October 4, 2017

Status Page

PROTOCOL #: 12-436

The following site(s) are **open to enrollment:**

MGH

Study closed to enrollment at DFCI

ClinicalTrials.gov Identifier: NCT01952730

Protocol Number: 12-436

Approval Date: 11/27/2012 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 09/12/2013 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
09/12/13	AM #1 revised the Front Sheet	02/26/13	n/a
09/12/13	AM #2 revised the Protocol and Front Sheet	03/28/13	n/a
10/04/13	Study renewal/Consent Form footer replaced due to Continuing Review #1	10/04/13	N/A
02/04/14	Suspended per FDA – Data Analysis for Toxicity May Continue	2/4/14	N/A
03/04/14	Front Sheet replace due to Amendment #3	02/18/14	N/A
09/22/14	Consent Form replaced/ Study renewal due to Continuing Review #2	09/16/14	09/16/14
06/12/15	Protocol and Front Sheet replaced due to Amendment #4	06/11/15	N/A
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
08/21/15	Protocol and Front Sheet replaced due to Amendment #5	08/05/15	N/A
09/16/15	ON HOLD: All research must stop due to lapsed Continuing Review. Study expired 09/16/15	N/A	N/A
09/30/15	Remove Hold. Study renewal/ Consent Form footer replaced due to Continuing Review #3	09/24/15	09/29/15
09/19/16	Study renewal/Consent Form footer replaced due to Continuing Review #4	09/08/16	09/19/16
08/17/17	Study renewal/Consent Form footer replaced due to Continuing Review # 5	08/10/17	08/16/17
10/04/17	Reopen at MGH site only (DFCI remains closed); eligibility checklist, front sheet, and protocol replaced per Amendment # 6	06/12/2017	n/a
10/04/17	Front sheet, protocol, and consent form replaced per Amendment #7	06/12/2017	10/04/2017
07/09/2018	Study renewal/Consent Form footer replaced per Continuing Review #6	06/28/2018	07/09/2018
06/10/2019	Study renewal/Consent Form footer replaced per Continuing Review #7	06/03/2019	06/10/2019

Protocol Version Date: May 31, 2017
NCI Protocol #: N/A

Local Protocol #: 12-436

Title: A Pilot Safety Study of Vaccination with Autologous, Lethally Irradiated Colorectal Cancer cells Engineered by Adenoviral Mediated Gene Transfer to Secrete Human Granulocyte-Macrophage Stimulating Factor in patients with Stage IV colorectal cancer

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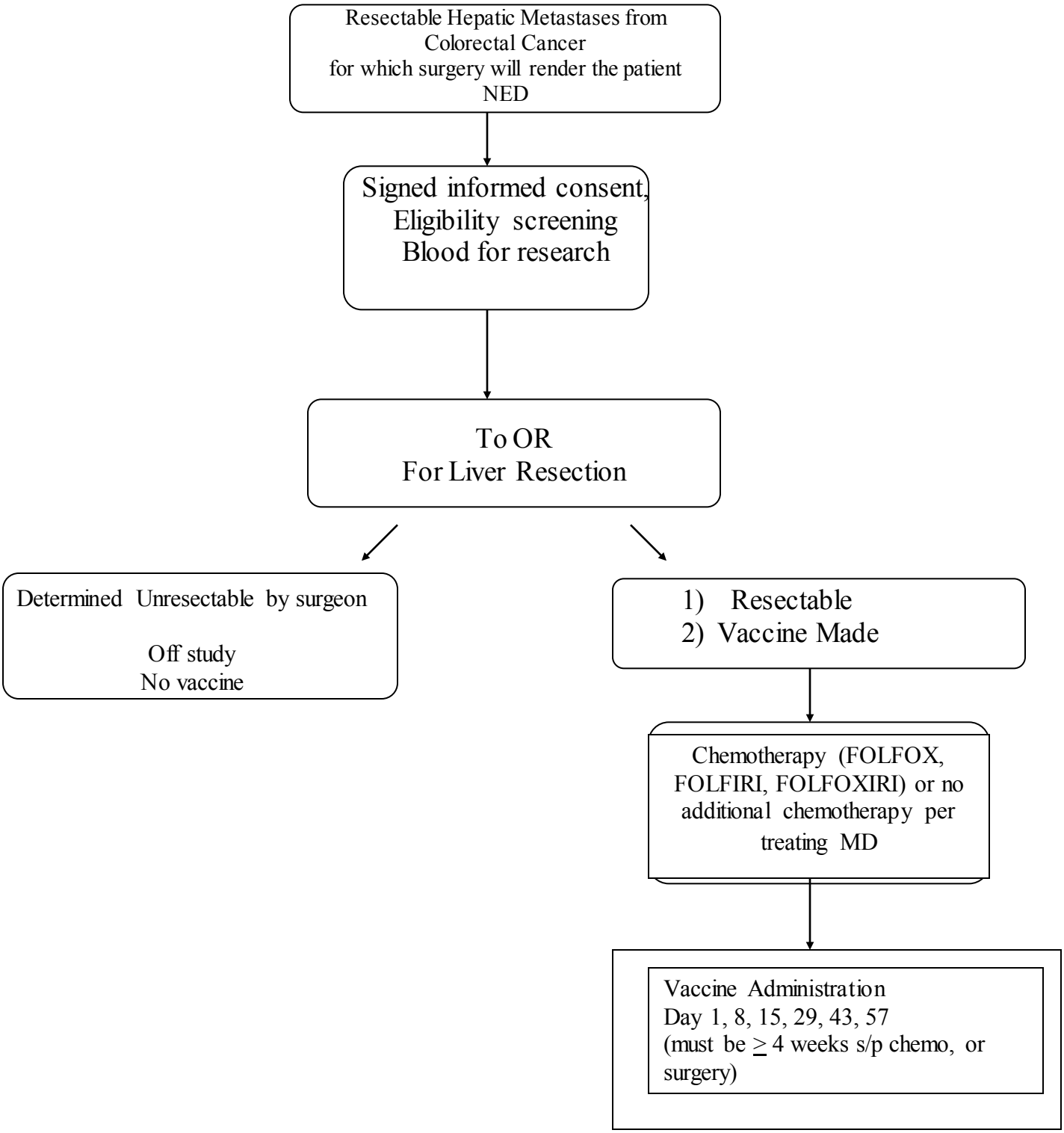
Agent(s):

Lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF

Schema



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1. OBJECTIVES

1.1 Study Design

The purpose of this study is to evaluate the feasibility, safety, and toxicity of an adenoviral mediated GMCSF vaccination strategy in stage IV colorectal cancer patients. The Phase I trial employing adenoviral mediated gene transfer in patients with metastatic melanoma demonstrated that a simplified method of vaccine production was feasible, safe and immunogenic. We now propose a Phase I study utilizing the same constructs and vaccine strategy aimed at treating patients who have undergone hepatic resection for their colorectal cancer metastases. Only patients with resectable liver metastases are eligible to participate since the resected liver metastasis will be used to construct the vaccine.

