

Adjuvant Active Specific Immunotherapy for Stage II and III Colon Cancer With an Autologous Tumor Cell Vaccine: Eastern Cooperative Oncology Group Study E5283

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Purpose: A randomized phase III clinical trial of adjuvant active specific immunotherapy (ASI) with an autologous tumor cell–bacillus Calmette-Guérin (BCG) vaccine was conducted to determine whether surgical resection plus ASI was more beneficial than resection alone in stage II and III colon cancer patients.

Patients and Methods: Patients (n = 412) with colon cancer (297 with stage II disease, 115 with stage III disease) were randomly allocated to an observation arm or to a treatment arm in which they received three weekly intradermal vaccine injections of 10^7 irradiated autologous tumor cells beginning approximately 4 weeks after surgery. The first two weekly injections also contained 10^7 BCG organisms. Patients were observed for determination of time to recurrence and disease-free and overall survival.

Results: This was a negative study in that after a 7.6-year median follow-up period, there were no statistically significant differences in clinical outcomes between the treatment arms. However, there were dis-

ease-free survival ($P = .078$) and overall survival ($P = .12$) trends in favor of ASI when treatment compliance was evaluated, ie, patients who received the intended treatment had a delayed cutaneous hypersensitivity (DCH) response to the third vaccination (induration ≥ 5 mm). Also, the magnitude of the DCH response correlated with improved prognosis. The 5-year survival proportion was 84.6% for those with indurations greater than 10 mm, compared with 45.0% for those with indurations less than 5 mm.

Conclusions: When all randomized patients were evaluated, no significant clinical benefit was seen with ASI in surgically resected colon cancer patients with stage II or III colon cancer. However, there was an indication that treatment compliance with effective immunization results in disease-free and overall survival benefits.

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IN THE LATE 1970s, Hanna and Peters¹ established a guinea pig hepatocarcinoma model for the study of adjuvant forms of therapy. Through a series of experimental investigations, their group demonstrated (1) the value of a

vaccine prepared from tumor cells mixed with bacillus Calmette-Guérin (BCG); (2) the importance of a correct ratio of BCG organisms to tumor cells²; (3) the need for viable, metabolically active tumor cells³; and (4) an optimal vaccination schedule.⁴ Using this vaccine, they showed that it was possible to control spontaneously occurring hematogenous and lymphatic metastases from surgically excised primary tumors.⁵

On the basis of these preclinical studies, Hoover et al^{6,7} initiated a trial of active specific immunotherapy using an autologous tumor cell–BCG vaccine in patients with stage II and III colorectal cancer. After surgical resection of their tumors, patients were randomized to either a control or treatment arm and stratified by both disease type and stage. Three to 4 weeks after surgical resection, treated patients received one intradermal vaccination with 10^7 irradiated autologous tumor cells and 10^7 BCG organisms per week for 2 weeks and then one vaccination of 10^7 tumor cells alone in the third week. Development of immunity to the tumor was measured by serial delayed cutaneous hypersensitivity (DCH) skin testing with autologous tumor cells, compared with normal colon mucosa cells. An analysis of recurrence and survival in the treated and control colon cancer patients suggested improved survival and fewer recurrences in the treated group. On the basis of these

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findings, the Eastern Cooperative Oncology Group (ECOG) conducted a randomized phase III trial comparing surgical resection alone versus postoperative immunotherapy with an autologous irradiated tumor cell plus BCG vaccine in patients with surgically resected stage II and III colon cancer. This article reports the final results of that trial. Reports on the preliminary results of this trial have been presented previously.^{8,9}

PATIENTS AND METHODS

Patient Selection and Randomization

Eligible patients must have had a curative resection for adenocarcinoma of the colon of tumor-node-metastasis stage II or III (Astler-Coller modified Dukes' classification, B₂ and B₃ [stage II] and C₁, C₂, and C₃ [stage III]). The primary tumor was required to be above the peritoneal reflection, thus excluding those patients with cancer of the rectum. Patients with direct extension of their tumor into the abdominal wall, a loop of small bowel, or an adjacent organ were eligible for this study, provided that an en bloc resection created a tumor-free margin, as shown on microscopic examination. Patients who had intestinal obstruction requiring that a colostomy be performed before definitive resection of the primary tumor were eligible, provided that they were able to enter onto the study within 28 to 35 days after the definitive surgical resection. Patients with evidence of residual or metastatic tumor were ineligible.

To be eligible, patients were required to have an ECOG performance status of 0 or 1, a normal carcinoembryonic antigen (CEA) level obtained more than 21 days after resection, and adequate hematologic, hepatic, and renal function, within the following parameters: WBC count of 4,000/mm³ or greater; platelet count of 150,000 or greater; hemoglobin value of 10 g/100 mL or greater; blood urea nitrogen value less than 25 mg/100 mL; serum bilirubin value less than 1.5 mg/100 mL; AST level less than 40 IU; and alkaline phosphatase level within the normal range for the participating institution.

Stratification factors included the location of the primary tumor (right colon [ascending colon, including hepatic flexure], transverse colon, and left colon [including splenic flexure and descending and sigmoid colon]) and stage (tumor-node-metastasis stage II or III). Patients were randomized postoperatively to either observation (control) or treatment (vaccine) by active specific immunotherapy with an autologous irradiated tumor cell vaccine, with BCG as an adjuvant. Patients in the vaccine arm of the study began treatment 28 to 35 days after surgical resection. Patients were not randomized until there was postoperative histopathologic confirmation of their tumor and stage of disease and until their tumor sample had been shown to yield enough viable tumor cells to allow vaccine preparation. Signed informed consent was obtained from all patients.

