Abbreviated Title: Ad5-based vaccines in mCRPC
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Title: Treatment of Patients with Castration Resistant Prostate Cancer using Multi-Targeted Recombinant Ad5 PSA/MUC1/Brachyury Based Immunotherapy Vaccines

NCI Principal Investigator: Marijo Bilusic, MD, PhD
Genitourinary Malignancies Branch (GMB), CCR, NCI
10 Center Drive
Building 10, Room B2L312
Bethesda, MD 20892
Phone: 240-760-6064
Email: marijo.bilusic@nih.gov

Investigational Agents:

Drug Name:	ETBX-071; adenoviral PSA vaccine	ETBX-061; adenoviral MUC1 vaccine	ETBX-051; adenoviral brachyury vaccine
IND Number:	17811	17811	17811
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 prostate 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 vaccines targeting three (3) human tumor associated antigens (TAA), PSA, MUC1, and brachyury, respectively have 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-071, ETBX-061, and ETBX-051) when administered subcutaneously (SC) to subjects with metastatic castration resistant prostate cancer

Eligibility:

- Subjects age 18 and older with cytologically or histologically confirmed prostate cancer for which no curative standard approved therapy is available.
- Metastatic Castration Resistant Prostate Cancer (mCRPC) patients with rising PSA or progressive disease despite castration levels of testosterone.
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- Prior treatment with immunotherapy, hormonal therapy, radiotherapy, chemotherapy, and/or other experimental therapy is allowed.
- ECOG performance status ≤ 1
- Adequate organ and bone marrow function.
- Subjects with a history of autoimmune disease (active or past) and subjects requiring systemic steroids are not eligible (physiologic doses of steroids for steroid replacement as well as nasal, topical and inhaled steroids are allowed). Autoimmune-related thyroid disease, type I diabetes and vitiligo are permitted if the condition is well controlled.

Design:

- This is a Phase I trial in subjects with mCRPC. A combination of three therapeutic vaccines (ETBX-071, ETBX-061, and ETBX-051) which use the same modified Adenovirus vector backbone, separately encoding three well studied TAA,PSA, MUC1, and brachyury, respectively) will be assessed. The vaccines will be tested at standard dose levels, with a dose de-escalation (if required) design employed. The dose level of each vaccine tested will be $5x10^{11}$ VP. This dose has been found in a prior phase 1 testing of a similar vaccine Ad5 [E1-, E2b-]-CEA(6D) (ETBX-011) to be well tolerated (with no DLT's or related SAE's), and be optimal for induction of immune responses.
- Up to six patients will be enrolled at dose level 1. If ≤ 1 of 6 patients experience a DLT, an 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 (-1) ($1x10^{11}$ 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 further dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-2) ($5x10^{10}$ 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 -2, then the study will be stopped.

- A dose expansion phase of study will be enrolled after the MTD of the vaccines have been determined. An additional 12 subjects will be enrolled in the dose expansion component of the trial, for a total of 18 subjects at the MTD.
- The ETBX-051, ETBX-61 and ETBX-71 vaccines will be administered subcutaneously (SC) at separate injection sites (proximal limb, preferably the thigh), will be administered SC every 3 weeks for 3 doses (dose de-escalation cohorts) followed by boosts every 8 weeks for 1 year (only patients enrolled in dose expansion cohort).

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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 (*E1*) and early 3 (*E3*) gene regions
Ad5 [E1-, E2b-]	Ad5 with deletions in the early 1 (*E1*), early 2b (*E2b*), and early 3 (*E3*) gene regions
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Brachyury	A transcription factor within the T-box complex of genes
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
CEA(6D)	Modified 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

Abbreviation or Specialist Term	Explanation
DLTs	Dose-limiting toxicities
E1	Adenovirus early 1 gene
E2b	Adenovirus early 2b gene
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-071	Ad5 [E1-, E2b-]-PSA
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
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

Abbreviation or Specialist Term	Explanation
LLD	Longest lesion diameter
LLN	Lower limit of normal
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
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
PSA	Prostate specific antigen
PT	Prothrombin
PTT	Partial thromboplastin time
aPTT	Activated partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious adverse event
SC	Subcutaneous
SD	Stable disease
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reactions
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-071, ETBX-061, and ETBX-051) when administered subcutaneously (SC) in subjects with Metastatic Castration Resistant Prostate Cancer (mCRPC).

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) and PSA doubling time (PSADT) in subjects with advanced cancer treated with the ETBX-071, ETBX-061, and ETBX-051 vaccines.

1.1.3 Exploratory Objectives

- To evaluate the immunogenicity of the ETBX-071, ETBX-061, and ETBX-051 vaccines platform
- To monitor circulating PSA serum/plasma levels.
- To evaluate CD8 and CD4 immunologic response as measured by an increase in PSA, MUC-1, and Brachyury-specific T cells.
- To evaluate tumors for expression of the target antigens, PSA, MUC-1, and Brachyury
- Correlations between immunogenicity and efficacy outcomes will be assessed.

1.1.4 Primary Endpoints

- Refer to section **8.1.1**

1.1.5 Secondary Endpoints

- Refer to section **8.1.2**

1.1.6 Exploratory Endpoints

- Immunogenicity of the ETBX-071, ETBX-061, and ETBX-051 vaccines by flow cytometric analysis of T-cell frequency, activation status, cytokine profiles, antibody levels and analysis in sera of sCD27 and sCD40L
- Evaluate circulating PSA levels and correlations with immunogenicity and/or efficacy.

