Advances in Brief

Active Specific Immunotherapy with a β-Human Chorionic Gonadotropin Peptide Vaccine in Patients with Metastatic Colorectal Cancer: Antibody Response Is Associated with Improved Survival[1]

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Abstract

Purpose: **Human chorionic gonadotropin (hCG) is produced by colorectal cancers and may play a role in its progression. The clinical and immunological effects of a synthetic vaccine targeting β-hCG composed of the COOH terminal peptide of β-hCG (CTP37) conjugated to diphtheria toxoid (DT) was investigated in patients with metastatic colorectal cancer.**

Experimental Design: **Seventy-seven patients from 12 centers were randomly divided into two vaccine dose groups. CTP37-DT was formulated in an emulsion and administered i.m. on days 0, 28, and 70. Patients were evaluated for toxicity, overall survival, and antibody response to hCG and to DT.**

Results: **Immunizations were well tolerated with no patients requiring cessation of the injections. Anti-hCG antibody was detected in 56 of the 77 patients treated. Significant differences in antibody response and survival were not observed between the two dose groups. An intention-to-treat analysis of all vaccinated patients showed a median survival of 34 weeks. Patients who developed anti-hCG antibody levels higher than or equal to the median value exhibited a median survival of 45 weeks compared with 24 weeks for patients who developed anti-hCG antibody levels lower than the median ($P = 0.0002$). In contrast, no significant difference was observed when comparing survival based upon the level of DT antibody that developed ($P = 0.80$).**

Conclusions: **Vaccination with CTP37-DT induced anti-hCG antibodies in most patients with advanced colorectal cancer. Anti-hCG antibody induction was associated with longer overall survival. CTP37-DT has an excellent safety profile and warrants further study in patients with colorectal cancer.**

Introduction

Colorectal cancer is one of the most commonly occurring malignancies in the world. It is a disease that kills nearly half of those afflicted within 5 years of initial diagnosis. Chemotherapy with 5-fluorouracil and leucovorin in the surgical adjuvant setting for patients with lymph node-positive, stage III colon cancer has been shown to improve survival (1, 2). Similar improvements in survival have been observed for patients with stage III rectal cancer administered 5-fluorouracil-based chemotherapy combined with radiotherapy in the surgical adjuvant setting (3, 4). A substantial proportion of these patients, 40–50%, will still develop recurrences and die of cancer. Virtually all patients with metastatic stage IV colorectal cancer will die of their cancer. Chemotherapy with 5-fluorouracil, leucovorin, and irinotecan has been shown to improve survival duration (5, 6). This improvement, however, is modest, and this chemotherapy program can be associated with significant toxicity, such as diarrhea, neutropenia, and vomiting. Novel treatment modalities are needed.

ASI[3] has been an attractive approach to the management of colorectal cancer, and a number of vaccine formulations are under investigation (7–10). Several lines of evidence support the use of hCG as a target. hCG, a glycoprotein hormone composed of two noncovalently linked polypeptide subunits, α and β, is frequently expressed by colorectal tumors. Although results have varied, tumor membrane-associated hCG, as determined by immunohistochemistry, has been reported in up to 52% of colorectal cancer patients (11–19), and elevated levels of circulating hCG have been observed in up to 41% (19–26). In contrast to other oncofetal antigens being targeted, such as carcinoembryonic antigen, hCG does not appear to be produced by normal colorectal cells. Positive staining for hCG was not found in normal colorectal epithelium/mucosa (12, 14, 16, 18, 19) or in benign colorectal lesion specimens (12). hCG production by nontrophoblastic tumors has been associated with a more aggressive course. In some studies, expression of hCG in colorectal cancer has been associated with reduced survival, directly (14, 18, 19) and indirectly through a correlation with poor histological differentiation (12), tumor invasiveness (11, 12, 18), and a higher incidence of metastases (15). hCG has been shown to promote tumor growth, and autocrine/paracrine stim-

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[3] The abbreviations used are: ASI, active specific immunization; hCG, human chorionic gonadotropin; CTP, COOH terminal peptide; DT, diphtheria toxoid.

ulation of tumor growth by endogenously produced hCG has been suggested (27–29). hCG can also promote neovascularization (30, 31) and suppress immune responses (32). These actions may be mediated by the cysteine-knot structural motif contained in β-hCG, which is nearly identical to that found in transforming growth factor-β2 and platelet-derived growth factor-β, factors that have been ascribed similar roles in oncogenesis (33). Thus, immunization against hCG may result in humoral and/or cellular immune effectors capable of directly lysing tumor cells expressing hCG. In addition, neutralization of soluble hCG with antibody may abrogate hCG-mediated tumor growth signals, angiogenesis, and immune escape phenomena (34).