Study Design

The original design of this study (EST 5283), as it was initiated in 1983, was a phase III adjuvant trial in patients with surgically resected stage III colon cancer. As a follow-up to the preclinical investigation of Hanna and Key,¹⁰ the original objective of the trial was to compare surgical resection alone with resection plus adjuvant treatment with the vaccine followed by fluorouracil (5-FU) therapy. Accrual onto this phase of the study was slow, and after 21 patients had entered onto it, a major revision was undertaken to include stage II patients and

eliminate 5-FU therapy. The study was then given a sequential design. According to the revised protocol, an evaluation of the two main end points of the study (disease-free survival and overall survival) was to be performed yearly, starting with the second year after the beginning of patient accrual. Because of the slower than anticipated accrual onto the study, the first interim analysis was performed in June 1989, 3 years into the revised study. Also, the North Central Cancer Group's fall 1989 publication of benefits from 5-FU treatment in combination with levamisole mandated closure of the study to stage III patients; accrual of stage II patients was also slower after that point. For this reason, a planned second interim analysis was delayed by 4 months and performed in November 1990. The third interim analysis was performed in June 1991 and the fourth in May 1992.

The results of all these first four interim analyses were inconclusive. A fifth interim analysis was performed in March 1993, and in June 1993, it was decided that accrual onto the trial stop. After continued follow-up until March 1997, a final planned analysis was performed. That analysis is the subject of this article. The results for the initial 21 patients entered onto the earlier design of EST 5283 are not presented because there were too few patients to allow any meaningful statistical evaluation.

Twenty-two ECOG member institutions contributed patients to this study. Surgical resection, tumor dissociation, and vaccine preparation and administration were performed at all these institutions. Initial patient evaluations included a medical history and physical examination; measurement of performance status, hemoglobin, WBC count, platelet count, blood urea nitrogen, creatinine, alkaline phosphatase, lactate dehydrogenase, AST, ALT, bilirubin, and CEA levels; prothrombin time; chest x-ray; proctosigmoidoscopy; and barium enema or colonoscopy. Patients underwent these evaluations at 3- to 6-month intervals over the next 1 to 5 years, with the exception of proctoscopy and barium enema or colonoscopy, which were performed at yearly intervals. Thereafter, all examinations were performed annually. Computed tomography (CT) scans of the abdomen or liver were obtained in cases in which clinical hepatomegaly was detected and/or when abnormal liver test results were reported. The existence of recurrent or metastatic disease (disease progression) was established if any of the following occurred: (1) any local recurrence detected by clinical or radiologic means and confirmed by biopsy; (2) hepatomegaly (after other nonmalignant causes were excluded) with a positive CT scan on two consecutive determinations performed 1 month apart; (3) hepatomegaly (once other nonmalignant causes were excluded) with a 50% or greater deterioration of liver function test results, including lactate dehydrogenase and alkaline phosphatase measurements, on two consecutive determinations performed 1 month apart; (4) pulmonary metastases, as determined by chest x-ray; (5) bone metastases, as determined by routine x-ray if previous x-rays of the suspected lesion were normal at the onset of the study (abnormal bone scans required confirmation by routine x-ray and/or biopsy); (6) ascites or pleural effusion with cytology results positive for malignant cells; and (7) a persistent rise in CEA titer of ten times the upper normal value, confirmed on two separate determinations performed at 1-month intervals in patients who had an initial normal CEA value on entering onto the study.

Vaccine Preparation

A description of the methods used for tumor procurement and vaccine preparation has previously been published.²⁻⁴ Approximately 3 to 4 g of fresh primary tumor tissue was generally adequate for vaccine preparation. The specimen, obtained under sterile conditions, was washed in sterile Hanks' balanced salt solution (HBSS) with gentami-

cin before being fragmented and dissociated enzymatically with collagenase type 1 and DNase. The tumor cells were concentrated in HBSS and suspended in an equal volume of chilled 15% dimethylsulfoxide and 1% human serum albumin-HBSS solution. The cells were frozen at a controlled rate of $-1^{\circ}\text{C}/\text{min}$ to the critical freezing point, flash-frozen through the heat of fusion and continued at $-5^{\circ}\text{C}/\text{min}$ to a final temperature of -80°C , and then stored in the vapor phase of liquid nitrogen. At the time of vaccination, tumor cells were rapidly thawed in a 37°C water bath, diluted in 1% DNase in HBSS, washed once with HBSS, and resuspended in HBSS for a total dose of x-irradiation of 20,000 rad. Cell viability was tested by the trypan blue exclusion test. TICE BCG (Organon Teknika Corp, Durham, NC) (10^7 organisms/0.1 mL) was added to the tumor cells (10^7 cells/0.1 mL) for a ratio of 1:1. Patients randomized to the treatment arm of the study received the vaccine intradermally in the right anterior thigh 28 to 35 days postoperatively. Vaccination was repeated 1 week later in the left thigh. The following week, 10^7 irradiated tumor cells without BCG were given intradermally in the right deltoid.

