ETUBICS CORPORATION

Protocol Version 1.1, December 21, 2009

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

Principal Investigator: Michael Morse, M.D.
Duke University Medical Center

Agents Used in This Study: Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)

Names and addresses of key study personnel:

<table>
<tr><td>Study Chairman and Principal Investigator
Name: Michael Morse, MD
Address: Duke University Medical Center
Box 3233, Durham, NC 27710
Telephone: (919) 681-3480
Fax: (919) 681-7970
Email: michael.morse@duke.edu</td><td></td></tr>
<tr><td>Bioassay Oversight/Laboratory Oversight
Name: Joseph P. Balint, PhD
Address: Etubics Corporation
410 West Harrison Street,
Seattle, Wa 98119
Telephone: (206) 838-5110
Fax: (206) 838-2978
Email: joe@etubics.com</td><td>Biostatistician
Name: Donna Niedzwiecki, PhD
Address: Duke University Medical Center
Box 3958, Durham, NC 27710
Telephone: (919) 681-5030
Fax: (919) 691-8028
E-mail: donna.niedzwiecki@duke.edu</td></tr>
<tr><td>Other Applicable Staff
Title: Acting Medical Director
Name: H. Kim Lyerly, MD
Address: 2424 Erwin Road, Suite 601
Durham, NC 27710
Telephone: (919) 684-5613
Fax: (919) 684-5653
Email: lyerl001@mc.duke.edu</td><td>Pharmacist or Person Responsible for Drug Accountability
Name: Cheryl Morgan
Address: Morris Pharmacy, Duke University
Medical Center, Trent Drive,
Durham, NC 27710
Telephone: (919) 668-0657
Fax: (919) 668-3895
Email: cheryl.morgan@duke.edu</td></tr>
<tr><td>Other Applicable Staff:
Title: CEO and CSO, Etubics Corporation
Name: Frank R. Jones, Ph.D.
Address: Etubics Corporation
410 West Harrison Street
Seattle, WA 981119
Telephone: 206-838-5110 Ext 101
Fax: 206-838-2978
Email: frank@etubics.com</td><td>Other Consultants:
Andrea Amalfitano, DO, PhD, Michigan State University
Elizabeth Gabitzsch, Etubics Corporation
Raj Dua, Ph.D., Etubics Corporation
Timothy Clay, PhD, Duke University</td></tr>
</table>

TABLE OF CONTENT

Page Number

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by any of the following: immunohistochemical staining, a CEA level in the peripheral blood greater than 5 ng/dL, or a tumor known to be universally CEA positive (*i.e.* colon and rectal cancer). Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, trastuzumab, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving steroid or immunosuppressive therapy. All patients must be >18 years old and have an ECOG Performance Status of 0 or 1. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase I component), and the maximally tolerated dose of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2B-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Peripheral blood draw of 90 mL for immune analysis 2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions or neurological toxicity, other Grade 3 or 4 allergic or major organ toxicity, or Grade 4 fever, that may possibly be associated with the immunization), then patients may

	begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles SQ in 0.7 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II cohort: An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the maximally tolerated dose every 3 weeks for 3 immunizations. 6) Patients will have 90 mL peripheral blood drawn prior to each immunization and at Week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 7) Time to progression will be measured using CT scans performed as per standard of care (approximately 3 month intervals).
Risks/toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 24 evaluable patients (plus up to 12 replacements); may require 30-42 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total
Criteria for Evaluation	Toxicity will be assessed using CTC toxicity criteria. CEA-specific immune response will be measured by ELISpot. Time to recurrence will be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a

	dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 1 immunization.

2. STUDY OBJECTIVES

a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

3. BACKGROUND AND SIGNIFICANCE

[redacted]

[redacted]

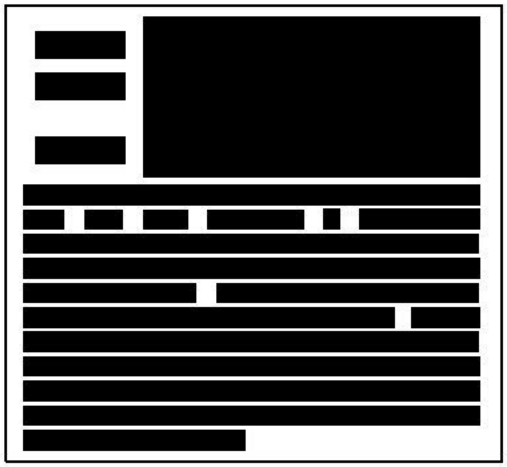
[redacted]

[redacted]

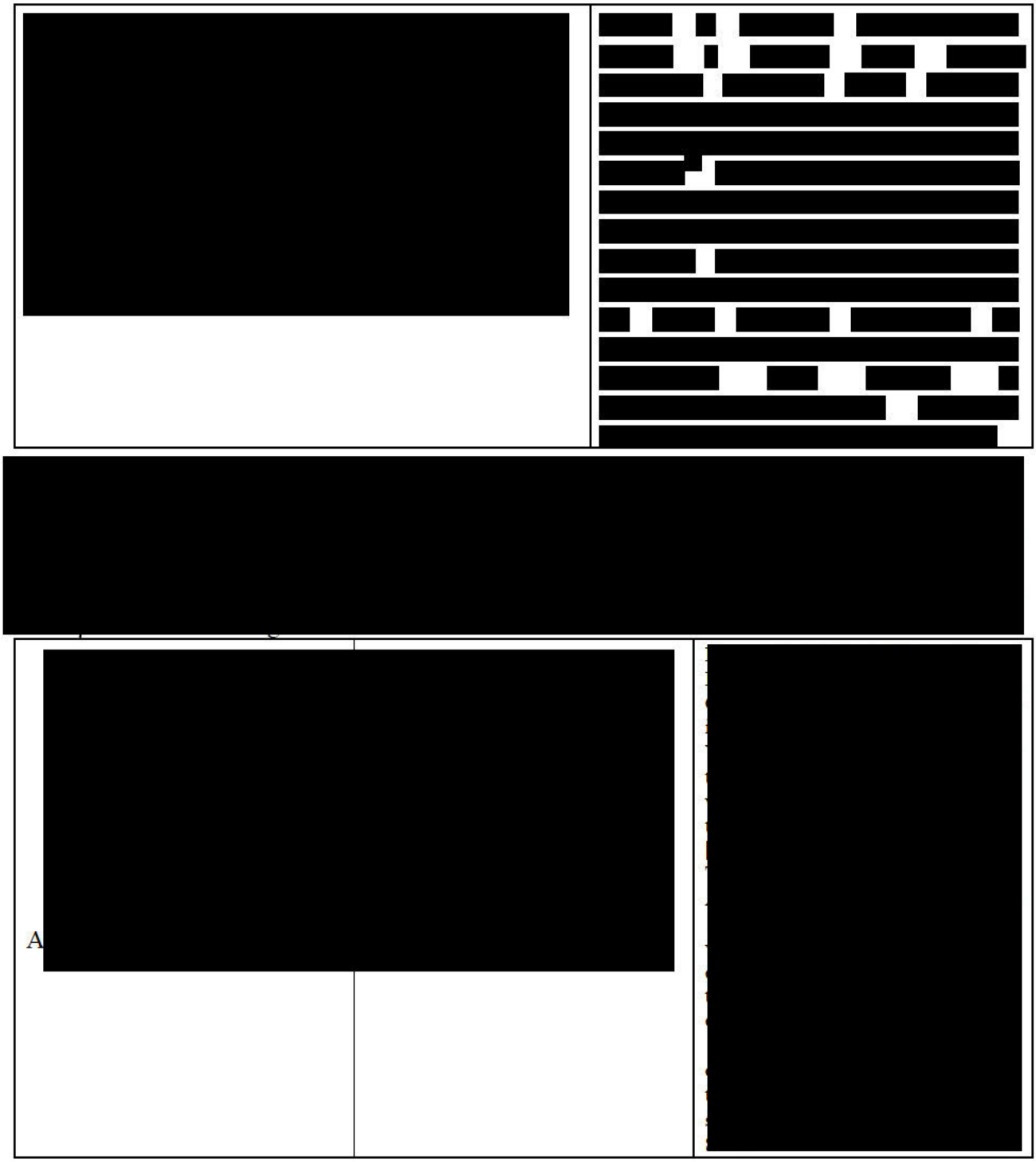
[redacted]

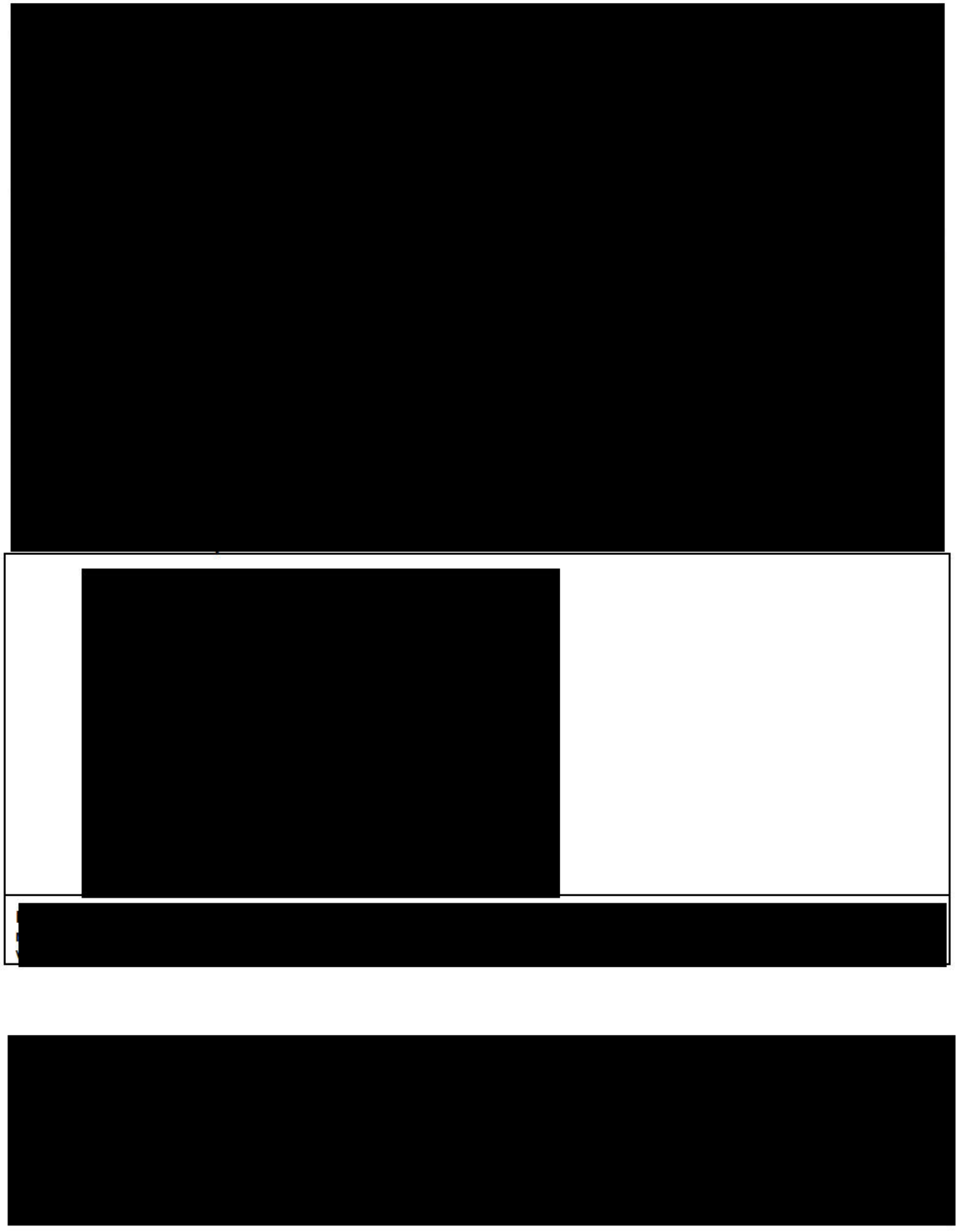
[redacted]

[redacted]



[redacted]





4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of metastatic malignancy due to a tumor expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. The tumor must express CEA as defined by any of the following: immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining), a CEA level in the peripheral blood greater than 5.0 ng/mL at any time prior to enrollment, or a tumor known to be universally CEA positive (i.e. colon and rectal cancer.)

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit or refused such therapy.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:

  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these therapies for at least 3 months).

- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these therapies for at least 3 months).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.
  - Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this therapies for at least 3 months).
- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.

- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. ECOG 0 or 1 performance status

4.1.5. Estimated life expectancy > 3 months..

4.1.6. Age ≥ 18 years, but ≤75.

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (may transfuse or use erythropoietin to achieve this level), platelets ≥ 75,000/microliter; PT-INR <1.5, PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosuppression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to Duke University Medical Center for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, trastuzumab, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered from all acute toxicities from prior treatment.

4.2.2. Patients with a history of brain metastases will not be permitted

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot) or viral hepatitis (as determined by HBsAg and Hepatitis C serology). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections. Patients with active CMV disease will be excluded, but CMV seropositive patients will be eligible.

4.2.8. Patients on steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies) prior to enrollment.

4.2.9. Patients with allergies to any component of the vaccine will be excluded from the protocol.

4.2.10. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.11. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.12. Patients will be allowed warfarin 1mg po qd for port prophylaxis but not full dose warfarin or low molecular weight heparin.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if DLT occur that necessitate re-dosing at a lower dosage levels (see section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number: Patients will be assigned study numbers in order of their screening using the following: ETBX-011-xxx.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

(See also Schema in Appendix 1.) The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment:

- History and physical exam, to include ECOG Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological, biochemical and immunological tests:

  CBC with differential

  PT INR and PTT

  Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose

  Urinalysis
- Infectious Disease

  HIV antibody, Hepatitis BsAg, Hepatitis C serology, CMV serology
- ECG
- Biological Markers:

  Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad vector, and other available serum markers (e.g., CEA or CA15-3) will be reviewed.
- Archive Samples:

  An additional 20 mL of blood may be drawn during the first clinic visit, when the patient history and physical exam are conducted, at the discretion of the immune monitoring laboratory, and serum stored for later analysis of immune responses.
- Imaging studies:

  CT or MRI scans of the chest, abdomen, brain, and/or pelvis will be requested within 1 month (±2 weeks) before starting study treatment to document the presence and size of any measurable metastatic disease that might present. CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI 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 in the chest, abdomen, and pelvis.

6. TREATMENT PLAN

In the phase I component, patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (based on CTCAE4.0 criteria) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, other Grade 3 or 4 allergic or major organ toxicity, or Grade 4 fever that may possibly be associated with the immunization.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component without the 1-week waiting period. Between dosage levels, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed by the Principle Investigator. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II, 12 additional patients will be enrolled at the MTD. In phase II, if at any time the rate of DLT in patients enrolled at the MTD (for the phase I and phase II cohorts combined) is ≥33%, the MTD will be re-defined as the next lower dosage level, and phase II will proceed with enrollment of additional patients at this lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be suspended and the study re-evaluated.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{11} particles in 0.7 mL SQ in the same thigh every 3 weeks for 3 immunizations.

Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then this dosage level will be considered the MTD and patients may begin enrolling into the phase II portion of the study. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

4) Phase II cohort: After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

   Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

6) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

7) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

9) The following safety events will trigger a temporary suspension of study vaccinations:

   a) If ≥66% of patients in cohort 1 experience DLT

   b) If ≥33% of patients in the phase II cohort experience DLT at dosage level 1 (i.e.,1 x 10^9 particles)

   c) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine

   d) If, at any time, more than 50% of patients experience a Grade 3 or 4 major organ toxicity

   e) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

The Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) will fully review all available safety data, consult with the principal investigator, medical

monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above.

Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

Figure 3A

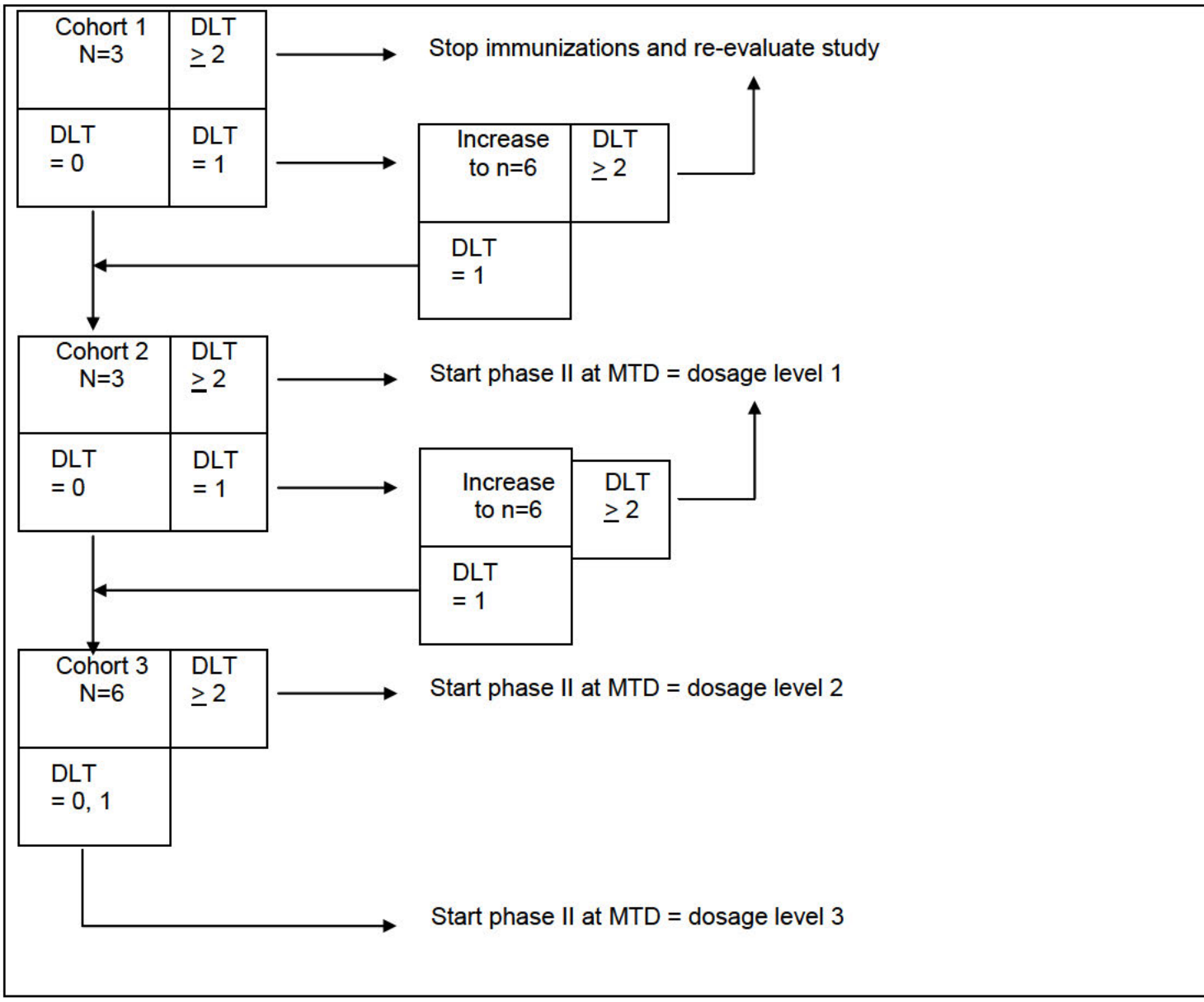


Figure 3B.

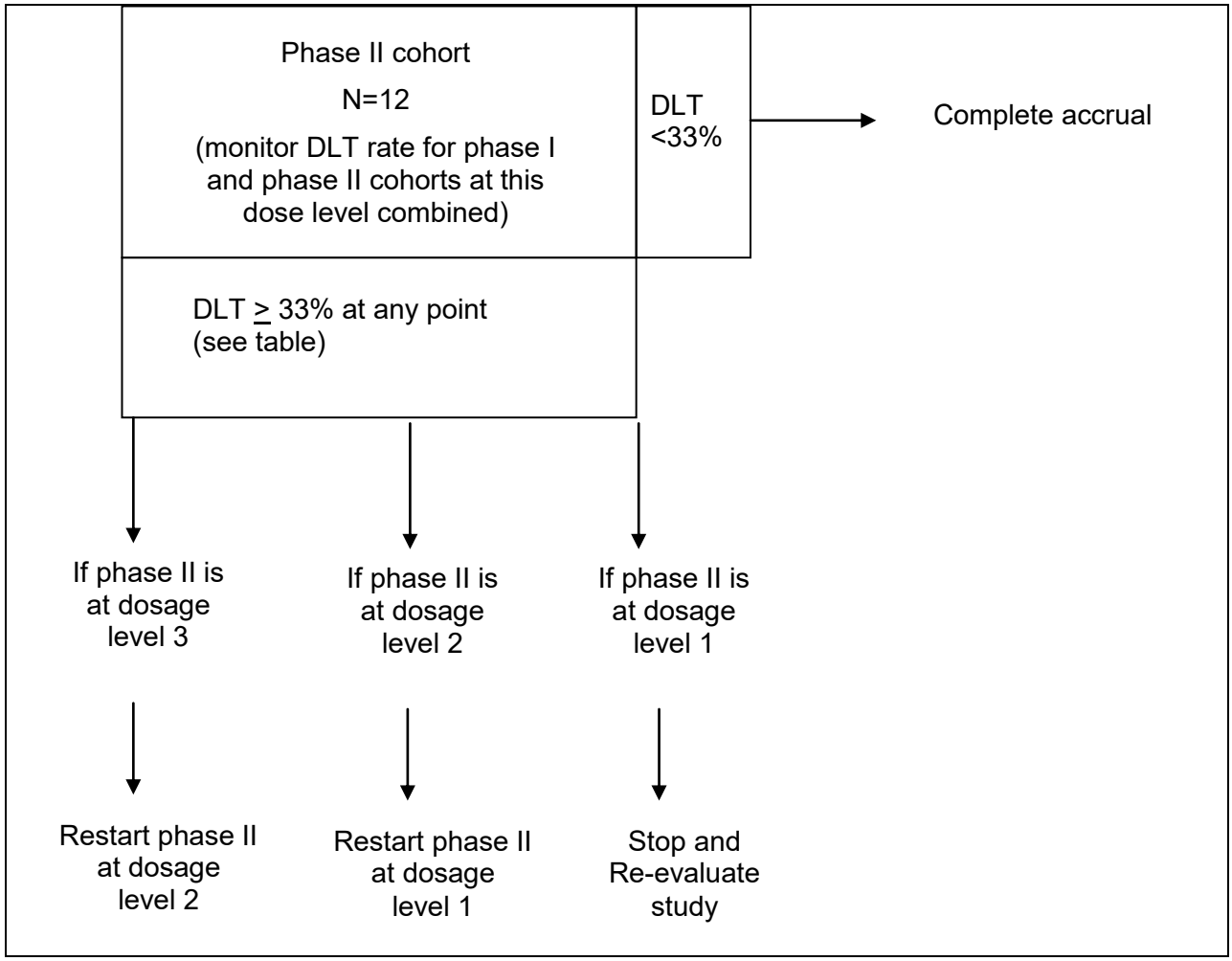


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.1. Pharmaceutical Information

6.1.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, or 1 x 10^{10}, particles subcutaneously (SQ) in 0.5 mL of a buffered saline solution every 3 weeks for 3 immunizations and patients who receive 1x10^{11} VP will receive Ad5[E1-, E2b-]-CEA(6D) in 0.7 mL of ARM formulation buffer SQ every 3 weeks for 3 immunizations.

6.1.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains 1X10^{11} total virus particles. The volume of injection for 1x10^{11} virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:

1. To administer 1x10e9 virus particles by subcutaneous injection:
From a 5.0mL vial of 0.9% sterile saline remove 0.05mL of fluid which leaves 4.95mL. Then remove 0.05mL from the vial labeled ETBX-011 and deliver this volume into the 5mL sterile saline vial. Mix the contents by inverting the 5mL diluted drug. Then with draw 0.5mL of diluted drug and deliver to the patient by subcutaneous injection (detailed description of dose preparation is described in the packaging insert).

2. To administer 1x10e10 virus particles by subcutaneous injection:
From a 5.0mL vial of 0.9% sterile saline remove 0.5mL of fluid which leaves 4.5mL. Then remove 0.5mL from the vial labeled ETBX-011 and deliver this volume into the 5mL sterile saline vial. Mix the contents by inverting the 5mL diluted drug. Then with draw 0.5mL of diluted drug and deliver to the patient by subcutaneous injection (detailed description of dose preparation is described in the packaging insert).

3. **To administer 1x10e11 virus particles by subcutaneous injection:**
Withdraw 0.5mL of contents from vial and administer each subject without any further manipulation.

6.1.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joseph P. Balint, Etubics Corporation, 410 West Harrison Street, Suite 100, Seattle, Wa 98119 for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations (each visit for immunization)

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

7.1.2 Hematological and Biochemical Assessment

Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose will be drawn at Week 0,3,6,9, at discontinuation of treatment (if treatment is discontinued early), and as clinically indicated.

7.1.3 Biological Markers

Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained at Week 0, approximately 3 weeks after the last immunization (approximately Week 12), and every 3 months thereafter.

7.1.4 Immunological Assessment

Peripheral blood (90 mL = 9 yellow tops = 62ml; 1 purple top = 7 ml; 3 red tops = 21ml) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Plasma will be archived from the initial and Week 9 blood draws for evaluating antibody levels.

7.1.5 Imaging Studies

CT or MRI scans of the chest, abdomen, and/or pelvis, will be requested (but not required) within 1-4 weeks after the third dose of vaccine. At a minimum, the same imaging modality of the same portion of the body should be performed at each time point.

7.1.6: Ad 5 neutralizing Ab assessment

Serum will be sent to Etubics for Ad5 neutralizing level determination

7.1.7: Monitoring for replication competent virus

If patients become febrile to >101.0 F or AST or ALT> 3 x ULN, then we will draw serum for replication competent virus analysis.

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up at Duke University Medical Center every 3 months for 1 year, while on the study (i.e., have not progressed or been removed from the study for other reasons). At each visit, a medical history and physical exam and labs (Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose) will be drawn.

At each visit, 40-90 mL of peripheral blood for immune analysis may be drawn, if there was previous evidence of an immune response or at the discretion of the investigator.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) Dose-limiting toxicity (DLT) is defined in section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia,

arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 Dose-limiting toxicity related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist at Duke University Medical Center for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations: In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

7.3.4.4 If non-dose-limiting toxicity occurs in a patient having at least a partial response to the vaccine (as defined below and including mixed responses), if the toxicity is not threatening to vital organs, and it appears to be reversible; then individual patients will be allowed to continue on the study, once the risks and benefits of continuing on the study have been discussed and documented.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety:

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosage level will be considered safe if <33% patients

treated at a dose level experience dose-limiting toxicity (i.e., 0 of 3, ≤ of 6, ≤ of 12, or ≤ of 18 patients). Dose-limiting toxicity is defined in section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response:

We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- Dose-limiting toxicity, as defined in section 6.
- Patient voluntarily decides to withdraw.

- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event that a patient withdraws, the patient will be asked to have "off treatment" evaluations performed, which may include 40-90 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator (Dr. Michael Morse) or in his absence, Dr. H. Kim Lyerly, immediately by telephone (Page operator 919 684-8111). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the IRB and the sponsor (Etubics) by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and the sponsor (Etubics) will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the Institutional Review Board (IRB). IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A Case Report Form must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. Case Report Forms must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on case report forms by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto case report forms, originals of laboratory and other test results must be kept on file with patient's case report form or clinical chart. Case report forms and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Experience Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

Protocol data and safety will be monitored by the Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) with the following plan:

1. Purpose: This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of AEs.
2. Monitoring: Up to 24 patients will be enrolled. The principal investigator will continuously monitor the study. The principal investigator will review the data and safety of the study after the enrollment of three patients in cohort 1, after enrollment of three patients in cohort 2, and after enrollment of six patients in cohort 3. Formal, independent monitoring by CPC will occur after enrollment of the three patients in cohort 1 and again after enrollment of three patients in cohort 2; then a scientific progress review will occur yearly, assuming a result of "satisfactory" on the initial review. The exceptions are as follows: If more than one patient experiences a Grade 4 or greater allergic reaction, the principal investigator will request a monitoring review by CPC. If at any time, more than 50% of patients experience a Grade 3 or 4 major organ toxicity, we will request a monitoring review by CPC.
3. Description of Monitoring: Adverse event reports will be reviewed with tabulation of all Grade 2, 3 or 4 toxicity.
4. Toxicity: see section 7.3.2 of the protocol.
5. Reporting Adverse Events: An adverse experience is any adverse change from the study patient's baseline (pre-treatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started. All adverse experiences that are classified as serious as described in section 10.7 of the protocol should be reported to the sponsor (Etubics) by telephone or fax within 24 hours, and reported in writing to the sponsor (etubics) within 72 hours. All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to the principal investigator, with a copy of the autopsy report and the death certificate. All adverse experiences will be recorded on the Adverse Experience Case Report Form. This report form should include severity, duration, outcome, and the investigator's judgment as to the relationship of the adverse experience to treatment.
6. Reporting of Pregnancy: If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the sponsor (Etubics), and to the IRB.

10.6. CTCAE Term (AE description) and Grade:

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 web site (http://ctep.cancer.gov).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Serious Adverse Event Reporting

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

Procedures for adverse event reporting

1. PI notified by medical staff of a SAE
   PI: Michael Morse, MD
   Address: Duke University Medical Center
   Seeley Mudd Building
   Box 3233, Durham, NC 27710
   Telephone: 1-919-681-3480
   Fax: 1-919-681-7970
   Pager 1-919-970-5626
   Email: michael.morse@duke.edu

2. PI calls sponsor to report an unexpected SAE associated with the use of the drug

   Sponsor: Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
   Telephone: 206-838-5110 ext. 102
   Cell: 1-206-818-2985
   Fax: 1-206-838-2978
   Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

   A. Chief Scientific Officer: Frank Jones, PhD
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 101
      Cell: 206-818-2857
      Fax: 206-838-2978
      Email: frj@etubics.com
   B. Laboratory Manager: Joseph Balint, PhD
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 107
      Fax: 206-838-2978
      Email: joe@etubics.com
   C. Vice President Research: Elizabeth S. Gabitzsch
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 103
      Cell: 970-402-2598
      Fax: 206-838-2978
      Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA on MedWatch 3200A form.
   a. If the SAE results in death or is life-threatening, report the SAE to the FDA within 7 days
   b. All other SAEs must be reported to FDA within 15 days

5. If the SAE requires input from a physician then the Acting Medical Director is consulted:
   Herbert K. Lyerly, MD
   Telephone: 919-684-5613
   Fax: 919-684-5653
   Email: rberenson@gmail.com

All SAE reports will be recorded on the Duke IRB Adverse Event Reporting form and will be reviewed and signed by the Principal Investigator. Only adverse events that are deemed to be serious, unexpected and related or possibly related to the research must be reported to the IRB (this is in accordance with Duke's IRB reporting policy). All reportable events will be forwarded to the IRB via campus mail or fax:

IRB: Duke Medical Center Institutional Review Board:
Hock Plaza, 4th floor
2424 Erwin Road
Box 2991
Durham, NC 27705
Fax: 919-668-5125

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the adverse event may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant Institutional Review Board (IRB) of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is also the responsibility of the investigator and the sponsor (Etubics) in conducting gene transfer research to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the sponsor (Etubics) to report these serious adverse events to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

Treatment regimen (dosing frequency, combination therapy)

Protocol description (and number, if assigned)

Description of event, severity, treatment, and outcome, if known

Supportive laboratory results and diagnostics

Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

10.8. Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.9. Safety Reporting Requirements for IND Holders

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report.

c. Data and Safety Monitoring

Data will be collected by: the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by the CPC and Etubics' CRA.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from

carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.

10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.
14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.

22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.
26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.

32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.
39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of

adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.

46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.
53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.

59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.
67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3 [a]	Week 6 [a]	Week 9	Months 6, 9, etc. [b]	Off Treatment
HLA Typing	X						
H & P	X	X	X	X	X	X	X
ECOG	X	X	X	X	X	X	X
β-HCG	X						
CBC & diff	X	X	X	X	X	X	X
PT/PTT	X						
Chemistries/LFTS	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
HbsAg, Hep C, CMV	X						
Immune Monitoring	X	X	X	X	X	X [c]	X
Biological Markers	X	X			X	X	X
MRI/CT Scan	X				X [d]	X	X
Immunization		X	X	X			

Notes: HLA = human leukocyte antigen, H & P = history & physical examination, ECOG= performance Status of 0 or 1, β-HCG = human chorionic gonadotrophin pregnancy test, CBC & diff = complete blood count and white blood cell differential, ANA = antinuclear antibody, HIV = human immunodeficiency virus antibody, HbsAg = hepatitis B surface antigen, Hep C = hepatitis C antibody, CMV = cytomegalovirus antibody, MRI/CT = magnetic resonance imaging/computed tomography.

[a] Immunizations may be performed -1 to +7 days after the specified week. Subsequent immunizations should be 3 weeks afterwards and keep to the every 3 week interval.

[b] Follow-up evaluations to be performed every 3 months after the Week 9 visit.

[c] Immune monitoring if there was evidence of an immune response or at the discretion of the immune monitoring laboratory.

[d] MRI/CT scan to be requested 1-4 weeks after the third immunization.

Protocol Version 2.0, 13 April 2010

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

Principal Investigator: Michael Morse, M.D.
Duke University Medical Center

Agents Used in This Study: Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)

Names and addresses of key study personnel:

<table>
<tr><td>Study Chairman and Principal Investigator
Name: Michael Morse, MD
Address: Duke University Medical Center
Box 3233, Durham, NC 27710
Telephone: (919) 681-3480
Fax: (919) 681-7970
Email: michael.morse@duke.edu</td><td></td></tr>
<tr><td>Bioassay Oversight/Laboratory Oversight
Name: Timothy Clay, PhD
Address: Duke University Medical Center
Box 2606, Durham, NC 27710
Telephone: (919) 684-0350
Fax: (919) 681-7970
Email: clay0017@c.duke.edu</td><td>Biostatistician
Name: Donna Niedzwiecki, PhD
Address: Duke University Medical Center
Box 3958, Durham, NC 27710
Telephone: (919) 681-5030
Fax: (919) 691-8028
E-mail: donna.niedzwiecki@duke.edu</td></tr>
<tr><td>Other Applicable Staff
Title: Acting Medical Director
Name: Ron Berenson, MD
Address: 410 West Harrison Street,
Seattle, WA. 98119
Telephone: 206-790-1094
Fax:(206) 838-2978
Email: rberenson@gmail.com</td><td>Pharmacist or Person Responsible for Drug Accountability
Name: Cheryl Morgan
Address: Morris Pharmacy, Duke University Medical Center, Trent Drive, Durham, NC 27710
Telephone: (919) 668-0657
Fax: (919) 668-3895
Email: cheryl.morgan@duke.edu</td></tr>
<tr><td>Other Applicable Staff:
Title: CEO and CSO, Etubics Corporation
Name: Frank R. Jones, Ph.D.
Address: Etubics Corporation
410 West Harrison Street
Seattle, WA 981119
Telephone: 206-838-5110 Ext 101
Fax: 206-838-2978
Email: frj@etubics.com</td><td>Other Consultants:
H. Kim Lyerly, MD, Duke University
Andy Amalfitano, DO, PhD, Michigan State University
Elizabeth Gabitzsch, Etubics Corporation
Raj Dua, Ph.D., Etubics Corporation</td></tr>
</table>

TABLE OF CONTENT

Page Number

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by any of the following: immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining), or a tumor known to be universally CEA positive (i.e. colon and rectal cancer). In addition, only colorectal adenocarcinomas (for those with colorectal cancers) can be enrolled. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, trastuzumab, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving steroid or immunosuppressive therapy. All patients must be ≥ 21 years old and have an ECOG Performance Status of 0 or 1. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase I component), and the maximally tolerated dose of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2B-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Peripheral blood draw of 90 mL for immune analysis 2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions or neurological toxicity, other Grade 3 or 4 allergic or major organ toxicity, or Grade 4

	fever, that may possibly be associated with the immunization), then patients may begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to 1 x 10^8 particles. 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{11} particles SQ in 0.7 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II cohort: An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the maximally tolerated dose every 3 weeks for 3 immunizations. 6) Patients will have 90 mL peripheral blood drawn prior to each immunization and at Week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 7) Time to progression will be measured using CT scans performed as per standard of care (approximately 3 month intervals).
Risks/toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 24 evaluable patients (plus up to 12 replacements); may require 30-42 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total
Criteria for Evaluation	Toxicity will be assessed using CTC toxicity criteria. CEA-specific immune response will be measured by ELISpot. Time to recurrence will

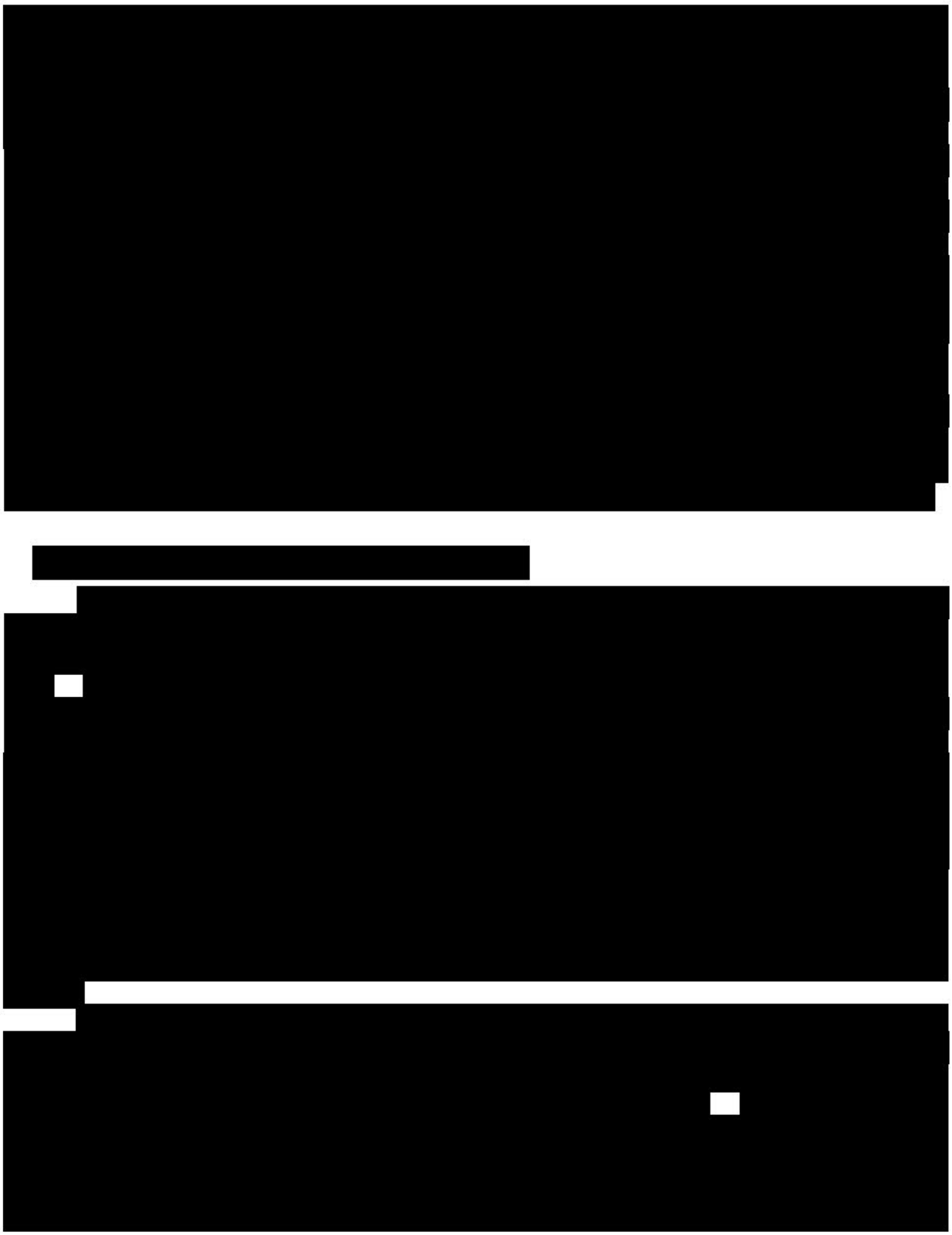
	be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed at the maximum tolerated dose (MTD). An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 1 immunization.

