

# **A pilot study for the preoperative establishment of specific anti-tumor immune response in patients with colorectal adenocarcinoma**

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## **A. Study Purpose**

Colorectal cancer claims the lives of 60,000 Americans annually, with more than 140,000 new cases diagnosed each year. While surgical resection cures some patients, many go on to develop recurrences. Conventional chemotherapy results in only modest improvements in survival, therefore, new treatment strategies are called for. One such strategy is to stimulate the patient's immune system to eradicate residual tumor after surgery. To date, immune therapies have been initiated months after tumor surgery and have met with modest success. Because cancer therapies are most effective when there are small numbers of tumor cells in the body and because the tumor cell burden is at its absolute lowest immediately after surgery, the use of immune therapy before and immediately after surgery may be more effective. The research proposal under consideration proposes to administer a tumor vaccine preoperatively in an effort to establish an anti-tumor immune response prior to surgical resection of the primary colorectal tumor.

This proposal is a single human pilot study that involves administration of an anti-tumor protein vaccine three times before surgery. The vaccine consists of a liposomal preparation of a single protein (GA733) that is found on the surface of most colon cancer cells. The liposomes themselves contain lipid A, which acts as a vaccine adjuvant to the GA 733 protein. Vaccine adjuvants improve host immune response to the vaccine and are commonly given together with other vaccines. The GA733 vaccine has been safely and successfully used in cancer patients. It is hoped that the preoperative vaccination regimen will result in specific and active immunity to the GA733 protein that will persist throughout the postoperative period. This anti-tumor immunity should provide the patient with the means of eliminating tumor cells that remain in the body after resection of the primary tumor. This study will assess the immunologic status of patients before and after the vaccination, as well as postoperatively. This study will also assess the safety of this vaccine regimen.

## **B. Study Subjects and Methods of Recruitment**

Patient recruitment will be done by the attending surgeon during the preoperative consultation in the doctor's office. If the patient is interested in participating, the details, requirements, risks, and benefits will be discussed with the patient by the coordinating Study nurse and consent will be obtained. Fifteen patients will receive the tumor vaccine according to the study protocol. Minors, and pregnant women will not be included.

## **C. Study Procedures**

A total of 3 vaccines will be given prior to surgery. Vaccination will be carried out weekly 3 weeks, 2 weeks, and 1 week prior to surgery. Patients will undergo 3 skin tests 6 times and 7 blood tests. Blood tests (including thyroid function tests) and skin tests (small skin injections) will be done for the sole purpose of investigation. Patients will be seen once weekly for 3 weeks before surgery for vaccine administration as well as for skin and blood testing. The postoperative blood samples will be taken at the time of standard postoperative blood sampling. Issues: This Study requires a 3 weeks period prior to surgery during which the tumor vaccine is administered and the patient, hopefully, develops an immune

response. In some cases, this may delay surgery by a week, which could be viewed as an ethical problem. However, in the vast majority of cases in the U.S. there is a delay of between two to six weeks between the diagnosis of large bowel cancer and the surgical resection. Further, the concept of delaying surgery to give a non-surgical cancer treatment is well established. Surgery is delayed by 9 to 12 weeks in rectal cancer patients that receive radiotherapy and chemotherapy before surgery. Because the estimated age of a moderate sized colon cancer is at least 5 years, a 1 to 2 week delay to surgery is not likely to have an impact on the outcome of the patient. Practical concerns center around patient compliance.

#### **D. Study Purpose and Rationale**

This proposal is a human pilot study. The purpose of this study is to determine if a peptide based tumor vaccine (plus adjuvant) given preoperatively to colon cancer patients will result in the establishment of specific and lasting immunity to the tumor associated antigen GA733. It is hoped that the vaccine would result in a specific cell mediated and humoral response to the GA733 protein. Active immunity against this protein may provide a specific means by which the patient can eliminate viable tumor cells that remain after the primary tumor has been surgically excised.