Vaccine Quality Control

All vaccines were subjected to a quality-control evaluation, which assessed the amount of tumor tissue obtained from surgery, total number of live and dead tumor cells, tumor characteristics, and percentage of viable cells. For a vaccine to be deemed "adequate," there must have been 6×10^7 viable tumor cells recovered from the dissociation, and the viability of the tumor cell component must have been $\geq 70\%$.

Statistical Analyses

Three outcomes were considered: time to recurrence, overall survival intervals, and disease-free survival intervals. Overall survival was determined as the time from randomization to death. Time to recurrence corresponded to time from randomization to confirmed recurrence of any malignancy. Disease-free survival interval was the time to recurrence or death of any cause. If a patient's disease recurred before they died, the time to recurrence was used in the analysis. Analyses were conducted in three different subsets of patients:

Intent-to-treat: All randomized patients (control group, $n = 207$; vaccine group, $n = 205$).

Analyzable: Eligible and participating patients who had any follow-up information regarding their disease statuses and whose vaccine preparations were classified as being "adequate" after quality-control review. All patients, including the controls, had vaccine preparations and were to be reviewed for quality control ($n = 153$, control; $n = 150$, vaccine).

Assessable: Patients in the treatment arm who were eligible, participating, had follow-ups, and whose vaccine preparations were classified as "adequate" after quality-control review. In addition, the dose of vaccine administered was adequate (all doses containing at least 10^7 irradiated viable tumor cells), and the manifestation of the effective vaccination for an immune response was demonstrated by a DCH reaction to the third vaccination of 5 mm or greater ($n = 153$, control; $n = 106$, vaccine).

Overall survival, disease-free survival, and time-to-recurrence curves were generated by the Kaplan-Meier method.¹¹ The log-rank test was used to compare the survival, disease-free survival, and time-to-recurrence distributions.¹² The k exact test was used to compare 5-year survival and disease-free survival proportions with the magnitude of the induration in response to the third vaccine's inocu-

lum.¹³ All statistical tests were two-sided and were performed using SASTM statistical software (SAS Institute Inc, Cary, NC).

RESULTS

Patient Characteristics

Four hundred twelve patients were randomized to the control (observation) arm ($n = 207$) or the vaccine arm ($n = 205$) of the study between March 1986 and June 1993. Two hundred ninety-seven patients had stage II colon cancer, and 115 patients had stage III. Eight patients assigned to the vaccine arm were removed from the study. One had stage III disease, and seven had stage II disease. Seven were removed because of patient refusals and one because the patient was found to be ineligible before treatment began. Thirty-seven (9%) of the remaining patients were declared ineligible. The most common reason for ineligibility was inadequacy of pretreatment laboratory tests.

The treatment arms were well-balanced with regard to patient characteristics, with the exception that 35% of subjects in the vaccine arm had more than three lymph nodes with histopathologic proof of tumor, compared with only 22% in the control arm (Table 1). There were no statistically significant differences between the treatment arms with respect to other prognostic factors, such as location of the primary tumor, tumor stage, and degree of differentiation.

Vaccine Quality Control

A case report form was completed for each enrolled patient describing the vaccine preparation for quality-control review. This form was submitted for 374 (91%) patients. Of these 374, 333 (89%) had vaccines that were deemed adequate after quality-control review. The percentage of viable tumor cells ranged from 72% to 99%, with a mean of 89% and an SD of 4.9%. The number of viable tumor cells cryopreserved per vial ranged from 1.0×10^7 to 8.6×10^7 , with a mean of 2.4×10^7 .

There was a total of 153 analyzable subjects (eligible and participating, with adequate vaccine preparation) in the control arm and 150 in the vaccine arm. Of the 150 in the vaccine arm, there were 106 who were also considered to be assessable (having had a DCH response to the third vaccine of ≥ 5 mm and having received all vaccine dosages of 10^7 irradiated viable tumor cells). The characteristics of the analyzable and assessable patient subsets are listed in Table 2. The distribution of the prognostic variables among these subsets was similar to that for the entire randomized population.

Table 1. Study Characteristics for All Randomized Subjects

Characteristic	Control Group		Vaccination Group		Total	
	No.	%	No.	%	No.	%
No. of subjects	207	50	205	50	412	100
Age, years						
Median	67		66		66	
Range	26-84		20-90		20-90	
Sex						
Male	110	53	106	52	216	53
Female	97	47	97	47	194	47
Unknown	0	0	2	1	2	1
Stage						
II	149	72	148	72	297	72
III	58	28	57	28	115	28
Location of primary tumor						
Right colon	100	48	94	46	194	47
Transverse colon	18	9	19	9	37	9
Left colon	89	43	92	45	181	44
Stage of differentiation						
Poor	26	13	21	10	47	11
Moderate	113	55	119	58	232	56
Well	34	16	29	14	63	15
Unknown	34	18	36	18	70	17
No. of positive nodes in stage III cancer						
≤3	45	78	36	63	81	70
>3	13	22	20	35	33	29
Unknown			1	2	1	1
No. of patients, by participation status						
Eligible	188	91	182	88	370	89
Ineligible	19	9	23	11	42	10
Removed	0		8*		8	

*Includes five ineligible patients.

Adverse Events

The only adverse event clearly attributable to the vaccine was a local reaction characterized by ulceration, drainage, and crusting, which was noted in 162 (79%) of 205 of the vaccinated patients and was described as mild, moderate, or severe in an equal number of patients in whom it occurred. In 15 patients, the vaccine site became infected; in four cases, the infection was described as mild, and in 11 cases, the infection was described as moderate. All patients with local infections responded to oral antibiotics. None of the infections became systemic.

