Therapeutic Vaccines for Gastrointestinal Cancers

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Abstract: Despite progress in the management of gastrointestinal malignancies, these diseases remain devastating maladies. Conventional treatment with chemotherapy and radiation is still only partially effective and highly toxic. In the era of increasing knowledge of the molecular biology of tumors and the interaction between the tumor and immune system, the development of targeted agents, including cancer vaccines, has emerged as a promising modality. In this paper, we discuss the principals of vaccine development, and we review most of the published trials on gastrointestinal cancer vaccines that have been conducted over the last decade. Many antigens and various treatment approaches have already been tested in colon, pancreatic, and other cancers. Some of these approaches have already shown some clinical benefit. In this paper, we discuss these different strategies and some of the future directions for targeting gastrointestinal malignancies with vaccines.

Keywords
Cancer vaccine, gastrointestinal, malignancies, trials, review

Gastrointestinal malignancies are some of the most common, devastating, and costly diseases. Colorectal cancer (CRC) is the second most common cancer in women and the third most common cancer in men worldwide. Although the incidence and mortality rates of CRC have decreased in historically high-risk areas such as the United States and Canada, 5-year survival rates remain low (28–42%) in developing countries.[1] Moreover, 5-year mortality rates for stage III–IV CRC are 25–35% despite advances in treatment.[2] Although hepatocellular cancer (HCC) and pancreatic cancer are less common, they have poor prognoses. HCC is the sixth most commonly diagnosed cancer and the third leading cause of cancer death worldwide.[1] Pancreatic cancer has an overall 5-year relative survival rate of 5.6%.[3] This low rate is due to pancreatic cancer's advanced stage at diagnosis and relative resistance to therapies. Esophageal cancer is the fourth most common gastrointestinal cancer worldwide (after colon, gastric, and liver cancer).[4] Although the incidence of gastric cancer has decreased in the United States, the incidence of gastroesophageal tumors has increased.[5]

Chemotherapy and radiation therapy—the conventional treatments for gastrointestinal malignancies—are associated with high toxicities and a significant negative effect on patients' quality of life. Over the past decade, significant advances in our understanding of the molecular biology of cancer—along with the development of molecular targeted agents such as cetuximab (Erbitux, Imclone) for CRC and sorafenib (Nexavar, Bayer) for HCC—have shown promise for cancer therapy. However, the advantages of these therapies to date are limited, and they come at the cost of additional side effects. Accordingly, there is an urgent need for novel strategies in the management of gastrointestinal malignancies. The past 2 decades have ushered in major developments in the understanding of the biologies of the immune system and the tumor immune microenvironment, as well as the dynamic interaction between these 2 entities. Thus, immunotherapy has emerged as a promising new strategy in cancer therapy, and cancer vaccines have become an attractive therapeutic option, one that holds hope of developing specific immune responses, achieving clinical efficacy, and having minimal side effects.

Cancer Vaccines

Cancer vaccines aim to actively stimulate the immune system against tumor cells by generating humoral and/or cellular immune responses. A humoral immune response generates antibodies that target extracellular antigens. On the other hand, a cell-mediated immune response targets intracellular antigens composed of either native proteins that are altered (eg, mutated or overexpressed) or foreign proteins. These antigens must be processed and presented by the antigen-presenting cells in order to stimulate T lymphocytes. There are 2 types of T cells: CD4+ cells (helper T cells) and CD8+ cells (cytotoxic T cells [CTLs]). Both types recognize intracellular antigens in the form of peptides via direct interaction between the T-cell receptor and peptide antigens displayed by the human leukocyte antigen (HLA) system on the surface of the target cells.

In general, vaccines are classified into 2 types. Cellular-based vaccines (autologous or allogeneic tumor cell–based vaccines) utilize whole cells or cell lysates as the source of antigens, which allows multiple antigens to be simultaneously targeted without being prospectively identified.[6] In contrast, antigen-based vaccines use antigens that are exclusively tumor-associated antigens (TAAs) of cancer cells. TAAs are specifically expressed by tumors and are caused by the alteration of cellular protein (via mutation or overexpression) or the acquisition of a foreign protein such as a virus oncogene.[7,8] TAAs can be classified into those that are self-antigens and those that are not. Self-antigens maintain their original amino acid sequences. Accordingly, epitopes presented by the HLA molecule are self (eg, carcinoembryonic antigen [CEA], mucin 1 [MUC1], Her-2/neu, and MART1). Of course, targeting a self-antigen runs the risk of breaking tolerance against antigens in normal cells and, therefore, inducing autoimmunity, as has been seen in melanoma vaccines.[9] However, it has been shown that this risk may not be a concern in the majority of clinical trials. In contrast, non–self-antigens acquire new amino acid sequences (eg, mutated proteins, RAS, and p53) or come from foreign antigens (eg, viruses such as human papillomavirus [HPV], hepatitis B virus, or Epstein-Barr virus). An ideal antigenic target for a cancer vaccine is uniquely expressed in the cancer cell, important for maintenance of the malignant phenotype, expressed on the cell surface, and immunogenic.[10]