1.2 BACKGROUND AND RATIONALE

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, efforts are focused on

developing multi-targeted immunotherapy approaches. The overall goal of the current project is to expand the immunotherapeutic approach for the treatment of mCRPC employing a multi-targeted approach. Previous studies have demonstrated safety, dose response, and favorable overall survival in metastatic colorectal cancer (mCRC) patients using Ad5 [E1-, E2b-]-based vaccine platform containing a modified carcinoembryonic antigen (CEA) (Ad5 [E1-, E2b-[-CEA(6D) referred to as ETBX-011.(1) This vaccine was employed as an immunotherapeutic tested as a single agent in a clinical setting.(2, 3)

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. 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 PSA, MUC1, and/or Brachyury.(4, 5)The objective is to develop a combination immunotherapeutic approach designed to induce broad anti-tumor immune responses directed against prostate cancer. In proof of concept research, published pre-clinical studies indicated that a multi-targeted vaccine based upon the Ad5 [E1-, E2b-] platform and containing CEA, MUC1, and brachyury TAA induces immune responses directed against all three target CEA, MUC1, and brachyury antigens with minimal to no "antigenic competition" in *in vitro* studies of human dendritic cells(6), or in murine vaccination studies.(6)

We plan to test a combination of multi-targeted PSA, MUC1, and brachyury targeted Ad5 [E1-, E2b-]-based vectored vaccines in a phase 1 clinical to test the safety and efficacy in patients with mCRPC. Subsequent trials will involve the use of these vaccines in combination with checkpoint inhibitor monoclonal antibodies (MAbs) and other immune modulators.

1.2.2 PSA Expression in Prostate Cancer and It's Use as an Immunotherapy Target

Prostate cancer is an ideal candidate for immunotherapy for several reasons. The slow growing nature of cancer within the prostate allows sufficient time to generate an anti-tumor immune response following a prime/boost or multiple immunization strategies.(7, 8) The presence of PSA in patient serum enables the malignancy to be detected early and, in some cases, before tumors are radiologically detectable.(9) This in turn can facilitate earlier treatment.(10) Circulating T cells that react with prostate TAAs have previously been detected, which suggests that self-tolerance to these antigens can be overcome.(10) The prostate is considered to be a non-essential organ and therefore induced immunological responses directed against specific prostate TAAs should not cause acute off target toxicity.(9, 10) Most importantly, the first prostate cancer specific immunotherapy, Sipuleucel-T (Provenge®, Dendreon Corporation, Seattle, WA), has been licensed by the US Food and Drug Administration (FDA) in 2010 for asymptomatic or minimally symptomatic mCRPC.(11, 12) Sipuleucel-T consists of autologous peripheral blood mononuclear cells with antigen presenting dendritic cells that have been activated ex vivo with a recombinant

fusion protein (PA2024) consisting of PAP linked to granulocyte-macrophage colony stimulating factor (GM-CSF).(12) In a phase III trial, mCPRC patients receiving Sipuleucel-T exhibited a 22% reduction in mortality.(11) The success of the therapeutic Sipuleucel–T has now paved the way for other immunotherapeutic prostate cancer vaccines to be granted regulatory approval and enter the market.

Prostvac™ vaccine offers an alternative strategy to Sipuleucel-T and employs genetically altered poxviruses to deliver targeting information to immune cells and generate an immune response. Administered subcutaneously, the poxviruses deliver the transgenes for the tumor associated antigens (PSA) to antigen presenting cells through cellular infection. Once these pox viruses are within the cellular cytoplasm, the transgenes are processed. The end result is an antigen presenting cell expressing a PSA peptide within the major histocompatibility complex, resulting in PSA-specific cytolytic T lymphocytes activation. Prostvac was evaluated in a randomized phase 2 trial. Subjects with minimally symptomatic mCRPC were randomized 2:1 (85/41) to vaccine therapy versus placebo. Treated subjects had prolonged median overall survival (OS) 26 vs. 18 months. Prostvac is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms.(13) This approach has been taken to pivotal randomized phase 3 trial, now fully enrolled (N=1297), and awaiting event driven results.(14)

In addition, a recombinant Ad-5 [E1-, E2b-] PSA vector (Ad-PSA) has now been developed. PSA has been incorporated into a replication incompetent early generation Ad5 [E1-]-based vector platform and tested in a phase 1 trial.(15) Sequential cohorts of subjects had increasing doses of a single injection of Ad5-PSA. Most subjects developed detectable cellular mediated anti-PSA responses 18/32 (67%). This approach is being evaluated in a phase 2 trial using multiple doses (3 injections) at 1 month intervals.(16) Infection of human dendritic cells with Ad-PSA induced high levels of expression of PSA. Moreover, Ad-PSA infected dendritic cells were shown to activate a PSA specific human T-cell line to produce >3,000 pg of IFN $_{g}$ in an MHC restricted manner, while control Ad5 infected dendritic cells expressed less than 1pg IFN-$_{g}$. Thus, PSA based vaccination approaches have preliminary evidence of clinical activity as well as an ability to induce anti-PSA directed cellular immunity. Improved vectors, such as the new Etubics Ad5 [E1-, E2b-]-based vector platform, should facilitate clinical development of this targeted approach. Non-replicating adenoviral vectors should improve the safety of this approach, and the ability to circumvent neutralizing anti-viral immune responses would enable sustained boosting to maximize immune responses. These features can be provided by Ad5 [E1-, E2b-]-based vectors.

1.2.3 MUC1 Expression in Cancer and It's Use as an Immunotherapy Target

MUC1 (CD227) is a tumor associated antigen (TAA) that is over-expressed on a majority of human carcinomas and several hematologic malignancies.(17-20) The MUC1 glycoprotein is normally expressed at the surface of glandular epithelial cells(21) and is over-expressed and aberrantly glycosylated in carcinomas.(17, 22) Thus, MUC1 has been recognized as a priority TAA that can be utilized as a target for tumor immunotherapy.(23, 24) Several clinical trials have been and are being performed to evaluate the use of MUC1 in immunotherapeutic vaccines.(25-30) Some of these trials have indicated that targeting MUC1 is safe and may provide survival benefit .(28-30)

Multiple enhancer agonist epitopes were previously identified, several of which are in the MUC1 C-terminus region. (31, 32) This is potentially important because studies 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.(24) Therefore, it is believed that MUC1 containing modified agonist epitopes has a greater potential as an immunogenic agent for vaccine development.

