than 1.5×10^7 target cells in one shot, combined with a magnetic separation of transfected cells. All ten patients enrolled had progressive disease

prior to enrollment. Patients received four subcutaneous injections of at least 1×10^6 of their cytokine modified tumor cells. The cytotoxic effects of treatment on patient-derived peripheral blood lymphocytes (PBLs) were monitored during the treatment, using autologous tumor cells as targets. PBLs were obtained from patients before, during, and after treatment, and were found to increase significantly during treatment (p = 0.01). One complete, one partial, and one mixed response with progression of abdominal metastases and regression of lung metastases were observed.[11]

1.2 Allogeneic Vaccines

Allogeneic whole tumor cell vaccines usually consist of one or more tumor cell lines and are relatively easy to prepare compared with autologous vaccines. This vaccine approach may also contain several tumor-specific antigens (TSAs) and/or TAAs. Moreover, the cell lines used in the preparations can be infected with vectors that express cytokine genes (such as GM-CSF)[29] or costimulatory molecule genes (such as B7-1)[30] to enhance the immunogenicity of the tumor cell. Allogeneic whole tumor cell vaccines modified to secrete GM-CSF have been used in a phase I trial in patients with pancreatic cancer.[31] Evidence of prolonged disease-free survival has been seen in some patients for at least 25 months after diagnosis. Increases in immune responses were also observed as measured by DTH.[31] Clinical studies are ongoing in patients with gastrointestinal carcinomas using either peptides or viral vector vaccines using the target antigens p53,[32] MAGE,[33] or the ras oncogene.[34,35]

An allogeneic tumor cell vaccine termed CancerVax® [1] was developed at the John Wayne Cancer Institute (Santa Monica, CA, USA) [see table I]. This is a polyvalent vaccine consisting of three live human melanoma cell lines chosen for their wide range of TAAs and MHC antigens. The immune response to CancerVax® has been shown to cross-react with nonvaccine tumor cells expressing some of the same immunogenic TAAs, such as gangliosides (GD2, GM2, GD3, and GM3), glycoproteins (fetal antigen, TA90), and/or proteins (MAGE-1, MAGE-3).[36] The use of this vaccine in melanoma patients has produced both immunologic as well as clinical responses.[37-42] IgM responses to a specific TAA, TA90, have been shown to predict survival in patients receiving CancerVax® adjuvant immunotherapy for stage III and stage IV melanoma.[43-45] Furthermore, the TA90 antigen has been shown to be expressed on a wide variety of solid tumors, including colorectal cancer. In a recently published study, 27 patients with stage IV colorectal adenocarcinoma were treated with CancerVax® (at weeks 0, 2, 4, 6, 8, and every fourth week successively for 1 year) co-administered with BCG (for the first 2 weeks of vaccine treatment). There was a significant (p = 0.0001) increase in anti-TA90 IgG and IgM titers and in DTH response to vaccine cells. The median overall survival was 21.9 months for the entire group. The authors correlated improved survival to immune responses.[12]

A number of clinical trials using whole cell vaccines are now actively accruing patients (see table II). Intracel Corporation is sponsoring a multicenter phase I/II study of adjuvant autologous tumor cell vaccination in patients with completely resected stage II or III colon cancer. After total surgical resection, patients receive autologous tumor cell vaccine intradermally once weekly for three vaccinations, with the first two vaccinations also containing BCG. Patients with stage III disease receive standard adjuvant chemotherapy with fluorouracil and leucovorin following vaccination. All patients receive a fourth vaccination about 6 months after surgical resection. Another phase II study is assessing autologous tumor cells (or, if unavailable, allogeneic) incubated with interferon, then irradiated and reinfused by dorsal pedal cannulation. Cyclophosphamide is given once 3 days prior to infusion, and GM-CSF daily for 9 days after infusion.

A disadvantage in the use of allogeneic vaccines prepared from whole tumor cells is that they do not constitutively express costimulatory molecules. Moreover, it is possible that since allogeneic cells are used, alloimmunity to non-self components may develop. However, this was not the case in early clinical trials, which include studies using oncolysates (i.e. tumor cell preparations that had been infected with vaccinia virus and then lysed in an effort to enhance their immunogenicity).[46]

Thus, in summary, the major advantage of using whole tumor cell vaccines is that several TSAs or TAAs, some of which have yet to be defined, may be present in the vaccine preparation. However, this approach has major disadvantages as well. The actual amount of any TAA or TSA in the vaccine composition may be diluted by normal cellular components of the tumor. The vast majority of solid tumors, including colorectal cancer, do not express costimulatory molecules, which are responsible for the activation of naive T cells to levels capable of inducing therapeutic responses. Finally, it is unclear which immunogenic proteins or epitopes are present in the vaccine, thus making it difficult to measure immune responses and to amplify those specific responses.

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1 The use of trade names is for product identification purposes only and does not imply endorsement.

