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A Safety and Feasibility Study of an Allogeneic Colon Cancer Cell Vaccine Administered with a Granulocyte–Macrophage Colony Stimulating Factor–Producing Bystander Cell Line in Patients with Metastatic Colorectal Cancer

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Abstract

Background—Despite recent advances in earlier detection and improvements in chemotherapy, the 5-year survival rate of patients with metastatic colorectal carcinoma remains poor.

Immunotherapy is a potentially effective therapeutic approach to the treatment of colorectal carcinoma. Preclinical studies have supported the antitumor activity of immunization with a granulocyte–macrophage colony-stimulating factor (GM-CSF) producing murine colon tumor cell vaccine.

Methods—A novel colorectal cancer vaccine composed of irradiated, allogeneic human colon cancer cells and GM-CSF-producing bystander cells was developed and tested in combination

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with a single intravenous low dose of cyclophosphamide in a phase 1 study of patients with metastatic colorectal cancer.

Results—A total of nine patients were enrolled onto and treated in this study. Six patients had a history of colorectal adenocarcinoma hepatic metastases and underwent curative metastasectomy, while three other patients had unresectable stage IV disease. This study demonstrates the safety and feasibility of this vaccine administered in patients with metastatic colorectal cancer. At last follow-up, the six patients who underwent curative metastasectomy survived longer than 36 months, and four of these six patients were without disease recurrence. Immunologic correlate results suggest that the GM-CSF-producing colon cancer vaccine enhances the production of anti-MUC1 antibodies.

Conclusions—This vaccine is feasible and safe. Future investigation of the efficacy and antitumor immunity of this vaccine is warranted.

Despite excellent screening and preventative strategies, colorectal carcinoma remains a major public health problem in industrialized countries and a rising health problem in developing countries.¹ Surgical resection of the primary colorectal lesions combined with adjuvant chemotherapy and radiation, when indicated, remain the mainstay of therapy.² Unfortunately, approximately 30 % of these patients will be diagnosed with metastatic disease at initial presentation, and an additional 25–30 % of patients will subsequently develop metastatic disease.^{3,4} Despite recent advances in earlier detection and improvements in chemotherapy, the median survival for all patients with metastatic colorectal carcinoma is approximately 22–24 months, with 5-year survival still <5 %.³

Immunotherapy has been proven to be effective in the treatment of melanoma and prostate cancer and is also a potentially effective therapeutic approach in the treatment of colorectal carcinoma.^{5–7} Preclinical studies demonstrate the antitumor activity of immunization with a granulocyte–macrophage colony-stimulating factor (GM-CSF) producing murine colon tumor cell vaccine.⁸ In a phase 3 colorectal cancer vaccine trial, vaccine-based immunotherapy using autologous tumor and Bacillus Calmette–Guérin (BCG) in subjects with stage II or III disease demonstrated a survival advantage in the vaccine group among the stage II patients, but not among stage III patients.⁹ Unfortunately, autologous colorectal tumor cells are usually unavailable or not technically feasible to produce. The characterization of tumor-associated antigens in melanoma revealed that most tumors share common antigens regardless of human leukocyte antigen type.¹⁰ Recently completed phase 1/2 trials evaluating irradiated GM-CSF-producing allogeneic pancreatic cancer tumor vaccines have demonstrated both clinical and immunologic responses, further adding to the rationale of this approach.^{11–13}

GM-CSF is an important growth and differentiation factor for dendritic cells, which are potent antigen-presenting cells that can take up cellular proteins encoding for tumor antigens.¹⁴ For effective antitumor activity, preclinical studies have demonstrated that GM-CSF secretion must be at the site of vaccination and high levels of the cytokine must be sustained for several days.¹⁵ The tumor cells themselves however do not need to be the source of GM-CSF production. This notion is supported by a B cell lymphoma mouse model showing that GM-CSF bystander production by an allogeneic lymphoma cell line mixed

with autologous lymphoma cells results in equivalent systemic immunity to the GM-CSF gene-transduced autologous lymphoma cell vaccine.¹⁶ Thus, a GM-CSF-producing bystander cell line significantly improves the feasibility when it is used as a source of GM-CSF in the human studies of autologous or allogeneic tumor vaccines.¹⁶