Most cancer vaccines are designed to generate a cellular immune response. To achieve this goal, antigens can be administered as peptides, whole proteins, recombinant proteins via viruses, and DNA and RNA vectors. In addition, antigens can be pulsed on dendritic cells (DCs). DCs are potent antigen-presenting cells that are powerful tools for generating specific cytotoxic immune responses via activation of naïve T cells.[11]

On the other hand, some cancer vaccines are designed to generate humoral immune responses via idiotopes that are structural antigenic determinants uniquely expressed on a few antibody populations. Induced anti-idiotypic antibodies have epitopes that mimic the antigen and can induce an anti–anti-idiopathic response against the real antigen.[12]

Immune Enhancers

In order to enhance the immune response, cancer vaccines are usually administered with adjuvants and are often combined with cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF) or interleukin (IL)-2.[13,14] Adjuvants are co-stimulatory molecules known for their capability to stimulate the immune system. GM-CSF is a protein that stimulates stem cells to produce granulocytes. IL-2 plays a crucial role in regulating the immune system.

Combined Modalities

Over the past decade, there has been great progress in generating immune responses against targeted tumor antigens. However, clinical responses have been lagging behind, perhaps suggesting that mechanisms of resistance downstream from initial T cells may be to blame. Therefore, recent attention has turned toward mechanisms of immune evasion, which could lead to the tumor escaping the activated immune system. Several immune modulators that play a crucial role in immune evasion have been

identified, including regulatory T cells, programmed cell death ligand 1, transforming growth factor-β, and indoleamine 2,3-dioxygenase.[15] Clinical trials are currently testing combination therapies consisting of cancer vaccines and these immune modulator inhibitors. Moreover, accumulating evidence has suggested that combining therapeutic vaccination with conventional chemotherapy can enhance the potential for an anti–tumor immune response.[16]

Colorectal Cancer

Mounting evidence has suggested that CRC is an immunogenic tumor. This evidence includes: the expression of TAAs by colorectal carcinoma; the strong correlation between the infiltration of memory T cells and the recurrence and metastases of CRC; and the spontaneous humoral and cellular immune responses against tumor antigens.[17-19] This hypothesis has been further explored in clinical trials using different cancer vaccination strategies.

Cellular-Based Vaccines

Autologous Tumor Cell–Based Vaccines The majority of phase II and III trials have used tumor cell–based vaccines consisting of irradiated autologous tumor cells (Table 1). A meta-analysis evaluated 3 phase III, prospectively randomized trials that examined autologous colorectal tumor cells mixed with bacillus Calmette-Guerin (BCG; OncoVAX, Vaccinogen) as an adjuvant in patients with resected stage II and III CRC. Looking at intent-to-treat analyses, there was a significant improvement in disease-free survival (DFS)—although none in overall survival (OS)—and minimal side effects.[20] In the first trial, a significant improvement was observed in OS and DFS in the vaccine arm compared to the surgery-alone arm in CRC patients. However, a subgroup analysis revealed no benefits in rectal cancer patients.[21] The second trial showed a 44% risk reduction for recurrence in the vaccine arm, with a more significant clinical benefit in stage II patients.[22] Although the third clinical trial failed to show any statistically significant difference in clinical

Table 1. Phase II and III Studies of Cellular-Based Therapeutic Vaccines for Colorectal Cancer

Study	Type of vaccine	Adjuvant used	Cancer stage	N	Study design	Immune response	Clinical response
Hoover HC Jr, Brandhorst JS, Peters LC, et al[21]	Autologous tumor cell	BCG	II–III	98	Surgery ± vaccine	Increase in DCH (67% vs 9%)	Increase in DFS and OS (HR, 3.97 and 0.39)*
Vermorken JB, Claessen AM, Van Tinteren H, et al[22]	Autologous tumor cell	BCG	II–III	254	Surgery ± vaccine	DCH in 92% of the vaccinated group	Decrease in recurrence risk (40%)**
Harris JE, Ryan L, Hoover HC Jr, et al[23]	Autologous tumor cell	BCG	II–III	412	Surgery ± vaccine	Correlation between DCH and prognosis†	No change in OS or DFS
Liang W, Wang H, Sun TM, et al[24]	Autologous tumor cell	NDV	I–IV	335	Surgery ± vaccine	Correlation between DCH and prognosis†	Increase in 5-year OS (66.5% vs 45.5%)
Schulze T, Kemmner W, Weitz J, et al[25]	Autologous tumor cell	NDV	IV	51	Liver metastases resection ± vaccine	N/A	Increase in OS and MFS (HR, 3.3 and 2.7)
Burgdorf SK[26]	Allogeneic tumor cell	DCs	IV	17	Single arm	Increase in Th1 cytokines in patients with SD	24% of patients had SD

BCG=bacillus Calmette-Guerin; DCH=delayed cutaneous hypersensitivity; DCs=dendritic cells; DFS=disease-free survival; HR=hazard ratio; MFS=metastases-free survival; NDV=Newcastle disease virus; OS=overall survival; SD=stable disease.

*But not in rectal cancer. **The major impact was seen in patients with stage II disease. †The magnitude of DCH was related to better prognosis in the vaccine group.