Subjects may be enrolled if they will be undergoing a hepatic resection for colorectal metastases at the MGH. We will analyze both toxicity and immunity in this patient cohort. If this trial reveals significant biologic activity without substantive toxicity, then these results would provide a foundation for undertaking a subsequent Phase III study testing the therapeutic efficacy of vaccination in the adjuvant setting.

Further, we will evaluate the biologic activity of this vaccination scheme in stage IV colorectal cancer patients with resected hepatic metastases. Blood, skin punch biopsies and tumor samples obtained during the course of immunization will function as critical reagents for detailed analysis of the vaccine-induced immune responses.

1.2 Primary Objectives

1.2.1 To determine the safety of 6 vaccinations with lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF in stage IV colorectal cancer patients who are completely resected**.**

1.3 Secondary Objectives

1.3.1 To determine the progression free survival and two-year survival of stage IV colorectal cancer patients vaccinated with lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF.

1.3.2 To evaluate the immune response elicited by the vaccine.

2. BACKGROUND

2.1 Study Agent(s)

A vaccine composed of cells from lethally irradiated, autologous colorectal cancer hepatic metastasis engineered by adenoviral mediated gene transfer to secrete GM-CSF (GVAX).

2.2 Study Disease

2.2.1 Background of Chemotherapy for Colorectal Cancer

In the United States, colorectal cancer is the fourth most common malignancy and the second most frequent cause of cancer-related death.[1] In 2011, an estimated 141,210 cases of colorectal cancer will be diagnosed and 49,380 people will die from this disease. Metastases to the liver are identified in up to 25% of patients at the time of diagnosis[1]. For the remaining patients anywhere from 50-70% of patients will develop hepatic metastases during follow-up[1]. Surgical resection continues to be the only modality which can provide patients with a long term cure. Multi drug chemotherapy and targeted therapies have improved patients' survival and have converted patients from unresectable to resectable, but usually cannot offer a cure. Additionally, chemotherapy often causes steatosis and steatohepatitis which increases the morbidity and mortality of hepatic resections. In addition to the hepatic damage caused by chemotherapy, different agents result in a multitude of side effects which can limit their utility.

Adjuvant therapy has evolved in the past two decades for stage IV colon cancer from just 5-FU to the addition of Oxaliplatin, Irinoetecan and numerous targeted agents. Current multi-drug regimens for metastatic colorectal cancer have improved the overall survival (OS) from 6 to 20 months or more[3]. One of the first positive phase III multi drug regimens added Irinotecan to 5FU (IFL), resulting in an improved overall survival from 14mo to 17mo[4]. Oxaliplatin was added to 5FU (FOLFOX) resulting in an improved progression free survival of nearly 3mo[5]. FOLFOX was found to be superior to IFL with an OS of 19.5months vs. 15months in the N9741 trial[6]. Subsequently FOLFOX and FOLFIRI were compared which demonstrated similar times to progression and overall survivals[7].

In the randomized trial by Nordlinger et al perioperative chemotherapy surrounding hepatic resection did offer a 9.2% longer progression free survival than an operation alone[2]. This data along with the now known hepatic toxicity of Oxaliplatin and Irinotecan, has prompted most clinicians to offer 6 cycles of chemotherapy followed by hepatic resection and an additional 6 cycles of chemotherapy post operatively. The majority of patients receive 5-FU with either Oxaliplatin and/or Irinotecan (FOLFOX, FOLFIRI, or FOLFOXIRI). Immunotherapy utilizing autologous hepatic colorectal cancer metastases has not been extensively explored. This clinical trial offers a novel immunotherapeutic strategy as an adjunct to conventional chemotherapy.

2.2.2 Anti-Colorectal Cancer Immune Responses

The role of immune cells in human neoplasia is unclear[8]. Tumor infiltrating lymphocytes in melanoma[9], colorectal cancers[10-12] and ovarian cancer[13] have been shown to inhibit tumor growth and are associated with improved prognoses. Recent data from Pages et al[14,15] demonstrated a significant difference in DFS and OS between patients who had primary tumors with vascular emboli, lymphatic invasion and perineural invasion (VELIPI) after adjustment for TNM and Duke's stage (VELIPI + and -: DFS3.3 mo vs. 26.9mo and 5yr OS 12% vs. 32.4%, $p<0.001$). Interestingly, VELIPI- tumors were associated with a strong immune infiltrate. Specifically, VELIPI- tumors had increased T cell differentiation (CD45R0, CD45RA, CD27, CD28, CCR7, and CD127), increased evidence of T cell migration (CD62L-, CCR7, CD103, CD49d, CXCR3), increased evidence of T cell activation (HLA-DR, CD98, CD80, CD86, CD134), and increased effector memory T cells (CD45RO+CCR7-CD28-CD27-) ($p<0.05$). Additionally, a high density of memory T cells (CD45RO+) was associated with tumors without lymph node involvement and metastases ($p<0.001$). Lymphocytic and memory T cell infiltrates are prognostic biomarkers associated with longer survival of patients independent of other clinicopathologic factors such as MSI status, Kras, BRAF, PIK3CA mutations, etc[16,17].

2.3 Rationale

This recent data supports the idea that after antigen stimulation a small population of antigen-specific memory T cells remains in the tissue and may "control" the primary cancer from recurring. Notwithstanding these provocative data, most patients fail to develop anti-colorectal cancer responses that are sufficiently potent to prevent lethal tumor progression. The understanding that tumor cells generally stimulate poor antigen presentation has motivated the design of several new strategies to increase anti-tumor immunity[18] [19]. Among the approaches using gene transfer, Dr. Dranoff's group at DFCI has demonstrated that vaccination with irradiated tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor (GM-CSF) generates potent, specific, and long-lasting anti-tumor immunity in multiple murine models, including the B16 melanoma[20]. Vaccination involves enhanced tumor antigen presentation by recruited dendritic cells and macrophages; the coordinated functions of $CD4^+$ and $CD8^+$ T cells, CD1d-restricted NKT cells, and antibodies mediate protective immunity[20] [21] [18] [22].

Further, Phase I trials conducted by Drs. Dranoff and Hodi at DFCI employing adenoviral mediated gene transfer in patients with metastatic melanoma demonstrated that a simplified method of vaccine production was feasible, safe and immunogenic. We now propose a Phase I study utilizing the same constructs and vaccine strategy aimed at treating patients who have undergone hepatic resection for their colorectal metastases.

First, we will evaluate the feasibility, safety, and toxicity of this vaccination strategy in stage IV colorectal cancer patients. Subjects may be enrolled if they will be undergoing a hepatic resection for colorectal metastases at MGH. We will analyze both toxicity and immunity in this patient cohort. If this trial reveals significant biologic activity without substantive toxicity, then these results would provide a foundation for undertaking a subsequent Phase III study testing the therapeutic efficacy of vaccination in the adjuvant setting.