Clinical Response

At the time of this report, a median follow-up period of 7.6 years has been completed by the 412 randomized patients. Table 3 summarizes the number of recurrences or deaths by treatment arm and stage in the three sets of patients evaluated.

On the basis of an intent-to-treat analysis of all randomized patients, there were no statistically significant differences between the two treatment arms in disease-free or overall survival or in time to recurrence (Fig 1). This intent-to-treat analysis was repeated within the two subsets of patients defined by tumor stage (tumor-node-metastasis stages II and III), with the same result (data not shown).

An analysis was conducted for analyzable patients (Fig 2). The Kaplan-Meier survival curves tended to separate, with vaccination being superior to observation, although they were not statistically significant. Because of the observed differences in the survival curves when the patients who had adequate vaccines were analyzed, there was concern that the quality of the vaccination might be impacting the results of the study. To test this hypothesis, we performed survival and disease-free survival explorative analyses between those assessable patients who had adequate vaccines as well as demonstrable antitumor immunity

Table 2. Study Characteristics for Analyzable and Assessable Patients

Characteristic	Analyzable				Assessable	
	Control Group		Vaccination Group		Vaccinated and With Effective Vaccination	
	No.	%	No.	%	No.	%
No. of subjects	153	51	150	49	106	41*
Age, years						
Median		67		65		63
Range		26-84		20-88		20-88
Sex						
Male	88	58	85	57	56	53
Female	65	42	65	43	50	47
Stage						
II	109	71	107	71	73	69
III	44	29	43	29	33	31
Location of primary tumor						
Right colon	71	46	71	47	47	44
Transverse colon	10	7	13	9	12	11
Left colon	72	47	66	44	47	45
Stage of differentiation						
Poor	17	12	17	11	16	14
Moderate	87	56	86	58	58	55
Well	28	18	20	13	11	10
Unknown	21	14	26	17	21	20
No. of positive nodes in stage III cancer						
≤3	39	89	27	63	19	58
>3	5	11	15	35	13	39
Unknown			1	2	1	3

NOTE. Analyzable patients were all eligible participating subjects who had had adequate vaccinations and follow-up visits. Assessable patients were those patients who had effective vaccinations with at least one diameter of induration ≥ 5 mm in the vaccine arm only.

*Percentage of analyzable controls. Percentages may not add up to 100% because of rounding.

(indurations to the third vaccine ≥ 5 mm) and the analyzable control patients whose vaccines were scored as adequate (Fig 3). In these explorative analyses, the curves tended to diverge even further, with treatment being superior to observation. Again, the results were not statistically significant, but by the log-rank test, the probability that the

vaccine regimen was superior to observation was .078 for disease-free survival and .12 for overall survival. This is in marked contrast to the intent-to-treat comparison of all randomized patients (Fig 1). When these explorative analyses were performed by tumor stage, no significant differences emerged (stage II overall survival and recurrence-free

Table 3. Summary of Events by Treatment Arm and Stage for All Group Comparisons

Group	All Subjects				Stage II				Stage III			
	Control		Vaccine		Control		Vaccine		Control		Vaccine	
	Events/Subjects*	%	Events/Subjects	%	Events/Subjects	%	Events/Subjects	%	Events/Subjects	%	Events/Subjects	%
All randomized patients												
Recurrence or death	84/207	40.6	85/205	41.5	50/149	33.6	53/148	35.8	34/58	58.6	32/57	56.1
Death	75/207	36.2	77/205	37.6	43/149	28.9	46/148	31.1	32/58	55.2	31/57	54.4
Analyzable patients												
Recurrence or death	63/153	41.2	54/150	36	35/109	32.1	32/107	29.9	28/44	63.6	22/43	51.2
Death	57/153	37.3	51/150	34	31/109	28.4	29/107	27.1	26/44	59.1	22/43	51.2
Assessable patients												
Recurrence or death	63/153	41.2	33/106	31.1	35/109	32.1	18/73	24.6	28/44	63.6	15/33	45.5
Death	57/153	37.3	31/106	29.2	31/109	28.4	16/73	21.9	26/44	59.1	15/33	45.5

*No. of events/no. of subjects.

