A randomized trial of ex vivo CD40L activation of a dendritic cell vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival


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Running title: Colorectal cancer dendritic cell vaccine

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Colorectal cancer dendritic cell vaccine

Statement of Translational Relevance

Several studies of the use of DC vaccines to attempt to treat measurable metastatic tumor deposits have been unsuccessful. There has been limited study of DC vaccines in the adjuvant setting after resection of metastatic tumors in general, and colorectal cancer in particular. We conducted a clinical trial in which patients were treated with a DC vaccine in the adjuvant setting after resection of metastatic disease. Observations in murine models in our lab were used to formulate one of the hypotheses we tested in the clinical trial and were used to determine the route of DC vaccination.

We demonstrate that an autologous tumor lysate pulsed DC vaccine can induce immune responses against autologous tumor in a high proportion of patients. The clinical relevance of these immune responses was evaluated. We found an association between induction of an autologous tumor specific immune response and improved recurrence free survival.
Abstract

**Purpose.** To determine whether an autologous dendritic cell (DC) vaccine could induce anti-tumor immune responses in patients after resection of colorectal cancer metastases and whether these responses could be enhanced by activating DCs with CD40L.

**Experimental Design.** Twenty six patients who had undergone resection of colorectal metastases were treated with intranodal injections of an autologous tumor lysate and control protein (KLH) pulsed DC vaccine. Patients were randomized to receive DCs that had been either activated or not activated with CD40L. All patients were followed for a minimum of 5.5 years.

**Results.** Immunization induced an autologous tumor-specific T-cell proliferative or IFNγ ELISPOT response in 15 of 24 assessable patients (63%) and a tumor specific DTH response in 61%. Patients with evidence of a vaccine induced, tumor specific T-cell proliferative or IFNγ response one week after vaccination had a markedly better recurrence free survival (RFS) at 5 years (63% vs. 18%, p=0.037) than non-responders. In contrast, no association was observed between induction of KLH-specific immune responses and RFS. CD40L maturation induced CD86 and CD83 expression on DCs but had no affect on immune responses or RFS.

**Conclusion.** Adjuvant treatment of patients after resection of colorectal metastases with an autologous tumor lysate pulsed DC vaccine induced tumor-specific immune responses in a high proportion of patients. There was an association between induction of tumor-specific immune responses and recurrence free survival. Activation of this DC vaccine with CD40L did not lead to increased immune responses.


**Introduction**

Of 147,000 patients who develop colorectal cancer in the US each year, approximately 50,000 die of metastatic cancer (1). Many colorectal cancer patients develop metastases, especially to the liver, which are amenable to surgical resection. However, despite resection of metastases, 70% to 80% of these patients die from recurrent colorectal cancer (2,3). Although recent studies have shown that postoperative (4) and perioperative (5) chemotherapy increases the recurrence free survival of patients after liver metastasis resection, over half the patients in both these studies recurred by 3 years after resection. New therapies are needed for treatment of these patients.

A tumor vaccine consisting of irradiated autologous tumor cells plus Bacillus Calmette-Guerin (BCG) has been used in phase III trials to treat patients after resection of primary colon cancer (6,7). In these trials, although overall survival was not increased, the patients who developed an immune response to their autologous tumor manifested improved survival compared with those who did not, suggesting that treatment with more potent vaccines could improve survival. In a murine model, we found that a tumor lysate pulsed DC vaccine generated an order of magnitude greater anti-tumor immunity than immunization with irradiated tumor cells plus the classic adjuvant, Corynebacterium parvum (8).

Although studies have demonstrated that DC vaccines can induce immune responses in cancer patients, optimal parameters for immunization remain under investigation (9). Here, we based the route of immunization on our observations in murine models of enhanced antigen specific T-cell proliferation and IFNγ production utilizing intranodal injections when compared to subcutaneous or intravenous routes of immunization (10). A clinical study subsequently confirmed that an intranodal DC injection was more likely to induce peptide-specific T-cell responses than intradermal or intravenous injections (11).

In murine models, we also observed that signaling through CD40, a molecule on the surface of DCs, is required for multiple different vaccines to induce anti-tumor immunity (12). Engagement of CD40 by CD40L promotes DC cytokine production and costimulatory
molecule expression, enabling DCs to effectively trigger T-cell activation and differentiation (13). In mice, activation of tumor lysate pulsed DCs with CD40L leads to more potent induction of systemic immunity than DCs prepared without CD40L activation (14).