This study is designed to evaluate a cancer vaccine consisting of a protein, GA733, which is a tumor associated antigen expressed on most colon cancer cells. GA733 is the 31 kDa extracellular portion of a 40kDa membrane glycoprotein referred to as C017-IA (8). An extracellular, membrane bound, protein is an excellent immune target since most lymphocytes and immune effector cells, have access to these proteins. As mentioned, it is hoped that both specific and humoral immune responses to the GA733 protein will be induced by the vaccine. This glycoprotein is over-expressed in several types of cancer, including colorectal carcinomas. An extracellular protein is an excellent immune target, if antibody production and a cellular response to this protein, can be induced with a vaccine. The vaccine will be given preoperatively in conjunction with lipid A in a liposomal emulsion to facilitate establishment of an immune response to the antigen, and hopefully to the patient's tumor (9). The goals of this study are to determine if an active immune response to this tumor antigen can be established preoperatively and maintained in the early postoperative period. The GA733 vaccine has been previously used in humans and was well tolerated without adverse effects. Further, it was demonstrated that the vaccine induced lymphocyte proliferation (1). Furthermore, in a human clinical trial that vaccinated Stage IV patients in a non-operative setting, significantly fewer vaccinated patients demonstrated disease progression than in the placebo group. The proposed study will assess the safety and efficacy of perioperative administration of the GA733 vaccine. Despite the fact that a variety of tumor vaccines have been tested in clinical trials during the last decade, researchers have not, thus far, attempted to use vaccines preoperatively. Presently, most vaccines are given at least 4 weeks after surgery. Although it has been established that humoral and cell mediated immune responses to tumor antigens can be established, the long term oncologic results, in many cases, have been disappointing (23). It has become clear that tumor vaccines are not very effective in the setting of a large tumor burden or well established tumors. To delay vaccination several months after surgery may permit small tumors to grow and free tumor cells to form metastases, thus decreasing the chances of success for immune based therapies (4). Immunotherapy may be most effective immediately following resection of the primary because the tumor burden is at its lowest level. In a murine study from our laboratory it was demonstrated that a tumor vaccine given two weeks prior to laparotomy was associated with a significantly lower incidence of lung metastases than laparotomy alone.(5) A second, more recent, murine study from our laboratory demonstrated To establish active immunity before surgery tumor vaccines must be given well in advance of the operation(6). The GA733 vaccine in a liposomal preparation with lipid A has been safely used in clinical trials without the development of untoward complications. Furthermore, as mentioned above, this vaccine has been shown to be effective in inducing a specific immune response(GA733) and to stimulate the immune system (lipid A) (9, 10).

#### **E. References**

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#### Study Design and Statistical Analysis

This pilot study will attempt to induce an active immune response to the GA733 vaccine prior to surgery. This is a non-randomized investigation that will involve only immunocompetent patients with colorectal cancers. This pilot study will include 15 patients who will be vaccinated preoperatively with the GA733 protein plus adjuvant. To verify that non-vaccinated patients with colorectal cancer do not have a naturally occurring immune response to the GA733 protein, all 15 patients will undergo a single DTH challenge to the GA733 protein prior to vaccination. There is a 0.79 probability of detecting at least one response among these non-vaccinated patients, if the response rate is 10%. The perioperative (post-vaccination) immune response rates will be estimated with a standard error of no more than 13%. Previously obtained and stored blood, from earlier patients, will serve as historical controls for blood tests, while five control patients will be used as controls for the DTH testing.

As stated, 15 subjects will be included in this study. This should be a sufficient number of patients to determine if the perioperative use of these agents is safe. With 15 subjects, we have an 80% chance of observing, at least once, any toxicity occurring at a rate of 10%. We have a 95% chance of observing, at least once, any toxicity occurring at a rate of 18%. If at any point during the study three subjects have experienced unacceptable toxicity, the study will be terminated. This corresponds to an unacceptable toxicity rate of 20% or higher. Toxicity will be graded according to CTC criteria. As already

mentioned, both of the agents involved have been shown to be safe when used after surgery or in the non-operative setting. Fifteen patients per group should also suffice to determine if this immune treatment strategy has any effect on the various immune parameters being considered.

In this study our principal goal is to demonstrate that it is possible to establish an immune response to the GA733 protein preoperatively. A secondary goal of this study is to assess the immune response to the GA733 protein in the early postoperative period (to determine the impact of surgery on the immune response). Serial DTH testing, lymphocyte proliferation assays, and determination of anti-GA733 antibody levels as well as histologic assessment of the actual tumor for immune response will be carried out in an attempt to verify if an active immune response has been established. The demonstration of positive DTH responses to the GA733 challenges or the finding of specific antibodies to GA733 in the serum would verify that an immune response had, indeed, been established. Of course the percentage of patients in whom an immune response is established is an important parameter that will also be determined.

**F. Study Procedures and Protocol** (being done only for research purposes and not required for clinical management)

The protocol and schedule of vaccinations, skin tests and blood sampling that comprise this study is found immediately below after which a brief description of the specific tests to be done is provided.