CTP37-DT is a synthetic subunit vaccine composed of a 37-amino acid peptide from the COOH terminal end of β-hCG conjugated to the carrier, DT. Clinical studies of CTP37-DT as an approach to block fertility began over a decade ago (35). More recently, clinical trials have evaluated CTP37-DT vaccine in patients with nontrophoblastic cancer. These initial Phase I studies demonstrated an excellent safety profile and established the immunological activity of the vaccine. ASI with CTP37-DT induced anti-hCG antibody in patients with a variety of advanced, nontrophoblastic cancers, including patients with colorectal cancer whose tumors expressed hCG (36, 37). We performed a randomized Phase II trial of CTP37-DT in patients with metastatic colorectal cancer. The objective of this study was to examine the clinical significance of the induction of anti-hCG antibody, specifically whether antibody induction was associated with longer survival duration. In addition, this clinical trial compared the clinical and immunological effects of two doses of vaccine to establish a dosing regimen for further study.

Materials and Methods

Eligibility Criteria. Eligibility criteria included a histological diagnosis of adenocarcinoma of the colon or rectum, resection of the primary tumor, stage IV disease, a Southwest Oncology Group performance status of 0, 1, or 2, and adequate bone marrow, renal, and hepatic function (total bilirubin less than the upper limit of normal range). If prior chemotherapy had been given, it had to be completed for at least 4 weeks. Patients were also required to manifest a positive skin test for delayed-type hypersensitivity to one or more of six common recall antigens (*Candida*, DT, tetanus toxoid, streptokinase, trichophytin, and tuberculin). Patients with positive immediate-type hypersensitivity skin tests to DT were excluded, as were patients receiving concurrent chemotherapy or corticosteroid therapy; patients who were pregnant, had cerebral metastasis, human immunodeficiency virus, hepatitis, or any concurrent active second malignancy except basal cell carcinoma of the skin or cervical squamous carcinoma and patients who had undergone splenectomy.

Vaccine Formulation and Immunization Schedule. The vaccine was prepared as a hand-made emulsion of squalene: mannide monooleate vehicle in a ratio of 4:1 (v/v) formulated with a CTP37-DT conjugate (25 molecules CTP37 per 10^5 Da of DT) to nor-muramyl dipeptide adjuvant ratio of 20:1 (w/w) dissolved in sterile saline. The injection volume was 0.4 ml for a 0.5-mg dose, 0.8 ml for a 1.0-mg dose, and 1.6 ml for a 2.0-mg dose (based upon conjugate weight). The low-dose regimen consisted of 0.5 mg of vaccine on days 0, 28, and 70. The high-dose regimen consisted of 2.0 mg of vaccine on day 0, followed by 1.0 mg on days 28 and 70. All vaccine doses were administered i.m. Patients who did not manifest progressive cancer at week 16 were eligible for vaccinations at weeks 16 and 24. Patients who did not manifest progressive cancer at week 24 were eligible for additional bimonthly vaccinations under an extension protocol approved by the Food and Drug Administration.

Study Design. The study was conducted at 12 clinical sites in the United States as an open, randomized trial. The study was approved by each center's Institutional Review Committee. All patients signed the approved informed consent form before study participation. Patients were randomly enrolled into one- of two-dose schedule regimens by a centralized randomization center. The patient was considered enrolled in the study when he/she first received treatment with the vaccine. Patients were monitored for clinical and laboratory evidence of toxicity, which was graded using National Cancer Institute Common Toxicity Criteria.