Therefore, we initiated a clinical trial whereby patients underwent resection of metastatic colorectal cancer and were then treated with intranodal injection of autologous tumor lysate pulsed DCs. Patients were randomized to receive DCs treated with or without CD40L. We hypothesized that this vaccine would induce tumor-specific immune responses, and that these responses would be enhanced by CD40L activation.

**Patients and Methods**

This study was approved by the Dartmouth Committee for the Protection of Human Subjects and performed according to US FDA IND 9554. Approximately 4 weeks after resection of all detectable metastatic colorectal cancer, patients signed their informed consent. Eligibility criteria included Karnofsky performance status >60%, recall DTH to either Candida or tetanus and enough tumor cells to form the vaccine and do the immunoassays (>75 x 10^6 cells). Patients were excluded if they were receiving immunosuppressive drugs, had active autoimmune disease, HIV, hepatitis B or C. A leukopheresis was scheduled 1-2 weeks after the patient consented. A CT scan was performed just prior to pheresis to determine if residual measurable disease was present. Eligible patients were randomized in a blinded fashion into two groups: one had CD40L added to their DC culture, the other did not. The data was unblinded following completion of all immunoassays. Patients received their first DC vaccination 8 days after pheresis. The second and third vaccinations were given 3 weeks and 6 weeks after the first vaccination, respectively.

**Preparation of Tumor Cells**

Tumors were transferred from the Operating Room to Pathology, where under sterile conditions the margins of resection were evaluated and a sample was removed for
histologic confirmation of adenocarcinoma. The tumor was then mechanically dissociated with a scalpel and filtered. The filtered cells were enumerated. Some were aliquotted for DTH testing, irradiated 10,000 cGy and cryopreserved at 2 million cells/mL in human AB serum (Gemini BioProducts, West Sacramento, Ca) containing 10% DMSO (Sigma, St. Louis, MO). The remaining cells were combined with tumor chunks that did not go through the filter and were enzymatically digested using 4 mg/mL collagenase (Sigma) and 0.2 mg/mL DNAase (Sigma). These cells were then washed, irradiated 10,000 cGy, and subjected to 3 freeze thaw cycles in liquid nitrogen to form the tumor lysate.

**Preparation of DC vaccines**

Patients underwent leukopheresis using the Cobe Spectra Apheresis System (Lakewood, CO) in the Cellular Therapy Center of the Norris Cotton Cancer Center. The leukopheresis product was enriched for monocytes by counter flow centrifugation elutriation using a Beckman J6-MI elutriation centrifuge (Palo Alto, CA). Monocytes were cultured in Life Cell culture bags (Baxter Nexell Therapeutics, Deerfield, Ill) in AIM-V media (Life Technologies, Grand Island, NY) at an initial concentration of 2.5 X 10^6 cells/mL with 10 ng/mL rhuGM-CSF (Immunex, Seattle, WA) and 20 ng/mL rhuIL-4 (Schering Plough, Kennilworth, NJ) added on days 0, 3 and 6 of culture. On culture day 6 autologous tumor lysate was added at a ratio of one tumor cell equivalent (TCE) to one DC. Keyhole limpet hemocyanin (KLH) (Vacmune, Biosyn Corp, Carlsbad, CA) was added at 25 ug/mL. On culture day 7 recombinant human CD40L (Amgen, Thousand Oaks, CA) was added at 1 ug/mL to the cultures of those patients who randomized to this treatment. On culture day 8 DCs were harvested, viable cells were enumerated and administered if they met the following release criteria: viability > 70%, no organisms on gram stain, cultured samples with no growth and endotoxin levels of < 5 EU/kg. Five million DCs were injected in a volume of 0.5 mL into each of 2 inguinal lymph nodes under ultrasound guidance. The remainder of the DCs were cryopreserved in autologous serum/10% DMSO at 1 X 10^7 DCs/mL for the second and third vaccinations, which were performed 3 weeks and 6 weeks after the initial vaccination in the same nodal basin.
Immonoassays

PBMCs were isolated from whole blood drawn from 3 different time points (pre-vaccine, one week after all vaccinations and 3 months after all vaccinations) using Ficoll-Paque Plus (GE Healthcare, Pittsburgh, PA). After separation, PBMCs were washed, then frozen in a 10% DMSO/FBS (Lonza, Portsmouth, NH) solution and stored at -140°C. PBMCs from all 3 time points were concurrently assayed.