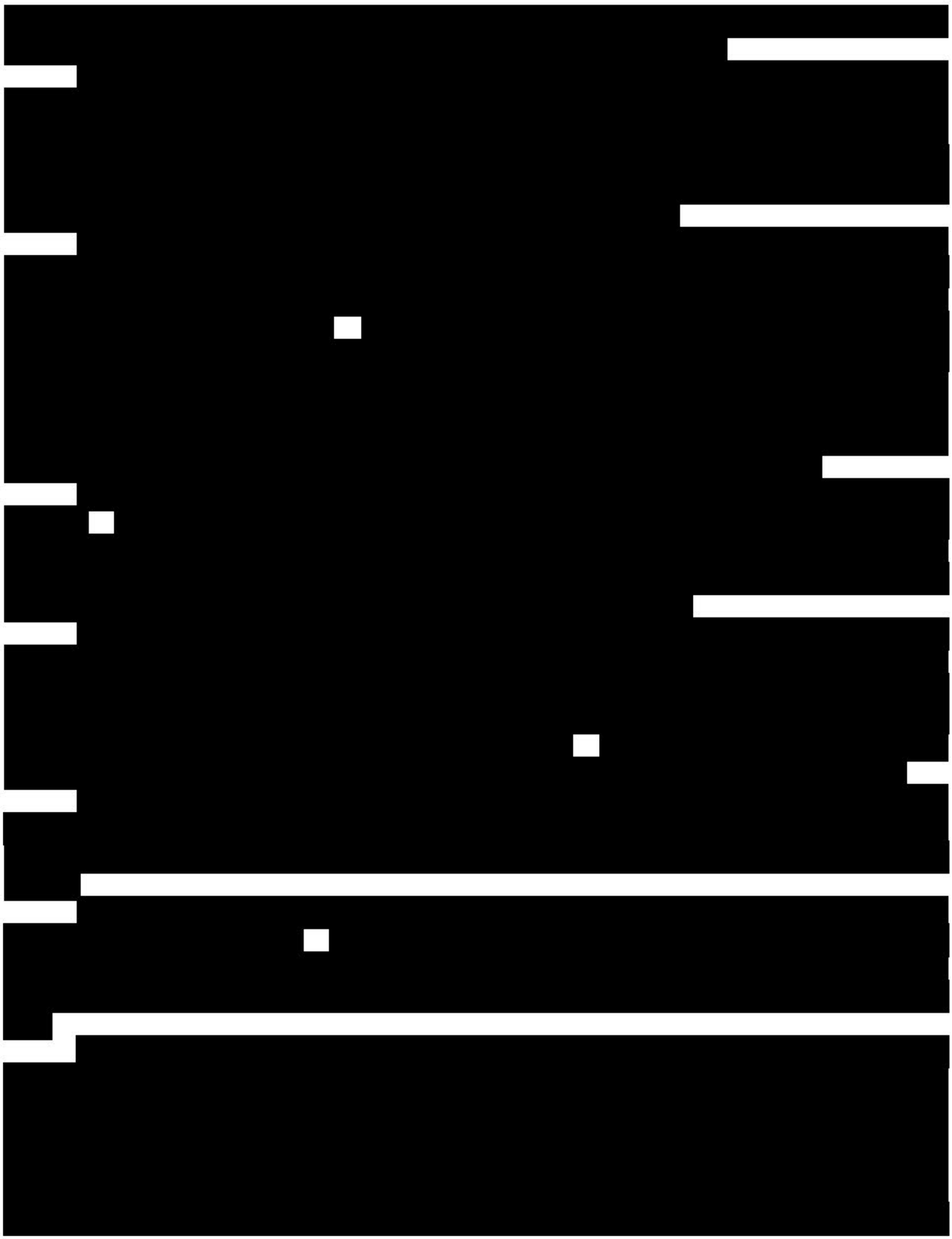
2. STUDY OBJECTIVES

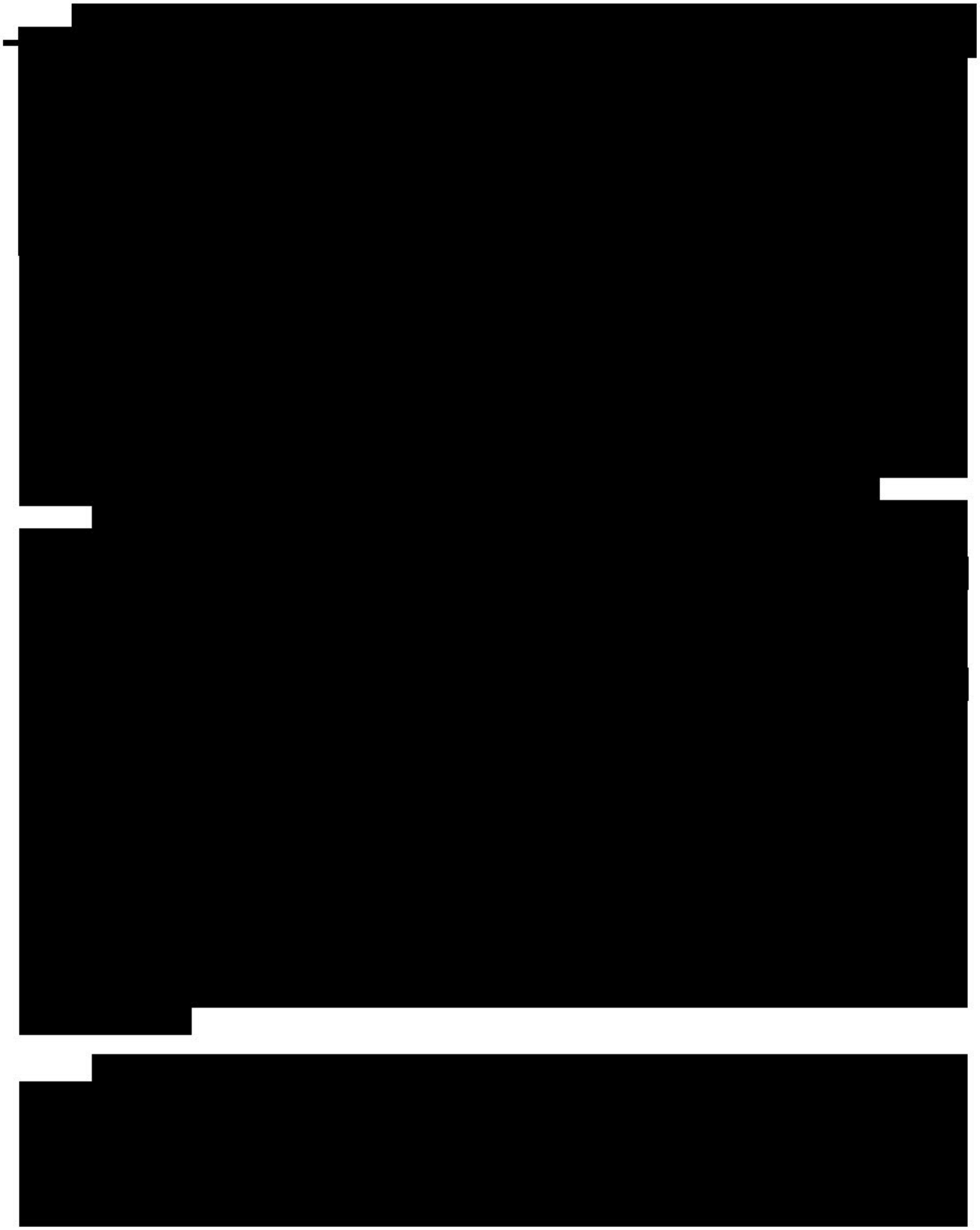
a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

3. BACKGROUND AND SIGNIFICANCE

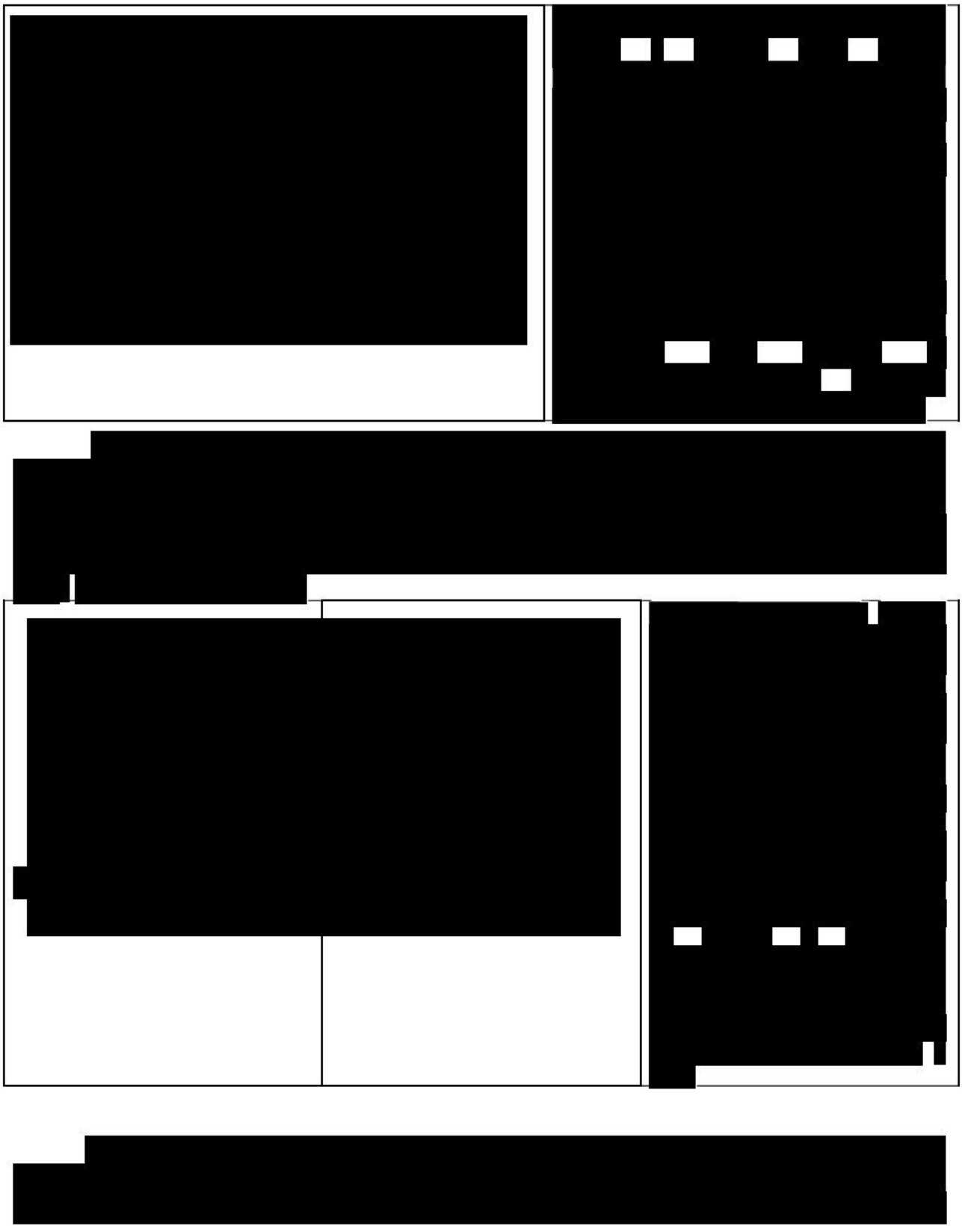
[redacted]

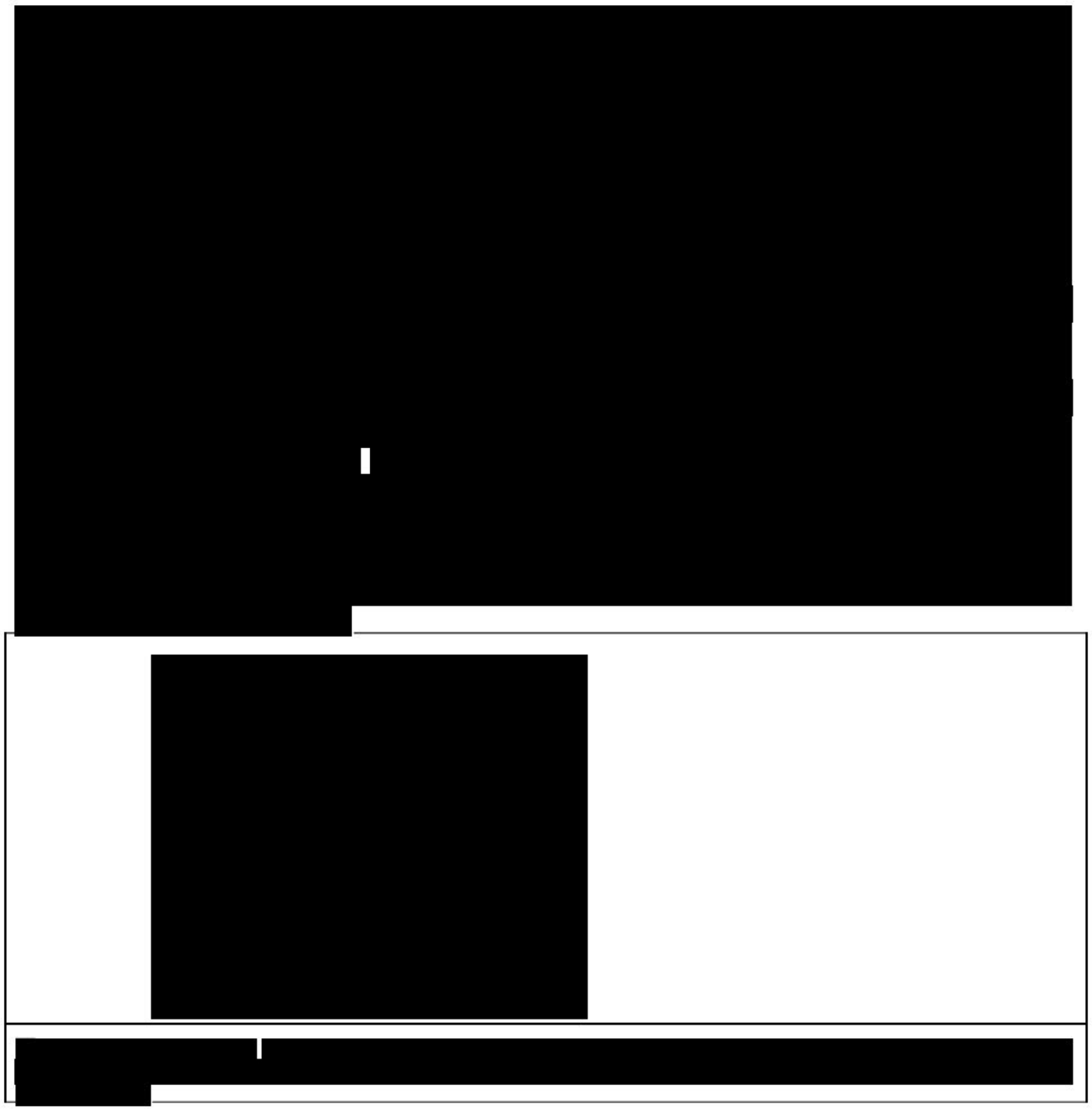






[redacted]





[redacted]

4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of metastatic malignancy due to a tumor expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. The tumor must express CEA as defined by any of the following: immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining), or a tumor known to be universally CEA positive (i.e. colon and rectal cancer.) In addition, only colorectal adenocarcinomas (for those with colorectal cancers) can be enrolled.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single therapies for at least 3 months).
- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single therapies for at least 3 months).
  - Patients who have been treated or offered the option of treatment with Bevacizumab (option clearly stated in the Consent Form).
  - Patients who have been treated or offered the option of treatment with Lapatinib (option clearly stated in the Consent Form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.

- Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single therapy for at least 3 months).

- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. ECOG 0 or 1 performance status

4.1.5. Estimated life expectancy > 3 months..

4.1.6. Age ≥ 21 years, but ≤75.

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (may transfuse or use erythropoietin to achieve this level), platelets ≥ 75,000/microliter; PT-INR <1.5, PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosuppression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to Duke University Medical Center for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, trastuzumab, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered from all acute toxicities from prior treatment.

4.2.2. Patients with a history of brain metastases will not be permitted

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot) or viral hepatitis (as determined by HBsAg and Hepatitis C serology). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections. Patients with active CMV disease will be excluded, but CMV seropositive patients will be eligible.

4.2.8. Patients on steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies) prior to enrollment.

4.2.9. Patients with allergies to any component of the vaccine will be excluded from the protocol.

4.2.10. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.11. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.12. Patients will be allowed warfarin 1mg po qd for port prophylaxis but not full dose warfarin or low molecular weight heparin.

4.2.13 Patients with metastatic disease that is determined to be resectable will be excluded.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if DLT occur that necessitate re-dosing at a lower dosage levels (see section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number: Patients will be assigned study numbers in order of their screening using the following: ETBX-011-xxx.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

(See also Schema in Appendix 1.) The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment:

- History and physical exam, to include ECOG Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological, biochemical and immunological tests:

  CBC with differential

  PT INR and PTT

  Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose

  Urinalysis
- Infectious Disease

  HIV antibody, Hepatitis BsAg, Hepatitis C serology, CMV serology
- ECG
- Biological Markers:

  Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad vector, and other available serum markers (e.g., CEA or CA15-3) will be reviewed.
- Archive Samples:

  An additional 20 mL of blood may be drawn during the first clinic visit, when the patient history and physical exam are conducted, at the discretion of the immune monitoring laboratory, and serum stored for later analysis of immune responses.
- Imaging studies:

  CT or MRI scans of the chest, abdomen, brain, and/or pelvis will be requested within 1 month (±2 weeks) before starting study treatment to document the presence and size of

any measurable metastatic disease that might present. CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI 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 in the chest, abdomen, and pelvis.

6. TREATMENT PLAN

In the phase I component, patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (based on CTCAE4.0 criteria) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitilgo or fever for less than 2 days and less than <101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component without the 1-week waiting period. Between dosage levels, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed by the Principle Investigator. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II, 12 additional patients will be enrolled at the MTD. In phase II, if at any time the rate of DLT in patients enrolled at the MTD (for the phase I and phase II cohorts combined) is ≥33%, the MTD will be re-defined as the next lower dosage level, and phase II will proceed with enrollment of additional patients at this lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will de-escalated to 1 x 10^8 particles.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{11} particles in 0.7 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then this dosage level will be considered the MTD and patients may begin enrolling into the phase II portion of the study. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

4) Phase II cohort: After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

   Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

6) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

7) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

9) The following safety events will trigger a temporary suspension of study vaccinations:

   a) If ≥33% of patients in the phase II cohort experience DLT at dosage level 1 (i.e.,1 x 10^9 particles)

b) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine

c) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

The Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above.

Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two Grade 4 toxicity events that are possibly/probably related to the study agent

Figure 3A

Figure 3B.

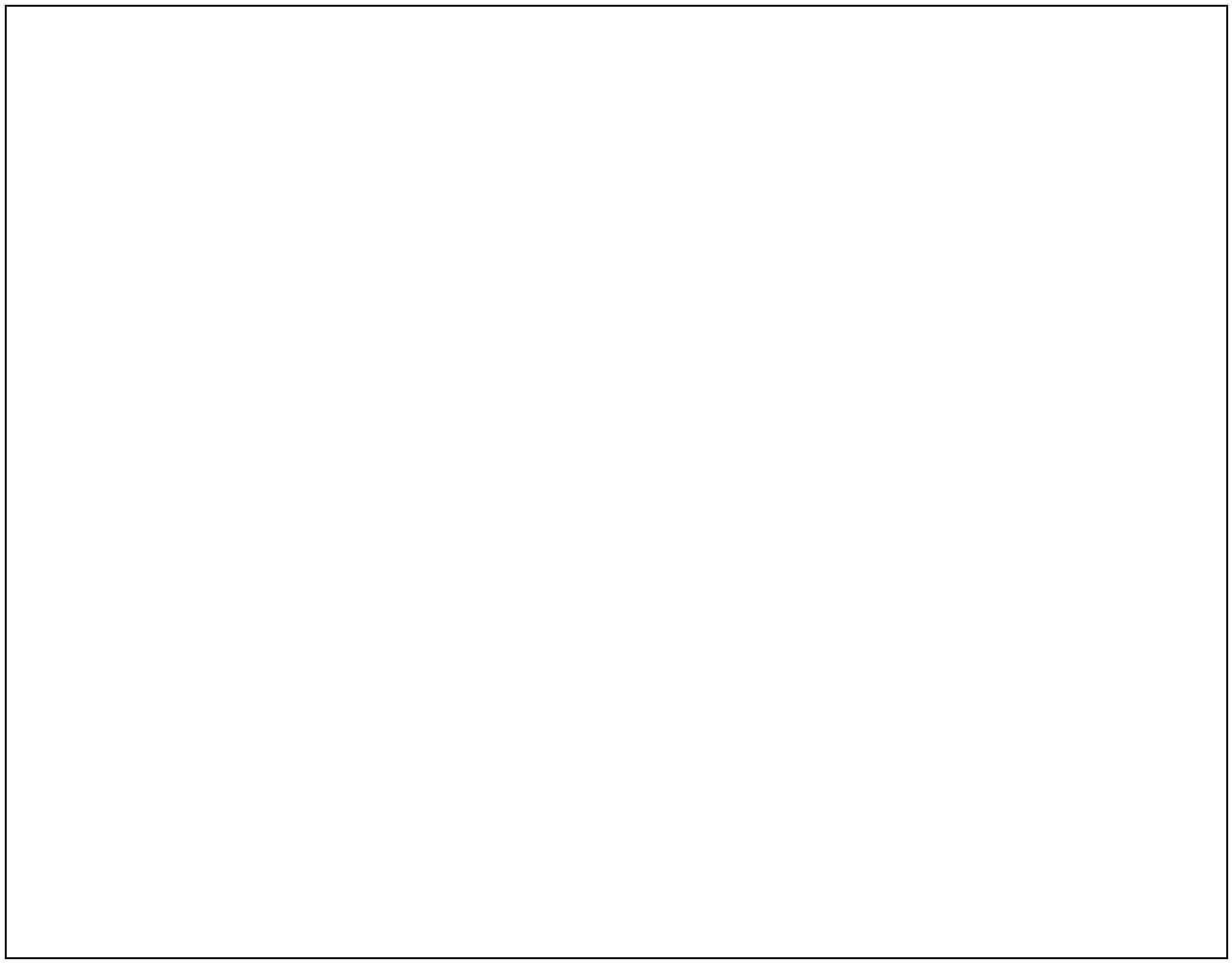


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.2. Pharmaceutical Information

6.1.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, or 1 x 10^{10}, particles subcutaneously (SQ) in 0.5 mL of a buffered saline solution every 3 weeks for 3 immunizations and patients who receive 1x10^{11} VP will receive Ad5[E1-, E2b-]-CEA(6D) in 0.7 mL of ARM formulation buffer SQ every 3 weeks for 3 immunizations.

6.1.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains 1X10^{11} total virus particles. The volume of injection for 1x10^{11} virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:

1. To administer 1x10e9 virus particles by subcutaneous injection:
From a 5.0mL vial of 0.9% sterile saline remove 0.05mL of fluid which leaves 4.95mL. Then remove 0.05mL from the vial labeled ETBX-011 and deliver this volume into the 5mL sterile saline vial. Mix the contents by inverting the 5mL diluted drug. Then with draw 0.5mL of diluted drug and deliver to the patient by subcutaneous injection (detailed description of dose preparation is described in the packaging insert).

2. To administer 1x10e10 virus particles by subcutaneous injection:
From a 5.0mL vial of 0.9% sterile saline remove 0.5mL of fluid which leaves 4.5mL. Then remove 0.5mL from the vial labeled ETBX-011 and deliver this volume into the 5mL sterile saline vial. Mix the contents by inverting the 5mL diluted drug. Then with draw 0.5mL of diluted drug and deliver to the patient by subcutaneous injection (detailed description of dose preparation is described in the packaging insert).

3. **To administer 1x10e11 virus particles by subcutaneous injection:**
Withdraw 0.5mL of contents from vial and administer each subject without any further manipulation.

6.1.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Tim Clay at Duke University Medical Center for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations (each visit for immunization)

General evaluations include medical history, ECOG performance status, and complete physical examination with weight. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

7.1.2 Hematological and Biochemical Assessment

Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose will be drawn at Week 0,3,6,9, at discontinuation of treatment (if treatment is discontinued early), and as clinically indicated.

7.1.3 Biological Markers

Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained at Week 0, approximately 3 weeks after the last immunization (approximately Week 12), and every 3 months thereafter.

7.1.4 Immunological Assessment

Peripheral blood (90) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Plasma will be archived from the initial and Week 9 blood draws for evaluating antibody levels.

7.1.5 Imaging Studies

CT or MRI scans of the chest, abdomen, and/or pelvis, will be requested (but not required) within 1-4 weeks after the third dose of vaccine. At a minimum, the same imaging modality of the same portion of the body should be performed at each time point.

7.1.6: Ad 5 neutralizing Ab assessment

Serum will be sent to Etubics for Ad5 neutralizing level determination

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up at Duke University Medical Center every 3 months for 1 year, while on the study (i.e., have not progressed or been removed from the study for other reasons). At each visit, a medical history and physical exam and labs (Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose) will be drawn.

At each visit, 40-90 mL of peripheral blood for immune analysis may be drawn, if there was previous evidence of an immune response or at the discretion of the investigator.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) Dose-limiting toxicity (DLT) is defined in section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine

studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 Dose-limiting toxicity related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist at Duke University Medical Center for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations: In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety:

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This will be performed by medical monitor and the principle investigator. A note to file will be generated following assessment and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience dose-limiting toxicity (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). Dose-limiting toxicity is defined in section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response:

We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- Dose-limiting toxicity, as defined in section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of a withdrawal due to toxicity, patients will be requested to have safety evaluations performed as per protocol for one year duration. This may include having up to 90 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator (Dr. Michael Morse) or in his absence, Dr. H. Kim Lyerly, immediately by telephone (Page operator 919 684-8111). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the IRB and the sponsor (Etubics) by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and the sponsor (Etubics) will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the Institutional Review Board (IRB). IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A Case Report Form must be completed and signed by the

principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. Case Report Forms must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on case report forms by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto case report forms, originals of laboratory and other test results must be kept on file with patient's case report form or clinical chart. Case report forms and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Experience Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

Protocol data and safety will be monitored by the Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) with the following plan:

1. Purpose: This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of AEs.
2. Monitoring: Up to 24 patients will be enrolled. The principal investigator will continuously monitor the study. The principal investigator will review the data and safety of the study after the enrollment of three patients in cohort 1, after enrollment of three patients in cohort 2, and after enrollment of six patients in cohort 3. Formal, independent monitoring by CPC will occur after enrollment of the three patients in

cohort 1 and again after enrollment of three patients in cohort 2; then a scientific progress review will occur yearly, assuming a result of "satisfactory" on the initial review. The exceptions are as follows: If more than one patient experiences a Grade 4 or greater allergic reaction, the principal investigator will request a monitoring review by CPC. If at any time, more than 50% of patients experience a Grade 3 or 4 major organ toxicity, we will request a monitoring review by CPC.

3. Description of Monitoring: Adverse event reports will be reviewed with tabulation of all Grade 2, 3 or 4 toxicity.
4. Toxicity: see section 7.3.2 of the protocol.
5. Reporting Adverse Events: An adverse experience is any adverse change from the study patient's baseline (pre-treatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started. All adverse experiences that are classified as serious as described in section 10.7 of the protocol should be reported to the sponsor (Etubics) by telephone or fax within 24 hours, and reported in writing to the sponsor (etubics) within 72 hours. All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to the principal investigator, with a copy of the autopsy report and the death certificate. All adverse experiences will be recorded on the Adverse Experience Case Report Form. This report form should include severity, duration, outcome, and the investigator's judgment as to the relationship of the adverse experience to treatment.
6. Reporting of Pregnancy: If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the sponsor (Etubics), and to the IRB.

10.6. CTCAE Term (AE description) and Grade:

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 web site (http://ctep.cancer.gov). The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Serious Adverse Event Reporting

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

Procedures for adverse event reporting

1. PI notified by medical staff of a SAE

   PI: Michael Morse, MD
   Address: Duke University Medical Center
   Seeley Mudd Building
   Box 3233, Durham, NC 27710
   Telephone: 1-919-681-3480
   Fax: 1-919-681-7970
   Pager 1-919-970-5626
   Email: michael.morse@duke.edu

2. PI calls sponsor to report an unexpected SAE associated with the use of the drug

   Sponsor: Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
   Telephone: 206-838-5110 ext. 102
   Cell: 1-206-818-2985
   Fax: 1-206-838-2978

Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

A. Chief Scientific Officer: Frank Jones, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 101
Cell: 206-818-2857
Fax: 206-838-2978
Email: frj@etubics.com

B. Laboratory Manager: Joseph Balint, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 107
Fax: 206-838-2978
Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 103
Cell: 970-402-2598
Fax: 206-838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA on MedWatch 3200A form.
   a. If the SAE results in death or is life-threatening, report the SAE to the FDA within 7 days
   b. All other SAEs must be reported to FDA within 15 days

5. If the SAE requires input from a physician then the Acting Medical Director is consulted:
Ron Berenson, MD
Telephone: 206-790-1094
Fax: 206-838-2978
Email: rberenson@gmail.com

All SAE reports will be recorded on the Duke IRB Adverse Event Reporting form and will be reviewed and signed by the Principal Investigator. Only adverse events that are deemed to be serious, unexpected and related or possibly related to the research must be reported to the IRB (this is in accordance with Duke's IRB reporting policy). All reportable events will be forwarded to the IRB via campus mail or fax:

IRB: Duke Medical Center Institutional Review Board:
Hock Plaza, 4th floor
2424 Erwin Road
Box 2991
Durham, NC 27705
Fax: 919-668-5125

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the adverse event may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant Institutional Review Board (IRB) of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is also the responsibility of the investigator and the sponsor (Etubics) in conducting gene transfer research to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the sponsor (Etubics) to report these serious adverse events to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

Treatment regimen (dosing frequency, combination therapy)

Protocol description (and number, if assigned)

Description of event, severity, treatment, and outcome, if known

Supportive laboratory results and diagnostics

Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

10.8. Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.9. Safety Reporting Requirements for IND Holders

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report.

c. Data and Safety Monitoring

Data will be collected by: the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by the CPC and Etubics' CRA.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.

13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.
14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.

25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.
26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.
32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.

36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.
39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.

49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.
53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII

expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.

62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.
67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3 [a]	Week 6 [a]	Week 9	Months 6, 9, etc. [b]	Off Treatment
H & P	X	X	X	X	X	X	X
ECOG	X	X	X	X	X	X	X
β-HCG	X						
CBC & diff	X	X	X	X	X	X	X
PT/PTT	X						
Chemistries/LFTS	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
Immune Monitoring	X	X	X	X	X	X [c]	X
Biological Markers	X	X			X	X	X
MRI/CT Scan	X				X [d]	X	X
Immunization		X	X	X			

Notes: H & P = history & physical examination, ECOG= performance Status of 0 or 1, β-HCG = human chorionic gonadotrophin pregnancy test, CBC & diff = complete blood count and white blood cell differential, ANA = antinuclear antibody, MRI/CT = magnetic resonance imaging/computed tomography.

[a] Immunizations may be performed -1 to +7 days after the specified week. Subsequent immunizations should be 3 weeks afterwards and keep to the every 3 week interval.

[b] Follow-up evaluations to be performed every 3 months after the Week 9 visit.

[c] Immune monitoring if there was evidence of an immune response or at the discretion of the immune monitoring laboratory.

[d] MRI/CT scan to be requested 1-4 weeks after the third immunization.

Protocol Version 2.1, May 14, 2010

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

Principal Investigator: Michael Morse, M.D.
Duke University Medical Center

Agents Used in This Study: Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)

Names and addresses of key study personnel:

Study Chairman and Principal Investigator **Name: Michael Morse, MD** **Address:** Duke University Medical Center Box 3233, Durham, NC 27710 **Telephone:** (919) 681-3480 **Fax:** (919) 681-7970 **Email:** michael.morse@duke.edu	
Name: Kent Weinhold, PhD **Address:** Duke Center for AIDS Research 204 SORF, LaSalle St. Box 2926 Duke University Medical Center Durham, NC 27710-2926 **Telephone:** (919) 684-5572 **Fax:** (919) 684-4288 **Email:** kjw@duke.edu	**Biostatistician** **Name:** Donna Niedzwiecki, PhD **Address:** Duke University Medical Center Box 3958, Durham, NC 27710 **Telephone:** (919) 681-5030 **Fax:** (919) 691-8028 **E-mail:** donna.niedzwiecki@duke.edu
Other Applicable Staff **Title:** Acting Medical Director **Name:** H. Kim Lyerly, MD **Address**: Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, NC 27710 **Telephone:** (919) 684-5613 **Fax:** (919) 684-5653 **Email**: lyerl001@mc.duke.edu	**Pharmacist or Person Responsible for Drug Accountability** **Name:** Cheryl Morgan **Address:** Morris Pharmacy, Duke University Medical Center, Trent Drive, Durham, NC 27710 **Telephone:** (919) 668-0657 **Fax:** (919) 668-3895 **Email:** cheryl.morgan@duke.edu
Other Applicable Staff: **Title:** CEO and CSO, Etubics Corporation **Name:** Frank R. Jones, Ph.D. **Address**: Etubics Corporation 410 West Harrison Street Seattle, WA 981119 **Telephone:** 206-838-5110 Ext 101 **Fax:** 206-838-2978 **Email:** frj@etubics.com	**Other Consultants:** Andy Amalfitano, DO, PhD, Michigan State University Elizabeth Gabitzsch, Etubics Corporation Raj Dua, Ph.D., Etubics Corporation Diane Lickey, RN, Etubics Coprporation

TABLE OF CONTENT

Page Number

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by any of the following: immunohistochemical staining, (at least 50% of the tumor with at least moderate intensity of staining) , or a tumor known to be universally CEA positive (*i.e.* colon and rectal cancer). In addition, only colorectal adenocarcinomas (for those with colorectal cancer) can be enrolled. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, trastuzumab, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving steroid or immunosuppressive therapy. All patients must be >21 years old and have an Karnofsky Performance Score of 70% or higher. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase I component), and the maximally tolerated dose of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2B-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Peripheral blood draw of 90 mL for immune analysis 2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions or neurological toxicity, other Grade 3 or 4 allergic or major organ toxicity, or Grade 4 fever, that

	may possibly be associated with the immunization), then patients may begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at the lowest dosage level, dosing will be de-escalated to $1x10^8$ particles. 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II cohort: An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the maximally tolerated dose every 3 weeks for 3 immunizations. 6) Patients will have 90 mL peripheral blood drawn prior to each immunization and at Week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 7) Time to progression will be measured using CT scans performed as per standard of care (approximately 3 month intervals).
Risks/toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 24 evaluable patients (plus up to 12 replacements); may require 30-42 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total
Criteria for Evaluation	Toxicity will be assessed using CTC toxicity criteria. CEA-specific immune response will be measured in the peripheral blood. Time to recurrence will be determined by RECIST criteria.

Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

2. STUDY OBJECTIVES

a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

3. BACKGROUND AND SIGNIFICANCE

[redacted]

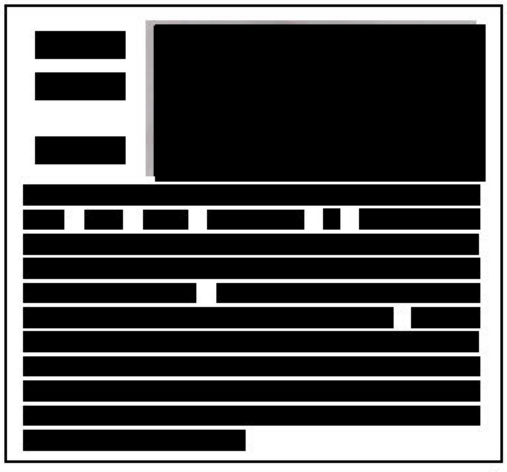
[redacted]

[redacted]

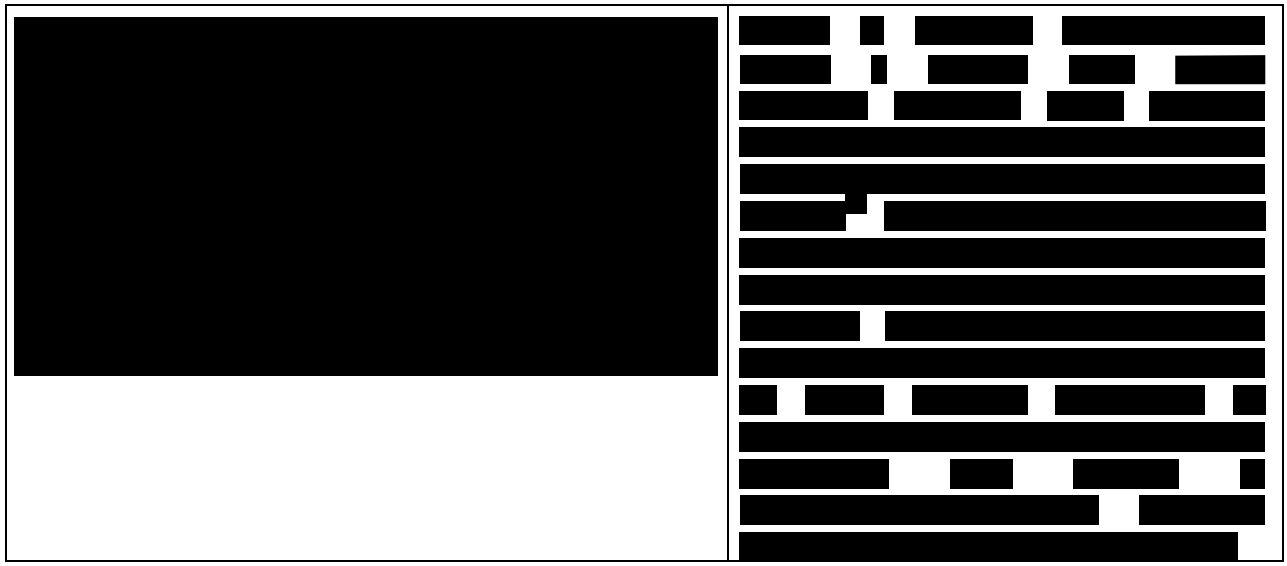
[redacted]

[redacted]

[redacted]



[redacted]



[redacted]

[redacted]

4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of metastatic malignancy due to a tumor expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. The tumor must express CEA as defined by any of the following: immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining), or a tumor known to be universally CEA positive (i.e. colon and rectal cancer). In addition, only colorectal patients must be confirmed as having an adenocarcinomas can be enrolled.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
  - Patients who have been treated or offered the options of treatment with Bevacizumab (option clearly stated in the consent form).
  - Patients who have been treated or offered the options of treatment with Lapatinib (option clearly stated in the consent form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.

- Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single agent therapy for at least 3 months).

- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. Karnofsky performance score of 70% or higher

4.1.5. Estimated life expectancy > 3 months..

4.1.6. Age ≥ 21 years, but ≤75.

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (it is acceptable to have had prior transfusion), platelets ≥ 75,000/microliter; PT-INR <1.5, PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosuppression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to Duke University Medical Center for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, trastuzumab, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered to grade 1 acute toxicities from prior treatment.

4.2.2. Patients with a history of or current brain metastases will not be permitted

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot) or viral hepatitis (as determined by HBsAg and Hepatitis C serology). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections. Patients with active CMV disease will be excluded, but CMV seropositive patients will be eligible.

4.2.8. Patients on steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies) prior to enrollment.

4.2.9. 4.2.9. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.10. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.11. Patients will be allowed warfarin 1mg po qd other than for port prophylaxis.

4.2.12. Patients with metastatic disease which is determined to be resectable will be excluded.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if

DLT occur that necessitate re-dosing at a lower dosage levels (see section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number: Patients will be assigned study numbers in order of their screening using the following: ETBX-011- 001, 002, 003 etc. including screened patients.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

(See also Schema in Appendix 1.) The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment:

- History and physical exam, to include Karnofsky Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological, biochemical and immunological tests:

  CBC with differential

  PT INR and PTT

  Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose

  Urinalysis
- Infectious Disease

  HIV antibody, Hepatitis BsAg, Hepatitis C serology, CMV serology
- Anti Nuclear Antibody (ANA)
- Biological Markers:

  Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad5 vector, and other available serum markers (e.g., CEA or CA15-3) may be reviewed.
- Archive Sample

  20 mL of blood may be drawn during the first clinic visit, when the patient history and physical exam are conducted, at the discretion of the immune monitoring laboratory, and serum stored for later analysis of immune responses.
- Imaging studies:

  CT or MRI scans of the chest, abdomen, brain, and/or pelvis will be requested within 1 month (±2 weeks) before starting study treatment to document the presence and size of any measurable metastatic disease that might present. CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed. This applies to tumors in the chest, abdomen, and pelvis.

6. TREATMENT PLAN

In the phase I component, patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (based on CTCAE4.0 criteria) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitilgo or fever for less than 2 days and less than <101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by version 4 CTC guide), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. The first patient will be called to check on their condition prior to enrolling the second patient since patients can be enrolled after 1 week of initiation of cohort 3. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component without the 1-week waiting period. Between dosage levels, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed by the Principle Investigator. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II, 12 additional patients will be enrolled at the MTD. In phase II, if at any time the rate of DLT in patients enrolled at the MTD (for the phase I and phase II cohorts combined) is ≥33%, the MTD will be re-defined as the next lower dosage level, and phase II will proceed with enrollment of additional patients at this lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. Diary card to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection) will be given to each patient at the first treatment along with a ruler. Patient can fax (# 919-684-2311) the completed diary form prior to next appointment date. The diary card will be placed in the patient file and a copy given to the Principal Investigator for his records. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to $1x10^8$ particles. and a new cohort instituted.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{11} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then this dosage level will be considered the MTD and patients may begin enrolling into the phase II portion of the study. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

4) Phase II cohort: After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

   Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in

phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.
6) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).
7) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.
9) The following safety events will trigger a temporary suspension of study vaccinations:
    a) If ≥33% of patients in the phase II cohort experience DLT at dosage level 1 (i.e.,1 x 10^9 particles)
    b) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine
    c) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

The Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above.

Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two patients having a Grade 4 toxicity event that is possibly/probably related to the study agent.

Figure 3A

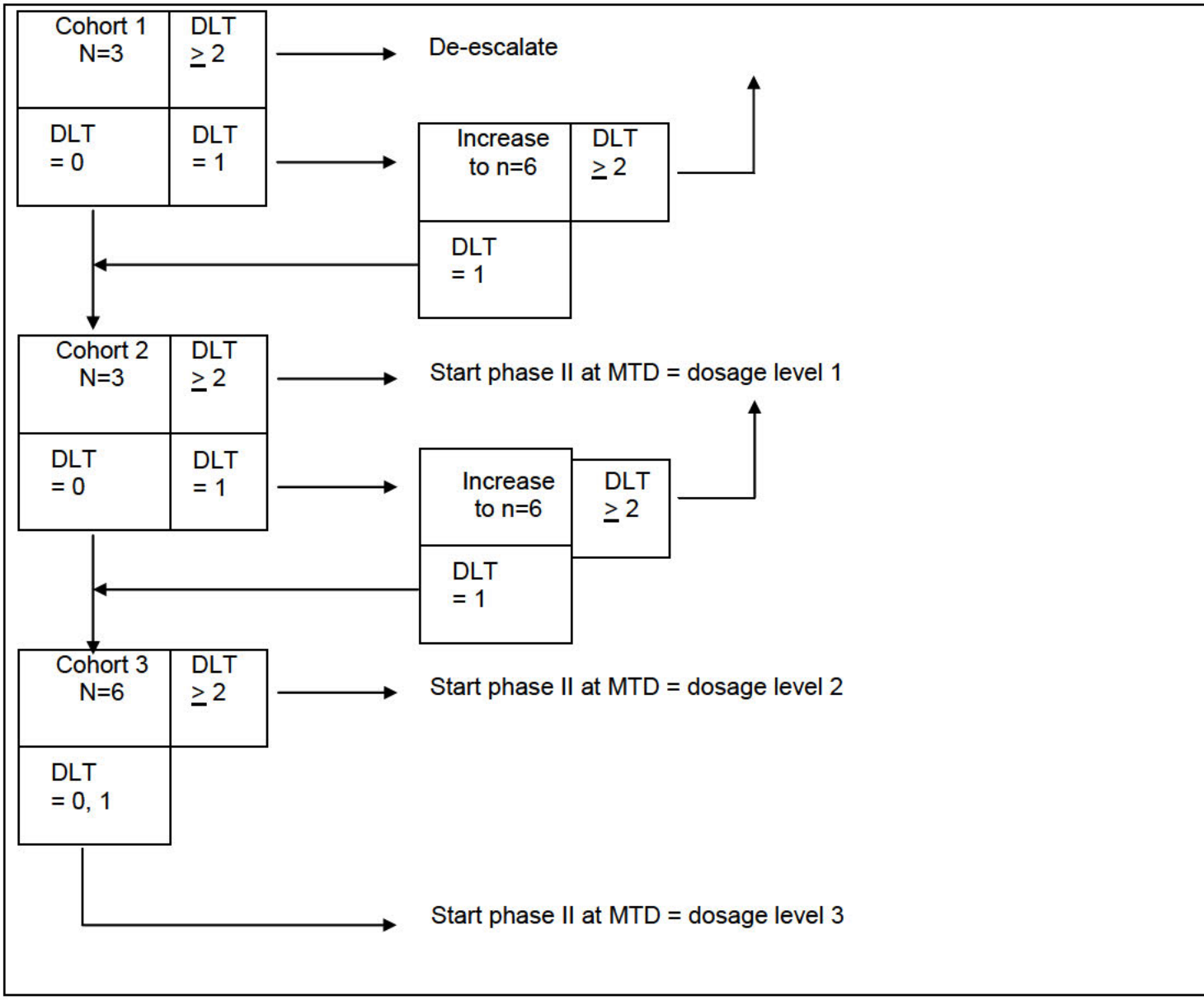

Cohort 1
N=3
DLT
≥ 2
De-escalate
DLT
= 0
DLT
= 1
Increase
to n=6
DLT
≥ 2
DLT
= 1
Cohort 2
N=3
DLT
≥ 2
Start phase II at MTD = dosage level 1
DLT
= 0
DLT
= 1
Increase
to n=6
DLT
≥ 2
DLT
= 1
Cohort 3
N=6
DLT
≥ 2
Start phase II at MTD = dosage level 2
DLT
= 0, 1
Start phase II at MTD = dosage level 3

Figure 3B.

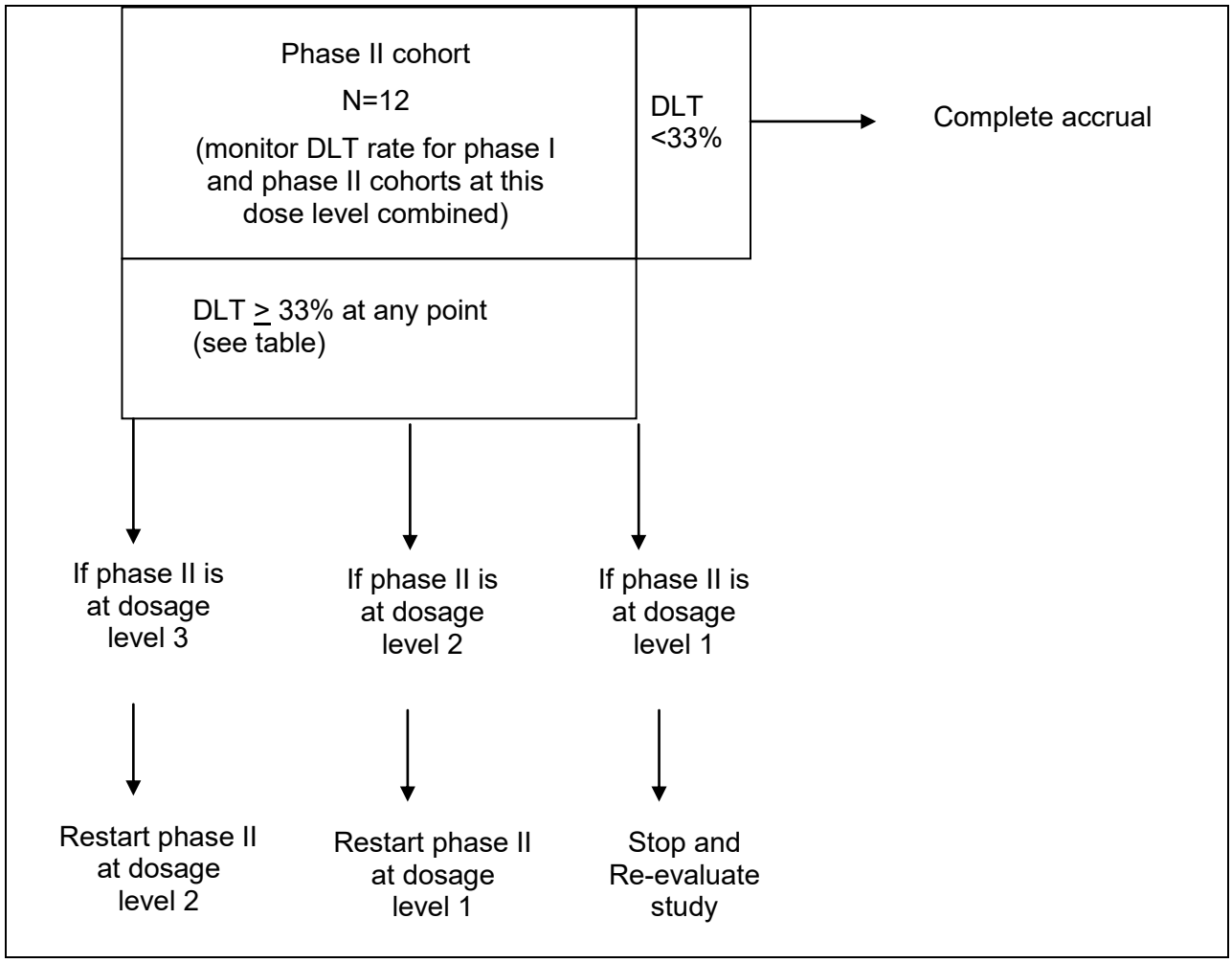


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.2. Pharmaceutical Information

6.1.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, or 1 x 10^{10}, particles subcutaneously (SQ) in 0.5 mL of a buffered saline solution every 3 weeks for 3 immunizations and patients who receive 1x10^{11} VP will receive Ad5[E1-, E2b-]-CEA(6D) in 0.5 mL of ARM formulation buffer SQ every 3 weeks for 3 immunizations.

6.1.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains 1X10^{11} total virus particles. The volume of injection for 1x10^{11} virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:
A detailed description of dose preparation is described in the study IPHP and clinic SOP.

1. To administer 1x10e9 virus particles by subcutaneous injection:
Perform 2 serial dilutions of vialed vaccine as follows:
Draw 4.5 mL of sterile saline into a syringe. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
Use a new sterile syringe with a needle and repeat above procedure. Withdraw 4.5 mL of sterile saline. In the second syringe withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/ml**. Remove the needle from the syringe containing the 4.5 mL saline, and inject the 0.5 mL of 2 x 10^{10}vp/mL from the second syringe.
Place a new needle on the 10-mL syringe (**Syringe C from Step 9 above**) and mix the two solutions. This solution now has a concentration **2 x 10^9 vp/mL (**1 x 10^9 vp per 0.5 mL)**.**
Label a new 1-mL sterile syringe ETBX-011, 1 x 10^9 vp and withdraw 0.5 mL from the syringe containing **2 x 10^9 vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^9 vp)** can be kept at room temperature for four hours prior to administering to the patient.

2. To administer 1x10e10 virus particles by subcutaneous injection:
Draw 4.5 mL of sterile saline into a syringe. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from

the second syringe into the syringe containing saline. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
Label a new 1-mL sterile syringe ETBX-011, 1 x 10^{10} vp and withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^{10} vp)** can be kept at room temperature for four hours prior to administering to the patient.

3. **To administer 1x10e11 virus particles by subcutaneous injection:**
Withdraw 0.5mL of contents from vial and administer each subject without any further manipulation.

6.1.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joe Balint, at Etubics Corporation for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations (each visit for immunization)

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

7.1.2 Hematological and Biochemical Assessment

Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose will be drawn at Week 0,3,6,9, at discontinuation of treatment (if treatment is discontinued early), and as clinically indicated.

7.1.3 Biological Markers

Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained at Week 0, approximately 3 weeks after the last immunization (approximately Week 12), and every 3 months thereafter.

7.1.4 Immunological Assessment

Peripheral blood (90mL) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Serum will be archived from each blood draw for evaluating antibody levels.

7.1.5: Ad5 neutralizing Ab assessment

Serum will be sent to Etubics for Ad5 neutralizing level determination

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up at Duke University Medical Center every 3 months for 1 year, while on the study (i.e., have not progressed or been removed from the study for other reasons). At each visit, a medical history and physical exam and labs (Blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose) will be drawn.

At each visit, 40-90 mL of peripheral blood for immune analysis may be drawn, if there was previous evidence of an immune response or at the discretion of the investigator.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from

the CTEP web site (http://ctep.cancer.gov).) Dose-limiting toxicity (DLT) is defined in section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 Dose-limiting toxicity related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist at Duke University Medical Center for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations: In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety:

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This decision will be made by the medical monitor and

the Principle Investigator. A note will be generated following the assessment decision and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience dose-limiting toxicity (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). Dose-limiting toxicity is defined in section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response:

Immune responses against CEA will be evaluated from the peripheral blood of patients from among the following studies at the discretion of the Principle Investigator (ELISpot, cytokine flow cytometry, and antibody responses). We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- Dose-limiting toxicity, as defined in section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of withdrawal due to toxicity, a patient will be requested to have safety evaluations performed as per the protocol for a one year duration post treatment. This may include having up to 90 mL of blood drawn for immunologic testing..

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator (Dr. Michael Morse) or in his absence, Dr. H. Kim Lyerly, immediately by telephone (Page operator 919 684-8111). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the IRB and the sponsor (Etubics) by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and the sponsor (Etubics) will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the Institutional Review Board (IRB). IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator

must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A Case Report Form must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. Case Report Forms must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on case report forms by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto case report forms, originals of laboratory and other test results must be kept on file with patient's case report form or clinical chart. Case report forms and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Experience Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if

the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

Protocol data and safety will be monitored by the Scientific Monitoring Subcommittee of the Cancer Protocol Committee (CPC) with the following plan:

1. Purpose: This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of AEs.
2. Monitoring: Up to 24 patients will be enrolled. The principal investigator will continuously monitor the study. The principal investigator will review the data and safety of the study after the enrollment of three patients in cohort 1, after enrollment of three patients in cohort 2, and after enrollment of six patients in cohort 3. Formal, independent monitoring by CPC will occur after enrollment of the three patients in cohort 1 and again after enrollment of three patients in cohort 2; then a scientific progress review will occur yearly, assuming a result of "satisfactory" on the initial review. The exceptions are as follows: If more than one patient experiences a Grade 4 or greater allergic reaction, the principal investigator will request a monitoring review by CPC. If at any time, more than 50% of patients experience a Grade 3 or 4 major organ toxicity, we will request a monitoring review by CPC.
3. Description of Monitoring: Adverse event reports will be reviewed with tabulation of all Grade 2, 3 or 4 toxicity.
4. Toxicity: see section 7.3.2 of the protocol.
5. Reporting Adverse Events: An adverse experience is any adverse change from the study patient's baseline (pre-treatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started. All adverse experiences that are classified as serious as described in section 10.7 of the protocol should be reported to the sponsor (Etubics) by telephone or fax within 24 hours, and reported in writing to the sponsor (etubics) within 72 hours. All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to the principal investigator, with a copy of the autopsy report and the death certificate. All adverse experiences will be recorded on the Adverse Experience Case Report Form. This report form should include severity, duration, outcome, and the investigator's judgment as to the relationship of the adverse experience to treatment.
6. Reporting of Pregnancy: If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the sponsor (Etubics), and to the IRB.

10.6. CTCAE Term (AE description) and Grade:

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 web site (http://ctep.cancer.gov). The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Serious Adverse Event Reporting

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

Procedures for adverse event reporting

1. PI notified by medical staff of a SAE

PI: Michael Morse, MD
Address: Duke University Medical Center
Seeley Mudd Building
Box 3233, Durham, NC 27710
Telephone: 1-919-681-3480
Fax: 1-919-681-7970
Pager 1-919-970-5626
Email: michael.morse@duke.edu

2. PI calls sponsor to report an unexpected SAE associated with the use of the drug

Sponsor: Carol Jones, Vice President of Administration
Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
Telephone: 206-838-5110 ext. 102
Cell: 1-206-818-2985
Fax: 1-206-838-2978
Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

A. Chief Scientific Officer: Frank Jones, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 101
Cell: 206-818-2857
Fax: 206-838-2978
Email: frj@etubics.com

B. Laboratory Manager: Joseph Balint, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 107
Fax: 206-838-2978
Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: 206-838-5110 ext. 103
Cell: 970-402-2598
Fax: 206-838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA on MedWatch 3200A form.
   a. If the SAE results in death or is life-threatening, report the SAE to the FDA within 7 days
   b. All other SAEs must be reported to FDA within 15 days

5. If the SAE requires input from a physician then the Acting Medical Director is consulted:

H. Kim Lyerly, MD
Telephone: (919) 684-5613
Fax: (919) 684-5653

Email: lyerl001@mc.duke.edu

All SAE reports will be recorded on the Duke IRB Adverse Event Reporting form and will be reviewed and signed by the Principal Investigator. Only adverse events that are deemed to be serious, unexpected and related or possibly related to the research must be reported to the IRB (this is in accordance with Duke's IRB reporting policy). All reportable events will be forwarded to the IRB via campus mail or fax:

IRB: Duke Medical Center Institutional Review Board:
Hock Plaza, 4th floor
2424 Erwin Road
Box 2991
Durham, NC 27705
Fax: 919-668-5125

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the adverse event may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant Institutional Review Board (IRB) of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is also the responsibility of the investigator and the sponsor (Etubics) in conducting gene transfer research to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the sponsor (Etubics) to report these serious adverse events to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

Treatment regimen (dosing frequency, combination therapy)

Protocol description (and number, if assigned)

Description of event, severity, treatment, and outcome, if known

Supportive laboratory results and diagnostics

Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

10.8. Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.9. Safety Reporting Requirements for IND Holders

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report.

c. Data and Safety Monitoring

Data will be collected by: the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by the CPC and Etubics' CRA.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted

potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.

9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.
14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.

21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.
26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating

factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.

32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.
39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.

45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.
53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.

58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.
67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment [a]	Week 0 [a]	Week 3 [b]	Week 6 [b]	Week 9	Months 6, 9, etc. [c]	Off Treatment
H & P	X	X	X	X	X	X	X
Karnofsky Status	X	X	X	X	X	X	X
β-HCG	X						
CBC & diff	X	X	X	X	X	X	X
PT/PTT	X						
Chemistries/LFTS	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
Immune Monitoring	X	X	X	X	X	X [d]	X
Biological Markers	X	X			X	X	X
MRI/CT Scan	X				X [e]	X	X
Immunization		X	X	X			

Notes: H & P = history & physical examination, Karnofsky = performance score of 70% or higher, β-HCG = human chorionic gonadotrophin pregnancy test, CBC & diff = complete blood count and white blood cell differential, ANA = antinuclear antibody, HIV = human immunodeficiency virus antibodyMRI/CT = magnetic resonance imaging/computed tomography.

[a] CEA and or other biological markers and with tumor that are universally CEA positive testing for biomarkers will be performed at the discretion of PI

[b] Immunizations may be performed -1 to +7 days after the specified week. Subsequent immunizations should be 3 weeks afterwards and keep to the every 3 week interval.

[c] Follow-up evaluations to be performed every 3 months after the Week 9 visit.

[d] Immune monitoring if there was evidence of an immune response or at the discretion of the immune monitoring laboratory.

[e] MRI/CT scan to be requested 1-4 weeks after the third immunization.

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

STUDY PRODUCT:	**Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)**
STUDY SPONSOR:	**Etubics Corporation** 410 West Harrison Street Seattle, WA 98119
PRINCIPLE INVESTIGATOR:	**Michael Morse** **Duke University Medical Center** Box 3233, Durham, NC 27710
ACTING MEDICAL DIRECTOR:	**H. Kim Lyerly, MD** **Duke Comprehensive Cancer Center** Duke University Medical Center Durham, NC 27710
CO-INVESTIGATOR:	**Arvind Chaudhry, MD** **Medical Oncology Associates, PS** 6001 N. Mayfair St. Spokane, WA 99208
Original Protocol:	Version 1.1, December 21, 2009
Amendment 1:	Version 2.1, May 14, 2010
Amendment 2:	Version 2.1, August 31, 2010

TABLE OF CONTENT

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (*i.e.* colon and rectal cancer). If colorectal cancer, pathologic or clinical confirmation of adenocarcinoma is required. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, trastuzumab, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving steroid or immunosuppressive therapy. All patients must be ≥21 years old and have a Karnofsky Performance Score of 70% or higher. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase I component), and the Maximally Tolerated Dose (MTD) of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2B-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Peripheral blood draw (90 mL) for immune analysis. 2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by NCI Common Terminology

	Criteria for Adverse Events (CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity, then patients may begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at the lowest dosage level, dosing will be de-escalated to $1x10^8$ particles. 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II Cohort: An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations. 6) Patients will have 90 mL peripheral blood drawn prior to each immunization and at week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 7) Time to progression will be measured using MRI/CT scans performed as per standard of care (approximately 3 month intervals).
Risks/Toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 24 evaluable patients (plus up to 12 replacements); may require 30-42 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total.
Criteria for Evaluation	Toxicity will be assessed using CTCAE version 4.0. CEA-specific immune response will be measured in the peripheral blood. Time to recurrence will be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a

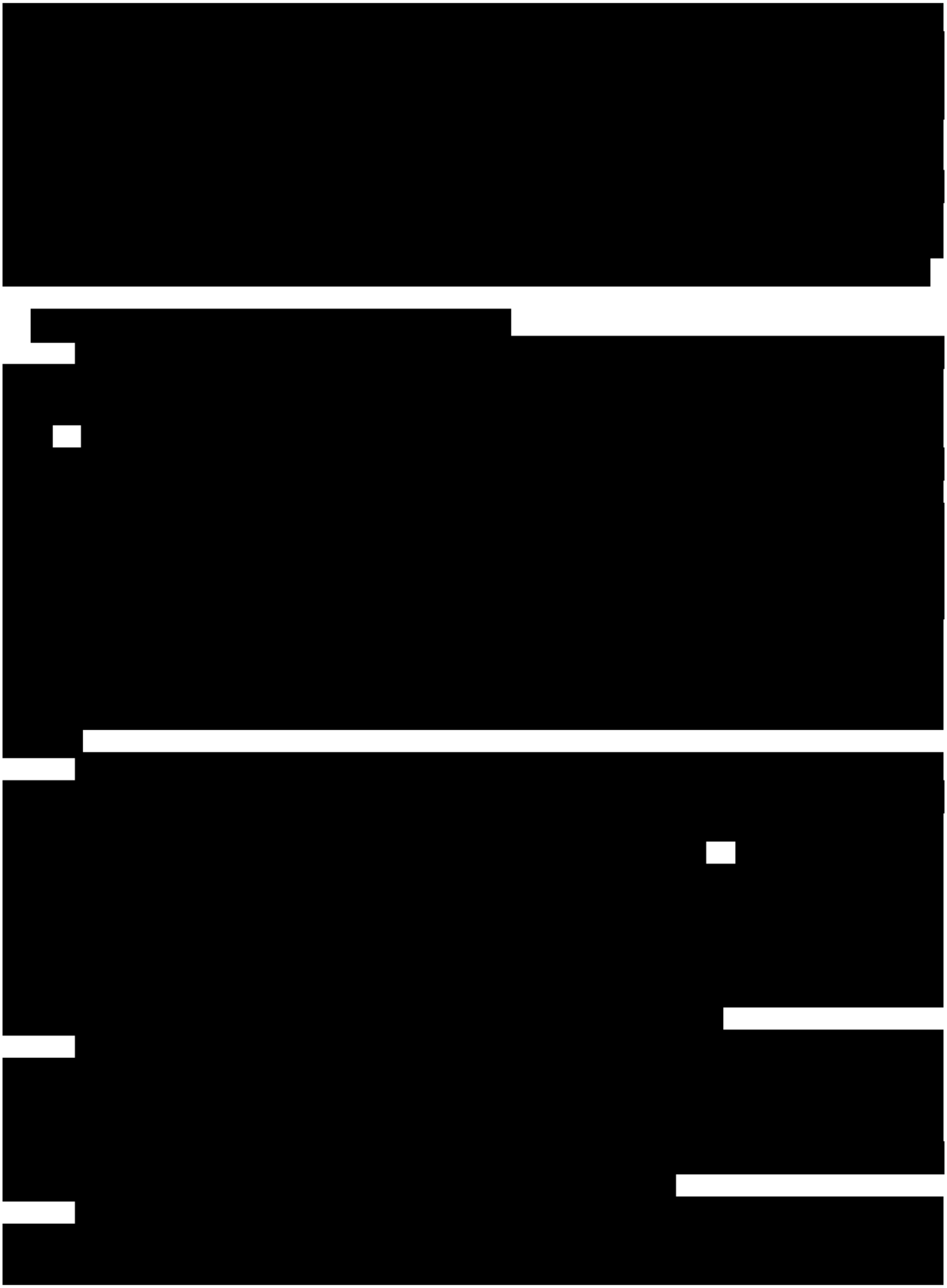
	dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 9. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

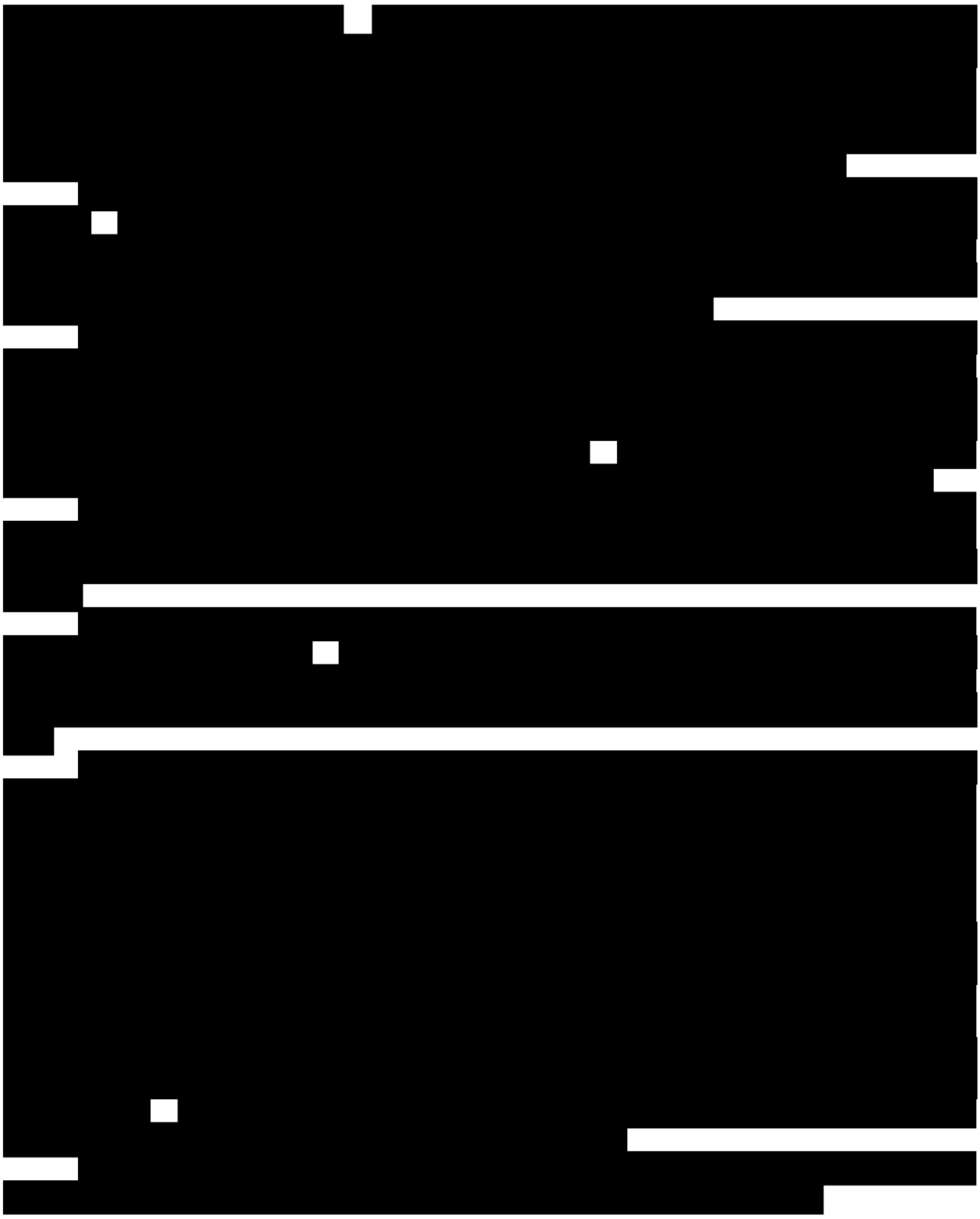
2. STUDY OBJECTIVES

a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

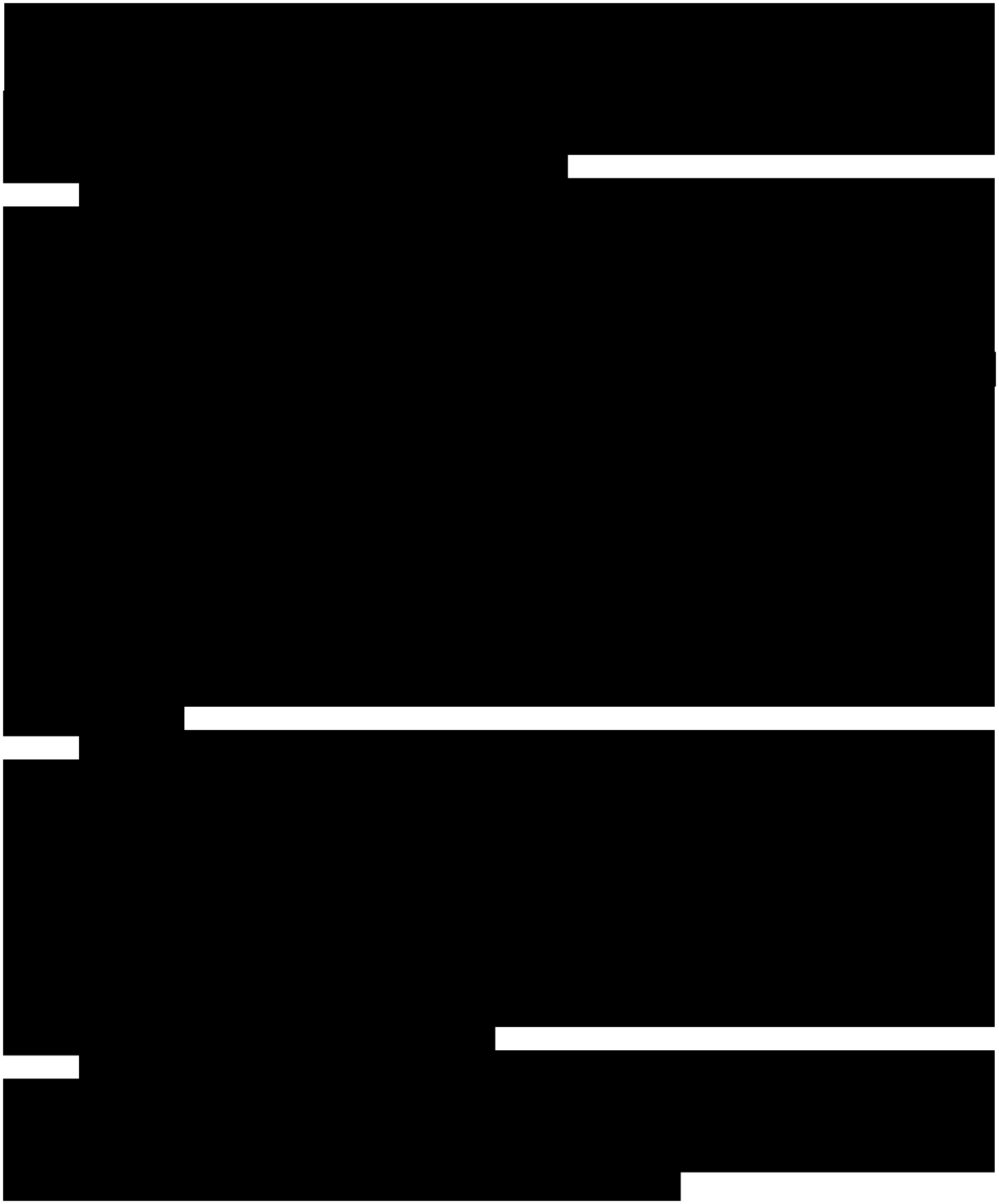
3. BACKGROUND AND SIGNIFICANCE

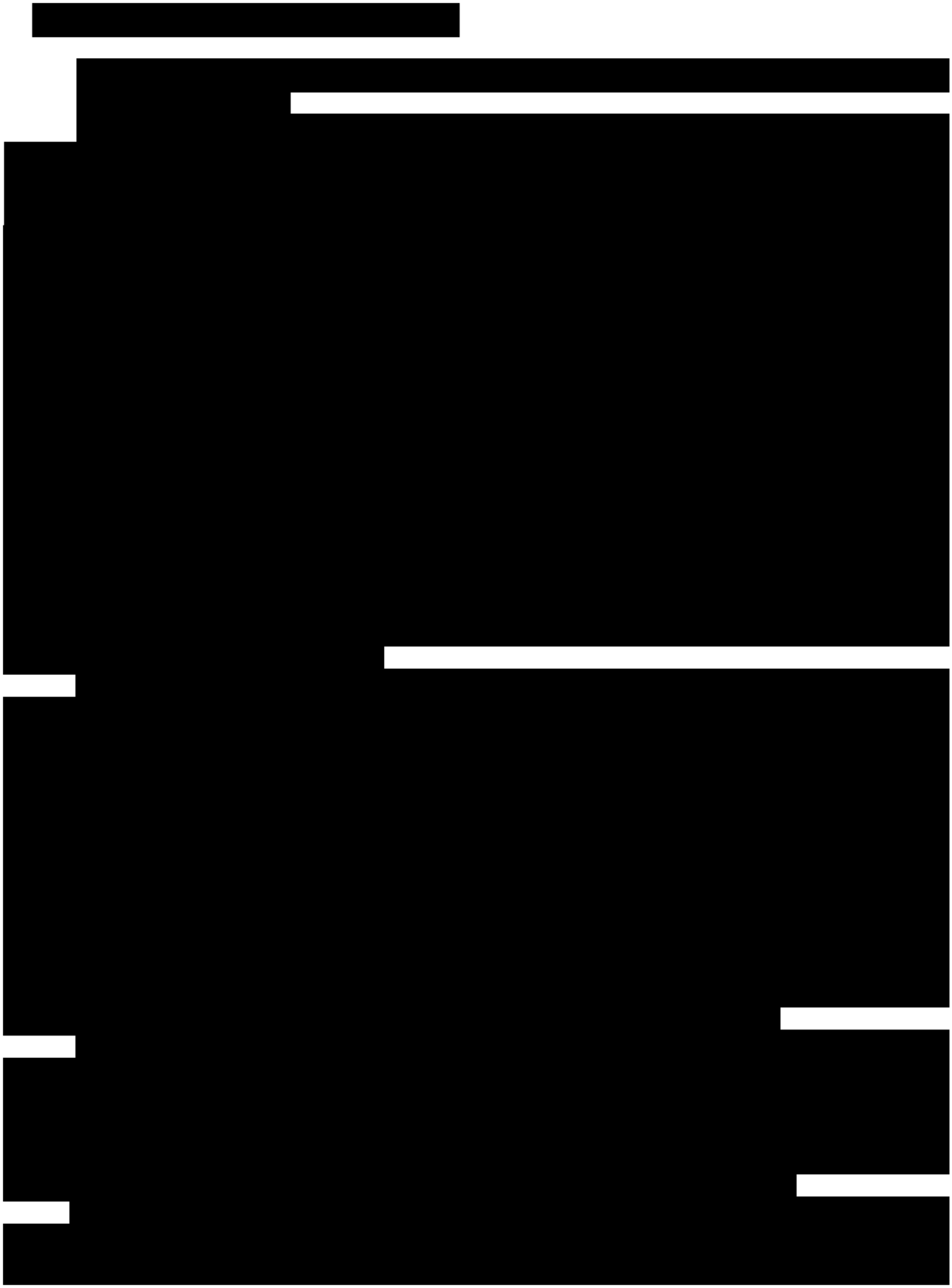
[redacted]