Efficient immunization against cancer requires a vaccine capable of eliciting potent CD4⁺ and CD8⁺ T cell responses. However, tumors have evolved mechanisms to escape immune recognition.¹⁷ One well-characterized mechanism in human cancers is down-regulation of effector CD4⁺ and CD8⁺ T cells by CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs).^{18,19} Furthermore, elimination of these Tregs elicits potent antitumor immune responses leading to tumor eradication in many preclinical tumor models.^{20,21} A single intravenous low dose of cyclophosphamide given before each vaccination is currently the most common approach for Treg depletion in patients, and the 250 mg/m² dose has been tested in a vaccine trial for advanced pancreatic cancer patients with some success.¹² Subsequently, Emens et al.²² reported that a 200 mg/m² cyclophosphamide dose given with a GM-CSF-producing breast cancer vaccine induced improved immune responses compared with a 250 mg/m² dose. It is important to note that this dose of cyclophosphamide is much lower than the dose used for cytotoxic purposes.

We tested a novel colorectal cancer vaccine composed of irradiated, allogeneic colon cancer cells and GM-CSF-producing bystander cells in combination with a single intravenous low dose of cyclophosphamide in a phase 1 study of patients with metastatic colorectal cancer.

METHODS

Patient Selection

This was a single-institution study approved by the Johns Hopkins Medical Institution Review Board. Nine patients were enrolled at the Johns Hopkins Hospital (Baltimore, MD) between January 2009 and May 2011. Patients were considered eligible if they met the following criteria including: histologically confirmed adenocarcinoma of colon and/or rectum; radiographic evidence of metastatic disease; adequate hematologic, hepatic, and renal function; an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. Additional eligibility criteria are described in the Supplementary Material.

Study Design

The primary objective of the study was to evaluate the safety and feasibility of vaccination with two irradiated allogeneic colorectal carcinoma cell lines administered with a GM-CSF-producing bystander cell line in sequence with an immunomodulatory dose of cyclophosphamide. Secondary objectives included evaluation of immune response, disease-free survival, and overall survival (OS) in vaccinated patients. Toxicities were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events, v3.0. Dose-limiting toxicity (DLT) was defined as grade 3/4 nonhematologic adverse events and grade 4 hematologic adverse events.

The total vaccine dose was escalated from 5×10^7 to 2.5×10^8 per tumor cell line, and the GM-CSF-producing erythroleukemia cell line K562 was escalated from 4×10^7 to 2×10^8

(Table 1). These two dose levels were chosen because the same two dose levels were shown to be associated with better immune responses in the phase 1 study of the GM-CSF-secreting pancreatic cancer vaccine.¹¹ Patients received up to four monthly vaccinations administered intradermally.

The first three patients received an immunomodulatory dose of cyclophosphamide (250 mg/m² intravenously) 1 day before each vaccination. After Emens et al.²² reported that a 200 mg/m² cyclophosphamide dose induced improved immune responses compared with a 250 mg/m² dose, the remaining six patients received cyclophosphamide at 200 mg/m².

Vaccine Production

The investigational colon cancer vaccine consisted of three components: (1) SW837 cell line derived from a primary colorectal adenocarcinoma, (2) SW620 cell line derived from a lymph node metastasis of colorectal adenocarcinoma, and (3) K562/GM-CSF cell line, which is a K562 cell line derived from a chronic myelogenous leukemia patient in a blast crisis and subsequently transfected with a plasmid vector encoding human GM-CSF.¹⁶ Cryopreserved, irradiated vaccine cells were thawed the day of vaccination and mixed in a vaccine formulation consisting of the two colon cancer cell lines, SW620 and SW837, admixed in a 1:1 ratio, then admixed with the GM-CSF-producing K562 cells in a ratio of 5:2. The ratio of cancer cells to K562/GM-CSF cells was chosen so as to achieve a GM-CSF level per 10⁶ cancer cells approximating similar GM-CSF-secreting vaccines of other cancer types.^{11,23} Additional vaccine production information is provided in the Supplementary Material.