1.2.4 Brachyury Expression in Cancer and It's Potential 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.(33) 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(34), aberrantly high levels of Brachyury have been observed in the primary and/or metastatic sites of non-small cell (NSCLC) and small cell (SCC) lung cancer(35, 36), colon(37), hepatocellular(38), prostate(39) and breast carcinomas(40), including triple negative breast cancer (TNBC)(41). High levels of Brachyury are also characteristic of the rare tumor type chordoma(42), 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")(43). Carcinoma cells undergoing this phenotypic transition exhibit enhanced motility and invasiveness *in vitro*, propensity to metastasize *in vivo*, and features of tumor stemness(44), including resistance to a range of therapeutics such as chemotherapy, radiation, small molecule therapies and, potentially, immunotherapy(45-48). 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(49), colon(37) breast(40), triple negative breast(41) and gastrointestinal stromal tumor (GIST)(50). Brachyury expression has also been shown to be correlated with advanced stage prostate cancer(39).

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*.(33, 51) 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.(33, 51)

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.(52) 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(53) or an MVA-Brachyury-TRICOM vaccine(54) also demonstrated the generation of Brachyury-specific T cells as well as safety in humans, thus providing further evidence of immunogenicity(55). 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.

1.2.5 Adenovirus-Based Vectors

Adenoviruses (Ads) have emerged as leading candidate vectors to deliver vaccines designed to induce CMI and antibody responses (56-58). Ad vectors infect multiple cell types, including dendritic cells which result in priming of a vigorous CMI response. Ads are a family of DNA viruses characterized by an icosahedral, non-enveloped capsid containing a linear double-stranded genome **(**57**,** 58**).** 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 (56-59). 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 (57). 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 (58). 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 **(**57**,** 59**)**. Most humans have antibodies against Ad5, with up to two-thirds having lymphoproliferative responses against Ad5 **(**60**,** 61**)**. 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 have 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-]) (62-65). 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 **(**1**,** 3**,** 6**,** 65-73**)**.

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.**(**2**,** 3**)** The Phase I/II study consisted of a dose-escalation study of four dosage levels ($1x10^9$, $1x10^{10}$, $1x10^{11}$, $5x10^{11}$ VP/dose) of ETBX-011 (Phase I component), and the maximally tolerated dose of ETBX-011 (Phase II and $5x10^{11}$ 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 Karnofsky 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.**(**2**,** 3**)**

1.2.6.2 Clinical Immune Response

A secondary objective of the study listed above was to evaluate CEA specific immune responses following immunization treatments with the product candidate. As determined by an ELISA technique,**(**2**,** 3**)** no antibody activity directed against CEA was observed. CMI responses were assessed 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 three weeks following the last treatment from patients. CEA specific ELISpot assays were performed on PBMC as previously described(1-3) to determine the numbers of interferon gamma (IFN-γ) secreting lymphocytes (SFC) after exposure to CEA peptides *in vitro*. The highest CMI responses were determined during immunizations, regardless of time point (weeks 3, 6, or 9) in the patients

treated in cohort 1, cohort 2, cohort 3/Phase II, and cohort 5. This analysis revealed a dose response to increasing levels of product (**Figure 1**). The highest CMI levels occurred in patients that received the highest dose of $5x10^{11}$ VP (Cohort 5).

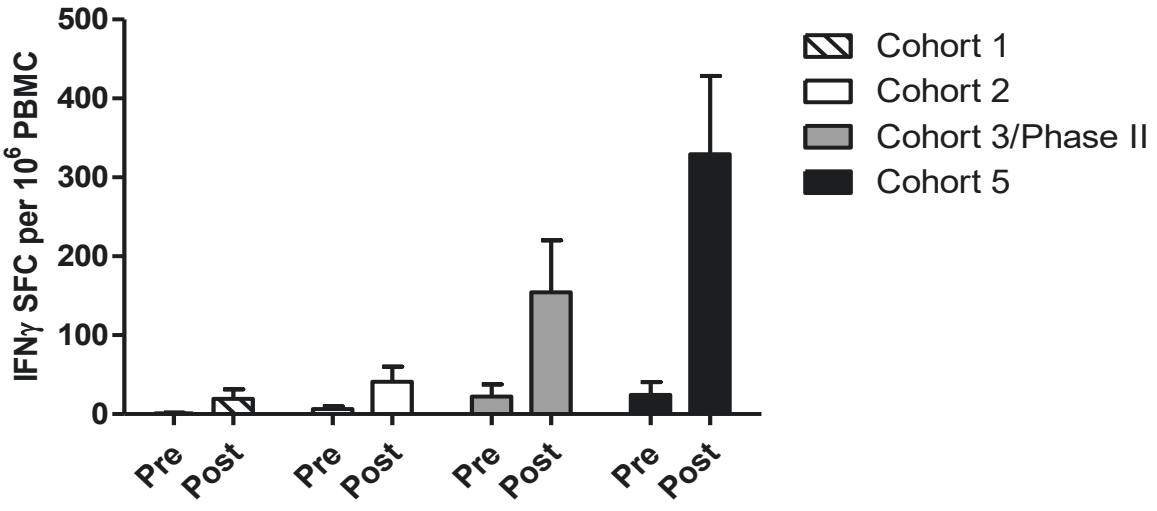


Figure 1. CMI responses in treated patients. CMI (IFN-γ secretion) was assessed at baseline (Pre) and after administrations of Ad5 [E1-, E2b-]-CEA(6D) (Post). The highest CMI responses (regardless of time point) observed in the patients after treatment revealed a dose response. The highest CMI levels occurred in patients that received the highest dose of $5x10^{11}$ VP (Cohort 5). The CMI responses for Cohort 3/Phase II and Cohort 5 were significantly elevated (P=0.0002 and P=0.0317, respectively; *Mann-Whitney test*) as compared to their baseline (Pre) values. Specificity of the responses was demonstrated by the lack of reactivity with the irrelevant antigens β-galactosidase and HIV-gag (data not shown). For positive controls, PBMCs were exposed to concanavalin A (data not shown). SFC: spot forming cells. Values = Mean ± SEM for each Cohort.

