

Abbreviated Title: Ad5-based combination vaccines
Version Date: 05.16.2018

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CC Protocol #: 18C0032 A

Version Date: May 16, 2018

IBC#: RD-17-VIII-13

OSP#: 1712-1703

NCT #: NCT03384316

Title: Phase I Trial Using a Multi-Targeted Recombinant Ad5 (CEA/MUC1/Brachyury) Based Immunotherapy Vaccine Regimen in Patients with Advanced Cancer

NCI Principal Investigator: Julius Strauss, MD
Laboratory of Tumor Immunology & Biology (LTIB),
CCR, NCI
10 Center Drive
Building 10, Room 13N240A
Bethesda, MD 20892
Phone: 301-480-0202
Email: julius.strauss@nih.gov

Investigational Agents:

Drug Name:	ETBX-011; adenoviral CEA vaccine	ETBX-061; adenoviral MUC1 vaccine	ETBX-051; adenoviral brachyury vaccine
IND Number:	17676	17676	17676
Sponsor:	Center for Cancer Research	Center for Cancer Research	Center for Cancer Research
Manufacturer:	Etubics	Etubics	Etubics

PRÉCIS

Background:

- The overall goal of the current project is to expand our immunotherapeutic approach for the treatment of advanced cancer employing a multi-targeted approach.
- Therapeutic cancer vaccines targeting overexpressed proteins offer a potential method to activate T cells against tumors.
- A novel adenovirus based vaccine targeting three (3) human tumor associated antigens (TAA), CEA, MUC1, and brachyury, respectively has demonstrated anti-tumor cytolytic T cell responses in pre-clinical animal models of cancer.

Objectives:

- To determine the overall safety and recommended phase 2 dose of a combination of three immunotherapeutic vaccines (ETBX-011/ETBX-061/ETBX-051), when administered subcutaneously (SC) to subjects with advanced solid tumors

Eligibility:

- Subjects age 18 and older with cytologically or histologically confirmed locally advanced or metastatic solid tumor malignancy who have completed or had disease progression on at least one prior line of disease-appropriate therapy or who are not candidates for therapy of proven efficacy for their disease.
- Subjects may have measurable or non-measurable but evaluable disease. Subjects with surgically resected metastatic disease at high risk of relapse are also eligible.
- ECOG performance status ≤ 1
- Adequate organ and bone marrow function
- Subjects with active autoimmune diseases requiring systemic treatment and subjects requiring systemic steroids (except for physiologic doses for steroid replacement) are not allowed

Design:

- This is a Phase I trial in subjects with advanced cancer. A combination of three therapeutic vaccines (ETBX-011, ETBX-51, EBX-61) using the same modified Adenovirus vector backbone, separately encoding three well-studied tumor-associated antigens will be assessed. The vaccine will be tested at a single dose level, and a dose de-escalation design (if required). The dose level of each vaccine tested will be 5×10^{11} VP. This dose has been found in prior phase 1 testing of Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011) to be well tolerated (with no dose-limiting toxicities (DLTs) or related Serious adverse events (SAEs), and optimal for induction of immune responses. Each of the three vaccines will be administered subcutaneously (SC) at separate injection sites (proximal limb, preferably the thigh), every 3 weeks for 3 doses, then bi-monthly (every 8 week) boosts for up to a year.
- Up to six patients will be enrolled at Dose Level 1. If ≤ 1 of 6 patients experience a DLT, initiation of the dose expansion phase will occur. If ≥ 2 of 6 experience DLT at Dose Level 1, then dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level Dose Level -1 (1×10^{11} VP). If ≤ 1 of 6 patients experience a DLT, then the maximum tolerated (MTD) will be declared at this dose, and initiation of the dose expansion phase

will occur. If ≥ 2 of 6 experience DLT at Dose Level -1, then a protocol amendment may be written to evaluate a further dose de-escalation.

- A dose expansion phase of study will be enrolled after the MTD of the combination vaccine has been determined. An additional 4 subjects will be enrolled in the dose expansion component of the trial, for a total of 10 subjects at the MTD.
- The ETBX-011, ETBX-51 and ETBX-61 vaccines will be administered SC every 3 weeks for 3 doses, and then bi monthly boosts for up to a year. Evaluations including immunological assessments will be carried out at baseline, on days of vaccination, and after the last vaccination.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
β-HCG	β-Human chorionic gonadotropin
Ad	Adenovirus
Ad5	Adenovirus serotype-5
Ad5 [E1-]	Ad5 with deletions in the early 1 (<i>E1</i>) and early 3 (<i>E3</i>) gene regions
Ad5 [E1-, E2b-]	Ad5 with deletions in the early 1 (<i>E1</i>), early 2b (<i>E2b</i>), and early 3 (<i>E3</i>) gene regions
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSI	BioSpecimen Inventory
BUN	Blood urea nitrogen
CAP	College of American Pathologists
CBC	Complete blood count
CCR	(NCI) Center for Cancer Research
CEA	Carcinoembryonic Antigen
CLIA	Clinical Laboratory Improvement Amendments
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
CR	Complete response
CRADA	Cooperative Research and Development Agreement
CRF	Case report form
CSC	Cancer stem cell
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DFS	Disease-free survival
DCR	Disease control rate
DLT	Dose-limiting toxicity
DLTs	Dose-limiting toxicities
E1	Adenovirus early 1 gene
E2b	Adenovirus early 2b gene

Abbreviation or Specialist Term	Explanation
E3	Adenovirus early 3 gene
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EOS	End of study
ETBX-011	Ad5 [E1-, E2b-]-CEA
ETBX-051	Ad5 [E1-, E2b-]-Brachyury
ETBX-061	Ad5 [E1-, E2b-]-MUC1
ETBX-081	Combination of ETBX-011 + ETBX-051 + ETBX-061 Vaccines
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM-CSF	Granulocyte-macrophage colony-stimulating factor
cGMP	Current Good Manufacturing Practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHS	Health and Human Services
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HTD	Highest tested dose
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
INR	International normalized ratio
IRB	Institutional Review Board
ISH	In situ hybridization
LLD	Longest lesion diameter
LLN	Lower limit of normal

Abbreviation or Specialist Term	Explanation
MDSC	Myeloid-derived suppressor cell
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
MUC1	A Transmembrane glycoprotein
MUC1c	A modified MUC1 with agonist epitope
NCI	National Cancer Institute
NIH	National Institutes for Health
NK	Natural killer (cell)
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
PT	Prothrombin
PTT	Partial thromboplastin time
aPTT	Activated partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious adverse event
SC	Subcutaneous
sCD27	Soluble CD27
sCD40L	Soluble CD40L
SD	Stable disease
SOC	System Organ Class
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reactions
TAA	Tumor-associated antigen
Treg	Regulatory T cells
ULN	Upper limit of normal
VP	Virus particles

1 INTRODUCTION

1.1 STUDY OBJECTIVES AND ENDPOINTS

1.1.1 Primary Objectives

To determine the overall safety and recommended phase 2 dose of a combination of three immunotherapeutic vaccines (ETBX-011, ETBX-061, and ETBX-051), when administered subcutaneously (SC) to subjects with advanced solid tumors.

1.1.2 Secondary Objectives

To make preliminary assessments of:

- Objective response rate (ORR),
- Disease control rate (DCR),
- Duration of response,
- Progression-free survival (PFS)
- Overall survival (OS)

in subjects with advanced solid tumors treated with the combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen.

1.1.3 Exploratory Objectives

To evaluate the ability of the combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen to generate T-cell responses specific for Brachyury, MUC1, and CEA.

1.1.4 Primary Endpoints

- Dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD) or highest tested dose (HTD).
- Treatment-emergent adverse event (AEs) and serious adverse events (SAEs).
- Clinically significant changes in safety laboratory tests, physical examinations, electrocardiograms (ECGs), and vital signs.

1.1.5 Secondary Endpoints

- Objective response rate (ORR; confirmed complete or partial response) according to the Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1.
- Disease control rate (DCR; confirmed response or stable disease [SD] lasting for at least 6 months).
- Duration of response.
- PFS
- OS

1.1.6 Exploratory Endpoints

- Immunogenicity via the induction of antigen-specific T cells of the combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen by flow cytometric analysis, and analysis in sera of sCD27 and sCD40L.

1.2 BACKGROUND AND RATIONAL

1.2.1 Overall Objective

As a result of the discovery of new biomarkers associated with tumor development and metastasis, many tumor-associated antigens (TAAs) are being utilized in immunotherapeutic modalities designed to induce anti-tumor directed cytotoxic immune responses. It is increasingly clear that not any one of these TAA is sufficient, as a single entity, by which one can develop a potent immunotherapeutic response. Furthermore, the addition of immune checkpoint inhibitors has augmented the immunotherapy approach against TAAs. Consequently, our efforts are focused on developing multi-targeted approaches. The overall goal of the current project is to expand our immunotherapeutic approach for the treatment of advanced cancer employing a multi-targeted approach. Safety and ability to generate CEA-specific T-cell responses in metastatic colorectal cancer (mCRC) patients using our Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011) immunotherapeutic as a single agent has been achieved in a clinical setting(1, 2). Patients in that study exhibited evidence of a favorable survival probability, with all 25 patients treated at least 2 times with vaccine exhibiting a 12-month overall survival probability of 48%, with a mean overall survival of 11 months. The phenotypic heterogeneity in terms of expression of different TAAs in a given primary or metastatic tumor mass is a well-established phenomenon(3-7). One can speculate that the use of an immunotherapeutic vaccine regimen targeting three distinct TAAs, each of which is widely expressed on the majority of human carcinomas, would be potentially therapeutically advantageous over the use of a vaccine targeting only one TAA. With the safety and immunogenicity of Ad5 [E1-, E2b-]-CEA established in patients as a single agent, a multi-target approach is now being investigated. The objective is to develop a combination immunotherapeutic approach designed to induce broad anti-tumor immune responses directed against tumors that over express CEA, MUC1, and/or Brachyury. Preclinical studies were recently published indicating that this multi-targeted CEA, MUC1, Brachyury Ad5 combination vaccine induces immune responses directed against all three target CEA, MUC1, and Brachyury antigens with minimal to no “antigenic competition” in human *in vitro* studies or in murine vaccination studies(8). Testing of a combination multi-targeted CEA, MUC1, and Brachyury targeted adenoviral vector-based vaccines in a phase 1 clinical trial is planned to test the safety, ability to generate T-cell responses to each tumor antigen, and efficacy of the immunotherapeutic. Subsequent trials will involve the use of this vaccine in combination with checkpoint inhibitor monoclonal antibodies (MAbs) and other immune modulators.

1.2.2 Brachyury Expression in Cancer and Its Use as an Immunotherapy Target

Brachyury is an embryonic transcription factor of the T-box family that regulates the formation of the posterior mesoderm in the developing embryo, a process that requires the conversion of epithelial cell layers into mesenchymal cells(9). While in the majority of adult normal tissues Brachyury is undetectable, with the exception of low levels found in normal testis, thyroid and a subset of B cells (10, 11), aberrantly high levels of Brachyury have been observed in the primary and/or metastatic sites of non-small cell (NSCLC) and small cell (SCLC) lung cancer (12, 13), colon (14), hepatocellular (15), prostate (16) and breast carcinomas (17), including triple negative breast cancer (TNBC) (18). High levels of Brachyury are also characteristic of the rare tumor type chordoma (19, 20), which is thought to originate from remnants of the embryonic notochord where Brachyury is normally found. Recent studies have now characterized the role of

Brachyury in the biology of carcinomas and demonstrated the ability of this transcription factor to drive the phenotypic conversion of tumor cells from an epithelial to a mesenchymal-like phenotype (also designated as an epithelial-mesenchymal transition, EMT, or carcinoma “mesenchymalization”) (21, 22). Carcinoma cells undergoing this phenotypic transition exhibit enhanced motility and invasiveness *in vitro*, propensity to metastasize *in vivo*, and features of tumor stemness (23), including resistance to a range of therapeutics such as chemotherapy, radiation, small molecule therapies and, potentially, immunotherapy (24-27). In agreement with a role for Brachyury in the progression of carcinomas, multiple studies have now shown that the level of Brachyury in the primary tumor correlates with poor patient prognosis in carcinomas of the lung (28), colon (14), breast (17), triple negative breast (18) and gastrointestinal stromal tumor (GIST) (29). Brachyury expression has also been shown to be correlated with advanced stage prostate cancer (16).

Transcription factors have been considered “difficult to drug” due to their primary location in the nucleus and lack of a hydrophobic groove for drug attachment. Studies have shown, however, that Brachyury-specific T cells can be generated both *in vitro* and *in vivo* (30, 31). Utilizing 9-mer peptides of the Brachyury protein, for example, Brachyury-specific CD8⁺ T cells have been expanded *in vitro* from the blood of cancer patients; these Brachyury-specific CD8⁺ T cells were utilized in cytotoxic assays for effective lysis of human tumor cells that endogenously express Brachyury (30, 31).

The inherent immunogenicity of Brachyury was also revealed from the analysis of immune responses in cancer patients immunized against CEA or PSA. In addition to generating responses against the tumor-associated antigens contained within their respective vaccines, development of Brachyury-reactive CD8⁺ T cells was also observed(32). This expansion of Brachyury-specific T cells may have been the result of cross-presentation of the antigen to the immune system, following tumor destruction in response to the vaccine. These studies demonstrated that Brachyury is immunogenic, and has the potential to function as a target for anticancer vaccination. In addition, two recently completed Phase I clinical studies with a recombinant yeast-Brachyury vaccine(33) or an MVA-Brachyury-TRICOM vaccine(33, 34) also demonstrated the generation of Brachyury-specific T cells as well as safety in humans, thus providing further evidence of immunogenicity(35). These combined properties, i.e., tumor-restricted expression, relevant function in tumor progression, and immunogenicity, make Brachyury a potential target for immunotherapy-mediated approaches against cancer. Preventing or reverting the EMT process in carcinomas via the use of Brachyury-based cancer vaccines represents an attractive modality to minimize tumor dissemination and the emergence of therapeutic resistance.

1.2.3 CEA Expression in Cancer and Its Use as an Immunotherapy Target

CEA represents an attractive target for immunotherapy since it is over expressed on all metastatic colorectal cancer (mCRC) adenocarcinomas and can be overexpressed in other cancers including breast, lung, gastric, pancreatic, bladder, medullary thyroid, head and neck, cervical, hepatic, lymphoma, and melanoma. It has also been identified as one of the priority cancer antigens most likely to be successful at generating a cancer immunotherapeutic(36). A reason that CEA is a good target for T cell-mediated immunity in humans is that it contains known epitopes recognized via an MHC-restricted fashion by human cytolytic T lymphocytes (CTL) that bind to MHC loci HLA-

A2, A3, and A24(8, 37). Of the HLA-A2 restricted epitopes of CEA, CAP1, a nine amino acid sequence, has been reported to stimulate CTL in cancer patients immunized with vaccinia-CEA. CAP1(6D) is a peptide analog of CAP1. Its sequence includes a heteroclitic (non-anchor position) mutation, resulting in an amino acid change from asparagine (Asn) to aspartic acid (Asp), to enhance recognition by the T-cell receptor without any change in binding to HLA A2. Compared with the non-mutated CAP1 epitope, CAP1(6D) has been shown to enhance sensitization of CTL by 100 to 1,000 times(37). The CAP1(6D) epitope has been incorporated into the vector platform (Ad5 [E1-, E2b-]-CEA(6D)) and has been safely tested in mCRC patients to induce active cell-mediated immunity (CMI) against CEA(1, 2).

1.2.4 MUC1 Expression in Cancer and Its Use as an Immunotherapy Target

MUC1 (CD227) is a TAA that is overexpressed on a majority of human carcinomas and several hematologic malignancies(38-41). MUC1 is normally expressed at the surface of glandular epithelial cells(42) and, in carcinomas, it is overexpressed and aberrantly hypoglycosylated(40, 42, 43). Several clinical trials have been and are being performed to evaluate the use of MUC1 in immunotherapeutic vaccines(2, 44-46). Some of these trials have indicated that targeting MUC1 is safe and may provide survival benefit(2, 45, 47). Multiple enhancer agonist epitopes were previously identified, several of which are in the MUC1 C-terminus region(48, 49). This is potentially important because studies(47) have demonstrated that the C-terminus of MUC1 has oncogenic potential, associates with poor prognosis and drug resistance, and induces “stemness” features in a range of human carcinomas. The human T-cell lines generated using these MUC1 agonist epitopes were more efficient than those generated with the corresponding native epitopes in terms of antigen-specific interferon (IFN)- γ production and lysis of tumor cells endogenously expressing native MUC1(48, 49). Therefore, it is believed that MUC1 containing modified agonist epitopes has a greater potential as an immunogenic agent for vaccine development.