Table 2. Phase II and III Studies of Antigen-Based Therapeutic Vaccines for Colorectal Cancer

Study	Type of vaccine	Adjuvant used	Cancer stage	N	Study design	Immune response	Clinical response
Lesterhuis WJ, de Vries IJ, Schuurhuis DH, et al[35]	CEA	DCs	IV	10	Liver metastases resection + vaccine	7 patients had CEA-specific T cells in DTH biopsies	5 patients had NED for a mFU of 14 months
Babatz J, Röllig C, Löbel B, et al[36]	CEA	DCs	IV	9	Single arm	5 patients had positive ELISPOT test results to CEA	1 patient had SD
Morse MA, Nair SK, Mosca PJ, et al[37]	CEA	DCs	IV	13	Liver metastases resection + vaccine	T-cell infiltration at vaccine site	3 patients had SD
Conry RM, Khazaeli MB, Saleh MN, et al[39]	CEA	Vaccinia	III–IV	32	Single arm	7 patients had positive antibodies to CEA	No clinical response
Ullenhag GJ, Frödin JE, Jeddi-Tehrani M, et al[42]	CEA	GM-CSF	I–III	24	Surgery + vaccine ± GM-CSF	100% of the GM-CSF group had CEA T cells and IgG vs 75% and 66%, respectively, in the vaccine-alone group	Increase in survival in patients with positive CEA and IgG results
Loibner H, Eckert H, Eller N, et al[44]	mAb 17-1A	Alum	III–IV	240	Vaccine vs placebo	100% of patients had positive antibodies to Ep-CAM	1-year survival rate doubled in the vaccine group
Mosolits S, Markovic K, Frödin JE, et al[45]	Ep-CAM	GM-CSF	II–IV	13	Ep-CAM vs anti-idiotypic antibody mimicking Ep-CAM	100% of patients in the Ep-CAM group had positive Ep-CAM and IgG results vs 0% in the anti-idiotypic antibody group	71% of the Ep-CAM group had NED for 2 years vs 67% of the anti-idiotypic antibody group
Neidhart J, Allen KO, Barlow DL, et al[46]	KSA	GM-CSF/ baculovirus	IV	11	KSA vs KSA + GM-CSF	7 patients had a cellular response; 8 patients had a humoral response	3 patients had SD for 4 months
Ullenhag GJ, Frödin JE, Mosolits S, et al[47]	Ep-CAM/ KSA	GM-CSF/ canarypox	I–III	12	Surgery + vaccine ± GM-CSF	5 of 6 patients in the GM-CSF group had positive ELISPOT test results vs 2 of 6 patients in the vaccine-alone group	N/A
Sadanaga N, Nagashima H, Mashino K, et al[55]	MAGE-3	DCs	Advanced*	12	Single arm	4 of 8 patients had peptide-specific CTLs	7 patients had decreases in tumor markers; 3 patients had minor tumor regressions
Tanaka F, Haraguchi N, Isikawa K, et al[56]	MAGE-3	DCs	Advanced**	28	Single arm	4 of 8 patients had peptide-specific CTLs	11 patients had decreases in tumor markers; 4 patients had minor tumor regressions

(Table continued on the following page.)

Table 2. *(Continued)* Phase II and III Studies of Antigen-Based Therapeutic Vaccines for Colorectal Cancer

Study	Type of vaccine	Adjuvant used	Cancer stage	N	Study design	Immune response	Clinical response
Maxwell-Armstrong CA, Durrant LG, Buckley TJ, et al[60]	Anti-idiotypic mAb		IV	162	Vaccine vs placebo	N/A	No change in OS
Ullenhag GJ, Spendlove I, Watson NF, et al[61]	Anti-idiotypic mAb	BCG/ alum	I–III	67	Surgery + adjuvant + neoadjuvant vaccine vs surgery alone	44% of patients in the vaccine group had positive ELISPOT test results	No clinical response
Durrant LG, Maxwell-Armstrong C, Buckley D, et al[62]	Anti-idiotypic mAb		I–IV	35	Neoadjuvant vaccine + surgery	Increase in CD4 and NK levels in tumors	65% of patients had NED for a mFU of 2 years
Kameshima H, Tsuruma T, Torigoe T, et al[65]	Survivin	IFA, IFN	IV	13	Vaccine + IFA ± IFN	4 of 8 patients who received IFN had increased CTL levels	1 of 5 patients in the IFA arm had SD vs 4 of 8 patients in the IFN arm
Kavanagh B, Ko A, Venook A, et al[66]	CEA, MAGE, HER-2, KLH, pan-DR	DCs	IV	13	Single arm	3 of 11 patients had positive ELISPOT test results to CEA	No clinical response
Hamilton J, Behrens RJ, Achtar M, et al[73]	RAS	Detox™	III–IV	11	Single arm	N/A	3 patients had NED for 14–42 months
Harrop R, Drury N, Shingler W, et al[76]	5T4	MVA	IV	19	Vaccine + IFL	11 of 12 patients had positive ELISPOT test results to 5T4	1 patient had CR; 5 patients had SD

CEA=carcinoembryonic antigen; CR=complete remission; CTL=cytotoxic T cell; DCs=dendritic cells; DTH=delayed-type hypersensitivity; ELISPOT=enzyme-linked immunosorbent spot; Ep-CAM=epithelial cell-adhesion molecule; GM-CSF=granulocyte macrophage colony-stimulating factor; IFA=incomplete Freund adjuvant; IFL=5-fluorouracil, leukovorin, and irinotecan; IFN=type I interferon; Ig=immunoglobulin; KLH=keyhole limpet hemocyanin; mAb=monoclonal antibody; MAGE=melanoma-associated antigen; mFU=median follow-up; MVA=modified vaccinia Ankara; NED=no evidence of disease; NK=natural killer cells; OS=overall survival; SD=stable disease.