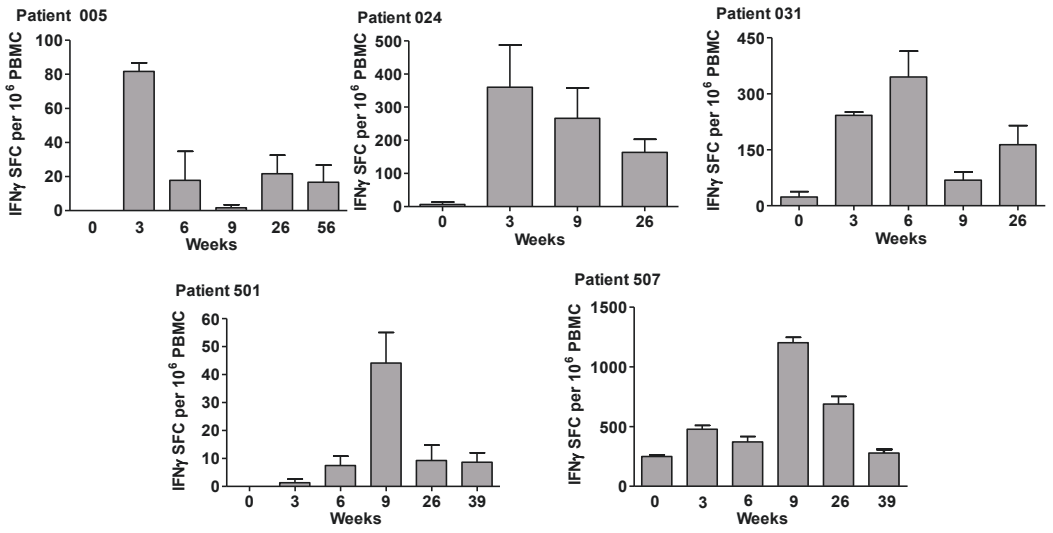


Figure 2. Profiles of CEA directed CMI responses in 5 mCRC patients during and after the course of immunotherapy. Note the decrease in CMI responses from their peak values after immunizations ended. Values are Mean ± SEM.

In a preliminary study,(2) a population of polyfunctional CD8+ T cells was observed (those that secrete more than 1 cytokine when activated) after immunizations that secreted multiple cytokines, a sign of greater functionality of T cells induced by the vaccine. In further follow-up analysis(2) 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.

Ad5 NAb and CMI were measured against Ad5 and 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 NAb titer value obtained among all patients was 1:189 ±1:71 SEM and the mean pre-treatment Ad5 NAb titer among seropositive patients was 1:308 ± 1:108. Analysis of serum samples from patients who received 3 immunizations revealed Ad5 NAb titers that were significantly increased ($P<0.0001$, *Mann-Whitney test*) by week 9 (mean 1:4767 ± 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 Clinical Evidence of Activity

The Ad5 [E1-, E2b-]-CEA(6D) treated colorectal cancer patients (total = 32) were followed for survival and Kaplan-Meier plots and survival proportions performed (PRISM software).(2, 3)

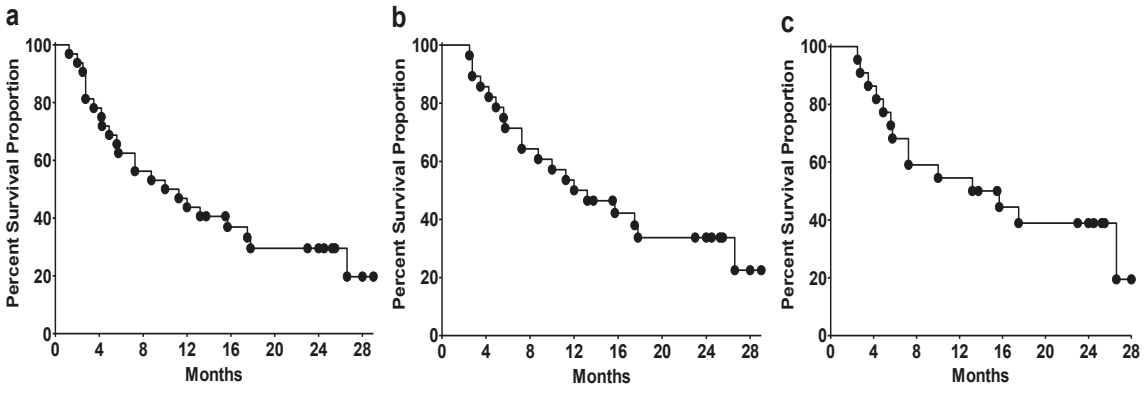


Figure 3. Kaplan-Meier survival plots on long-term overall survival of treated mCRC patients. Panel **a** represents all treated patients. Panel **b** represents patients that received all 3 treatments. Panel **c** represents patients immunized 3 times with the 2 highest doses of vaccine. There were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

Events were determined by information from the social security death index (SSDI) database, clinical charts and telephone calls (**Figure 3**).

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 3a**) 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 3b**) with a median survival time of 13 months. For the 22 patients optimally dosed with the two highest doses of vaccine (1 and 5 x 10^{11}) and receiving all 3 immunizations, the 28-month overall survival was 19% (**Figure 3c**). 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 (immunizations).

1.2.7 ETBX-071 Pre-clinical Studies

Pre-clinical studies were performed in mice based upon immunization and treatment schedules used in earlier murine model studies. In a pre-clinical study with the Ad5 [E1-, E2b-]-HER2/neu immunotherapy vaccine to treat HER2/neu expressing tumors(74), a dose response study was performed using doses of 10^8, 10^9, or 10^{10} VP injected 3 times subcutaneously (SC) at weekly intervals. A dose response effect was observed on the induced CMI responses with the highest CMI response level being observed after immunizations with 10^{10} VP of vaccine. In addition, a study was performed to assess CMI induction following a single or multiple homologous immunizations with Ad5 [E1-, E2b-]-HER2/neu, groups of Ad5 naive BALB/c mice (n=5/group) were immunized once, twice, or three times at weekly intervals with 10^{10} VP of Ad5 [E1-, E2b-]-HER2/neu. Two weeks following the last immunization, splenocytes were exposed to HER2/neu peptides or irrelevant antigens and analyzed by ELISpot for the number of IFN-and IL-2 secreting