2.4 Correlative Studies Background

We will evaluate the biologic activity of this vaccination scheme in stage IV colorectal cancer patients with resected hepatic metastases. Prior to liver resection, blood samples will be collected and fresh frozen tissue from the hepatic metastases will be stored. Blood and skin punch biopsies obtained during the course of immunization, and pre-immunization tumor samples will function as critical reagents for detailed analysis of the vaccine-induced immune responses. Biopsies will be obtained two days after the first and fifth vaccinations. Specifically, we will evaluate the immune cell composition (CD4+ and CD8+ T cells, T regulatory cells, macrophage, etc) in the resected specimens and in the circulating blood. ELISPOTs will be performed to evaluate the specificity of the CD8+ T cell response to known colorectal cancer antigens. In patients with recurrent disease, biopsy of recurrence may also be evaluated when clinically appropriate.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination prior to their planned liver resection to be eligible to participate in the study:

3.1.1 Stage IV patients must have:
A) Histologically documented hepatic colorectal cancer metastasis
B) Resectable hepatic lesion(s) as judged by PI or surgeon with the intent of an R0 resection.
C) Primary tumor previously resected or planned resection at time of hepatic surgery

3.1.2 ECOG Performance Status 0 or 1

3.1.3 Age $\geq$ 18 years

3.1.4 Patients may receive chemotherapy (FOLFOX, FOLFIRI, FOLFOXIRI) after their hepatic resection, but must be $\geq$4 weeks from the last dose of chemotherapy in order to be eligible to receive vaccine.

3.1.5 Patients must be fully recovered from their hepatic resection per their surgeon or the study PI.

3.1.6 Participants must be ≥ 4 weeks from chemotherapy, radiotherapy, immunotherapy, systemic glucocorticoid therapy or the operation to receive the first vaccine dose.

3.1.7 Must be able to understand and willing to sign a written informed consent document.

3.1.8 The primary colorectal cancer must be resected before hepatic resection or at the time of hepatic resection.

3.1.9 Laboratory values must demonstrate:

A. Total bilirubin ≤2.0mg/dl

B. AST (SGOT) ≤ 9 x ULN

C. ALT (SGPT) ≤ 9 x ULN

D. Alkaline Phosphatase ≤ 3 x ULN

E. Creatinine ≤2.0 mg/dl

3.1.10 Negative Serum pregnancy test in women of childbearing potential.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Uncontrolled active infection

3.2.2 Infection with HIV, Hepatitis B or C

3.2.3 Other current malignancies except any in situ cancer or basal or squamous cell carcinoma

3.2.4 Active autoimmune disease including but not limited to: ALS, MS, SLE

3.2.5 Active corticosteroid therapy

3.2.6 Hepatic metastases involving both branches of the portal vein (right and left) or all three hepatic veins (left, middle and right)

3.2.7 Peritoneal metastases identified at the time of attempted resection

3.2.8 Residual/recurrent disease

3.2.9 Pregnant and or nursing women are excluded from this study because GM-CSF based vaccines have not been performed in animal or human reproduction studies. It may have potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with GM-CSF, breastfeeding should be discontinued if the mother is treated with GM-CSF. These potential risks may also apply to other agents used in this study.

3.2.10 Greater than 1 month since resection of liver metastasis for vaccine production.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be**

eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.

Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

5. TREATMENT PLAN

5.1 Tumor procurement

At the time of the liver resection at MGH the specimen will be handled according to CVC SOP B1.01 (Appendix) and transferred to DFCI CMCF by an authorized study team member, for example Bethyl Rose (617-643-6189 or 617-771-2975) or Matteo Ligorio, MD (617-583-3640).

5.1.2Vaccine administration

Treatment will be administered on an outpatient basis. It is expected that patients will have fully recovered from surgery and chemotherapy prior to administration of vaccine, per treating surgeon. In addition, patients may receive postoperative chemotherapy at the discretion of the treatment team. Treatment with GVAX may not start prior to 4 weeks after the last dose of chemotherapy or the operation. GVAX treatment must begin within 6 months of the date of operation. If enough vaccine is made patients may elect to have an additional 6 vaccines if they still meet the eligibility criteria.

Following notification by the treating provider that vaccine may be administered, the CMCF staff will be notified to thaw the vaccine. Vaccines will be retrieved from the CMCF by an authorized study team member as noted, according to CMCF policy and brought to the MGH on ice via the Partners shuttle or in a taxi between 1-4 hours prior to vaccine administration. The vaccine will be stored on ice until it is ready to be administered.

Expected toxicities and potential risks as well as dose modifications for GVAX are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No

investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Vaccination Plan					
Agent	Pre-medications; Precautions	Range	Route	Schedule	Cycle Length
GVAX	No premedications	$1x10^5$ to $1x10^7$ cells/vaccine	Subcutaneous and Intradermal	Days 1,8,15, 29,43, 57	57 days

5.1.2.1 Vaccinations will be administered on days 1, 8, 15, and every two weeks thereafter until 6 vaccines have been administered or the patient is removed from study. As indicated in 5.2.2.4, individual vaccine cell dose will vary depending on the final cell yield from vaccine production. The total cell yield will be divided in 6 equal aliquots, and the minimal dose will be $1x10^5$ cells/vaccine and maximal dose will be $1x10^7$ cells/vaccine. This variation in cell dosage is designed to maximize each patient's opportunity of receiving vaccinations and is not based on any expectation of significant differences in toxicity as a function of cell number.

5.1.2.2 All patients receiving at least one vaccination will be evaluable for toxicity. Patients will be considered evaluable for biologic activity if at least six vaccinations have been administered at a given dose level. There is no requirement for a particular number of patients at each cell dose level.

5.1.2.3 Vaccinations may be administered within 24 hours of the scheduled vaccine administration date (e.g. day 8, 15, 29) beginning with the day 8 vaccine.

5.1.2.4 Patients must be greater than 28 days from any chemotherapy, radiotherapy, immunotherapy, systemic glucocorticoid therapy, or resection of hepatic colorectal metastasis.

5.2 Pre-treatment Criteria

5.2.1 Primary Tumor Procurement

5.2.1.1 Patients will undergo a surgical procedure (hepatic resection) to obtain tissue for vaccine preparation.

5.2.1.2 The responsible surgeon will send a portion of the tumor for standard of care pathology review. Then, the surgeon will place the research tumor specimen in sterile media in a sterile container. After gross evaluation by the pathologist of

the entire specimen in the operating room, the tissue will be transferred to the authorized study team member for vaccine processing at the DFCI CMCF.

5.2.1.3 Patients will receive routine post-operative care.

5.2.1.4At the discretion of the treating physician patients may receive post operative chemotherapy prior to vaccine administration. Participants will undergo a separate standard of care chemotherapy consent session with their treating physician as the chemotherapy is not a part of the investigational agent in this study.

5.2.1.5If recurrent disease is identified on the restaging scans prior to vaccine, vaccine will not be administered.

5.2.2 Cell Preparation

5.2.2.1Tumors will be transferred to the CMCF at the Dana-Farber Cancer Institute by approved personnel and processed to single cell suspension by mechanical and enzymatic digestion as required; short-term tumor cultures may be established if necessary to obtain sufficient cells.

5.2.2.2Tumor cells will be transduced with a replication defective adenoviral vector encoding human GM-CSF.

5.2.2.3After transduction, the tumor cells will be washed extensively and irradiated with 10,000 cGy.