1.2.5 Adenovirus-Based Vectors

Adenoviruses (Ads) have emerged as leading candidate vectors to deliver vaccines designed to induce CMI and antibody responses (50-52). Ad vectors infect multiple cell types, including dendritic cells which result in priming of a vigorous e response. Ads are a family of DNA viruses characterized by an icosahedral, non-enveloped capsid containing a linear double-stranded genome (51, 52). None of the human Ads are associated with neoplastic disease and only cause relatively mild, self-limiting illness in immunocompetent individuals. Ad serotype-5 (Ad5) is the most widely used subtype for human vaccines. The wild-type Ad5 genome is approximately 36 kilobases and encodes genes that are divided into early and late viral functions, depending on whether they are expressed before or after DNA replication. Ad5 vectors do not integrate (i.e., their genomes remain episomal), so the risk for insertional mutagenesis and/or germ-line transmission is extremely low if at all.

1.2.5.1 Early Generation Ad5 Vectors

Early generation Ad5 vectors (Ad5 [E1-]) contain deletions in the early 1 (*E1*) gene and early 3 (*E3*) gene regions (50-53). The *E1* gene is required for DNA synthesis, capsid protein expression, and viral replication, and the *E3* gene is required for anti-host immunity. Ad5 [E1-] vectors have a decreased ability to replicate and cannot produce infectious virus in cells that do not express the Ad5 *E1* genes. Recombinant Ad5 [E1-] vectors are propagated in human cells (typically human embryonic kidney 293 cells), allowing for Ad5 [E1-] vector replication and packaging (51). There

have been over 300 human clinical trials that utilized Ad5 [E1-] vectors, with more than 2000 subjects given the virus SC, intramuscularly, or intravenously (52). Ad5 [E1-] vectors have a number of positive attributes; one of the most important is their relative ease for scale up and current good manufacturing practices (cGMP) production. Furthermore, recombinant Ad5 [E1-] vectors have a large carrying capacity that approaches 7 kilobases. However, preclinical and clinical studies have demonstrated that pre-existing immunity against Ad5 can be an inhibitory factor to the use of Ad5 [E1-] vaccines in practice (51, 53). Most humans have antibodies against Ad5, with up to two-thirds having lymphoproliferative responses against Ad5 (54, 55). CMI directed against pre-existing or newly synthesized Ad5 cell surface proteins interfere with Ad5 [E1-] vaccines by eliminating the vaccine and vector-infected cells. This reduces the effectiveness of the early generation Ad5 [E1-]-based vaccines.

1.2.5.2 New Generation Ad5 Vectors (Ad5 [E1-, E2b-])

A new and advanced generation of Ad5 vectors has been developed that, in addition to deletions in the *E1* and *E3* gene regions, have deletions in the early 2b (*E2b*) gene regions (Ad5 [E1-, E2b-]) (56-59). The *E2b* genes are required for viral replication and encode viral DNA polymerase as well as the preterminal protein. In addition, the deletion of the *E2b* genes drastically reduces late gene expression (capsid type proteins), which decreases anti-vector immune responses and enables longer term transgene expression with enhanced immunogenicity. Thus, the Ad5 [E1-, E2b-] vector overcomes limitations of early generation vectors, as it permits the immunization of people who have been previously exposed to Ad5. In preclinical studies of cancer and infectious disease, Ad5 [E1-, E2b-] vector-based vaccines were used in multiple homologous immunization regimens and induced immune responses despite the presence of pre-existing Ad5 immunity (2, 8, 59-68).

1.2.6 Clinical Experience with an Ad5 [E1-, E2b-]-based Cancer Immunotherapy Vaccine

1.2.6.1 Clinical Safety

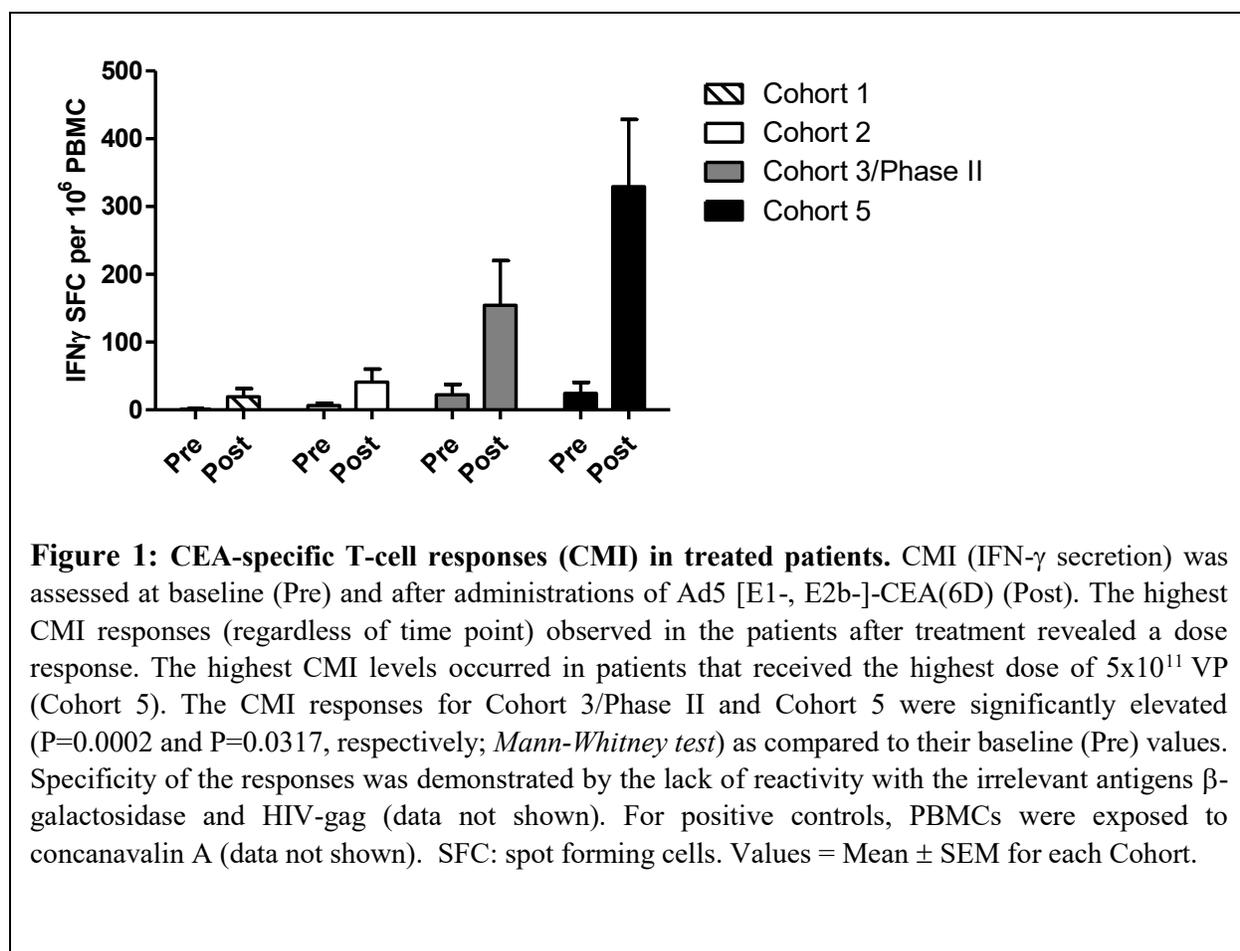
Etubics Corporation has performed a Phase I/II clinical trial (IND#14325) with an Ad5 [E1-, E2b-]-based vector containing a modified carcinoembryonic antigen (Ad5 [E1-, E2b-]-CEA(6D)) for the immunotherapy of CEA expressing cancer(1, 2). The Phase I/II study consisted of a dose-escalation study of four dosage levels (1×10^9 , 1×10^{10} , 1×10^{11} , 5×10^{11} VP/dose) of ETBX-011 (Phase I component), and the maximally tolerated dose of ETBX-011 (Phase II and 5×10^{11} VP/dose components). Ad5 [E1-, E2b-]-CEA(6D) was administered by SC injection every 3 weeks. Thirty-two patients with metastatic colorectal cancer (mCRC), median age 57.5 (range 38–77) who had failed a median of three prior chemotherapeutic regimens (range 2–5), had a performance status of 90% (range 70–100%), and had three sites of metastatic disease (range 1–4), were enrolled. The majority of patients were able to receive all three immunizations. Four patients who stopped immunizations early did so due to significant disease progression. A total of 94 immunization treatments was administered to all patients. There was no dose-limiting toxicity and no serious adverse effects (SAE) that resulted in treatment discontinuation at any vaccine dose level. The most common toxicity was a self-limited, injection site reaction. Other reactions occurred with less than a 10% incidence of all adverse effects (AE) reported and included fever, flu-like symptoms, anorexia, chills, nausea, and headache. These symptoms were also self-limiting and did not require intervention other than symptomatic measures such as acetaminophen(1, 2).

1.2.6.2 Immune Responses

A secondary objective of the Ad5 [E1-, E2b-]-CEA(6D) Phase I trial was to evaluate CEA-specific immune responses following vaccination. As determined by an ELISA technique(1, 2), no antibody activity directed against CEA was observed. CEA-specific cell-mediated immunity (CMI) responses were observed in colorectal cancer patients treated in cohort 1, cohort 2, cohort 3/Phase II, and cohort 5. PBMCs were isolated prior to immunotherapy treatment and after all treatments as well as 3 weeks following the last treatment from patients. CEA-specific ELISpot assays were performed on PBMC as previously described(1, 2, 61) to determine the numbers of interferon gamma (IFN- γ) secreting lymphocytes (SFC) after exposure to CEA peptides *in vitro*. This analysis revealed a dose response to increasing levels of vaccine (Figure 1). The highest CMI levels occurred in patients that received the highest dose of 5×10^{11} VP (Cohort 5).

In(1), a population of polyfunctional CD8+ T cells (those that secrete more than 1 cytokine when activated) was identified that after immunizations secreted multiple cytokines, a sign of greater functionality of T cells induced by the vaccine. In further follow-up analysis(1) of a few patient blood samples, a decrease in CEA-directed immune responses was noted after immunotherapy immunizations was stopped. This observation supports a rationale for booster immunizations to maintain immune responses.

Anti-Ad5 antibody (Ab) and CMI against Ad5 were correlated with CEA-specific CMI. Each patient had their serum and PBMC sample tested at baseline (prior to treatment) and at 9 weeks after completion of 3 treatments. Nineteen of 31 colorectal cancer patients (61%) tested in this study had Ad5 neutralizing activity in serum samples prior to the onset of treatment with Ad5 [E1-, E2b-]-CEA(6D). The mean pre-treatment Ad5 Ab titer value obtained among all patients was $1:189 \pm 1:71$ SEM and the mean pre-treatment Ad5 Ab titer among seropositive patients was $1:308 \pm 1:108$. Analysis of serum samples from patients who received 3 immunizations revealed Ad5 Ab titers that were significantly increased ($P < 0.0001$, *Mann-Whitney test*) by week 9 (mean $1:4767 \pm 1:1225$ SEM) when compared with their respective baseline values. Analysis of PBMC for CMI responses to Ad5 also revealed a significant increase ($P < 0.01$, *Mann-Whitney test*) in Ad5-directed CMI responses after immunizations with Ad5 [E1-, E2b-]-CEA(6D) (22.6 ± 9.3 SEM IFN- γ secreting SFC at week 0 versus 191.1 ± 83.7 IFN- γ SFC at week 9).



1.2.6.3 Analysis of Clinical Activity

The Ad5 [E1-, E2b-]-CEA(6D) vaccinated heavily pretreated colorectal cancer patients (total=32) were followed for survival and Kaplan-Meier plots and survival proportions performed (PRISM software)([1](#), [2](#)). Events were determined by information from the social security death index (SSDI) database, clinical charts and telephone calls ([Figure 2](#)).

The seven patients in cohorts 1 and 2 experienced a 12-month survival proportion of 29%. The 21 patients in cohort 3 and Phase II experienced a 12-month survival proportion of 48%. The six patients in cohort 5 experienced a 12-month survival proportion of 50%. Twenty-nine-month overall survival of the intent-to-treat population (32 patients) was 20% ([Figure 2a](#)) with a median survival time of 11 months from informed consent/first injection. For the subset of 28 patients that received all 3 immunizations, the 29-month survival was 23% ([Figure 2b](#)) with a median survival time of 13 months. For the 22 patients optimally dosed with the two highest doses of vaccine (1 and 5×10^{11}) and receiving all 3 immunizations, the 28-month overall survival was 19% ([Figure 2c](#)). Median overall survival was 13 months in the optimally treated patients. Since there was no active control group in the study, comparisons for significance in survival time cannot be made. There were 3 stable disease events observed immediately after completion of treatment.