Detection of Antibodies against hCG. The level of anti-hCG antibodies produced was measured by determining the binding of ^{125}I-labeled hCG to various dilutions of sera collected at intervals after immunization, as described previously (35). In brief, purified hCG was obtained from the United States National Institute of Child Health and Human Development as batch CR-121 and iodinated. Dilutions of preimmune and postimmune sera were prepared in PBS containing 0.05 M edetic acid and 20% normal FCS. These dilutions were tested for binding to 5 ng/ml hCG. Quadruplicate samples of 50 μl of each dilution, 50 μl of labeled antigen, and 50 μl of 1% BSA/PBS were incubated at 4°C for 96 h before the addition of 50 μl of 50% FCS/PBS and 200 μl of 25% polyethylene glycol. After centrifugation at 1500 × *g* for 15 min, the supernatants were decanted, and the precipitates were counted. The binding in preimmune sera from each patient was used to calculate nonspecific binding for hCG. Results are expressed as the amount of hCG bound in nmol/l of undiluted serum (nM). Antibody titers with ≥1 nM were considered as positive for hCG. All patient serum samples were coded, and clinical information was not made available to the individuals conducting the assay until the assay was completed. This was the case for all subsequent assays.

Detection of Anti-DT Antibodies. A standard solid phase, indirect ELISA was used for analysis of patient antisera for anti-DT antibodies. Ninety-six-well microtiter plates were coated with purified DT (Pasteur Merieux Connaught, Toronto, Ontario, Canada) at a concentration of 1 μg/ml in PBS. Serial 2-fold dilutions of patient antisera were added to the wells in PBS/1% BSA and 0.1% Tween 20 and incubated for 1 h at room temperature. Wells were washed with PBS/0.1% Tween 20, followed by the addition of a goat antihuman IgA, IgG, IgM horseradish peroxide conjugate (Kierkegaard and Perry Laboratories, Gaithersburg, MD) for 45 min. After well washing with PBS/0.1% Tween 20 (twice) and distilled water (twice), plates were developed by the addition of chromogen (ABTS; Kierkegaard and Perry Laboratories) for 15 min and subsequently analyzed for absorbance at 405 nm. The results for all patient samples are reported as absorbance at a selected dilution (1:

Table 1 Patient characteristics of the low- and high-dose treatment groups

	Low-dose group	High-dose group
n	36	41
Sex		
Male	24 (67%)	24 (59%)
Female	12 (33%)	17 (41%)
Age		
Median (yr)	62	64
Range (yr)	33–78	29–83
<65 yr	21	21
>65 yr	15	20
Primary site		
Colon	24	26
Rectum	10	11
Not available	2	4
Performance status		
0	26 (72%)	24 (59%)
1	9 (25%)	14 (34%)
2	1 (3%)	3 (7%)
Stage at time of diagnosis		
III	14 (39%)	17 (42%)
IV	12 (33%)	16 (39%)
Other/Not available	10 (28%)	8 (20%)
Prior chemotherapy	32 (89%)	34 (83%)
Recall antigen		
Number positive (mean)	2.2	2.2
Induration (mean in mm)	13	14
Serum hCG		
<5 mIU/ml	31 (86%)	37 (90%)
>5 mIU/ml	5 (14%)	4 (10%)

32,000), which occurred in a linear titration range. A pool of normal human sera (Sigma) from different lots was used as the background sample. Antisera with absorbance value <0.1 at 1:32,000 dilution were considered to have anti-DT antibody titer below the background level.

Statistical Analysis. Clinical sample handling and coding and data management were done by Arkios BioDevelopment International (Cincinnati, OH), an independent organization. GraphPad PRISM software was used for analysis of antibody response data, which involved Kaplan-Meier curves to display survival and log-rank tests to compare survival. χ^2 tests were used to evaluate patient characteristics and toxicity incidence. $P < 0.05$ was considered to be statistically significant.

Results

Patient Characteristics. Seventy-seven patients with stage IV colorectal cancer were randomly assigned to a low-dose or high-dose CTP37-DT vaccination regimen between July 11, 1996 and November 18, 1997 (Table 1). There were no significant differences between the two dose groups in terms of patient sex, age, primary site, performance status, stage at the time of diagnosis, response to recall antigens, or serum hCG levels. Prior chemotherapy was also similar in both groups. The majority of patients had been treated with 5-fluorouracil, frequently in combination with leucovorin and/or levamisole. Eight patients in the low-dose group and seven in the high-dose group had regimens that included irinotecan. Mitomycin C, floxuridine, trimetrexate, and melphalan were used in 7 patients.