5.2.2.4A small aliquot of the transduced cells will be placed into culture and GM-CSF secretion will be determined by ELISA. The target level for GM-CSF production will be at least 40 ng/10^6 cells/24 hours, although achieving this secretion will not be required for vaccine administration. Routine sterility cultures and testing for endotoxin and mycoplasma contamination will be performed

5.2.2.5Individual vaccine cell dose will vary depending on the final cell yield from vaccine production. The total cell yield will be divided in 6 equal aliquots, and the minimal dose will be $1x10^5$ cells/vaccine and maximal dose will be $1x10^7$ cells /vaccine. This variation in cell dosage is designed to maximize each patient's opportunity of receiving vaccinations and is not based on any expectation of significant differences in toxicity as a function of cell number.

5.3 Subsequent Vaccines

5.3.1 If in the judgment of the treating physician, the clinical status of the patient following completion of the first six vaccinations permits, the option for expansion of tumor cells previously cryopreserved, vaccine production, and vaccination will be offered to the patient. The patient will need to satisfy the same eligibility criteria as for entry on the first round of treatment, except for the requirement that greater than four weeks have elapsed since prior GVAX immunotherapy. The decision to enter this second phase can be made at any time following completion of the six initial vaccinations required for toxicity evaluation.

It is recognized that initiation of a second round of treatment may obscure the ability to evaluate potential delayed toxicities associated with the first round of vaccination. The likelihood of such toxicities given the experience with earlier melanoma trials seems remote, however, and additional data regarding the safety of repeat courses of vaccine procurement and vaccination is a higher priority. Additional rounds of vaccination may be considered as long as the patient continues to meet eligibility criteria.

5.3.2 For patients proceeding to one additional round of vaccine, the procedures outlined in sections 5.1-5.4 will be followed. It is anticipated that the cell dosage for additional rounds of vaccination may be different from that used in the first round.

5.1.3 Patients may opt to discontinue treatment after the first round of vaccines to undergo another type of treatment or participate in another clinical trialPatients also have the option of a second round of vaccination if enough vaccine is available. The subject will need to satisfy the same eligibility criteria for entry as done with the first round of vaccines.

5.4 Agent Administration

5.4.1 Vaccinations will be administered on days 1, 8, 15, and every two weeks thereafter until 6 vaccines have been administered or the patient is removed from study. As indicated in 5.2.9, individual vaccine cell dose will vary depending on the final cell yield from vaccine production. The total cell yield will be divided in 6 equal aliquots, and the minimal dose will be $1x10^5$ cells/vaccine and maximal dose will be $1x10^7$ cells/vaccine. This variation in cell dosage is designed to maximize each patient's opportunity of receiving vaccinations and is not based on any expectation of significant differences in toxicity as a function of cell number.

5.4.2 Vaccinations may be administered within 24 hours of the scheduled vaccine administration date (e.g. day 8, 15, 29) beginning with the day 8 vaccine.

5.4.3 Vaccination Procedure

5.4.3.1 Cells will be thawed in a dedicated laminar flow biosafety cabinet in the CMCF and washed. Cells will be resuspended in a volume of 1 ml. for administration. 2 injections (1/2 dose subcutaneously and 1/2 dose intradermally at the same site with repositioning of the needle) will be given and will constitute a single vaccination. The volume of each injection will be 0.5 ml.

5.4.3.2 Injections will be administered according to standard procedures in the patient's arms, thighs, or trunk on a rotation basis.

5.4.3.3 A treating physician, NP, PA, or RN designated by the PI will administer the injections in the outpatient facilities of the Massachusetts General Hospital.

5.5 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue for a total of 6 vaccines or until one of the following criteria applies:

- Disease progression, intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Participant demonstrates an inability or unwillingness to comply with the protocol requirements.
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.
  If enough vaccine is available and patients meet eligibility criteria he/she may be a candidate for an additional 6 vaccines.

5.6 Duration of Follow Up

Participants will be followed for 3 months after the last vaccine then every 6 months for 3 years, then yearly for up to 5 years. Since they are receiving a gene modified product, each patient will remain in long term follow up and followed annually for survival for a period of fifteen years for surveillance and toxicity evaluation.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

6.1 Anticipated Toxicities

6.1.1 GM-CSF is widely used for accelerating hematopoietic recovery and its spectrum of toxicity well established. It is likely that the highest dose level used in this study will generate far lower amounts of GM-CSF than the usual starting dose for systemic application (350 μg/day). While we cannot predict the exact toxicities attributable to local secretion of GM-CSF delivered by adenoviral mediated gene transfer, toxicities observed from the previous trial were restricted to errythema, pruritus, and swelling. Local toxicities of GM-CSF protein injection have included pustular eruption, necrotizing vasculitis, errythema, pruritus, recall errythema at previous injection sites, general papular rash, and phlebitis. Systemic toxicities have included fever, bone pain, malaise and diarrhea, transient liver function test (LFT) abnormalities, leukopenia, leukocytosis, arthralgia, dyspnea, fluid retention, serous effusions, and recrudescence of various auto-immune diseases.

6.1.2 A theoretical potential exists for the induction of auto-immune disease by the vaccination, although no evidence of vitiligo or visual disturbances was noted in the previous melanoma studies. Mechanisms which allow the immune system to recognize tumor antigens in principle could also lead to the breakdown of tolerance to normal self antigens. Because of this concern, patients entered on study will be closely monitored for any clinical or laboratory evidence of auto-immune phenomena.

6.1.3 A final source of toxicity in this study relates to the use of adenoviral vectors. Wild type adenovirus serotype 5 (from which the vector used in this study is derived) is associated with an upper respiratory infection and conjunctivitis. There is controversy regarding a role in infantile intussusceptions. Other adenovirus serotypes are associated with cystitis, gastroenteritis, and pneumonia in military recruits during basic training. The adenoviral vector used in this study will be certified to contain less than one replication competent serotype 5 viral particle per vaccine inoculum. In the event that recombination between a latent wild type adenovirus and the introduced adenoviral vector occurs in the tumor cell, this will not lead to production of a replication competent GM-CSF expressing virus, as the GM-CSF cDNA is inserted in the E1 deleted region of the vector. Transduced cells will be injected into the skin; thus in the absence of an open wound, there is no significant risk of transmitting the virus to close contacts. If skin breakdown does occur, the skin should be covered with an adherent bandage until healing has occurred.

6.1.4 Lethal irradiation represents an important safeguard against some toxicities associated with vaccinating cells. Irradiation will prevent the outgrowth of tumor cells potentially rendered more virulent by virtue of in vitro manipulation or insertional mutagenesis, as well as the autonomous proliferation of any non-neoplastic elements in the vaccinating inoculum induced by the autocrine secretion of GM-CSF. Toxicities observed from the previous adenovirus-based melanoma trial were restricted to erythema, pruritus, and swelling (all easily treated with either ice packs or topical lotions such as Aloe cream). It is possible,

although unlikely, that additional toxicities including blister formation or ulceration may occur.

6.2 Toxicity Management

6.2.1 Treatment of toxicities will be supportive. If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used. This may include for example, cold compresses and or moisturizing lotions applied to localized vaccine site reactions.