Statistical Analysis

This study was not designed to determine a maximum tolerated dose; however, a safety evaluation was performed following the standard 3 + 3 dose escalation approach with the two dose levels defined (Table 1). Study design and details of statistical analysis are provided in the Supplementary Material.

RESULTS

Patient Characteristics

A total of nine patients were enrolled and treated in this study. Three patients who had a history of hepatic metastases of colorectal adenocarcinoma and underwent curative metastasectomy were enrolled to the cohort of dose level 1 (DL1) (Table 1). None of the three patients experienced DLT. Six patients were subsequently enrolled to the cohort of dose level 2 (DL2). In this second cohort, three patients underwent curative metastasectomy for hepatic metastases of colorectal adenocarcinoma. To assess the feasibility and toxicity of administering the vaccine and low-dose cyclophosphamide on patients with unresectable metastatic colorectal adenocarcinoma, an additional three patients with unresectable metastatic colorectal adenocarcinoma were enrolled to the second cohort. None of these six patients experienced DLT.

Clinical characteristics of the total nine patients are summarized in Table 2. All six patients who underwent curative hepatic metastasectomy completed all four planned vaccinations. Their treatment schema is delineated in Fig. 1. One of the three patients with unresectable metastatic diseases completed all four vaccinations; the remaining two patients were taken off the study as a result of disease progression after receiving one vaccination.

Toxicity

No DLTs were observed. Local reactions at vaccination sites including erythema, induration, and pruritus were observed with every vaccination in all patients, and tenderness in five of nine patients, similar to those reported in patients who received GM-CSF-producing tumor vaccine of other disease types. Minimal systemic toxicities were observed (Table 3). The most common adverse effects were transient, low-grade fever/chill (55 %), fatigue (33 %), nausea (22 %), and headache (22 %). Fever and chills were likely due to vaccine treatment, while nausea was likely the result of cyclophosphamide administration. Headache may have been the result of ondansetron, which was provided as an optional and prophylactic antiemetic before the administration of cyclophosphamide. Grade 3 lymphopenia was transiently observed in one patient, which we attributed to the cyclophosphamide treatment. Thus, the higher dose level is considered to have an acceptable degree of safety and warrants subsequent trials to further assess vaccine associated clinical outcomes. In addition, cyclophosphamide at 200 mg/m² intravenously was well tolerated.

Serologic Response

Although a number of tumor-associated antigens are expressed in the colorectal cancer cell components of the vaccine, it is not known whether the vaccine targets these antigens and exactly which T cell epitopes are activated by the vaccine. Therefore, we first examined whether antibody response to these putative antigens were induced by the vaccine. Antigens with existing recombinant proteins, including EpCAM, S100A4, galectin-3, and MUC1, were chosen to test against the pre- and postvaccination serum by ELISA. As shown in Fig. 2, the titer of anti-MUC1 antibodies was significantly enhanced by the vaccine treatments in three of eight patients ($p < 0.05$) whose pre- and postvaccination sera were available. One patient (patient 1.009) showed a trend of enhancement in anti-MUC1 IgG production ($p = 0.10$). No enhanced antibody responses to EpCAM, S100A4, or galectin-3 were detected (data not shown).