*Including 3 colorectal cancer patients. **Including 7 colorectal cancer patients.

outcomes, a correlation was found between the magnitude of the delayed cutaneous hypersensitivity response of the autologous tumor cells and OS and/or DFS.[23]

Liang and colleagues conducted a large, randomized, controlled, phase III trial that demonstrated a statistically significant improvement in OS in the intent-to-treat population of patients with stage I–IV malignant digestive tract tumors who received autologous tumor cell vaccines following surgical tumor resection.[24] The 5-year survival rate was 66.5% in patients who had surgery and then received an adjuvant vaccine compared to 45.5% in the surgery-alone arm. Once again, the magnitude of the delayed cutaneous hypersensitivity response was related to the patient's prognosis.[24]

In the metastatic setting, patients with resected liver metastases from CRC were randomized in a phase III trial to receive adjuvant Newcastle disease virus (NDV) modified tumor cell vaccine or negative control. The vaccine was an autologous tumor cell vaccine that was modified by the nonlytic, low pathogenic Ulster strain of NDV. Unlike the lytic strains of NDV, the Ulster strain has a potent immune-stimulating property that stimulates

the adaptive immune system. An intent-to-treat analysis revealed a significant advantage in OS and metastases-free survival for vaccinated patients.[25]

Although promising results have been observed with autologous tumor vaccination, this method has many limitations. Autologous tumor vaccines are difficult to develop due to the limited amount of available tissue, the invasiveness of the procedure for obtaining this tissue, and the poor performance status caused by the advanced stage of disease. Furthermore, these vaccines are costly and time-consuming to develop.

Allogeneic Tumor Cell–Based Vaccines In 2 trials, allogeneic tumor cell lysates were loaded on DCs and administered to patients with disseminated CRC. In the first trial, increased levels of Th1 cytokines—such as GM-CSF, tumor necrosis factor-α, interferon (IFN)-γ, and IL-2—were observed in patients who achieved stable disease (24%).[26] In the second trial, specific immune responses were detected with transient stabilization or even reduction of CEA levels in some patients.[27]

Antigen-Based Vaccines

Self-Antigen–Targeted Vaccines Many TAAs—such as CEA, epithelial cell-adhesion molecule (Ep-CAM), MUC1, CD55, SART3, and human chorionic gonadotropin—have been identified in CRC.[28] Natural T-cell responses against TAAs occur in approximately one half of CRC patients who have involvement of lymph nodes or distant metastases.[19] Therefore, targeting these antigens presents an attractive strategy that has been explored in clinical trials. Some of the attempts to specifically target several of the known CRC antigens are discussed below and in Table 2.

Carcinoembryonic Antigen–Targeted Vaccines CEA is the most targeted antigen in CRC vaccines. The presence of circulating anti-CEA antibodies is associated with better prognosis and a significant increase in survival in patients with CRC.[29] The anti-idiotype monoclonal antibody 3H1, which mimics CEA, was able to break immune tolerance in patients with advanced CEA-positive CRC who failed standard therapies.[30] The majority of clinical trials have used DCs pulsed with CEA peptides or loaded with CEA messenger RNA (mRNA).[31-37] All of these findings come from phase I and II trials in patients with metastatic disease who failed standard chemotherapy. Immune responses were demonstrated by an increase in the level of CEA-specific T cells postvaccination. However, clinical responses were less significant, as stable disease was seen in few patients. Importantly, these clinical responses correlated with immune responses with no significant side effects.

Other phase I and II clinical trials have used recombinant vaccinia virus encoding CEA in metastatic CRC patients and have found similar outcomes. Conry and associates investigated the effect of this method in patients with CEA-expressing colorectal adenocarcinomas.[38,39] CEA-specific antibodies were induced in 7 of 32 patients who were vaccinated with recombinant vaccinia virus encoding human CEA complementary DNA.[38,39] Marshall and coworkers also showed an increase in CEA-specific CTLs with a replication-defective avipox vaccine containing the gene for human CEA in 2 phase I trials.[40,41] However, limited clinical activity was observed. These investigators have also shown that local administration of GM-CSF and low-dose IL-2 in combination with vaccines enhances specific immune responses.[41] Ullenhag and colleagues described a similar effect with GM-CSF when 24 resected CRC patients without macroscopic disease were immunized with recombinant CEA with or without GM-CSF. Anti-CEA immunoglobulin G titers were associated with increased survival rates.[42] However, other trials were not able to show the same positive effect of GM-CSF in combination with CEA vaccine. The addition of GM-CSF to ALVAC-CEA B7.1—a canarypox virus encoding the gene for CEA and for the T-cell co-stimulatory molecule B7.1—did not enhance the induction of CEA-specific T cells.[43]