All adverse events experienced by participants will be collected from the time of the first vaccine administration, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit will be followed until the toxicity has resolved, stabilized, determined to be irreversible or the participant is lost to follow-up.

6.2.2 General Concomitant Medication and Supportive Care Guidelines

Patients are prohibited from taking steroids, NSAIDS, antihistamines (by mouth or IV) but may use local measures such as ice packs or topical moisturizers between the time of the first vaccination and two weeks after the sixth vaccination. Use of topical anesthetics such as EMLA © and topical antihistamines are prohibited at injection sites.

6.3 Dose Modifications/Delays

6.3.1 There will be no dose adjustments for Grade 1 and 2 toxicities seen on this study.

6.3.2 If a patient develops grade 3 toxicity attributable to the vaccine, additional treatment will be withheld until all toxicity has resolved, then he or she may be retreated at monthly intervals. If the toxicity recurs with resumption of vaccine then the patient will be removed from study. If a patient develops a grade 4 toxicity attributable to the vaccine, he or she will be removed from study. If three grade 4 or greater toxicities attributable to the vaccine are observed at any time, the trial will be suspended to investigate the causes of these unexpected toxicities.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Description

GVAX is a vaccine composed of cells from lethally irradiated, autologous colorectal or hepatic metastasis engineered by adenoviral mediated gene transfer to secrete GM-CSF

7.2 Form

Cells will be thawed in a dedicated laminar flow biosafety cabinet in the CMCF and washed. Cells will be resuspended in a volume of 1 ml. of sterile saline for administration.

7.3 Storage and Stability

Tumor cells for vaccination and immunologic evaluation will be cryopreserved and stored in liquid nitrogen. A minimum of six individual vaccine aliquots will be prepared for each patient. Cell dose per aliquot will be fixed for an individual patient and will range from a minimum of $1x10^5$ cells per aliquot to $1x10^7$ cells per aliquot. The dosage will be determined by dividing the total cell yield following transduction into six aliquots (after QC removal). Thus, a minimum of $6.5x10^5$ cells total will be required to prepare six aliquots of $1x10^5$ cells. For total cell yields greater than $6x10^7$cells total, individual aliquots will remain at $1x10^7$ cells per dose.

7.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.5 Availability

GVAX is an investigational agent and will be supplied free-of-charge from CMCF.

7.6 Preparation

Tissue obtained from a patient by hepatic resection will transferred to the Cell Manipulation Core Facility (CMCF) at the Dana Farber Cancer Institute in a sterile container containing sterile media. The tissue will be processed to single cell suspension by mechanical and enzymatic digestion as required; short-term tumor cultures may be established if necessary. Tumor cells will be transduced with a replication defective adenoviral vector encoding human GM-CSF.

After transduction, the tumor cells will be washed extensively and irradiated with 10,000 cGy.

A small aliquot of the transduced cells will be placed into culture and GM-CSF secretion will be determined by ELISA. The target level for GM-CSF production will be at least 40 ng/10^6 cells/24 hours, although achieving this secretion will not

be required for vaccine administration. Routine sterility cultures and testing for endotoxin and mycoplasma contamination will be performed.

Individual vaccine cell dose will vary depending on the final cell yield from vaccine production. The total cell yield will be divided in 6 aliquots, and the minimal dose will be $1x10^5$ cells/vaccine and maximal dose will be $1x10^7$ cells/vaccine. This variation in cell dosage is designed to maximize each patient's opportunity of receiving vaccinations and is not based on any expectation of significant differences in toxicity as a function of cell number.

7.7 Administration

Vaccinations will be administered on days 1, 8, 15, and every two weeks thereafter until 6 vaccines have been administered or the patient is removed from study.

Individual vaccine cell dose will vary depending on the final cell yield from vaccine production. The total cell yield will be divided in 6 aliquots, and the minimal dose will be 1x105 cells/vaccine and maximal dose will be 1x107 cells/vaccine. Cells will be thawed in a dedicated laminar flow biosafety cabinet at CMCF and washed. Cells will be resuspended in a volume of 1 ml. for administration. 2 injections (1/2 dose subcutaneously and 1/2 dose intradermally) will be given and will constitute a single vaccination. The volume of each injection will be 0.5 ml.

A study team physician, PA, NP or RN will administer the injections. Injections will be administered in the outpatient setting according to standard procedure in the patient's arms and thighs on a rotating basis.

7.8 Ordering

The vaccine will be manufactured at the Cell Manipulation Core Facility, (CMCF). Once an Order for Processing has been received by CMCF, cells will be thawed for administration.

7.9 Accountability

Study reagent accountability is maintained by the Cell Manipulation Core Facility (CMCF).

7.10 Destruction and Return

CMCF will receive reagents. If they are not used the reagents will be held for future use until they are out of date at which point they will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

Immunologic studies will be performed on peripheral blood at designated time points as well as the resected hepatic metastasis and skin punch biopsies. Punch skin biopsies will be obtained 2 days after the first and fifth vaccinations. A piece of the resected hepatic metastasis will be fresh frozen and stored at -80 degrees at MGH. The remainder of the specimen will be stored in paraffin at MGH. If the patient had a colorectal resection at MGH in the past we may analyze the immune infiltrate of the primary cancer which is banked in paraffin (see 8.1.3); however, resection of the primary tumor at a hospital other than MGH is not an exclusion criterion.

If the patient develops recurrent disease, and undergoes clinically indicated resection, biopsies may also be obtained for immune analysis as well.

8.1.1 Vaccine site reactions: Punch skin biopsies will be obtained 2 days after the first and fifth vaccinations. Histological analysis for macrophages, dendritic cells, eosinophils, and lymphocytes will be performed as a research test. Specimen's will be transported in 10% formalin to the CMCF where they will be logged in for research.

8.1.2 Tumor infiltrates: The primary and hepatic lesions will be scored, as a research test, for cellular infiltrates (CD8+ T cells, CD4+ T cells, Treg, macrophages, etc), inflammation, edema, fibrosis, and percentage tumor cell death. Ten high power fields will be evaluated and the numbers summed. The character and type of infiltrating cells will be evaluated by routine histological and appropriate immunohistochemical analysis. Attempts will be made to obtain discarded tissue from biopsies performed to document disease recurrence as part of the patients' standard of care. The discarded tissue from the recurrent disease biopsies will also be assessed for immune infiltrates as part of the correlative science in this protocol.

8.1.3 Blood analyses: Serum and circulating mononuclear cells will be frozen prior to beginning vaccination and at regular intervals thereafter as described in Section 9.0. These samples will be used in research studies aimed at identifying the target antigens of vaccination. We may also use these samples, in research tests to measure immune responses against adenoviral proteins expressed in the vector used for vaccine preparation.