Table 2 Incidence of adverse events related to CTP37-DT vaccine ($n = 77$)

Adverse event	Low-dose group *n* (%)	High-dose group *n* (%)
Injection site reaction	15 (42%)	22 (54%)
Nausea and/or vomiting	5 (14%)	6 (15%)
Fatigue	6 (17%)	9 (22%)
Fever	5 (14%)	9 (22%)
Diarrhea	1 (3%)	5 (12%)
Flu-like symptoms	7 (19%)	3 (7%)
Myalgia	6 (17%)	4 (10%)
Rigors (chills)	2 (6%)	6 (15%)
Bone pain	1 (3%)	1 (2%)
Headache	2 (6%)	4 (10%)
Malaise	1 (3%)	2 (5%)
Abdominal pain	0 (0%)	2 (5%)
Asthenia	1 (3%)	3 (7%)
Dizziness	2 (6%)	2 (4%)

Only 2 patients, both in the low-dose group, received specific cancer treatment after randomization within the follow-up period of the study. One patient received irinotecan, and another had radiation therapy for a metastatic lesion.

Toxicity Profile. In general, the vaccinations were well tolerated with no patients requiring cessation because of toxicity. Fifty-three of the 77 (69%) patients reported an adverse experience or multiple adverse experiences possibly or probably related to CTP37-DT vaccination. Table 2 summarizes the events attributable to the study drug for low- and high-dose groups. None of the differences between the low- and high-dose groups were statistically significant. Injection site reactions were, for the most part, mild (grade 1) and consisted of pain and erythema. Two patients treated in the high-dose group, however, did develop abscesses at the injection site (grade 2). These resolved without specific treatment. One elevated WBC count (grade 1) was attributed to the vaccination treatment. Other adverse experiences reported were also mild (grade 1) and consisted primarily of constitutional symptoms normally associated with immunotherapy, such as fever, chills, malaise, and myalgia. None of the patients developed immediate-type hypersensitivity reactions to DT.

Antibody Response and Survival of the Low- and High-Dose Vaccination Groups. Significant hCG antibody response was not observed during the first 10 weeks of immunization in either the low- or high-dose group. At week 10, after the third immunization, antibody levels increased in both dose groups. The range of peak antibody production was 1–184 nmol of hCG bound per liter of undiluted serum (nM). The mean levels in the high-dose group were generally higher than in the low-dose group, although the difference was not statistically significant ($P = 0.09$ for week 1, $P = 0.56$ for week 12; Fig. 1). The same trend was seen in the response to DT in comparing the low- and high-dose groups (data not shown). Comparison of Kaplan-Meier survival curves demonstrated no significant difference in median survival between the low-dose (38.7 weeks) and high-dose (33.4 weeks) vaccination groups ($P = 0.17$; Fig. 2).

We investigated whether survival correlated with anti-hCG antibody levels for low-dose and high-dose groups. We found

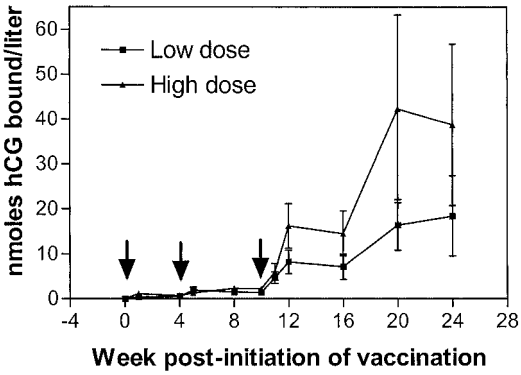

Fig. 1 Antibody response in the low- and high-dose groups after vaccination. Values are expressed as the means; *bars*, SE. *Arrows*, vaccination time points.

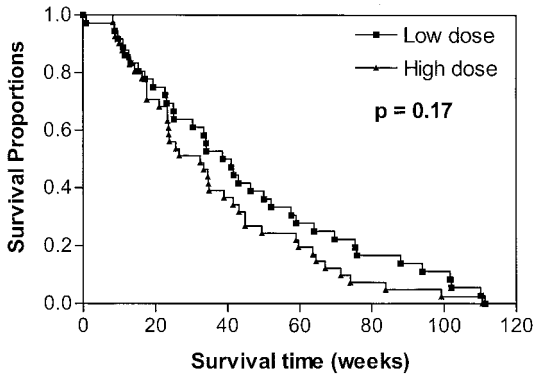

Fig. 2 Comparison of patient survival curves for the low- and high-dose groups.

that there is no clear relationship between survival and antibody production in either the low-dose or high-dose group. Poor correlation between these two parameters was seen in both groups; the correlation coefficients (r) were 0.29 and 0.08 for low- and high-dose groups, respectively.