Autologous DCs, cryopreserved after 6 days of culture, were thawed and washed in serum free AIMV (Gibco, Carlsbad, CA) medium, then re-suspended in AIMV supplemented with rhuGM-CSF at 500 IU/mL and rhuIL-4 at 20 ng/mL. DCs were incubated in 6 well Ultra Low Adhesion plates (Costar, Lowell, MA) at 37°C/5% CO₂. After 24 hours of incubation, DCs were pulsed with antigens for 24 hours. The following antigens were used: KLH at 25 ug/mL and autologous tumor lysate at a ratio of 1 tumor cell equivalent (TCE) to 1 DC. DCs were then harvested and washed in serum free AIMV medium, then re-suspended in CM and used as stimulators. For peptide antigens DCs were first activated with rhuCD40L (1 ug/mL), then pulsed with peptides and B₂microglobulin (Calbiochem, Gibbstown, NJ) at 3 ug/mL for 4hrs at 37°C. The HLA-A2 restricted peptides Ep-Cam p263-271 (GLKAGVIAV), CEA p571-579 (YLSGANLNL) and her-2/neu p654-662 (IISAVVGIL) (Peptide Technologies Corp. Gaithersburg, MD) were used at 20 ug/mL.

ELISPOT Assay

The ELISPOT assay was performed on 96 well Immobilon-P acrylic PVDF plates (Millipore, Billerica, MA) which were coated with 5 ug/mL mouse-anti human IFN-γ antibody, 1-DIK (Mabtech, Mariemont, OH) and incubated at 4°C overnight. After thawing, PBMCs were washed and transferred to tissue culture treated polystyrene flasks (Corning, Corning, NY) for a 10 minute adherence depletion at 37°C. Cells were harvested from flasks and then 3 x 10⁶ cells were added to each well in triplicate. Antigen-pulsed DCs were added at 20,000 per well. PHA (Sigma) was used as a control at 20 ug/mL. After an 18 hour incubation, the plates were washed with PBS/0.05% Tween 20 (Sigma). The biotinylated detection antibody (IFNγ: Mab 7-B6-1 Biotin, Mabtech) was added at 1 ug/mL.
and incubated at 37°C for 2 hours. The plates were then washed, Avidin-Peroxidase-Complex (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) was added and plates were incubated at room temperature for 1 hour. AEC substrate (3-amino-9-ethylcarbazole, Sigma) was added for 4 minutes, and the plates were washed and analyzed using the KS ELISPOT Axioplan 2 Imaging system (Carl Zeiss, Hallbergmoos, Germany).

**Restimulation ELISPOT Assay**

PBMCs were thawed, washed and plated at 1 x 10⁶/well in 24 well plates. DCs were antigen loaded as previously described and added to wells at 1 x 10⁵/well. IL-12 (Peprotech, Rocky Hill, NJ) was added to each well at 1 ng/mL. On culture day 2, IL-2 (Peprotech) was added at 10 U/mL. Cells were incubated for an additional 5 days. On Day 6, the cells were harvested and plated onto a pre-coated IFN-γ ELISPOT plate (as described above) at a concentration of 3 x 10⁴/well. Antigen-pulsed DCs were added to these cells and the ELISPOT plate was developed and analyzed as described above.

**Dye Dilution Proliferation Assay (DDPA)**

The DDPA was performed as previously described (15,16). Briefly, PBMCs were thawed, washed, centrifuged and resuspended in Diluent C (Sigma) at a concentration of 2 X 10⁷/mL. An equal volume of Diluent C containing 4 uM PKH-67 (Sigma) was added. Cells were incubated for 3 minutes in the dark at room temperature. The PKH-67 was quenched by adding an equal volume of FBS for 1 minute. PBMCs were then washed, aliquotted at 1 X 10⁶ cells/mL and stimulated with medium or DCs at a ratio of 1:10 DC to PBMC. After 7 days of culture at 37 C, 5% CO2 the cells were harvested, transferred to 12 x 75 mm tissue culture tubes (USA Scientific, Waltham, MA) and blocked with H-IgG (Sigma). Cells were stained with CD4 APC (Becton Dickson, Franklin Lakes, NJ) and CD8 PE (Becton Dickson), incubated on ice for 30 minutes and fixed with 1% paraformaldehyde. Cells were acquired on a Becton Dickson FacsCalibur and analyzed with Winlist and Modfit (Verity, Topsham, MA).