9.0 STUDY CALENDAR

Data Collection

Test	Day -21*	Day 1	Days 8, 15	Days 29, 43, 57	Restage[1]	Additional Vaccines
History/PE	X	X	X	X	X	
Weight/VS	X	X[9]	X	X	X	X
CBC with Diff	X	X	X	X	X	X
PT, PTT	X					X[7]
Lytes/BUN/Cr	X	X		X	X	X
LFT	X	X		X	X	X
Glucose	X					X
β-HCG[9]	X					X
HIV/ID profile[6]	X					X[8]
Staging Scans[2]	X				X	X
Immune[3]	X	X		X	X	X[3]
Biopsy		X[4]		X[5]		X[4]
CEA		X			X	X

* or later

1. Participants will be monitored before the first vaccine and every 3 months with a CEA level (1 tsp) for the first 3 years and then every 6-12 months for a total of 5 years. Restaging CT scans (chest, abdominal, pelvic CT) will be performed at 3 months (or sooner if clinically indicated) following completion of vaccine and then every 6 months for the first three years and then yearly to year 5. After 5 years imaging will be at the discretion of the treating physician.
2. Staging scans should be a chest, abdominal, and pelvic CT and must be obtained within three weeks (21 days) of tumor harvest.
3. Approximately 50 cc of blood on a monthly basis during vaccination and every 3 months after completing all vaccinations for two years.
4. Biopsies of vaccination reaction (vaccine #1) will be taken 2 days after administration.
5. Biopsies of vaccination reaction (vaccine #5) will be taken 2 days after administration.
6. Testing for Infectious Disease Markers (IDM testing) should be performed within seven days of liver resection. IDM testing requires: Syphillis, CMV, HepBSAg, Hep B Core, HCV, HIV ½, HTLV ½, WNV.
7. Repeat if Tumor Harvest is required for second round of vaccine.
8. Serum pregnancy tests only required of women of childbearing potential
9. Vital signs are to be recorded 20-30 minutes after vaccine administration.

10. MEASUREMENT of EFFECT

Although response is not the primary endpoint of this trial, participants with measurable and/or non-measurable disease will be assessed by RECIST criteria. Restaging CT scans (chest, abdominal, pelvic CT) will be performed 3 months (or sooner if clinically indicated) following completion of vaccine and then every 6 months for the first three years and then yearly to year 5. After 5 years imaging will be at the discretion of the treating physician. In addition to a baseline scan, confirmatory scans should also be obtained twelve weeks following initial documentation of an objective response

10.1 Antitumor Effect– Solid Tumors

Restaging CT scans (chest, abdominal, pelvic CT) will be performed 3 months (or sooner if clinically indicated) following completion of vaccine and then every 6 months for the first three years and then yearly to year 5. After 5 years imaging will be at the discretion of the treating physician. In addition to a baseline scan, confirmatory scans should also be obtained every 12 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline. Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria

Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of the first 6 vaccines will also be considered evaluable.}

10.1.1 Disease Parameters

Measurable disease.

Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed

Non-measurable disease.

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

10.1.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

FDG PET and PET/CT. The acquisition of FDG PET and FDG PET/CT scans should follow the NCI Guidelines for using FDG PET as an indicator of

therapeutic response {Shankar, 2006 #3452}, Patients should avoid strenuous exercise and be on a low carbohydrate diet for 24 hours prior to the scan. Patients should fast for 4 hours or longer prior to the FDG injection and should have a serum glucose of less than 200 mg/dL at the time of FDG injection. A 10-20 mCi dose of FDG should be injected for typical adult patients. For longitudinal studies with multiple scans, particular attention should be paid to ensure consistent patient preparation and acquisition parameters between the follow-up scan and the baseline scan. When designing a study where PET scans are going to be utilized as one of the modalities to evaluate efficacy, it is important to consult with physicians in nuclear medicine in designing the appropriate criteria to be utilized.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.1.3 Response Criteria

10.1.3.1 Evaluation of Target Lesions

Complete Response (CR):

Disappearance of all target lesions. Any pathological lymph node must have reduction in short axis to < 10 mm.

Partial Response (PR):

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD):

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5

mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

Stable Disease (SD):
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.3.2 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for when Confirmation is Required:
CR	CR	No	CR	≥4 wks confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	
SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	NonCR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

10.1.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time of operation until the first date that recurrence is objectively documented or death.

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.1.5 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the length of time during and after the treatment of a cancer, that a patient lives with the disease but it does not get worse, as per the NCI. For this clinical trial it is measured as the duration of time between the hepatic resection to the time of objective disease progression (recurrence) or death.

10.1.6 Response Review

Central review of staging scan will not be performed.

11 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

Adverse Event (AE)

Dana-Farber Cancer Institute/Harvard Cancer Care guidelines for reporting SAEs should be followed, including the guidelines for gene transfer studies. (http://www.dana-farber.org/res/OPRS/advevent/default.asp). These guidelines require the following events to be reported to the DFCI IRB: (1) Grade 2 (moderate) and Grade 3 (serious) events that are unexpected and possibly, probably, or definitely related/associated with the intervention; (2) all Grade 4 (life threatening) events, and (3) all Grade 5 (fatal) events, occurring while the subject is enrolled and actively participating in the trial, or when the event occurred within 30 days of the last study intervention. In accordance to these guidelines, the following steps will be taken to report a SAE:

Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more sever form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- In a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

All life-threatening events which may be due to vaccine administration

All fatal events while on study

All such events will be reported to the DFCI Office for the Protection of Research Subjects by the protocol chairperson within 48 hours.

11.4 Reporting to the Principal Investigator

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 9.1, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Cristina Ferrone, M.D.
Tel : 617-643-6189
cferrone@partners.org
Fax: 617-643-6116

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the IND Sponsor

Adverse events meeting the following criteria, regardless of relatedness or expectedness, must be reported to the IND Sponsor (or designee) for those subjects who received at least 1 vaccine:

- Grade ≥3 Non-Hematologic
- Grade ≥3 Hematologic
- All Grade 5 events

Reporting to the Sponsor begins at time of first vaccine and goes until 15 years after the subject's last vaccine.

All events meeting the criteria above must be forwarded to the IND Sponsor (or designee) within 2 business days of knowledge of the event using an FDA Form 3500A.

11.6 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

11.7 Reporting to the Food and Drug Administration (FDA)

Robert Soiffer, MD as holder of the IND (IND Sponsor), will be responsible for all communication with the FDA. The IND Sponsor will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.8 Reporting to the NIH Office of Biotechnology Activities (OBA)

The IND Sponsor will be responsible for all communication with OBA. The IND Sponsor will report to OBA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the gene transfer product.

Unexpected fatal or life-threatening experiences associated with the use of the gene transfer product will be reported to OBA as soon as possible but in no event later than 7 calendar days after initial receipt of the information (i.e., at the same time the event is reported to FDA).

All other serious unexpected experiences associated with the use of the gene transfer product will be reported to OBA as soon as possible but in no event later than 15 calendar days after initial receipt of the information (i.e., at the same time the event is reported to FDA).

The serious adverse event report must include: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type , e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo, ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported on the Form FDA 3500A, or other means provided that all of the above elements are specifically included.

11.9 Reporting to the Institutional Biosafety Committee (IBC)

The Overall Principal Investigator will report to the IBC any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the gene transfer product, provided that the event occurred at their investigative site.

Unexpected fatal or life-threatening experiences associated with the use of the gene transfer product will be reported to the IBC as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the gene transfer product will be reported the IBC as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Copies of all IBC submissions should be emailed to the IND Sponsor (or designee) within 2 business days of submission.