**a. Study Protocol**

Consenting immunocompetent patients are to be vaccinated with a protein vaccine consisting of 400 mcg of GA733 in a liposomal emulsion with 800mcg lipid A. All vaccinations will be administered via intradermal injection, in the volar aspect of either upper arm. The vaccine will be given weekly for 3 weeks prior to surgery. The schedule for the vaccines, DTH challenges, and blood tests follows:

**i. Initial Pre-vaccination Testing** (prior to acceptance into trial)

1. DTH testing: 3 intradermal injections (mumps, tetanus, & GA733)
2. Blood draw (7 cc heparinized, 7 cc EDTA)

**ii. Vaccination Schedule**

Three weeks before surgery	1) vaccination with a liposomal preparation of lipid A and GA733 vaccine
Two weeks before surgery	1) vaccination with a liposomal preparation of lipid A and GA733 vaccine
One week before surgery	1) vaccination with liposomal preparation of lipid A and GA733 vaccine

**iii. Preoperative Testing** (2-7 days prior to surgery)

1. DTH testing: 3 intradermal injections (mumps, tetanus, & GA733)
2. Blood draw (7 cc heparinized, 7 cc EDTA)

**iv. Postoperative day 3**

1. DTH testing: 3 (intradermal injections (mumps, tetanus, & GA733)
2. Blood draw (7 cc heparinized, 7 cc EDTA)

**v. Second Postoperative week** (POD 8-14)

1. DTH testing: (3 intradermal injections (mumps, tetanus, & GA733)
2. Blood draw (7 cc heparinized, 7 cc EDTA)

**vi. Fifth Postoperative Week** (POD 29-35)

1. DTH testing: (3 intradermal injections (mumps, tetanus, & GA733)
2. Blood draw (7 cc heparinized, 7 cc EDTA)

During the course of this study patients will be closely monitored for signs and symptoms of adverse reactions and side effects; a list of these follows.

- 1) Generalized allergic reaction to the GA733/lipid A vaccine (hives, difficulty breathing, etc.)
- 2) Changes in body temperature after vaccination

- 3) Local skin reaction to the vaccine/lipid A combination
- 4) Changes in pulse rate, blood pressure, or respiration rate after vaccination
- 5) Blood tests-leukocyte count, white blood cell differential, ESR
- 6) Urine analysis
- 7) Signs and symptoms of hypothyroidism

#### vii. Study Tests

The effectiveness of GA733 vaccine (plus lipid A adjuvant in a liposomal emulsion) administration will be evaluated via the following tests:

- 1) Delayed-type hypersensitivity(DTH) testing,
- 2) detection of specific anti-GA733 antibodies.
- 3) assessment of the specificity of the antibodies.
- 4) determination of the percentage of  $\gamma$  interferon expressing circulating CD8+ (cytotoxic) T cells,
- 5) determination of the percentage of activated circulating T cells in response to vaccine, and
- 6) assessment of the resected tumor for percentage of apoptotic tumor cells and tumor infiltrating CD8+ T-cells. All but the DTH tests will be carried out on the blood samples that will be obtained from each patient. For a complete description of all tests, please see appendix A:

#### G. Study Drugs

Two drugs will be utilized in this study: 1) GA733 protein vaccine and 2) lipid A in a liposomal emulsion. In regards to the safety of these agents, GA733 has been safely used in humans for purposes similar to ours; while liposomal lipid A has proven safety and efficacy as a vaccine adjuvant( 4,5). Because the doses and routes of administration have been proven safe and efficacious by other investigators, the focus of the proposed study will be to administer these drugs at a different time (preoperatively instead of postoperatively. The group size, 15 patients, should be large enough to reveal major side effects or toxicities (although none are anticipated). A study of this scope, and pilot studies in general, are not likely to reveal rare side effects, however. That information would be gathered in the anticipated Phase I, II or III studies to follow.