T-cells. Significantly elevated numbers of IFN-γ and IL-2 secreting cells were observed in mice after one, two, or three immunizations with Ad5 [E1-, E2b-]-HER2/neu. The numbers of IFN-γ and IL-2 secreting cells observed after two or three immunizations were significantly higher than those observed after one immunization ($p<0.02$ and $p<0.002$, respectively). However, the differences were not statistically significant between two and three immunizations. This provided us with a rationale for using a dose of 10^{10} VP injected 3 times SC in order to induce significant tumor associated antigen (TAA) directed immune responses. In cancer immunotherapy studies with Ad5 [E1-, E2b-]-based cancer vaccines directed against CEA, HER2/neu, or HPV-E6/E7 to treat CEA, HER2/neu, or HPV-E6/E7 expressing tumors, respectively, the induced immune responses resulted in anti-tumor activity as evidence by inhibition of tumor growth in treated mice as compared to control mice(1, 71, 72, 74). For the CEA murine tumor model, immunotherapy treatment had to be initiated within one day of tumor implantation due to the fast-growing aggressive nature of the tumor. For the HER2/neu and HPV-E6/E7 murine tumor models, the tumors were not as fast growing and allowed for establishment of implanted tumor cells before tumor immunotherapy was initiated. The antitumor activity observed in all 3 tumor models indicated that the vaccines could exhibit activity against small or palpable (larger) tumors. The pre-clinical studies using ETBX-071 (Ad5 [E1- E2b-]-PSA) are described below.

ETBX-071 is a PSA targeting vaccine comprising the Ad5 [E1-, E2b-] vector and a PSA gene insert (Ad5 [E1-, E2b-]-PSA). Studies were performed to assess the use of Ad5 [E1-, E2b-]-PSA as a cancer vaccine in a BALB/c mouse model. Ad5 [E1-, E2b-]-PSA induced potent CMI against PSA in mice. Studies were also performed to show anti-tumor activity of the vaccine in a murine model of PSA expressing cancer. These data indicate that in vivo delivery of Ad5 [E1-, E2b-]-PSA can induce PSA directed anti-tumor immunity against PSA expressing cancers.

1.2.7.1 Immunogenicity

To assess immune responses generated following multiple homologous immunizations with Ad5 [E1-, E2b-]-PSA (ETBX-071), initial studies were performed using a BALB/c mouse model. Groups of BALB/c mice (n = 5/group) were immunized three times SC at 1-week intervals with 10^{10} VP of Ad5 [E1-, E2b-]-PSA. Control mice were injected with buffer solution only. Two weeks following the last immunization, splenocytes were harvested and exposed to PSA protein and assessed for CMI responses by ELISpot for IFN-γ or IL-2 secreting splenocytes. As shown in **Figure 4**, PSA directed CMI responses were induced in vaccinated but not control mice. Specificity of the CMI responses was demonstrated by a lack of reactivity against irrelevant HIV-gag or cytomegalovirus virus (CMV) antigens in ELISpot assays.

Antibody responses were also tested using a previously described quantitative assay(74) and PSA directed antibody responses were detected in immunized but not control mice (**Figure 5**).

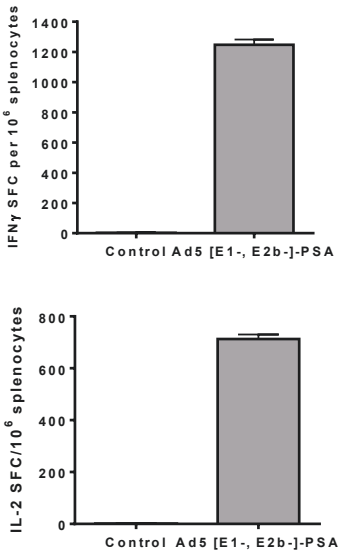


Figure 4. PSA induced CMI responses as assessed by ELISpot assays for IFN-γ or IL-2 secreting splenocytes (spot forming cells; SFC) were detected in vaccine immunized mice but not in control mice. Specificity of responses was confirmed by lack of reactivity to irrelevant HIV-gag or CMV peptide pools. A positive control included cells exposed to concanavalin A (Con A, data not shown). Values = Mean ± SEM.

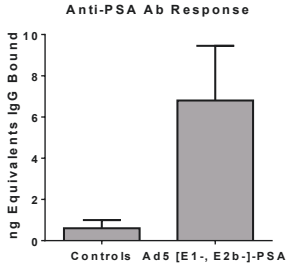


Figure 5. Antibody responses. BALB/c mice were immunized three times with either injection buffer (control group) or 10^{10} VP of Ad5 [E1-, E2b-]-PSA at 7 day intervals. Sera from individual mice were assessed 14 days after the final immunization for PSA directed antibody responses using a quantitative ELISA. Values = Mean ± SEM.

To determine if infected human DC could stimulate human antigen-specific T cell lines to secrete IFN-γ, infected dendritic cells (DC) were incubated with antigen-specific T cell lines and tested for IFN-γ secreting activity as a measure of stimulation. Human DC were infected with Ad5 vector, incubated for 48 hours, washed, and used for stimulation of human antigen-specific T cells. As shown in **Table 1** below, infection of human dendritic cells (from a HLA-A2 donor) with recombinant Ad5-PSA vectors encoding transgenes can activate PSA-specific T cell lines to produce IFN-γ.

Additional studies were performed to assess immune responses generated by the Ad5 [E1-, E2b-]-PSA (ETBX-071) component of a multi-targeted PSA, MUC1, brachyury vaccine regimen. In these studies, groups (n = 5) of C57Bl/6 mice were used. The mice were injected SC 3 times at 2-week intervals with $3X10^{10}$ VP containing a 1/1/1 mixture of Ad5 [E1-, E2b-]-PSA, Ad5 [E1-, E2b-]-MUC1, and Ad5 [E1-, E2b-]-brachyury (tri-immunization). Two weeks after the last immunization CMI activity was determined employing ELISpot assays for IFN-γ secreting cells (SFC) after exposure of splenocytes to PSA peptide pools, as previously described.(1-3, 6, 65, 67-

70, 73, 74) As shown in **Figure 6**, significant CMI responses to were detected in immunized mice. Flow cytometry intracellular cytokine staining was performed as previously described(70, 72) on spleen cells after exposure to PSA peptides to assess the quantity of activated CD8+ T cells. As shown in **Figure 7**, IFN-γ and IFN-γ/TNF-α expressing polyfunctional CD8+ cells were detected in immunized but not control splenocytes. These above results demonstrate that the Ad5 [E1-, E2b-]-PSA vaccine component in the multi-targeted vaccine is effective at inducing PSA directed immune responses.