Participating investigators should obtain from the IND Sponsor a copy of the adverse event report submitted to the FDA and OBA, and forward this information to their respective IBC as soon as possible.

11.10 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.11 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12. 2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13 REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

    - Title 21 Part 11 – Electronic Records; Electronic Signatures
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

    - Title 21 Part 312 – Investigational New Drug Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws

- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-center Guidelines

N/A

13.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A

14 STATISTICAL CONSIDERATIONS

The study consists of a single stage of accrual of patients with hepatic metastases from colorectal cancer.

Patient safety will be assured by monitoring the number of patients who fail to receive the first six scheduled vaccinations because of toxicity. If three or more patients experience grade 4 or worse toxicity due to the vaccine before completing six immunizations, the study will be terminated. If the unknown true rate of grade 4 or worse toxicity is 0.05, then the probability of early termination is 0.04. Similarly, if the unknown true rate is 0.25, then the probability of early termination is 0.76.

Feasibility will be assessed by the ability to make vaccine from resected hepatic metastases in quantities sufficient for six vaccinations. Fifteen patients will be entered. If fewer than 9 patients receive six vaccinations, the study will cease. A success rate under 65% (true rate) may imply that the method needs further development or that the amount of tissue available in this population limits the feasibility of this strategy. This study will have 75% power against the null of 45% success rate, testing at the one-sided 0.18 significance level and using exact methods.

The percentage of patients who successfully receive 6 vaccinations and the number of vaccinations received by patients will be described. Any late occurrences of grade 3 or worse toxicity and the vaccination number at which they occur will be reported.

For the correlative science it is unclear which changes will occur in the circulating immune cells since a study like this has never been done in patients with resected hepatic metastases from colorectal cancer. Currently we do not have estimates to present a statistical model or plan. No changes in the correlative science should influence administration of the vaccine in the patients.

Table 1. 90% confidence intervals for the true proportion of patients who receive all six injections

Observed number of	Observed rate of patients	90% confidence interval for

patients who receive all six injections	who receive all six injections	the true rate of patients who receive all six injections
8	53%	(30%, 76%)
10	67%	(42%, 86%)
11	73%	(49%, 90%)
12	80%	(56%, 94%)

14.1 Study Design/Endpoints

Primary

14.1.1To determine the feasibility of manufacturing lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF from hepatic metastases.

14.1.2 To determine the safety and biologic activity of vaccination with lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF in stage IV colorectal cancer patients.

Secondary

14.1.3 To determine the progression free survival and two-year survival of stage IV colorectal cancer patients vaccinated with lethally irradiated, autologous colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF

14.2 Sample Size/Accrual Rate

The plan is to enroll 15 patients with in two (2) years.

14.3 Reporting and Exclusions

14.3.1 **Feasibility**- Feasibility will be defined by the capacity for patients in this specific population to initiate the vaccination process, i.e., to receive at least one vaccination This will be governed by the ability to successfully harvest a minimum number of viable tumor cells at the time of surgery.

At the treating physician's discretion, patients with progressive disease or treatment-related complications between surgery and initiation of vaccination who are deemed incapable of or inappropriate for treatment will not initiate vaccination and will be included in the feasibility analysis.

We will consider this treatment plan feasible in this patient population if at

least 60% of initially enrolled patients go on to begin vaccination.

If an enrolled patient withdraws from the study for a reason that is not associated with surgical morbidity or disease progression, that patient will not be counted in the feasibility analysis.

14.3.2 **Safety and Biologic Activity**: All participants who receive at least one vaccine will be considered evaluable for toxicity. All participants who receive all 6 vaccines will be evaluable for biologic response.

14.3.3 **Evaluation of response.** All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

15 PUBLICATION PLAN

The Primary Investigator, Cristina Ferrone, M.D., will submit the results for the Publication with in twenty-four months of closing the trial.

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17 APPENDICES

17.1 Certification of Vector and Transduced Tumor Cells

17.1.1 Vector Manufacturing Process

Description

A portion of the resected hepatic metastasis measuring between 1-2 cm^2 will be placed in sterile saline and transported on ice to the DFCI by one of the study staff members.

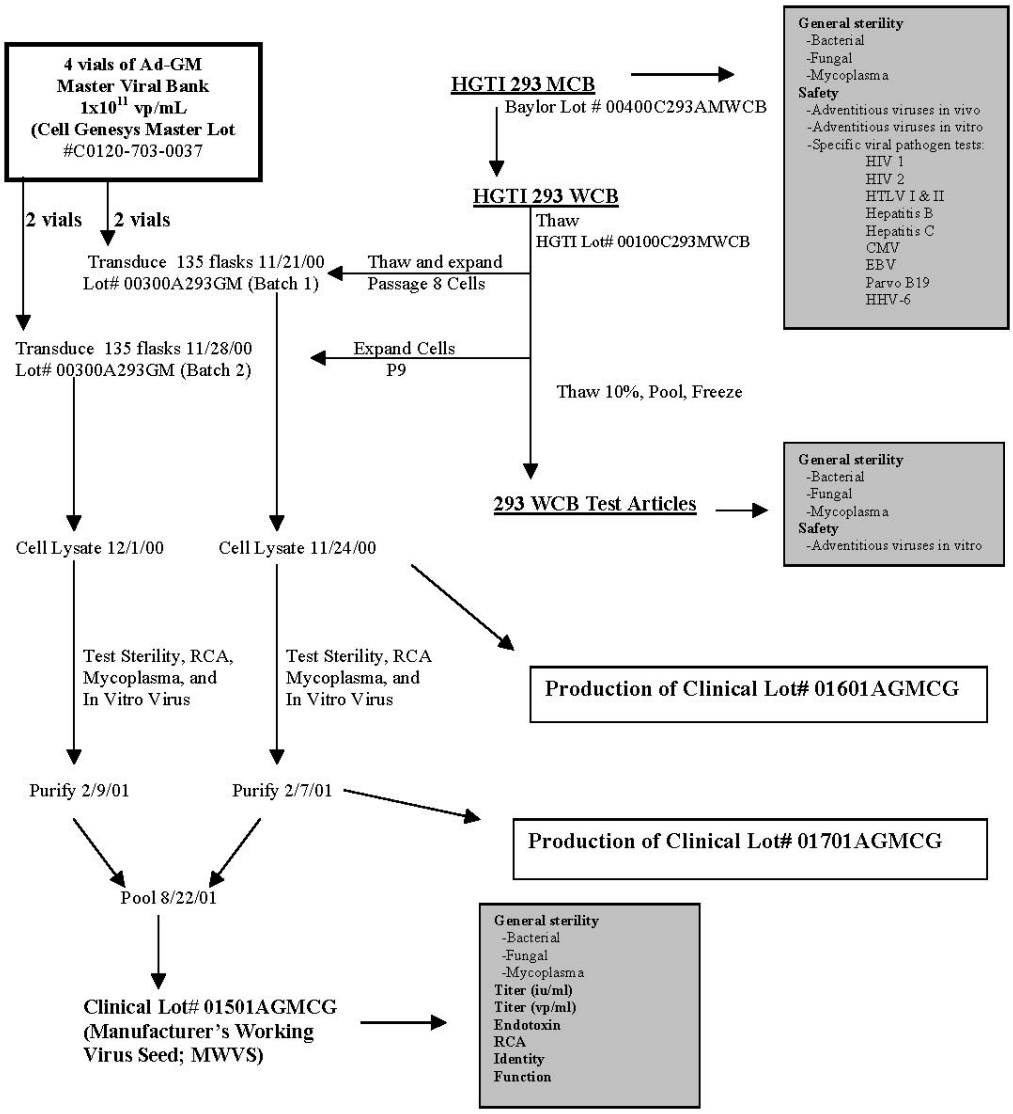
The CG6444 vector is a replication-defective, E1/E3-deleted type 5 adenoviral vector containing a full-length, human genomic GM-CSF expression cassette inserted in the E1 region.