The vaccine will consist of the GA733 protein. GA733 is the 31 kDa extracellular portion of a 40 kDa membrane glycoprotein referred to as Ep-CAM or CO 17- 1 A that is over-expressed in several cancer types including colorectal carcinoma. Murine monoclonal antibodies(mAb's) to the complete antigen (CO17-IA) that includes the GA733 protein have been used to treat Stage III colorectal cancer patients in a phase III randomized and controlled trial. A significant survival benefit was demonstrated for treated patients(1). Immunization with GA733 resulted in tumor regression, development of specific antibodies and tumor specific cytotoxic activity in a murine colorectal cancer model(2). GA733 vaccine in combination with lipid A (as adjuvant) has also been administered to human colorectal cancer patients after surgery(3). The preliminary results documented that vaccination induced a specific T cell proliferative response and no adverse side effects. Furthermore, in a human clinical trial that vaccinated Stage IV patients in a non-operative setting, significantly fewer vaccinated patients demonstrated disease progression than in the placebo group (8). The proposal under consideration would use this protein vaccine in a similar manner(dose of GA733) as in these human studies. In animal and human studies GA733 was well tolerated: no dangerous side effects have been reported (7).

Lipid A is the immunomodulating agent that will be given with each GA733 vaccination in this study. Synthetic lipid A, although a component of LPS, and lipid A subunits do not have an endotoxin-like effect, however, they do continue to activate macrophages(6). These activated macrophages release IL-1, which induce immature macrophages to become mature IL- and IL-2 producing cells. It is well known that IL-2 stimulates helper T cell function and proliferation, an important step in humoral immunity, and that activated macrophages are excellent antigen presenting

cells. The use of the emulsion form of the vaccine is due to the high immunogenicity, as emulsions are powerful inducers of antibodies to the emulsified antigen (5). Although emulsions are not noted for inducing cytotoxic T-lymphocyte responses, liposomes are among the most powerful agents in eliciting this response(5). Therefore, an emulsion of liposomes containing lipid A should cause a strong antibody and cytotoxic T-lymphocyte response to the emulsified antigen, GA733. The safety of lipid A is demonstrated by the fact that DetoX™, a similar substance consisting of lipid A and cell wall skeleton, has been safely given in over 800 patients without major adverse reactions (7). The doses GA733 and lipid A we propose to use fall well within the range of doses used, and well tolerated in previous human studies (7).

These two agents are combined by the manufacturer under the IND number 6927 and was kindly supplied by Jenner Biopharmaceuticals. The vaccine consists of 100mcg in 0.3ml of alum. Included in the suspension is 200mcg of lipid A. Each 1 mL vial contains 0.3mL vaccine. The intended dose is 400mcg of GA733 protein and 800mcg of lipid A injected intradermally on the volar aspect of the upper arm. Major toxicities include the possibility of immune reaction to normal tissue, however, in previous trials involving injections of preformed anti-GA733 monoclonal antibodies, this was not observed in over 1000 treated patients (7). Also, duodinitis has occurred in two of 15 patients treated with the anti-GA733 monoclonal antibody, KS 1/4. However, it was combined with a vinblastine derivative DAVLB in that study(7). Diarrhea and abdominal cramping occurred, but each was self-limited. Two patients reported nausea and vomiting associated with a transient rise in AST and ALT. Minor reactions include local erythema, edema, pain at injection site, fever, low grade lethargy, headache, and myalgia.

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#### I. Medical Devices

none

**J. Study Questionnaires**

None

**K. Study Subjects**

A total of 15 patients will receive the vaccine according to the protocol. Non-vaccinated colon cancer patients who underwent surgical resection of their tumor and underwent a delayed-type hypersensitivity skin test once, prior to surgery, to document that unvaccinated patients do not have specific immunity to the GA733 protein.

**a. Inclusion criteria:**

Patients must be between 18 and 85 years of age and have been proven to have adenocarcinoma of the large bowel on histologic analysis of colonoscopically obtained biopsies. Patients may be of any race and either gender. All patients will be assessed for metastatic disease with an abdominal and pelvic C.T. scan (preferably with IV and oral contrast) as well as standard chest X-rays. Patients with colorectal tumors located between 10 cm (from the anal verge) and the cecum will be eligible for these studies. Patients with mid or distal rectal cancers (<10cm from anus) will not be eligible for entry into these studies. Only those patients found to be immunocompetent by DTH recall antigen testing will be considered for entry. Also patients must have normal renal, thyroid, and hepatic function and be hematologically stable as defined by (values according to CPMC clinical chemistry lab):

Hemoglobin	≥8.0g/dL
Neutrophils	≥1.5 x 10 <sup>9</sup> /L
Lymphocytes	≥0.5 x 10 <sup>9</sup> /L
Platelets	≥100 X 10 <sup>9</sup> /L
Serum creatinine	≤1.8 mg/dL
Serum bilirubin	≤2mg/dL
SGOT	≤41 mg/dL
SGPT	≤41 mg/dL
TSH	≤10mcu/ml
Thyroxine	5-20mcg/dL