Relationship between Antibody Levels and Survival. Because there was no significant difference in anti-hCG antibody levels between the two dose groups, all 77 vaccinated patients were examined as a single, intention-to-treat population to ascertain the relationship between antibody response and survival. Overall, 56 of the 77 patients (73%) responded to the vaccine by producing anti-hCG antibodies. Median survival of all vaccinated patients in both dose groups was 34 weeks. The median antibody level was used as a cutoff point to delineate between two populations: a "low responder" group composed of patients with antibody levels less than the median, which included antibody nonresponders, and a "high responder" group with antibody levels greater than, or equal to, the median value. Using this analysis, it was found that the high responder group experienced a significantly longer median survival time of 44.9 weeks compared with the low responder group at 23.6 weeks ($P = 0.0002$; Fig. 3*A*). In contrast, when antibody response to the DT carrier portion of the vaccine was analyzed in an identical manner, median survival time was not found to be significantly different between high and low anti-DT antibody responders ($P = 0.80$; Fig. 3*B*). The clinical characteristics of the low and high anti-hCG antibody responders are displayed in Table 3. Relatively more females were in the low-responder group ($P < 0.05$). Low and high responders did not differ with regard to the other parameters evaluated, including prior chemotherapy and performance status.

An additional analysis was performed that excluded patients who did not survive to the 16-week vaccination time point and as a result were not afforded enough time and/or boosts to produce a significant antibody response against hCG. The 62 patients who did survive past the 16-week time point were analyzed as above. Those patients who developed anti-hCG antibody levels higher than or equal to the median value exhibited a median survival of 44.9 weeks compared with 38.7 weeks for patients who developed anti-hCG antibody levels lower than the median ($P = 0.01$; Fig. 4*A*). As in the intention-to-treat analysis, no significant association was observed when comparing the antibody response against DT ($P = 0.48$; Fig. 4*B*).

Relationship between serum β-hCG Level and the Development of Anti-β-hCG Antibodies. Of the 77 patients, 9 had serum β-hCG above what is normally considered background levels, 5 mIU/ml, before vaccination (range, 10–34 mIU/ml). Anti-β-hCG antibody was induced in 7 of these patients. The β-hCG levels of 2 patients that did not respond were both 10 mIU/ml. One patient's serum β-hCG dropped from 12 mIU/ml to below background level as the anti-β-hCG antibody level increased. Levels of serum β-hCG did not increase/decrease in the other six patients that developed anti-β-hCG antibody with vaccination.

Relationship between Antibody Responses to hCG and to DT. A positive but not significant correlation ($r = 0.38$) was seen between the antibody response to DT and to hCG. There were 16 (21%) patients who had anti-DT antibody titer below the background level, but 9 of the same 16 patients produced a measurable amount of anti-hCG antibodies. Eleven of the 21 patients who did not produce antibodies to hCG produced antibodies to DT.

Discussion

We examined the clinical and immunological effects of ASI with a β-hCG peptide vaccine in patients with metastatic colorectal cancer. ASI with CTP37-DT generated a humoral immune response against hCG protein in 73% of the patients enrolled. Two analyses were performed to examine the relationship between antibody response and patient survival. That anti-hCG antibody induction was associated with an improved survival was evident with both. The first involved an intention-to-treat analysis of all vaccinated patients, and the second, a subset analysis that excluded those patients who did not survive to the 16-week vaccination time point. Because at least three vaccinations and ~16 weeks are required to generate substantial anti-hCG levels (Fig. 1), these patients did not have adequate time (and/or boosts) to generate a response. Inclusion of these patients in the analysis has the potential of associating low antibody level and low median survival, independent of any mode of action of the vaccine. After exclusion of this subpopu-