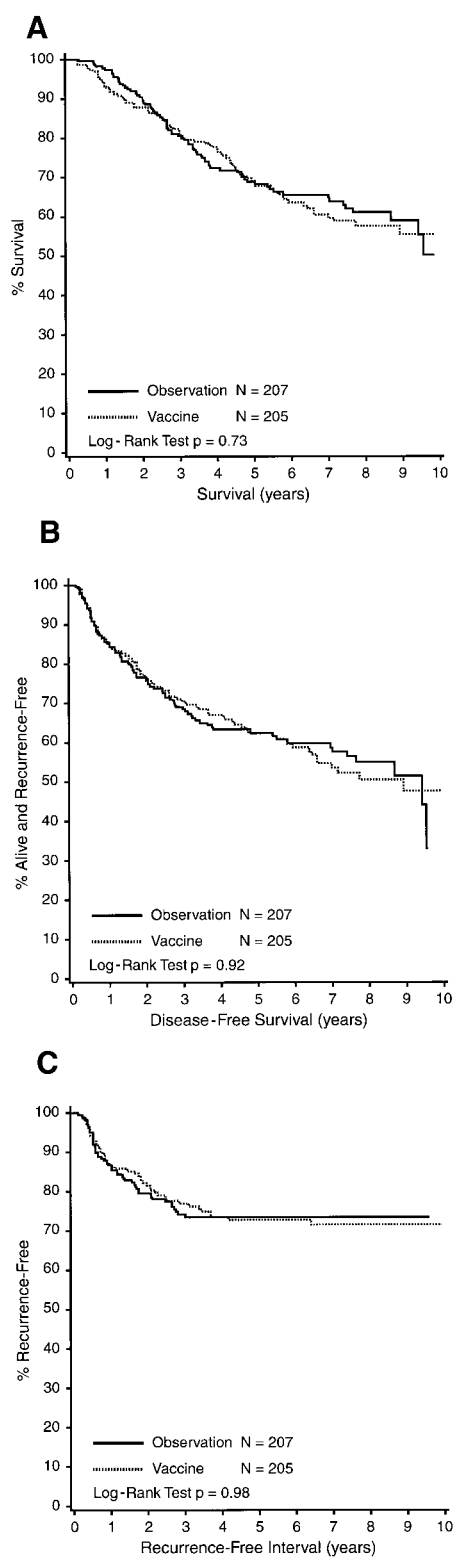


Fig 1. Survival (A), disease-free survival (B), and recurrence-free interval (C) for analyzable patients. Analyzable patients were eligible, participating patients (C) for all randomized patients.

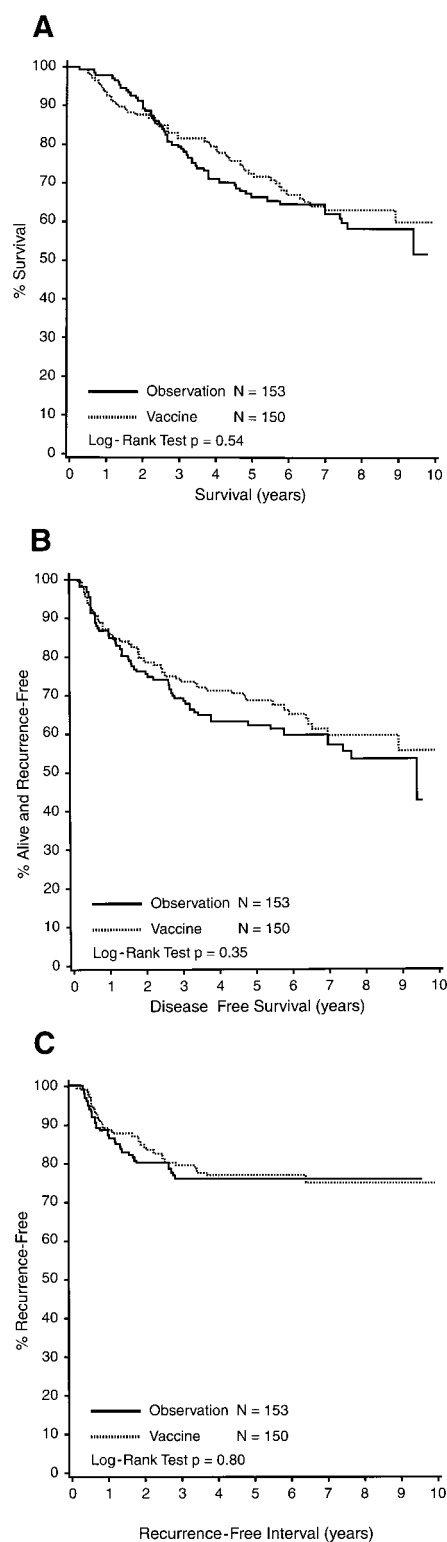


Fig 2. Survival (A), disease-free survival (B), and recurrence-free interval (C) for eligible patients. Eligible patients were eligible, participating patients who had follow-up information and adequate vaccine preparation.