Table II. Ongoing vaccine clinical trials in colorectal cancer

Vaccine	Phase	Patient population	Sponsor
Whole cell vaccines			
Autologous tumor cell + BCG	I/II	n = 30, adjuvant colon	Intracel Corporation
Autologous or allogeneic tumor cell + IFN + GM-CSF given with cyclophosphamide	II	n = 20–40, high-risk adjuvant/metastatic solid tumors	NCI
Peptide-based vaccines			
MAGE-12	I	n = 112, MAGE-12 positive solid tumor, HLA-Cw*0702	NCI
Ras peptide pulsed DC ± IL-2	I/II	n = 49, locally advanced or metastatic colorectal cancer, HLA-A2-1 positive	NCI, Vanderbilt
Ras peptide + Detox™ adjuvant with IL-2 + GM-CSF[a]	II	n = 42–90, metastatic ras positive cancer	NCI
Anti-idiotype vaccines			
105AD7 [mimics gp72 glycoprotein (CD55)] with allogeneic tumor cells	I/II	n = 45, metastatic colorectal cancer	Onyvax, Ltd.
3H1 [mimics CEA] and 11D10 [mimics high molecular weight human milk fat globule antigen]	II	n = 63, adjuvant vaccine in patients with minimal metastatic colon cancer after complete hepatic resection	CALGB
Vector-based vaccines			
Fowlpox-CEA with costimulatory molecules such as B7-1 and GM-CSF	I	n = 48, advanced or metastatic CEA-expressing cancer[b]	NCI, Fox Chase Cancer Center
ALVAC-CEA-B7-1 + irinotecan + tetanus toxoid (3 arms with chemo, chemo + vaccine or chemo, vaccine and tetanus toxoid)	II	n = 90, HLA-A2-positive patients with metastatic colorectal cancer	NCI, Aventis
rF-CEA(6D)/TRICOM alone, or in combination with rV-CEA(6D)/TRICOM and GM-CSF	I	n = 55, metastatic CEA-expressing tumor	NCI, Georgetown University Hospital
DC infected rF-CEA/TRICOM	I	n = 18, metastatic CEA-expressing tumor	NCI, Duke University Medical Center

a Khleif S, unpublished data.

b Not specifically colorectal.

ALVAC = avipox vector; **BCG** = bacillus Calmette Guerin; **CALGB** = Cancer and Leukemia Group B; **CEA** = carcinoembryonic antigen; **chemo** = chemotherapy; **DC** = dendritic cell; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **HLA** = human leukocyte antigen; **IFN** = interferon; **IL** = interleukin; **NCI** = National Cancer Institute; **rF** = recombinant fowlpox; **rV** = recombinant vaccinnia; **TRICOM** = triad of costimulatory molecules.

2. Tumor-Associated Antigen (TAA) Strategies

2.1 Early Strategies

In the early 1970s, using colony inhibition assays, Hellstrom et al.[47] demonstrated that lymphocytes from colorectal cancer patients would prevent colony formation by autologous as well as homologous tumor cells. This work suggested the presence of cross-reactive TAAs in colorectal cancer as well as the presence of individual specific TSA. Elias et al.[48] further demonstrated specific cell-mediated immunity in patients with Dukes B and C colon cancer as measured by an autologous leukocyte migration inhibition assay (MIA). The principle behind this assay is that the migration of peripheral human blood leukocytes is inhibited when

leukocytes are exposed *in vitro* to an antigen to which they have previously been sensitized. Immunity was observed prior to surgery and in the immediate postoperative period, but disappeared shortly afterwards. Furthermore, immunity could not be demonstrated by MIA against colon cancer TAA, when lymphocytes were tested from patients with Dukes D colon cancer, patients with other cancers, or from healthy volunteers.[48] This suggested that these antigens were specific for the tumor; however, more advanced disease may suppress the immune responses.

A phase I study by Hollinshead et al.[49] explored the use of TAA therapy in patients with adenocarcinoma of the colon. The TAA was derived from the patient's colon cancer, which was obtained post-operatively. Cell membranes were separated from the tumor, and soluble membrane proteins were removed by

Sephadex B-200 chromatography. Semipurified TAAs were identified by *in vitro* and *in vivo* testing in colon cancers and controls for cell-mediated immunoreactivities, and colon TAAs were identified in fetal intestine cell membranes and on tumor cell membranes. Using discontinuous, gradient gel electrophoresis, both colon TAA and carcinoembryonic antigen (CEA) were separated and eluted and cross-compared, with TAA shown to be separate from CEA. The TAA consisted of two stable polypeptides with approximate molecular weights of 72kD and 88kD, respectively, as compared with the 180kD molecular weight of CEA. For the phase I trial, TAAs were prepared from the tumors of 70 selected hepatitis-free donors; they were tested for standard potency after sterility, and general safety tests were performed. The final product was dispersed in 200, 300, and 500μg TAA protein concentrations per 0.2mL. Vaccines were prepared by mixing TAA 0.2mL with complete Freund's adjuvant (CFA) 0.2mL.

Twenty-two patients received vaccinations with follow-up ranging from 3 months to 3 years (median 21 months). Seven patients had Dukes B2, seven had Dukes C, and eight had Dukes D stage colon cancer. All patients underwent surgical resection, with six of eight patients with Dukes D clinically disease free at the time of vaccination. Each patient received three 1-monthly vaccinations. Two patients received 200μg doses of TAA, two received 500μg doses of TAA, and 18 received 300μg doses. All patients developed skin ulcers at the vaccination site, all of which healed and formed a scar. Two patients developed fever and chills during the first day post-vaccination, which resolved with acetaminophen. There were no clinical or biochemical manifestations of any type of systemic toxicity. Patients were tested for DTH reactions to skin tests with 50μg protein TAA alone. A reaction of >5mm in duration was considered a positive test. One patient had a negative response to serial skin testing; three patients showed a positive response, but this decreased during months 6, 9, and 10, respectively. The remaining patients showed steady positive responses; in general, the greatest responses were noted between 5 and 6 months post-therapy. MIA was also performed in patients with Dukes B and C stages. A patient receiving the 200μg protein TAA did not show as pronounced a reaction as patients receiving 300μg or 500μg doses. Furthermore, at 4–5 months post-vaccination, there was a switch from ± to positive reactions in all patients. This concurred with the DTH results, which demonstrated that strongest responses occurred approximately 5 months post-vaccination. Although this was a small phase I trial indicating the safety of the vaccine, at the median follow-up of 21 months, 82% of the patients were still alive, and 59% of the patients were without evidence of disease.