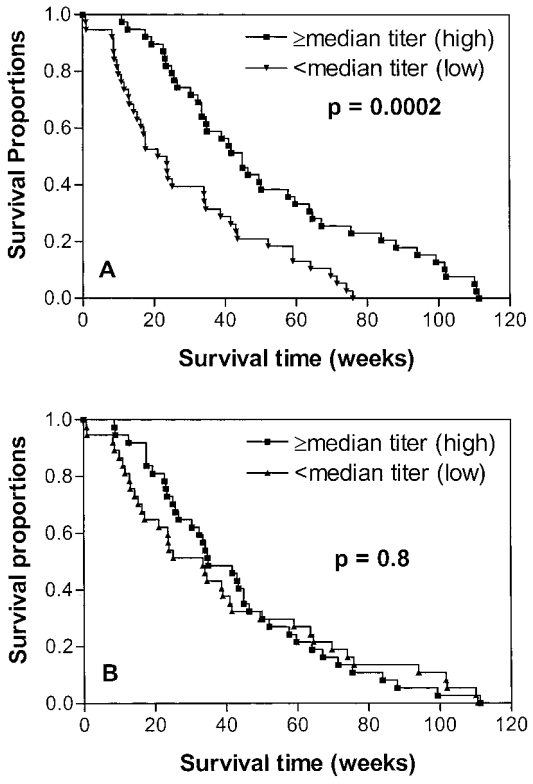

Fig. 3 Comparison of patient survival curves for low and high hCG (*A*) and DT (*B*) antibody responders, intent-to-treat population.

Table 3 Patient characteristics of low- and high-antibody responders

	Low responders	High responders
n	38	39
Sex ($P < 0.05$)		
Male	19 (50%)	29 (74%)
Female	19 (50%)	10 (26%)
Age		
Median (yr)	64	61
Range (yr)	29–83	33–83
<65 yr	21 (55%)	21 (54%)
>65 yr	17 (45%)	18 (46%)
Primary site		
Colon	23 (60%)	27 (69%)
Rectum	11 (29%)	10 (26%)
Not available	4 (11%)	2 (5%)
Performance status		
0	22 (58%)	28 (72%)
1	13 (34%)	10 (26%)
2	3 (8%)	1 (2%)
Stage at time of diagnosis		
III	17 (45%)	14 (36%)
IV	13 (34%)	15 (38%)
Other/Not available	8 (21%)	10 (26%)
Prior chemotherapy	33 (87%)	33 (85%)
Recall antigen		
Number positive (mean)	2.4	2.2
Induration (mean in mm)	13	14
Serum hCG		
<5 mIU/ml	34 (89%)	34 (87%)
>5 mIU/ml	4 (11%)	5 (13%)

lation from the analysis, it was found that a similar association existed between antibody levels and survival as with the intention-to-treat analysis. With the exception of gender, the clinical characteristics of the high and low antibody responders were not different with regard to factors that may influence prognosis. Relatively more females were among the low antibody responders. Although gender has not been consistently correlated with survival, there are data that the disease-free and overall survival of females is greater and not less, as characterized the low antibody responders (38). In contrast to the hCG response, a higher antibody response to the DT portion of the vaccine conjugate was not associated with increased survival. ASI with DT alone has been proposed as a therapeutic approach to stimulate the immune system in cancer patients (39). Our results indicate that an anti-DT humoral response is not associated with prolonged survival; however, we cannot rule out the potential role for a cellular immune response to DT.

A clear dose response was not observed in previous clinical studies of CTP37-DT, although the limited sample sizes of the Phase I studies performed precluded any conclusion (35–37). We examined the impact of a low- and a high-dose regimen in a randomized fashion. In comparing the two dose groups, it was found that the antibody levels generated after vaccination did not differ significantly. The difference in survival between the two dose groups was also not found to be significant. In addition, the incidence of clinical toxicity was also equivalent. Although the incidence of local reactions was not statistically different between the low- and high-dose groups, more severe local reactions were observed in the high-dose group. These finding supported the selection of the low-dose regimen, 0.5 mg, for future clinical trials.

Trophoblastic cells during pregnancy normally produce hCG, and the pituitary and other normal tissues, such as bladder, prostate, and testis, can also produce small amounts of hCG (40–42). The immunological tolerance resulting from this normal exposure to hCG can be overcome by CTP37-DT vaccine, including those patients with higher than normal circulating levels of hCG. More females were low antibody responders. Whether this represents an increased tolerance to hCG based on prior pregnancy is not known. Prior pregnancy was not noted to influence the ability to respond to CTP37-DT in previous studies (35–37). Tumor expression of hCG was not assessed in this study, and how it influences the clinical and immunological responses observed will require further study. How frequently hCG is overexpressed by human colorectal cancers will also require further study. hCG occurs in a number of molecular forms, including intact hCG, free subunits, and fragments. Previous studies in colorectal cancer using clinical specimens have used primarily reagents to detect the intact molecule/subunits. Studies applying reagents directed at hCG fragments as well as more sensitive techniques have suggested that the expression by nontrophoblastic tumors may be higher than that reported in earlier studies (23). Expression of the β-hCG CTP is more frequent than the intact molecule/subunit on human colorectal cell lines (43). In a previous study of CTP37-DT vaccine, tumor membrane-associated expression of the β-hCG CTP, as deter-