Epithelial Cell-Adhesion Molecule–Targeted Vaccines Another antigen that is commonly targeted in CRC due to its overexpression in tumor cells is Ep-CAM (also known as GA733 antigen, CO17-1A, EGP, KS1-4, and KSA). In an attempt to induce anti-idiopathic antibodies to Ep-CAM, the anti–Ep-CAM murine monoclonal antibody (mAb) 17-1A has been used as an active vaccine in conjunction with alum as an adjuvant in a randomized, placebo-controlled, phase II trial.[44] Fifty percent of patients with stage III or IV epithelial cancer—mainly CRC but also upper gastrointestinal tract cancer—had an immune response to the vaccine. Interim analysis of data from 45 stage IV CRC patients showed a significant survival benefit for patients who had an immune response. Furthermore, vaccination with recombinant Ep-CAM protein was compared to vaccination with anti-idiotypic antibody in a randomized phase I/II trial in patients with resected stage II–IV CRC without residual macroscopic disease. Ep-CAM protein in combination with GM-CSF induced long-lasting humoral and cellular immune responses compared to anti-idiotypic antibody.[45]

Similar to CEA, KSA has been delivered via recombinant virus encoding the full-length antigen in metastatic CRC patients (using baculovirus-derived KSA) and in patients with no evidence of disease (using the avipox

virus ALVAC-KSA).[46,47] In both settings, the vaccine was administered with and without GM-CSF and elicited significant Ep-CAM–specific cellular immune responses. Interestingly, patients who received GM-CSF had the highest levels of cellular immune responses.

Mucin-Targeted Vaccines Mucins are glycoproteins present on the luminal surface of ductal epithelial cells and derived tumors such as CRC. MUC1 expression in CRC correlates with a worse prognosis.[48] MUC1 is hypoglycosylated and nonpolarized on tumors, exposing epitopes that can stimulate CTLs and thus making it an attractive antigen for cancer vaccines to target.[49] In several studies, patients with advanced CRC received peptides derived from MUC1 directly mixed with BCG, combined with cyclophosphamide, or pulsed on DCs.[50-52] Cellular responses to MUC1 stimulation in vitro were found in 28% of patients with or without cyclophosphamide, and strong T-cell IFN-γ responses were identified—via enzyme-linked immunosorbent spot (ELISPOT) assay results—in the DC-based vaccine. However, no objective clinical response was observed in CRC patients.

Cancer-Testis Antigen–Targeted Vaccines Cancer-testis antigens (CTAs) are selectively expressed in various types of human neoplasms, but not in normal tissues other than testis, making these antigens attractive targets for cancer vaccines.[53] Melanoma-associated antigens (MAGE) are CTAs selectively expressed in gastrointestinal carcinomas.[54] Patients with advanced gastrointestinal carcinomas (in the stomach, esophagus, and colon) were immunized with autologous DCs pulsed with MAGE-3 peptides in 2 clinical trials. After vaccination, minor tumor regressions were observed in some patients in both trials, in addition to the induction of peptide-specific CTL responses.[55,56]

Other Antigen-Targeted Vaccines Other expressed antigens in CRC have been identified, including CD55, SART3, and survivin. CD55 is a glycoprotein that protects CRC cells from attack by complement.[57] SART3 is an antigen that possesses 2 epitopes able to induce HLA-A24–restricted CTLs in CRC patients, and survivin is a member of the inhibitor of apoptosis protein family also expressed in CRC.[58,59] These antigens have been targeted in phase I and II trials with promising results.[60-65]

Multiple Antigen–Targeted Vaccines Targeting multiple antigens carries promise not only for inducing tumor immune response but also for achieving clinical efficacy. This approach has been investigated using both DC-based vaccines and co-stimulatory molecules engineered into vaccinia. Vaccination of metastatic CRC patients with DCs loaded with multiple major histocompatibility complex class I peptides derived from CEA, MAGE, and HER-2—as well as keyhole limpet hemocyanin protein and pan-DR epitope peptides—was able to induce immune responses to multiple TAAs. However, all patients showed progressive disease.[66] In another approach, 25 patients (including 10 patients with CRC) were treated with a poxviral vaccine regimen consisting of the genes for CEA and MUC1, along with a triad of co-stimulatory molecules (TRICOM) engineered into vaccinia (PANVAC-V, Therion) or fowlpox (PANVAC-F, Therion). Immune responses to MUC1 and/or CEA were seen in 9 of 16 tested patients. However, no clinical response was seen in any of the CRC patients.[67]