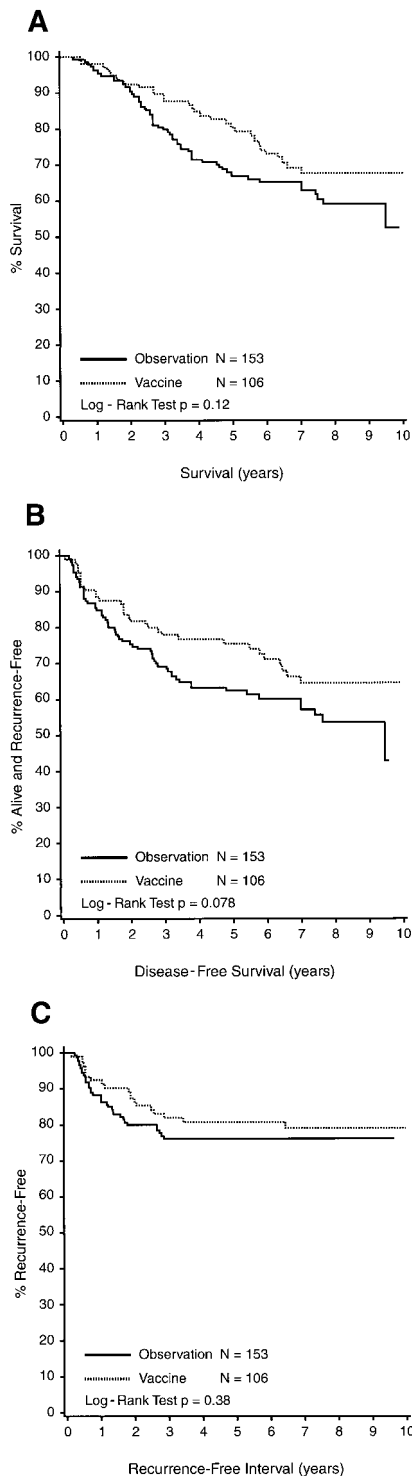


Fig 3. Survival (A), disease-free-survival (B), and recurrence-free interval (C) for assessable vaccine patients, compared with analyzable control patients. Assessable patients were eligible, participating patients who had follow-up information, adequate vaccines, and indurations to the third vaccine of 5 mm or greater in diameter.

survival log-rank $P = .23$ and $P = .19$, respectively; stage III overall survival and recurrence-free survival log-rank $P = .22$ and $P = .14$, respectively).

With the observation that effective immunization appeared to be associated with a survival benefit, an analysis was performed to determine whether clinical outcome correlated with the degree of the induration to the third vaccination. Disease-free and overall survival were compared for patients in three groups: those with indurations to the third vaccination of less than 5 mm, between 5 and 10 mm, and greater than 10 mm (Fig 4). Outcomes clearly improved in relation to increasing indurations in response to the third vaccine. The proportion of patients surviving 5 years with an induration response of less than 5 mm was 45.0%. With an induration response between 5 mm and 10 mm, it was 74.7%. With an induration response of greater than 10 mm, it was 84.6%. The ordered differences in the 5-year survival and disease-free survival proportions for these three subsets of subjects were statistically significant (k exact test $P = .003$ for overall survival and $P = .006$ for disease-free survival). The overall survival and the recurrence-free survival of the treated patients with indurations of less than 5 mm were not significantly different from those of the nontreated controls. These 15 subjects had characteristics similar to those of the entire population of 412 subjects. The only minor difference was that 80% (12 of 15) of these subjects had stage II disease, whereas 72% of the entire population had stage II disease. This would argue against a bias toward a poorer outcome in this subset, on the basis of population selection for this major prognostic factor (tumor stage).

The percentage of subjects who developed an induration to the third vaccine was essentially the same for stage II (85%) and stage III patients (92%). However, when overall survival in vaccinated patients whose induration responses to the third vaccine were less than 5 mm was compared with that in patients whose responses were 5 mm or greater, a statistically significant difference was seen in stage II patients (log-rank $P = .032$) but not in stage III patients.

DISCUSSION

The concept of using vaccines to induce specific immunity against carcinomas has been actively pursued over the last two decades. Early attempts to induce tumor regression in cancer patients by inducing tumor-specific immunity with autologous or allogeneic tumor cell vaccines were not successful. These early trials lacked adequate controls and were not always based on relevant preclinical studies. In addition, there was no demonstration that patients had been successfully immunized by either in vitro or in vivo specific immunologic responses. However, more recently, there

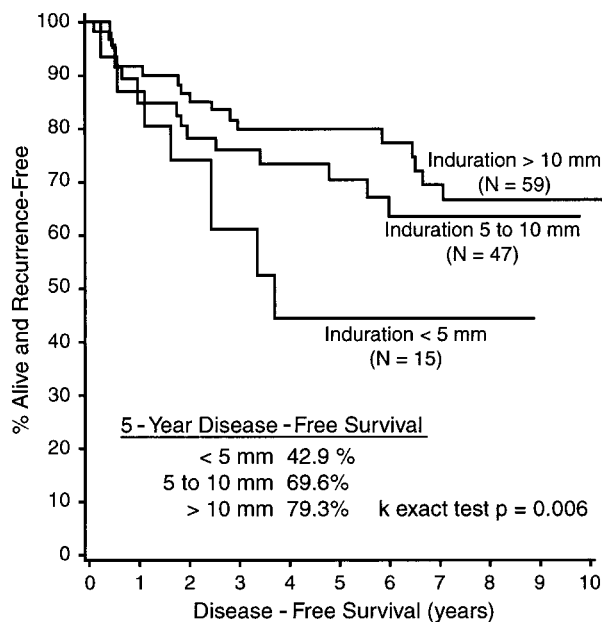
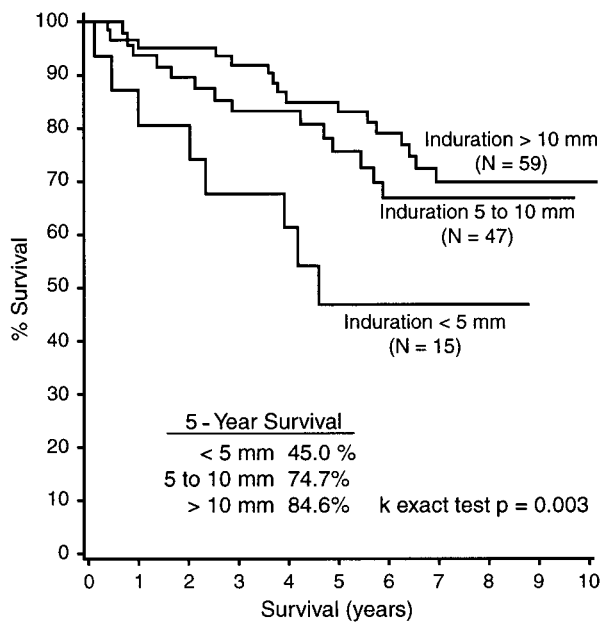


Fig 4. Survival and disease-free survival in patients grouped according to their DCH response to the third vaccine.

have been numerous, well-documented demonstrations of vaccine-induced immunity against cancer in animal models and evidence of clinical responses in humans that correlate with the development of specific antitumor immunity. This suggests that active specific immunotherapy could be a useful modality of treatment for cancer.