**DTH testing**
DTH tests were done by intradermal injection of 1 million mechanically dissociated, irradiated viable autologous tumor cells or 5 ug of KLH. Tests were read 2 days later and were considered positive if > 5 mm of induration was observed.

Statistics

For the DDPA and ELISPOT assays, all samples were run in triplicate to calculate the mean percentage of proliferating cells and spots (+ SD). A response for a particular patient at one time point was considered positive if the mean value obtained as a result of a specific antigenic stimulus (e.g. tumor lysate-pulsed DCs) was significantly greater (by t-test) than the mean value derived from the control stimulus (e.g. unpulsed DCs). Values reported are the response to the antigenic stimulus minus the response to the control stimulus.

The study was designed with a sample size of 24 patients to achieve a power of 80% to detect a difference in vaccine-induced immune responses in the DDPA assay between the CD40L and non-CD40L groups. The Kaplan-Meier product limit method was used to estimate relapse-free survival and overall survival. The log rank test was used to compare the survival of vaccine responders to non-responders.

Results

Between December 2001 and December 2003, 26 patients were accrued to this trial. Six additional patients were assessed but were not enrolled due to the following reasons: tumor cell yield too low (3), patient declined enrollment (3). Twelve patients received DCs that were stimulated with CD40L; 14 patients received DCs not treated with CD40L. There was no statistically significant difference between the two groups (CD40L vs no CD40L) when variables of potential prognostic importance were considered (Table 1). The Clinical Risk Score (CRS), a prognostic indicator based on a large database of patients who underwent hepatic resection (2), was similar for each group. When calculating the CRS we excluded the 2 patients who had lung metastases resected to form the vaccine and the 3 patients who had previously had liver metastases resected, since such patients were not
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included in the database used to derive the CRS. All patients had all detectable tumor resected at the time of the operation with negative margins. All patients had a positive DTH test to Candida or tetanus after tumor resection. Two patients had new measurable metastases detected on the CT scan done just prior to their vaccinations. The remainder of the patients had no measurable tumor and normal CEA levels at the time of initiation of adjuvant immunotherapy.

Addition of CD40L to the cultures changed the phenotype of the DCs. When DCs grown in the presence of CD40L were compared to DCs grown without CD40L there was no change in expression of MHC Class I, MHC Class II or the costimulatory molecule CD80. Ninety eight percent of DCs expressed MHC Class I, 90% expressed MHC Class II and 52% expressed CD80. However, the percentage of DCs expressing the costimulatory molecule CD86 were higher in the CD40L group (87% vs 68%, p=0.01) and the mean fluorescence intensity (MFI) was higher in the CD40L group (390 vs 165, p=0.03). In addition, the percentage of DCs expressing the maturation marker CD83 were higher in the CD40L group (30% vs 10%, p=0.01) and the MFI was higher in the CD40L group (61 vs 29, p=0.007).

No toxicity was observed in any of the patients. The two patients with measurable disease progressed at 6 months and died of colorectal cancer 11 months after vaccination. The 24 patients with no measurable disease treated with an adjuvant vaccine were followed for a minimum of 5.5 years. Eight are alive and disease free, 4 are alive with recurrent disease and 12 have died of colorectal cancer. The recurrence free survival (RFS) of patients treated with adjuvant vaccine was 58% at one year, 42% at 2 years and 38% at 5 years. The overall survival was 96% at 1 year, 83% at 2 years and 58% at 5 years.

Tumor specific immunoassay results

Twenty four patients had cells available for the dye dilution proliferation assay. Detectable tumor-reactive CD4 or CD8 T-cells were present prior to vaccination in 5 patients (Table 2). In 4 of 5 patients, that response continued to be detected at 1 week and
at 3 months after vaccination, but the magnitude of response was not increased by vaccination. CD4/CD8 T cells that proliferated in response to tumor were first detected 1 week after vaccination in 6 patients. In these 6 patients, the mean percentage of tumor reactive CD4 and CD8 T cells was 1.3% and 1.6%, respectively. A response continued to be detectable 3 months after vaccination in 3 of these 6 patients. Two patients were first noted to have a tumor-specific proliferative response 3 months after vaccination. Overall, immunization induced an autologous tumor-specific proliferative T-cell response in 33% (8 of 24) patients.