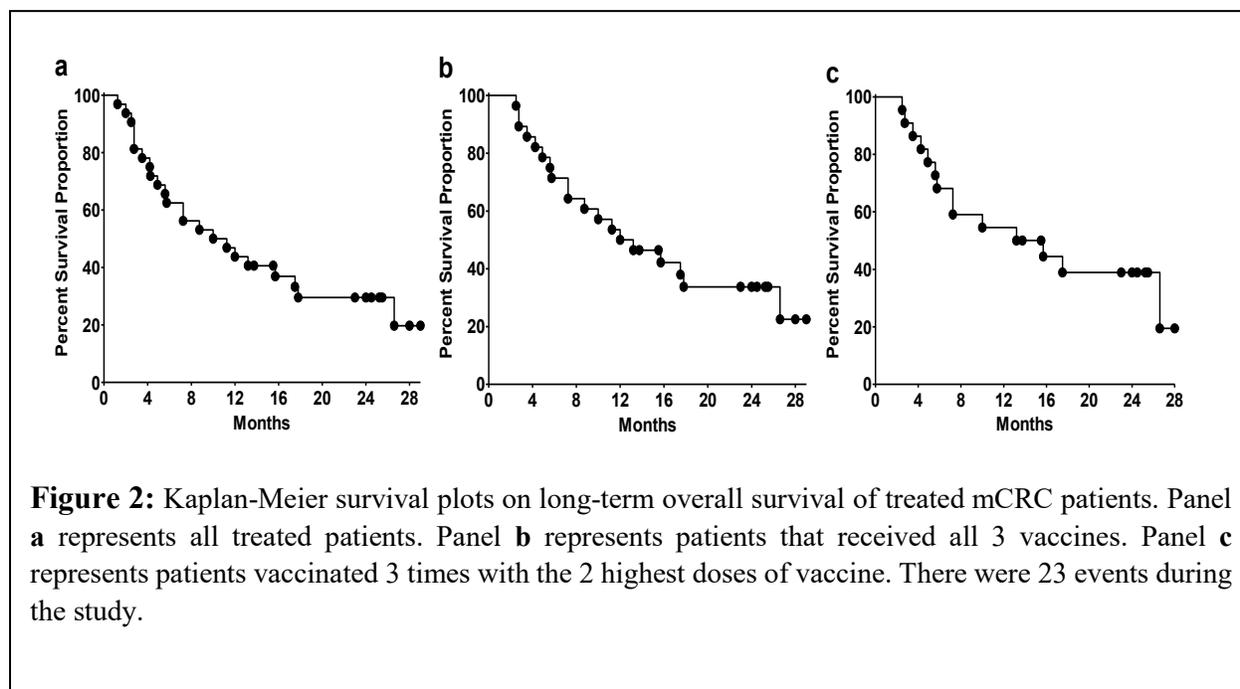


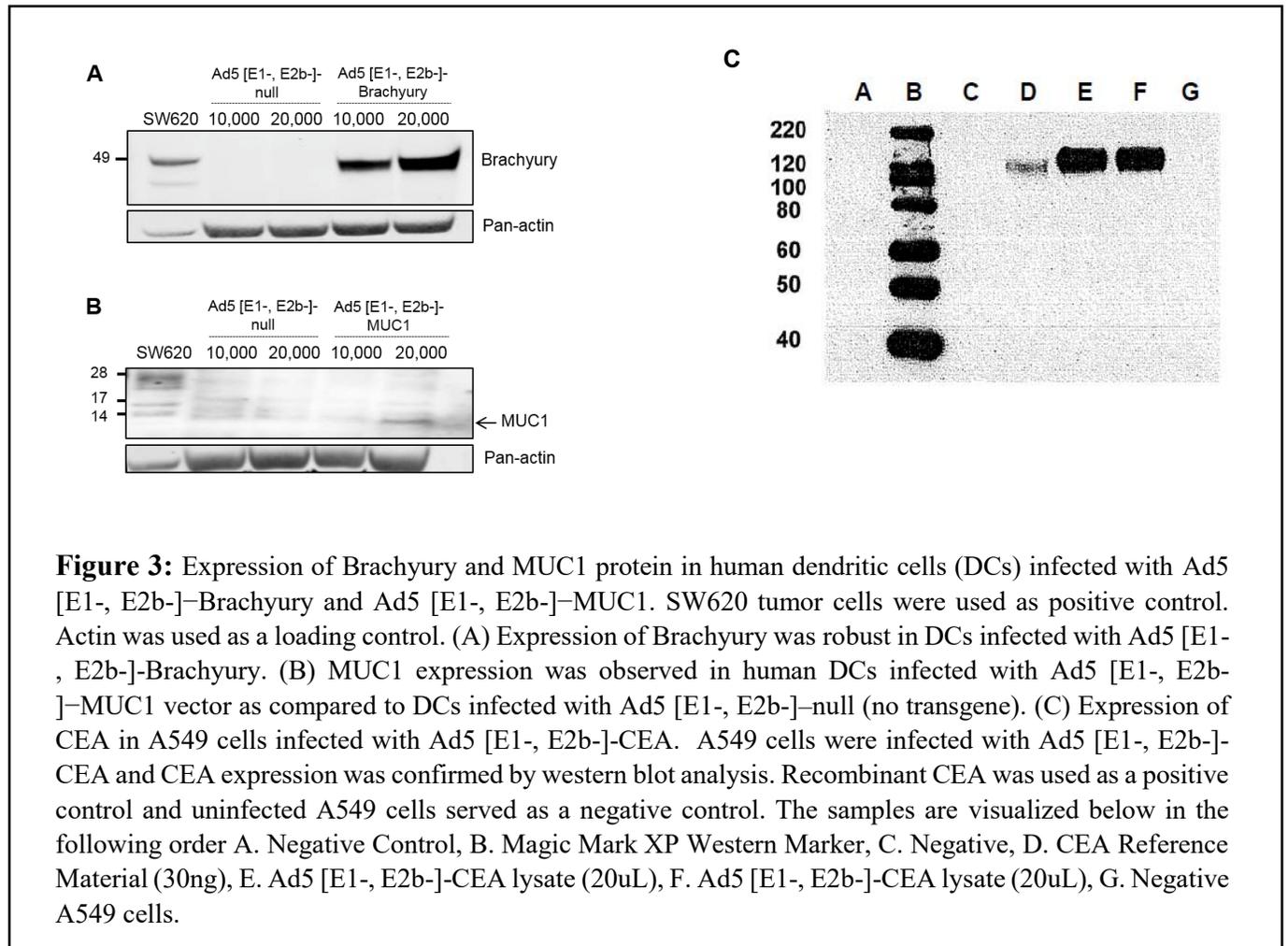
Figure 2: Kaplan-Meier survival plots on long-term overall survival of treated mCRC patients. Panel **a** represents all treated patients. Panel **b** represents patients that received all 3 vaccines. Panel **c** represents patients vaccinated 3 times with the 2 highest doses of vaccine. There were 23 events during the study.

1.2.7 ETBX-081 Preclinical Studies

Recombinant Ad5 [E1-, E2b-]-CEA, recombinant Ad5 [E1-, E2b-]-MUC1 and recombinant Ad5 [E1-, E2b-]-Brachyury were generated and characterized as previously described(8). As seen in **Figure 3A**, Western blot analysis using an anti-Brachyury-specific monoclonal antibody (MAb 54-1)(11) revealed Brachyury expression when human dendritic cells (DCs) were infected with Ad5 [E1-, E2b-]-Brachyury. An Ad5 [E1-, E2b-] vector devoid of any transgene (Ad5 [E1-, E2b-]-null) was used as a negative control and SW620 human colon carcinoma cells that endogenously express Brachyury were used as a positive control. An anti-MUC1-specific MAb was used to detect the expression of MUC1 in Ad5 [E1-, E2b-]-MUC1-infected human DCs (**Figure 3B**). SW620 cells, which also express MUC1 endogenously, were used as a positive control. The difference in molecular weights seen in the human DCs versus the SW620 human carcinoma cells is most likely due to the differential glycosylation of the MUC1 protein. As has been previously shown by others(69-72), it would appear that MUC1-C is being expressed in the human DCs predominantly as the unglycosylated 17 or 15 kDa form and not the 25-20 glycosylated species. A Western blot of Ad5 [E1-, E2b-]-CEA infected human cells is shown in **Figure 3**.

The generation of Brachyury-, CEA-, and MUC1-specific human CD8⁺ T cells employing the corresponding peptide for each TAA was previously reported(30, 31, 37, 48, 49, 73). As shown in **Table 1**, Ad5 [E1-, E2b-]-null did not activate any of the T cells to produce IFN- γ . Ad5 [E1-, E2b-]-Brachyury-infected DCs activated Brachyury-specific T cells and not CEA-specific T cells (as a negative control). This demonstrates that the Ad5 [E1-, E2b-]-Brachyury-infected DCs could process Brachyury in a manner that generates Brachyury-MHC Class I complexes capable of specific T-cell activation. Similarly, Ad5 [E1-, E2b-]-CEA-infected DCs specifically activated CEA-specific T cells but not MUC1-specific T-cell lines. Both Class I HLA-A2 and -A24 MUC1-specific T-cell lines have been previously generated(49) and the Ad5 [E1-, E2b-]-MUC1-infected

DCs were capable of activating both of these T-cell lines but not the CEA-specific T-cell line (**Table 1**). Human DCs were similarly infected with the ETBX-081 vector. As seen in **Table 1 A and Table 1 B**, T cells specific for CEA, MUC1, and Brachyury were each activated to induce similar levels of IFN- γ as seen with the use of the individual Ad-5 vectors.



Studies were also undertaken to determine whether simultaneous infection of human DCs with the CEA/MUC1/Brachyury mixture of ETBX-081 could generate T-cell lines specific for all three TAAs. As seen in **Table 2**, when the T cells were activated by incubation with autologous B cells pulsed with the corresponding peptide, and not a control peptide, specific T-cell activation was observed. For example, the Brachyury-specific T-cell line, generated by infecting human DCs with ETBX-081, was stimulated to produce IFN- γ when incubated with autologous DCs pulsed with Brachyury peptide, but was not activated with the same autologous DCs pulsed with a CEA peptide. Similar results were seen with CEA and MUC1 T-cell lines generated with ETBX-081-infected DCs. These results indicate the lack of so-called “antigenic competition” in the *in vitro* use of ETBX-081.

Table 1

Table 1 A: Infection of Human Dendritic Cells with Recombinant Adenovirus Vectors Encoding CEA, MUC1 or Brachyury Can Activate Antigen-specific T-cell Lines

Dendritic cells (DCs) infected with	Antigen-specific T-cell lines			
	CEA	MUC1 (HLA-A2)	MUC1 (HLA-A24)	Brachyury
Ad5 [E1-, E2b]-null	<15.6	<15.6	<15.6	<15.6
Ad5 [E1-, E2b]-Brachyury	<15.6	--	--	351.9
Ad5 [E1-, E2b]-MUC1	<15.6	335.2	806.4	--
Ad5 [E1-, E2b]-CEA	350.0	<15.6	<15.6	--
Uninfected DCs	<15.6	<15.6	<15.6	<15.6
T cells only	<15.6	<15.6	<15.6	<15.6

Human DCs (6-day culture in IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) 2×10^4 cells/well in 0.5 ml of AIM-V) were infected with indicated adenovirus vectors at 20,000 multiplicity of infection (MOI). After 48 hours, DCs were washed and used for stimulation of human antigen-specific T cells. Results are expressed in pg/ml of IFN- γ per 1×10^5 T cells/ml. Numbers in bold indicate a significant enhancement of IFN- γ secretion compared to corresponding wells with uninfected DCs. [-- indicates that the assay was not performed.]

Table 1 B: Infection of Human Dendritic Cells with ETBX-081 Vectors Encoding Transgenes Can Activate Antigen-specific T-cell Lines to Produce IFN- γ

Dendritic cells (DCs) infected with	Antigen-specific T-cell lines			
	CEA (HLA-A2)	MUC1 (HLA-A2)	MUC1 (HLA-A24)	Brachyury (HLA-A2)
ETBX-081	480	236	763	496
Ad5 [E1, E2b]-null	<15.6	<15.6	<15.6	<15.6
Uninfected DCs	<15.6	<15.6	<15.6	<15.6
T cells only	<15.6	<15.6	<15.6	<15.6

Human DCs (6-day culture in IL-4 and GM-CSF) from an HLA-A2 and -A24 donor were infected with ETBX-081 vector at 2×10^4 /well (24-well plate) in 0.5 ml of AIM-V. ETBX-081 vectors were used at 20,000 MOI for 1 hour and then 1.5 ml of AIM-V were added to each well. Infected DCs were incubated for 48 hours and then washed and used for stimulation of human antigen-specific T cells. Results are expressed in pg of IFN- γ per 1×10^5 T cells/ml. Numbers in bold indicate a significant enhancement of IFN- γ secretion compared to corresponding wells with uninfected DCs.

Table 2: Infection of Human Dendritic Cells with ETBX-081 Can Generate Antigen-specific T Cells to Brachyury, MUC1 and CEA and Produce IFN- γ When Stimulated with Autologous B Cells Pulsed with the Corresponding Peptides

Antigen-specific T-cell lines	Peptides (10 μ g/ml)			
	CEA	MUC1 (A2)	MUC1 (A24)	Brachyury
T-Brachyury	<15.6	--	--	243
T-MUC1 (A2)	<15.6	174	--	--
T-MUC1 (A24)	<15.6	--	206	--
T-CEA	211	<15.6	--	--

Human dendritic cells (DCs) from a prostate cancer patient (6-day culture in IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) 2×10^4 cells/well in 0.5 ml of AIM-V) were infected with ETBX-081 at 20,000 MOI. After 48 hours, infected DCs were washed and used to generate specific cytotoxic T lymphocytes (CTLs) using autologous peripheral blood mononuclear cells (PBMCs) as effectors. Following 3 cycles of in vitro stimulations, autologous peptide-pulsed B cells were used as antigen-presenting cells. Results are expressed in pg/ml of IFN- γ . [-- indicates that the assay was not performed.]

Table 3: Infection of Human DCs with ETBX-081 Can Generate Brachyury-, MUC1- and CEA-specific CTLs That Efficiently Lyse Tumor Cells Expressing All Three Antigens

Antigen-specific T-cell lines	SW620	ASPC-1
	Brachyury ⁺ MUC1 ⁺ CEA ⁺ (HLA-A2 ⁺ /A24 ⁺)	Brachyury ⁺ MUC1 ⁺ CEA ⁺ (HLA-A1 ⁺ /A26 ⁺)
T-Brachyury	64.4 (3.6)	8.3 (2.7)
T-MUC1 (P93L)	28.5 (1.3)	2.0 (1.6)
T-MUC1 (C6A)	49.3 (3.3)	5.0 (1.8)
T-CEA	42.4 (3.7)	4.3 (1.9)

Human dendritic cells (DCs) were infected with ETBX-081 at 20,000 MOI. Infected DCs were used to generate specific cytotoxic T lymphocytes (CTLs) using autologous peripheral blood mononuclear cells (PBMCs). Autologous DCs were used as antigen-presenting cells for three in vitro stimulations (IVS). Autologous peptide-pulsed B cells pulsed were used to re-stimulate antigen-specific CTLs for two additional IVS. The effector-to-target ratio used was 30:1; CTLs were used at IVS 5. Results are expressed in % specific lysis (SD).

Studies also investigated whether Brachyury-, MUC1-, and CEA-specific human T cells generated using DCs infected with ETBX-081 could lyse human carcinoma cells that endogenously express these TAAs. SW620 human colon carcinoma cells express all three TAAs and possess the HLA-

A2 and -A24 Class I alleles. ASPC-1 human pancreatic carcinoma cells were used as a negative control since they express the three TAAs but in the context of HLA-A1 and -A26 molecules. The results (**Table 3**) demonstrated that ETBX-081–infected human DCs can generate T cells capable of lysing, in an MHC-restricted manner, human tumor cells that endogenously express Brachyury, CEA, and MUC1.

Studies were also undertaken to determine whether Ad5 [E1-, E2b-]–Brachyury, Ad5 [E1-, E2b-]–MUC1, and Ad5 [E1-, E2b-]–CEA could each generate TAA-specific T-cell responses *in vivo*, and whether the ETBX-081 mixture could generate comparable responses. C57Bl/6 mice (n=5 per group) were injected subcutaneously (s.c.) three times at 2-week intervals with 10^{10} viral particles (VP) of Ad5 [E1-, E2b-]–CEA, Ad5 [E1-, E2b-]–MUC1, Ad5 [E1-, E2b-]–Brachyury, or ETBX-081 (1:1:1 mixture of 10^{10} VP each). An additional group of mice (n=5) received 3×10^{10} VP of Ad5 [E1-, E2b-]–null (an empty vector control). Two weeks after the final vaccination, splenocytes from vaccinated mice were stimulated with corresponding Brachyury, CEA, or MUC1 peptide pools and analyzed for IFN- γ and IL-2 secreting cells by the enzyme-linked immunospot (ELISPOT) assay. Mice vaccinated with singular constructs or with ETBX-081 responded to Brachyury, CEA, and MUC1 peptides, respectively, with significant increases in IFN- γ and IL-2 spot forming cells (SFCs) as compared to control mice (**Figure 4A and B**). There was no significant difference in the average number of IFN- γ SFCs in mice vaccinated with Ad5 [E1-, E2b-]–Brachyury or Ad5 [E1-, E2b-]–CEA individually as compared with the ETBX-081 vaccine. There was a significant decrease in IFN- γ SFCs in mice treated with the ETBX-081 vaccine as compared to Ad5 [E1-, E2b-]–MUC1 alone, although the MUC1–specific immune response induced by ETBX-081 remained significantly elevated over control mice ($p < 0.0001$) (**Figure 4A**). IL-2 responses were similar in mice treated with ETBX-081 versus single vaccine constructs; moreover, there was a significant increase ($p = 0.004$) in CEA-specific IL-2 SFCs when mice were vaccinated with the ETBX-081 vaccine versus the Ad5 [E1-, E2b-]–CEA vaccine alone (**Figure 4B**). Taken together, these data indicate that combining Ad5 [E1-, E2b-]–Brachyury, Ad5 [E1-, E2b-]–CEA, and Ad5 [E1-, E2b-]–MUC1 in a ETBX-081 vaccine admixture has the effect of generating antigen-specific IFN- γ – and IL-2–producing cells similar to that achieved when using each vaccine alone.