Non–Self-Antigen–Targeted Vaccines

p53 The most commonly mutated gene in human cancers (including CRC) is *p53*, which provides a unique target for immune therapy and vaccines.[68] We have demonstrated that immunologic responses can be generated against mutant p53 peptides in a population of heavily pretreated patients. Twenty-four patients with advanced malignancies that expressed mutant *p53* were vaccinated with the specific mutant *p53* (including 10 patients with CRC). Progression-free survival and postvaccination survival were longer than expected in 9 of 20 evaluable patients, with these individuals showing a statistically significant increase in the level of IFN-γ–producing specific T cells.[69] In another phase I/II trial, Van der Burg and coworkers evaluated the immune response to a recombinant canarypoxvirus vaccine (ALVAC, Aventis) encoding wild-type human *p53* in 15 patients with advanced CRC.[70] IFN-γ–secreting T cells against both ALVAC and *p53* were detected by ELISPOT assay without significant clinical response.[70]

RAS Another commonly mutated oncogene in CRC—found in 40% of cases—is *RAS*. The mutant oncogene produces an abnormal RAS protein distinct from the wild-type protein, making it another attractive oncogene to target in cancer vaccines.[71] In a phase I trial, administering a 13-mer peptide reflecting the patient's mutant RAS to 5 pancreatic cancer patients and 7 CRC patients with no evidence of disease demonstrated that the patient's mutant RAS was safe and that it produced specific immunologic responses in 5 of the 11 evaluable patients.[72] Therefore, a follow-up phase II trial was conducted using mutant RAS peptide as an adjuvant in fully resected Dukes C or D CRC patients. Three patients with Dukes C CRC have remained disease-free for 14–42 months.[73]

Combined Immunotherapy and Chemotherapy

There is now strong evidence that the immune system can be activated by chemotherapy via different mechanisms. Chemotherapy may enhance cross-presentation of tumor antigens in vivo, induce production of cytokines, and trigger immunomodulatory activities.[74] The synergetic clinical efficacy of this combination was evident in CRC whether the antigen was delivered by recombinant vaccinia virus or DCs. In patients with stage III CRC, the combination of oxaliplatin, capecitabine (Xeloda, Genentech), and CEA peptide–pulsed DCs induced nonspecific T-cell reactivity.[75] In the metastatic setting, potent 5T4-specific immune responses were induced in all 12 evaluable patients who were vaccinated with TroVax (Oxford BioMedica), which consists of modified vaccinia Ankara and encoded tumor antigen 5T4; this vaccine was administered before, during, and after treatment with 5-fluorouracil, leucovorin, and irinotecan.[76] Although these trials revealed encouraging results, a randomized trial comparing the combination of ALVAC-CEA/B7.1 vaccine and chemotherapy (5-fluorouracil, leucovorin, and irinotecan) to chemotherapy alone in patients with metastatic CRC failed to show differences in clinical or immune responses.[77]

Moreover, in an attempt to deplete regulatory T cells—which are thought to be responsible for down-modulating the immune response—cyclophosphamide was administered prior to vaccination with a plasmid DNA vaccine encoding CEA fused to a helper T-cell epitope (CEA66 DNA), which was combined with GM-CSF. The investigators found the vaccine to be well tolerated, with no signs of autoimmunity.[78]

Esophageal and Gastric Cancers

It has been reported that CTAs—such as GAGE, NY-ESO-1, and MAGE—are expressed in the upper gastrointestinal tract.[79,80] Of these CTAs, NY-ESO-1 has been targeted the most often in clinical trials. Vaccination with NY-ESO-1 protein in esophageal cancer patients increased specific CD4+ and CD8+ T-cell responses whether it was administered as a single peptide or in combination with other peptides, such as HER-2.[81-85] Furthermore, several clinical trials have been conducted in patients with advanced esophageal squamous-cell carcinoma using a combination of CTAs. Specific T-cell immune responses were observed in patients with esophageal squamous-cell carcinoma who were vaccinated with 3 HLA-A24–restricted epitope peptide CTAs; these antigens included TTK protein kinase (TTK), lymphocyte antigen 6 complex locus K (LY6K), and insulin-like growth factor–II mRNA binding protein 3. Vaccination was able to induce clinical responses in 5 of the 10 vaccinated patients.[86] In a similar approach, vaccination with LY6K and TTK in combination with CpG-7909 (VaxImmune, Hokkaido System Science) as an adjuvant successfully induced antigen-specific CD8+ T-cell responses, and stable disease remained in 5 of the 9 patients.[87]

In gastric cancer, targeting the gastrin peptide has been investigated in a multicenter, phase II study. Patients with untreated metastatic or unresectable gastric or gastroesophageal adenocarcinoma received G17DT (Aphton) vaccination—containing a 9-amino-acid epitope derived from the amino-terminal sequence of gastrin-17—and cisplatin plus 5-fluorouracil. Sixty-five of 94 patients were deemed to be immune responders based on 2 consecutive antigastrin antibody titers of at least 1 unit; these patients had a longer time to progression and a longer median survival rate compared to nonresponders.[88] In another trial, peripheral blood mononuclear cells (PBMCs) from gastric cancer patients were tested in vitro against 14 peptides on HLA-A24 and 16 peptides on HLA-A2. Patients were only vaccinated with peptides (a maximum of 4) that had proven their ability to induce HLA-A24–restricted or HLA-A2–restricted and tumor-specific CTL activity in PBMCs. Four of the 8 vaccinated patients had increased cellular and humoral immune responses to the vaccinated peptides in postvaccination PBMCs.[89]

Finally, increasing evidence has suggested that high-risk HPV infection is closely associated with esophageal squamous-cell carcinoma.[90] This discovery is worth further investigation that could aid in the development of a therapeutic HPV vaccine.