2.2 Vector-Encoded TAA

Vectors have been studied extensively as a means of vaccine delivery and a number of review articles have been published describing the use of these vectors for cancer vaccines.[50-55] Strategies incorporating the use of both viral and bacterial vectors are now in use in the clinic. Each of these vectors has its own advantages and disadvantages. The advantages to using a vector-based vaccine are: (i) the entire tumor antigen gene or parts of that gene can be inserted; (ii) multiple genes (including genes for costimulatory molecules and cytokines) can be inserted into some types of vectors; (iii) the relative cost of this type of production is low compared with the preparation and purification of proteins or whole tumor cell vaccines; and (iv) many vectors have the ability to infect 'professional' APC so that the antigens they express can be processed.

Viral vectors including poxvirus (vaccinia and avipox) and adenovirus have been extensively used as delivery vehicles for TAA vaccines. Vaccinia virus, which was derived from a benign cutaneous disease in cows, has been administered to more than 1 billion people and is responsible for the worldwide eradication of smallpox.[56] To date, as many as seven transgenes have been expressed in one vaccinia virus vector. Another major advantage is that proteins expressed in vaccinia virus tend to be more immunogenic than the native protein, which is most likely to be attributable to the inflammatory responses triggered against highly immunogenic vaccinia virus proteins. Other advantages of poxviruses, such as vaccinia virus, modified vaccinia Ankera (MVA) and avipox viruses include: (i) a wide host range; (ii) stable recombinants; (iii) accurate replication; and (iv) efficient post-translational processing of the inserted gene.

Adenovirus as a vector for the development of recombinant vaccines is attractive because its viral genome can be altered to accept foreign genes that are stably integrated. To improve the quality of recombinant adenovectors, endogenous viral DNA sequences are typically deleted from replication-competent regions, which results in an attenuated form of the virus. Recombinant adenoviruses have been widely used in gene therapy protocols, and a number of vaccine protocols for the induction of immune responses have already been carried out.[57-60]

One TAA that has been extensively studied in viral vector vaccines is CEA, a 180 000-glycoprotein member of the immunoglobulin super-gene family. Several functions have been attributed to CEA, including homotypic and heterotypic intercellular adhesion. It has also been reported that CEA can cooperate in cellular transformation with several proto-oncogenes, such as BCL2 and c-Myc. CEA is overexpressed on >90% of colorectal cancers and other gastrointestinal tumors, as well as other tumors.

A disadvantage to using CEA as a target in immunologic-based therapies is that CEA is a normal protein expressed in the body; thus it is likely that tolerance will exist to this protein. Vaccination with a live recombinant vaccinia virus can help to overcome the problem of tolerance. It allows for the expression of foreign antigens encoded by a transgene directly in various cells of the host, including professional APCs. This method of vaccination enables antigen processing and presentation of antigenic peptides along with host histocompatibility antigens and other necessary co-factors found on the APCs. One of the main advantages of using recombinant vaccinia viruses to develop cancer vaccines, as demonstrated by numerous investigators, is that when a gene for a protein is inserted into recombinant vaccinia and used as an immunogen, the recombinant protein is much more immunogenic than the use of that protein with adjuvant.[61-63] This concept was exemplified by Kass et al.,[62] who showed that two injections of CEA protein in adjuvant generated little, if any, immune response to CEA in a CEA transgenic (CEA-Tg) mouse. This would be expected because the host is seeing CEA as a 'self' antigen. However, when the recombinant vaccinia virus containing the CEA transgene (designated rV-CEA) is administered one or two times, a strong CEA-specific T-cell response is elicited.[62] The likely reason for this is that a strong inflammatory response is generated by the host against vaccinia proteins. In turn, this inflammatory process apparently leads to an environment of cytokine production and T-cell proliferation that may further amplify the immune response to the transgene antigen. This process favors induction of both cell-mediated and humoral responses to the transgene antigen. Because vaccinia actively replicates in the host, it can present high levels of transgene antigen to the immune system over a period of approximately 1 week, substantially increasing the potential for immune stimulation. The host-immune response to the vaccinia vector then eliminates the virus.

Several clinical trials have demonstrated the immunogenicity of CEA. Tsang et al.[64] and Cole et al.[65] demonstrated that administration of rV-CEA to advanced carcinoma patients can lead to the induction of CEA-specific immune responses.[64,65] The CTL lines generated were shown to be capable of lysing CEA peptide-pulsed targets and CEA-expressing tumor cells. These studies also demonstrated that as long as ≥10^7 plaque-forming units (pfu) of rV-CEA were administered, a good 'take' (erythema and pustule formation) was observed in all patients who had previously received a childhood smallpox vaccination. These studies also demonstrated the safety of administering a live recombinant vector in patients with advanced cancer. They also showed that while rV-CEA could be administered once, and at most twice, by the third vaccination there was little or no 'take'; these findings correlated with the lack of increases in CEA-specific responses by

the third vaccination.[64,65] Furthermore, no significant antineoplastic effect was observed. Possible reasons for the lack of clinical efficacy in these trials were: (i) the prior exposure to the vaccinia virus in all patients treated, which led to the development of an anti-vaccinia immune response; (ii) the advanced state of the tumors in patients; and (iii) the potentially decreased immune status of patients attributable to prior chemotherapy regimens.