Figure 4: Analysis of IFN- γ – and IL-2–expressing splenocytes following vaccination of mice with Ad5 [E1-, E2b-]–Brachyury, Ad5 [E1-, E2b-]–CEA, Ad5 [E1-, E2b-]–MUC1, ETBX-081, and Ad5 [E1-, E2b-]–null. C57Bl/6 mice (n = 5/group) were vaccinated three times at 2-week intervals with 1010 VP (viral particle) of Ad5 [E1-, E2b-]–Brachyury (white bar), Ad5 [E1-, E2b-]–CEA (grey bar), Ad5 [E1-, E2b-]–MUC1 (black bar) or ETBX-081 (1:1:1 mixture of 1010 VP each of Ad5 [E1-, E2b-]–Brachyury, Ad5 [E1-, E2b-]–CEA, Ad5 [E1-, E2b-]–MUC1) (diagonal hatched bar). Controls received 3×10^{10} VP of Adeno-null (horizontal striped bar). Splenocytes were collected 14 days after the final vaccination and assessed for IFN- γ –secreting cells (A) or IL-2–secreting cells (B) by ELISPOT assay. For positive controls, splenocytes were exposed to Concanavalin A (Con A) (data not shown). Data reported as the number of spot forming cells (SPFs) per 106 splenocytes. The error bars depict the SEM. Significant differences ($p < 0.05$) between columns are reported in p-values, not significant = ns.

Studies were then undertaken to determine whether the ETBX-081 vaccine regimen was as effective as the use of a single recombinant adenovirus construct in eliciting an anti-tumor effect. It should be noted that unlike human carcinomas of which the majority overexpress the human TAAs CEA, MUC1 and Brachyury, no mouse models express all three of these antigens. C57BL/6 mice (n = 7/group) were implanted s.c. with 1×10^6 MC38 cells expressing MUC1 (MC38-MUC1) in the left flank. Mice were vaccinated weekly with s.c. injections in the opposite flank using 10^{10} VP of Ad5 [E1-, E2b-]-MUC1 or ETBX-081, respectively. A control group of mice received 3×10^{10} VP of Ad5 [E1-, E2b-]-null (no transgene). Mice vaccinated with Ad5 [E1-, E2b-]-MUC1 or ETBX-081 had significantly smaller tumors than control mice on days 25 ($p < 0.01$) and 29 ($p < 0.05$) (Figure 5). There was no significant difference ($p > 0.1$) in anti-tumor effect for the groups of mice vaccinated with Ad5 [E1-, E2b-]-MUC1 vs. ETBX-081 at all time points.

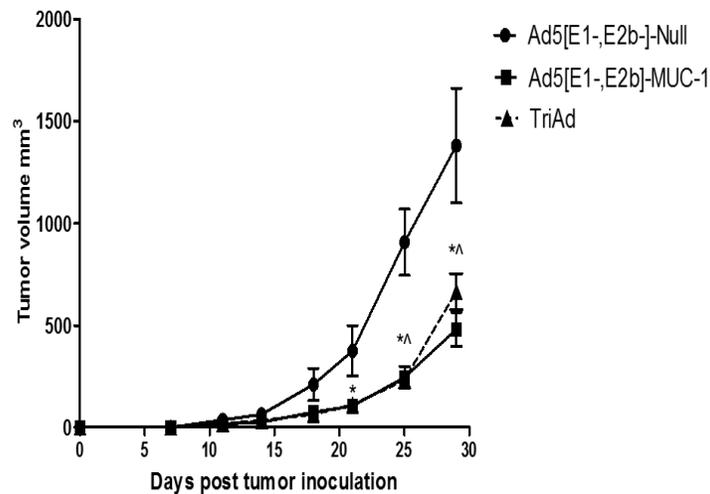


Figure 5: Comparison of immunotherapy of MUC1-expressing tumors using Ad5 [E1-, E2b-]-MUC1 vs ETBX-081. C57Bl/6 mice (n=7/group) were inoculated with 10^6 MC-38-MUC1 cells subcutaneously in the left flank. Mice were administered 10^{10} VP (viral particle) of Ad5 [E1-, E2b-]-MUC1 or ETBX-081 (1:1:1 mixture of 10^{10} VP each of Ad5 [E1-, E2b-]-CEA, Ad5 [E1-, E2b-]-MUC1, and Ad5 [E1-, E2b-]-Brachyury, 3×10^{10} VP total). A control group of mice received 3×10^{10} VP of Ad5 [E1-, E2b-]-null (no transgene). Tumor growth was monitored and volumes calculated. (*) indicates days when Ad5 [E1-, E2b-]-MUC1 treated mice had significantly smaller ($p < 0.05$) tumors than control mice and (^) indicates days when ETBX-081-treated mice had significantly smaller ($p < 0.05$) tumors than control mice. There was no significant difference ($p > 0.1$) between Ad5 [E1-, E2b-]-MUC1 vs. ETBX-081-treated mice at any time point. Error bars

1.2.8 Clinical Experience

The combination of ETBX-011, ETBX-061, and ETBX-051 vaccine has not been tested in humans.

This Phase I study is the first clinical study of this vaccine combination in subjects with advanced cancer.

1.2.9 Rationale

The preclinical data with the combination ETBX-011, ETBX-061, and ETBX-051 vaccine support this Phase I study to evaluate the safety, preliminary efficacy, and immunogenicity of the vaccine. Subsequent trials will involve the use of this vaccine in combination with checkpoint inhibitor monoclonal antibodies (MAbs) and other immune modulators.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

- 2.1.1.1 Age \geq 18 years (male and female).
- 2.1.1.2 Ability to understand and provide signed informed consent that fulfills Institutional Review Board (IRB)'s guidelines.
- 2.1.1.3 Subjects with cytologically or histologically confirmed locally advanced or metastatic solid tumor malignancy.
- 2.1.1.4 Subjects must have completed or had disease progression on at least one prior line of disease-appropriate therapy or not be candidates for therapy of proven efficacy for their disease.
- 2.1.1.5 Subjects may have measurable or nonmeasurable but evaluable disease as defined in section 6.3.1. Subjects with surgically resected locally advanced or metastatic disease at high risk of relapse are also eligible.
- 2.1.1.6 Eastern Cooperative Oncology Group (ECOG) performance status \leq 1 ([Appendices](#)
- 2.1.1.7 [Appendix A](#))
- 2.1.1.8 Subjects who have received prior CEA, MUC1, and/or Brachyury-targeted immunotherapy (vaccine) are eligible for this trial if this treatment was discontinued at least 4 weeks prior to enrollment.
- 2.1.1.9 Resolution of clinically significant side effects of prior chemotherapy, radiotherapy, immunotherapy or surgical procedures to NCI CTCAE Grade \leq 1 or grade \leq 2 for neuropathy.
- 2.1.1.10 Adequate hematologic function at screening, as follows:
 - 2.1.1.10.1 Absolute neutrophil count (ANC) \geq 1 x 10⁹/L
 - 2.1.1.10.2 Hemoglobin \geq 9 g/dL
 - 2.1.1.10.3 Platelets \geq 75,000/mcL.

2.1.1.11 Adequate renal and hepatic function at screening, as follows:

2.1.1.11.1 Serum creatinine ≤ 1.5 x upper limit of normal (ULN) OR creatinine clearance (CrCl) ≥ 40 mL/min (if using the Cockcroft-Gault formula below):

i. *Female* CrCl = [(140 - age in years) x weight in kg x 0.85] / [72 x serum creatinine in mg/dL]

ii. *Male* CrCl = [(140 - age in years) x weight in kg x 1.00] / [72 x serum creatinine in mg/dL]

2.1.1.11.2 Bilirubin ≤ 1.5 x ULN OR in subjects with Gilbert's syndrome, a total bilirubin ≤ 3.0 x ULN

2.1.1.11.3 Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN, unless liver metastases are present, then values must be ≤ 3 x ULN)

2.1.1.12 The effects of the combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen on the developing human fetus are unknown. For this reason, female subjects of childbearing potential defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy or tubal ligation) or who is not postmenopausal (menopause being defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes) and male patients who are not surgically sterile (vasectomy etc.), must agree to use acceptable contraceptive methods for the duration of the study and for one month after the last vaccination. Acceptable forms of contraception include oral contraceptives, intrauterine device, condom or vaginal diaphragm plus spermicidal (gel/foam/cream/vaginal suppository), or total abstinence.

2.1.1.13 Ability to attend required study visits and return for adequate follow up, as required by this protocol.

2.1.2 Exclusion Criteria

2.1.2.1 Pregnant and nursing women. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with combination ETBX-011, ETBX-051, ETBX-061, breastfeeding should be discontinued if the mother is treated with combination ETBX-011, ETBX-051, ETBX-061. These potential risks may also apply to other agents used in this study.

2.1.2.2 There should be a minimum of 4 weeks from any prior investigational drug, chemotherapy, immunotherapy, with the exception of hormonal therapy for prostate and breast cancers, HER2-directed therapy for HER2+ breast or stomach cancer (3+ IHC or FISH+), drugs targeting EGFR, ALK or ROS1 in EGFR, ALK, ROS1-mutated lung cancer, respectively, or standard maintenance therapies for any solid tumor under the condition that subjects are on these therapies for at least two months before start of trial treatment.

2.1.2.3 There should also be a minimum of 4 weeks from any prior radiotherapy except for palliative bone directed therapy.

2.1.2.4 Known active brain or central nervous system metastasis (less than 1 month out from definitive radiotherapy or surgery), or seizures requiring anticonvulsant treatment, or clinically significant cerebrovascular accident or transient ischemic attack (<3 months).

- 2.1.2.5 Subjects with active autoimmune disease requiring systemic immunosuppressive treatment within the past 4 weeks such as but not restricted to inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. A history of autoimmune disease which is not active nor has required recent systemic immunosuppressive therapy (< 4 weeks prior to enrollment) is not reason for exclusion.
- 2.1.2.6 Subjects with serious intercurrent chronic or acute illness, such as cardiac or pulmonary disease, hepatic disease, or other illness considered by the Investigator as high risk for investigational drug treatment.
- 2.1.2.7 Subjects with clinically significant heart disease, such as congestive heart failure (class II, III, or IV defined by the New York Heart Association functional classification), history of unstable or poorly controlled angina, or history (< 1 year) of ventricular arrhythmia.
- 2.1.2.8 Subjects with a medical or psychological impediment that would impair the ability of the subject to receive therapy per protocol or impact ability to comply with the protocol or protocol-required visits and procedures.
- 2.1.2.9 History of second malignancy within 3 years prior to enrollment except for the following: adequately treated non-melanoma skin cancer, cervical carcinoma in situ, superficial bladder cancer or other localized malignancy after discussion with the medical monitor.
- 2.1.2.10 Presence of a known active acute or chronic infection, including human immunodeficiency virus (HIV, as determined by enzyme-linked immunosorbent assay [ELISA] and confirmed by western blot) and hepatitis B and hepatitis C virus (HBV/HCV, as determined by HBsAg and hepatitis C serology).
- 2.1.2.11 Subjects on systemic intravenous or oral corticosteroid therapy with the exception of physiologic doses of corticosteroids (\leq the equivalent of prednisone 10 mg/day) or other immunosuppressives such as azathioprine or cyclosporin A are excluded on the basis of potential immune suppression. For these subjects these excluded treatments must be discontinued at least 2 weeks prior to enrollment for recent short course use (\leq 14 days) or discontinued at least 4 weeks prior to enrollment for long term use ($>$ 14 days). In addition, the use of corticosteroids as premedication for contrast-enhanced studies is allowed prior to enrollment and on study.
- 2.1.2.12 Subjects with known allergy or hypersensitivity to any component of the investigational product will be excluded.
- 2.1.2.13 Subjects 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.
- 2.1.2.14 Subjects vaccinated with a live (attenuated) vaccine (e.g., FluMist[®]) or a killed (inactivated)/subunit vaccine (e.g., PNEUMOVAX[®], Fluzone[®]) within 28 days or 14 days, respectively, of the first planned dose of ETBX vaccine.

2.1.3 Recruitment Strategies

This study will be listed on available websites (www.clinicaltrials.gov, <https://ccr.cancer.gov/clinical-trials-search-start>) and participants will be recruited from the current patient population at NIH.

2.2 SCREENING EVALUATION

All screening tests and procedures must be performed within 28 days prior to the first planned dosing of the study drug, unless otherwise noted. The following procedures and evaluations will be performed and documented in the subject's source records.

- History and physical exam including ECOG performance status and vital signs
- 12-Lead ECG
- Clinical laboratory tests (within 16 days prior to drug administration)
 - Chemistry: sodium, potassium, chloride, bicarbonate, calcium, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total protein, albumin, and total and direct bilirubin.
 - Hematology: complete blood count (CBC) with differential and platelets
 - Coagulation panel: PT, INR, and PTT.
 - Urinalysis.
- Serum pregnancy test (β -HCG) for females of childbearing-potential and women < 12 months since the onset of menopause (within 16 days prior to enrollment)
- HBV (HBsAg), HCV (anti-HCV), HIV (anti-HIV) screening (within 3 months prior to enrollment)
- Appropriate tumor imaging and assessment. All baseline tumor measurements should be performed based on the subject's qualifying scan obtained within 28 days prior to the start of treatment.
- Confirmation of diagnosis. A report from any CAP or CLIA certified laboratory is acceptable.

2.3 REGISTRATION PROCEDURES

Registration will be a two-part process as patients are screened on this protocol. Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. To initially register a subject after the participant has signed the consent, complete the top portion of the registration Eligibility Checklist from the website (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) indicating that the patient is being registered for screening and send via encrypted email to: NCI Central Registration Office ncicentralregistration-1@mail.nih.gov. Once eligibility is confirmed after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and email the completed registration checklist to the CRO at NCI Central Registration Office ncicentralregistration-1@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

Subjects that do not meet screening criteria should be removed from the study following the procedure in section 3.7.3.

2.3.1 Treatment Assignment Procedure

Cohorts

Number	Name	Description
1	Dose De-Escalation	Subjects enrolled to dose de-escalation cohorts
2	Dose Expansion	Subjects enrolled at the MTD after the MTD is established

Arms

Number	Name	Description
1	Dose De-Escalation	Combination ETBX-011, ETBX-051, ETBX-061 adenoviral vaccine regimen dose de-escalation
2	Dose Expansion	Combination ETBX-011, ETBX-051, ETBX-061 adenoviral vaccine regimen dose

Arm Assignment

Subjects in cohort 1 will be directly assigned to arm 1.
Subjects in cohort 2 will be directly assigned to arm 2.

2.4 BASELINE EVALUATION

All subjects are required to complete baseline evaluations within 1 day prior to the first planned dosing of the study drug (Any screening evaluation done on D-1 of treatment can also serve for the baseline evaluation). The following procedures and evaluations will be performed and documented in the subject's source records.

- History and physical exam including ECOG performance status and vital signs
- Serum pregnancy test (β -HCG) for females of childbearing-potential and women < 12 months since the onset of menopause.
- Chemistry: sodium, potassium, chloride, bicarbonate, calcium, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total protein, albumin, and total and direct bilirubin.
- Hematology: CBC with differential and platelets

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase I trial in subjects with advanced cancer.

The combination vaccine regimen is three different adenoviral vaccines (ETBX-011, ETBX -061 and ETBX-051), administered to patients at the same time. All utilize the same second generation E1(-), E2 (-) vector.

ETBX-011 is a CEA-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector and a modified CEA (CEA(6D)) gene insert. The investigational product ETBX-061 is a MUC1-targeting vaccine

that comprises the Ad5 [E1-, E2b-] vector and a modified MUC1 (MUC1c) gene insert. The investigational product ETBX-051 is a Brachyury-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector and a modified Brachyury gene insert. (Table 8).

3.1.1 Dose Limiting Toxicity

For the purposes of dose escalation a dose limiting toxicity (DLT) is defined as:

- Any Grade 3 or greater toxicity that is possibly related to the vaccine and as defined by Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 with the exception of transient (≤ 24 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management or transient (≤ 24 hours) Grade 3 fatigue, skin reactions or rash, headache, nausea, emesis that resolves to Grade ≤ 1 or asymptomatic grade 3 amylase/lipase elevation. For purposes of dose escalation the DLT evaluation period will be for 3 weeks following the first dose of drug.

Subjects experiencing a DLT will be removed from protocol therapy.