Patients found to be anemic or to have other abnormal results for the parameters mentioned will be appropriately evaluated as per the judgment of their internist or primary care physician. Also, patients must have a performance status <2 (ECOG Scale) and a life expectancy of >3 months. Childbearing potential must be terminated by surgery, radiation, menopause, or use of a contraceptive (oral contraceptive, barrier device, or IUD). Only patients to be operated on by the principal investigator, Dr. Kenneth Forde, or Dr. Emina Huang will be eligible for inclusion in this study. Exclusion criteria: Patients with a history of prior malignancy or immune compromise (current steroid usage or the use of any known immunosuppressing drug) will be excluded. Patients Hepatitis B or C or HIV positive will be excluded. Patients with a history that puts them at risk (sexual practices, intravenous drug abuse, history of blood transfusion) would be advised to undergo HIV testing. However, patients without risk factors will not be routinely tested. Patients who have received chemotherapy or radiotherapy within the 3 months preceding the anticipated surgery will not be eligible. Also ineligible will be any patients with abnormal thyroid function tests or any history of hypo/hyperthyroidism. Patients found to be anergic via preoperative DTH skin testing to mumps or tetanus will be excluded. A positive pregnancy test, given to all premenopausal women, will also be criteria for exclusion, as will nursing.

**L. Recruitment of Subjects**

Patients with colorectal cancer, under the care of the three surgeons mentioned above, who meet the inclusion criteria and are determined to be appropriate candidates, and who are scheduled to undergo surgical resection will be invited to enroll in the study. The surgeon in question will be the first to discuss the study with the patient and raise the possibility of the patient becoming involved in the study. Following this, provided the patient has expressed an interest in the study, the Study coordinator or a Study RN will have a more detailed discussion with the patient and family regarding the study. All questions will be answered. It will be made clear that involvement in the study is voluntary and that a decision to decline entry into the study will in no way impact on the surgery or care that the patient would receive. The evaluation and recruitment process would occur in the physician's office at the time of the patient's initial visit or on a follow up preoperative visit (if other preoperative tests are needed to stage the patient at the time of the initial visit).

#### **M. Confidentiality of Study Data**

Each patient will be assigned a unique identifier. Data will be stored in a secure location, accessible only to the principal investigators and study coordinating nurse.

#### **N. Potential Conflict of Interest**

None

#### **O. Location of the study**

Atchley Pavilion, Milstein hospital, or East 60<sup>th</sup> Columbia Offices (Columbia Presbyterian Eastside)

#### **P. Potential Risks**

Because both of the preparations (GA733 protein vaccine, and lipid A) that we plan to use in this investigation have already been used in the human setting with minimal side effects and adverse reactions, we believe that the risk to the patients will be minimal. The risks will closely mirror the adverse reactions that were outlined in section D. M. Potential Benefits: The short range hypothesis of this study is that a series of preoperative vaccinations with the GA733 protein and lipid A combination is safe, will be well tolerated, and will result in a specific anti-GA733 immune response detectable perioperatively. The long range hypothesis of this study is that induction of a specific anti-tumor immune response will enable the patient's immune system to eradicate minimal residual disease after surgery. The potential gain for patients in this study is a reduction in the rate of tumor recurrence after surgery and also the possibility of a greater chance of survival.

#### **Q. Alternative Therapies**

At present, the standard of care for patients with colon cancer does not include preoperative neoadjuvant immunotherapy. Conventional adjuvant therapy is offered to selected patients starting 6 to 8 weeks postoperatively. Many investigational immune based therapies are currently in clinical trials. To our knowledge, no other protocols aim to induce immunity preoperatively. It should be stressed that involvement in this study does not preclude patients from undergoing conventional postoperative chemotherapy or postoperative immunotherapy at the usual time that such treatments are presently initiated. Therefore, patients will have the same adjuvant treatment opportunities as patients who decide not to enroll in the studies.

#### **R. Compensation to Subjects**

None

**S. Cost to Subjects**

Additional cost to the patient will be incurred due to travel expenses related to additional visits to the outpatient office during the preoperative period.