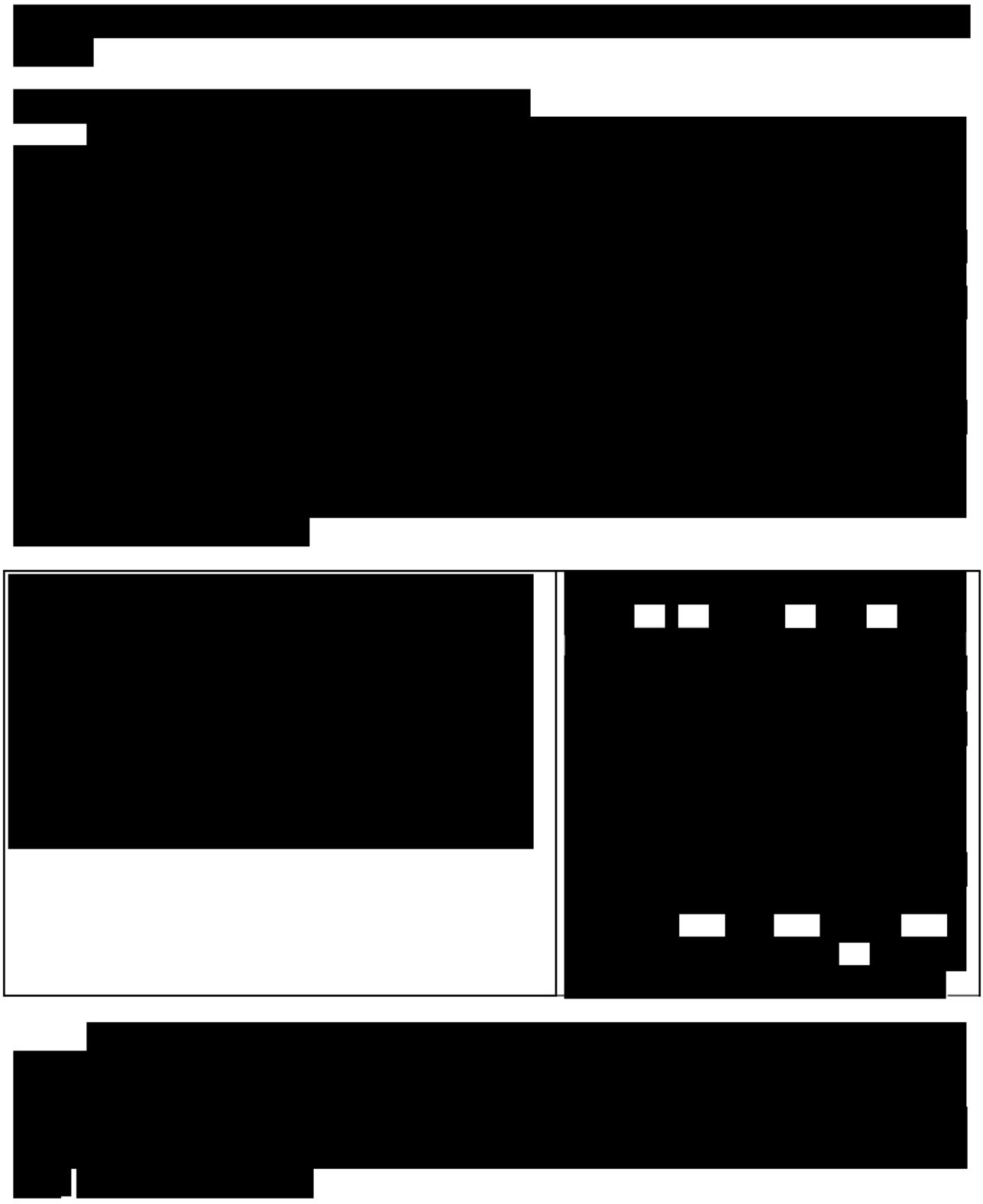


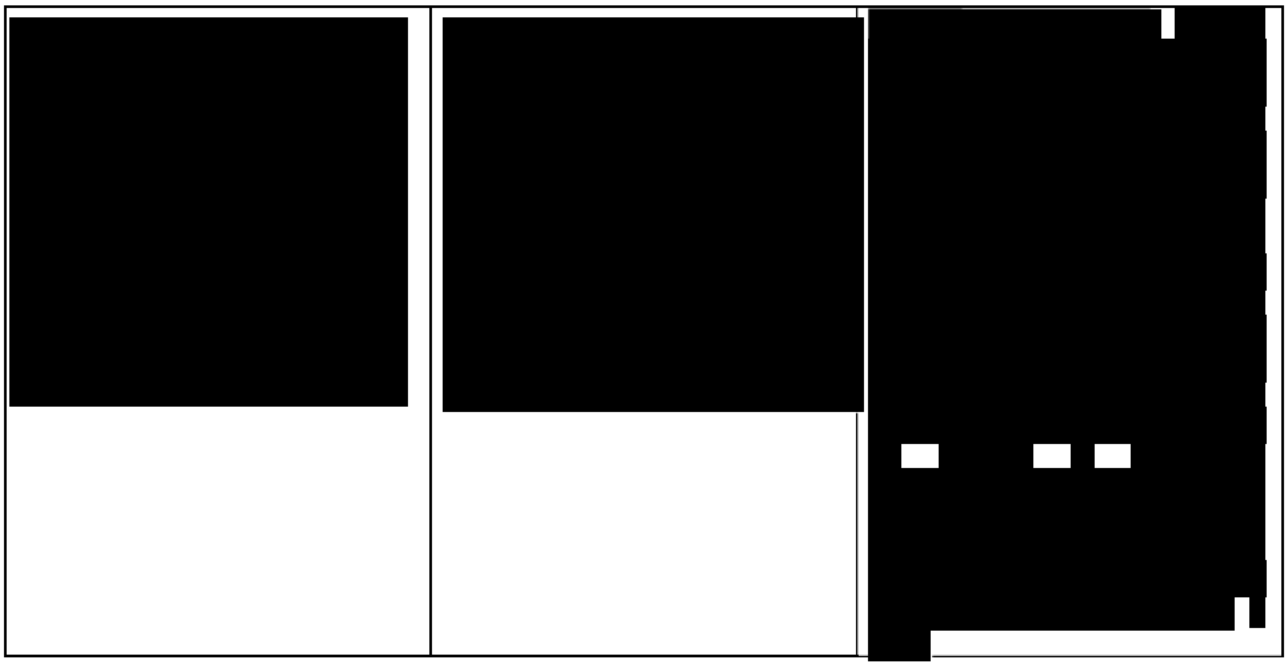


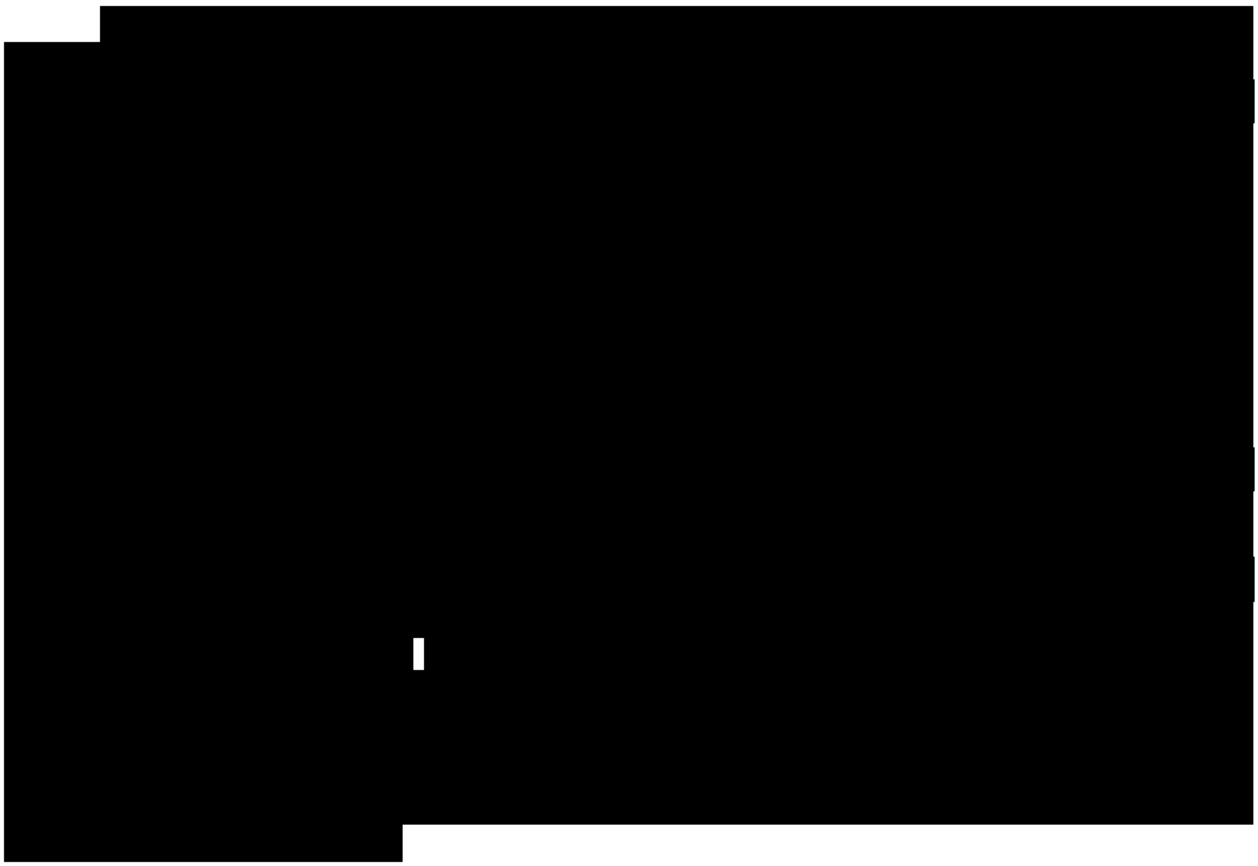
[redacted]











4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of malignancy expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. For all tumor types other than colorectal, the tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (i.e. colon and rectal cancer)**.** If colorectal cancer then, pathologic or clinical confirmation of adenocarcinoma is required.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
  - Patients who have been treated or offered the options of treatment with Bevacizumab (option clearly stated in the consent form).
  - Patients who have been treated or offered the options of treatment with Lapatinib (option clearly stated in the consent form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.

  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.
  - Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single agent therapy for at least 3 months).
- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. Karnofsky performance score of 70% or higher

4.1.5. Estimated life expectancy > 3 months

4.1.6. Age ≥ 21 years, but ≤ 75

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (it is acceptable to have had prior transfusion), platelets ≥ 75,000/microliter; PT-INR <1.5, PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosupression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to the clinical site for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, trastuzumab, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including

radiotherapy) and study treatment. Patients must have recovered to grade 1 acute toxicities from prior treatment.

4.2.2. Patients with a history of or current brain metastases will not be permitted.

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections.

4.2.8. Patients on steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies) prior to enrollment.

4.2.9. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.10. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.11. Patients will be allowed warfarin 1mg po qd other than for port prophylaxis.

4.2.11. Patients with metastatic disease which is determined to be resectable will be excluded.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if DLT occur that necessitate re-dosing at a lower dosage levels (Refer to Protocol Section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number

Patients will be assigned study numbers in order of their screening using the following: ETBX-011- 001, 002, 003 etc. including screened patients.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment: (Refer to Appendix 1 - Schema)

- History and physical exam, to include Karnofsky Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological and Biochemical Tests:
  - CBC with differential
  - PT, INR and PTT
  - Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose
- Urinalysis
- HIV Antibody
- Anti Nuclear Antibody (ANA)
- Biological Markers:
  - Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad5 vector and sent to Etubics Corporation for determination.

  Other available serum markers (e.g., CEA or CA15-3) will be reviewed if determined and available.
  -
- Immunologic Evaluation/Archive Sample:
  - Blood should be drawn during the first clinic visit for immunologic evaluation and/or archiving. .
- Imaging Studies:
  - Brain CT scan or MRI is required within 6 weeks of starting study treatment.
  - Available CT scans or MRI of the chest, abdomen, and/or pelvis will be reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, but is not required.

6. TREATMENT PLAN

In the phase I component, patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (Based on NCI CTCAE version 4.0) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and

less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. The first patient will be called to check on their condition prior to enrolling the second patient since patients can be enrolled after 1 week of initiation of cohort 3. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component without the 1-week waiting period. Between dosage levels, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II, 12 additional patients will be enrolled at the MTD. In phase II, if at any time the rate of DLT in patients enrolled at the MTD (for the phase I and phase II cohorts combined) is ≥33%, the MTD will be re-defined as the next lower dosage level, and phase II will proceed with enrollment of additional patients at this lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to $1x10^8$ particles and a new cohort of 3 patients instituted.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit

at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then this dosage level will be considered the MTD and patients may begin enrolling into the phase II portion of the study. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to phase II.

4) Phase II cohort: After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

   Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

6) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

7) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

8) The following safety events will trigger a temporary suspension of study vaccinations:

   a) If ≥33% of patients in the phase II cohort experience DLT at dosage level 1 (i.e.,1×10^9 particles)

b) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine
c) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

Etubics Corporation will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above. Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two patients having a Grade 4 toxicity event that is possibly/probably related to the study agent.

Figure 3A.

Figure 3B.

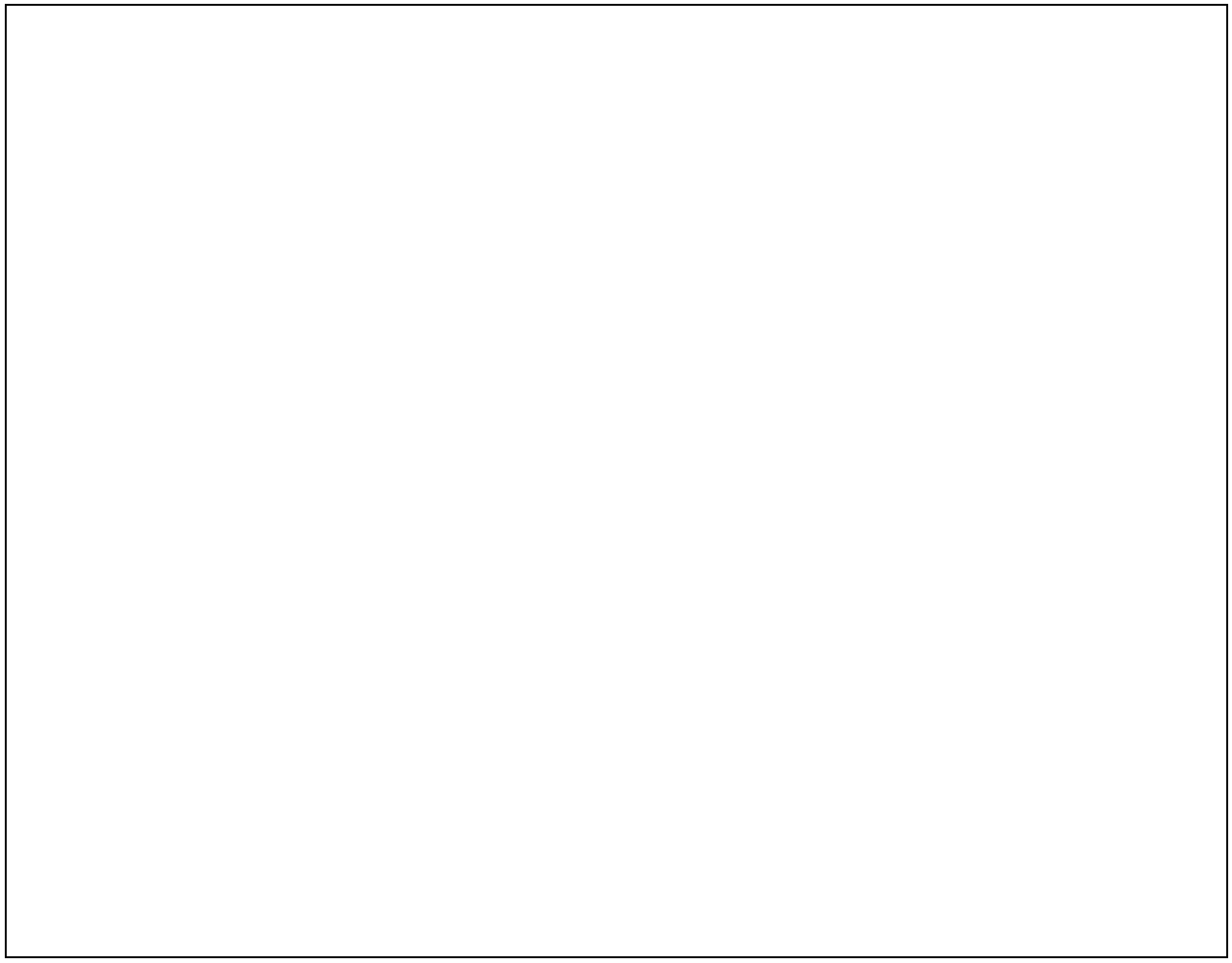


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.2. Pharmaceutical Information

6.1.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, or 1 x 10^{10}, or $1x10^{11}$ virus particles (vp) per immunization ain 0.5 mL of sterile saline SQ every 3 weeks for 3 immunizations.

6.1.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains $1X10^{11}$ total virus particles. The volume of injection for $1x10^{11}$ virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:

Cohort 1: To administer 1x10e9 virus particles by subcutaneous injection, perform 2 serial dilutions of vialed vaccine as follows:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Use a new sterile syringe with a needle and repeat above procedure.
6. Withdraw 4.5 mL of sterile saline.
7. In the second syringe withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/ml**.
8. Remove the needle from the syringe containing the 4.5 mL saline, and inject the 0.5 mL of 2 x 10^{10}vp/mL from the second syringe.
9. Place a new needle on the 10-mL syringe from Step 8 above and mix the two solutions. This solution now has a concentration **2 x 10^9 vp/mL** (1 x 10^9 vp per 0.5 mL)**.**
10. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^9 vp and withdraw 0.5 mL from the syringe containing **2 x 10^9 vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^9 vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 2: To administer 1x10e10 virus particles by subcutaneous injection:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^{10} vp and withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^{10} vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 3: To administer 1x10e11 virus particles by subcutaneous injection:

1. Withdraw 0.5mL of contents from a previously thawed ETBX-011 from the supplied vial and administer to each subject without any further manipulation.

6.1.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joe Balint, at Etubics Corporation for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations

Weeks 0, 3, 6 (Immunization Visits):

General evaluations include medical history, Karnofsky performance status , and complete physical examination with weight by the attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

Week 9:

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight by attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Immunization injection sites will be assessed.

7.1.2 Hematological and Biochemical Assessment

Weeks 0, 3, 6 (Immunization Visits):

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

Week 9:

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, glucose and ANA.

7.1.3 Biological Markers

Weeks 0 and 9:

Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained and send to Etubics Corporation for determination. Biomarkers (e.g., CEA or CA15-3) will be reviewed if determined and available.

7.1.4 Immunological Assessment

Weeks 0, 3, 6, and 9:

Peripheral blood (90mL) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Serum from each blood draw will be archived and sent to Etubics Corporation for Ad5 neutralizing level determination.

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up every 3 months for 1 year, while on the study (i.e., have not progressed or been removed from the study for other reasons). At each visit, a medical history and physical exam and the following lab tests will be performed: blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose. At each visit, peripheral blood (40-90 mL) should be drawn for immune analysis if there is previous evidence of an immune response or at the discretion of the investigator.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) DLT is defined in Protocol Section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity,

manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 DLT related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations:

In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This decision will be made by the medical monitor and the Principal Investigator. A note will be generated following the assessment decision and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience DLT (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). DLT is defined in Protocol Section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response

Immune responses against CEA will be evaluated from the peripheral blood of patients from among the following studies at the discretion of the Principal Investigator (ELISpot, cytokine flow cytometry, and antibody responses). We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- DLT as defined in Protocol Section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of withdrawal due to toxicity, a patient will be requested to have safety evaluations performed as per the protocol for a one year duration post treatment. This may include having up to 90 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator, or in his absence, Dr. H. Kim Lyerly, Acting Medical Director, immediately by telephone (919) 684-8111 (paging operator). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the Institutional Review Board (IRB) and the Etubics Corporation by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and Etubics Corporation will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the site's IRB. IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients signing consent. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A CRF must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. CRFs must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on CRFs by name; appropriate coded identification and

patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto CRFs, originals of laboratory and other test results must be kept on file with patient's CRFs or clinical chart. CRFs and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Event Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

A Etubics Corporation representative will conduct a site initiation visit and will review protocol documents with the investigators and their staff. During the study, the Etubics monitor will visit the site regularly to check the completeness of study files, the accuracy of data entries on the CRFs, and adherence to the protocol and to Good Clinical Practice (GCP). Key study personnel must be available to assist the monitor during these visits. The investigator must maintain source documents for each subject in the study. The investigator must also keep the original informed consent form signed by every patient. The investigator must give the monitor access to all relevant source documents to verify data entries on CRFs. No information in source documents about the identity of subjects will be disclosed.

This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of adverse events (AEs). The principal investigator will continuously monitor the data and safety of all subjects enrolled. All grades of toxicities will be recorded.

Safety assessments will consist of monitoring all AEs including serious adverse events (SAEs), the regular monitoring of hematology, serum chemistry, and routine monitoring of vital signs and physical condition. AE monitoring should occur from the time of informed consent to 30

days after the last dose of study drug. For those subjects who discontinue study participation prior to receiving study drug, AE and SAEs will be collected through the time of discontinuation.

Toxicity will be assessed using the NCI CTCAE version 4.0. Refer to Protocol Section 7.3.2.

An AE is any adverse change from the study patient's baseline (pretreatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started.

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

10.6. CTCAE Term (AE description) and Grade

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for AE reporting. A copy can be downloaded from the CTEP web site (http://ctep.cancer.gov).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE

Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Assessing Causality

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.8. Serious Adverse Event Reporting

All AEs that are classified as serious, unexpected and related or possibly related, will be recorded on the MedWatch 3500A form and reported within 24 hours of learning of the event to Etubics Corporation. The form should be completed as much as possible but should not be held until all information is available. Additional information and/or corrections may be submitted as they are obtained. The investigator will follow SAEs until resolution, a return to baseline condition or stabilization or 30 days after the last subject is enrolled whichever occurs first. SAEs that are ongoing at the time of clinical database closure will be recorded as unresolved. All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to Etubics Corporation, with a copy of the autopsy report and the death certificate.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form

- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

Reporting of Pregnancy:

If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the Etubics Corporation and to the IRB.

Procedures for SAE Reporting:

1. Medical staff/study team notifies principal investigator of SAE. MedWatch 3500A is completed.

2. Principal Investigator calls Etubics Corpration to report the SAE and faxes the completed MedWatch 3500A to:

   Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
   Telephone: 206-838-5110 ext. 102
   Cell: (206) 818-2985
   Fax: (206) 838-2978
   Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

   A. Chief Scientific Officer: Frank Jones, PhD
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
   Telephone: 206-838-5110 ext. 101
   Cell: (206) 818-2857
   Fax: (206) 838-2978
   Email: frj@etubics.com

   B. Laboratory Manager: Joseph Balint, PhD
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
   Telephone: (206) 838-5110 ext. 107
   Fax: (206) 838-2978
   Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 103
Cell: (970) 402-2598
Fax: (206) 838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA via MedWatch 3500A form.

   a. If the SAE results in death or is life-threatening, SAE report will be submitted to the FDA within 7 days
   b. All other SAEs must be reported to the FDA within 15 days

5. All SAE will require input from a physician, the Acting Medical Director will be consulted:

   H. Kim Lyerly, MD
   Telephone: (919) 684-5613 or (919)684-8111 (paging operator)
   Fax: (919) 684-5653
   Email: lyerl001@mc.duke.edu

6. SAEs will be reported to the IRB according to the site's IRB guidelines.

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the AE may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant IRB of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is the responsibility of Etubics Corporation to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the Etubics Corporation to report these SAEs to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

10.9. Safety Reporting Requirements

In accordance with 21 CFR 212.32, the sponsor of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation.

c. Data and Safety Monitoring

Data will be collected by the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by Etubics Corporation.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte

antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.

14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.

26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.
32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of

melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.

39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based

vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.

53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.

67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3	Week 6	Week 9	Months 6, 9, etc. [i]	Off Treatment
H & P	X	X	X	X	X	X	X
Karnofsky Status	X	X	X	X	X	X	X
β-HCG [a]	X						
CBC with diff	X	X	X	X	X	X	X
PT, INR, PTT	X						
Chemistries	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
Immune Monitoring [b]	X [b]	X	X	X	X	X [b]	X [b]
Antibodies to CEA/Ad5 Vector [c]		X			X		
Biological Markers [d] (i.e. CEA, CA15-3)	X [d]				X [d]	X [d]	X [d]
Brain MRI/CT Scan [e]	X						
MRI/CT Scan [f]	X [f]				X [f]	X [f]	X [f]
Immunization [g]		X	X	X			
AE Assessment [h]	X	X	X	X	X		

Key: H & P = history & physical examination, **KPS** = Karnofsky Performance Scale, **β-HCG** = human chorionic gonadotrophin pregnancy test; **CBC & diff** = complete blood count and white blood cell differential, **Chemistries** = Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose; **ANA** = antinuclear antibody, **HIV** = human immunodeficiency virus antibody, **MRI/CT** = magnetic resonance imaging/computed tomography; **AE** = Adverse Event

Notes:

[a] β-HCG performed on women of childbearing potential

[b] Immune monitoring drawn during the first clinic visit at the discretion of the investigator/immune monitoring laboratory, prior to each immunization and approximately 3 weeks after the last immunization, and will be repeated at the discretion of the investigator/immune monitoring laboratory.

[c] Serum obtained for antibodies to CEA and Ad5 vector weeks 0 and 9.

[d] Available serum markers (e.g., CEA or CA15-3) reviewed as available.

[e] Brain CT scan or MRI required within 6 weeks of starting study treatment.

[f] Available CT scans or MRI of the chest, abdomen, and/or pelvis reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, however is not required. Repeat imaging conducted according to standard of care.

[g] Immunizations may be modified so that the interval is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

[h] AE monitoring from the time of informed consent to 30 days after the last dose of study drug.

[i] Follow-up evaluations requested every 3 months for 1 year, while on the study.

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

STUDY PRODUCT:	**Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)**
STUDY SPONSOR:	**Etubics Corporation** 410 West Harrison Street Seattle, WA 98119
PRINCIPAL INVESTIGATOR:	**Michael Morse** **Duke University Medical Center** Box 3233, Durham, NC 27710
Original Protocol:	Version 1.1, December 21, 2009
Amendment 1:	Version 2.0, 13 April 2010
Amendment 2:	Version 2.1, May 14, 2010
Amendment 3:	Version 2.1, August 31, 2010
Amendment 4:	Version 3.1, June 20, 2011

TABLE OF CONTENT

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (*i.e.* colon and rectal cancer). If colorectal cancer, pathologic or clinical confirmation of adenocarcinoma is required. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving chronic steroid or immunosuppressive therapy. All patients must be ≥21 years old and have a Karnofsky Performance Score of 70% or higher. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase I component), and the Maximally Tolerated Dose (MTD) of Ad5 [E1-, E2B-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2B-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Peripheral blood draw (90 mL) for immune analysis. 2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by NCI Common Terminology

	Criteria for Adverse Events (CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity, then patients may begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at the lowest dosage level, dosing will be de-escalated to $1x10^8$ particles. 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II Cohort (Cohort 4): An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations. 6) Cohort 5: Beginning after the third dose of ETBX-011 for the 12^{th} patient in cohort 4, enrollment in an additional higher dose cohort will be permitted. Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of $5x10^{11}$ particles SQ in 2.5 mL every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had all 3 injections and been observed for at least 30 days after the last injection. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then 1×10^{11} particles will be declared the MTD. 7) Patients will have 90 mL peripheral blood drawn prior to each immunization and at week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 8) Time to progression will be measured using MRI/CT scans performed as per standard of care (approximately 3 month intervals).
Risks/Toxicities	Potential risks associated with the vaccine include anaphylaxis, fever,

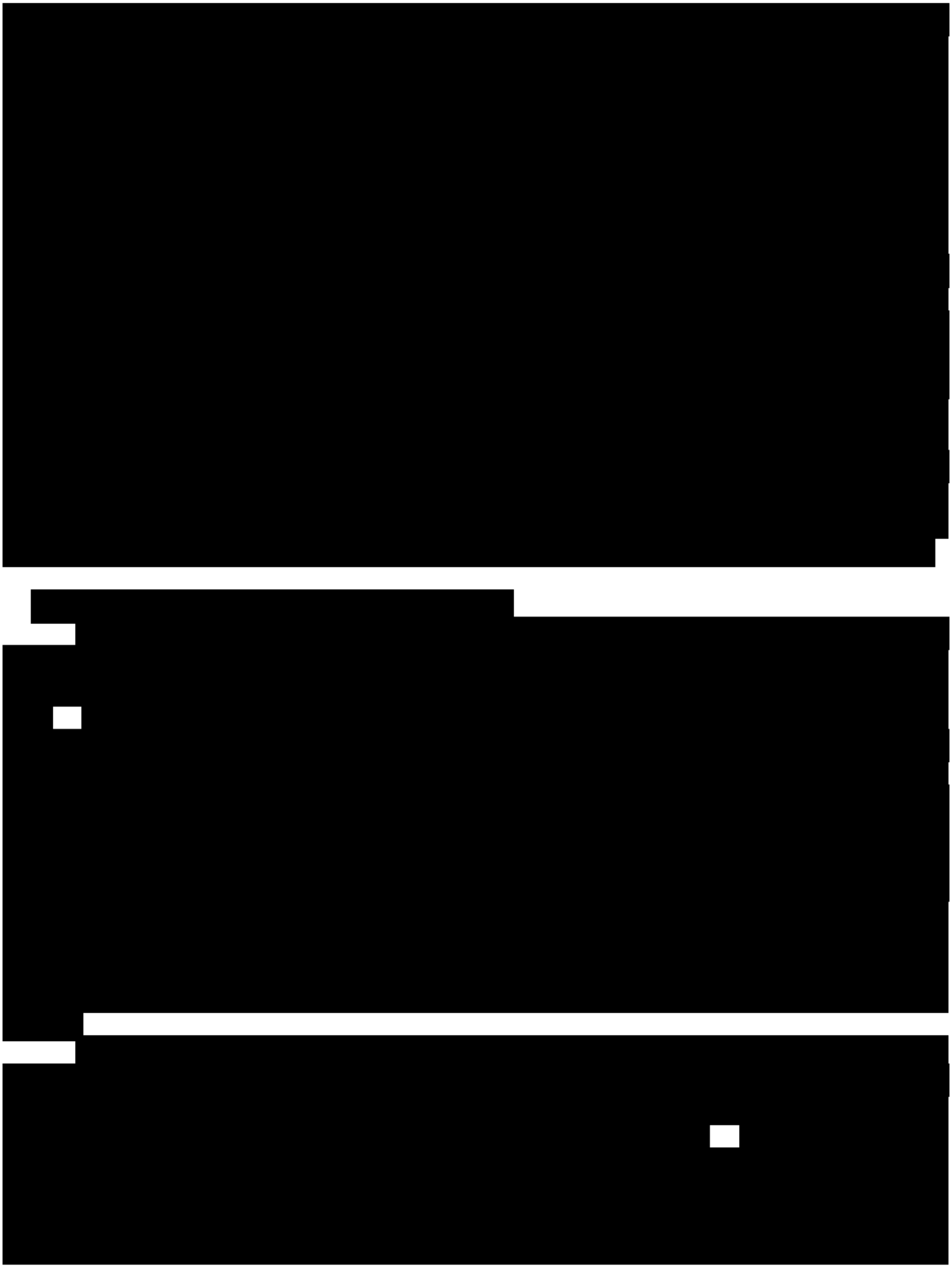
	skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 30 evaluable patients (plus up to 12 replacements); may require 30-42 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total.
Criteria for Evaluation	Toxicity will be assessed using CTCAE version 4.0. CEA-specific immune response will be measured in the peripheral blood. Time to recurrence will be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 9. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

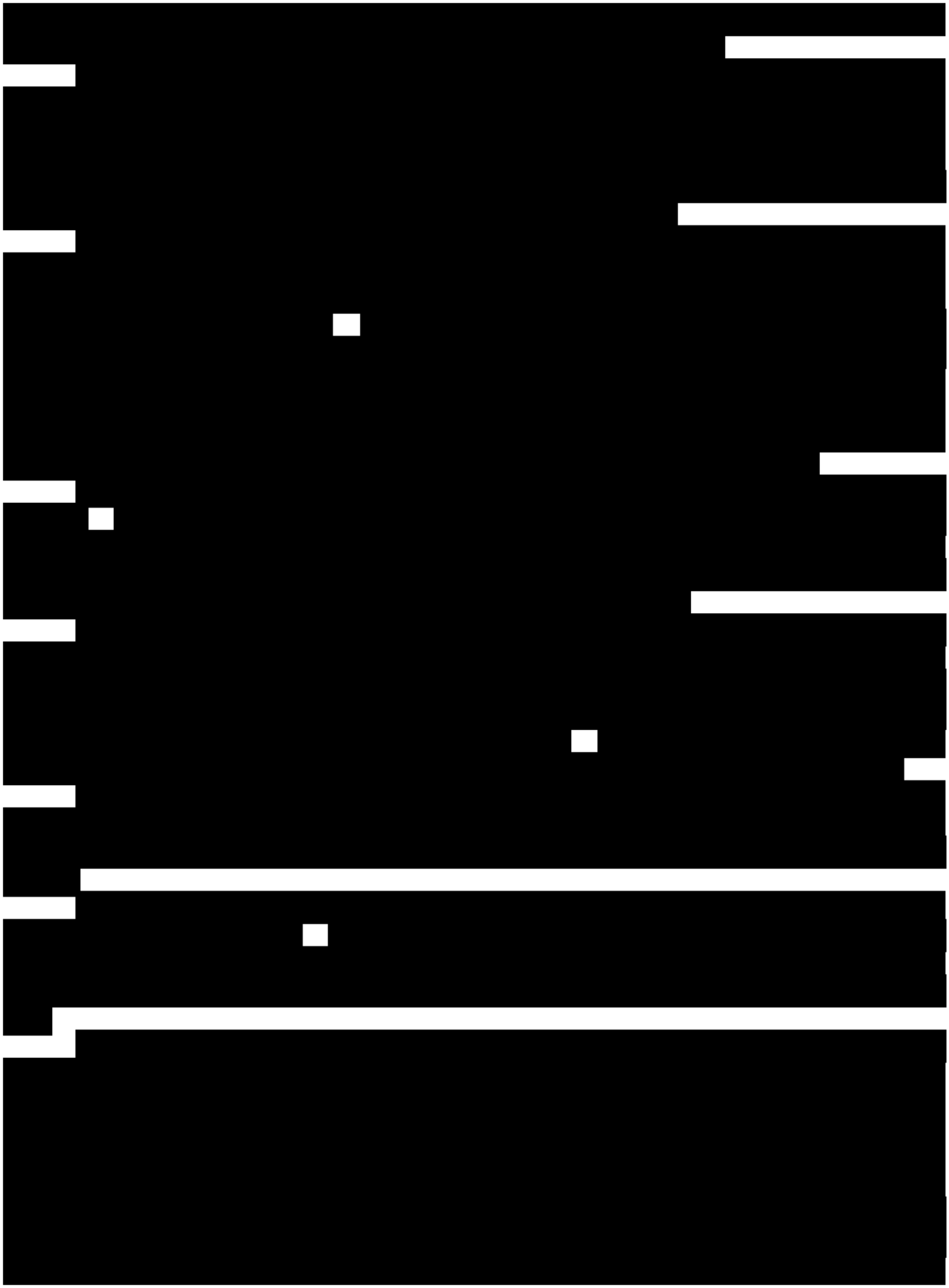
2. STUDY OBJECTIVES

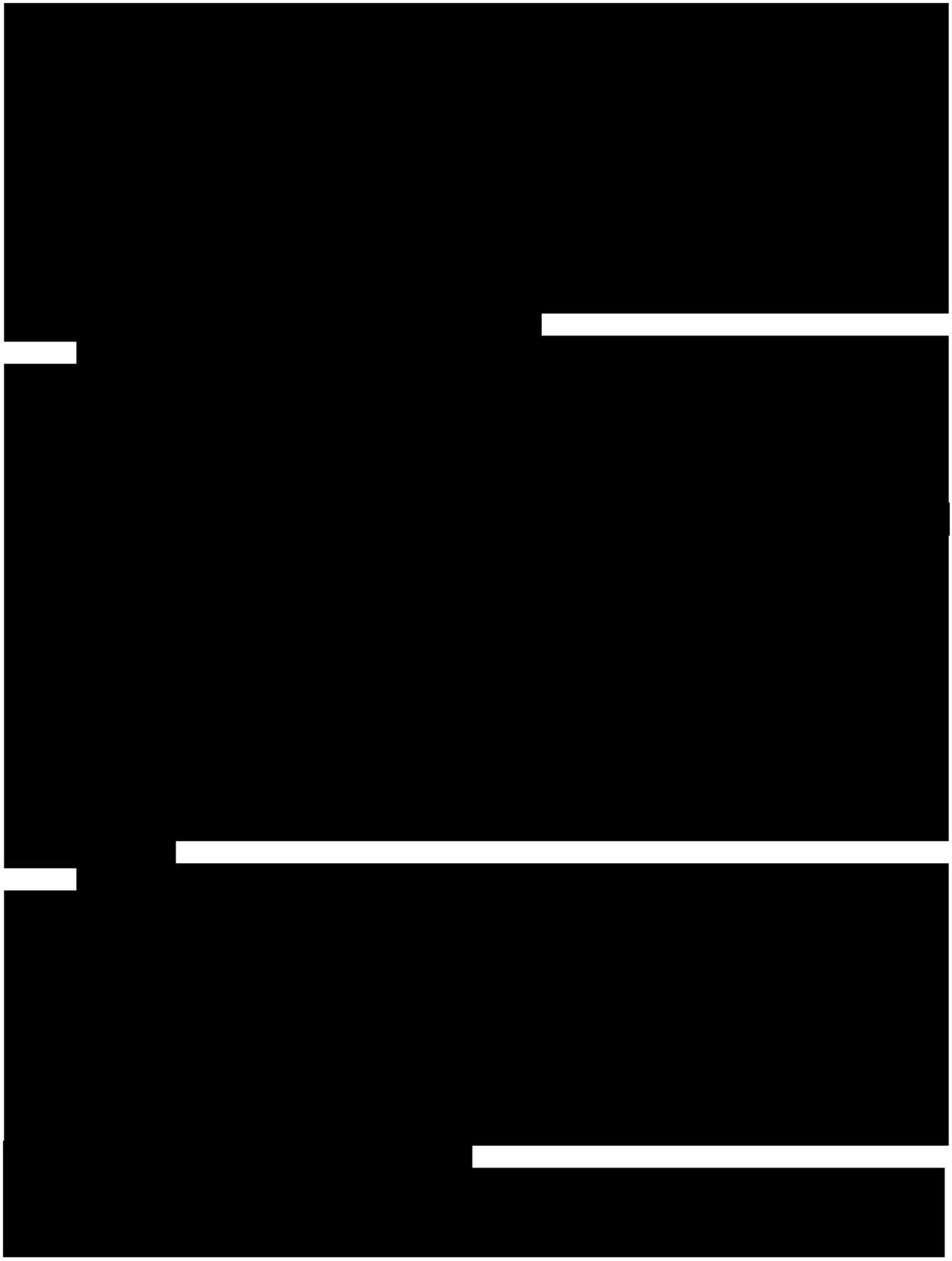
a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