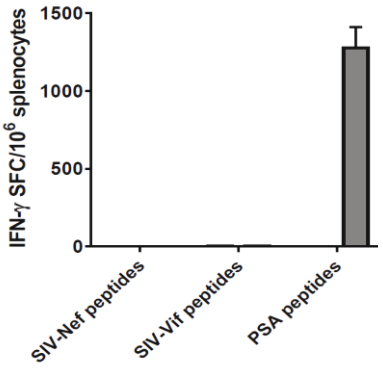


Figure 6. PSA induced CMI responses as assessed by ELISpot assays for IFN-γ secreting splenocytes (spot forming cells; SFC) were detected in vaccine immunized (grey bars) mice but not in control (white bars) mice (injected with Ad5-null empty vector). Specificity of the responses was confirmed by lack of reactivity to irrelevant SIV-nef or SIV-vif peptides. A positive control included cells exposed to concanavalin A (Con A, data not shown). Values = Mean ± SEM.

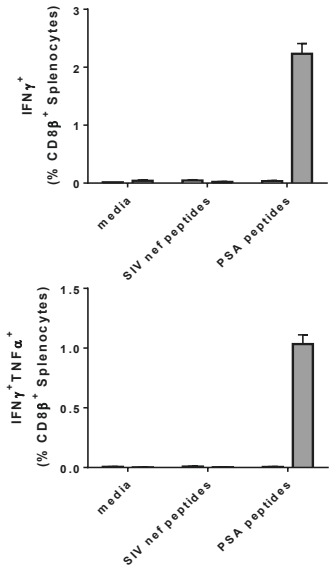


Figure 7. PSA induced stimulation of CD8+ cells expressing IFN-γ (Top) and IFN-γ /TNF-α (Bottom) in mice immunized with vaccine (grey bars) but not in controls injected with Ad5-null (white bars). Specificity of the responses was confirmed by lack of reactivity to media alone or irrelevant SIV-nef peptides. Values = Mean ± SEM.

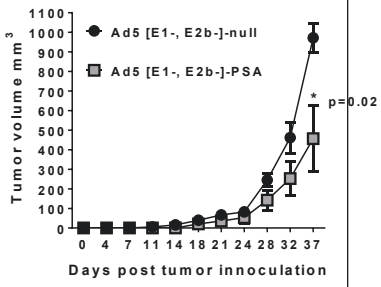


Figure 8. Anti-tumor responses. BALB/c mice were immunized three times with either 10^{10} VP of Ad5 [E1-, E2b-]-null (control group) or 10^{10} VP of Ad5 [E1-, E2b-]-PSA at 7 day intervals. Mice were implanted with PSA expressing tumors cells and monitored for tumor growth. Note the significant inhibition of tumor growth in the immunized mice as compared to controls. Values = Mean ± SEM.

1.2.7.2 Anti-tumor Activity

The anti-tumor activity of the Ad5 [E1-, E2b-]-PSA vaccine was tested in a murine model of PSA expressing cancer. BALB/c mice were immunized three times SC at weekly intervals with $1X10^{10}$ VP of Ad5 [E1-, E2b-]-null (empty vector controls) or $1X10^{10}$ VP of Ad5 [E1-, E2b-]-PSA vaccine. Two weeks after the last immunization (vaccination), mice were implanted with $1x10^{6}$ PSA expressing murine tumor cells. All mice were monitored for tumor growth and tumor volumes

were calculated to determine if pre-immunization with Ad5 [E1-, E2b-]-PSA inhibited growth of tumors in immunized but not control mice. As shown in **Figure 8**, mice immunized with Ad5 [E1-, E2b-]-PSA experienced slower tumor growth as compared to control mice injected with Ad5-null (empty vector). These studies indicate that the Ad5 [E1-, E2b-]-PSA component in the multi-targeted vaccine regimen can induce PSA directed anti-tumor responses in immunized animals.

Table 1

Dendritic cells infected with	**Antigen Specific T Cells**	
	T-PSA-(HLA-A2)	**T-CEA-(HLA-A2)**
Ad5 [E1-, E2b-]-PSA (20,000 MOI)	>3,000	<0.732
Ad5 [E1-, E2b-]-PSA (10,000 MOI)	>3,000	0.84
Ad5 [E1-, E2b-]-Null (20,000 MOI)	0.9	0.89
DCs	4.24	0.78
No DCs (PSA T Cells only)	<0.732	ND
No DCs (CEA T Cells only)	ND	<0.732

Results are expressed in picograms of IFN-γ per $5X10^5$ T cells/mL. DC only = <0.732. MOI: multiplicity of infection.

1.2.8 ETBX-061 Pre-clinical Studies

ETBX-061 is a MUC1 targeting vaccine comprising the Ad5 [E1-, E2b-] vector and a modified MUC1 (MUC1c) gene insert. The best MUC1c modified for immune enhancer capability has been selected and incorporated into the recombinant Ad5 [E1-, E2b-] platform to produce a new and more potent immunotherapeutic vaccine (Ad5 [E1-, E2b-]-MUC1) for treating MUC1 expressing cancers.

1.2.8.1 Immunogenicity

Studies were performed to assess immune responses generated by the Ad5 [E1-, E2b-]-MUC1 (ETBX-061) component of a multi-targeted PSA, MUC1 and brachyury vaccine regimen. In these studies, groups (n = 5) of C57Bl/6 mice were used. The mice were injected SC 3 times at 2-week intervals with $3X10^{10}$ VP containing a 1/1/1 mixture of Ad5 [E1-, E2b-]-PSA, Ad5 [E1-, E2b-]-MUC1, and Ad5 [E1-, E2b-]-brachyury (tri-immunization). Two weeks after the last immunization CMI activity was determined employing ELISpot assays for IFN-γ secreting cells (SFC) after exposure of splenocytes to MUC1 peptide pools, as previously described.(1-3, 6, 65, 67-70, 73, 74) As shown in **Figure 9**, significant CMI responses to were detected in immunized mice. Flow cytometry intracellular cytokine staining was performed as previously described (70, 72) on spleen cells after exposure to MUC1 peptides to assess the quantity of activated CD8+ T cells. As shown in **Figure 10**, IFN-γ and IFN-γ/TNF-α expressing polyfunctional CD8+ cells were detected in immunized but not control splenocytes. Testing was also performed to determine if immunization with the Ad5 [E1-, E2b-]-MUC1 vaccine induced MUC1 directed antibody. Using a previously described quantitative ELISA assay(74) with purified MUC1 glycoprotein, antibody responses to MUC1 were detected in sera from immunized but not control mice. (**Figure 11**). These studies indicate that the Ad5 [E1-, E2b-]-MUC1 component in the multi-targeted vaccine can induce MUC1 directed immune responses in immunized animals.