**T. Minors as Research Subjects**

n/a

**U. Radiation of Radioactive Substances**

None

## Appendix A 1.

Delayed-type hypersensitivity (DTH) testing: Serial DTH challenges with both standard recall antigens (mumps and tetanus) and the GA733 protein will be carried out several times to assess overall immune competence and the presence or absence of a specific immune response to the GA733 protein. The initial mumps and tetanus DTH challenges will be given to assess whether or not consenting patients are immunocompetent. Only those able to mount a DTH response to either the mumps or the tetanus challenge will be eligible for vaccination and entry into the trial.

DTH testing assess a number of different elements of cell-mediated immune system including: antigen presentation, CD4+ T lymphocyte proliferation and elaboration of cytokines, and effector cell function. In order to respond to a challenge the patient needs to have been exposed to the antigen in question in the past. To determine baseline levels of delayed-type hypersensitivity responses, all patients are skin tested by intradermal injections of mumps (40 complement units per cc) and tetanus (10 limits of flocculation per cc) each in a total volume of 0.1 cc. Forty-eight hours after the challenge, two perpendicular diameters are measured with calipers. The magnitude of the response is calculated by using the formula for the area of an ellipse ( $A = (D1/2 \times D2/2) \times 7t$ ). Patients that are able to mount a significant response to at least one of the recall antigens (defined as response area  $>20 \text{ mm}^2$  diameter) are eligible for the study.

As mentioned above, study patients will be challenged with the mumps, tetanus, and GA733 protein after consenting to be assessed for entry into the trial. Those found to respond to either both or one of the mumps and tetanus challenges will be entered into the study and will begin the vaccination regime. Study patients will undergo additional challenges to mumps, tetanus, and the GA733 protein: 1) during the week prior to surgery (2-7 days before operation), 2) on the third postoperative day (POD 3), 3) during the second postoperative week (POD 8-14), and 4) during the 5<sup>th</sup> postoperative week (POD 29-35). If a patient responds to only 1 of the 2 recall antigens (mumps and tetanus) at the initial challenge then when re-challenged subsequently they will only be injected with the antigen to which they responded, to avoid unnecessary injection with an agent to which they have proven to be anergic.

The preoperative post-vaccination and post-operative DTH results for the mumps, tetanus, and GA733 protein will be compared to the pre-vaccination results. The change in absolute area of the responses will be calculated as will the percent change from baseline (pre-vaccination results). 2. Production of anti-GA733 antibodies in ELISA: The finding of specific antibodies to the GA733 protein will prove that preoperative vaccine induced a specific immune response. Method: 96-well plates will be coated with GA733. After blocking and several washes, serial dilutions of patients' serum will be added and incubated. The plates will be then washed and incubated with peroxidase labeled antibody to human IgG or IgM, respectively. After applying substrate, the intensity of the color will be evaluated using the ELISA reader. 3. Specificity of the Antibodies will be determined via Western Blot analysis. Recombinant GA733 protein and human GA733 expressing colon cancer cell line (SW480, HT29) lysates will be subjected to electrophoreses on SDS-PAGE and transferred to nitrocellulose membranes. After blocking and several washes, membranes will be incubated with diluted patients' serum, washed again and incubated with peroxidase labeled antibody to human immunoglobulins. The membranes will be then washed, developed with ECL reagent and evaluated using an X-ray Film. 4. The percentage of 7 interferon expressing CD8+ (cytotoxic) T cells in PBMC isolated from patient blood samples. An indirect and non-specific assessment of the impact of the vaccinations on the T-cell population will be carried out by determining the percentage of CD8+ T-cells that are producing  $\gamma$  interferon. Specific CD8+ T lymphocytes (cytotoxic) T cells produce  $\gamma$  interferon after antigen activation. The percent of  $\gamma$  interferon producing cells found in each patients preoperative and pretreatment blood sample will serve as the baseline against which the postoperative results will be compared.

Isolated peripheral blood mononuclear cells will be incubated with FITC labeled anti-CD8 antibody and then washed and fixed in paraformaldehyde-ethanol. After several washes and incubation with PE labeled anti- $\gamma$  interferon antibody, the percentage of  $\gamma$  interferon expressing CD8+ cells will be estimated using flow cytometry analysis. 5. The Percentage of Activated Circulating T cells in response to