3.1.2 Dose De-Escalation

Up to six patients will be enrolled at dose level 1. If ≤ 1 of 6 patients experience a DLT, initiation of the dose expansion phase will occur. If ≥ 2 of 3 or 6 experience DLT in the initial dose level, then dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-1) (1×10^{11} VP). If ≤ 1 of 6 patients experience a DLT, then the maximum tolerated (MTD) will be declared at this dose, and initiation of the dose expansion phase will occur. If ≥ 2 of 3 or 6 experience DLT at dose level -1, then an amendment may be written to evaluate a further dose de-escalation.

In the second part, dose expansion will occur when the MTD has been determined. An additional 4 subjects will be enrolled in the dose expansion component of the trial, for a total of 10 subjects treated at the MTD.

A schematic overview of the study is shown in Figure 6.

In the initial component of the study, 3 to 6 subjects will be sequentially enrolled starting at the standard dose at dose level 1. During enrollment at each dose level, there will be a minimum of 3 days between enrolling successive subjects. DLTs will be monitored continuously.

Dose levels are shown in Table 4. No intra-patient dose escalations are permitted.

Table 4: Dose Levels for Each ETBX VP

Dose Level (DL)	ETBX VP
Standard (1)	5×10^{11}
-1	1×10^{11}

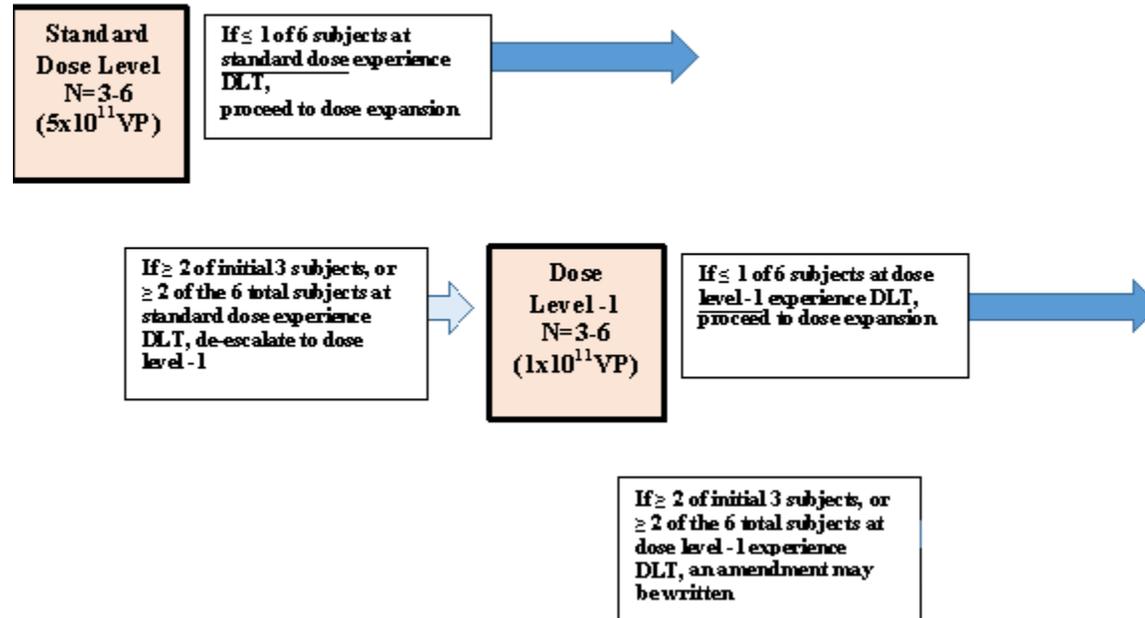
Standard dose (DL 1) (5×10^{11} VP):

- If ≤ 1 of the initial 6 subjects experience a DLT, dose expansion will commence.
- If >1 of the initial 3 subjects, or if ≥ 2 of the 6 total subjects experience a DLT, enrollment at DL -1 will commence.

Dose de-escalation DL -1 (1×10^{11} VP):

- If ≤ 1 of the initial 6 subjects experience a DLT, this dose level will be defined as the MTD and dose expansion will commence.
- If >1 of the initial 3 subjects, or if ≥ 2 of the 6 total subjects experience a DLT, then a protocol amendment may be written to evaluate a further dose de-escalation..

Figure 6: Study Design and Treatment Schema



3.2 ADMINISTRATION AND DURATION OF TREATMENT

Each of the three ETBX vaccines will be administered on Weeks 0, 3, and 6 followed by booster vaccines every 8 weeks for up to a year. Treatment may be shorter for patients who experience progressive disease (unequivocal or confirmed) or unacceptable toxicity, withdraw consent, or if the Investigator feels it is no longer in their best interest to continue treatment. For specifics on drug administration see section [11.1.5](#).

Vital sign assessments (temperature, heart rate, blood pressure, respiratory rate) are to be obtained after the subject has been in a seated resting position for at least 5 minutes. For the first injection, vital signs must be assessed 30 and 60 minutes after the injection. Vital signs must be assessed 30 minutes after the subsequent injections.

3.3 DOSE DELAY OR DISCONTINUATION

Applies to events occurring outside of the DLT evaluation period

- Patients must have recovered to \leq grade 2 for injection site reaction or grade \leq 1 for any other toxicity related to the vaccines for the parameters used to assess levels of organ function required for eligibility (see Section [2](#)) after each vaccination in order to receive a subsequent vaccination. Patients will receive a diary card to record injections site reactions (see [Appendix B](#)).
If \geq grade 3 nonautoimmune toxicity attributable to the vaccines persists for $>$ 42 days, the patient will not receive further vaccine inoculations and will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.
- Patients who develop any \geq grade 3 autoimmunity, not related to a therapeutic response, will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.
- Patients who develop any grade 4 toxicity attributable to the vaccines will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.
- Dosing of the vaccines injections should be given on schedule every 3 weeks (Week 0, 3, and 6), and then bi-monthly (every 8 weeks) boosts for up to a year. In the event of conflicts, a +/- 1 week window is acceptable.
- For unrelated acute illnesses present at the time of a scheduled vaccination, dosing can be delayed until symptoms subside, or the subject may be withdrawn at the discretion of the Investigator; delays up to 4 weeks are considered acceptable in this setting.

3.4 DOSE MODIFICATIONS

No dose modifications are allowed with these vaccines except for dose de-escalation as described in section [3.1](#).

3.5 STUDY CALENDAR

Assessment	Screening	Treatment (Every 3-Week Dosing)			Bimonthly_Boosters (Every 8-Week Dosing)						End of Treatment (within 90 days after the last vaccine) ^m	Follow Up ^l
		Baselin e/0	3	6	14	22	30	38	46	54		
Study Week	Day -28 to -1ⁿ											
Clinic Visit	X	X ^c	X	X	X	X	X	X	X	X	X	
Informed Consent	X ^a											
Inclusion/Exclusion	X											
Demographics	X											
Confirmation of diagnosis (path report)	X											
Physical Examination, Height ^b , Weight, ECOG	X ^c	X ^c	X	X	X	X	X	X	X	X	X	
Medical History ^d	X	X ^c										
Concomitant Medications	X	X ^c	X	X	X	X	X	X	X	X	X	
Vital Signs ^e	X	X ^c	X	X	X	X	X	X	X	X	X	
12-Lead ECG	X				X	X	X	X	X	X	X	
Confirm Contraceptive Measures ^f	X											
Study Drug Injection/ Injection Site Reaction Monitoring ^g		X	X	X	X	X	X	X	X	X	X	

Assessment	Screening	Treatment (Every 3-Week Dosing)			Bimonthly_Boosters (Every 8-Week Dosing)						End of Treatment (within 90 days after the last vaccine) ^m	Follow Up ^l
		Baseline e/0	3	6	14	22	30	38	46	54		
Study Week	Day -28 to -1ⁿ	Baseline e/0	3	6	14	22	30	38	46	54		
Dispensation of Subject Diary Card ^h		X										
Review of Subject Diary Card ^h			X									
Tumor Imaging ⁱ	X			X	X	X	X	X	X	X		
Pregnancy Test ^j	X ^{c,n}	X ^c	X	X	X	X	X	X	X	X	X	
Urinalysis	X ⁿ										X	
Chemistry Panel	X ^{c,n}	X ^c	X	X	X	X	X	X	X	X	X	
CBC, Differential, Platelets	X ^{c,n}	X ^c	X	X	X	X	X	X	X	X	X	
Coagulation	X ⁿ										X	
Serum Virology (HIV, HBV, HCV) ^k	X ⁿ											
Adverse Events		X	X	X	X	X	X	X	X	X	X	
Exploratory Immune Analysis		X		X	X		X				X	
Telephone Follow Up												X

^a Informed consent may be obtained on D0 after eligibility is confirmed and other screening protocols may be used to obtain screening labs and procedures as per NCI's internal SOP

^b Height will only be assessed at screening.

^c If the assessment is performed within 24 hours prior to the first dosing, a second assessment at baseline/week 0 can be omitted.

^d Complete medical history will be evaluated at screening and includes current and past cardiac and pulmonary history, documentation of diagnosis including history of current and prior cancers, prior treatment(s), and prior radiologic studies. Any new events in the medical history will be evaluated at baseline..

^e Vital signs include temperature, heart rate, blood pressure, and respiratory rate. Vital sign assessments are to be obtained after the subject has been in a seated resting position for at least 5 minutes. For the first injection, vital signs must be assessed 30 and 60 minutes after the injection. Vital signs must be assessed 30 minutes after the subsequent injections.

^f Acceptable contraceptive measures are described in Section [2.1.1.12](#).

^g Injection site reactions will be monitored

^h Subjects will be given a diary card for the self-evaluation and reporting of injection site reactions.

ⁱ All baseline tumor measurements should be performed based on the subject's qualifying scan obtained within 28 days prior to the start of treatment. Tumor imaging and assessments will be performed

^j Serum pregnancy test will be performed on females of childbearing-potential and women < 12 months since the onset of menopause. The pregnancy test will be performed at each dosing visit with the result confirmed as negative prior to dosing.

^k Serum virology test for HIV (as determined by ELISA and confirmed by western blot), and HBV and HCV (as determined by HBsAg and hepatitis C serology).

^l After the subject completes or withdraws from study therapy, the study team will contact the subject approximately every 3 months for 12 months and then approximately every 6 months for 24 months and then every 12 months thereafter for another 24 months to collect follow-up information, including survival status and any current cancer treatment regimen.

^m If the patient cannot return to the Clinical Center within 90 days after the last vaccine, the patient will be contacted by phone to AEs and laboratory assessments will be deferred until a later point if at feasible.

ⁿ See section [2.2](#) for screening criteria which are exceptions to the D-28 to D-1 screening window period.

3.6 INJECTION SITE REACTIONS

Local injection site reactions are expected to occur over several days after vaccination. Erythema and some soreness are possible. Injection site reactions will be monitored by clinic staff prior to discharge and by subject self-evaluation and reporting. Subjects will be given a diary card with reporting fields for diameter of erythema and duration for the seven days after the first set of vaccine injections (see [Appendix B](#)). In addition, pain at the injection site, fever, and chills will be collected as Yes/No and medications (over-the-counter ibuprofen, acetaminophen, aspirin, etc.) taken for injection site pain or discomfort as Yes/No. The site staff will review the diary cards with the subject during the next clinic visit and record responses in the case report form.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Regardless of reason for removal from study therapy, patients will be asked to have follow up safety visit within 90 days after the last vaccine. Patients who refuse to return for this visit will be asked to review any safety concerns by phone within this time period.

3.7.1 Criteria for removal from protocol therapy

- Completion of protocol therapy.
- Clinical or radiographic progression of disease Patients with serological progression alone will remain on treatment at the discretion of the PI.
- Unacceptable Toxicity as defined as any serious adverse event that is unexpected relative to the known safety profile of the investigational agents in the opinion of the investigator or as described in sections [3.1.1](#) and [3.3](#)
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

Also see section [3.3](#)

3.7.2 Off-Study Criteria

- PI decides to end study
- Participant requests to be withdrawn from study. Reasons for withdrawal will be documented.
- Noncompliance with protocol guidelines (patient removed at discretion of Principal Investigator)
- Death
- Screen failure

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-1@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Subjects must inform the investigators of the current or planned use or all other medications during the study (including prescription medications, vitamins, herbal and nutritional supplements, and over-the-counter medications).

For the administration of ETBX vaccines, antiemetics, stool softeners, and antidiarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically on the first cycle. The selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens will not include steroids.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any patients taking antibiotics for infection must complete that course of therapy and be free of evidence of further infection before receiving any dose of vaccine. Use of prophylactic antibiotics is allowed.

Concurrent systemic corticosteroid use (daily or every other day for continued use > 14 days) should be avoided within 28 days before the first planned dose of ETBX vaccines. Use of physiologic doses of systemic steroids, inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm³. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm³.

4.1 CONCURRENT MEDICATIONS/INTERVENTIONS

4.1.1 Anticancer Therapy

If a subject requires additional systemic anticancer treatment with the exception of treatments described in section 3.1 then study treatment must be discontinued. Local intervention is discouraged unless medically unavoidable. Subjects receiving local intervention (*e.g.*, palliative radiation) are allowed to continue to receive study treatment at the investigator's discretion.

4.1.2 Other Medications

Subjects must be instructed to inform the investigators of the current or planned use or all other medications during the study (including prescription medications, over-the-counter medications, vitamins and herbal and nutritional supplements). It is the responsibility of the investigator to ensure that details regarding all medications are documented. Bisphosphonates started prior to screening activities or initiated during the course of the study to control bone pain may be used with caution.

Colony stimulating factors (*e.g.*, erythropoietin and granulocyte colony-stimulating factors) administered as dictated by safety purposes are acceptable while the subject is enrolled on study.

Pain medications administered as dictated by standard practice are acceptable while the patient is enrolled on the study.

No concurrent investigational agents are permitted.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Peripheral Blood Collection

Subjects will have approximately 70 mL of peripheral blood drawn to evaluate the study drug's effect on the immune response at specific time points during the study and/or after a specified injection. Blood draws will be done at baseline and prior to each injection (baseline, week 6, 14, 30 and EOT).

5.1.2 Samples Collected

Six 10-mL green top sodium heparin tubes for PBMC samples and one 8-mL serum-separating tube for serum samples will be drawn. Samples will be picked up by courier and delivered to the Leidos Biomedical Research facility in Frederick, MD..

5.1.3 Sample Processing

Blood samples will be processed at:

Leidos Biomedical Research
Attn: Dr. Ludmila Krymskaya /Theresa Burks
1050 Boyles Street
Bldg. 496/Room 121
Frederick, MD 21702

5.1.4 Sample Tracking, and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside the National Institutes for Health (NIH) without IRB notification and an executed material transfer agreement.

5.1.5 Pharmacodynamic Sample Management and Storage

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80°C freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure,

limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing patient specimens. The subcontractor does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects. The subcontractor's IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of the subcontractor to accept only de-identified samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input, and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.1.6 Sample Analysis

Immune assessments will be performed at the Laboratory of Tumor Immunology and Biology at the NCI's Center for Cancer Research (CCR) and include flow cytometry-based and serum assays.

Analyses of PBMCs:

1. Pre- and post-therapy PBMCs, separated by Ficoll-Hypaque density gradient separation, will be analyzed for antigen-specific immune responses using an intracellular cytokine staining assay. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools encoding the tumor-associated antigens CEA, MUC1, and Brachyury. Control peptide pools will involve the use of human leukocyte antigen peptide as a negative control and CEFT peptide mix as a positive control. CEFT is a mixture of peptides of CMV, Epstein-Barr virus, influenza, and tetanus toxin. Post-stimulation analyses of CD4 and CD8 T cells

will involve the production of IFN- γ , IL-2, tumor necrosis factor, and CD107a. If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens. A detailed description of this assay has been previously reported (35).

2. If sufficient PBMCs are available, PBMCs from selected subjects may be analyzed for changes in standard immune cell types (CD4 and CD8 T cells, natural killer [NK] cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as 123 immune cell subsets, as described elsewhere (74), and for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs.

Analyses of soluble factors:

Sera will be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand, and selected patients may be analyzed for cytokines and antibodies to human tumor antigens. For selected patients, sera may be analyzed for antibodies to CEA, MUC1, or Brachyury and adenovirus.