Pancreatic Cancer

Spontaneous immune response to pancreatic cancer has been suggested. CTL precursors that reacted to 13 peptides encoded by tumor-rejection antigens (SART1, SART2, SART3, and SART4) in HLA-A2+ or HLA-A24+ pancreatic cancer patients were detected in prevaccination PBMCs.[91] Furthermore, studies have identified several unique antigens that could potentially be targeted in pancreatic cancer (Table 3).[92,93]

Cellular-Based Vaccines

Jaffee and colleagues developed a novel allogeneic tumor cell–based vaccine expressing GM-CSF.[94] In a phase I trial, this vaccine increased delayed-type hypersensitivity responses to autologous tumor cells in 3 of 14 patients with resected pancreatic cancer. Immune responsive patients showed DFS for at least 25 months after diagnosis.[94] In a follow-up phase II study, a median survival of 26 months was reported when the same vaccine was used in combination with 5-fluorouracil and radiation therapy.[95]

Table 3. Phase II and III Studies of Therapeutic Vaccines for Pancreatic Cancer

Study	Type of vaccine	Adjuvant used	Cancer stage/ vector	N	Study design	Immune response	Clinical response
Lutz E, Yeo CJ, Lillemoe KD, et al[95]	Allogeneic tumor cell expressing GM-CSF		Resected	60	Surgery + vaccine + 5-FU + radiotherapy	Correlation between levels of mesothelin-specific CD8+ T cells and DFS	Median DFS of 17.3 months; median OS of 24.8 months
Brett BT, Smith SC, Bouvier CV, et al[96]	Antigastrin-17 mAb (G17DT)		Advanced	30	3 different doses	67% of patients have positive antibodies for G17DT	Median OS of 217 days for responders vs 121 days for nonresponders
Gilliam AD, Topuzov EG, Garin AM, et al[97]	Antigastrin-17 mAb (G17DT)		Advanced	154	Randomized to vaccine or placebo	N/A	Median OS of 151 days with vaccine vs 82 days with placebo
Kondo H, Hazama S, Kawaoka T, et al[103]	MUC1	DCs, CTLs	Unresectable	20	MUC1 peptide–pulsed DCs and MUC1-activated CTLs	Correlation between OS and CD83 level*	1 patient had CR; 5 patients had SD
Nordqvist C[104]	CEA, MUC1	GM-CSF, TRICOM, vaccinia, and fowlpox	Advanced	255	Randomized to vaccine or palliative chemotherapy	N/A	No change in OS
Middleton G[107]	Telomerase peptides (GV1001)	GM-CSF	Advanced	520	Randomized to vaccine or gemcitabine	N/A	No change in OS
Wedén S, Klemp M, Gladhaug IP, et al[109]	Mutant KRAS		Resected	23	Single arm	3 patients had memory response for up to 9 years	10-year OS rate of 20%
Shapiro J, Marshall J, Karasek P, et al[110]	Antigastrin-17 mAb (G17DT)		Advanced	383	Randomized to vaccine or placebo	Correlation between anti-G17 titers and OS	No clinical benefit
Wright JA, Osterlee J, Fekete S, et al[111]	Virulizin		Advanced	434	Randomized to vaccine or placebo	N/A	No change in OS

5-FU=fluorouracil; CEA=carcinoembryonic antigen; CR=complete response; CTLs=cytotoxic T cells; DCs=dendritic cells; DFS=disease-free survival; GM-CSF=granulocyte macrophage colony-stimulating factor; mAb=monoclonal antibody; MUC1=mucin1; OS=overall survival; SD=stable disease; TRICOM=triad of co-stimulatory molecules.

*The group with longer survival had higher expression of CD83 (a mature DC marker).

Antigen-Based Vaccines

Self-Antigen–Targeted Vaccines Antigen-specific immune responses have been elicited in pancreatic cancer patients vaccinated with different antigens, such as gastrin, Ep-CAM, CEA, and MUC1.[96-101] Moreover, clinical responses were demonstrated by using a MUC1 peptide–loaded DC-based vaccine as an adjuvant in 12 patients with resected pancreatic and biliary cancer; all of the patients remained without evidence of disease for a 1-year follow-up period.[102]

In the metastatic setting, the combination of MUC1 peptide–loaded DCs and CTLs sensitized with pancreatic

cancer cells expressing MUC1 also showed promising results. Five of 20 patients had stable disease, and 1 patient with multiple lung metastases had a complete response.[103] However, there were disappointing results in a phase III trial in which patients were treated with PANVAC-VF (Therion)—a vaccine composed of recombinant vaccinia virus and fowlpox virus expressing CEA, MUC1, and TRICOM—followed by GM-CSF. Vaccinating 255 patients with advanced pancreatic cancer failed to show an advantage in OS over standard palliative chemotherapy.[104]