ECOG clinical trial EST-5283 was a technologically challenging study to be performed within the cooperative group setting. The goal of this clinical study was to compare the effects of autologous tumor cell vaccination after surgical resection with surgery alone in patients with stage II or III colon cancer. There is no record of a clinical cooperative group's attempting such a technologically challenging experimental trial for the management of colon cancer. The trial was established to attempt to confirm the earlier findings of Hoover et al,^{5,6} in which a similar regimen of autologous tumor cell vaccines was shown, in a limited number of patients, to have a statistically significant benefit in disease-free survival among a subset of assessable patients with colon cancer and adequate follow-up.

In an intent-to-treat analysis, which included all randomized patients, we found no significant difference in overall survival, disease-free survival, or in time to recurrence. The analyzable patients were a subset of 303 patients, which excluded ineligible patients, patients with no follow-up, and patients who were removed from the study, and included those patients whose vaccines met quality-control specifications. Although there was a trend toward treatment being superior to resection only, again there was no statistically significant difference. However, the study demonstrated that successful adjuvant active specific immunotherapy with an autologous tumor cell-BCG vaccine may be dependent on the quality and dosage of the vaccine. Also, there was a prognostic correlation with the ability of the vaccine to induce a significant tumor-associated immune response, as determined by DCH to a challenge of irradiated autologous tumor cells (third vaccine). In the subset of assessable patients, the improvement in disease-free survival of the vaccinated patients approached statistical significance ($P = .078$). Of course, these comparisons must be interpreted cautiously because the size of the induration is a post-randomization variable. Also, because patients on the observation arm were not vaccinated, there was no opportunity to assess induration in this group. Hence, it is not possible to rule out selection bias as an explanation of the results.

The most interesting finding of the ECOG study was the correlation of the magnitude of the DCH response to autologous tumor cells with survival and disease-free survival in the group of patients in whom the vaccine had adequate tumor-cell viability and tumor-cell dose to mediate an effective immune response. The 5-year survival and disease-free survival proportions correlated with the order of the induration response to the third vaccine of less than 5, 5 to 10, or greater than 10 mm (45.0%, 74.7%, and 84.6%, and 42.9%, 69.6% and 79.3%, respectively). These results provide two options for interpretation. Option 1 is that use of a vaccine that induces an immune response improves

patient survival and that this is just a prognostic factor of general immune competence. Option 2 is that use of a vaccine that does not produce substantial induration substantially decreases patient survival. With respect to the first option, the results of a study conducted by Hoover et al¹⁴ ruled against the immune response as a general prognostic factor. In their study, 29 patients were tested against standard recall antigens 1 week before vaccination and 6 weeks after vaccination. All patients reacted initially to at least one of the standard recall antigens. There was no significant change in reactivity in the follow-up period, except that all but two of the immunized patients converted to purified protein derivative–positive. This, plus the required performance status of 0 or 1 for patient eligibility, rules against anergy or overall health status accounting for prognosis being associated with immune status. With respect to the second option, a comparison of outcomes between patients with indurations of less than 5 mm with nontreated control patients showed no significant difference. Thus a reasonable conclusion is that correlation of outcomes with magnitudes of DCH responses is the cause-and-effect relationship because this correlation was statistically significant (overall survival *k* exact test $P = .003$; disease-free survival $P = .006$). Although the percentages of stage II and stage III subjects who developed indurations to the third vaccine were essentially the same, only vaccinated stage II patients—but not stage III patients—who developed indurations of 5 mm or greater to the third vaccination had a statistically significant survival benefit. This suggests that both tumor stage and the degree of tumor immunity are important factors in a therapeutic effect.

A number of other tumor-specific or tumor-associated immunotherapy studies of melanoma, B-cell lymphoma, and a variety of other cancers have shown a correlation of tumor-specific immunity with prognosis.¹⁵⁻²¹ It is important to consider that in vaccine studies, a relevant surrogate end point, such as DCH, will benefit investigational plans by determining whether patients received treatment in the manner conceived for the study. It is extremely relevant that there is a correlation between the cell-mediated immune response to the autologous tumor cell vaccine and survival and disease-free survival of the immunized patients. This correlation suggests that the immune response induced to the primary tumor is cross-reactive with tumor metastases.

It is possible that treatment compliance was a major factor related to the disparate results from this study and those reported by Vermorken et al.²² In the latter study, a centralized manufacturing laboratory supported the 11 participating institutions, in contrast to the 22 institutions that individually prepared vaccines for this ECOG trial. That the

vaccine preparation in the ECOG study was not optimal was evident from quality-control assessments. In the study presented here, 12% of all vaccines failed to meet quality-control specifications, and 15% of the patients who were vaccinated failed to have demonstrable antitumor immunity. In the Vermorken et al study, 94% of all vaccines administered (including all four vaccines in each patient) met quality-control specifications, and 98% of subjects developed an induration of 5 mm or greater to the third vaccine. This indicates that the decentralized method of manufacturing the vaccine in this cooperative group setting was problematic. It is also possible that a vaccine regimen which included a larger number of vaccinations over a longer period of time may have achieved a more positive result, as was the case in the Vermorken et al study.