vaccine: This test is another indirect and nonspecific assessment of the impact of the vaccines on the lymphocyte population. Mononuclear cells will be isolated from peripheral blood by gradient centrifugation on lymphocyte separation medium. These cells will be then washed and incubated with FITC labeled anti-CD3 antibody and PE labeled anti-CD25 (IL-2 receptor  $\alpha$  chain) antibody. After several washes, the percentage of activated (CD25+) cells out of total T lymphocytes will be calculated using flow cytometry analysis. 6. Induction of apoptosis of autologous cancer cells and the number of tumor infiltrating  $\gamma$  interferon expressing CD8+ T cells in the tumor: The resected tumor will be assessed histologically via special methods in an effort to determine the effect of the tumor vaccine on tumor cell apoptosis and on tumor infiltrating lymphocytes. Colon cancer tissue will be obtained during surgery and fixed in buffered formalin. Subsequently, tissue will be embedded in paraffin, 5 $\mu$ m thick sections of tissue will be prepared and mounted on slides. The slides will be then treated in xylene, ethanol and re-hydrated. The number of apoptotic cancer cells will be estimated using TUNEL assay (TdT-mediated dUTP nick-end labelling). Slides will be incubated with TdT and digoxigenin labeled dUTP. After several washes, FITC labeled anti-digoxigenin antibody will be applied. After incubation, several washes and counter-staining of nuclei with propidium iodide, the number of apoptotic cells (green fluorescence) and necrotic cancer cells (small picnotic nuclei) will be evaluated using fluorescent microscopy. The number of tumor infiltrating  $\gamma$  interferon expressing CD8+

T cells will be evaluated using monoclonal antibodies (anti-CD8-FITC, anti- $\gamma$  interferon-PE) for tissue slides and fluorescent microscopy. 7 Proliferation of CD8+ lymphocytes in response to GA733. The purpose of this test is to indirectly document the existence of a sub-population of T cells that are specific for the GA733 protein, thus documenting the effectiveness of the vaccine. Exogenous GA733 should stimulate these specific cells to replicate much as a mitogen would. Method: PBMC's (10<sup>6</sup>/well) obtained from each of the blood samples will be cultured for 3 days with 1 pg/ml of GA733. Four hours before harvesting, cells will be cultured in 10  $\mu$ M BrdU. Cells will be then harvested, ethanol fixed, denatured with HCl and stained with anti-BrdU-FITC and anti-CD8-PE. During the flow cytometry analysis, CD8+ cells will be gated and their BrdU incorporation analyzed.

All of the above tests are not routinely performed before or after surgery outside of the setting of a study. No special instrumentation or catheters are required. The invasiveness of the study procedures is limited to skin pricks and phlebotomy. Although direct patient participation and duration of study procedures will persist for no more than 37 days postoperatively, the evaluation of safety parameters will continue for 6 months

### Columbia Presbyterian Medical Center Consent to Participate in a Research Study

The purpose of this consent form is to provide you with the information you need to consider in deciding whether to participate in the research study.

Study Title: The preoperative establishment of a specific anti-tumor immune response in patients with colorectal adenocarcinoma.

#### **Study Purpose**

You are invited to participate in a research study designed to bolster your body's immune system before your surgery in an effort to reduce the chances of the tumor recurring after the primary tumor has been removed surgically. A large body of evidence now suggests that the body's own immune defenses are instrumental in the fight against cancer. It has been well established that surgery is the only known cure for colon and rectal cancer. However, in some patients a relatively small number of tumor cells may remain either in the bloodstream or at other sites in the body immediately following the surgery. These tumor cells that remain may give rise to recurrent tumors called metastases. Participants in this investigation will receive a series of vaccinations before surgery that, hopefully, will enable their immune systems to better deal with and eliminate tumor cells that may be present in the patient's body after surgery. You qualify as a possible participant in this study because you have been diagnosed with colorectal cancer and are to undergo an operation to remove the cancer.

#### **Study Procedures**

If you decide to participate in this study you will receive a series of vaccinations that contain an anti-tumor vaccine plus a drug which maximizes the body's response to the vaccine. A total of 3 vaccinations will be given in the 3 weeks before your operation. You will also undergo skin testing (similar to the skin test commonly performed for tuberculosis) on a number of occasions before and after surgery to evaluate your body's response to the vaccinations. Small blood samples (1 1/2 teaspoons each) will also be taken on a number of occasions during the study to evaluate your response to the treatment. Administration of the study medications and skin testing will require extra visits to the doctor's office or a visit to your home by a registered nurse of the Study Coordinator. These extra visits will also allow your physician to monitor your progress more closely and detect any adverse reactions to the study medications. You will be provided with a schedule of visits.