Derivation of the CG6444 Vector

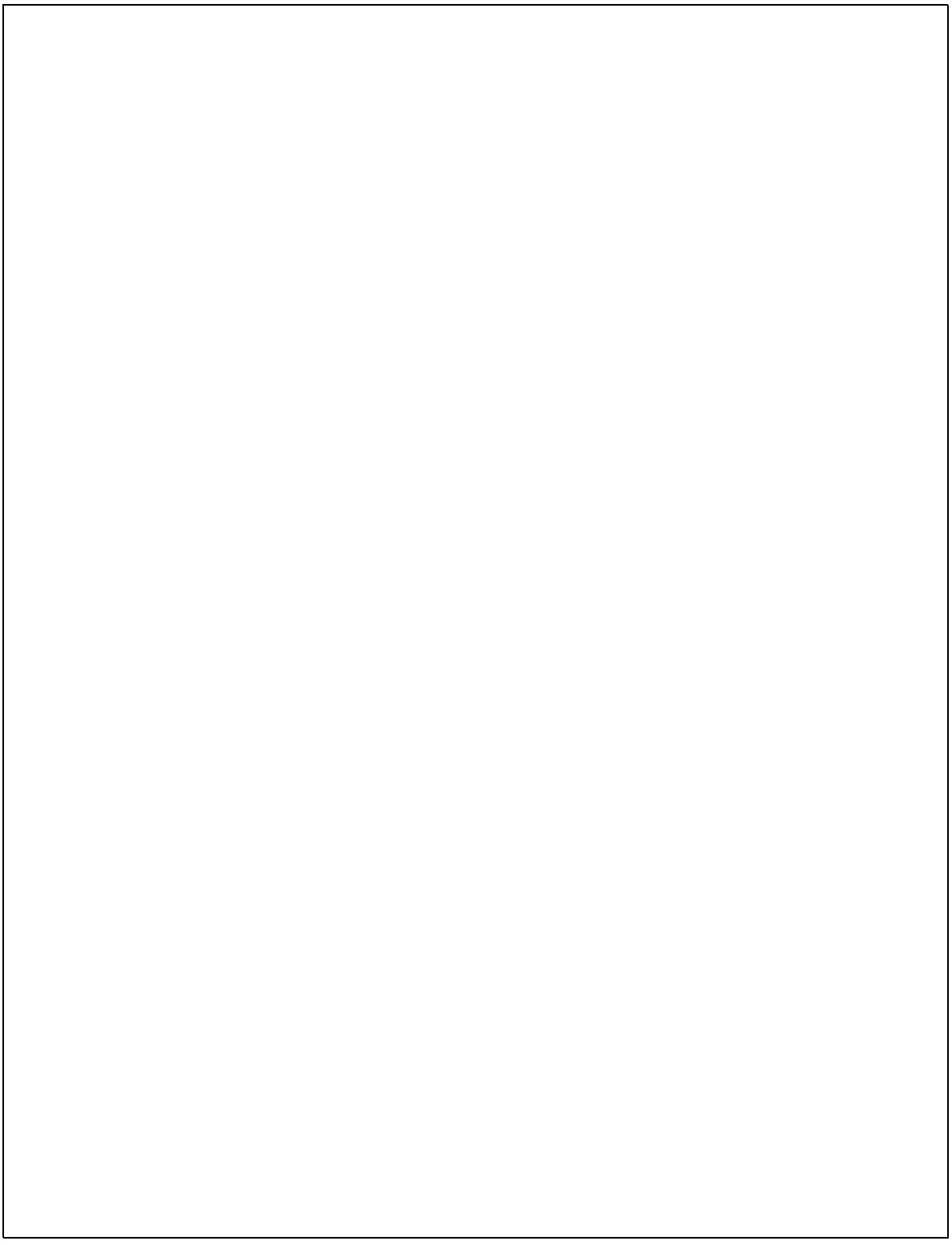
The human genomic GM-CSF (hgGM-CSF) gene was amplified by polymerase chain reaction (PCR) from a plasmid artificial chromosome (PAC) (PAC282; Genome Systems, St. Louis, MO) using forward and reverse primers complementary to genomic nucleotide sequences immediately flanking the hgGM-CSF gene. To aid in recombinant DNA cloning of the PCR-amplified product, EcoRI restriction endonuclease recognition sequences were included at the 5-prime ends of both primers. Following EcoRI digestion, the amplified product was non-directionally cloned into the EcoRI site of an Ad E1-targeting shuttle vector, plox III-4 CMV. Resulting clones were screened by restriction endonuclease digestion for proper orientation of the insert, relative to the promoter. One properly oriented clone (plox III-4 CMV/hgGM-CSF # 5) was identified and the insert sequenced. Exon coding regions for the cloned hgGM-CSF insert were identical to sequences published in GenBank.

An infectious adenovirus clone was generated by homologous recombination in yeast. For recombination, transfer vector plox III-4 CMV/hgGM-CSF #5 and PAC A/U E1E3, E4+ (containing the remainder of the adenovirus genome) were chemically transformed into yeast strain, YPH857. Yeast clones were screened by restriction digestion and Southern blot analysis using probes for both total adenovirus genomic- and hgGM-CSF-DNA. One clone E1E3 hgGM-CSF #8, with proper DNA fragment size and identity profile was isolated. DNA fragment migration and hybridization patterns confirmed insertion of hgGM-CSF in the E1 region, and maintenance of the E3 deletion from the adenovirus backbone. Presence of adenoviral E4 genomic sequences was confirmed by PCR analysis. Infectious virus was rescued from cloned E1E3 hgGM-CSF #8 DNA by transfection into 293 cells. Viral clones were triple plaque-purified on 293 cells and screened by DNA restriction analysis. A plaque-purified CG6444 clone was isolated and the E1 region was sequenced. Inserted hgGM-CSF coding sequences were identical to sequences published in GenBank. The CG6444 virus construct, derived from the Master Virus bank, has been sequenced in its entirety. (This sequence is presented as Attachment 3 in the Master File CMC BB-IND 8793).

SCHEMATIC REPRESENTATION FOR PRODUCTION OF AD-GM MASTER VIRAL SEED
LOT #01501AGMCG



Certificates of Analysis for Master and Working Cell Banks and Virus Banks



Appendix (*letter*): Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.