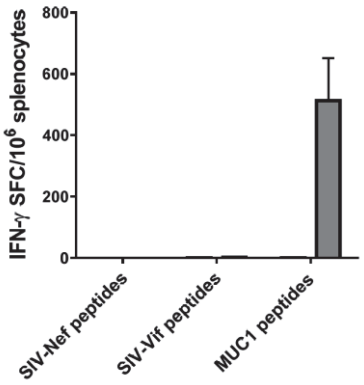


Figure 9. MUC1 induced CMI responses as assessed by ELISpot assays for IFN-γ secreting splenocytes (spot forming cells; SFC) were detected in vaccine immunized (grey bars) mice but not in control (white bars) mice (injected with Ad5-null empty vector). Specificity of the ELISpot assay responses was confirmed by lack of reactivity to irrelevant SIV-nef or SIV-vif peptides. A positive control included cells exposed to concanavalin A (Con A, data not shown). Values = Mean ± SEM.

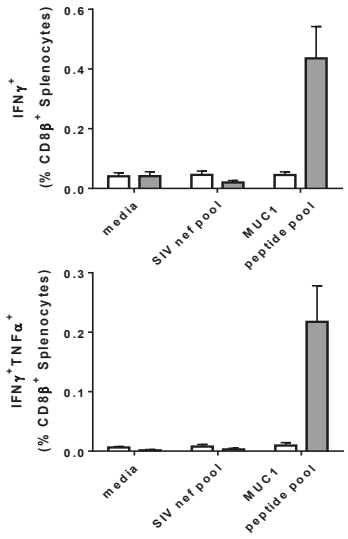


Figure 10. MUC1 induced stimulation of CD8+ cells expressing IFN-γ (Top) and IFN-γ /TNF-α (Bottom) in mice immunized with vaccine (grey bars) but not in controls injected with Ad5-null (white bars). Specificity of the responses was confirmed by lack of reactivity to media alone or irrelevant SIV-nef peptides. Values = Mean ± SEM.

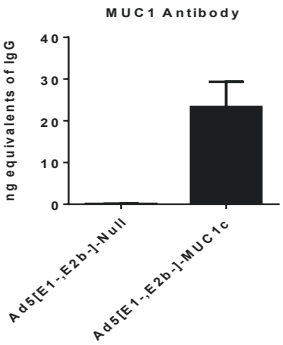


Figure 11. MUC1 directed antibody responses induced in vaccine immunized mice but not in control mice. Values = Mean ± SEM.

1.2.8.2 Anti-tumor Activity

A study was conducted to test the anti-tumor capability of the Ad5 [E1-, E2b-]-MUC1 (ETBX-061) component of the multi-targeted vaccine regimen. Immunotherapy studies were performed in mice with established MUC1 expressing tumors. Groups (n = 7) of C57Bl/6 mice were injected SC in the right flank with $5X10^5$ MC38-MUC1 expressing murine tumor cells. After palpable tumors were detected, mice were treated by 3 SC injections with $1X10^{10}$ VP of Ad5 [E1-, E2b-]-null (no transgene empty vector) or Ad5 [E1-, E2b-]-MUC1, respectively. Tumor volumes were calculated and tumor growth curves were plotted as previously described.(1, 6, 74) As published previously, numbers of 7-10 mice/group are sufficient for statistical evaluation of treatment.(1, 6, 74) Significant anti-tumor activity was observed in MUC1 expressing tumor-bearing mice treated by immunotherapy with Ad5 [E1-, E2b-]-MUC1 but not in control mice injected with Ad5-null (empty vector). **Figure 12** shows the significant anti-tumor activity and increased survival observed in MUC1 expressing tumor-bearing mice treated by immunotherapy. This study indicates

that the Ad5 [E1-, E2b-]-MUC1 component of the vaccine regimen can induce anti-tumor activity against MUC1 expressing tumor cells.

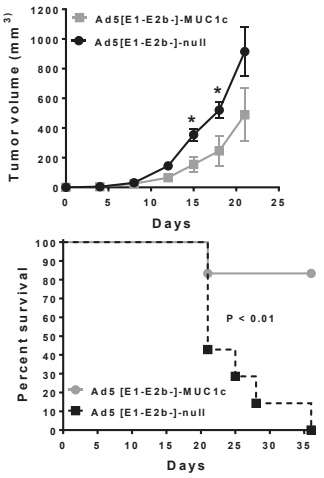


Figure 12. C57Bl/6 mice were inoculated SC with MC38-MUC1 expressing tumor cells in the left flank and administered 10^{10} VP of Ad5-null (empty vector) or 10^{10} VP of Ad5 [E1-, E2b-]-MUC1 in the right flank on days 0, 7, 14. Mice treated with Ad5 [E1-, E2b-]-MUC1 had significantly (P<0.05) smaller tumors on days 15 and 18 as compared to controls (top) and significantly (P<0.01) longer survival (bottom). Values = Mean ± SEM. Experiment was terminated on day 36.

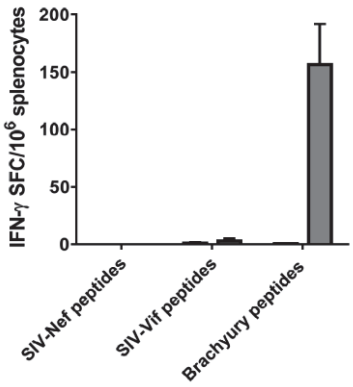


Figure 13. Brachyury induced CMI responses as assessed by ELISpot assays for IFN-γ secreting splenocytes (spot forming cells; SFC) were detected in immunized (grey bars) mice but not in control (white bars) mice (injected with Ad5-null empty vector). Specificity of the ELISpot assay responses was confirmed by lack of reactivity against irrelevant SIV-nef or SIV-vif peptides. A positive control included cells exposed to concanavalin A (Con A, data not shown). Values = Mean ± SEM.