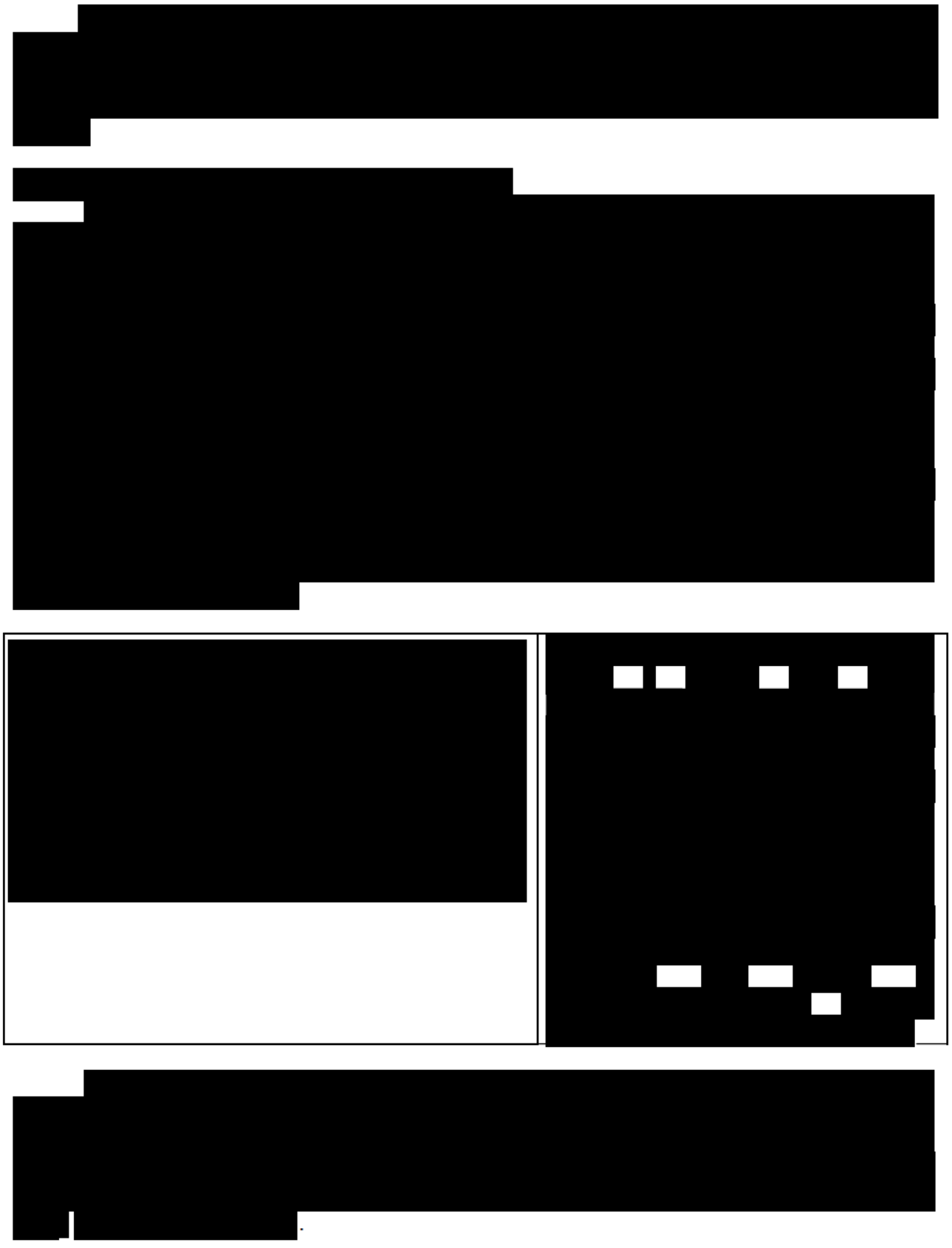
3. BACKGROUND AND SIGNIFICANCE

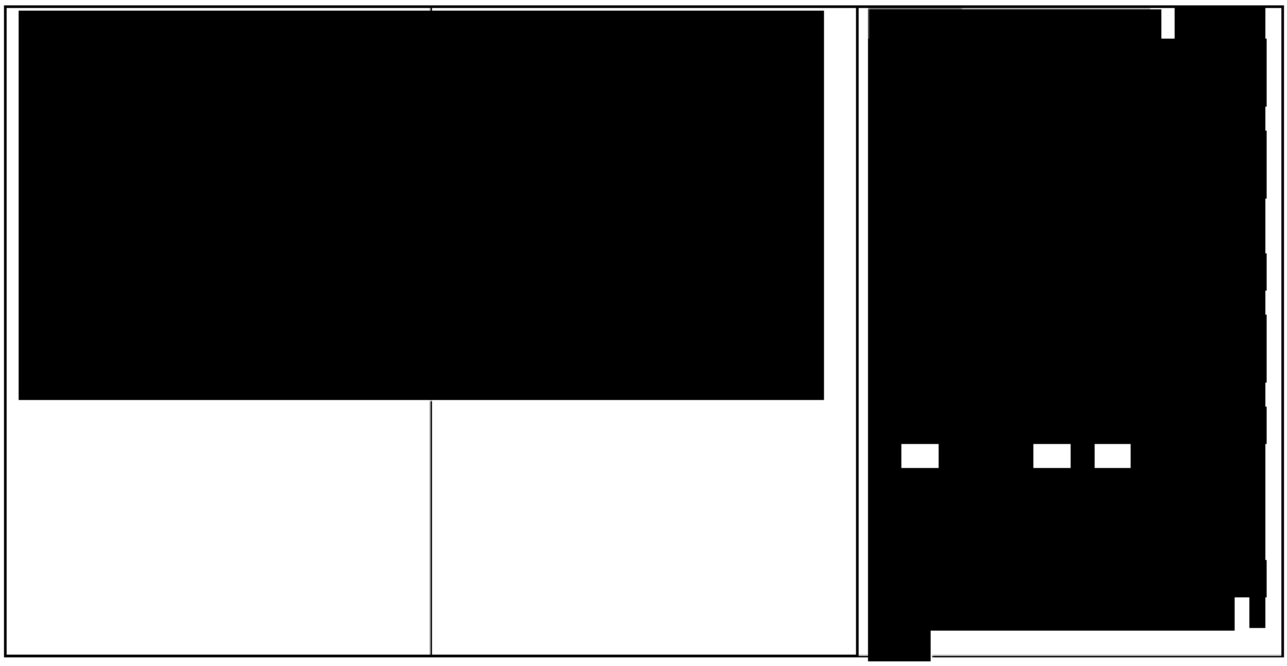
[redacted]

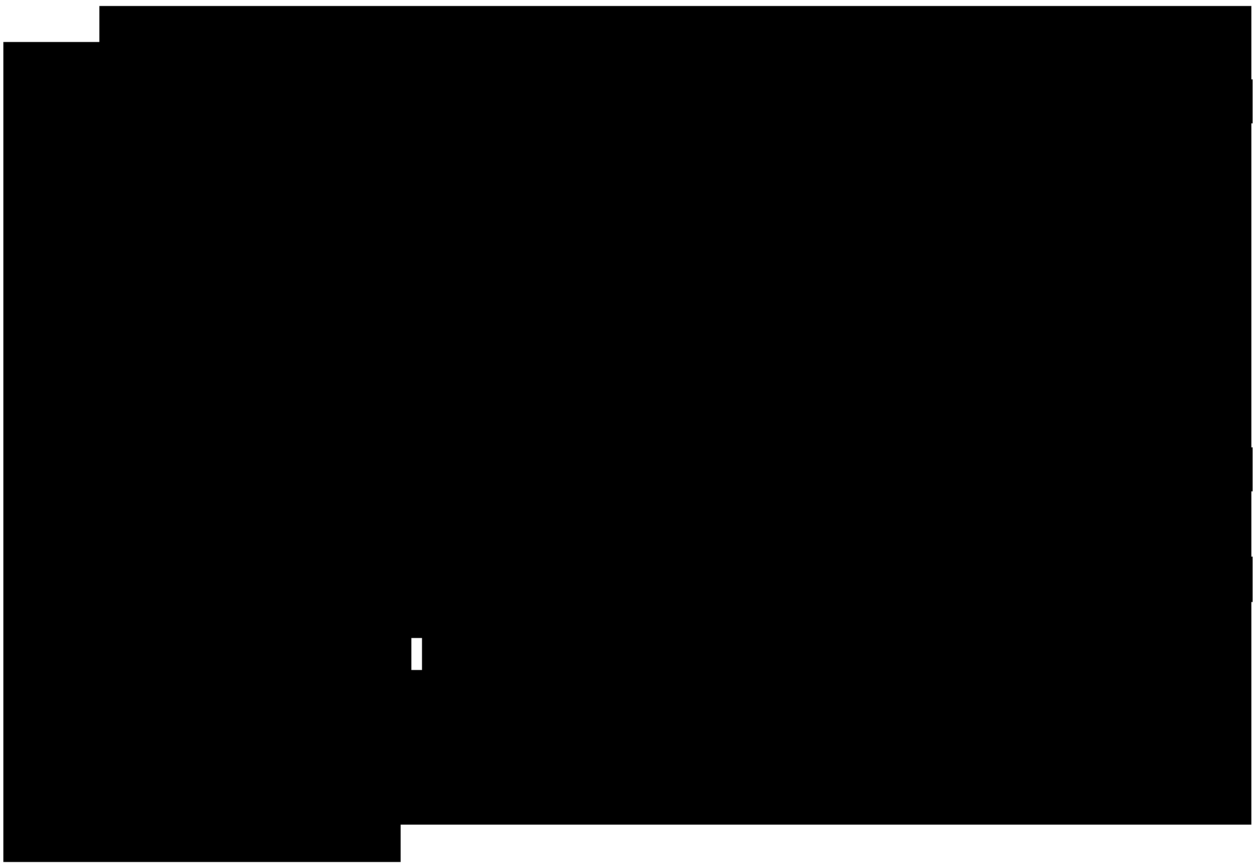












[redacted]

4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of malignancy expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. For all tumor types other than colorectal, the tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (i.e. colon and rectal cancer)**.** If colorectal cancer then, pathologic or clinical confirmation of adenocarcinoma is required.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
  - Patients who have been treated or offered the options of treatment with Bevacizumab (option clearly stated in the consent form).
  - Patients who have been treated or offered the options of treatment with Lapatinib (option clearly stated in the consent form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.

  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.
  - Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single agent therapy for at least 3 months).
- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. Karnofsky performance score of 70% or higher

4.1.5. Estimated life expectancy > 3 months

4.1.6. Age ≥ 21 years, but ≤ 75

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (it is acceptable to have had prior transfusion), platelets ≥ 75,000/microliter; PT-INR <1.5 (unless patient is receiving warfarin in which case PT-INR must be <3), PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosupression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to the clinical site for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered to grade 1 acute toxicities from prior treatment.

4.2.2. Patients with a history of or current brain metastases will not be permitted.

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections.

4.2.8. Patients on chronic steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies or for acute treatment (<5 days) of intercurrent medical condition such as a gout flare) prior to enrollment.

4.2.9. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.10. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.11. Patients with metastatic disease which is determined to be resectable must have been referred to a surgeon for consideration of resection.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if DLT occur that necessitate re-dosing at a lower dosage levels (Refer to Protocol Section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number

Patients will be assigned study numbers in order of their screening using a site specific sequential numbering system, e.g.,: ETBX-011- 001, 002, 003 etc. including screened patients.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment: (Refer to Appendix 1 - Schema)

- History and physical exam, to include Karnofsky Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological and Biochemical Tests:
  - CBC with differential
  - PT, INR and PTT
  - Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose
- Urinalysis
- HIV Antibody
- Anti Nuclear Antibody (ANA)
- Biological Markers:
  - Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad5 vector and sent to Etubics Corporation for determination.
  - Other available serum markers (e.g., CEA or CA15-3) will be reviewed if determined and available.
- Immunologic Evaluation/Archive Sample:
  - Blood may be drawn during the first clinic visit (after consent is signed) for immunologic evaluation and/or archiving.
- Imaging Studies:
  - Brain CT scan or MRI is required within 6 weeks of starting study treatment.
  - Available CT scans or MRI of the chest, abdomen, and/or pelvis will be reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, but is not required.

6. TREATMENT PLAN

Patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (Based on NCI CTCAE version 4.0) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and

less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. The first patient will be called to check on their condition prior to enrolling the second patient since patients can be enrolled after 1 week of initiation of cohort 3. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component (Cohort 4) without the 1-week waiting period. Between dosage levels, through Cohort 3, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed. Patients in cohort 5 may begin injections once all patients in cohort 4 have had at least their first injections and all patients in cohort 3 have had a study visit at least 3 weeks after receiving their first dose of vaccine. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II (Cohort 4) and Cohort 5, if at any time the rate of DLT in patients enrolled is ≥33%, the MTD will be re-defined as the next lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to $1x10^8$ particles and a new cohort of 3 patients instituted.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of

DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to the next cohort.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for proceeding to phase II enrollment (Cohort 4) will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then subjects may enroll in Cohort 4. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to the next level.

4) Phase II cohort (Cohort 4): After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

   Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Cohort 5: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 5×10^{11} particles SQ in 2.5 mL every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had all 3 injections and been observed for at least 30 days after the last injection. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then 1×10^{11} particles will be declared the MTD.

6) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

7) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

8) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third

immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

9) The following safety events will trigger a temporary suspension of study vaccinations:

   a) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine
   b) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

Etubics Corporation will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above. Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two patients having a Grade 4 toxicity event that is possibly/probably related to the study agent.

Figure 3A.

Figure 3B.

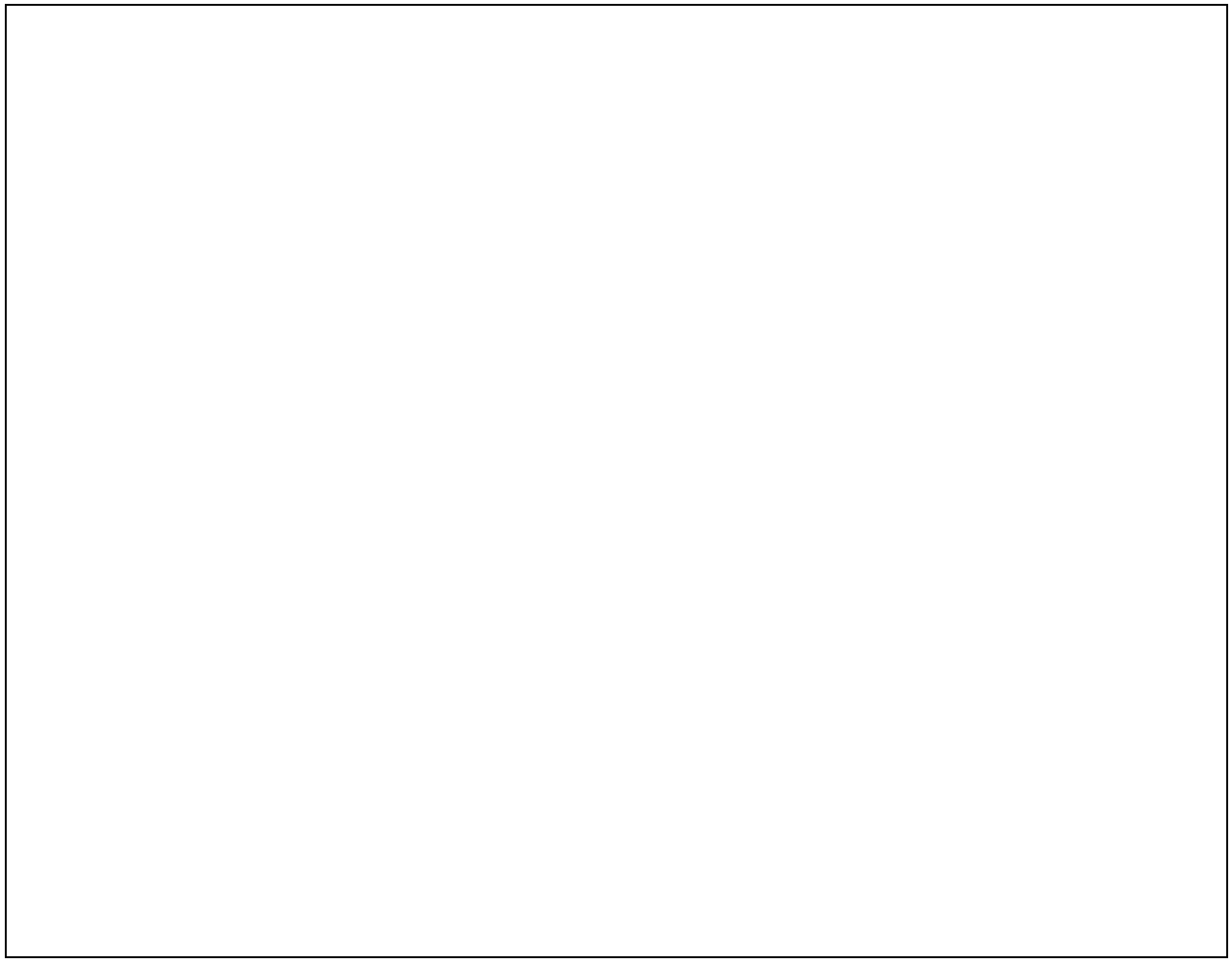


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.2. Pharmaceutical Information

6.2.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, 1 x 10^{10}, or 1 x 10^{11} virus particles (vp) per immunization in 0.5 mL of sterile saline SQ every 3 weeks for 3 immunizations. The 5th cohort will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, or 1 x 10^{10}, or 1 x 10^{11} virus particles (vp) per immunization in 0.5 mL of sterile saline SQ every 3 weeks for 3 immunizations.

6.2.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains 1x10^{11} total virus particles. The volume of injection for 1x10^{11} virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:

Cohort 1: To administer 1x10e9 virus particles by subcutaneous injection, perform 2 serial dilutions of vialed vaccine as follows:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Use a new sterile syringe with a needle and repeat above procedure.
6. Withdraw 4.5 mL of sterile saline.
7. In the second syringe withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/ml**.
8. Remove the needle from the syringe containing the 4.5 mL saline, and inject the 0.5 mL of 2 x 10^{10}vp/mL from the second syringe.
9. Place a new needle on the 10-mL syringe from Step 8 above and mix the two solutions. This solution now has a concentration **2 x 10^9 vp/mL** (1 x 10^9 vp per 0.5 mL)**.**
10. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^9 vp and withdraw 0.5 mL from the syringe containing **2 x 10^9 vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^9 vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 2: To administer 1x10e10 virus particles by subcutaneous injection:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^{10} vp and withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^{10} vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 3 and 4: To administer 1x10e11 virus particles by subcutaneous injection:

1. Withdraw 0.5mL of contents from the previously thawed, supplied ETBX-011 from the vial and administer to each subject without any further manipulation.

Cohort 5: To administer 5x10e11 virus particles by subcutaneous injection:

1. Withdraw 0.5mL of contents from each of 5 previously thawed ETBX-011 vials (total volume 2.5ml) and administer to each subject without any further manipulation.

6.2.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joe Balint, at Etubics Corporation for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations

Weeks 0, 3, 6 (Immunization Visits):

General evaluations include medical history, Karnofsky performance status , and complete physical examination with weight by the attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

Week 9:

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight by attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Immunization injection sites will be assessed.

7.1.2 Hematological and Biochemical Assessment

Weeks 0, 3, 6 (Immunization Visits):

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

Week 9:
Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, glucose and ANA.

7.1.3 Biological Markers

Weeks 0 and 9:
Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained and send to Etubics Corporation for determination. Biomarkers (e.g., CEA or CA15-3) will be reviewed if determined and available.

7.1.4 Immunological Assessment

Weeks 0, 3, 6, and 9:
Peripheral blood (90mL) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Serum from each blood draw will be archived and sent to Etubics Corporation for Ad5 neutralizing level determination.

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up every 3 months for 1 year. At each visit, a medical history and physical exam and the following lab tests will be performed: blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

At each visit, 40-90 mL of peripheral blood should be drawn for immune analysis, if there was previous evidence of an immune response or at the discretion of the investigator. Available serum markers (e.g., CEA, CA15-3) will be reviewed.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) DLT is defined in Protocol Section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 DLT related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations:

In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This decision will be made by the medical monitor and the Principal Investigator. A note will be generated following the assessment decision and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience DLT (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). DLT is defined in Protocol Section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response

Immune responses against CEA will be evaluated from the peripheral blood of patients from among the following studies at the discretion of the Principal Investigator (ELISpot, cytokine flow cytometry, and antibody responses). We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- DLT as defined in Protocol Section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of withdrawal due to toxicity, a patient will be requested to have safety evaluations performed as per the protocol for a one year duration post treatment. This may include having up to 90 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator, or in his absence, Dr. H. Kim Lyerly, Acting Medical Director, immediately by telephone (919) 684-8111 (paging operator). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the Institutional Review Board (IRB) and the Etubics Corporation by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and Etubics Corporation will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the site's IRB. IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients signing consent. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A CRF must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. CRFs must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on CRFs by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto CRFs, originals of laboratory and other test results must be kept on file with patient's CRFs or clinical chart. CRFs and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Event Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

A Etubics Corporation representative will conduct a site initiation visit and will review protocol documents with the investigators and their staff. During the study, the Etubics monitor will visit the site regularly to check the completeness of study files, the accuracy of data entries on the CRFs, and adherence to the protocol and to Good Clinical Practice (GCP). Key study personnel must be available to assist the monitor during these visits. The investigator must maintain source documents for each subject in the study. The investigator must also keep the original informed consent form signed by every patient. The investigator must give the monitor

access to all relevant source documents to verify data entries on CRFs. No information in source documents about the identity of subjects will be disclosed.

This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of adverse events (AEs). The principal investigator will continuously monitor the data and safety of all subjects enrolled. All grades of toxicities will be recorded.

Safety assessments will consist of monitoring all AEs including serious adverse events (SAEs), the regular monitoring of hematology, serum chemistry, and routine monitoring of vital signs and physical condition. AE monitoring should occur from the time of informed consent to 30 days after the last dose of study drug. For those subjects who discontinue study participation prior to receiving study drug, AE and SAEs will be collected through the time of discontinuation.

Toxicity will be assessed using the NCI CTCAE version 4.0. Refer to Protocol Section 7.3.2.

An AE is any adverse change from the study patient's baseline (pretreatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started.

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

10.6. CTCAE Term (AE description) and Grade

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for AE reporting. A copy can be downloaded from the CTEP web site (http://ctep.cancer.gov).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Assessing Causality

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.8. Serious Adverse Event Reporting

All AEs that are classified as serious, unexpected and related or possibly related, will be recorded on the MedWatch 3500A form and reported within 24 hours of learning of the event to Etubics Corporation. The form should be completed as much as possible but should not be held until all information is available. Additional information and/or corrections may be submitted as they are obtained. The investigator will follow SAEs until resolution, a return to baseline condition or stabilization or 30 days after the last subject is enrolled whichever occurs first. SAEs that are ongoing at the time of clinical database closure will be recorded as unresolved.

All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to Etubics Corporation, additional information may be requested from the treating institution. Deaths believed to be related to disease progression will not be reported to the FDA as SAEs, as these deaths are an anticipated outcome of the disease; however, all deaths will be reported to the FDA in the annual reports.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

Reporting of Pregnancy:

If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the Etubics Corporation and to the IRB.

Procedures for SAE Reporting:

1. Medical staff/study team notifies principal investigator of SAE. MedWatch 3500A is completed.

2. Principal Investigator calls Etubics Corpration to report the SAE and faxes the completed MedWatch 3500A to:

   Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
   Telephone: 206-838-5110 ext. 102
   Cell: (206) 818-2985
   Fax: (206) 838-2978
   Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

   A. Chief Scientific Officer: Frank Jones, PhD
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 101

Cell: (206) 818-2857
Fax: (206) 838-2978
Email: frj@etubics.com

B. Laboratory Manager: Joseph Balint, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 107
Fax: (206) 838-2978
Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 103
Cell: (970) 402-2598
Fax: (206) 838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA via MedWatch 3500A form.

   a. If the SAE results in death or is life-threatening, SAE report will be submitted to the FDA within 7 days
   b. All other SAEs must be reported to the FDA within 15 days

5. All SAE will require input from a physician, the Acting Medical Director will be consulted:

   H. Kim Lyerly, MD
   Telephone: (919) 684-5613 or (919)684-8111 (paging operator)
   Fax: (919) 684-5653
   Email: lyerl001@mc.duke.edu

6. SAEs will be reported to the IRB according to the site's IRB guidelines.

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the AE may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant IRB of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is the responsibility of Etubics Corporation to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the Etubics Corporation to report these SAEs to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

10.9. Safety Reporting Requirements

In accordance with 21 CFR 312.32, the sponsor of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation.

c. Data and Safety Monitoring

Data will be collected by the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by Etubics Corporation.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte

antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.

14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.

26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.
32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of

melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.

39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based

vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.

53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.

67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3	Week 6	Week 9	Months 6, 9, etc. [i]	Off Treatment
H & P	X	X	X	X	X	X	X
Karnofsky Status	X	X	X	X	X	X	X
β-HCG [a]	X						
CBC with diff	X	X	X	X	X	X	X
PT-INR, PTT	X						
Chemistries	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
Immune Monitoring [b]	X [b]	X	X	X	X	X [b]	X [b]
Antibodies to CEA/Ad5 Vector [c]		X			X		
Biological Markers [d] (i.e. CEA, CA15-3)	X [d]				X [d]	X [d]	X [d]
Brain MRI/CT Scan [e]	X						
MRI/CT Scan [f]	X [f]				X [f]	X [f]	X [f]
Immunization [g]		X	X	X			
AE Assessment [h]	X	X	X	X	X		

Key: H & P = history & physical examination, **KPS** = Karnofsky Performance Score, **β-HCG** = human chorionic gonadotrophin pregnancy test; **CBC & diff** = complete blood count and white blood cell differential, **Chemistries** = Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose; **ANA** = antinuclear antibody, **HIV** = human immunodeficiency virus antibody, **MRI/CT** = magnetic resonance imaging/computed tomography; **AE** = Adverse Event

Notes:

[a] β-HCG performed on women of childbearing potential

[b] Immune monitoring drawn during the first clinic visit at the discretion of the investigator/immune monitoring laboratory, prior to each immunization and approximately 3 weeks after the last immunization, and will be repeated at the discretion of the investigator/immune monitoring laboratory.

[c] Serum obtained for antibodies to CEA and Ad5 vector weeks 0 and 9.

[d] Available serum markers (e.g., CEA or CA15-3) reviewed as available.

[e] Brain CT scan or MRI required within 6 weeks of starting study treatment.

[f] Available CT scans or MRI of the chest, abdomen, and/or pelvis reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, however is not required. Repeat imaging conducted according to standard of care.

[g] Immunizations may be modified so that the interval is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

[h] AE monitoring from the time of informed consent to 30 days after the last dose of study drug.

[i] Follow-up evaluations requested every 3 months for 1 year, while on the study.

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

STUDY PRODUCT:	**Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)**
STUDY SPONSOR:	**Etubics Corporation** 410 West Harrison Street Seattle, WA 98119
PRINCIPAL INVESTIGATOR:	**Michael Morse** **Duke University Medical Center** Box 3233, Durham, NC 27710
Original Protocol:	Version 1.1, December 21, 2009
Amendment 1:	Version 2.0, April 13, 2010
Amendment 2:	Version 2.1, May 14, 2010
Amendment 3:	Version 2.1, August 31, 2010
Amendment 4:	Version 3.1, June 20, 2011
Amendment 5:	Version 4.2, September 21, 2011

TABLE OF CONTENT

1. PROTOCOL SUMMARY

<table>
<tr><td>Title</td><td colspan="2">A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA</td></tr>
<tr><td>Objectives</td><td colspan="2">a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.</td></tr>
<tr><td>Major Inclusion/ Exclusion Criteria</td><td colspan="2">Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (i.e. colon and rectal cancer). If colorectal cancer, pathologic or clinical confirmation of adenocarcinoma is required. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving chronic steroid or immunosuppressive therapy. All patients must be ≥21 years old and have a Karnofsky Performance Score of 70% or higher. Pregnant women and nursing mothers are excluded.</td></tr>
<tr><td>Study Design</td><td colspan="2">Phase I/II study with four dosage levels of the study drug Ad5 [E1-, E2B-]-CEA(6D) vaccine given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations.</td></tr>
<tr><td></td><td colspan="2">Phase I Component</td></tr>
<tr><td></td><td>Cohort</td><td>Dosage</td></tr>
<tr><td></td><td>1</td><td>1 x 10^9 virus particles (vp) in 0.5 mL</td></tr>
<tr><td></td><td>2</td><td>1 x 10^{10} virus particles (vp) in 0.5 mL</td></tr>
<tr><td></td><td>3</td><td>1 x 10^{11} virus particles (vp) in 0.5 mL</td></tr>
<tr><td></td><td colspan="2">Phase II Component:</td></tr>
<tr><td></td><td>Cohort</td><td>Dosage</td></tr>
<tr><td></td><td>4 (MTD from Phase I)</td><td>1 x 10^{11} virus particles (vp) in 0.5 mL</td></tr>
<tr><td></td><td>5</td><td>5x10^{11} virus particles (vp) in 2.5 mL</td></tr>
<tr><td></td><td colspan="2">The following procedures will occur:
1) Peripheral blood draw (100 mL) for immune analysis.
2) Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 virus particles (vp) subcutaneously in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort</td></tr>
</table>

	have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity, then patients may begin enrolling into cohort 2. If there is 1 DLT, then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at the lowest dosage level, dosing will be de-escalated to $1x10^8$ virus particles (vp). 3) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{10} virus particles (vp) subcutaneously in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 4) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} virus particles (vp) subcutaneously in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 5) Phase II Component - Cohort 4: An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations. MTD determined as Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1×10^{11} virus particles (vp)subcutaneously in 0.5 mL every 3 weeks for 3 immunizations. 6) Cohort 5: Upon completion of Cohort 4 and in the absence of DLT, enrollment in cohort 5 will be permitted. Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of $5x10^{11}$ virus particles (vp) subcutaneously in 2.5 mL in the thigh every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving

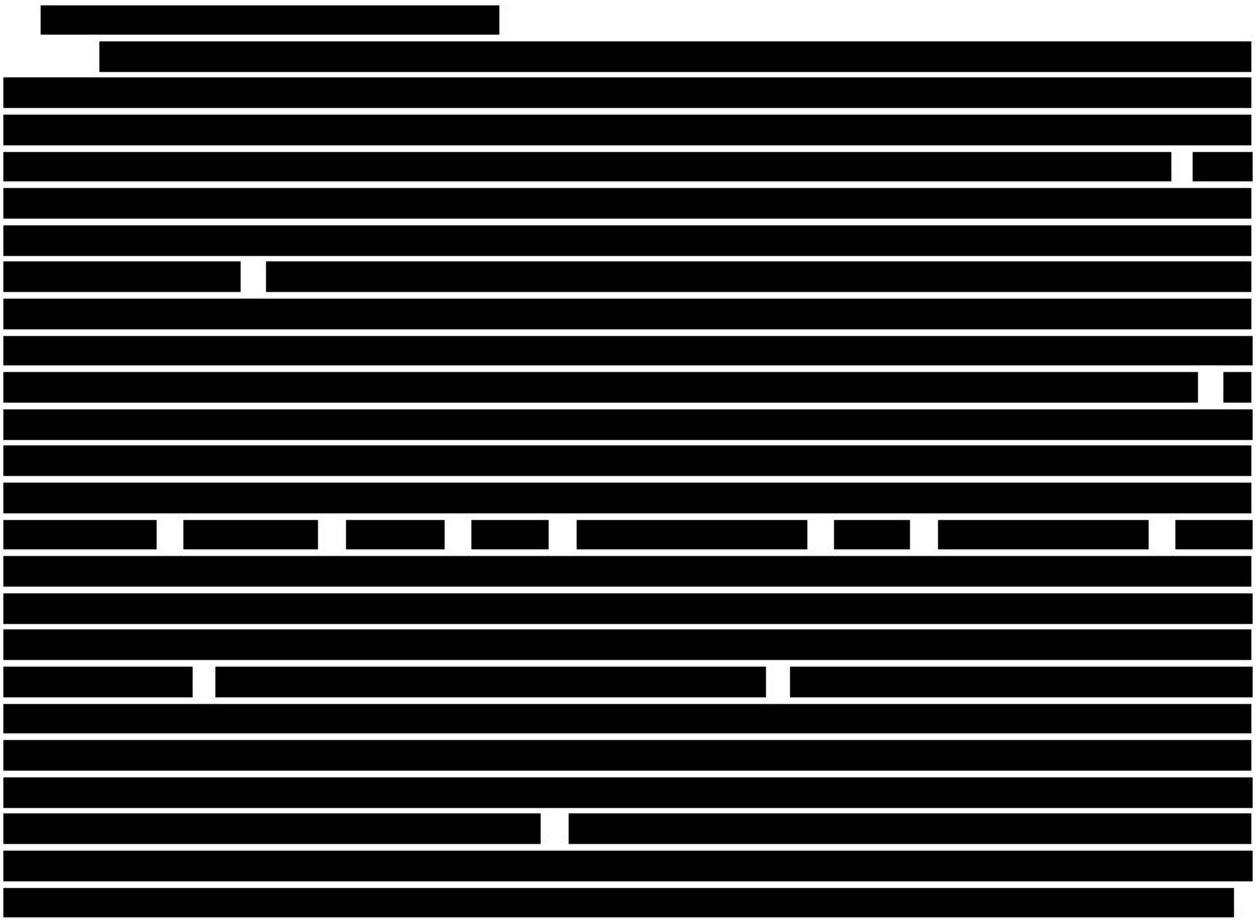
	their first dose of vaccine. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then 1 x 10^{11} virus particles (vp) will be declared the MTD. 7) Patients will have 100 mL peripheral blood drawn prior to each immunization and at week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 8) Time to progression will be measured using MRI/CT scans performed as per standard of care (approximately 3 month intervals).
Risks/Toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 30 evaluable patients (plus up to 12 replacements); may require 40-45 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total.
Criteria for Evaluation	Toxicity will be assessed using CTCAE version 4.0. CEA-specific immune response will be measured in the peripheral blood. Time to recurrence will be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 9. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

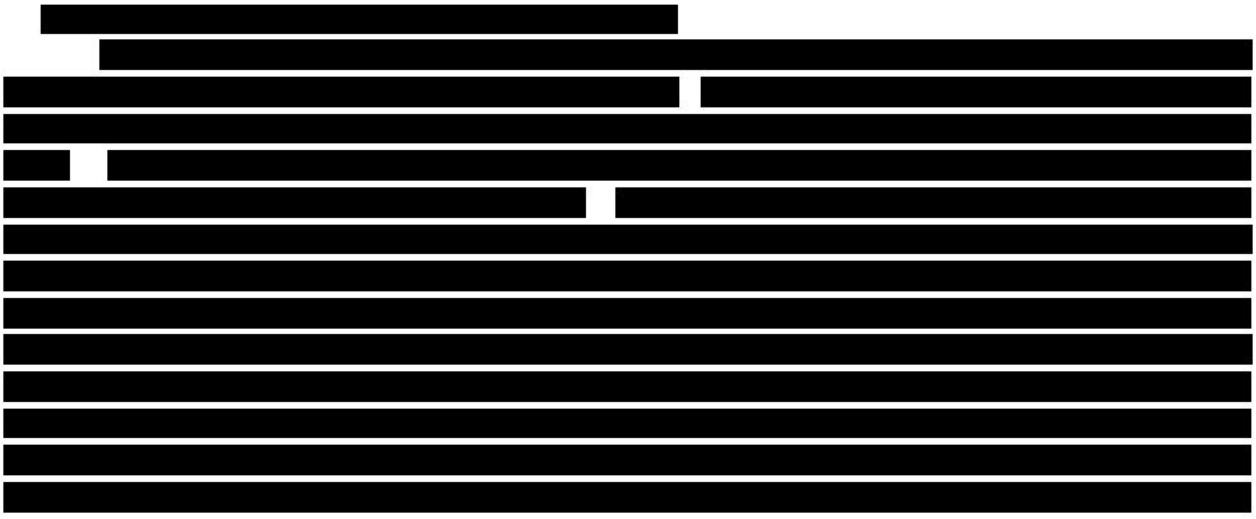
2. STUDY OBJECTIVES

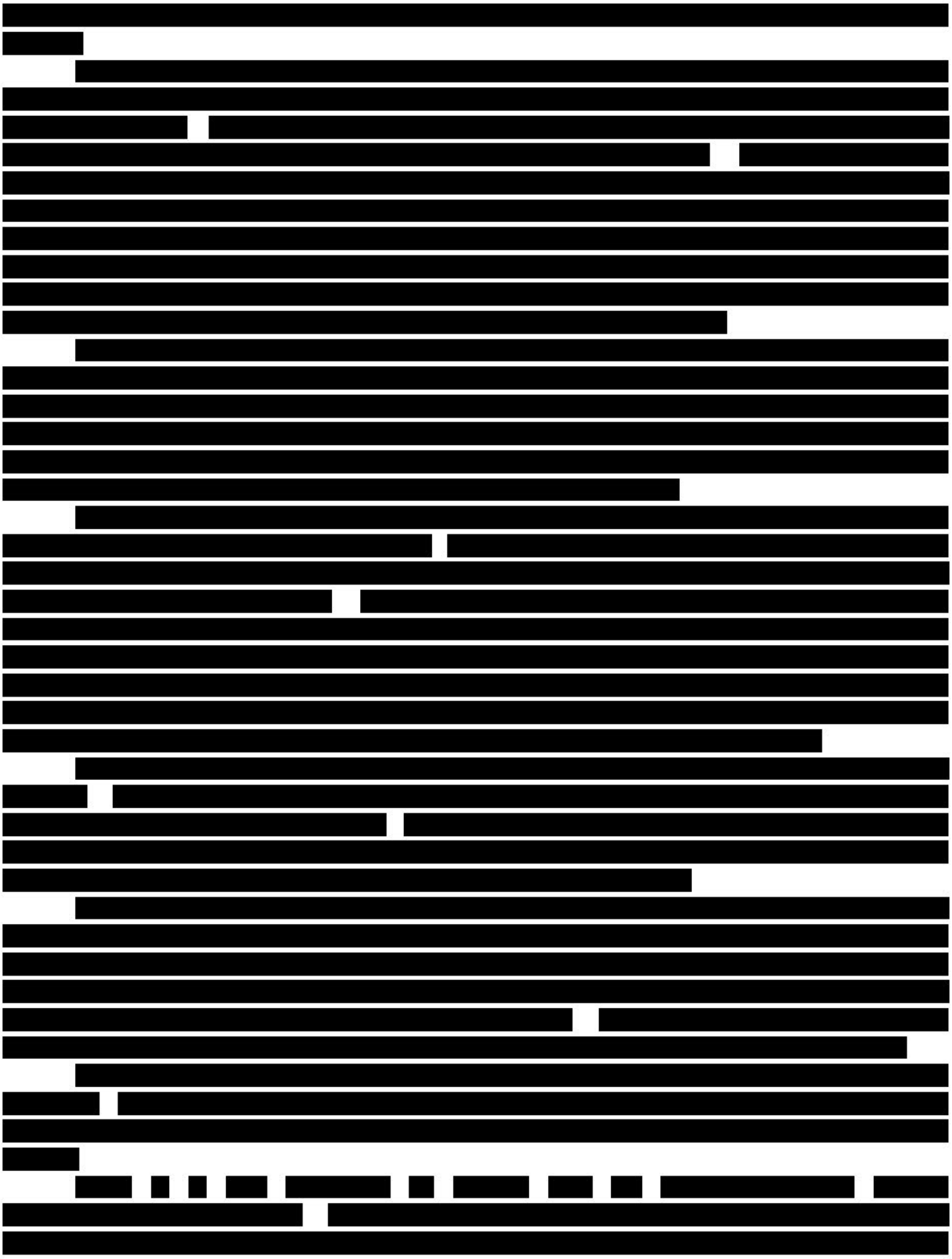
a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2B-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.