We evaluated PBMC cytokine secretion in response to tumor lysate pulsed DCs in a primary ELISPOT immunoassay and after a single round of in vitro stimulation. In the primary ELISPOT assay, 2 patients had evidence of induction of tumor specific IFNγ secreting cells one week after vaccination. In the restimulated ELISPOT assay (Table 3), a tumor specific IFNγ response was detectable in 4 patients prior to vaccination. In three of these four patients the response was significantly increased one week after vaccination, and in 5 additional patients a new tumor specific IFNγ response was noted. Therefore, 33% (8/24) of patients had evidence of induction of a tumor specific IFNγ response one week after vaccination. In 5 of these 8 patients this response was still detectable 3 months after vaccination. An additional 2 patients first manifested a tumor specific IFNγ response 3 months after vaccination. Overall, immunization induced a tumor specific IFNγ response in 42% (10 of 24) patients.

Two of 25 patients had a positive DTH reaction to autologous tumor prior to vaccination. After vaccination, 64% of patients developed a positive DTH test to autologous tumor cells.

Association between tumor specific immune responses and survival

Tumor specific immune responses prior to vaccination were not associated with improved RFS. In contrast, the 6 patients who had evidence of a vaccine induced anti-tumor proliferative response one week after vaccination had a markedly better RFS at 5 years (67% vs. 31%, p= 0.057) than non-responding patients (Figure 1). There was a trend
towards increased RFS at 5 years (63% vs 28%, p=NS) in the group of patients who developed a tumor specific IFNγ response one week after immunization. The eleven patients who developed a tumor specific proliferative or IFNγ response one week after vaccination had a significantly greater RFS at 5 years (63% vs 18%, p= 0.037) than those who did not develop a response (Figure 2).

There was no significant difference in RFS between the patients who had evidence of a vaccine induced anti-KLH proliferative or IFNγ response and patients with no evidence of a response. Thus, it is the induction of an autologous tumor specific immune response, rather than simply the ability to be immunized, that is associated with improved RFS.

There were no significant differences in the clinical characteristics of the 11 patients who developed tumor specific proliferative or IFNγ immune responses one week after vaccination and the patients who did not. Similar numbers of patients in each group had prior surgery for liver metastases, chemotherapy prior to liver metastasis resection, or concomitant resection of extra-hepatic intra-abdominal metastases. There was no significant difference in the mean Clinical Risk Scores (2) of the immune responders compared to the non-responders. Thus, the improved RFS observed in patients who developed a tumor specific immune response cannot be explained by clinical prognostic factors.

There was no difference in the RFS of patients who developed a positive tumor specific DTH response compared with patients who had a negative DTH test.

Three patients developed tumor specific proliferative and IFNγ secretory responses at 1 week after vaccination and had a positive tumor DTH test. Two of these patients are free of recurrence. The third patient developed a recurrence 3.5 years after vaccination, which we resected, and remains free of disease 7 years after vaccination.

Immune responses to KLH

Of 24 patients, 13 (54%) had KLH specific proliferative responses induced by vaccination (Table 2). The mean percentage of KLH reactive CD4 and CD8 T cells was 2.8 and 1.7, respectively. In all but one of these patients, KLH reactive T-cells continued to be
detectable 3 months after vaccination. In the primary IFNγ ELISPOT assay, vaccination induced a KLH specific IFNγ response in 38% (9 of 24) patients (Table 3). In the restimulation IFNγ ELISPOT assay 83% (20 of 24) patients had evidence of a KLH specific response one week after vaccination. The mean response was 473 spots/ 3 X 10^5 PBMCs. Sixty percent of patients developed a positive DTH response to KLH.

**Effect of CD40L on immune responses**

CD40L activation of DCs did not significantly affect the percentage of patients with positive anti-tumor or anti-KLH immune responses as measured by ELISPOT, DDPA or DTH assay. Patients treated with CD40L matured DCs and those treated with DCs that were not exposed to CD40L had a similar RFS.