To overcome this problem, non-replicating poxviruses were examined in clinical trials. The poxvirus family contains MVA, a derivative of vaccinia virus.[50] This is a virus that has been passaged in chick embryo fibroblasts over 350 times to decrease the virulence of the virus, and thus has the theoretical advantage from a patient safety standpoint of being able to infect mammalian cells but not to replicate in them. Other replication-defective members of the poxvirus family are the avipox vectors (fowlpox and canarypox/ALVAC).[51] These avipox vectors infect human cells and express their transgenes for 2–3 weeks before undergoing cell death. They are incapable of reinfecting cells. Marshall et al.[13] and Zhu et al.[66] designed a phase I study (n = 18) to define the safety of avipox CEA recombinant in patients with advanced CEA-expressing carcinoma. This study also constituted the first trial of any avipox recombinant vaccine in cancer patients. Safety was demonstrated, as was the generation of statistically significant increases in CEA-specific CTL precursors from peripheral blood mononuclear cells (PBMC) from seven of nine HLA-A2-positive patients after vaccination.[13,66] However, preclinical and recent clinical[14] studies have shown that optimal use of recombinant vaccinia viruses may be to prime the immune response, followed by boost vaccinations with other vectors (such as replication-defective avipox vectors), peptides or proteins.

3. Prime and Boost

Priming with one type of immunogen and boosting with another may be advantageous because some of the most effective methods of vaccination, such as the use of recombinant vaccinia virus or adenoviruses, can be used only for a limited number of times because of host anti-vector responses. Numerous preclinical studies have demonstrated the advantages of diversified prime and boost protocols.[67-71] In an effort to determine which heterologous prime and boost regimen to use, a small randomized trial was conducted to compare the rV-CEA as the initial priming vaccination followed by boosting with avipox-CEA (VAAA) with the three vaccinations with avipox-CEA first, followed by rV-CEA (AAAV).[14] In each group, patients were evaluated for immunologic responses using the enzyme-linked immunosorbent spot (ELISPOT) assay. The ELISPOT assay is relatively sensitive and quantitative. By measuring cytokine release on a single-cell basis,

the assay can detect a peptide-specific T-cell response against specific HLA class I binding peptides.[72] This study showed that the immune responses seen in the VAAA arm were much better than those in the AAAV arm. Furthermore, continued follow-up of these patients revealed that although there were only nine patients in each arm, five of nine patients (at the time of a recent presentation) were alive on the VAAA arm (2-year survival estimate 67 ± 19%), whereas in the AAAV arm, zero of nine patients were alive (2-year survival estimate $0 \pm 0\%$).[73] This survival difference was related to the immune response; thus, those patients who had at least a 2.5-fold increase in their CEA-specific T cells lived longer (p = 0.03).

3.1 Costimulatory Molecules

Destruction of immunologic targets (such as tumors) requires T-cell lymphocyte recognition (via the TCR) of antigenic peptides presented in the context of MHC molecules on APCs. Costimulatory molecules are critical in the generation of potent T-cell responses. The initiation of an immune response requires at least two signals for the activation of naive T cells by APCs. The first signal is antigen specific, delivered through the TCR via the peptide/MHC, and causes the T cell to enter the cell cycle. The second 'costimulatory' signal is required for cytokine production and proliferation. The most extensively studied pathway of costimulation is that involving the interaction of the costimulatory molecule B7-1 (CD80) expressed on APC with cd28 and ctla4 on the T cell.[74-77] A second B7 family member, B7-2 (CD86), has also been identified that interacts with the same T-cell ligands as B7-1. However, B7-2 is upregulated earlier in APC stimulation, and then decreases as B7-1 levels increase. During DC maturation, another important molecule is strongly upregulated, together with costimulatory molecules such as B7-1 and B7-2. This molecule known as leukocyte function-associated antigen (LFA)-3 (CD83) is one of the best-known maturation markers for human DCs. The fact that CD83 is strongly upregulated together with costimulatory molecules such as B7-1and B7-2 during DC maturation suggests that it plays an important role in the induction of immune responses. One mechanism proposed for the ability of tumor cells to evade destruction by the immune system is their failure to express adequate levels of costimulatory molecules, resulting in a failure to induce T-cell responses.[78-81] A corollary of this hypothesis is that the introduction of proper costimulatory molecules into tumors that express TAAs should enhance their ability to elicit specific anti-tumor immune responses. Several studies have demonstrated that transfected tumor cells expressing B7-1 induce potent responses against both modified and unmodified tumor cells.[82,83] B7-1-transfected tumors either failed to grow or, after initial growth, regressed. Furthermore, the immune response induced by B7-1-positive tumors protected animals from re-challenge with untransfected tumor.

The proper engagement of the TCR and costimulatory receptor requires the expression of both antigen and costimulatory molecules, respectively, in the same cell. Therefore, co-expression of costimulatory molecules using a single recombinant vector presents the potential for cooperation among these proteins to enhance T-cell activation.

In a recently completed clinical trial by von Mehren et al.[16] 39 patients with CEA-expressing cancers were treated with ALVAC-CEA/B7-1 alone and 30 patients received ALVAC-CEA/B7-1 and GM-CSF (see table I[16,84]). Patients received 4.5×10^8 pfu intradermally every other week, for a total of four injections. The vaccine was again found to be safe, with the major toxicity limited to local erythema and swelling at the vaccine sites. Disease stabilization was seen in 26% of the patients who received the vaccine alone, and in 37% of the patients who received the vaccine in combination with GM-CSF. Increases in the T-cell precursor frequency to a CEA peptide were seen in 10 of 12 patients treated with the vaccine alone and T-cell precursor frequencies as high as 1 in 13 000 were documented.[16] No change in the T-cell precursor frequency recognizing an unrelated flu matrix peptide was documented.