Telomerase-Targeted Vaccines Recently, telomerase has been identified as an important target in pancreatic cancer due to its high expression and crucial role in the immortalization of cancer cells. Telomeres are noncoding repetitive DNA sequences at the end of chromosomes that are lost in most somatic cells but continue to be expressed in tumor cells, leading to synthesis of new nucleotide repeats.[105] GV1001 (Pharmexa)—a vaccine consisting of immunogenic telomerase peptides—induced immune responses in 24 of 38 evaluable patients with nonresectable pancreatic cancer when it was used in combination with GM-CSF.[106] GV1001 was further evaluated in a phase III, randomized, controlled, open-label trial (the PrimoVax trial) of 520 patients with advanced nonresectable pancreatic cancer. Patients were randomly assigned to 1 of 2 groups: gemcitabine or GV1001 and GM-CSF (followed by gemcitabine if the disease progressed). Preliminary data based on 174 deaths showed no survival advantage in the GV1001 group.[107] Another clinical trial (TeloVac) is currently investigating the combination of gemcitabine and capecitabine with concurrent and sequential chemoimmunotherapy using GV1001 in patients with locally advanced or metastatic pancreatic cancer.[108]

Non–Self-Antigen–Targeted Vaccines Similar to CRC, oncogenes such as *KRAS* have also been targeted in pancreatic cancer, as *KRAS* mutations are frequently found in adenocarcinomas of the pancreas (in 90% of cases). In the adjuvant setting, vaccinating pancreatic cancer patients with mutant RAS peptide corresponding to the tumor's *RAS* mutation has been shown to be feasible and has potential immunologic and clinical efficacy.[72] This efficacy was further investigated by Wedén and associates in patients who were vaccinated with mutant RAS peptide after surgical resection of pancreatic adenocarcinoma.[109] Seventeen of 30 patients responded immunologically; 4 of these patients survived for 10 years, with 3 of these patients retaining memory response for up to 9 years after vaccination.[109]

Combined Immunotherapy and Chemotherapy

In the metastatic setting, 2 phase III trials using combined modalities failed to show clinical efficacy. Gemcitabine monotherapy was compared to gemcitabine combination therapy with gastrin in the first trial; Virulizin (Lorus Therapeutics)—a mixture of proteins that have been extracted from bovine reticuloendothelial tissue that activates macrophages—was examined in the second trial.[110,111]

Hepatocellular Cancer

It has been suggested that immunotherapy may represent a valuable therapeutic option for HCC based on several factors: the spontaneous regression of the tumor in some patients, the reduction of HCC risk in chronic hepatitis B and C virus patients being treated with IFN-α, and the correlation between immune response and clinical outcome.[112] However, the majority of clinical trials have focused on prophylactic hepatitis B virus vaccination due to the established role of this virus in the development of liver cirrhosis that could lead to HCC. Vaccinating against hepatitis B virus infection has resulted in a significant reduction in the incidence of HCC.[113]

As for therapeutic approaches, no major clinical benefit has been found thus far. The expression of α-fetoprotein (AFP) in up to 80% of HCC patients has made it a classic target in clinical trials. Vaccination with AFP peptides pulsed onto autologous DCs in HLA-A*0201 patients with AFP-positive HCC elicited an AFP-specific T-cell response.[114] Other tumor antigens have also been identified in HCC, including MAGE, NY-ESO-1, human telomerase reverse transcriptase (hTERT), and glypican-3 (GPC3). It has been shown that patients with HCC have high values of serum hTERT mRNA, which correlate with tumor size and the degree of differentiation.[115] No GV1001-specific immune responses were detected after treating 40 advanced HCC patients with cyclophosphamide followed by GM-CSF and telomerase peptide (GV1001); 17 patients demonstrated stable disease 6 months after the initiation of treatment, with a decrease in regulatory T cells.[116] GPC3 is another overexpressed antigen in HCC; this antigen has been associated with an increase in the frequency of GPC3 peptide–specific CTLs after vaccinating HCC patients with HLA-A2–restricted GPC3 peptide.[117] Furthermore, the expression of MAGE-C1/CT-7 and GAGE in HCC and its correlation with reduced OS is worth further investigation.[118]

Conclusion

This paper reviews clinical trials conducted over the past 2 decades that have examined therapeutic vaccines for gastrointestinal malignancies. As outlined above, many strategies have been used, including autologous and allogeneic tumor cell–based vaccines and TAA-targeted vaccines, as well as different methods and routes of administration.

Specific immune responses were constantly generated in these trials and correlated with clinical responses in some cases. However, the vast majority of these trials were phase I or II and failed to show a significant improvement in clinical outcomes. The lack of significant clinical efficacy could be attributed to immune suppression or modulation by the host or to advanced disease on patient entry, which could prevent there being adequate time for the development of clinical efficacy. Clearly, the current direction of cancer vaccine development is combining the vaccine with immune modulators (to overcome immune suppression) and/or conventional chemotherapy to further enhance clinical efficacy. Nevertheless, our achievements thus far show promise for the future in terms of translating immune response into significant clinical efficacy.

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