Clinical Outcomes

At the time of last follow-up on April 30, 2014, the median OS of all nine patients after the first vaccination is 51.2 months, with a range of 1.2–64.0 months. All six patients who had liver-restricted metastases received adjuvant chemotherapy after partial hepatectomy. Four of these six patients remain free of disease at 36.2–53.5 months after their first vaccination. Although one patient was suspected to have disease recurrence as pulmonary metastases as early as 3.7 months, he died 38.4 months after the first vaccination. Another patient was found to have a new liver metastasis at 21.0 months after the first vaccination and therefore underwent partial hepatectomy. He remains free of disease at last follow-up. All three patients who had unresectable metastatic disease had received at least two lines of

chemotherapy, and had both hepatic and nonhepatic metastases. Two patients continued to have disease progression after receiving one or two vaccinations. The third patient was found to have disease progression after completion of all four vaccinations.

DISCUSSION

This study demonstrates the safety and feasibility of an allogeneic colon cancer cell vaccine administered with a GM-CSF-producing bystander cell line for patients with metastatic colorectal cancer. To our knowledge, this is the first study of a GM-CSF-producing colorectal cancer vaccine.

GM-CSF-producing whole cell vaccines, made from either autologous or allogeneic tumor cells, have been widely tested in preclinical models and clinical trials.^{8,21,22,24} Although a GM-CSF-producing vaccine did not demonstrate survival benefit in phase 3 studies of metastatic prostate cancer, antitumor responses have been observed in association with vaccine-induced immune responses in various preclinical cancer models and in clinical trials testing a similar vaccine in pancreatic cancer patients.^{8,12,13,21,27}

Although autologous tumor vaccine alone as an adjuvant therapy showed some promise in stage II/III colon cancer patients, its role as an adjuvant therapy when combined with chemotherapy remains to be determined. Of note, the majority of patients with stage IV disease are not resection candidates and therefore present without an easily accessible source of autologous tumor. Our study describes a novel vaccine formula using an allogeneic tumor vaccine for colorectal cancer immunotherapy. Also different from the previously reported vaccine approaches, a GM-CSF-producing bystander cell line was used as a source of local GM-CSF.^{12,13,28} It has been demonstrated that a sustained high level of GM-CSF at the vaccination site is important for the activation of antitumor immune response.¹⁵ This study adds to the experience with K562/GM-CSF and finds it to be safe and well tolerated. Moreover, this study has combined vaccine therapy with the administration of immunomodulating doses of cyclophosphamide and demonstrated the safety of this combination approach. It remains to be demonstrated in future clinical studies whether cyclophosphamide can deplete Tregs within colorectal tumors and enhance the infiltration of effector T cells into the tumors.

Our study initially targeted patients who underwent curative resection of hepatic metastases and demonstrated the safety of vaccine therapy in this patient population. Immunotherapy may play a bigger role in the treatment of stage IV disease with minimal residual disease rather than stage II/III colorectal cancer. Our study later expanded to unresectable metastatic disease, and all three patients had disease progression after receiving vaccine treatment. Induction of effective antitumor immune response may require multiple vaccinations. After the patients' disease fails to respond to multiple lines of chemotherapy, it may be more resistant to any antitumor immunotherapeutic interventions. Patients with a large burden of metastatic disease may not be ideal candidates for vaccine studies. This pilot phase 1 study reinforces that patients with minimal residual disease either after surgical resection of metastatic diseases or after effective chemotherapy may be better candidates for immunotherapy.