**Anti-peptide immune responses**

Eight HLA-A2 positive patients had a sufficient number of cells for restimulation ELISPOT assay. We evaluated 3 peptides from colorectal cancer associated proteins that had previously been shown to induce T-cell responses in patients with colorectal cancer (17). Prior to vaccination, CEA, Her 2-neu and Ep-cam reactive cells were detectable in 3, 4 and 1 patient, respectively (Table 4). An IFNγ secreting anti-peptide response was either induced or significantly increased by vaccination in 3 patients for the CEA and Her 2-neu peptides, and in 4 patients for Ep-cam. Thus, use of a whole cell antigen source can induce peptide specific immune responses.

**Discussion**

Our study is the first to evaluate the role of CD40L induced maturation as a component of the DC manufacturing process in a prospectively randomized trial. At the time we began this trial, it was known that CD40 activation of murine and human DCs led to enhanced generation of cytotoxic T lymphocytes (CTLs) by increasing DC expression of
the costimulatory molecules CD80 and CD86 and by inducing IL-12 secretion (14,18). It had also been demonstrated that activating murine tumor lysate-pulsed DCs with CD40L led to more potent induction of systemic immunity than DCs prepared without CD40L activation (14). Therefore, we hypothesized that CD40L activated human DCs would induce a more potent immune response than DCs grown without a specific maturation factor. However, treatment of DCs with CD40L did not increase tumor or KLH specific immune responses in our patients. It is unlikely that this was due to inactive or inadequate amounts of CD40L, as we observed significant upregulation of CD86 and CD83 expression. One possible explanation for this finding is that inadequate toll-like receptor (TLR) signaling was present to optimize CD40-induced IL-12 expression in our vaccine. Subsequent to the initiation of our trial, it was demonstrated that DC stimulation with the combination of CD40L and LPS increased IL-12 secretion several fold compared to CD40L alone (19,20). We did not measure DC IL-12 secretion.

Others have attempted to induce anti-tumor responses in colorectal cancer patients with measurable metastatic tumors using DC vaccines. Chang treated 2 patients with an autologous tumor lysate pulsed, intradermally injected DC vaccine and demonstrated a tumor specific response in one patient (21). Tamir observed tumor specific responses in 2 of 6 patients treated with an autologous tumor lysate pulsed DC vaccine (22). Mazzolini treated 5 patients with intra-tumoral injections of IL-12 transfected DCs and observed a transient decrease in CEA in one patient (23). In two studies using CEA peptide as the antigenic source, minimal anti-peptide responses were observed, but in another study, 2 of 12 patients had tumor regression and induction of peptide specific CD8 cells (24-6).

We focused our efforts in the adjuvant setting because of the paucity of data showing objective responses to vaccination in patients with measurable metastases (27,28). In a review of 527 patients with measurable metastatic colorectal cancer treated on 32 studies with active specific cancer vaccination, the response rate was only 0.9% (27). Effective immunologic treatment of patients with measurable disease burdens has required adoptive transfer of large numbers of tumor reactive T-cells into lymphodepleted hosts, resulting in a high percentage of circulating anti-tumor T-cells that persist over time (29). It is unclear, however, whether one would need to generate such a high proportion of anti-
tumor T-cells to eliminate or control micrometastatic tumor deposits that exist after resection of macrometastatic disease.

We treated patients with a DC vaccine in the adjuvant setting with no measurable disease after colorectal metastasis resection. These patients are an attractive group to attempt to immunize because they are immunocompetent (as we have now demonstrated by recall antigen DTH testing) and approximately 70% will die from eventual tumor growth despite being treated with best current therapies. Only one previous study has reported adjuvant treatment of colorectal cancer patients after metastasis resection. In this study, 9 of 13 patients treated with a CEA mRNA loaded DC vaccine relapsed at a median of 122 days, and few anti-peptide responses were detected (30). Using a tumor lysate pulsed, intranodally injected DC vaccine we were able to induce an autologous tumor specific T cell proliferative or IFNγ secretory response in 15 of 24 patients (63%), and a DTH response to autologous tumor cells in 14 of 23 patients (61%). In those patients who developed a vaccine induced proliferative T-cell response one week after vaccination, the mean percentage of tumor reactive CD4 and CD8 T-cells was 1.3% and 1.6%, respectively. Also, 7 of the 11 patients (64%) who developed a tumor specific proliferative or IFNγ response 1 week after vaccination had evidence of a persistent response 3 months after vaccination. Patients who developed a vaccine induced tumor specific response had a significantly better RFS at 5 years (63% vs. 18%, p= 0.037) than the remainder of the patients. The similarity in the standard prognostic clinical characteristics of the tumor specific immune responders and non-responders increases the possibility that a causal relationship exists between DC vaccination and improved RFS. This study also suggests that a modest level of persistent anti-tumor T-cell reactivity may be sufficient to eliminate residual micrometastases, or at the least, keep them in a state of equilibrium with the immune system (31).