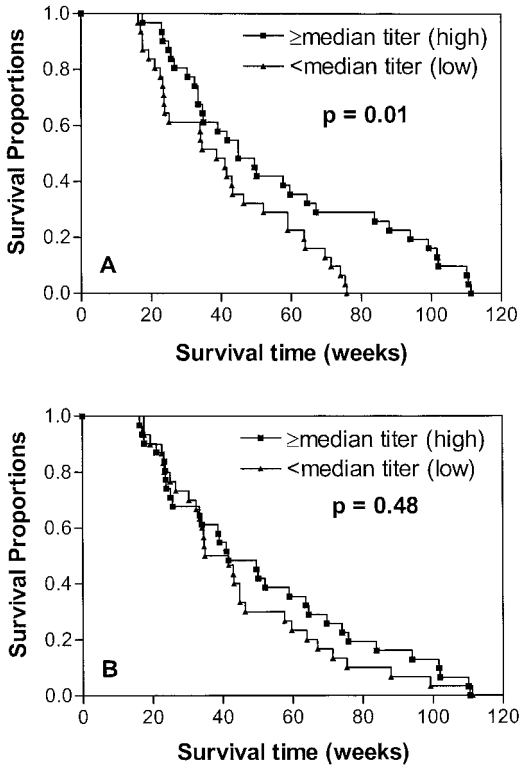

Fig. 4 Comparison of patient survival curves for low and high hCG (*A*) and (*DT*) antibody responders, subset of patients surviving >16 weeks.

mined immunohistochemically, was observed in 11 of 13 patients (85%) with colorectal cancer. Tumor expression of the β-hCG CTP did not influence the generation of anti-hCG antibody with CTP37-DT, because antibody induction was observed in all 13 (36). Recently reported studies have indicated that the transcription of specific hCG genes is a common feature of malignant cells, including colorectal cancers, and may also have prognostic significance (44, 45).

Although many cancer vaccine studies have focused on cellular immune responses and T-cell activation to elicit antitumor effects, there is a substantial body of data to indicate that antibody may also result in antitumor activity (46). Similar to what was observed in this clinical trial, antibody induction in melanoma patients after vaccination with a vaccine targeting the ganglioside, G_{M2}, was associated with an increase in survival (47). It should also be noted that an antibody-based approach to cancer therapy has been validated by the clinical efficacy of monoclonal antibodies specific for tumor-associated molecules (48, 49). Cellular immune responses were not measured in this study. In previous studies of this CTP37-DT formulation, which had the same adjuvant, the same carrier protein, and the same peptide:carrier ratio, no detectable T-helper or cytolytic T lymphocyte responses were observed (36). This observation is consistent with the design of the original contraceptive vaccine, which was formulated to induce a neutralizing humoral response and not a cellular immune response.

In summary, this study demonstrates that induction of anti-hCG antibody with CTP37-DT does have clinical significance in patients with colorectal cancer. Patients with metastatic disease who develop higher antibody levels manifest a longer survival. ASI targeting hCG with CTP37-DT offers a number of potential advantages over other cancer vaccine formulations being applied in patients with colorectal cancer. hCG is produced by colorectal cancers and may play a role in its progression. CTP37-DT is well characterized chemically without the complexity imposed by glycosylation, folding, and disulfide linkages of other vaccine formulations, and without biologically active contaminants that might appear as a result of incomplete purification of tissues or fluids. Furthermore, it is a stable formulation that does not require special handling. Importantly, injections of CTP37-DT have been well tolerated and do not produce the adverse effects that can limit chemotherapy programs. The results of this randomized Phase II study support the development of Phase III clinical trials to determine whether ASI with CTP37-DT can improve the outcome of patients with colorectal cancer.

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Clinical Cancer Research

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