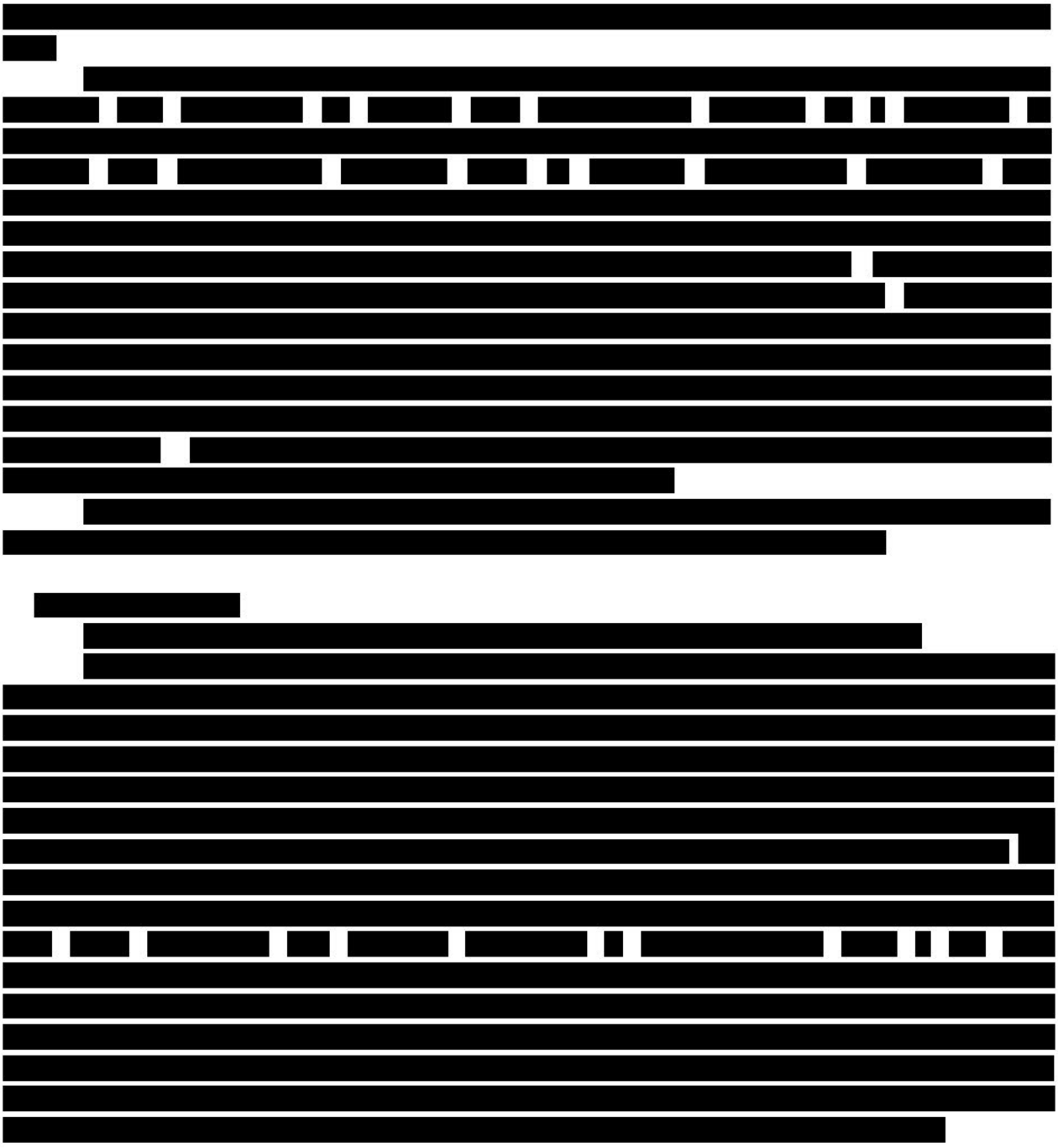
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

3. BACKGROUND AND SIGNIFICANCE





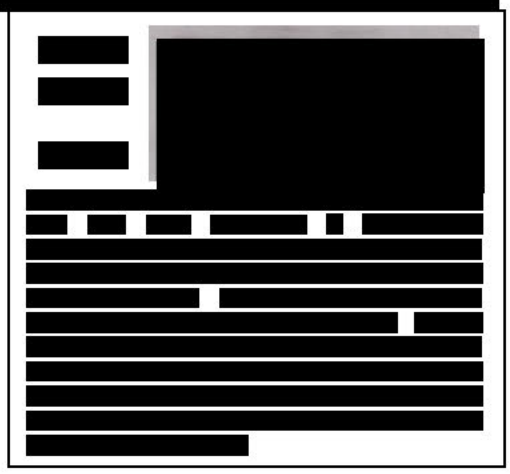




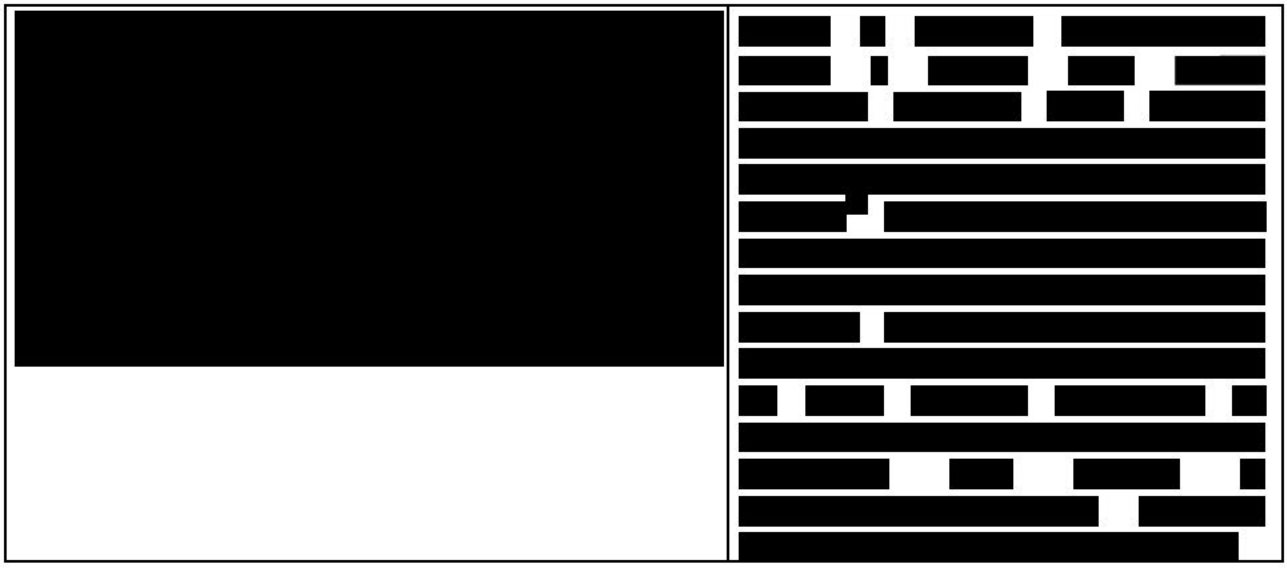
[redacted]

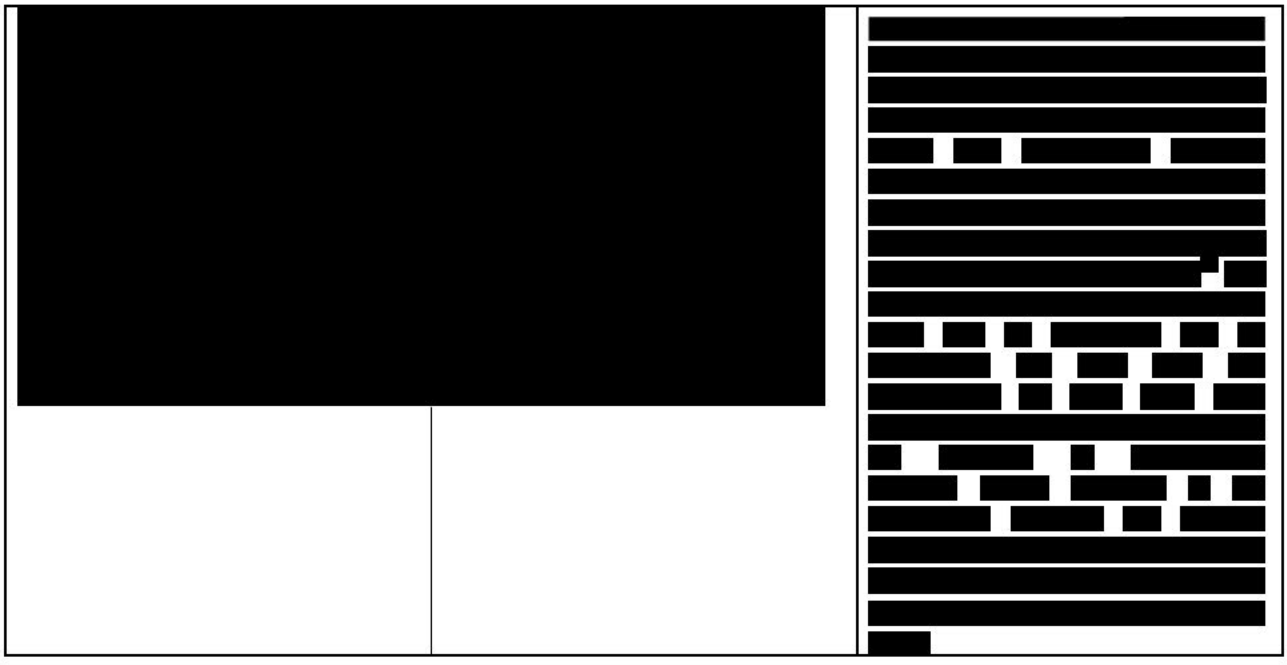
[redacted]

[redacted]



[redacted]





[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of malignancy expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. For all tumor types other than colorectal, the tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (i.e. colon and rectal cancer). If colorectal cancer then, pathologic or clinical confirmation of adenocarcinoma is required.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may

continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).

- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
  - Patients who have been treated or offered the options of treatment with Bevacizumab (option clearly stated in the consent form).
  - Patients who have been treated or offered the options of treatment with Lapatinib (option clearly stated in the consent form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.
  - Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single agent therapy for at least 3 months).
- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. Karnofsky performance score of 70% or higher

4.1.5. Estimated life expectancy > 3 months

4.1.6. Age $\geq$ 21 years, but $\leq$ 75

4.1.7. Adequate hematologic function, with WBC $\geq$ 3000/microliter, hemoglobin $\geq$ 9 g/dL (it is acceptable to have had prior transfusion), platelets $\geq$ 75,000/microliter; PT-INR <1.5 (unless patient is receiving warfarin in which case PT-INR must be <3), PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosupression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to the clinical site for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered to grade 1 acute toxicities from prior treatment.

4.2.2. Patients with a history of or current brain metastases will not be permitted.

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections.

4.2.8. Patients on chronic steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-

enhanced studies or for acute treatment (<5 days) of intercurrent medical condition such as a gout flare) prior to enrollment.

4.2.9. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.10. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.11. Patients with metastatic disease which is determined to be resectable must have been referred to a surgeon for consideration of resection.

4.3. Accrual

We expect to accrue a minimum of 30 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 40-45 patients if DLT occur that necessitate re-dosing at a lower dosage levels (Refer to Protocol Section 6 for a description of the dose escalation criteria.)

4.4 Assignment of study number

Patients will be assigned study numbers in order of their screening using a site specific sequential numbering system, e.g.,: ETBX-011- 001, 002, 003 etc. including screened patients.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment: (Refer to Appendix 1 - Schema)

- History and physical exam, to include Karnofsky Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological and Biochemical Tests:
  - CBC with differential
  - PT, INR and PTT
  - Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose
- Urinalysis
- HIV Antibody
- Anti Nuclear Antibody (ANA)
- Biological Markers:

  - Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad5 vector and sent to Etubics Corporation for determination.
  - Other available serum markers (e.g., CEA or CA15-3) will be reviewed if determined and available.
- Immunologic Evaluation/Archive Sample:
  - Blood may be drawn during the first clinic visit (after consent is signed) for immunologic evaluation and/or archiving.
- Imaging Studies:
  - Brain CT scan or MRI is required within 6 weeks of starting study treatment.
  - Available CT scans or MRI of the chest, abdomen, and/or pelvis will be reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, but is not required.

6. TREATMENT PLAN

Patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (Based on NCI CTCAE version 4.0) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. The first patient will be called to check on their condition prior to enrolling the second patient since patients can be enrolled after 1 week of initiation of cohort 3. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and the phase II component, cohort 4 without the 1-week waiting period. Between dosage levels, through cohort 3, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3. Upon completion of cohort 4 and in the absence of DLT, enrollment in cohort 5 will be permitted.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9 virus particles (vp) in 0.5 mL subcutaneously in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to $1x10^8$ virus particles (vp) and a new cohort of 3 patients instituted.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{10} virus particles (vp) in 0.5 mL subcutaneously in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to the next cohort.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^{11} virus particles (vp) in 0.5 mL subcutaneouslyin the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for proceeding to phase II enrollment (Cohort 4) will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then subjects may enroll in Cohort 4. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to the next level.

4) Phase II - Cohort 4: After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

Note: If during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Cohort 5: Upon completion of cohort 4 and in the absence of DLT, six patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of $5x10^{11}$ virus particles (vp) subcutaneously in 2.5 mL in the thigh every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then 1×10^{11} virus particles (vp) will be declared the MTD.

6) Patients will have 100 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

7) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

8) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

9) The following safety events will trigger a temporary suspension of study vaccinations:

   a) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine
   b) Death not attributed to disease.

   Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

   Etubics Corporation will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above. Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two patients having a Grade 4 toxicity event that is possibly/probably related to the study agent.

Figure 3A.

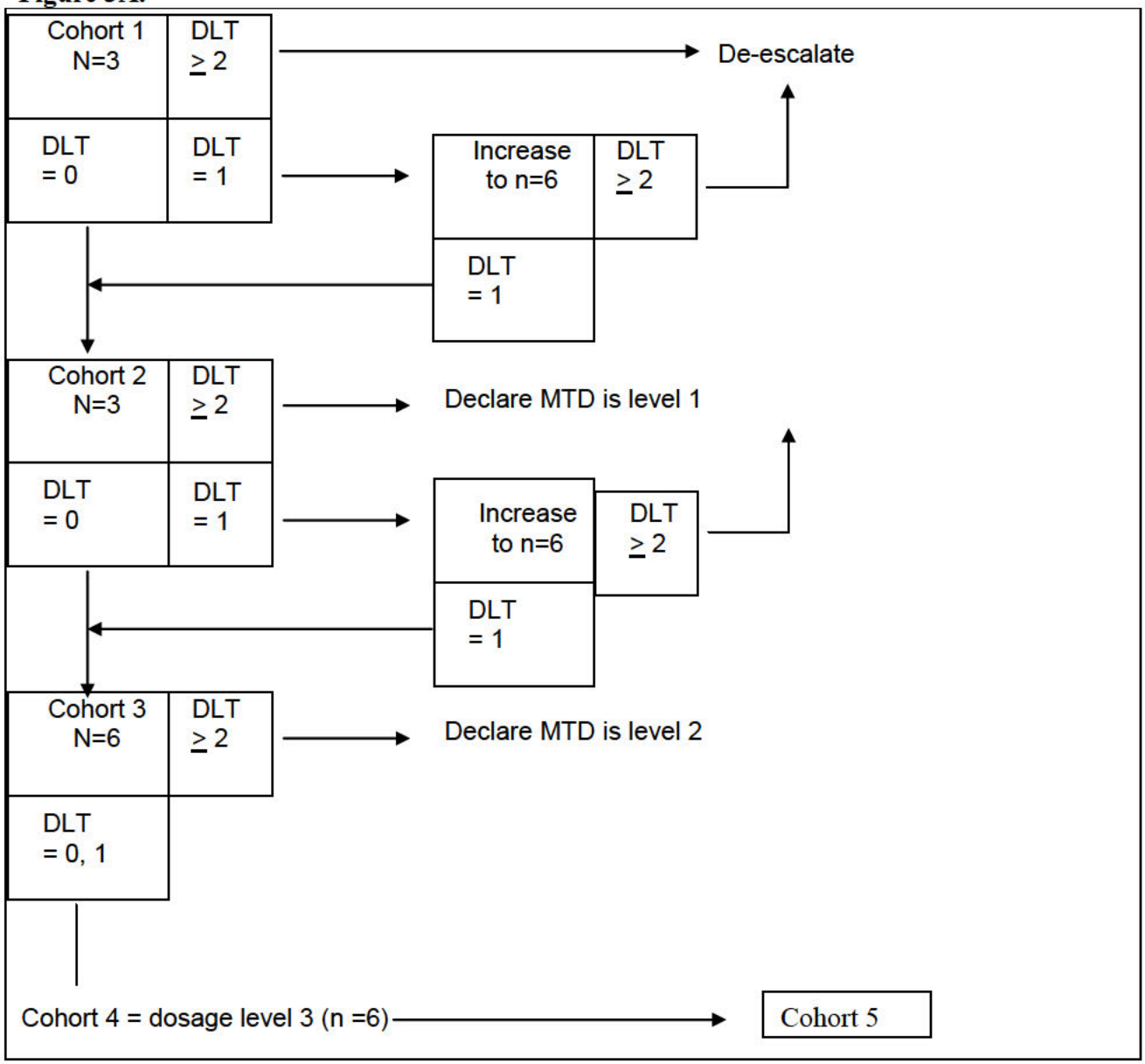


Figure 3B.

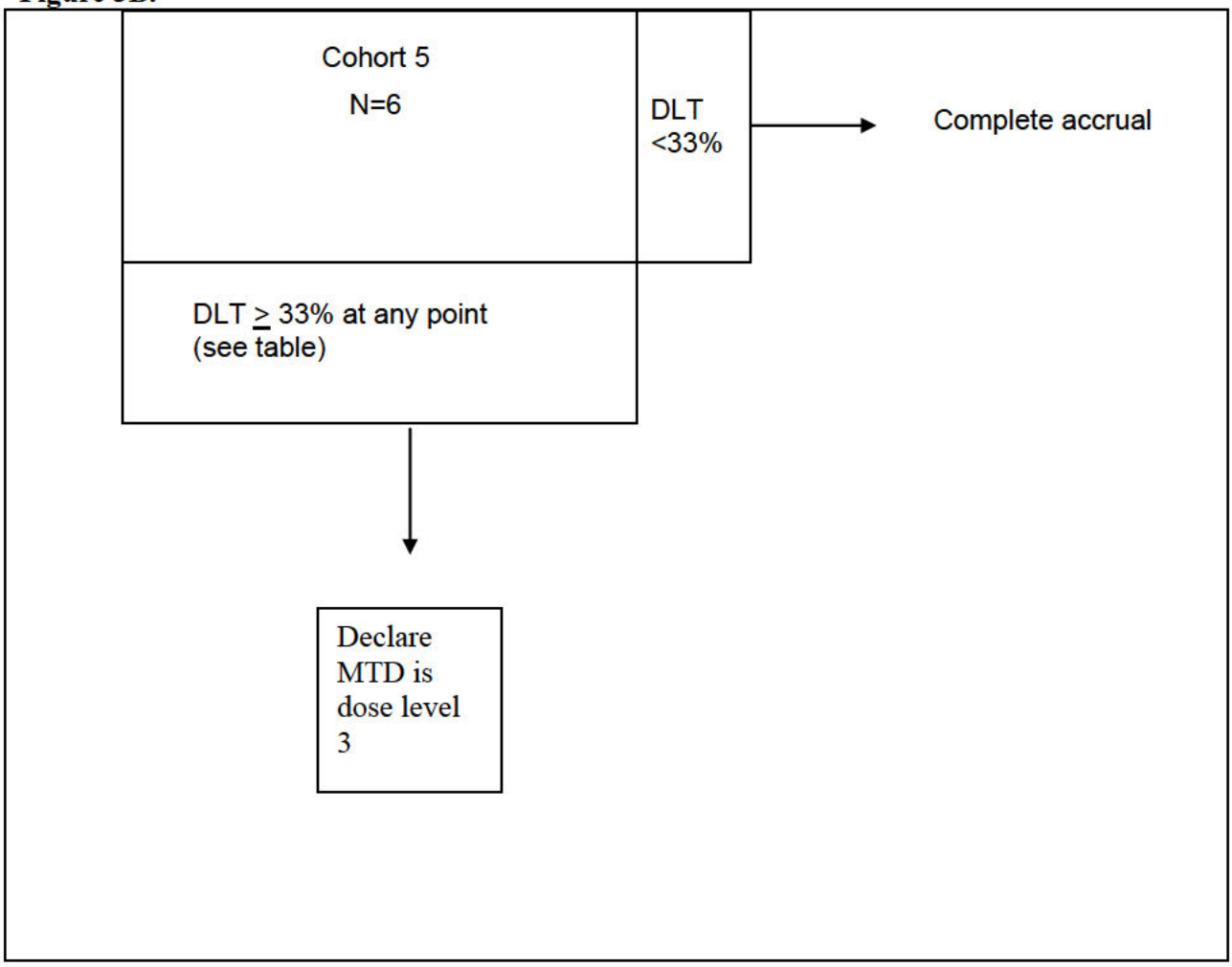


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18
# with DLT to be ≥33%	1	2	3	4	5	6

6.2. Pharmaceutical Information

6.2.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 1 x 10^9, 1 x 10^{10}, or 1 x 10^{11} virus particles (vp) per immunization in 0.5 mL of sterile saline subcutaneously every 3 weeks for 3 immunizations (cohorts 1, 2, 3, and 4). The 5th cohort will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 5 x 10^{11} virus particles (vp) per immunization in 2.5 mL of sterile saline subcutaneously in the thigh every 3 weeks for 3 immunizations.

6.2.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains 1x10^{11} total virus particles (vp). The volume of injection for 1x10^{11} virus particles (vp) is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. The product should be stored at </= –20°C until used.

Instructions for dose preparation:

Cohort 1: To administer 1x10e9 virus particles (vp) by subcutaneous injection, perform 2 serial dilutions of vialed vaccine as follows:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Use a new sterile syringe with a needle and repeat above procedure.
6. Withdraw 4.5 mL of sterile saline.
7. In the second syringe withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/ml**.
8. Remove the needle from the syringe containing the 4.5 mL saline, and inject the 0.5 mL of 2 x 10^{10}vp/mL from the second syringe.
9. Place a new needle on the 10-mL syringe from Step 8 above and mix the two solutions. This solution now has a concentration **2 x 10^9 vp/mL** (1 x 10^9 vp per 0.5 mL).
10. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^9 vp and withdraw 0.5 mL from the syringe containing **2 x 10^9 vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^9 vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 2: To administer 1x10e10 virus particles (vp) by subcutaneous injection:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2 x 10^{10} vp/ml.**
5. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^{10} vp and withdraw 0.5 mL from the syringe containing **2 x 10^{10} vp/mL**. This prepared vaccine (**ETBX-011, 1 x 10^{10} vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 3 and 4: To administer 1x10e11 virus particles (vp) by subcutaneous injection:

1. Withdraw 0.5mL of contents from the previously thawed, supplied ETBX-011 from the vial and administer to each subject without any further manipulation.

Cohort 5: To administer 5x10e11 virus particles (vp) by subcutaneous injection:

1. Withdraw 0.5mL of contents from each of 5 previously thawed ETBX-011 vials (total volume 2.5ml) and administer to each subject without any further manipulation.

6.2.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joe Balint, at Etubics Corporation for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations

Weeks 0, 3, 6 (Immunization Visits):

General evaluations include medical history, Karnofsky performance status , and complete physical examination with weight by the attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

Week 9:

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight by attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Immunization injection sites will be assessed.

7.1.2 Hematological and Biochemical Assessment

Weeks 0, 3, 6 (Immunization Visits):

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

Week 9:
Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, glucose and ANA.

7.1.3 Biological Markers
Weeks 0 and 9:
Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained and send to Etubics Corporation for determination. Biomarkers (e.g., CEA or CA15-3) will be reviewed if determined and available.

7.1.4 Immunological Assessment
Weeks 0, 3, 6, and 9:
Peripheral blood (90mL) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Serum from each blood draw will be archived and sent to Etubics Corporation for Ad5 neutralizing level determination.

7.2. Long-Term Follow-Up
Patients will be requested to continue long-term follow-up every 3 months for 1 year. At each visit, a medical history and physical exam and the following lab tests will be performed: blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

At each visit, 40-100 mL of peripheral blood should be drawn for immune analysis, if there was previous evidence of an immune response or at the discretion of the investigator. Available serum markers (e.g., CEA, CA15-3) will be reviewed.

7.3. Management of Intercurrent Events
7.3.1 Concomitant Medications
Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) DLT is defined in Protocol Section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 DLT related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations:

In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort.

8. STATISTICAL CONSIDERATIONS

8.1. Safety

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This decision will be made by the medical monitor and the Principal Investigator. A note will be generated following the assessment decision and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience DLT (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). DLT is defined in Protocol Section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II (cohort 4) and in cohort 5. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all cohorts.

8.2. Rate of Immune Response

Immune responses against CEA will be evaluated from the peripheral blood of patients from among the following studies at the discretion of the Principal Investigator (ELISpot, cytokine flow cytometry, and antibody responses). We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 12. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- DLT as defined in Protocol Section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of withdrawal due to toxicity, a patient will be requested to have safety evaluations performed as per the protocol for a one year duration post treatment. This may include having up to 100 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator, or in his absence, Dr. H. Kim Lyerly, Acting Medical Director, immediately by telephone (919) 684-8111 (paging operator). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the Institutional Review Board (IRB) and the Etubics Corporation by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and Etubics Corporation will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the site's IRB. IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients signing consent. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A CRF must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. CRFs must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on CRFs by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto CRFs, originals of laboratory and other test results must be kept on file with patient's CRFs or clinical chart. CRFs and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Event Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

A Etubics Corporation representative will conduct a site initiation visit and will review protocol documents with the investigators and their staff. During the study, the Etubics monitor will visit the site regularly to check the completeness of study files, the accuracy of data entries on the CRFs, and adherence to the protocol and to Good Clinical Practice (GCP). Key study personnel must be available to assist the monitor during these visits. The investigator must maintain source documents for each subject in the study. The investigator must also keep the original informed consent form signed by every patient. The investigator must give the monitor

access to all relevant source documents to verify data entries on CRFs. No information in source documents about the identity of subjects will be disclosed.

This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of adverse events (AEs). The principal investigator will continuously monitor the data and safety of all subjects enrolled. All grades of toxicities will be recorded.

Safety assessments will consist of monitoring all AEs including serious adverse events (SAEs), the regular monitoring of hematology, serum chemistry, and routine monitoring of vital signs and physical condition. AE monitoring should occur from the time of informed consent to 30 days after the last dose of study drug. For those subjects who discontinue study participation prior to receiving study drug, AE and SAEs will be collected through the time of discontinuation.

Toxicity will be assessed using the NCI CTCAE version 4.0. Refer to Protocol Section 7.3.2.

An AE is any adverse change from the study patient's baseline (pretreatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started.

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

10.6. CTCAE Term (AE description) and Grade

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for AE reporting. A copy can be downloaded from the CTEP web site (http://ctep.cancer.gov).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Assessing Causality

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.8. Serious Adverse Event Reporting

All AEs that are classified as serious, unexpected and related or possibly related, will be recorded on the MedWatch 3500A form and reported within 24 hours of learning of the event to Etubics Corporation. The form should be completed as much as possible but should not be held until all information is available. Additional information and/or corrections may be submitted as they are obtained. The investigator will follow SAEs until resolution, a return to baseline condition or stabilization or 30 days after the last subject is enrolled whichever occurs first. SAEs that are ongoing at the time of clinical database closure will be recorded as unresolved.

All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to Etubics Corporation, additional information may be requested from the treating institution. Deaths believed to be related to disease progression will not be reported to the FDA as SAEs, as these deaths are an anticipated outcome of the disease; however, all deaths will be reported to the FDA in the annual reports.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

Reporting of Pregnancy:

If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the Etubics Corporation and to the IRB.

Procedures for SAE Reporting:

1. Medical staff/study team notifies principal investigator of SAE. MedWatch 3500A is completed.

2. Principal Investigator calls Etubics Corpration to report the SAE and faxes the completed MedWatch 3500A to:

   Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 981119
   Telephone: 206-838-5110 ext. 102
   Cell: (206) 818-2985
   Fax: (206) 838-2978
   Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

   A. Chief Scientific Officer: Frank Jones, PhD
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 101

Cell: (206) 818-2857
Fax: (206) 838-2978
Email: frj@etubics.com

B. Laboratory Manager: Joseph Balint, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 107
Fax: (206) 838-2978
Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 103
Cell: (970) 402-2598
Fax: (206) 838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA via MedWatch 3500A form.

   a. If the SAE results in death or is life-threatening, SAE report will be submitted to the FDA within 7 days
   b. All other SAEs must be reported to the FDA within 15 days

5. All SAE will require input from a physician, the Acting Medical Director will be consulted:

   H. Kim Lyerly, MD
   Telephone: (919) 684-5613 or (919)684-8111 (paging operator)
   Fax: (919) 684-5653
   Email: lyerl001@mc.duke.edu

6. SAEs will be reported to the IRB according to the site's IRB guidelines.

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the AE may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant IRB of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is the responsibility of Etubics Corporation to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the Etubics Corporation to report these SAEs to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

10.9. Safety Reporting Requirements

In accordance with 21 CFR 312.32, the sponsor of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation.

c. Data and Safety Monitoring

Data will be collected by the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by Etubics Corporation.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte

antigen class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.

14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.

26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.
32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of

melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.

39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based

vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.

53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.

67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

11. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3	Week 6	Week 9	Months 6, 9, etc. [i]	Off Treatment
H & P	X	X	X	X	X	X	X
Karnofsky Status	X	X	X	X	X	X	X
β-HCG [a]	X						
CBC with diff	X	X	X	X	X	X	X
PT-INR, PTT	X						
Chemistries	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
Immune Monitoring [b]	X [b]	X	X	X	X	X [b]	X [b]
Antibodies to CEA/Ad5 Vector [c]		X			X		
Biological Markers [d] (i.e. CEA, CA15-3)	X [d]				X [d]	X [d]	X [d]
Brain MRI/CT Scan [e]	X						
MRI/CT Scan [f]	X [f]				X [f]	X [f]	X [f]
Immunization [g]		X	X	X			
AE Assessment [h]	X	X	X	X	X		

Key: H & P = history & physical examination, **KPS** = Karnofsky Performance Score, **β-HCG** = human chorionic gonadotrophin pregnancy test; **CBC & diff** = complete blood count and white blood cell differential, **Chemistries** = Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose; **ANA** = antinuclear antibody, **HIV** = human immunodeficiency virus antibody, **MRI/CT** = magnetic resonance imaging/computed tomography; **AE** = Adverse Event

Notes:

[a] β-HCG performed on women of childbearing potential

[b] Immune monitoring drawn during the first clinic visit at the discretion of the investigator/immune monitoring laboratory, prior to each immunization and approximately 3 weeks after the last immunization, and will be repeated at the discretion of the investigator/immune monitoring laboratory.

[c] Serum obtained for antibodies to CEA and Ad5 vector weeks 0 and 9.

[d] Available serum markers (e.g., CEA or CA15-3) reviewed as available.

[e] Brain CT scan or MRI required within 6 weeks of starting study treatment.

[f] Available CT scans or MRI of the chest, abdomen, and/or pelvis reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, however is not required. Repeat imaging conducted according to standard of care.

[g] Immunizations may be modified so that the interval is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

[h] AE monitoring from the time of informed consent to 30 days after the last dose of study drug.

[i] Follow-up evaluations requested every 3 months for 1 year, while on the study.