Additional assays:

Blood samples may be used for additional research studies, which may include phenotypic and functional analysis of immune-cell subsets and analyses for cytokines (IFN- γ , IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers. In addition, assays for antibody or neutralizing antibody titer to adenovirus (type 5) and cell-mediated immunity to adenoviral antigens may be assessed.

Samples will be tracked according to Section 5.1.4.

5.1.7 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open as long as sample or data analysis continues. Samples will be stored until they are no longer of scientific value or until a subject withdraws consent for their continued use, at which time they will be destroyed. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and subject consent or an exemption from the Office of Human Research Protections.

The Investigator will report any loss or destruction of samples to the NCI IRB as soon as he/she is made aware of such loss. The Investigator will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors such as a broken freezer or lack of dry ice in a shipping container, or if a subject withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems or lost samples will also be reported to the IRB, the NCI Clinical Director, and the office of the NCI CCR.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be entered in C3D. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for a minimum of 30 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE only if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- De-identified data in an NIH-funded or approved public repository.
- De-identified data in BTRIS (automatic for activities in the Clinical Center)
- De-identified or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository. Insert name or names: clinicaltrials.gov .
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.3 EFFICACY ASSESSMENTS

6.3.1 Antitumor Response

Tumor assessments will be done at week 6 and then every 8 weeks thereafter and may include the following evaluations: physical examination (with photograph and measurement of skin lesions, as applicable); cross-sectional imaging using computed tomography (CT) or magnetic resonance imaging (MRI) scan of the chest, abdomen, and pelvis (pelvis scan is optional unless known pelvic disease is present at baseline); nuclear bone scan for subjects with known/suspected bone lesions; and CT or MRI scan of the brain (only as clinically warranted based on symptoms/findings). The preferred method of disease assessment is CT with contrast. If CT with contrast is contraindicated, CT of the chest without contrast and MRI scan of the abdomen/pelvis with contrast is preferred. At baseline, tumor lesions will be selected and categorized as target (measurable disease) or non-target lesions (non-measurable disease).

6.3.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
 - Scan slice thickness 5 mm or under: as ≥ 10 mm
 - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial

effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible and repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions above the 5 target lesions should be identified as non-target lesions and should be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. The same mode of assessment (e.g., CT) used to identify/evaluate lesions at baseline should be used throughout the course of the study unless subject safety necessitates a change (e.g., allergic reaction to contrast media).

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published. (75-77) In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer. (78)

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

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

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 RECIST Response Criteria

Antitumor activity will be evaluated with target and/or non-target lesions according to RECIST Version 1.1 ([79](#)) as summarized below.

6.3.5 Target Response

Percentage change in target lesion size will be evaluated by the following formulae:

1. When determining complete response or partial response:

$$[(\text{Post value} - \text{Baseline value})/\text{Baseline value}] \times 100$$

2. When determining progressive disease:

$$[(\text{Post value} - \text{Smallest value since treatment started})/(\text{Smallest value since treatment started})] \times 100$$

Target response will be classified according to the RECIST Version 1.1 Target Lesion Response Criteria in [Table 5](#).

Table 5: RECIST Target Response Criteria

Target Response Criteria	Definition
Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum diameters while on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of 5 mm. (Note: the appearance of one or more lesions is also considered progression).

Non-target response will be classified according to the RECIST Version 1.1 Non-Target Lesion Response Criteria in [Table 6](#).

Table 6: RECIST Non-Target Response Criteria

Non-Target Response Criteria	Definition
CR	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Non-CR / Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
PD	Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

Overall response will be classified according to the RECIST Version 1.1 Overall Response Criteria in [Table 7](#).

Table 7: RECIST Overall Response Criteria

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR / Non-PD	No	PR
CR	Not Evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	Inevaluable
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

6.3.6 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

6.3.7 Progression-Free Survival (PFS)

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site:

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/etc.htm)

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
 - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
 - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NCI-IRB and CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI CD:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems

- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
 - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
 - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
 - All Grade 5 events regardless of attribution;
 - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

During the first 30 days after the subject receives investigational agent/intervention, the investigator must report within 1 business day to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form “Describe Event or Problem”.

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3 “Pregnancy, puerperium and perinatal conditions - Other (pregnancy)”** under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for one month after the last dose of combination ETBX-011, ETBX-051, ETBX-061 .

Pregnancy of the patient’s partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until one month after the last dose should, if possible, be followed up and documented.

7.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

7.4.1 Etubics Corporation

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

In the event of any new SAE (of any Grade) occurring during the reporting period (from first dose until decision made to discontinue treatment), the investigator must within 2 business days inform the Manufacturer (Etubics Corporation) or designee by telephone, by fax, or by email.

When an event (or follow-up information) is reported by telephone, a written report must be sent immediately thereafter by fax or email.

Reporting procedures and timelines are the same for any new information on a previously reported SAE (=follow-up). Any new SAE is reported to:

Jelena Berglund, PhD
VP Operations
401 West Harrison Street, Seattle, WA 98119
Telephone: 206-838-5110
Cell: 919-265-7089 (primary number)
Fax: 206-838-2978
Email: jelena@etubics.com

All Medwatch 3500a forms and written reports should be transmitted, which must be completed by the investigator following specific completion instructions. Relevant pages from the CRF may be provided in parallel (e.g., medical history, concomitant drugs).

In all cases, the information provided in the SAE Report Form must be consistent with the data on the event that is recorded in the corresponding sections of the CRF.

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of ETBX vaccines as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the ETBX vaccines, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.5.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.5.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);

- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.5.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.6 DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient, enrollment and data management issues. Decisions about dose level enrollment and dose escalation will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.6.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.6.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL METHODS

8.1 STATISTICAL HYPOTHESIS

This is a Phase I trial in subjects with advanced cancer. A combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen using the same modified Adenovirus vector backbone, separately encoding three well-studied TAAs will be assessed. The vaccine will be tested at standard dose level, and a dose de-escalation (if required) design will be employed. The primary endpoint will be to determine the overall safety of combined ETBX-011/ETBX-061/ETBX-051 vaccine when administered SC to subjects with advanced solid tumors and establish a recommended phase 2 dose of vaccine. This will be reported when an MTD is determined (expected to be less than 1 year from trial start). A secondary endpoint will be to obtain preliminary data on ORR, DCR, duration of response, PFS, and OS in treated subjects (preliminary data will be assessed around two years following trial start). An exploratory hypothesis (objective) will be to evaluate the immunogenicity of the combined ETBX-011/ETBX-061/ETBX-051 vaccine.

8.2 SAMPLE SIZE DETERMINATION

Up to six patients will be enrolled in an initial dose level 1. If ≤ 1 of 6 patients experience a DLT, then the MTD will be declared, and initiation of the dose expansion phase will occur. If ≥ 2 of 6 experience DLT in the initial dose level 1, then dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-1) (1×10^{11} VP). If ≤ 1 of 6 patients experience a DLT, then the maximum tolerated (MTD) will be declared at this dose, and initiation of the dose expansion phase will occur. If ≥ 2 of 6 (or ≥ 2 of the first 3) experience DLT in this second dose level -1, then an amendment may be written to further evaluate dose de-escalation. Once the MTD is identified, the dose expansion component will commence. An additional 4 subjects will be

enrolled at the MTD for a total of 10 subjects to gain additional safety data and exploratory data on vaccine immunogenicity.

We assume 16 patients will have screen failure. Therefore, up to 32 subjects will be enrolled in the study. Initial dose evaluation and or dose finding will require 6-12 subjects, followed by 4 additional subjects treated at a defined dose.

8.3 POPULATION FOR ANALYSES

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with the combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

8.4 STATISTICAL ANALYSES

8.4.1 General Approach

Descriptive analyses of toxicity results and exploratory description of time-to-event and response based results. The planned analyses for each objective is described in the subsections below.

8.4.2 Analysis of Primary Efficacy Endpoints

Not applicable in this Phase 1 Study. The primary objective of evaluating safety is described in Section [8.4.4](#) below.

8.4.3 Analyses of Secondary Endpoint

Analyses of secondary endpoint to obtain preliminary data on objective response rate (ORR), disease control rate (DCR), duration of response, progression-free survival (PFS), and overall survival (OS) in treated subjects.

Since this is a Phase I trial, only preliminary data will be obtained for evidence of vaccine efficacy. Preliminary analyses of vaccine efficacy are described in the Sections [8.4.3.1](#) to [8.4.3.4](#) below.

8.4.3.1 Objective Tumor Response and Disease Control Rate

The percentage of subjects that achieve an objective confirmed complete or partial overall tumor response using RECIST Version 1.1 will be evaluated by dose level and overall enrollment. If anti-tumor responses are observed, the 95% confidence interval of the response rate will be evaluated.

Disease control (confirmed response or SD lasting for at least 6 months) will be analyzed in a similar manner.

8.4.3.2 Duration of Response

The duration of overall response will be evaluated by dose level and overall enrollment. The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or PD is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

8.4.3.3 Progression-Free Survival

PFS will be evaluated by dose level and overall using Kaplan-Meier methods. PFS will be defined as the time from the date of first treatment to the date of disease progression or death (any cause) whichever occurs first. Subjects who do not have disease progression or have not died at the end of follow up will be censored at the last known date the subject was progression free.

8.4.3.4 Overall Survival

OS will be evaluated by dose level and overall using Kaplan-Meier methods. OS will be defined as the time from the date of first treatment to the date of death (any cause). Subjects who are alive at the end of follow up will be censored at the last known date alive.

8.4.4 Safety Analysis

DLTs will be evaluated continuously in a dose level. An overall assessment of whether to de-escalate to the next dose level will be made at least 1 week after the last subject in the previous dose level has received their first injection. A dose level will be considered safe if $< 33\%$ of subjects treated at a dose level experience a DLT (i.e., 0 of 3, ≤ 1 of 6, ≤ 2 of 9, ≤ 3 of 10). Safety will be evaluated in 3 or 6 subjects at each dose level in the dose escalation component of the study. Safety will continue to be monitored among additional subjects treated at the MTD or HTD in the dose expansion component of the study. A subject will be considered evaluable for safety if treated with at least one injection. DLTs will be observed for 1 week.

Safety endpoints will be analyzed as summary statistics during treatment and/or as change scores from baseline assessments. AEs will be coded as defined in the Medical Dictionary for Regulatory Activities (MedDRA). All AEs will be recorded and tabulated following each treatment (vaccine injection). AEs will be recorded by severity, frequency, and relationship to the study intervention and will be presented by System Organ Class (SOC) designations and preferred term groupings. Information on each AE will include start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be presented either in a Table or a Listing.

In addition, overall safety will be assessed by descriptive analyses using tabulated frequencies of AEs by grade using CTCAE Version 5.0 within dose levels and for the overall study population in terms of treatment-emergent AEs, SAEs, and clinically significant changes in safety laboratory tests, physical examinations, ECGs, and vital signs.

The safety events listed in section [8.4.6.2](#) will trigger a temporary suspension of the study injections.:

8.4.5 Baseline Descriptive Statistics

Not applicable for this Phase I study.

8.4.6 Planned Interim Analyses and Halting Guidelines

8.4.6.1 Planned interim analysis

Not applicable for this Phase I study.

8.4.6.2 Halting guidelines

The following safety events will trigger a temporary suspension of the study injections:

- Death possibly related to the study agent.
- Two Grade 4 toxicity events that are possibly related to the study agent.
- At any time during the expansion phase > 33% of subjects experience a Grade 3 or 4 major organ toxicity possibly related to study injections.

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 treatment schedule of any individual subject.

Treatment may also be suspended for safety concerns other than those described above if, in the judgment of the Investigator, participant safety is threatened

8.4.7 Sub-Group Analyses

None will be performed because of the small size of the study.

8.4.8 Tabulation of Individual Participant Data

No individual participant data will be tabulated.

8.4.9 Exploratory Analyses (Immune Responses)

Exploratory immune analyses will be conducted to evaluate anti-tumor immune responses induced by vaccine injections. Immune response will be assessed among all subjects treated in each dose level. The magnitude of immune responses will also be described. A subject will be considered evaluable for immune response if they receive at least three vaccine injections. The percentage of subjects with a positive immune response will be evaluated by dose levels and overall enrollment. A positive immune response is defined by CMI reactivity in *ex vivo* stimulation using a flow cytometric readout (cytokine production or CD107 expression). Antigen-specific peptide challenge assays require a readout of >250 reactive T-cells/million cells above the background (35).

If more than one dose level of patients is enrolled and at least three patients are treated in the dose level, Statistical analyses of data will be performed. For flow cytometry analyses on PBMC samples, Student T tests will be performed on percentages of TNF- α and/or IFN- γ expressing cells among the different dose levels to determine any significant differences in cell populations.

9 COLLABORATIVE AGREEMENTS

9.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA is in place between the Laboratory of Tumor Immunology and Biology (LTIB), CCR NCI and Etubics Corporation, the manufacturer of ETBX-011 (adenoviral CEA vaccine); ETBX-061 (adenoviral MUC1 vaccine); and ETBX-051 (adenoviral brachyury vaccine), CRADA# 02997.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

10.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all genders and racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with gender and ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.1.2 Strategies/Procedures for Recruitment

Patient accrual for this protocol will be facilitated by Web-based recruitment strategies. This protocol will be listed on www.clinicaltrials.gov.

10.1.3 Justification for Exclusions

Due to impaired cellular immunity with the concomitant increased risk of serious side effects from vaccinations with infectious agents, the Centers for Disease Control and Prevention recommends that HIV infected patients be excluded, in addition, patients with chronic hepatitis infection, including B and C, because of potential immune impairment.

10.2 PARTICIPATION OF CHILDREN

Because no dosing or adverse event data are currently available on the use of the study vaccines in patients <18 years of age, children are excluded from this study.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 10.4), all subjects \geq age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have

pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients will receive evaluation of their disease at the National Cancer Institute's Clinical Center. This protocol may or may not benefit an individual, but the results may help the investigators learn more about the disease and develop new treatments for patients with this disease.

Potential adverse reactions attributable to the administration of the vaccine utilized in this trial are discussed in section 11. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. Patients will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of patients will be recorded in the patient chart. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland.

Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. In all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) or other regulatory authorities may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration to register and verify patients' eligibility.

10.5 CONSENT PROCESS AND DOCUMENTATION

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient, and a signed informed consent document will be obtained by a study investigator prior to entry onto the study.

The PI or associate investigator will meet with the patient to discuss the protocol treatment and alternative options in detail. It will be stated clearly that participation in the research study is voluntary and that participants can withdraw from the study without losing benefits they would otherwise be entitled to. The patient will be encouraged to ask questions, and additional meetings to discuss the treatment options will be arranged as necessary.

If there is an optional biopsy for research in the protocol, then the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record.

10.5.1 Telephone re-consent procedure

Re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent. The original informed consent document will be sent back to the consenting

investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator.

10.5.2 Consent process for non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 COMBINATION ETBX-011, ETBX-051, ETBX-061 VACCINE REGIMEN

The combination ETBX-011, ETBX-051, ETBX-061 vaccine regimen refers to the combination of three investigational adenoviral vaccines. All utilize the same second generation E1(-), E2 (-) vector. Subjects will each receive the three vaccines at the same time, at the same doses, through three separate injections administered in different limbs (proximal limb areas) or separate locations.

ETBX-011 is a CEA-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector and a modified CEA (CEA(6D)) gene insert. The investigational product ETBX-061 is a MUC1-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector and a modified MUC1 (MUC1c) gene insert. The investigational product ETBX-051 is a Brachyury-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector and a modified Brachyury gene insert. (**Table 8**). Refer to the current version of the Investigator's Brochure for detailed investigational product information.