This study also provides limited evidence to support that the GM-CSF-producing colon cancer vaccine can moderately enhance the production of anti-MUC1 antibodies. In this study, the detection of anti-MUC1 antibodies using anti-IgG antibodies suggests that the production of anti-MUC1 antibodies involves T cells. However, it is not known whether the generation of anti-MUC1 antibodies accounts for antitumor immune responses. Interestingly, according to the literature, most of the colorectal cancer survivors have detectable, peripheral CD8⁺ T cells directed against two MUC1-derived epitopes tested, demonstrating that this protein represents one dominant target for CD8⁺ T cells in colorectal cancer.²⁹ MUC1-specific immunotherapy has also been proposed and tested in the preclinical models of colorectal cancer.³⁰ More recently, a multinational, multicenter, phase 2, randomized, double-blinded, placebo-controlled clinical trial testing a MUC1 targeting vaccine (L-BLP25) in patients with stage IV colorectal adenocarcinoma was initiated.³¹ Another recently completed phase 2 study compared immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 with and without GM-CSF in resected metastatic colorectal cancer.³² They reported that both vaccinate groups had similar survival; however, survival for vaccinated patients was longer than for a contemporary unvaccinated group. Our study, which is limited by the small sample size, was not able to assess the correlation between any immunologic measurement and clinical outcome. Whether MUC1 is a T cell target of our GM-CSF-producing colon cancer vaccine and whether there are other immune dominant antigens, particularly those whose immune responses correlate with clinical outcomes, require further investigation.

In summary, we present the first-in-human study of a GM-CSF-producing colon cancer vaccine and demonstrate the safety and feasibility of administering this vaccine to patients with metastatic colorectal cancer. Our findings indicate that future studies to investigate the efficacy and antitumor immunity of this vaccine are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIG. 1.
Study schema for patients who had metastasectomy

FIG. 2.

Enhanced production of anti-MUC1 IgG in postvaccination sera from 4 of 8 patients. ELISA assays to detect anti-MUC1 IgG antibodies were done with prestudy sera and the sera after the last vaccination from 8 individual patients. Three of 8 patients demonstrated significantly enhanced production of anti-MUC1 IgG in postvaccination sera compared to that in prevaccination sera, including patients 1.002 ($p < 0.01$), 1.004 ($p < 0.01$), and 1.008 ($p = 0.04$). One patient, patient 1.009, showed a trend of enhancement in anti-MUC1 IgG production ($p = 0.10$).

TABLE 1

Dose levels of colon cancer vaccine cells and GM-CSF-producing bystander cells

Dose level	No. of subjects	Total vaccine dose, colon cancer cells + GM-CSF-producing cells
1	3–6	$1 \times 10^8 + 4 \times 10^7$
2	6	$5 \times 10^8 + 2 \times 10^8$

TABLE 2

Demographic and clinical characteristics

Subject ID	Dose level	Age, y	Gender	Metastasectomy	No. of prior chemotherapy sessions ^a	No. of vaccines received	Disease status after vaccination	Survival, mo ^b	Enhanced anti-MUC1 antibody response
1.001	1	71	Male	Yes	1	4	Disease recurrence	Alive at 64.0 mo	No
1.002	1	57	Male	Yes	1	4	Disease recurrence	Died at 38.4 mo	Yes
1.003	1	30	Male	Yes	1	4	Disease-free	Alive at 53.5 mo	No
1.004	2	41	Female	Yes	1	4	Disease-free	Alive at 43.2 mo	Yes
1.005	2	50	Male	Yes	1	4	Disease-free	Alive at 37.8 mo	No
1.006	2	64	Male	No	4	1	Disease progression	Died at 1.2 mo	NA
1.007	2	51	Male	No	2	1	Disease progression	Died at 8.1 mo	No
1.008	2	68	Male	No	5	4	Disease progression	Died at 9.1 mo	Yes
1.009	2	43	Female	Yes	1	4	Disease-free	Alive at 36.2 mo	Yes ^c

^aNumber of prior chemotherapy sessions received after diagnosis of metastatic disease

^bCalculated from the date of the first vaccination

^cWith a non-statistically significant trend of enhancement in anti-MUC1 antibody response

TABLE 3

Vaccine- and cyclophosphamide-related systemic adverse events

Adverse event	CTC grade	No. of events	Patients with events, %
Fever/chill	1	7	55
Headache	1	6	22
Fatigue	1	4	33
Myalgia	1	1	11
Lymphopenia	3	1	11
Nausea	1	3	22

CTC common terminology criteria for adverse events