A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA

STUDY PRODUCT:	**Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011)**
STUDY SPONSOR:	**Etubics Corporation** 410 West Harrison Street, Suite 100 Seattle, WA 98119
PRINCIPAL INVESTIGATOR:	**Gerald Messerschmidt, MD** **Etubics Corporation** 410 West Harrison Street, Suite 100 Seattle, WA 98119

Original Protocol:	Version 1.1, December 21, 2009
Amendment 1:	Version 2.0, 13 April 2010
Amendment 2:	Version 2.1, May 14, 2010
Amendment 3:	Version 2.1, August 31, 2010
Amendment 4:	Version 3.1, June 20, 2011
Amendment 5:	Version 3.2, September 23, 2015

TABLE OF CONTENT

1. PROTOCOL SUMMARY

Title	**A PHASE I/II STUDY OF ACTIVE IMMUNOTHERAPY WITH Ad5 [E1-, E2b-]-CEA(6D) VACCINE (ETBX-011) IN PATIENTS WITH ADVANCED OR METASTATIC MALIGNANCIES EXPRESSING CEA**
Objectives	a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2b-]-CEA(6D), in patients with advanced or metastatic CEA-expressing malignancies. b) The secondary objectives are to evaluate CEA-specific immune responses to the immunizations and to obtain preliminary data on clinical response rate.
Major Inclusion/ Exclusion Criteria	Patients with a histologically confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit or refused such therapy. The tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (*i.e.* colon and rectal cancer). If colorectal cancer, pathologic or clinical confirmation of adenocarcinoma is required. Patients will not be treated until 4 or more weeks after any prior chemotherapy or radiation therapy, but may be receiving non-cytotoxic targeted therapy (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, or gefitinib) or hormonal therapy. They must not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV, or be receiving chronic steroid or immunosuppressive therapy. All patients must be ≥21 years old and have a Karnofsky Performance Score of 70% or higher. Pregnant women and nursing mothers are excluded.
Study Design	Phase I/II study with three dosage levels of Ad5 [E1-, E2b-]-CEA(6D) vaccine (phase I component), and the Maximally Tolerated Dose (MTD) of Ad5 [E1-, E2b-]-CEA(6D) vaccine (phase II component). The study drug is Ad5 [E1-, E2b-]-CEA(6D) given by subcutaneous (SQ) injection every 3 weeks for 3 immunizations. The following procedures will occur: 1) Cohort 1: Three patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1 x 10^9 particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of dose-limiting toxicities (DLT) for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity, then patients may begin enrolling into cohort 2. If there is 1 DLT, then an

	additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT (i.e., 1 DLT in the 6 total patients), then patients may begin enrolling into cohort 2. If 2 patients have DLT at the lowest dosage level, dosing will be de-escalated to $1x10^8$ particles. 2) Cohort 2: Three patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1×10^{10} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. 3) Cohort 3: Six patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1×10^{11} particles SQ in 0.5 mL every 3 weeks for 3 immunizations. Assessment of DLT for proceeding to phase II enrollment will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then patients may begin enrolling into the phase II portion of the study. 4) Phase II Cohort (Cohort 4): An additional 12 patients will receive Ad5 [E1-, E2B-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations. 5) Cohort 5: Beginning after the third dose of ETBX-011 for the 12^{th} patient in cohort 4, enrollment in an additional higher dose cohort will be permitted. Six patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of $5x10^{11}$ particles SQ in 2.5 mL every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had all 3 injections and been observed for at least 30 days after the last injection. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then 1×10^{11} particles will be declared the MTD. 6) Cohort 6: Up to ten (10) will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of $5x10^{11}$ particles SQ in 1.0 mL every 3 weeks for 3 immunizations. Patients will be permitted to receive booster doses of Ad5 [E1-, E2b-]-CEA(6D) every 3 months for a period of one year following the first 3 treatments. In the event that a qualified patient undergoes reimaging prior to the completion of their initial 3 study immunizations and is found to have disease progression, the patient should continue on the immunization protocol because this could be a "flare reaction" associated with the vaccine which may be an inflammatory response. Patients will be required to have MRI/CT scans performed prior to initiation of treatment, at 3 month intervals +/- 1

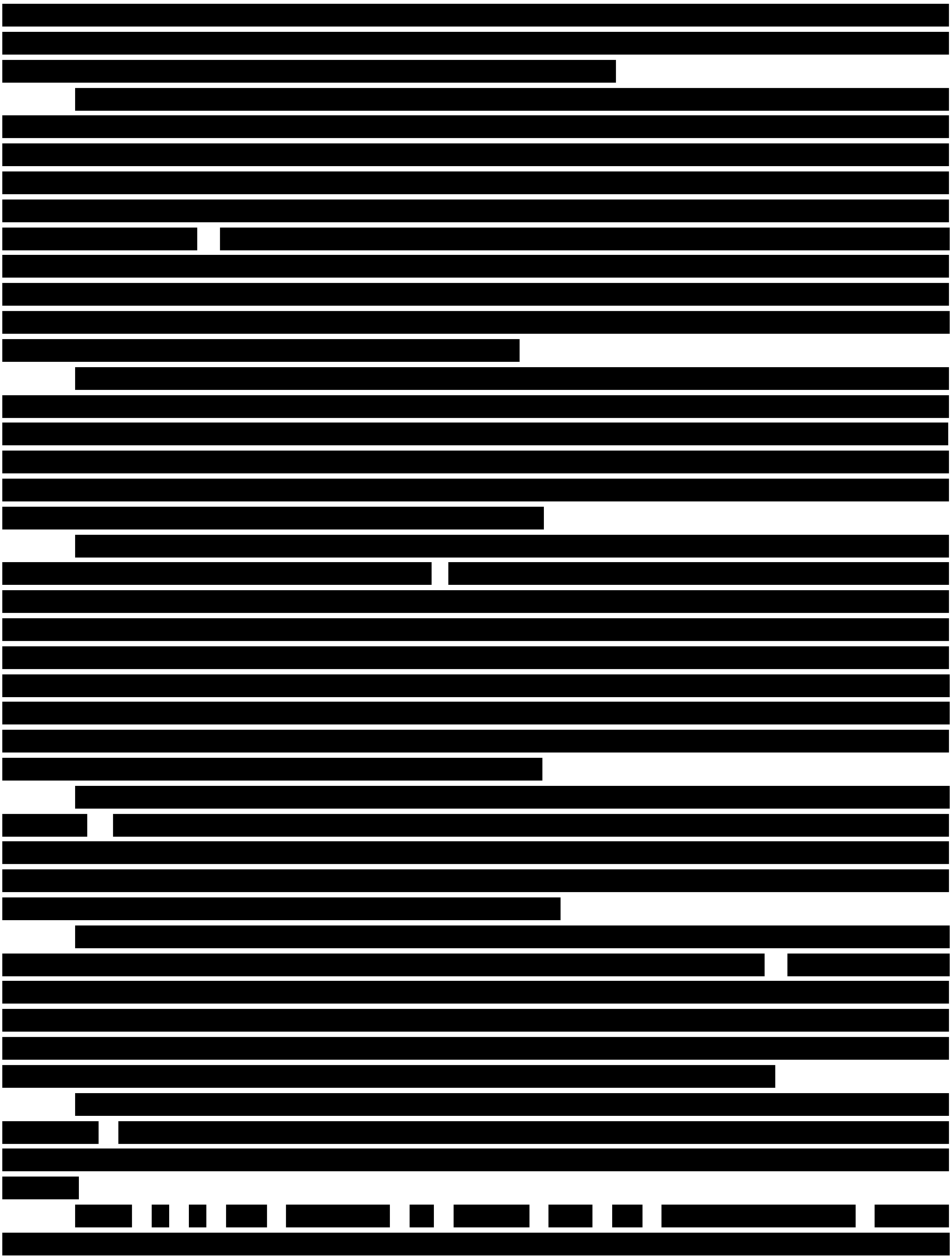
	month for 12 months or if the investigator believes signs or symptoms are suggestive of progression or relapse. All patients will be followed for overall survival for a period of 24 months from initiation of treatment. 7) Patients will have 90 mL peripheral blood drawn prior to each immunization and at week 9 to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. 8) Time to progression will be measured using MRI/CT scans performed as per standard of care (approximately 3 month intervals).
Risks/Toxicities	Potential risks associated with the vaccine include anaphylaxis, fever, skin reaction, autoimmunity (colitis), and hepatic insufficiency.
Number of Patients	Planned: 30 evaluable patients (plus up to 12 replacements) plus an additional 10 patients from cohort 6; may require 40-52 patients if DLT occur.
Duration of Study	6 months after the last patient is enrolled; approximately 2 years total.
Criteria for Evaluation	Toxicity will be assessed using CTCAE version 4.0. CEA-specific immune response will be measured in the peripheral blood. Time to recurrence will be determined by RECIST criteria.
Statistical Analysis	Safety: We will evaluate safety in each cohort at least 3 weeks after the last patient in the previous cohort has received their first injection. A dosing scheme will be considered safe if <33% of patients treated at a dosage level experience DLT (e.g., 0 of 3, ≤1 of 6, ≤3 of 12 or ≤5 of 18 patients). A patient will be considered evaluable for safety if treated with at least one immunization. Rate of immune response: We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 9. Immune response will be assessed at the MTD. An observed immune response in 9 of 18 patients will be considered sufficient evidence of immune response to justify further investigation. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

2. STUDY OBJECTIVES

a) The primary objective of this protocol is to determine the safety of immunization with Ad5 [E1-, E2b-]-CEA(6D) in patients with advanced or metastatic CEA-expressing malignancies.
b) The secondary objectives are to evaluate CEA-specific immune response to the immunizations and obtain preliminary data on response rate.

3. BACKGROUND AND SIGNIFICANCE

[redacted]

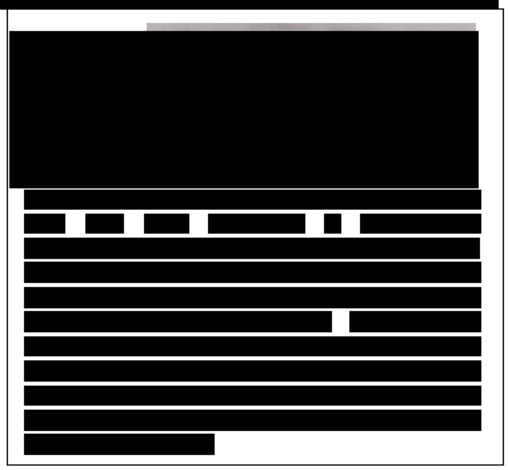


[redacted]

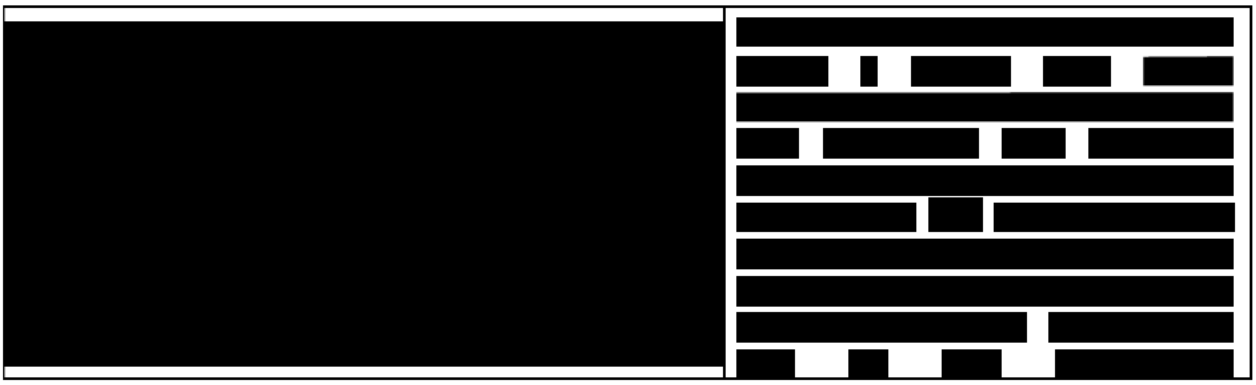
[redacted]

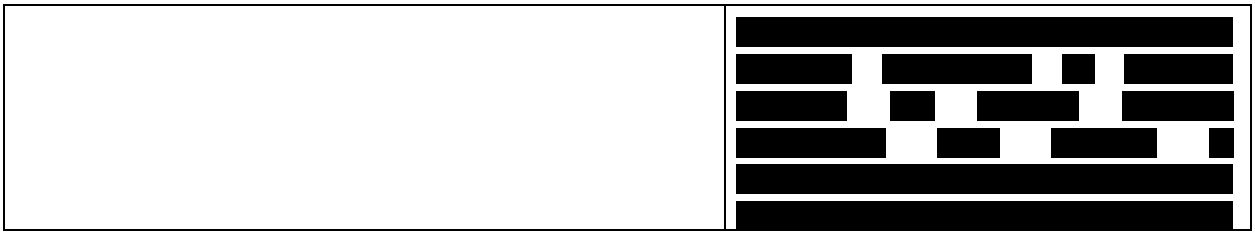
[redacted]

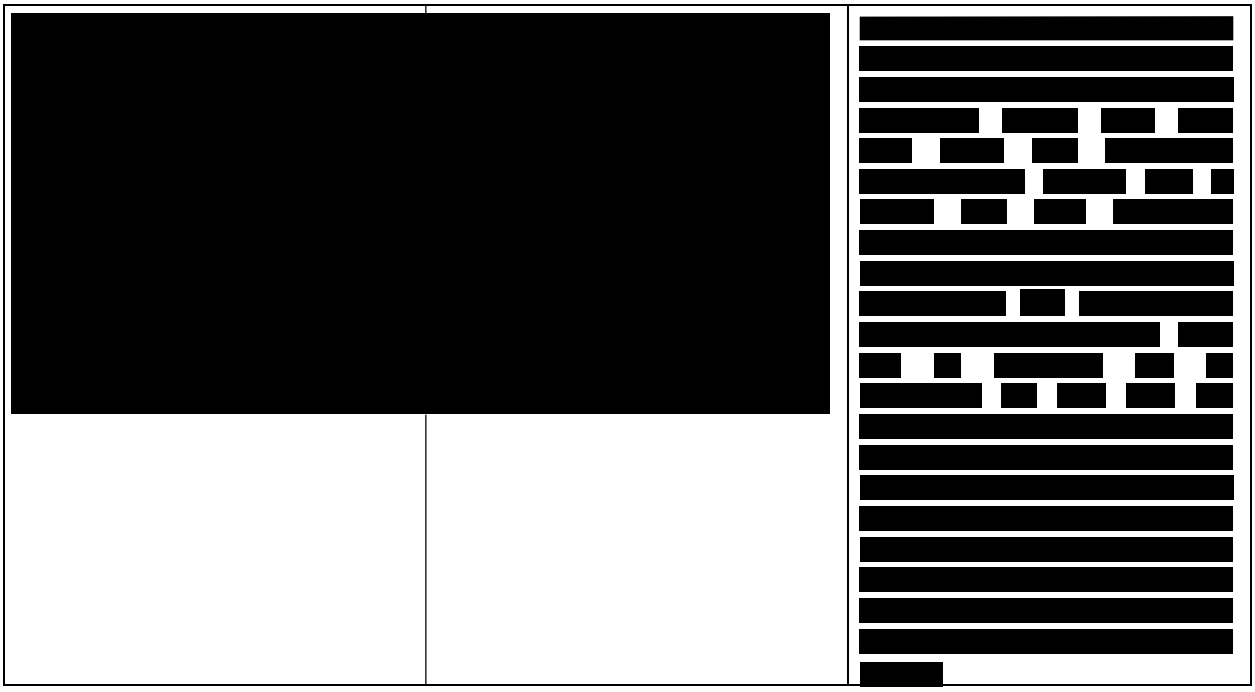
[redacted]

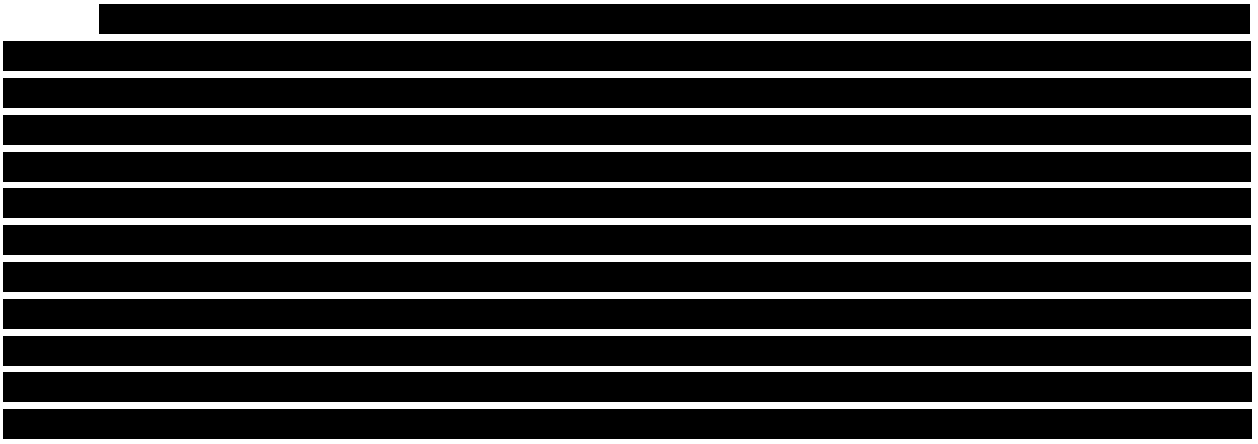


[redacted]

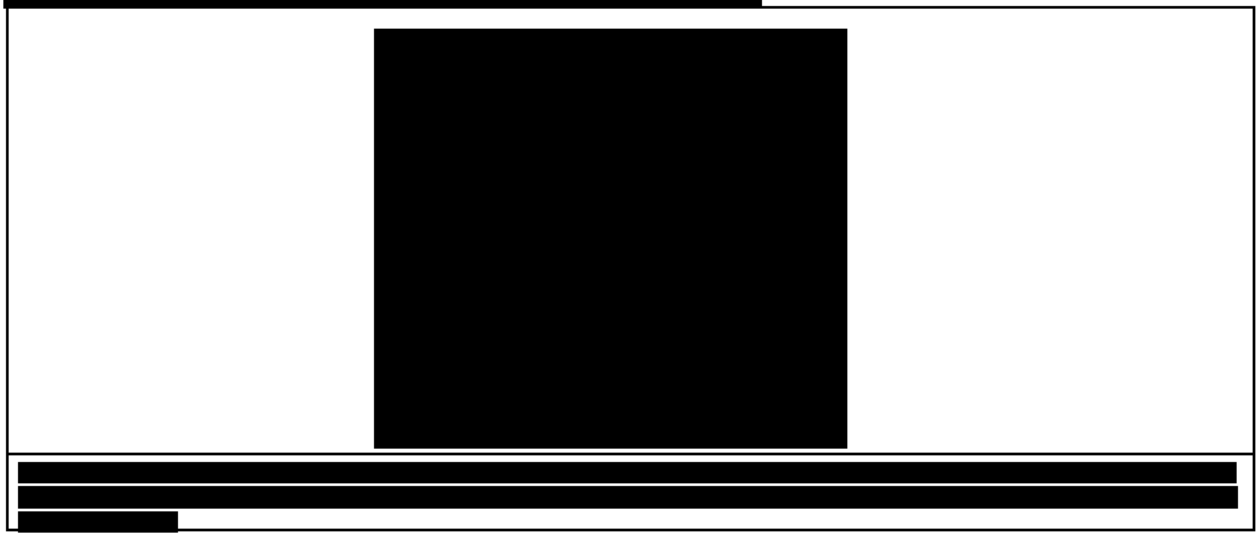








[redacted]



[redacted]

[redacted]

[redacted]

[redacted]

4. PATIENT SELECTION

4.1. Criteria for Patient Eligibility

4.1.1. Histologically confirmed diagnosis of malignancy expressing CEA. Because this is a safety and immunogenicity study, patients are NOT required to have measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST).

4.1.2. For all tumor types other than colorectal, the tumor must express CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a tumor known to be universally CEA positive (i.e. colon and rectal cancer)**.** If colorectal cancer then, pathologic or clinical confirmation of adenocarcinoma is required.

4.1.3. Patients must have received treatment with standard therapy known to have a possible overall survival benefit.

For the following common cancers, the following eligibility criteria apply:

- Colorectal cancer: Must have received and progressed through at least one line of palliative chemotherapy consisting of one of the following regimens:
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and oxaliplatin.
  - Palliative chemotherapy for metastatic colorectal cancer with 5-fluorouracil (or capecitabine) and irinotecan.
  - Palliative chemotherapy regimen for metastatic colorectal cancer that includes bevacizumab.
  - Colorectal cancer patients currently receiving palliative single-agent bevacizumab or cetuximab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Breast cancer: Must have received and progressed through at least one line of chemotherapy for metastatic breast cancer consisting of one of the following regimens:
  - Palliative anthracycline- or taxane-based chemotherapy
  - Patients with tumors that over express HER2 (IHC 3+ or FISH+) must have received and progressed through at least one line of palliative therapy that combines trastuzumab with chemotherapy.
  - Breast cancer patients currently receiving palliative endocrine therapy or single-agent trastuzumab will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).

  - Patients who have been treated or offered the options of treatment with bevacizumab (option clearly stated in the consent form).
  - Patients who have been treated or offered the options of treatment with lapatinib (option clearly stated in the consent form).
- Lung cancer: Must have received and progressed through chemotherapy for metastatic disease consisting of one of the following regimens:
  - Palliative platinum-based (cisplatin or carboplatin) chemotherapy if the patient has not received chemotherapy previously.
  - Palliative taxane-based (docetaxel or paclitaxel) or vinorelbine chemotherapy if the patient has received chemotherapy previously.
  - Lung cancer patients currently receiving palliative single-agent erlotinib or gefitinib will be eligible for this trial and may continue these therapies concomitant with study treatment (if they have been on these single agent therapies for at least 3 months).
- Pancreatic cancer: Must have received and progressed through chemotherapy including gemcitabine.
  - Pancreatic cancer patients currently receiving palliative single-agent erlotinib will be eligible for this trial and may continue this therapy concomitant with study treatment (if they have been on this single agent therapy for at least 3 months).
- For other malignancies, if a first line therapy with survival or palliative benefit exists, it should have been administered and there should have been progressive disease.
- Patients who have received and progressed through first-line palliative chemotherapy must be advised regarding second-line therapy before being enrolled on this investigational study.

4.1.4. Karnofsky performance score of 70% or higher

4.1.5. Estimated life expectancy > 3 months

4.1.6. Age ≥ 21 years, but ≤ 75

4.1.7. Adequate hematologic function, with WBC ≥ 3000/microliter, hemoglobin ≥ 9 g/dL (it is acceptable to have had prior transfusion), platelets ≥ 75,000/microliter; PT-INR <1.5 (unless patient is receiving warfarin in which case PT-INR must be <3), PTT <1.5X ULN

4.1.8. Adequate renal and hepatic function, with serum creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL (except for Gilbert's syndrome which will allow bilirubin ≤ 2.0 mg/dL), ALT and AST ≤ 2.5 x upper limit of normal.

4.1.9. Patients who have received prior CEA-targeted immunotherapy are eligible for this trial, if this treatment was discontinued at least 3 months prior to enrollment.

4.1.10. Patients who are taking medications that do not have a known history of immunosupression are eligible for this trial.

4.1.11. Ability to understand and provide signed informed consent that fulfills Institutional Review Board's guidelines.

4.1.12. Ability to return to the clinical site for adequate follow-up, as required by this protocol.

4.2. Criteria for Patient Exclusion

4.2.1 Patients with concurrent cytotoxic chemotherapy or radiation therapy should be excluded. There are no exclusions based on the number of prior chemotherapy, biologic, hormonal, or experimental regimens. Except for the permitted concomitant therapies (bevacizumab, cetuximab, panitumumab, trastuzumab, lapatinib, erlotinib, gefitinib, or hormonal therapy which patients must have been on for at least 3 months at the time of enrollment if they intend to continue them with the vaccine), there must be at least 3 months between any prior CEA-targeted immunotherapy and study treatment and at least 4 weeks between any other prior therapy (including radiotherapy) and study treatment. Patients must have recovered to grade 1 acute toxicities from prior treatment.

4.2.2. Patients with a history of or current brain metastases will not be permitted.

4.2.3. Patients with a history of autoimmune disease, such as but not restricted to, inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune related thyroid disease and vitiligo are permitted.

4.2.4. Patients with serious intercurrent chronic or acute illness, such as cardiac disease (NYHA class III or IV), hepatic disease, or other illness considered by the Principal Investigator as unwarranted high risk for investigational drug treatment.

4.2.5. Patients with a medical or psychological impediment to probable compliance with the protocol should be excluded.

4.2.6. Concurrent (or within the last 5 years) second malignancy other than non-melanoma skin cancer, cervical carcinoma *in situ*, controlled superficial bladder cancer, or other carcinoma in situ that has been treated.

4.2.7. Presence of an active acute or chronic infection including: a urinary tract infection, HIV (as determined by ELISA and confirmed by Western Blot). Patients with HIV are excluded based on immuno-suppression, which may render them unable to respond to the vaccine; patients with chronic hepatitis are excluded because of concern that hepatitis could be exacerbated by the injections.

4.2.8. Patients on chronic steroid therapy (or other immuno-suppressives, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Patients must have had 6 weeks of discontinuation of any steroid therapy (except that used as pre-medication for chemotherapy or contrast-enhanced studies or for acute treatment (<5 days) of intercurrent medical condition such as a gout flare) prior to enrollment.

4.2.9. Pregnant and nursing women should be excluded from the protocol since this research may have unknown and harmful effects on an unborn child or on young children. If the patient is sexually active, the patient must agree to use a medically acceptable form of birth control while receiving treatment and for a period of 4 months following the last vaccination therapy. It is not known whether the treatment used in this study could affect the sperm and could potentially harm a child that may be fathered while on this study.

4.2.10. Patients with acute or chronic skin disorders that will interfere with injection into the skin of the extremities or subsequent assessment of potential skin reactions will be excluded.

4.2.11. Patients with metastatic disease which is determined to be resectable must have been referred to a surgeon for consideration of resection.

4.3. Accrual

We expect to accrue a minimum of 24 evaluable patients (plus up to 12 replacements for patients removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity). The study may require 30-42 patients if DLT occur that necessitate re-dosing at a lower dosage levels (Refer to Protocol Section 6 for a description of the dose escalation criteria).

4.4 Assignment of study number

Patients will be assigned study numbers in order of their screening using a site specific sequential numbering system, e.g.,: ETBX-011- 001, 002, 003 etc. including screened patients.

- Assignment of study day: Day 0 is the day of the first immunization. The second immunization is at Day 21.

5. PRE-TREATMENT EVALUATION

The following pre-treatment evaluations will be completed within 1 month (+/- 2 weeks) before starting study treatment: (Refer to Appendix 1 - Schema)

- History and physical exam, to include Karnofsky Performance Score and review of inclusion and exclusion criteria
- ß-HCG for women with childbearing potential
- Hematological and Biochemical Tests:
  - CBC with differential
  - PT, INR and PTT
  - Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose
- Urinalysis
- HIV Antibody
- Anti Nuclear Antibody (ANA)
- Biological Markers:
  - Serum will be obtained to measure antibodies to CEA and the neutralizing antibodies to Ad5 vector and sent to Etubics Corporation for determination.
  - Other available serum markers (e.g., CEA or CA15-3) will be reviewed if determined and available.
- Immunologic Evaluation/Archive Sample:
  - Blood may be drawn during the first clinic visit (after consent is signed) for immunologic evaluation and/or archiving.
- Imaging Studies:
  - Brain CT scan or MRI is required within 6 weeks of starting study treatment.

- Available CT scans or MRI of the chest, abdomen, and/or pelvis will be reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, but is not required.

6. TREATMENT PLAN

Patients will be enrolled into successive dosage level cohorts of 3 or 6 patients and monitored for dose-limiting toxicity (DLT).

DLT (Based on NCI CTCAE version 4.0) is defined as any Grade 2, 3 or 4 immediate hypersensitivity reactions, Grade 3 or 4 fever that may possibly be associated with the immunization, Grade ≥2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade ≥2 allergic reactions (grade 2 is defined as generalized urticaria as defined by CTCAE version 4.0), or Grade ≥3 non-hematologic toxicity.

During dose escalation through the first three patients of cohort 3, there will be a minimum of 1 week between enrolling successive patients. The first patient will be called to check on their condition prior to enrolling the second patient since patients can be enrolled after 1 week of initiation of cohort 3. If no DLT have been observed at this point, then further enrollment can occur in cohort 3 and phase II component (Cohort 4) without the 1-week waiting period. Between dosage levels, through Cohort 3, assessment of DLT for dose escalation will be made after all patients in a cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine and all the available safety data and laboratory results have been reviewed. Patients in cohort 5 may begin injections once all patients in cohort 4 have had at least their first injections and all patients in cohort 3 have had a study visit at least 3 weeks after receiving their first dose of vaccine. If DLT occurs in <33% of patients in a given dosage level cohort, progression to the next dosage level will proceed. If DLT occurs in ≥33% of patients in a given cohort, the next lower dosage level will be defined as the maximum tolerated dose (MTD). If DLT occurs in <33% of patients in the highest dosage level tested, that dosage level will be defined as the MTD. In phase II (Cohort 4) and Cohort 5, if at any time the rate of DLT in patients enrolled is ≥33%, the MTD will be re-defined as the next lower dosage level. Additional details of this dose escalation and de-escalation plan are provided below and in Figures 3A, 3B and Table 3.

1) Phase I: Cohort 1: Three patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1 x 10^9 particles in 0.5 mL subcutaneously (SQ) in the same thigh every 3 weeks for 3 immunizations. Immunizations should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT (as defined above), then patients may begin enrolling into cohort 2. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 2. If 2 patients have DLT at this lowest dosage level, dosing will be de-escalated to $1x10^8$ particles and a new cohort of 3 patients instituted.

2) Cohort 2: Three patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1 x 10^{10} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for dose escalation will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there are no DLT, then patients may begin enrolling into cohort 3. If there is 1 DLT then an additional 3 patients will be enrolled at this dosage level. Assessment of DLT for dose escalation will be made after the 3 additional patients have had a study visit at least 3 weeks after receiving their first dose of vaccine. If none of these latter 3 patients have DLT, then patients may begin enrolling into cohort 3. If 2 patients have DLT at this dosage level, the dosage level in cohort 1 will be considered the MTD. If only 3 patients were enrolled in cohort 1, an additional 3 patients will be enrolled at that dosage before proceeding to the next cohort.

3) Cohort 3: Six patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1 x 10^{11} particles in 0.5 mL SQ in the same thigh every 3 weeks for 3 immunizations. Immunizations site should be separated by 5 cm. A diary card will be given to each subject at the first treatment along with a ruler to record any adverse reactions for 2 days after treatment (24 hours and 48 hours post injection). The diary card will be placed in the study file. Assessment of DLT for proceeding to phase II enrollment (Cohort 4) will be made after all patients in this cohort have had a study visit at least 3 weeks after receiving their first dose of vaccine. If there is 0 or 1 DLT, then subjects may enroll in Cohort 4. If 2 patients have DLT at this dosage level, the dosage level in cohort 2 will be considered the MTD. If only 3 patients were enrolled in cohort 2, an additional 3 patients will be enrolled at that dosage before proceeding to the next level.

4) Phase II cohort (Cohort 4): After the MTD is established, an additional 12 patients will receive Ad5 [E1-, E2b-]-CEA(6D) at the MTD every 3 weeks for 3 immunizations.

Note: if during accrual of patients in the phase II cohort, DLT occurs at a sufficient rate such that the rate of DLT amongst the total number of patients (those treated at MTD in phase I and those in phase II) is ≥33%, then phase II will be restarted at the dosage level which is one level below the prior MTD.

5) Cohort 5: Six patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of $5x10^{11}$ particles SQ in 2.5 mL every 3 weeks for 3 immunizations. Assessment of DLT will be made after all patients in this cohort have had all 3 injections and been observed for at least 30 days after the last injection. If there are 0-1 DLT, then this will be declared the maximum feasible dose. If there are 2 or more DLT, then $1x10^{11}$ particles will be declared the MTD.

6) Cohort 6: Up to ten (10) patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of $5x10^{11}$ particles SQ in 1.0 mL every 3 weeks for 3 immunizations. Patients will be permitted to receive booster doses of Ad5 [E1-, E2b-]-CEA(6D) every 3 months for a period of two years following the first 3 treatments. In the event that a qualified patient undergoes reimaging prior to the completion of their initial 3 study immunizations and is found to have disease progression, the patient should continue on the immunization protocol because this could be a "flare reaction" associated with the vaccine which may be an inflammatory response. Patients will be required to have MRI/CT scans performed prior to initiation of treatment, at 3 month intervals +/- 1 month for 12 months or if the investigator believes signs or symptoms are suggestive of progression or relapse. All patients will be followed for overall survival for a period of 24 months from initiation of treatment.

7) Patients will have 90 mL peripheral blood drawn prior to each immunization and approximately 3 weeks after the third immunization (Week 9) to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations.

8) Time to progression will be measured using CT scans performed at approximately 3 month intervals (based on clinical standard of care).

9) For all patients, if scheduling conflicts arise, the scheduled 3-week interval between immunizations may be modified so that the interval between immunizations is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

10) The following safety events will trigger a temporary suspension of study vaccinations:

    a) If one or more patients develop a Grade 4 allergic reaction without a clear attributable cause, other than study vaccine
    b) Death not attributed to disease.

Assessment of these halting rules is a review of cumulative events for all study participants, and should not be confused with reasons for delaying or terminating the immunization schedule of any individual patient.

Etubics Corporation will fully review all available safety data, consult with the principal investigator, medical monitor and the FDA as needed, before determining if resuming vaccinations is appropriate. If it is determined that study vaccinations can resume, the halting rules will apply to each subsequent event that meets the criteria described above. Vaccinations may also be suspended for safety concerns other than those described above if, in the judgment of the principal investigator or sponsor, participant safety is threatened.

6.1. Study Stopping Rules

- Death possibly related to the study agent.
- Two patients having a Grade 4 toxicity event that is possibly/probably related to the study agent.

Figure 3A.

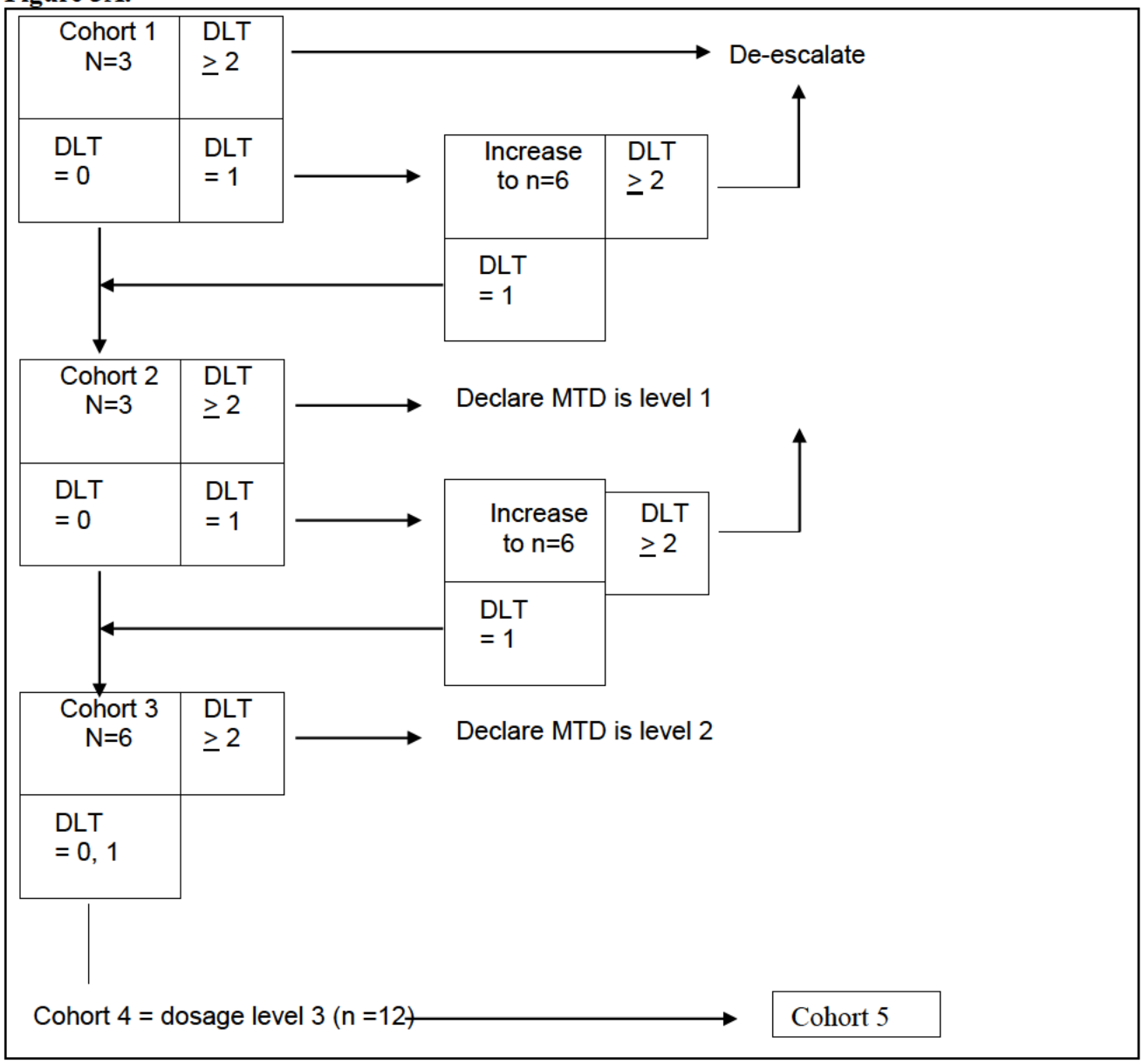

Cohort 1
N=3
DLT
≥ 2
De-escalate
DLT
= 0
DLT
= 1
Increase
to n=6
DLT
≥ 2
DLT
= 1
Cohort 2
N=3
DLT
≥ 2
Declare MTD is level 1
DLT
= 0
DLT
= 1
Increase
to n=6
DLT
≥ 2
DLT
= 1
Cohort 3
N=6
DLT
≥ 2
Declare MTD is level 2
DLT
= 0, 1
Cohort 4 = dosage level 3 (n =12)
Cohort 5

Figure 3B.

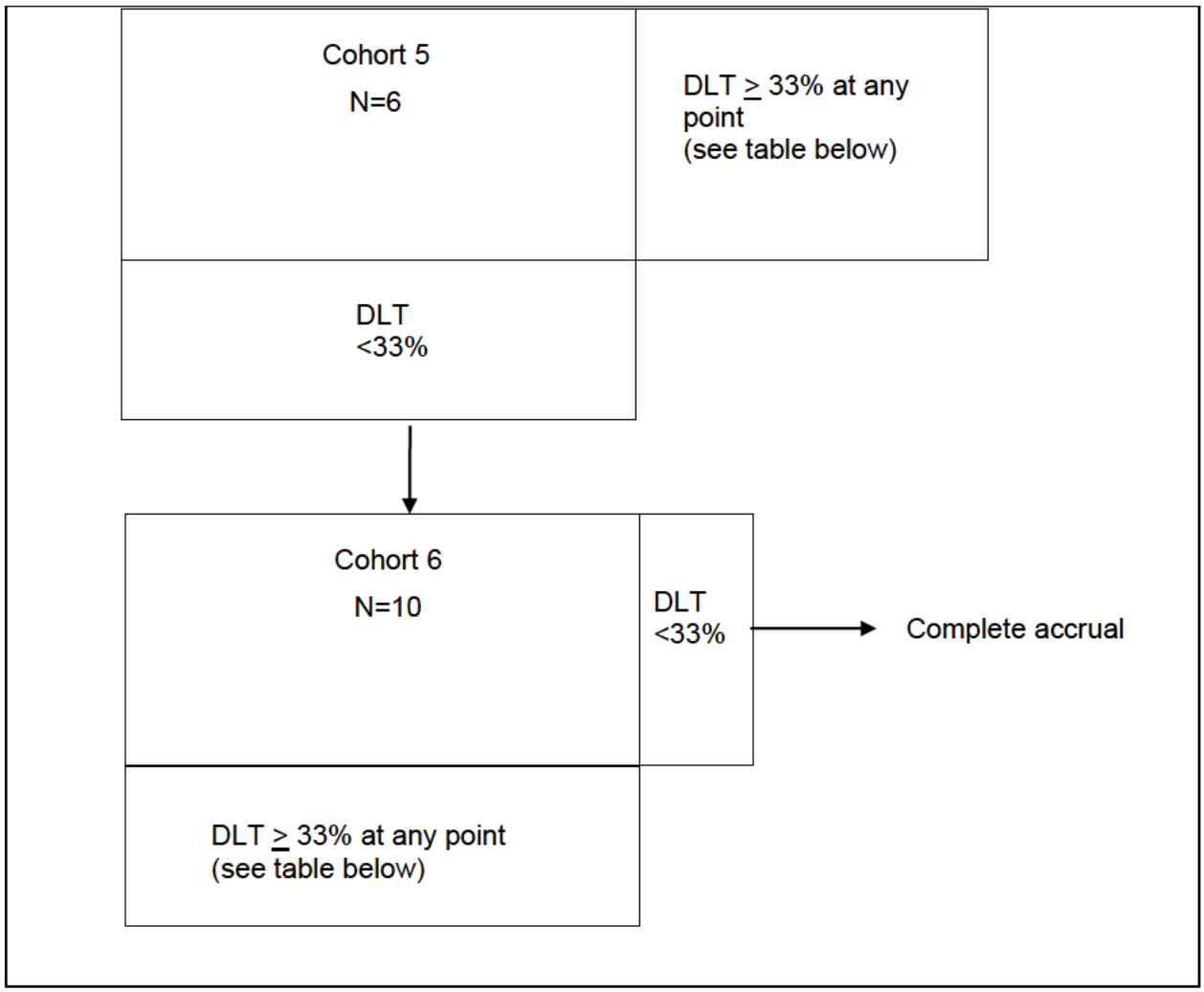


Table 3. Calculation of rates of DLT during patient accrual in phase I and phase II

# evaluable for toxicity	Cohort 1 (n=3)	Cohort 2 (n=3)	Cohort 3 (n=6)	Cohort 4 (n=12)	Cohort 5 (n=6)	Cohort 6 (n=10)
# with DLT to be ≥33%	1	2	4	8	10	13

6.2. Pharmaceutical Information

6.2.1 Dosage and Administration

Patients will receive Ad5 [E1-, E2b-]-CEA(6D) at a dose of 1 x 10^9, 1 x 10^{10}, or 1 x 10^{11} virus particles (vp) per immunization in 0.5 mL of sterile saline SQ every 3 weeks for 3 immunizations. The 5th cohort will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 5 x 10^{11} virus particles (vp) per immunization in 2.5 mL of sterile saline SQ every 3 weeks for 3 immunizations. The 6th cohort will receive Ad5 [E1-, E2B-]-CEA(6D) at a dose of 5 x 10^{11} virus particles (vp) per

immunization in 1.0 mL SQ every 3 weeks for 3 immunizations and each additional boost will be delivered at the same dose.