A number of additional costimulatory molecules on APCs have been identified; these include ICAM-1 and LFA-3, whose ligands are LFA-1 and cd2, respectively, on the surface of T cells.[85] Both ICAM-1 and LFA-3 are also capable of conferring similar levels of costimulation of T cells against tumor cells in mouse models.[86,87] Multigene constructs using poxviral vectors (avipox and vaccinia) have been generated. These vectors contain a triad of costimulatory molecule transgenes consisting of B7-1, ICAM-1, and LFA-3, and have been given the designation TRICOM, i.e. rV-TRICOM and avipox-TRICOM. Preclinical studies using TRICOM constructs have shown them to be superior to those constructs that contain one or two of the costimulatory molecules.[69,88,89]

An ongoing phase I clinical trial at Georgetown University (Washington, DC, USA) is evaluating the safety of CEA-TRICOM vectors (see table II). To date, 51 patients have been accrued, completing all six dose-escalation cohorts as well as a seventh cohort with GM-CSF. Only mild treatment-related toxicity has been observed to date. Evidence of clinical activity (resolution of a lung tumor) has been observed in at least one patient treated with only two injections of avipox-CEA/TRICOM. These studies thus indicate the safety profile of the TRICOM vectors.[90,91]

In another phase I trial that is currently accruing, patients with locally advanced or metastatic CEA-expressing cancer are given a

pox-vector-based vaccine with CEA/TRICOM genes with GM-CSF or avipox GM-CSF. A third trial evaluating an avipox-CEA based vaccine also contains a T-cell costimulatory molecule (B7-1). In this Aventis-sponsored trial, patients with metastatic colorectal cancer are randomized into three arms with irinotecan alone, irinotecan with vaccine, or the combination of irinotecan with vaccine and tetanus toxoid (see table II).

3.2 Peptides

Peptides are characterized by their ability to induce an immune response by interacting with the appropriate class of the MHC molecule on the APC surface. Peptides of approximately 8–11 amino acids in length, if they possess the appropriate binding motifs, will bind to MHC class I molecules. These peptide-MHC complexes will interact with the TCR to activate CD8+ T cells. These CD8+ T cells, termed cytotoxic T lymphocytes, are usually responsible for lytic destruction of tumors. Peptides of approximately 11–15 amino acids in length, if they contain the appropriate binding motifs, will bind to MHC class II molecules on the surface of T cells. This will lead to the activation of CD4+ or 'helper' T cells that produce cytokines and help to promote activation of CD8+ T cells. These 'MHC-restricted' responses are thus effective only if the appropriate MHC allele is present in a patient.

The most studied MHC restriction element in the human population is the MHC class I allele, known as HLA-A2, which is found in approximately 50% of all Caucasians. Numerous peptide-binding motifs to HLA-A2 molecules have been identified, and clinical trials are being carried out in which a given cohort of individuals possess the HLA-A2 allele; thus T-cell responses to a particular defined peptide can be quantified. Preclinical studies have shown that both CD8 and CD4+ T cells are usually activated for a vigorous antitumor effect.[92-94] However, studies do exist in which a CD8+ T-cell response alone or a CD4+ T-cell response alone can provide an antitumor effect.[17,95,96] Oligopeptides that contain both class I and class II epitopes can also be used. In a unique set of circumstances, an oligopeptide of the repeat sequences of the mucin (MUC)-1 has been shown to cross-link TCRs in an MHC-unrestricted manner to activate T cells.[97]

This approach is currently being evaluated in a phase I/II study of a ras peptide cancer vaccine with or without IL-2 in HLA-A2-1-positive patients with locally advanced or metastatic colorectal cancer (see table II). Patients are assigned to one of two treatment groups according to extent of disease. Patients with prior locally advanced disease are assigned to treatment group A, while those with metastatic disease are assigned to treatment group B. In group A, patients are vaccinated against influenza on day −6. Patients undergo collection of PBMC on day −4. The PBMC are cultured with sargramostim (GM-CSF) and IL-4 for 5 days and CD40 ligand for 24 hours to differentiate them into DCs and then pulsed for 2 hours with the appropriate peptide (ras) to form a vaccine. Patients receive peptide-pulsed DC vaccine intravenously (IV) over 5 minutes on days 1, 15, 29, 43, and 57. In group B, patients undergo collection of PBMC and receive vaccination as in group A. Patients also receive IL-2 subcutaneously on days 2–6 and 9–13. Treatment repeats every 2 weeks for up to five courses in the absence of disease progression or unacceptable toxicity. Patients are followed up on days 75, 90, 120, and 365.

Using peptides as immunogens has many advantages, including: (i) whole proteins may contain parts of the molecule that are shared with normal cellular proteins, and the use of peptides minimizes the potential for induction of autoimmunity; (ii) preparation is relatively easy and affordable; (iii) because the immunogen is extremely well defined, the immune response can be analyzed in several ways and quantitated; (iv) tetramers, which are molecules that contain specific peptides bound to MHC components, can be used to bind to and isolate antigen-specific T cells induced and amplified in the host; (v) tumors can be stripped of peptide-MHC complexes, and those peptides displayed on the surface of tumors can be identified; and (vi) peptides can be modified to be more immunogenic in the generation of peptide agonists.