This trial clearly supports the idea that to be immunologically effective, control of the vaccine preparation and the quality assurance that the vaccine meets specifications are of the highest priority and must be considerations in any future tumor cell vaccine study. With the limitations in methods that were adopted for this ECOG trial, no statistically significant clinical benefit was observed with the dose and schedule of the autologous tumor cell vaccine that was used. There was a clear indication that the DCH response may provide an estimation of efficacy in these studies during the first few weeks of treatment. This hypersensitivity response to the autologous tumor cell vaccine could be an effective surrogate end point in monitoring the treatment potential of the immunizations so that an indication of efficacy is obtained early in the treatment process.

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REFERENCES

1. Hanna MG Jr, Peters LC: Specific immunotherapy of established visceral micrometastases by BCG-tumor cell vaccine alone or as an adjunct to surgery. *Cancer* 42:2613-2625, 1978
2. Hanna MG Jr, Brandhorst JS, Peters LC: Active-specific immunotherapy of residual micrometastases: An evaluation of sources, doses and ratios of BCG with tumor cells. *Cancer Immunol Immunother* 7:160-173, 1979
3. Peters LC, Hanna MG Jr: Active-specific immunotherapy of established metastases: Effect of cryopreservation procedures on tumor cell immunogenicity in guinea pigs. *J Natl Cancer Inst* 64:521-525, 1980
4. Peters LC, Brandhorst JS, Hanna MG Jr: Preparation of immunotherapeutic autologous tumor cell vaccines from solid tumors. *Cancer Res* 39:1353-1360, 1979
5. Hoover HC Jr, Peters LC, Brandhorst JS, et al: Therapy of spontaneous metastases by an autologous tumor vaccine. *J Surg Res* 30:409-415, 1981
6. Hoover HC Jr, Surdyke MG, Dangel RB, et al: Prospectively randomized trial of adjuvant active-specific immunotherapy for human colorectal cancer. *Cancer* 55:1236-1243, 1985
7. Hoover HC Jr, Brandhorst JS, Peters LC, et al: Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-Year median follow-up of a phase III prospectively randomized trial. *J Clin Oncol* 11:390-399, 1993
8. Harris JE, Ryan L, Benson A, et al: Results of the ECOG trial of autologous colon carcinoma vaccine. *J Immunother* 14:358, 1993
9. Harris JE, Ryan L, Adams G, et al: Survival and relapse in adjuvant autologous tumor vaccine therapy for Dukes B and C colon cancer: EST 5283. *Proc Am Soc Clin Oncol* 13:295a, 1994 (abstr)
10. Hanna MG Jr, Key ME: Immunotherapy of metastases enhances subsequent chemotherapy. *Science* 217:367-369, 1982
11. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53:457-481, 1958
12. Peto R, Peto J: Asymptotically efficient rank invariant test procedures. *J Royal Stat Soc* 135:185-206, 1972 (series A; with discussion)
13. Mehta CR, Patel NR, Tsiatis AA: Exact significance testing to establish treatment equivalence with ordered categorical data. *Biometrics* 40:819-825, 1984
14. Hoover HC Jr, Surdyke M, Dangel RB, et al: Delayed cutaneous hypersensitivity to autologous tumor cells in colorectal cancer patients immunized with an autologous tumor cell: Bacillus Calmette-Guérin vaccine. *Cancer Res* 44:1671-1676, 1984
15. Bystryn JC, Oratz R, Harris MN, et al: Immunogenicity of a polyvalent melanoma antigen vaccine in humans. *Cancer* 61:1065-1070, 1988
16. McCune CS, O'Donnell RW, Marquis DM, et al: Renal cell carcinoma treated by vaccines for active specific immunotherapy: Correlation of survival with skin testing by autologous tumor cells. *Cancer Immunol Immunother* 32:62-66, 1990
17. Adler A, Gillon G, Hedwig L, et al: Active specific immunotherapy of renal cell carcinoma patients: A prospective randomized study of hormono-immuno-versus hormonotherapy—Preliminary report of immunological and clinical aspects. *Biol Res Mod* 6:610-624, 1987
18. Berd D, Maguire HC Jr, McCue P, et al: Treatment of metastatic melanoma with an autologous tumor-cell vaccine: Clinical and immunologic results in 64 patients. *Clin Oncol* 8:1858-1867, 1990
19. Restifo NP, Sznol M: Cancer vaccines, in DeVita VT Jr, Hellman S, Rosenberg SA (eds): *Cancer: Principles and Practice of Oncology* (ed 5). Philadelphia, PA, Lippincott-Raven, 1997, pp 3023-3044
20. Hsu FJ, Caspar CB, Czerwinski D, et al: Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma: Long-term results of a clinical trial. *Blood* 89:3129-3135, 1997
21. Morton DL, Foshag LJ, Hoon DSB, et al: Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. *Ann Surg* 216:463-482, 1992
22. Vermorken JB, Claessen AM, van Tinteren H, et al: Active specific immunotherapy for stage II and stage III human colon cancer: A randomised trial. *Lancet* 353:345-350, 1999