6.2.2 How Supplied

ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 0.5 ml of extractable vaccine which contains $1x10^{11}$ total virus particles. The volume of injection for $1x10^{11}$ virus particles is 0.5 mL. The lower doses will be produced by dilution in 0.9% saline using the following directions. For cohort 6, ETBX-011 will be provided in a frozen state in a 2ml vial with a fill volume of 1.0 ml of extractable vaccine which contains $5x10^{11}$ total virus particles. The volume of injection for $5x10^{11}$ virus particles is 1.0 mL. The product should be stored at –20°C until used.

Instructions for dose preparation:

Cohort 1: To administer $1x10^9$ virus particles by subcutaneous injection, perform 2 serial dilutions of vialed vaccine as follows:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2×10^{10} vp/ml.**
5. Use a new sterile syringe with a needle and repeat above procedure.
6. Withdraw 4.5 mL of sterile saline.
7. In the second syringe withdraw 0.5 mL from the syringe containing **2×10^{10} vp/ml**.
8. Remove the needle from the syringe containing the 4.5 mL saline, and inject the 0.5 mL of 2 x 10^{10}vp/mL from the second syringe.
9. Place a new needle on the 10-mL syringe from Step 8 above and mix the two solutions. This solution now has a concentration **2×10^9 vp/mL** (1 x 10^9 vp per 0.5 mL)**.**
10. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^9 vp and withdraw 0.5 mL from the syringe containing **2×10^9 vp/mL**. This prepared vaccine **(ETBX-011, 1 x 10^9 vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 2: To administer $1x10^{10}$ virus particles by subcutaneous injection:

1. Draw 4.5 mL of sterile saline into a syringe.
2. Using a second syringe, withdraw 0.5 mL of previously thawed ETBX-011 from the supplied vial.
3. Remove the needle from syringe containing the 4.5 mL saline, and inject the 0.5 mL of ETBX-011 from the second syringe into the syringe containing saline.
4. Mix. This new solution has a concentration **2×10^{10} vp/ml.**
5. Label a new 1-mL sterile syringe ETBX-011, 1 x 10^{10} vp and withdraw 0.5 mL from the syringe containing **2×10^{10} vp/mL**. This prepared vaccine **(ETBX-011, 1 x 10^{10} vp)** can be kept at room temperature for four hours prior to administering to the patient.

Cohort 3 and 4: To administer $1x10^{11}$ virus particles by subcutaneous injection:

1. Withdraw 0.5mL of contents from the previously thawed, supplied ETBX-011 from the vial and administer to each subject without any further manipulation.

Cohort 5: To administer $5x10^{11}$ virus particles by subcutaneous injection:

1. Withdraw 0.5mL of contents from each of 5 previously thawed ETBX-011 vials (total volume 2.5ml) and administer to each subject without any further manipulation.

Cohort 6: To administer $5x10^{11}$ virus particles by subcutaneous injection:

1. Withdraw 1.0mL of contents from the previously thawed, supplied ETBX-011 from the vial and administer to each subject without any further manipulation.

6.2.3 Disposal of Unused Vaccine

Unless other arrangements are agreed in writing, all unused vaccine should be delivered to Dr. Joe Balint, at Etubics Corporation for disposal at or before the completion of the clinical study.

7. TREATMENT EVALUATION

7.1. Short-Term Evaluation During and After Active Immunotherapy

On vaccine administration days, blood will be drawn before administration. Evaluations will also be conducted for patients who discontinue from the study if they have received any treatment. The investigator will determine the degree of evaluation based on the patient's condition and/or reason for discontinuation from the study.

7.1.1 General Evaluations

Weeks 0, 3, 6 (Immunization Visits):

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight by the attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Patients will remain in the clinic for approximately 30 minutes following receipt of vaccine to monitor for any adverse reactions. Local and systemic reactogenicity after each dose of vaccine will be assessed daily for 3 days (on the day of immunization and 2 days thereafter) using diary cards to report symptoms and a ruler to measure local reactogenicity.

Week 9:

General evaluations include medical history, Karnofsky performance status, and complete physical examination with weight by attending physician. Any other treatments, medications, biologics, or blood products that the patient is receiving or has received since the last visit will be recorded. Immunization injection sites will be assessed.

7.1.2 Hematological and Biochemical Assessment

Weeks 0, 3, 6 (Immunization Visits):

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

Week 9:

Blood will be drawn to perform chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, glucose and ANA.

7.1.3 Biological Markers

Weeks 0 and 9:

Serum (5ml) to measure antibodies to CEA and the Ad5 vector will be obtained and send to Etubics Corporation for determination. Biomarkers (e.g., CEA or CA15-3) will be reviewed if determined and available.

7.1.4 Immunological Assessment

Weeks 0, 3, 6, and 9:

Peripheral blood (90mL) will be drawn prior to each immunization and approximately 3 weeks after the last immunization to determine whether there is an effect on the immune response at specific time points during the study and/or after a specific number of immunizations. Peripheral blood mononuclear cells (PBMC) will be assayed for T cell responses to CEA using ELISPOT at all time points, plus proliferation assays, multi-parameter flow cytometric analysis, and cytoxicity assays, if possible and at the discretion of the investigator. Serum from each blood draw will be archived and sent to Etubics Corporation for Ad5 neutralizing level determination.

7.2. Long-Term Follow-Up

Patients will be requested to continue long-term follow-up every 3 months for 1 year. At each visit, a medical history and physical exam and the following lab tests will be performed: blood chemistry and hematology, including CBC with differential, Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose.

At each visit, 40-90 mL of peripheral blood should be drawn for immune analysis, if there was previous evidence of an immune response or at the discretion of the investigator. Available serum markers (e.g., CEA, CA15-3) will be reviewed.

7.3. Management of Intercurrent Events

7.3.1 Concomitant Medications

Patients will be removed from the protocol treatment if they initiate concomitant chemotherapeutic agents, corticosteroids, or other immunosuppressive agents, or other forms of immunotherapy. After meeting the inclusion criteria, all other medications deemed appropriate for the patient, by the investigator, may be administered to the patient. All medications and changes in medication during treatment will be recorded. Hormonal therapy and bisphosphonates may be continued in breast cancer patients if they have been stable on the agents for at least 1 month prior to enrollment.

7.3.2 Adverse Events

7.3.2.1 Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (which can be downloaded from the CTEP web site (http://ctep.cancer.gov).) DLT is defined in Protocol Section 6.

7.3.2.2 Possible side effects from immunization may include local effects (pain, tenderness, rednesss or swelling), systemic effects (malaise, fatigue, myalgia, arthralgia, headache, nausea, vomiting, chills or fever), and allergic reactions such as hives, rash or anaphylactic reactions. Induction of auto-immunity, manifest as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc., is theoretically possible, but has not been observed in our prior CEA vaccine studies. Also, liver function test abnormalities and liver failure are theoretically possible.

7.3.3 Treatment of Toxicity

For acute allergic reactions, use of diphenhydramine 25-50 mg IV, corticosteroids (Solumedrol 100 mg), and epinephrine (1:1000, 0.3-1 mL sq) should be considered. These medications are available in the clinical setting where the vaccine will be administered. For auto-immunity, corticosteroids (prednisone 0.5-1 mg/kg/d) should be considered. Clinical assessment of other targets, such as thyroid, arthritis, urticaria, or proteinuria, for auto-immunity and serum sickness like antigen-antibody complex disease, will also be performed as indicated.

7.3.4 Active Immunotherapy will be Discontinued for:

7.3.4.1 Life-threatening anaphylactic reactions related to active immunotherapy

7.3.4.2 DLT related to active immunotherapy

7.3.4.3 Disease progression (by RECIST criteria). Patients will be offered referral to a medical oncologist for discussion of other treatment options, and for continued medical care.

7.3.4.3.1 Disease progression prior to completing the 3 study immunizations:

In the event that a patient undergoes reimaging studies prior to the completion of their 3 study immunizations and is found to have disease progression, they will be permitted to continue on the study as long as the progression has been 50% or less by RECIST criteria.

If a patient is removed from the study prior to completion of the assigned vaccine schedule for any reason other than toxicity, that patient will be replaced, in order to obtain data to help determine the toxicity of the immunizations. We will allow up to 3 replacements per cohort (Phase I, Dose levels 1 and 2; Phase II, MTD).

8. STATISTICAL CONSIDERATIONS

8.1. Safety

We will evaluate safety continuously in a cohort. We will make our overall assessment of whether to escalate to the next dose level at least 3 weeks after the last patient in the previous cohort has received their first injection. This decision will be made by the medical monitor and the Principal Investigator. A note will be generated following the assessment decision and filed in study binder. A dosage level will be considered safe if <33% patients treated at a dose level experience DLT (i.e., 0 of 3, ≤1 of 6, ≤3 of 12, or ≤5 of 18 patients). DLT is defined in Protocol Section 6. Safety will be evaluated in 3 or 6 patients at each dosage level in phase I. Safety will continue to be monitored among additional patients treated at the MTD in phase II. A patient will be considered evaluable for safety if treated with at least one immunization. DLTs will be observed through 9 weeks to accommodate safety evaluation of all 3 product doses.

8.2. Rate of Immune Response

Immune responses against CEA will be evaluated from the peripheral blood of patients from among the following studies at the discretion of the Principal Investigator (ELISpot, cytokine flow cytometry, and antibody responses). We will determine the percentage of patients with a positive immune response. We define a positive immune response by ELISpot as described at the 2002 Society of Biologic Therapy Workshop on "Immunologic Monitoring of Cancer Vaccine Therapy", i.e. a T cell response is considered positive if the mean number of spots adjusted for background in six wells with antigen exceeds the number of spots in six control wells by 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of $p \leq 0.05$ using the Student's t test. Immunogenicity assays will occur prior to each immunization and at week 9. Immune response will be assessed among the 18 patients treated at the MTD (12 in phase I and 12 in Phase II). The therapy will be considered of further interest if 9 of 18 patients treated at the MTD dose exhibit an immune response as defined above. Meeting this criterion establishes that the immune response rate is at least 33% with approximately 90% confidence. At significance level 0.1 there is 82% power to test the null hypothesis that the immune response rate is ≤ 0.33 versus the alternative that the immune response rate is ≥ 0.58. The magnitude of response will also be described. A patient will be considered evaluable for immune response if they receive at least 3 immunizations.

8.3. Determination of Clinical Response

Among patients with measurable/evaluable disease, response determination will be made according to the RECIST criteria:

Complete Response (CR):	Disappearance of target lesion, confirmed at 4 weeks
Partial Response (PR):	30% decrease in longest dimension of target lesion, confirmed at 4 weeks
Stable Disease (SD):	Neither PR nor PD
Progressive Disease (PD):	20% increase in longest dimension of target lesion; no CR, PR, or SD documented before increased disease.

The exact binomial confidence interval for the proportion of subjects with a clinical response of CR or PR will be calculated.

9. PATIENT WITHDRAWAL

Patients may be removed from the study for the following reasons:

- DLT as defined in Protocol Section 6.
- Patient voluntarily decides to withdraw.
- Patient non-compliance with the study protocol.
- Intercurrent disease which in the opinion of the patient's treating physician would affect the ability of the patient to continue on the clinical study.

In the event of withdrawal due to toxicity, a patient will be requested to have safety evaluations performed as per the protocol for a one year duration post treatment. This may include having up to 90 mL of blood drawn for immunologic testing.

10. STUDY CONDUCT AND ETHICAL AND REGULATORY CONSIDERATIONS

10.1. Departure from the Protocol

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the principal investigator, or in his absence, Dr. Gerald Messerschmidt, Medical Director, immediately by telephone (610) 613-3882 (cell phone). Such contacts with the principal investigator will be made to permit a decision as to whether or not the patient will be continued on the study. Such departures need to be clearly documented and reported to the Institutional Review Board (IRB) and the Etubics Corporation by the principal investigator.

10.2. Informed Consent

In accordance with guidelines in the Federal Register, Vol. 48, No. 17, 1982, pp. 8951-2, all patients are required to sign a statement of informed consent. This phase I/II study involves research that presents risk, but holds the prospect of direct benefit to the individual patient (46.405-45 Code of the Federal Regulations part 46). The investigator will report to the IRB and Etubics Corporation will report to FDA changes in the research protocol and all unanticipated problems involving risks to human patients and others, and no changes will be made in the research activity without IRB approval.

10.3. Institutional Review

This study must be approved by the site's IRB. IRB approval of the protocol and the informed consent form for this study must be given in writing. The IRB must also approve any significant changes to the protocol as well as a change of principal investigator. Records of all study review and approval documents must be kept on file by the investigator and are subject to FDA inspection during or after completion of the study. Adverse events must be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

10.4. Documentation and Monitoring

Data will be collected for all patients signing consent. Accurate completion of the computer data forms for all patients is the responsibility of the investigator.

10.4.1 Case Report Forms

Case Report Forms (CRFs) are used to record study data and are an integral part of the study and subsequent reports. Therefore, all reports must be legible and complete. All forms should be filled out using a black ballpoint pen. Errors should be lined out but not obliterated and the correction inserted, initialed, and dated by the principal investigator, co-investigators, study coordinator, or data manager. A CRF must be completed and signed by the principal investigator for each patient enrolled, including those removed from the study for any reason. The reason for removal must be noted on the Final Report Form by the investigator for each patient. CRFs must be kept current to reflect patient status at each phase during the course of the study. Patients are not to be identified on CRFs by name; appropriate coded identification and patient initials must be used. The investigator must keep a separate log of patient names and addresses. This log is subject to FDA inspection. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto CRFs, originals of laboratory and other test results must be kept on file with patient's CRFs or clinical chart. CRFs and copies of test results must be available at all times for inspection by the FDA.

10.4.2 Maintenance of Study Documentation

The following will be maintained:

a. Case Report Forms - which must be kept legible, accurate, and up-to-date.
b. Patient Files/Signed Informed Consent - which substantiates the data entered on the case report forms for all required test and evaluation procedures and verifies that the patient has signed an informed consent to enter the study.
c. Patient Exclusion Record - which should reflect the reason any patient was screened and found ineligible for the study.
d. Monitoring Log - listing dates of monitor visits.
e. Regulatory Documents - including protocol, investigator brochure, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.
f. Adverse Event Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The sponsor will notify the investigator if a marketing application is approved or if the investigation is discontinued and the FDA notified.

10.5. Monitoring of the Protocol

An Etubics Corporation representative will conduct a site initiation visit and will review protocol documents with the investigators and their staff. During the study, the Etubics monitor will visit the site regularly to check the completeness of study files, the accuracy of data entries on the CRFs, and adherence to the protocol and to Good Clinical Practice (GCP). Key study personnel must be available to assist the monitor during these visits. The investigator must maintain source documents for each subject in the study. The investigator must also keep the original informed consent form signed by every patient. The investigator must give the monitor access to all relevant

source documents to verify data entries on CRFs. No information in source documents about the identity of subjects will be disclosed.

This is a phase I/II clinical study with more than minimal risk and as such will be monitored for the occurrence of a greater frequency of adverse events (AEs). The principal investigator will continuously monitor the data and safety of all subjects enrolled. All grades of toxicities will be recorded.

Safety assessments will consist of monitoring all AEs including serious adverse events (SAEs), the regular monitoring of hematology, serum chemistry, and routine monitoring of vital signs and physical condition. AE monitoring should occur from the time of informed consent to 30 days after the last dose of study drug. For those subjects who discontinue study participation prior to receiving study drug, AE and SAEs will be collected through the time of discontinuation.

Toxicity will be assessed using the NCI CTCAE version 4.0. Refer to Protocol Section 7.3.2.

An AE is any adverse change from the study patient's baseline (pretreatment) condition, including any clinical or laboratory test abnormality that occurs during the course of the proposed clinical study after treatment has started.

Events are classified as SERIOUS if they meet any of the following criteria [per the US Code of Federal Regulations (CFR) 21 CFR 312.32 and the recommendations of the International Conference on Harmonization (ICH)]:

An SAE is any sign, symptom or medical condition that emerges during the study or during a post-study follow-up period that 1) was not present at the start of the study and is not a chronic condition that is part of the patient's medical history, OR 2) was present at the start of the study or as part of the patient's medical history but worsened in severity and/or frequency during study participation, AND that meets any of the following regulatory serious criteria:

- any death
- any life-threatening event, i.e., an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity
- any congenital anomaly/birth defect diagnosed in a child of a patient who participated in this study and received investigational drug
- other medically important events that in the opinion of the investigator may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g. allergic bronchospasm requiring intensive treatment in an emergency room, convulsions occurring at home that do not require in-patient hospitalization, any blood dyscrasias, or the development of drug dependency or drug abuse).

10.6. CTCAE Term (AE description) and Grade

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for AE reporting. A copy can be downloaded from the CTEP web site (http://ctep.cancer.gov).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the following guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

10.7. Assessing Causality

Investigators are required to assess whether there is a reasonable possibility that study medications caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

10.8. Serious Adverse Event Reporting

All AEs that are classified as serious, unexpected and related or possibly related, will be recorded on the MedWatch 3500A form and reported within 24 hours of learning of the event to Etubics Corporation. The form should be completed as much as possible but should not be held until all information is available. Additional information and/or corrections may be submitted as they are obtained. The investigator will follow SAEs until resolution, a return to baseline condition or stabilization or 30 days after the last subject is enrolled whichever occurs first. SAEs that are ongoing at the time of clinical database closure will be recorded as unresolved.

All study-related deaths should be reported to the IRB within 24 hours; all other serious adverse experiences should be reported to the IRB within 5 business days. All deaths, whether considered study-related or not, must also be reported immediately to Etubics Corporation, additional information may be requested from the treating institution. Deaths believed to be related to disease progression will not be reported to the FDA as SAEs, as these deaths are an anticipated outcome of the disease; however, all deaths will be reported to the FDA in the annual reports.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B., initials, patient number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report.)

Reporting of Pregnancy:

If a participant becomes pregnant during the study, treatment will be discontinued (*i.e.*, no additional dose of study vaccine will be given) and the participant will be encouraged to continue to have regularly scheduled follow-up visits and evaluations. The occurrence of pregnancy, and the outcome of any pregnancy, in a subject treated with study vaccine, must be reported to the Etubics Corporation and to the IRB.

Procedures for SAE Reporting:

1. Medical staff/study team notifies principal investigator of SAE. MedWatch 3500A is completed.

2. Principal Investigator calls Etubics Corpration to report the SAE and faxes the completed MedWatch 3500A to:

   Carol Jones, Vice President of Administration
   Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
   Telephone: 206-838-5110 ext. 2
   Cell: (206) 818-2985
   Fax: (206) 838-2978
   Email: cj@etubics.com

3. Carol Jones reports the SAE to one of the following in this order:

   A. Chief Scientific Officer: Frank Jones, PhD
      Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
      Telephone: 206-838-5110 ext. 1
      Cell: (206) 818-2857

Fax: (206) 838-2978
Email: frj@etubics.com

B. Laboratory Manager: Joseph Balint, PhD
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 7
Fax: (206) 838-2978
Email: joe@etubics.com

C. Vice President Research: Elizabeth S. Gabitzsch
Address: 410 West Harrison Street, Suite 100, Seattle, WA 98119
Telephone: (206) 838-5110 ext. 3
Cell: (970) 402-2598
Fax: (206) 838-2978
Email: beth@etubics.com

4. Contacted person in three (3) above notifies the FDA via MedWatch 3500A form.

   a. If the SAE results in death or is life-threatening, SAE report will be submitted to the FDA within 7 days
   b. All other SAEs must be reported to the FDA within 15 days

5. All SAE will require input from a physician, the Acting Medical Director will be consulted:

   Gerald Messerschmidt, MD
   Telephone: (610) 613-3882 or (206) 838-5110
   Fax: (206) 838-2978
   Email:gerry@etubics.com

6. SAEs will be reported to the IRB according to the site's IRB guidelines.

In accordance with FDA regulations and ICH guidelines, investigators will be notified of the occurrence of new, serious, unexpected adverse events associated with the use of the study medication (i.e. there is a reasonable possibility that the AE may have been caused by the drug) within 15 calendar days via a written report. It is the responsibility of the investigator to promptly inform the relevant IRB of these new adverse events/risks to patients, in accordance with 21 CFR 312.66. It is the responsibility of Etubics Corporation to promptly inform the NIH Office of Biotechnology Activities (OBA) and relevant Scientific Review Board of these new adverse events/risks to patients, in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules, in particular Appendix M. It is the responsibility of the Etubics Corporation to report these SAEs to the FDA. The SAE report will be forwarded to the FDA after recording the event data via the FDA MedWatch form 3500A.

10.9. Safety Reporting Requirements

In accordance with 21 CFR 312.32, the sponsor of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

***7 Calendar-Day Telephone or Fax Report*:**

The sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:**

The sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the investigational product. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation.

c. Data and Safety Monitoring

Data will be collected by the principal investigator, co-investigators, and the protocol coordinator. The protocol coordinator under the supervision of the principal investigator will report the AEs. The data will be audited by Etubics Corporation.

11. REFERENCES

1. Berinstein, N.L. 2002. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J Clin Oncol* 20:2197-2207.
2. Tsang, K.Y., Zaremba, S., Nieroda, C.A., Zhu, M.Z., Hamilton, J.M., and Schlom, J. 1995. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 87:982-990.
3. Tsang, K.Y., Zhu, M., Nieroda, C.A., Correale, P., Zaremba, S., Hamilton, J.M., Cole, D., Lam, C., and Schlom, J. 1997. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. *Clin Cancer Res* 3:2439-2449.
4. Ras, E., van der Burg, S.H., Zegveld, S.T., Brandt, R.M., Kuppen, P.J., Offringa, R., Warnarr, S.O., van de Velde, C.J., and Melief, C.J. 1997. Identification of potential HLA-A *0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA). *Hum Immunol* 53:81-89.
5. Zaremba, S., Barzaga, E., Zhu, M., Soares, N., Tsang, K.Y., and Schlom, J. 1997. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 57:4570-4577.
6. Kawashima, I., Hudson, S.J., Tsai, V., Southwood, S., Takesako, K., Appella, E., Sette, A., and Celis, E. 1998. The multi-epitope approach for immunotherapy for cancer: identification of several CTL epitopes from various tumor-associated antigens expressed on solid epithelial tumors. *Hum Immunol* 59:1-14.
7. Zhu, M.Z., Marshall, J., Cole, D., Schlom, J., and Tsang, K.Y. 2000. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 6:24-33.
8. Bremers, A.J., van der Burg, S.H., Kuppen, P.J., Kast, W.M., van de Velde, C.J., and Melief, C.J. 1995. The use of Epstein-Barr virus-transformed B lymphocyte cell lines in a peptide-reconstitution assay: identification of CEA-related HLA-A*0301-restricted potential cytotoxic T-lymphocyte epitopes. *J Immunother Emphasis Tumor Immunol* 18:77-85.
9. Kawashima, I., Tsai, V., Southwood, S., Takesako, K., Sette, A., and Celis, E. 1999. Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. *Cancer Res* 59:431-435.
10. Kim, C., Matsumura, M., Saijo, K., and Ohno, T. 1998. In vitro induction of HLA-A2402-restricted and carcinoembryonic-antigen-specific cytotoxic T lymphocytes on fixed autologous peripheral blood cells. *Cancer Immunol Immunother* 47:90-96.
11. Nukaya, I., Yasumoto, M., Iwasaki, T., Ideno, M., Sette, A., Celis, E., Takesako, K., and Kato, I. 1999. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. *Int J Cancer* 80:92-97.
12. Schirle, M., Keilholz, W., Weber, B., Gouttefangeas, C., Dumrese, T., Becker, H.D., Stevanovic, S., and Rammensee, H.G. 2000. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol* 30:2216-2225.
13. Tangri, S., Ishioka, G.Y., Huang, X., Sidney, J., Southwood, S., Fikes, J., and Sette, A. 2001. Structural features of peptide analogs of human histocompatibility leukocyte antigen

class I epitopes that are more potent and immunogenic than wild-type peptide. *J Exp Med* 194:833-846.

14. Samanci, A., Yi, Q., Fagerberg, J., Strigård, K., Smith, G., Rudén, U., Wahren, B., and Mellstedt, H. 1998. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 47:131-142.
15. Foon, K.A., John, W.J., Chakraborty, M., Das, R., Teitelbaum, A., Garrison, J., Kashala, O., Chatterjee, S.K., and Bhattacharya-Chatterjee, M. 1999. Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *J Clin Oncol* 17:2889-2885.
16. Foon, K.A., John, W.J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. 1997. Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin Cancer Res* 3:1267-1276.
17. Paoletti, E. 1996. Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* 93:11349-11353.
18. Cox, W.I., Tartaglia, J., and Paoletti, E. 1993. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 195:845-850.
19. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9:190-193.
20. Hodge, J.W., McLaughlin, J.P., Kantor, J.A., and Schlom, J. 1997. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15:759-768.
21. Kass, E., Schlom, J., Thompson, J., Guadagni, F., Graziano, P., and Greiner, J.W. 1999. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 59:676-683.
22. Kass, E., Panicali, D.L., Mazzara, G., Schlom, J., and Greiner, J.W. 2001. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 61:206-214.
23. Conry, R.M., Allen, K.O., Lee, S., Moore, S.E., Shaw, D.R., and LoBuglio, A.F. 2000. Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin Cancer Res* 6:34-41.
24. Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. 1999. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17:332-337.
25. von Mehren, M., Arlen, P., Tsang, K.Y., Rogatko, A., Meropol, N., Cooper, H.S., Davey, M., McLaughlin, S., Schlom, J., and Weiner, L.M. 2000. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 6:2219-2228.

26. von Mehren, M., Davey, M., and McLaughlin, S. 2000. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF in patients with advanced CEA-expressing cancers (abstr 1883). In *Proc Am Soc Clin Oncol* 480a.
27. von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H.S., Meropol, N.J., Alpaugh, R.K., Davey, M., McLaughlin, S., Beard, M.T., et al. 2001. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7:1181-1191.
28. Hörig, H., Lee, D.S., Conkright, W., Divito, J., Hasson, H., LaMare, M., Rivera, A., Park, D., Tine, J., Guito, K., et al. 2000. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother* 49:504-514.
29. Marshall, J.L., Hoyer, R.J., Toomey, M.A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J.E., Gehan, E., Peck, R.A., Arlen, P., et al. 2000. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18:3964-3973.
30. Slack, R., Ley, L., and Chang, P. 2001. Association between CEA-specific T cell responses following treatment wiht vaccinia CEA and survival in patients with CEA bearing cancers (abstr 1086). In *Proc Am Soc Clin Oncol* 272a.
31. Marshall, J.L., Gulley, J.L., Arlen, P.M., Beetham, P.K., Tsang, K.Y., Slack, R., Hodge, J.W., Doren, S., Grosenbach, D.W., Hwang, J., et al. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 23:720-731.
32. Hu, H., Serra, D., and Amalfitano, A. 1999. Persistence of an [E1-, polymerase-] adenovirus vector despite transduction of a neoantigen into immune-competent mice. *Hum Gene Ther* 10:355-364.
33. Hodges, B.L., Evans, H.K., Everett, R.S., Ding, E.Y., Serra, D., and Amalfitano, A. 2001. Adenovirus vectors with the 100K gene deleted and their potential for multiple gene therapy applications. *J Virol* 75:5913-5920.
34. Hodges, B.L., Serra, D., Hu, H., Begy, C.A., Chamberlain, J.S., and Amalfitano, A. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *J Gene Med* 2:250-259.
35. Arthur, J.F., Butterfield, L.H., Roth, M.D., Bui, L.A., Kiertscher, S.M., Lau, R., Dubinett, S., Glaspy, J., McBride, W.H., and Economou, J.S. 1997. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther* 4:17-25.
36. Diao, J., Smythe, J.A., Smyth, C., Rowe, P.B., and Alexander, I.E. 1999. Human PBMC-derived dendritic cells transduced with an adenovirus vectorinduce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845-853.
37. Brossart, P., Goldrath, A.W., Butz, E.A., Martin, S., and Bevan, M.J. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J Immunol* 158:3270-3276.
38. Butterfield, L.H., Jilani, S.M., Chakraborty, N.G., Bui, L.A., Ribas, A., Dissette, V.B., Lau, R., Gamradt, S.C., Glaspy, J.A., McBride, W.H., et al. 1998. Generation of melanoma-

specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus. *J Immunol* 161:5607-5613.

39. Bregni, M., Shammah, S., Malaffo, F., Di Nicola, M., Milanesi, M., Magni, M., Matteucci, P., Ravagnani, F., Jordan, C.T., Siena, S., et al. 1998. Adenovirus vectors for gene transduction into mobilized blood CD34+ cells. *Gene Ther* 5:465-472.
40. Dietz, A.B., and Vuk-Pavlovic, S. 1998. High efficiency adenovirus-mediated gene transfer to human dendritic cells. *Blood* 91:392-398.
41. Ishida, T., Chada, S., Stipanov, M., Nadaf, S., Ciernik, F.I., Gabrilovich, D.I., and Carbone, D.P. 1999. Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. *Clin Exp Immunol* 117:244-251.
42. Ribas, A., Butterfield, L.H., McBride, W.H., Jilani, S.M., Bui, L.A., Vollmer, C.M., Lau, R., Dissette, V.B., Hu, B., Chen, A.Y., et al. 1997. Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 57:2865-2869.
43. Wan, Y., Bramson, J., Carter, R., Graham, F., and Gauldie, J. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum Gene Ther* 8:1355-1363.
44. Wan, Y., Emtage, P., Foley, R., Carter, R., and Gauldie, J. 1999. Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *Int J Oncol* 14:771-776.
45. Miller, P.W., Sharma, S., Stolina, M., Butterfield, L.H., Luo, J., Lin, Y., Dohadwala, M., Batra, R.K., Wu, L., Economou, J.S., et al. 2000. Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum Gene Ther* 11:53-65.
46. Lu, L., Gambotto, A., Lee, W.C., Qian, S., Bonham, C.A., Robbins, P.D., and Thomson, A.W. 1999. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6:554-563.
47. Rea, D., Schagen, F.H., Hoeben, R.C., Mehtali, M., Havenga, M.J., Toes, R.E., Melief, C.J., and Offringa, R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245-10253.
48. Hirschowitz, E.A., Weaver, J.D., Hidalgo, G.E., and Doherty, D.E. 2000. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther* 7:1112-1120.
49. Loser, P., Jennings, G.S., Strauss, M., and Sandig, V. 1998. Reactivation of the previously silenced cytomegalovirus major immediate-early promoter in the mouse liver: involvement of NFkappaB. *J Virol* 72:180-190.
50. Morelli, A.E., Larregina, A.T., Ganster, R.W., Zahorchak, A.F., Plowey, J.M., Takayama, T., Logar, A.J., Robbins, P.D., Falo, L.D., and Thomson, A.W. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappaB-dependent pathway. *J Virol* 74:9617-9628.
51. Jonuleit, H., Tüting, T., Steitz, J., Brück, J., Giesecke, A., Steinbrink, K., Knop, J., and Enk, A.H. 2000. Efficient transduction of mature CD83+ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther* 7:249-254.
52. Tillman, B.W., Hayes, T.L., DeGruijl, T.D., Douglas, J.T., and Curiel, D.T. 2000. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based

vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res* 60:5456-5463.

53. Shiver, J.W., Fu, T.M., Chen, L., Casimiro, D.R., Davies, M.E., Evans, R.K., Zhang, Z.Q., Simon, A.J., Trigona, W.L., Dubey, S.A., et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415:331-335.
54. Yang, Z.Y., Wyatt, L.S., Kong, W.P., Moodie, Z., Moss, B., and Nabel, G.J. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J Virol* 77:799-803.
55. Casimiro, D.R., Chen, L., Fu, T.M., Evans, R.K., Caulfield, M.J., Davies, M.E., Tang, A., Chen, M., Huang, L., Harris, V., et al. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 77:6305-6313.
56. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. 1999. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 6:1574-1583.
57. Hartigan-O'Connor, D., Barjot, C., Salvatori, G., and Chamberlain, J.S. 2002. Generation and growth of gutted adenoviral vectors. *Methods Enzymol* 346:224-246.
58. Harui, A., Roth, M.D., Kiertscher, S.M., Mitani, K., and Basak, S.K. 2004. Vaccination with helper-dependent adenovirus enhances the generation of transgene-specific CTL. *Gene Ther* 11:1617-1626.
59. Morral, N., Parks, R.J., Zhou, H., Langston, C., Schiedner, G., Quinones, J., Graham, F.L., Kochanek, S., and Beaudet, A.L. 1998. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Hum Gene Ther* 9:2709-2716.
60. DelloRusso, C., Scott, J.M., Hartigan-O'Connor, D., Salvatori, G., Barjot, C., Robinson, A.S., Crawford, R.W., Brooks, S.V., and Chamberlain, J.S. 2002. Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc Natl Acad Sci U S A* 99:12979-12984.
61. Reddy, P.S., Sakhuja, K., Ganesh, S., Yang, L., Kayda, D., Brann, T., Pattison, S., Golightly, D., Idamakanti, N., Pinkstaff, A., et al. 2002. Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther* 5:63-73.
62. Amalfitano, A., Hauser, M.A., Hu, H., Serra, D., Begy, C.R., and Chamberlain, J.S. 1998. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 72:926-933.
63. Hartigan-O'Connor, D., Kirk, C.J., Crawford, R., Mulé, J.J., and Chamberlain, J.S. 2001. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 4:525-533.
64. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. 1999. A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *J Virol* 73:1046-1053.
65. Nazir, S.A., and Metcalf, J.P. 2005. Innate immune response to adenovirus. *J Investig Med* 53:292-304.
66. Schaack, J. 2005. Induction and inhibition of innate inflammatory responses by adenovirus early region proteins. *Viral Immunol* 18:79-88.

67. Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A.V., Clayton, G.H., Ornelles, D., and Moorhead, J. 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. *Proc Natl Acad Sci U S A* 101:3124-3129.

12. APPENDIX 1 – SCHEMA

Procedure / Test	Pre-treatment	Week 0	Week 3	Week 6	Week 9	Months 6, 9, etc. [i]	Off Treatment
H & P	X	X	X	X	X	X	X
Karnofsky Status	X	X	X	X	X	X	X
β-HCG [a]	X						
CBC with diff	X	X	X	X	X	X	X
PT-INR, PTT	X						
Chemistries	X	X	X	X	X	X	X
Urinalysis	X						
ANA	X				X		X
HIV	X						
Immune Monitoring [b]	X [b]	X	X	X	X	X [b]	X [b]
Antibodies to CEA/Ad5 Vector [c]		X			X		
Biological Markers [d] (i.e. CEA, CA15-3)	X [d]				X [d]	X [d]	X [d]
Brain MRI/CT Scan [e]	X						
MRI/CT Scan [f]	X [f]				X [f]	X [f]	X [f]
Immunization [g]		X	X	X			
AE Assessment [h]	X	X	X	X	X		

Key: H & P = history & physical examination, **KPS** = Karnofsky Performance Score, **β-HCG** = human chorionic gonadotrophin pregnancy test; **CBC & diff** = complete blood count and white blood cell differential, **Chemistries** = Na, K, Cl, CO_2, BUN, creatinine, Ca, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT and glucose; **ANA** = antinuclear antibody, **HIV** = human immunodeficiency virus antibody, **MRI/CT** = magnetic resonance imaging/computed tomography; **AE** = Adverse Event

Notes:

[a] β-HCG performed on women of childbearing potential

[b] Immune monitoring drawn during the first clinic visit at the discretion of the investigator/immune monitoring laboratory, prior to each immunization and approximately 3 weeks after the last immunization, and will be repeated at the discretion of the investigator/immune monitoring laboratory.

[c] Serum obtained for antibodies to CEA and Ad5 vector weeks 0 and 9.

[d] Available serum markers (e.g., CEA or CA15-3) reviewed as available.

[e] Brain CT scan or MRI required within 6 weeks of starting study treatment.

[f] Available CT scans or MRI of the chest, abdomen, and/or pelvis reviewed. Imaging studies within 6 weeks of starting study treatment is preferred, however is not required. Repeat imaging conducted according to standard of care.

[g] Immunizations may be modified so that the interval is between 20 and 28 days (3 weeks -1 day to 3 weeks +7 days). If the second and/or third immunization is delayed, the subsequent immunizations should occur no earlier than 20 days after the previous immunization.

[h] AE monitoring from the time of informed consent to 30 days after the last dose of study drug.

[i] Follow-up evaluations requested every 3 months for 1 year, while on the study.