3.3 Agonist Peptides

Formation of peptide agonists involves modifying the amino acids of the peptide that bind to either the MHC on the APCs or the TCR. More vigorous MHC binding (i.e. higher affinity for the MHC molecule) often leads to the generation of a more vigorous T-cell response. The advantage of using agonist epitopes has now been demonstrated in clinical trials. An agonist peptide epitope to CEA has been shown to have clinical activity in patients with CEA-expressing tumors.[98] In a recent trial by Fong et al.,[18] patients with CEA-expressing tumors received two 1-monthly vaccinations with DCs loaded with the CEA agonist peptide; 2 of 12 patients experienced complete responses, one patient had a mixed response, and two had stable disease. Clinical response in this trial correlated with CEA-specific T-cell responses.

The agonist CEA epitope is being studied in a phase II randomized trial in HLA-A2-positive patients with CEA-producing adenocarcinomas of gastrointestinal tract origin. Patients are randomized to one of two treatment arms. In arm I, patients receive CEA peptide (CAP 1-6D) emulsified in Montanide ISA-51 adjuvant subcutaneously on day 1. In arm II, patients receive CAP 1-6D dissolved in sargramostim (GM-CSF) intradermally on day 1. Treatment repeats in both arms every 3 weeks for six courses in the

absence of disease progression or unacceptable toxicity. Patients are followed up at 3 weeks and then as necessary.

The specificity that a peptide possesses can also be disadvantageous to its use as a cancer vaccine. It is possible that the peptide identified has a dominant CTL epitope, which may induce a CTL response. However, this response may be short-lived because of the lack of 'help' provided by helper peptides not present in the vaccine. Furthermore, the use of a peptide vaccine is limited, because patients who do not have that specific allele (e.g. only 50% of the population has the HLA-A2 allele for an HLA-A2-reactive peptide) would not be eligible to receive the vaccine. Combinations of antigenic peptides with reactivities for multiple HLA alleles would circumvent this limitation. Clinical experience with the use of peptides as cancer vaccines is now emerging. Some peptides under study include human papillomavirus (HPV),[99] ras,[92,93] HER-2/*neu*,[94] MAGE,[100] MART-1, tyrosinase,[101] gp100,[102,103] CEA,[17] MUC-1,[96] PSMA,[104,105] among others.

3.4 Anti-Idiotypes

The concept of vaccinating with anti-idiotypic antibodies is based on the immune network approach described by Jerne.[106] According to this hypothesis, the variable antigen-binding regions of antibodies (Ab1) contain idiotypic determinants that are immunogenic and induce the formation of so-called anti-idiotypic antibodies (Ab2). Some of these Ab2 ('internal-image') antibodies are able to functionally mimic the 3-dimensional structure of the original antigen. Thus, selective vaccination with Ab2 could induce a specific immune reaction directed against the original antigen.[107-111] Several small clinical studies using murine Ab2 for the treatment of patients with advanced colorectal carcinoma have demonstrated the induction of antitumoral humoral and cellular immune responses leading to improved clinical responses and tumor regression.[109,112-115] Other studies (discussed in this section) have evaluated anti-idiotype antibodies that mimic TAAs on colorectal cancer cells (see table I).

An anti-idiotypic antibody was generated against murine 17-1A antibody. Six patients with colorectal cancer who underwent surgery were vaccinated with this human anti-idiotype antibody that mimics GA773-2 antigen.[19] All of the patients developed specific T-cell responses against GA733-2, and five of six developed specific IgG antibody response against GA733. Other investigators designed a similar trial, using a rat anti-idiotype antibody generated to the 17-1A antibody.[116] Nine colorectal cancer patients were evaluated in this study following vaccination with aluminum hydroxide precipitated 17-1A. Although four of the nine patients developed a DTH response, no specific antibodies were detected post-vaccination.

The human monoclonal antibody 105AD7 mimics the gp72 antigen that is expressed in 80% of colorectal cancer cells, and can induce a DTH reaction against human tumor cells. In a phase I study with 13 colorectal cancer patients, 105AD7 was administered intramuscularly, and increased levels of IL-2 and a lymphocyte-proliferative response were observed after stimulation *in vitro* by gp72 antigen-positive cells. When these results were compared with historical controls, the authors suggested that there was a clinical benefit seen with this vaccine.[20] Small trials have demonstrated the ability of 105AD7 to induce significant infiltration of CD4+ cells and natural killer (NK) cells into tumors in vaccinated patients,[117] a significant increase in median CD25+ lymphocytes within the tumor[118] and a significant increase in apoptosis in tumor cells.[119] However, in order to evaluate these promising results more definitively, a prospective, randomized, double-blind, placebo-controlled survival study in patients with advanced colorectal cancer was performed.[21] Patients (n = 162) were randomized to receive three treatments with either 105AD7 or placebo at the time of enrollment, and at 6 and 12 weeks. Study groups were comparable in terms of patient demographics and time from diagnosis of advanced colorectal cancer (277.1 vs 278.6 days). Patient demographics, time of diagnosis of advanced colorectal cancer, and baseline disease were similar, with 50% of patients having malignancy in at least two anatomic sites. Compliance with treatment was poor, with only 50% of the patients receiving three planned vaccinations. Median survival from randomization date was 124 and 184 days in 105AD7 and placebo arms, respectively (p = 0.38), and 456 and 486 days from the date of diagnosis of advanced disease (p = 0.82). In this trial, 105AD7 vaccination did not demonstrate any improvement in survival in patients with advanced colorectal cancer. Although the reason for this was not entirely clear, according to the authors, the high tumor burden and the lack of compliance may have played a role in the poor results.[21]

CeaVac® (Titan Pharmaceuticals, San Francisco, CA, USA) is an anti-idiotype murine monoclonal antibody that mimics a highly tumor-restricted CEA epitope.[22] Polyclonal antibody responses were demonstrated in 17 of 23 patients with advanced colorectal cancer and, in 13 of these patients, anti-CEA responses were detected. Five patients had specific T-cell responses to CEA. None of these patients had objective clinical responses, but overall, median survival for 23 evaluable patients was 11.3 months, with a 44% 1-year survival. Toxicity was limited to a local reaction at the vaccine site with minimal pain incurred.