Table 8: Investigational Products

Table 8a

Product Name(s):	ETBX-011 (Ad5 [E1-, E2b-]-CEA(6D) Vaccine)
Dosage Form:	Suspension for injection

Dose	5 x 10 ¹¹ VP (standard dose), or 1 x 10 ¹¹ VP (DL-1).
Route of Administration	SC injection
Physical Description	ETBX vaccine is supplied as a sterile, clear solution in a 2-mL single-dose vial. The vaccine is provided at a concentration of 5 x 10 ¹¹ VP per 1 mL and contains ARM formulation buffer (20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0). Each vial contains approximately 1.3 mL of the vaccine. The product should be stored at -20+10°C until ready for use.
Manufacturer	Etubics

Table 8b

Product Name(s):	ETBX-061 (Ad5 [E1-, E2b-]-MUC1 Vaccine)
Dosage Form:	Suspension for injection
Dose	5 x 10 ¹¹ VP (standard dose), or 1 x 10 ¹¹ VP (DL-1).
Route of Administration	SC injection
Physical Description	ETBX vaccine is supplied as a sterile, clear solution in a 2-mL single-dose vial. The vaccine is provided at a concentration of 5 x 10 ¹¹ VP per 1 mL and contains ARM formulation buffer (20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0). Each vial contains 1 mL of extractable vaccine. The product should be stored at ≤ -60°C until ready for use.
Manufacturer	Etubics

Table 8c

Product Name(s):	ETBX-051 (Ad5 [E1-, E2b-]-Brachyury Vaccine)
Dosage Form:	Suspension for injection
Dose	5 x 10 ¹¹ VP (standard dose), or 1 x 10 ¹¹ VP (DL-1).
Route of Administration	SC injection
Physical Description	ETBX vaccine is supplied as a sterile, clear solution in a 2-mL single-dose vial. The vaccine is provided at a concentration of 5 x 10 ¹¹ VP per 1 mL and contains ARM formulation buffer (20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0). Each vial contains 1 mL of extractable vaccine. The product should be stored at ≤ -60°C until ready for use.
Manufacturer	Etubics

11.1.1 Source

The ETBX vaccines will be supplied by the manufacturer, Etubics Corporation, through a Cooperative Research and Development Agreement (CRADA). The manufacturing department of Etubics will supply each vaccine in 2-mL single-dose vials. Each single-dose vial contains a sterile, clear suspension of the ETBX vaccine at 5×10^{11} VP per 1 mL intended for single dose administration and contains ARM formulation buffer (20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0).

Upon receipt of the investigational product, the Investigator or delegated designee will verify that an appropriate shipping temperature was maintained, conduct an inventory, and sign the drug receipt form and send a scanned copy to the Sponsor contact as designated on the form. If a temperature excursion occurs, the Sponsor should be notified immediately, and the investigational product must be quarantined and maintained under the correct storage conditions until further instructions are provided by the Sponsor. The temperature excursion form will need to be completed and returned to the Sponsor. The original drug receipt form and packing slip must be retained in the Investigator's pharmacy records.

11.1.2 Toxicity

The safety of immunizations (injections) with the ETBX-011, ETBX-051, ETBX-061 vaccines has not been established and will be determined in this phase I clinical trial.

A Phase I/II clinical trial of ETBX-011 (Ad5 [E1-, E2b-]-CEA(6D)) (IND#14325) that expresses the tumor-associated antigen carcinoembryonic antigen (CEA) has been performed. A summary of the study results on the clinical trial is presented below.

Schedule, Dosing, and Safety of ETBX-011: The primary objective of the Phase I/II dosing trial was to assess safety and a secondary objective was to evaluate CEA-specific immune responses to CEA and to obtain preliminary data on response rate. The study was performed under an FDA-approved IND (IND14325) and registered at ClinicalTrials.gov (NCT01147965). Participants were recruited from oncology clinics at Duke University Medical Center and Medical Oncology Associates, Spokane, WA and provided informed consent that was approved by IRB's. Patients with a histological confirmed diagnosis of metastatic malignancy who were previously treated with standard therapy known to have a possible survival benefit were enrolled into the study. For this study, the carcinoma must have had over expression of CEA as defined by immunohistochemical staining (at least 50% of the tumor with at least moderate intensity of staining) or a carcinoma known to be universally CEA positive *i.e.* colorectal adenocarcinoma). Patients were not treated until 4 or more weeks after any prior chemotherapy or radiation therapy. They could not have a history of autoimmune disease, serious intercurrent chronic or acute illness, active hepatitis, serologic evidence for HIV infection, or be receiving steroid or immunosuppressive therapy. All patients were ≥ 21 years old and had a Karnofsky Performance Score of 70% or higher and a life expectancy of at least 3 months. Pregnant women and nursing mothers were excluded. Dose Limiting Toxicities (DLTs) were defined as any Grade 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 °F, Grade ≥ 2 allergic

reactions (Grade 2 is defined as generalized urticaria as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE version 5.0), or Grade ≥ 3 non-hematologic toxicity. The Ad5 [E1-, E2b-]-CEA(6D) injections were given subcutaneously in the same thigh. The doses were administered every 3 weeks for 3 treatments as follows: Cohort 1 (3 patients) 10^9 VP in 0.5 ml; Cohort 2 (3 patients): 10^{10} VP; Cohort 3 (6 patients) 10^{11} VP. Following establishment of safety in Phase I, 12 additional patients were entered into a Phase II using the 10^{11} VP/dose. To evaluate a higher dose, 6 additional patients (cohort 5) received 5×10^{11} VP/dose.

Patient Demographics: One patient with CEA expressing bladder and one patient with lung carcinoma was enrolled. Thirty-two patients, median age 57.5 (range 38-77) with metastatic colorectal cancer, who had failed a median of three prior chemotherapeutic regimens (range: 2–5), had a median performance status of 90% (range 70-100%), and had a median of three sites of metastatic disease (range 1-5), were enrolled. The majority was able to receive all three immunizations. Five patients who stopped immunizations early did so due to significant disease progression. The colorectal adenocarcinoma patient demographics compares favorably with previously published studies of patients with chemotherapy-refractory colorectal cancer [44-46].

Adverse Effects: A total of 94 immunization treatments were administered to all patients. There was no dose limiting toxicity and no serious adverse events that resulted in treatment discontinuation at any dose level. The most common toxicity was a self-limited, injection site reaction. Other reactions that occurred at a low frequency include fever, flu-like symptoms, anorexia, chills, nausea, and headache. These symptoms were also self-limiting and did not require intervention other than symptomatic measures such as acetaminophen. There were no SAE associated with immunizations. A summary of the adverse events reported on 34 patients treated and evaluated for safety are presented below.

Adverse Events: $\geq 5\%$ Frequency Based on Incidence of Total Treatments

Adverse Events as of 07/31/12	# of Events	Unrelated/Unlikely	Possible	Probably/Definite	Incidence % (based on 94 total treatments)
Injection Site Reaction	21	2		21	22.3
Flu-like symptoms	10	4	5	3	10.6
Fever	9	6	1	4	9.5
Fatigue	8	6	2		8.5
Shortness of Breath	6	6			6.3
Pain	6	4			6.3
Anorexia	5		1		5.3
Chills	5	5	1	4	5.3
Constipation	5	5			5.3
Edema	5	4			5.3
Nausea	5	4	1		5.3

Grade 3 and Grade 4 Adverse Events in \geq 2% of Patients*

Adverse Events	Number of Grade 3 (G3)	Number of Grade 4 (G4)	Percent (Based on 34 patients evaluated for safety)
Pain (all types)	2		5.6
Fatigue	1		2.9
Anemia	1		2.9
Pleural effusion	1		2.9
Alkaline Phosphatase Increase	1		2.9
Abdominal Bloating	1		2.9
Bowel obstruction	1		2.9
GI disorder	1		2.9
Acute renal failure	1		2.9

*Represents reported adverse events whether or not related to ETBX-011 vaccine

Of 34 total patients entered into the trial, 28 received all three treatments and the blood hematology, chemistry, and ANA values at week 0 (prior to first treatment) were compared with those obtained at week 9 (three weeks after the third treatment). There were no biologically significant changes in chemistry, hematology, or ANA values.

11.1.3 Formulation and Preparation

Each vaccine is supplied as a clear colorless liquid filled in a 2-mL amber vial at a concentration of 5×10^{11} total virus particles (VP) per 1.0 mL. Each vial is sealed with a rubber stopper and has a white flip off seal. End user of the product will need to flip the white plastic portion of the cap up/off with their thumb to expose the rubber stopper, and then puncture the stopper with an injection needle to withdraw the liquid. The rubber stopper is secured to the vial with an aluminum crimped seal.

11.1.4 Stability and Storage

Individual vials (in the desired number) of vaccine will be packaged in a cardboard box and will be shipped over dry ice by overnight courier with a temperature monitoring device included. Upon receipt, one will inspect contents of package for any noticeable damages or defects. Unpack the shipment contents and place the cardboard box containing vaccine vials into a freezer with a monitored temperature control. ETBX-011 vials should be stored at $-20 \pm 10^\circ\text{C}$; ETBX-051 and ETBX-061 vials should be stored at $\leq -60^\circ\text{C}$. Receiver must stop the temperature monitoring device by turning off the power switch (instructions for handling and operation of temperature monitoring device will be provided with the package).

11.1.5 ETBX Vaccine Dose Preparation and Administration

The dose of ETBX vaccine to be injected is 5×10^{11} VP per 1 mL. A dose level of 1×10^{11} VP per mL (dose level -1) is available for de-escalation. Prior to injection, the appropriate vial should be removed from the freezer and allowed to thaw at controlled room temperature ($20\text{--}25^\circ\text{C}$, $68\text{--}77^\circ\text{F}$) for at least 20 minutes and not more than 30 minutes, after which it should be kept at $2\text{--}8^\circ\text{C}$ (35--

46°F). The vaccine is stable in the vial for at least 8 hours after removal from the freezer when kept refrigerated at 2-8°C (35-46°F). Once the vaccine has been thawed, it must not be refrozen.

Each vial is sealed with a rubber stopper and has a white flip-off seal. The end user of the product will need to flip the white plastic portion of the cap up/off with their thumb to expose the rubber stopper and then puncture the stopper with an injection needle to withdraw the liquid. The rubber stopper is secured to the vial with an aluminum-crimped seal.

The thawed vial should be swirled and then, using aseptic technique, the pharmacist or pharmacist designee should withdraw the appropriate volume from the appropriate vial using an appropriately sized syringe.

The vaccine dose should be injected as soon as possible (i.e. within 1 hour) using a 1 to 1/2 inch, 20 to 25-gauge needle. If the vaccine cannot be injected within 1 hour, the syringe should be returned to the pharmacy and properly disposed in accordance with institutional policy and procedure, and disposition must be recorded on the investigational product accountability record.

11.1.5.1 Instructions for Dose Preparation – 5×10^{11} Virus Particles

Withdraw 1 mL of contents from the vial, prepare the injection site with alcohol, and administer to the subject by SC injection without any further manipulation (refer to the vial label for a description of the vial ETBX vaccine concentration).

11.1.5.2 Instructions for Dose Preparation – 1×10^{11} Virus Particles

Withdraw 0.2 mL of contents from the vial, prepare the injection site with alcohol, and administer to the subject by SC injection in the thigh without any further manipulation (refer to the vial label for a description of the vial ETBX vaccine concentration).

11.1.5.3 Administration

Each of the three ETBX vaccines will be administered on Weeks 0, 3, and 6 followed by bi-monthly booster vaccines for up to a year. All study drug administration treatments should occur within ± 7 days of the planned visit date.

ETBX-011 will be administered by subcutaneous injection in the upper arm after preparation of the site with alcohol. A 1 to ½ inch, 20- to 25- gauge needle should be used for administration.

ETBX-051 will be administered by subcutaneous injection in the anterolateral upper thigh after preparation of the site with alcohol. A 1 to ½ inch, 20- to 25- gauge needle should be used for administration. It is preferred to administer ETBX-051 and ETBX-061 in separate thighs. However, if administered in the same thigh, injection sites should be separated by at least 5 cm.

ETBX-061 will be administered by subcutaneous injection in the anterolateral upper thigh after preparation of the site with alcohol. A 1 to ½ inch, 20- to 25- gauge needle should be used for administration. It is preferred to administer ETBX-051 and ETBX-061 in separate thighs. However, if administered in the same thigh, injections sites should be separated by at least 5 cm.

11.1.6 Incompatibilities

The vaccines are administered as separate subcutaneous injections, with no known incompatibilities.

11.1.7 Other Considerations

Abbreviated Title: Ad5-based combination vaccines
Version Date: 05.16.2018

The Ad5 [E1-, E2b-] vector is non-replicating and its genome does not integrate into the human genome. However, since this is a non-replicating recombinant virus, it is recommended that it be handled under Biosafety Level-2 conditions. Any vial of ETBX vaccine material that has been used in the study should be autoclaved or incinerated after use according to institutional policy and according to local, state and federal regulations. Refer to the Material Safety Data Sheets for additional handling instructions.

12 REFERENCES

1. Balint JP, Gabitzsch ES, Rice A, Latchman Y, Xu Y, Messerschmidt GL, et al. Extended evaluation of a phase 1/2 trial on dosing, safety, immunogenicity, and overall survival after immunizations with an advanced-generation Ad5 [E1-, E2b-]-CEA(6D) vaccine in late-stage colorectal cancer. *Cancer Immunol Immunother.* 2015;64(8):977-87.
2. Morse MA, Chaudhry A, Gabitzsch ES, Hobeika AC, Osada T, Clay TM, et al. Novel adenoviral vector induces T-cell responses despite anti-adenoviral neutralizing antibodies in colorectal cancer patients. *Cancer Immunol Immunother.* 2013;62(8):1293-301.
3. Horan Hand P, Colcher D, Salomon D, Ridge J, Noguchi P, Schlom J. Influence of spatial configuration of carcinoma cell populations on the expression of a tumor-associated glycoprotein. *Cancer Res.* 1985;45(2):833-40.
4. Szpak CA, Johnston WW, Lottich SC, Kufe D, Thor A, Schlom J. Patterns of reactivity of four novel monoclonal antibodies (B72.3, DF3, B1.1 and B6.2) with cells in human malignant and benign effusions. *Acta Cytol.* 1984;28(4):356-67.
5. Greiner JW, Hand PH, Noguchi P, Fisher PB, Pestka S, Schlom J. Enhanced expression of surface tumor-associated antigens on human breast and colon tumor cells after recombinant human leukocyte alpha-interferon treatment. *Cancer Res.* 1984;44(8):3208-14.
6. Hand PH, Nuti M, Colcher D, Schlom J. Definition of antigenic heterogeneity and modulation among human mammary carcinoma cell populations using monoclonal antibodies to tumor-associated antigens. *Cancer Res.* 1983;43(2):728-35.
7. Lottich SC, Szpak CA, Johnston WW, Thor A, Schlom J. Phenotypic heterogeneity of a tumor-associated antigen in adenocarcinomas of the colon and their metastases as demonstrated by monoclonal antibody B72.3. *Cancer Invest.* 1986;4(5):387-95.
8. Gabitzsch ES, Tsang KY, Palena C, David JM, Fantini M, Kwilas A, et al. The generation and analyses of a novel combination of recombinant adenovirus vaccines targeting three tumor antigens as an immunotherapeutic. *Oncotarget.* 2015;6(31):31344-59.
9. Wilkinson DG, Bhatt S, Herrmann BG. Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature.* 1990;343(6259):657-9.
10. Hamilton DH, Litzinger MT, Fernando RI, Huang B, Palena C. Cancer vaccines targeting the epithelial-mesenchymal transition: tissue distribution of brachyury and other drivers of the mesenchymal-like phenotype of carcinomas. *Semin Oncol.* 2012;39(3):358-66.
11. Hamilton DH, Fernando RI, Schlom J, Palena C. Aberrant expression of the embryonic transcription factor brachyury in human tumors detected with a novel rabbit monoclonal antibody. *Oncotarget.* 2015;6(7):4853-62.
12. Roselli M, Fernando RI, Guadagni F, Spila A, Alessandroni J, Palmirota R, et al. Brachyury, a driver of the epithelial-mesenchymal transition, is overexpressed in human lung tumors: an opportunity for novel interventions against lung cancer. *Clin Cancer Res.* 2012;18(14):3868-79.
13. Miettinen M, Wang Z, Lasota J, Heery C, Schlom J, Palena C. Nuclear Brachyury Expression Is Consistent in Chordoma, Common in Germ Cell Tumors and Small Cell Carcinomas, and Rare in Other Carcinomas and Sarcomas: An Immunohistochemical Study of 5229 Cases. *Am J Surg Pathol.* 2015;39(10):1305-12.
14. Kilic N, Feldhaus S, Kilic E, Tennstedt P, Wicklein D, Wasielewski R, et al. Brachyury expression predicts poor prognosis at early stages of colorectal cancer. *European journal of cancer (Oxford, England : 1990).* 2011;47(7):1080-5.