#### **Study Risks**

Rarely, patients may develop a local skin reaction at the site of injection of the medication. This may consist of redness, swelling, or a hematoma ("black and blue" mark). It is possible but highly unlikely that a vaccination might cause a more generalized allergic reaction such as hives (a generalized skin rash) or difficulty breathing. After vaccination, patients will be observed in the office for 10 to 15 minutes until it is clear that an adverse reaction is not occurring. Very rarely, patients may develop a small ulcer at the site of the skin test(s) that is due to the assessment of the patient's response to the vaccine. This type of ulcer should fully heal over a 4-8 week period and leave a small scar. In a human study carried out by the same investigator (involving over 400 patients) with the same type of skin tests, none of the patients developed a scar at the injection site. Also, hypothyroidism is a theoretical possibility as a result of treatment, although it is very unlikely to occur. Patients with hypothyroidism experience cold intolerance, lethargy, weight gain and constipation. You will be monitored for any early signs of this via blood tests and nurse's exam.

Participation in this study requires 3 weeks of time before your operation during which the vaccines are given and during which your body responds to the vaccines. The average length of time between the diagnosis of a colorectal tumor and cancer surgery in the U.S. ranges between 2 and 6 weeks. Therefore, for the majority of patients, involvement in this study would not result in a delay to surgery since they would have waited at least 3 weeks regardless. However, for the small percentage of patients

who would otherwise have their operation within 2 weeks of discovery of the tumor, participation in the study would result in a brief delay to surgery. It has been estimated that, at the time of discovery, most colon tumors have been growing in the body for about 3 to 7 years. Therefore, a delay of one or two weeks is most probably not a significant delay. Furthermore, if the vaccine is effective it will provide the immune system of the vaccinated patient with a means of eliminating tumor cells that remain after surgery.

**Study benefits**

It is hoped that the induction of specific immunity against your tumor with vaccines will enable your immune system to eradicate any remaining tumor cells after surgery. The potential gain is a reduction in the risk of tumor recurrence after surgery and, possibly, a greater chance of survival. It may also be found that the vaccine program does not result in any discernible benefit in regards to cancer recurrence or survival.

**Alternatives**

At present, the standard of care for patients with colon cancer does not include preoperative or perioperative adjuvant immunotherapy or perioperative adjuvant chemotherapy or radiotherapy. Therefore, colon cancer patients do not currently receive the type of treatment this study is investigating. Adjuvant therapy(chemotherapy, radiotherapy, or immunotherapy) is offered to selected patients about 6 weeks postoperatively. Although numerous clinical trials investigating different immunotherapies are underway in the U.S., to our knowledge, no other protocols aim to induce immunity preoperatively. Involvement in this vaccination study does not prevent you from undergoing any of the standard postoperative adjuvant therapies(chemotherapy, radiotherapy, or immunotherapy) that are typically started about 6 to 8 weeks after surgery.

**Costs**

You will be responsible for additional travel expenses. Additional office visits and laboratory tests will be provided at no cost. Study drugs will be administered free of charge.

**Compensation**

You will not be compensated for participating in this study.

**Confidentiality**

Any information obtained during this study and identified with you will remain confidential. The FDA may have access to medical records related to this study.

**Participation is Voluntary**

Your participation in this study is completely voluntary. You can refuse to participate, or withdraw from the study at any time, and such a decision will not affect your medical care at Columbia-Presbyterian Medical Center, now or in the future. Signing this form does not waive any of your legal rights.

**Questions**

If you have any questions, please ask, and we will do our best to answer them. If you have additional questions in the future, you can reach Dr. Whelan at 212 305 6136. If you have any questions on your rights as a research subject, you can call the Institutional Review Board at 212 305 5883 for information.

**Statement of Consent**

I have discussed this study with Dr. Whelan to my satisfaction. I understand that my participation is voluntary and that I can withdraw from the study at any time without prejudice. I have read the above and agree to enter this research study. Signing this form does not waive any of my legal rights.

I have been informed that if I believe I have sustained injury as a result of participating in a research study, I may contact the Principal Investigator, Dr. Whelan at 212 305 6136 or the Institutional Review Board at 212 305 5883, so that I can review the matter and identify the medical resources which may be available to me.

I understand that:

- a) The Presbyterian Hospital will furnish that emergency medical care determined to be necessary by the medical staff of this hospital;
- b) I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage;
- c) No monetary compensation for wages lost as a result of injury will be paid to me by Columbia-Presbyterian Medical Center, and;
- d) I will receive a copy of this consent form.

Signatures:

Participant Date