In a colorectal cancer trial using CeaVac® post-resection, 32 patients were randomized to treatment with aluminum hydroxide-precipitated CeaVac® 2mg intracutaneously or CeaVac® 2mg mixed with QS-21 adjuvant 100μg subcutaneously every other

week, then monthly until disease recurrence. Patients participating in this trial had differing stages of disease.[120] Four patients with Dukes stage B2, 11 with Dukes stage C, and eight with Dukes stage D had their tumors completely resected. Nine patients with Dukes stage D carcinoma had their tumors incompletely resected; positive margins post-operatively. Fourteen patients underwent chemotherapy with fluorouracil given concomitantly with CeaVac®. Ten patients relapsed or had progressive disease at time points ranging from 6–30 months. Two patients died at 14 and 20 months, respectively. All 32 patients demonstrated idiotype-specific T-cell responses, of which 75% were CEA specific. These T-cell responses were measured by proliferation of patients' PBMC in response to CEA. The concomitant use of chemotherapy did not impair this immune response.[120,121] Even though this trial was not designed to examine survival, the authors noted that several of the high risk patients appeared to do better than expected as measured by historical controls. Although this strategy of anti-idiotype vaccines for colorectal cancer may provide patients with some clinical benefit, larger phase III trials are necessary to confirm the clinical benefit of such an approach in the treatment of colorectal cancer.

A number of ongoing trials are currently accruing patients using the approach of anti-idiotype vaccines (table II). One multicenter phase II trial will study the efficacy of adjuvant monoclonal antibody 3H1 anti-idiotype vaccine and monoclonal antibody 11D10 anti-idiotype vaccine in patients with minimal metastatic colorectal cancer after complete hepatic resection. Beginning 6–12 weeks after curative hepatic resection, patients receive monoclonal antibody 3H1 anti-idiotype vaccine and monoclonal antibody 11D10 anti-idiotype vaccine intracutaneously at separate sites on days 1, 15, 29, and 45, then subcutaneously monthly for 2 years beginning on day 73, and then every other month for 3 years.

3.5 Dendritic Cell Vaccines

Antigen presentation is a crucial step in the initiation of an effective immune response that requires the presentation of antigens to sensitize naive T cells and to restimulate primed T cells. The DC is considered the most potent APC, and therefore, one of the most attractive means of vaccination.[122,123] DCs are capable of activating naive CD4+ and CD8+ T lymphocytes by antigen presentation through an MHC-restricted manner. DCs are found in most tissues where they exist in an immature state; in this state they are unable to stimulate T cells but possess an exceptional ability to capture and process antigens. These captured antigens can be presented efficiently by both class I and class II MHC molecules. Antigen capture acts as a signal for the cell to mature and mobilize to regional lymph nodes. These cells undergo extensive transformation, in which antigen capturing decreases and T-cell stimulatory functions increase. The unique capacity of these 'mature' DCs to activate T cells is probably related to the presence of an exceptionally high number of MHC, and costimulatory and adhesion molecules.

A number of different immunologic strategies using DCs have been investigated.[123-128] DC vaccines can be employed by: (i) loading with a peptide, protein or anti-idiotype antibody; (ii) infecting with a viral vector; or (iii) loading with apoptotic bodies from tumor cells. The major disadvantage of this strategy is the great cost and effort involved. One must obtain large amounts of peripheral PBMC from patients via leukapheresis. The PBMC must then be cultured for several days in the presence of cytokines such as GM-CSF, IL-4 and/or tumor necrosis factor (TNF)-α, and then reinfused into the patient. This must be done for each patient.

However, DC biology is a rapidly growing field with much promise. In clinical trials, DCs loaded with anti-idiotype antibodies have proved quite successful, resulting in clinical remissions in patients with B-cell lymphoma.[124,125] Other clinical studies using peptide-loaded DCs are under way in a range of human malignancies.[17,105,123,126-128] Generally, DC-based vaccinations have been found to be safe, and can induce antigen-specific T-cell responses and remissions, at least in subsets of patients with advanced disease. A particularly intriguing strategy involves vaccination with DCs that have been 'fused' with the host's own tumor cells, resulting in a DC-tumor cell hybrid vaccine. Significant antitumor responses were observed in both preclinical animal studies,[129,130] as well as in patients with metastatic renal cell carcinoma in a small phase I trial.[131]

As mentioned in section 3.2, Fong et al.[18] used DCs loaded with a CEA-agonist peptide as a vaccine strategy for patients with advanced CEA tumors. Although only 12 patients were vaccinated, this approach produced clinical responses that correlated with immunologic response to the vaccine. However, the drawback for loading DCs with a particular peptide is that patients are restricted to the HLA haplotype expressed on that peptide. For CTL activation, the MHC I complex on DCs can be loaded with immunogenic peptides that correspond to the patient-specific HLA haplotype.[132] Therefore, patients must be classified by their HLA type, and binding properties of the immunogenic peptides for the specific HLA have to be characterized. One way to avoid this problem is to use TAA encoded by DNA.[133] For this purpose, the patient-specific TAA gene has to be cloned into a vector before transfer into the DC, a labor-intensive and time-consuming process. Additionally, at present, there is no optimal expression system for human DCs.