References

2. Fong, Y, Fortner J, Sun R, Brennan M, Blumgart L. Clinical score for predicting


autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA.


Table 1. Patient characteristics

<table>
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<th>No CD40L</th>
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<td>57 (41-76)</td>
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abdominal met resected 1 2 3 (11%)
Mean number of mets resected 1.4 1.7 1.6
Number of pts with liver met> 5 cm 4 4 8 (31%)
Mean disease free interval, months 17 12 15
Pts with CEA level > 200 0 0 0
Mean Clinical Recurrence Score (2) 2.1 2.3 2.2

Table 2. Dye dilution proliferation assay.
Numbers represent the percentage of CD4 and CD8 T-cells that proliferated in response to the antigenic stimulus (DCs pulsed with autologous tumor lysate or DCs pulsed with KLH), using the dye dilution proliferation assay. A “-“ indicates that there was no proliferation significantly higher than that observed when unpulsed DCs were used as the stimulus. Values in bold are significantly higher than pre-vaccine values, indicating a response induced by the vaccine. Nd indicates the assay was not done, due to lack of cells.

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Table 3. Frequency of PBMCs secreting IFNγ in response to tumor and KLH.

Numbers represent the number of cells per 3 X 10^5 PBMCs that secreted IFNγ in response to the antigenic stimulus in an ELISPOT assay. To measure tumor specific responses, PBMCs were exposed to autologous tumor lysate loaded DCs and cultured for one week prior to stimulus by DCs pulsed with autologous tumor lysate, the restimulated assay described in Materials and Methods. KLH responses were measured without prior culture. A "-" indicates that the number of spots was not significantly higher than that observed when unpulsed DCs were used as the stimulus. Values in bold are significantly higher than pre-vaccine values, indicating a response induced by the vaccine. Nd indicates the assay was not done, due to lack of cells.

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Frequency of PBMCs secreting IFNγ in response to tumor and KLH
Table 4. Frequency of PBMCs secreting IFNγ in response to tumor peptides.
Numbers represent the number of cells per 3 X 10^5 PBMCs that secreted IFNγ in response to the antigenic stimulus in an ELISPOT assay. To measure tumor peptide specific responses, PBMCs were exposed to peptide loaded DCs and cultured for one week prior to stimulus by DCs pulsed with autologous peptide, the restimulated assay described in Materials and Methods. A “-” indicates that the number of spots was not significantly higher than that observed when unpulsed DCs were used as the stimulus. Values in bold are significantly higher than pre-vaccine values, indicating a response induced by the vaccine.

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Frequency of PBMCs secreting IFNγ in response to tumor peptides

Table 4. Frequency of PBMCs secreting IFNγ in response to tumor peptides.
Numbers represent the number of cells per 3 X 10^5 PBMCs that secreted IFNγ in response to the antigenic stimulus in an ELISPOT assay. To measure tumor peptide specific responses, PBMCs were exposed to peptide loaded DCs and cultured for one week prior to stimulus by DCs pulsed with autologous peptide, the restimulated assay described in Materials and Methods. A “-” indicates that the number of spots was not significantly higher than that observed when unpulsed DCs were used as the stimulus. Values in bold are significantly higher than pre-vaccine values, indicating a response induced by the vaccine.
Figure Legends

1. Autologous tumor-specific proliferative response one week after DC immunization is associated with an improved recurrence free survival (log rank p = 0.057).

2. Autologous tumor-specific proliferative or IFNγ ELISPOT response one week after DC immunization is associated with an improved recurrence free survival (log rank p = 0.037).
Recurrence Free Survival

Responders (n = 6)
Non-responders (n = 16)

p = 0.057

Month Since Pheresis
Recurrence Free Survival

- Responders (n = 11)
- Non-responders (n = 11)

p = 0.037

Months Since Pheresis
A randomized trial of ex vivo CD40L activation of a DC vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival

Richard J Barth, Jr., Dawn A Fischer, Paul K Wallace, et al.

Clin Cancer Res  Published OnlineFirst September 30, 2010.

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