15. Du R, Wu S, Lv X, Fang H, Wu S, Kang J. Overexpression of brachyury contributes to tumor metastasis by inducing epithelial-mesenchymal transition in hepatocellular carcinoma. *Journal of experimental & clinical cancer research* : CR. 2014;33:105.
16. Pinto F, Pertega-Gomes N, Pereira MS, Vizcaino JR, Monteiro P, Henrique RM, et al. T-box transcription factor brachyury is associated with prostate cancer progression and aggressiveness. *Clin Cancer Res*. 2014;20(18):4949-61.
17. Palena C, Roselli M, Litzinger MT, Ferroni P, Costarelli L, Spila A, et al. Overexpression of the EMT driver brachyury in breast carcinomas: association with poor prognosis. *J Natl Cancer Inst*. 2014;106(5).
18. Hamilton DH, Roselli M, Ferroni P, Costarelli L, Cavaliere F, Taffuri M, et al. Brachyury, a vaccine target, is overexpressed in triple-negative breast cancer. *Endocr Relat Cancer*. 2016;23(10):783-96.
19. Tirabosco R, Mangham DC, Rosenberg AE, Vujovic S, Bousdras K, Pizzolitto S, et al. Brachyury expression in extra-axial skeletal and soft tissue chordomas: a marker that distinguishes chordoma from mixed tumor/myoepithelioma/parachordoma in soft tissue. *Am J Surg Pathol*. 2008;32(4):572-80.
20. Vujovic S, Henderson S, Presneau N, Odell E, Jacques TS, Tirabosco R, et al. Brachyury, a crucial regulator of notochordal development, is a novel biomarker for chordomas. *The Journal of pathology*. 2006;209(2):157-65.
21. Fernando RI, Litzinger M, Trono P, Hamilton DH, Schlom J, Palena C. The T-box transcription factor Brachyury promotes epithelial-mesenchymal transition in human tumor cells. *J Clin Invest*. 2010;120(2):533-44.
22. Savagner P. Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity. *Curr Top Dev Biol*. 2015;112:273-300.
23. Nieto MA, Cano A. The epithelial-mesenchymal transition under control: global programs to regulate epithelial plasticity. *Seminars in cancer biology*. 2012;22(5-6):361-8.
24. Huang B, Cohen JR, Fernando RI, Hamilton DH, Litzinger MT, Hodge JW, et al. The embryonic transcription factor Brachyury blocks cell cycle progression and mediates tumor resistance to conventional antitumor therapies. *Cell Death Dis*. 2013;4:e682.
25. Larocca C, Cohen JR, Fernando RI, Huang B, Hamilton DH, Palena C. An autocrine loop between TGF-beta1 and the transcription factor brachyury controls the transition of human carcinoma cells into a mesenchymal phenotype. *Mol Cancer Ther*. 2013;12(9):1805-15.
26. Hamilton DH, Huang B, Fernando RI, Tsang KY, Palena C. WEE1 inhibition alleviates resistance to immune attack of tumor cells undergoing epithelial-mesenchymal transition. *Cancer Res*. 2014;74(9):2510-9.
27. David JM, Hamilton DH, Palena C. MUC1 upregulation promotes immune resistance in tumor cells undergoing brachyury-mediated epithelial-mesenchymal transition. *Oncoimmunology*. 2016;5(4):e1117738.
28. Haro A, Yano T, Kohno M, Yoshida T, Koga T, Okamoto T, et al. Expression of Brachyury gene is a significant prognostic factor for primary lung carcinoma. *Annals of surgical oncology*. 2013;20 Suppl 3:S509-16.
29. Pinto F, Campanella NC, Abrahao-Machado LF, Scapulatempo-Neto C, de Oliveira AT, Brito MJ, et al. The embryonic Brachyury transcription factor is a novel biomarker of GIST aggressiveness and poor survival. *Gastric Cancer*. 2016;19(2):651-9.

30. Palena C, Polev DE, Tsang KY, Fernando RI, Litzinger M, Krukovskaya LL, et al. The human T-box mesodermal transcription factor Brachyury is a candidate target for T-cell-mediated cancer immunotherapy. *Clin Cancer Res.* 2007;13(8):2471-8.
31. Tucker JA, Jochems C, Boyerinas B, Fallon J, Greiner JW, Palena C, et al. Identification and characterization of a cytotoxic T-lymphocyte agonist epitope of brachyury, a transcription factor involved in epithelial to mesenchymal transition and metastasis. *Cancer Immunol Immunother.* 2014;63(12):1307-17.
32. Madan RA, Mohebtash M, Arlen PM, Vergati M, Rauckhorst M, Steinberg SM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. *Lancet Oncol.* 2012;13(5):501-8.
33. NCT01519817. An Open Label Phase I Study to Evaluate the Safety and Tolerability of GI-6301 Vaccine Consisting of Whole, Heat-Killed Recombinant *Saccharomyces Cerevisiae* (Yeast) Genetically Modified to Express Brachyury Protein in Adults With Solid Tumors.
34. NCT02179515. An Open Label Phase I Study to Evaluate the Safety and Tolerability of a Modified Vaccinia Ankara (MVA) Based Vaccine Modified to Express Brachyury and T-Cell Costimulatory Molecules (MVA Brachyury-TRICOM).
35. Heery CR, Singh BH, Rauckhorst M, Marté JL, Donahue RN, Grenga I, et al. Phase I Trial of a Yeast-Based Therapeutic Cancer Vaccine (GI-6301) Targeting the Transcription Factor Brachyury. *Cancer Immunol Res.* 2015;3(11):1248-56.
36. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res.* 2009;15(17):5323-37.
37. Zaremba S, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res.* 1997;57(20):4570-7.
38. Yin L, Ahmad R, Kosugi M, Kufe T, Vasir B, Avigan D, et al. Survival of human multiple myeloma cells is dependent on MUC1 C-terminal transmembrane subunit oncoprotein function. *Mol Pharmacol.* 2010;78(2):166-74.
39. Yin L, Kufe D. MUC1-C Oncoprotein Blocks Terminal Differentiation of Chronic Myelogenous Leukemia Cells by a ROS-Mediated Mechanism. *Genes Cancer.* 2011;2(1):56-64.
40. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer.* 2004;4(1):45-60.
41. Kawano T, Ito M, Raina D, Wu Z, Rosenblatt J, Avigan D, et al. MUC1 oncoprotein regulates Bcr-Abl stability and pathogenesis in chronic myelogenous leukemia cells. *Cancer Res.* 2007;67(24):11576-84.
42. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem.* 1990;265(25):15286-93.
43. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma.* 1984;3(3):223-32.
44. Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. *Clin Cancer Res.* 2008;14(10):3060-9.
45. Limacher JM, Quiox E. TG4010: A therapeutic vaccine against MUC1 expressing tumors. *Oncoimmunology.* 2012;1(5):791-2.

46. Mohebtash M, Tsang KY, Madan RA, Huen NY, Poole DJ, Jochems C, et al. A pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine in patients with metastatic breast and ovarian cancer. *Clin Cancer Res.* 2011;17(22):7164-73.
47. Kufe DW. Functional targeting of the MUC1 oncogene in human cancers. *Cancer Biol Ther.* 2009;8(13):1197-203.
48. Tsang KY, Palena C, Gulley J, Arlen P, Schlom J. A human cytotoxic T-lymphocyte epitope and its agonist epitope from the nonvariable number of tandem repeat sequence of MUC-1. *Clin Cancer Res.* 2004;10(6):2139-49.
49. Jochems C, Tucker JA, Vergati M, Boyerinas B, Gulley JL, Schlom J, et al. Identification and characterization of agonist epitopes of the MUC1-C oncoprotein. *Cancer Immunol Immunother.* 2014;63(2):161-74.
50. Imler JL. Adenovirus vectors as recombinant viral vaccines. *Vaccine.* 1995;13(13):1143-51.
51. Tatsis N, Ertl HC. Adenoviruses as vaccine vectors. *Mol Ther.* 2004;10(4):616-29.
52. Campos SK, Barry MA. Current advances and future challenges in Adenoviral vector biology and targeting. *Curr Gene Ther.* 2007;7(3):189-204.
53. Seregin SS, Amalfitano A. Overcoming pre-existing adenovirus immunity by genetic engineering of adenovirus-based vectors. *Expert Opin Biol Ther.* 2009;9(12):1521-31.
54. Chirmule N, Probert K, Magosin S, Qian Y, Qian R, Wilson J. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther.* 1999;6(9):1574-83.
55. Barouch DH, Kik SV, Weverling GJ, Dilan R, King SL, Maxfield LF, et al. International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations. *Vaccine.* 2011;29(32):5203-9.
56. Amalfitano A, Hauser MA, Hu H, Serra D, Begy CR, Chamberlain JS. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol.* 1998;72(2):926-33.
57. Amalfitano A, Chamberlain JS. Isolation and characterization of packaging cell lines that coexpress the adenovirus E1, DNA polymerase, and preterminal proteins: implications for gene therapy. *Gene Ther.* 1997;4(3):258-63.
58. Hartigan-O'Connor D, Amalfitano A, Chamberlain JS. Improved production of gutted adenovirus in cells expressing adenovirus preterminal protein and DNA polymerase. *J Virol.* 1999;73(9):7835-41.
59. Gabitzsch ES, Jones FR. New recombinant Ad5 vector overcomes Ad5 immunity allowing for multiple safe, homologous immunizations. *J Clin Cell Immunol.* 2011;S4:001.
60. Osada T, Yang XY, Hartman ZC, Glass O, Hodges BL, Niedzwiecki D, et al. Optimization of vaccine responses with an E1, E2b and E3-deleted Ad5 vector circumvents pre-existing anti-vector immunity. *Cancer Gene Ther.* 2009;16(9):673-82.
61. Gabitzsch ES, Xu Y, Balint Jr JP, Hartman ZC, Lysterly HK, Jones FR. Anti-tumor immunotherapy despite immunity to adenovirus using a novel adenoviral vector Ad5 [E1-, E2b-]-CEA. *Cancer Immunol Immunother.* 2010;59(7):1131-5.
62. Gabitzsch ES, Xu Y, Yoshida LH, Balint J, Gayle RB, Amalfitano A, et al. A preliminary and comparative evaluation of a novel Ad5 [E1-, E2b-] recombinant-based vaccine used to induce cell mediated immune responses. *Immunol Lett.* 2009;122(1):44-51.
63. Gabitzsch ES, Xu Y, Yoshida LH, Balint J, Amalfitano A, Jones FR. Novel Adenovirus type 5 vaccine platform induces cellular immunity against HIV-1 Gag, Pol, Nef despite the presence of Ad5 immunity. *Vaccine.* 2009;27(46):6394-8.

64. Gabitzsch ES, Balint-Junior JP, Xu Y, Balcitis S, Sanders-Beer B, Karl J, et al. Control of SIV infection and subsequent induction of pandemic H1N1 immunity in rhesus macaques using an Ad5 [E1-, E2b-] vector platform. *Vaccine*. 2012;30(50):7265-70.
65. Jones FR, Gabitzsch ES, Xu Y, Balint JP, Borisevich V, Smith J, et al. Prevention of influenza virus shedding and protection from lethal H1N1 challenge using a consensus 2009 H1N1 HA and NA adenovirus vector vaccine. *Vaccine*. 2011;29(40):7020-6.
66. Wieking BG, Vermeer DW, Spanos WC, Lee KM, Vermeer P, Lee WT, et al. A non-oncogenic HPV 16 E6/E7 vaccine enhances treatment of HPV expressing tumors. *Cancer Gene Ther*. 2012;19(10):667-74.
67. Rice AE, Latchman YE, Balint JP, Lee JH, Gabitzsch ES, Jones FR. An HPV-E6/E7 immunotherapy plus PD-1 checkpoint inhibition results in tumor regression and reduction in PD-L1 expression. *Cancer Gene Ther*. 2015;22(9):454-62.
68. Gabitzsch ES, Xu Y, Balint Jr JP, Balcitis S, Sanders-Beer B, Jones FR. Induction and comparison of SIV immunity in Ad5 naïve and Ad5 immune non-human primates using an Ad5 [E1-, E2b-] based vaccine. *Vaccine*. 2011;29(45):8101-7.
69. Abe M, Kufe DW. Identification of a family of high molecular weight tumor-associated glycoproteins. *J Immunol*. 1987;139(1):257-61.
70. Poland PA, Kinlough CL, Rokaw MD, Magarian-Blander J, Finn OJ, Hughey RP. Differential glycosylation of MUC1 in tumors and transfected epithelial and lymphoblastoid cell lines. *Glycoconj J*. 1997;14(1):89-96.
71. Friedman EL, Hayes DF, Kufe DW. Reactivity of monoclonal antibody DF3 with a high molecular weight antigen expressed in human ovarian carcinomas. *Cancer Res*. 1986;46(10):5189-94.
72. Ramasamy S, Duraisamy S, Barbashov S, Kawano T, Kharbanda S, Kufe D. The MUC1 and galectin-3 oncoproteins function in a microRNA-dependent regulatory loop. *Mol Cell*. 2007;27(6):992-1004.
73. Salazar E, Zaremba S, Arlen PM, Tsang KY, Schlom J. Agonist peptide from a cytotoxic t-lymphocyte epitope of human carcinoembryonic antigen stimulates production of tc1-type cytokines and increases tyrosine phosphorylation more efficiently than cognate peptide. *Int J Cancer*. 2000;85(6):829-38.
74. Lepone LM, Donahue RN, Grenga I, Metenou S, Richards J, Heery CR, et al. Analysis of 123 peripheral human immune cell subsets: defining differences with age and between healthy donors and cancer patients not detected in analysis of standard immune cell types. *J Circ Biomark*. In press.
75. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol*. 2008;26(7):1148-59.
76. Rustin GJ, Quinn M, Thigpen T, du Bois A, Pujade-Lauraine E, Jakobsen A, et al. Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). *J Natl Cancer Inst*. 2004;96(6):487-8.
77. Bublej GJ, Carducci M, Dahut W, Dawson N, Daliani D, Eisenberger M, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. *J Clin Oncol*. 1999;17(11):3461-7.

78. Vergote I, Rustin GJ, Eisenhauer EA, Kristensen GB, Pujade-Lauraine E, Parmar MK, et al. Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup. *J Natl Cancer Inst.* 2000;92(18):1534-5.
79. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England : 1990).* 2009;45(2):228-47.

13 APPENDICES

13.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

13.2 APPENDIX B: INJECTION SITE REACTIONS DIARY CARD

Please answer all questions below once per day for the first 7 days

		Day 0 (Day of Injection)	Day +1	Day +2	Day +3	Day +4	Day +5	Day +6
1. Is there redness at the injection site?	Circle: Yes / No If yes measure longest diameter in millimeters (mm)	Yes / No _____mm						
2. Is there firmness or swelling at the injection site?	Circle: Yes / No If yes measure longest diameter in millimeters (mm)	Yes / No _____mm						
3. Is there soreness at the injection site?	Circle: Yes / No If yes tell us if the soreness is mild, moderate, or severe	Yes / No Mild Moderate Severe						
4. Have you experienced pain at the injection site?	Circle: Yes / No If yes tell us if the pain is mild, moderate, or severe	Yes / No Mild Moderate Severe						
5. Have you taken any medication for	Circle: Yes / No Provide name and	Yes / No Name: _____						

		Day 0 (Day of Injection)	Day +1	Day +2	Day +3	Day +4	Day +5	Day +6
injection site pain?	dose of medication	Dose: _____						
6. Have you experienced chills?	Circle: Yes / No If yes tell us if the chills are mild, moderate, or severe	Yes / No Mild Moderate Severe						
7. Record your daily temperature (Do not drink anything within 5 minutes prior to taking your temperature)	Circle: Yes / No If your temperature is 104°F for more than 24 hours, call your study doctor	Record Temp: _____						