Another approach is the introduction of mRNA encoding TAA into DCs.[134] RNA can be extracted from a small amount of tumor and amplified *in vitro*.[135] The mRNA extracted from the tumor is

Table III. Summary of advantages and disadvantages of vaccine strategies in colorectal cancer

Vaccine	Advantages	Disadvantages
Whole cell vaccines: autologous	Tumor antigens specific to patient present in vaccine preparation	Preparation is a laborious process Adequate number of tumor cells required Central laboratory required to prepare
Whole cell vaccines: allogeneic	Easier to prepare than autologous vaccines Vaccine may contain several tumor antigens May be infected with viral vectors expressing cytokine or costimulatory genes	Do not constitutively express costimulatory molecules Alloimmunity to non-self components may develop
Vector-encoded TAA	Choice and number of genes inserted Low cost for production	Potential safety issue with viral vectors Possible autoimmunity using self antigens
Peptide-based vaccines	Well defined immunogens, easy to evaluate immune responses Preparation relatively easy and affordable	Adequate immune response may require multiple epitopes not contained within the peptide sequence
Anti-idiotype vaccines	Target well defined specific antigen on colorectal cancer cells	Specificity may limit its activity on cells not expressing target Can be cumbersome to produce
DC vaccines	Most potent APC Numerous approaches, i.e. loading DC with peptide strategies	Great cost and effort to make vaccine Requires leukapheresis and cytokine culturing of cells for days

APC = antigen-presenting cell; **DC** = dendritic cell; **TAA** = tumor-associated antigen.

patient-specific and encodes all TAA expressed by the tumor sample. Few studies have used DCs transfected with mRNA.[15,134-137] Among these studies, some used mRNA encoding human CEA.[135,137,138] Transfection of DC with *CEA*-mRNA performed by lipofection, a technique used to transfer functional genes into a cell, successfully induced CEA-specific CD4+ and CD8+ T lymphocytes *in vitro*.[135,137,138] Moreover, in mice, specific *in vivo* CTL response and regression of lung metastases were achieved.[135] These promising results using DCs transfected with TAA-encoding RNA justify further investigations to obtain more experience with this important immunotherapeutic approach. In a recent study by Morse et al.,[17] performed at Duke University Medical Center (Durham, NC, USA), 21 patients with advanced CEA-expressing tumors received vaccinations with *in vitro*-generated DCs, loaded with an HLA-A2-restricted peptide of CEA to test the safety, feasibility, and clinical response. The DCs were loaded with the CEA peptide CAP-1 and cryopreserved. Groups of between three and six patients received four weekly or biweekly IV infusions of the CAP-1-loaded DCs, which were administered in escalating dose levels of 1×10^7, 3×10^7, and 1×10^8 cells/dose. A subset of the patients in the last group also received intradermal injections of 1×10^6 DCs. There were no toxicities directly related to the treatments. One patient had a minor response, and one had stable disease. Skin punch biopsy at DC injection sites demonstrated pleomorphic infiltrates in the three patients evaluated. The authors concluded that it was safe and feasible to administer cryopreserved DC-expressing CEA to patients with advanced metastatic CEA-expressing tumors.[17]

Rains et al.[139] conducted a study in which 15 patients with advanced colorectal cancer were treated with vaccines prepared from autologous DCs pulsed with tumor RNA and keyhole limpet hemocyanin. Although no radiologic objective responses have been seen to date, no major adverse effects were observed, and in seven patients, CEA levels fell, suggesting some activity of the vaccine. Furthermore, 11 of 13 patients tested developed a positive keyhole limpet hemocyanin skin test.[139]

A recent phase I/II study of active immunotherapy with CEA RNA-pulsed DCs in patients with resected hepatic metastases from adenocarcinoma of the colon has been initiated at Duke University Medical Center (Durham, NC, USA). Patients undergo leukapheresis for up to 4.5 hours to collect DCs. The separated DCs are then pulsed with CEA RNA. Patients receive CEA RNA-pulsed DCs IV every 2 weeks for a total of four doses. Patients then undergo a second leukapheresis 2 weeks after the last DC infusion to obtain specimens for immunologic tests. Patients with extra samples of DCs available may receive additional doses of CEA RNA-pulsed DCs every 2 months in the absence of unacceptable toxicity. Patients are followed up at weeks 12, 24, 36, and 48, and every 6 months thereafter.

4. Conclusion

Recent advances in the field of tumor immunology have provided insight into the mechanisms by which T cells can be activat-

ed against TAAs. The preclinical proofs of principle have allowed for the translation of this work into clinical vaccine trials for the treatment of colorectal cancer. A number of different approaches have now been examined in the clinic, which include the modification of whole tumor cell vaccine to a number of different strategies for the delivery of TAAs as vaccines (table III).

Approaches that incorporate 'off-the-shelf' technology avoid many of the technical hurdles presented by the more time-consuming manipulation of patient tumors or blood cells. As our knowledge of this field continues to expand, the combination of one or more of these approaches, as well as the integration of vaccines with more traditional therapeutics such as chemotherapy (some of which may also decrease suppressor cell activity) and radiation (which may also upregulate MHC, fas or TAA) may improve upon the success for the treatment of colorectal cancer if given at the proper dose and schedule. Further studies of dose and scheduling regimens will help to determine how vaccines can be combined with traditional anticancer therapeutic modalities.

Acknowledgments

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