1.2.9 ETBX-051 Pre-clinical Studies

ETBX-051 is a is a brachyury targeting vaccine comprising the Ad5 [E1-, E2b-] vector and a modified brachyury gene insert. A modified brachyury gene was incorporated into the recombinant Ad5 [E1-, E2b-] platform to produce an immunotherapeutic vaccine (Ad5 [E1-, E2b-]-brachyury) for treating brachyury expressing cancers.

1.2.9.1 Immunogenicity

Studies were performed to assess immune responses generated by the Ad5 [E1-, E2b-]-brachyury (ETBX-051) vaccine component of a multi-targeted PSA, MUC1, brachyury vaccine regimen. Groups (n = 5) of C57Bl/6 mice were used. The mice were injected SC 3 times at 2-week intervals

with $3X10^{10}$ VP containing a 1/1/1 mixture of Ad5 [E1-, E2b-]-PSA, Ad5 [E1-, E2b-]-MUC1, and Ad5 [E1-, E2b-]-brachyury (tri-immunization) or with $3X10^{10}$ VP Ad5 [E1-, E2b-]-null (empty vector controls). Two weeks after the last immunization CMI activity was determined employing ELISpot assays for IFN-γ secreting cells (SFC) after exposure of splenocytes to a brachyury peptide pool as previously described.(6) As shown in **Figure 13**, significant CMI responses to were detected in immunized mice. Flow cytometry intracellular cytokine staining was performed as previously described(70, 72) on spleen cells after exposure to brachyury peptides to assess the quantity of activated CD8+ T cells. As shown in **Figure 14**, IFN-γ and IFN-γ/TNF-α expressing polyfunctional CD8+ cells were detected in splenocytes from immunized but not control mice. These studies indicate that the Ad5 [E1-, E2b-]-brachyury component in the multi-targeted vaccine regimen can induce brachyury directed immune responses in immunized animals.

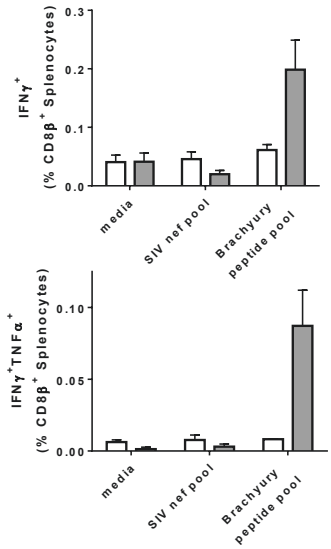


Figure 14. Brachyury induced stimulation of CD8+ cells expressing IFN-γ (Top) and IFN-γ/ TNF-α (Bottom) in vaccine immunized (grey bars) mice but not in control mice injected with Ad5-null (white bars). Specificity of the responses was confirmed by lack of reactivity to media alone or irrelevant SIV-nef peptides. Values = Mean ± SEM.

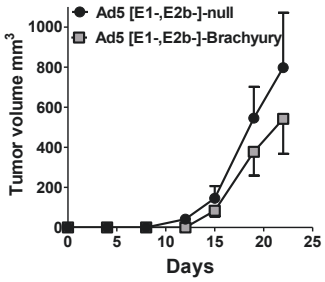


Figure 15. C57Bl/6 mice were inoculated with MC38-brachyury expressing tumor cells sc in the left flank and administered 10^{10} VP of Ad5-Null (empty vector) (N=4) or 10^{10} VP of Ad5 [E1-, E2b-]-brachyury (N=5) in the right flank on days 5, 11, and 17. Tumors were smaller in treated mice on days 15, 19, and 22. Values = Mean ± SEM

1.2.9.2 Anti-tumor Activity

A preliminary study was conducted to test the anti-tumor capability of the Ad5 [E1-, E2b-]-brachyury (ETBX-051) component of the multi-targeted vaccine regimen. Groups (n = 7) of

C57Bl/6 mice were injected SC in the right flank with $5X10^5$ brachyury expressing murine tumor cells. After palpable tumors were detected, mice were treated by 3 SC injections with $1X10^{10}$ VP of Ad5 [E1-, E2b-]-brachyury or with Ad5 [E1-, E2b-]-null. Tumor volumes were calculated and tumor growth curves were plotted as previously described.(6) Data in **Figure 15** shows that immunotherapy of mice with Ad5 [E1-, E2b-]-brachyury resulted in smaller tumors as compared to control mice injected with Ad5 [E1-, E2b-]-null. This study indicates that the Ad5 [E1-, E2b-]-brachyury component of the vaccine regimen can induce anti-tumor activity against brachyury expressing tumor cells.

1.2.10 Clinical Experience

The combination of 3 different vaccines: ETBX-071, ETBX-061, and ETBX-051 have not been tested in humans. This Phase I study is the first clinical study of this vaccines combination in subjects with mCRPC.

1.2.11 Rationale

The preclinical data merit a Phase I study to evaluate the safety, preliminary efficacy, and immunogenicity of the combination ETBX-071, ETBX-061, and ETBX-051 vaccines. 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 ≥ 18 years (male).

2.1.1.2 Ability to understand and provide signed informed consent that fulfills Institutional Review Board (IRB)'s guidelines.

2.1.1.3 Cytologically or histologically confirmed prostate cancer for which no curative standard approved therapy is available by either the Laboratory of Pathology at the NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda prior to starting this study. If no pathologic specimen is available, patients may enroll with a pathologist's report showing a histological diagnosis of prostate cancer and a clinical course consistent with the disease.

2.1.1.4 Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.

2.1.1.5 Subjects who have received prior PSA, MUC1, and/or brachyury-targeted immunotherapy (e.g. vaccine) are eligible for this trial if this treatment was discontinued at least 3 months prior to enrollment.

2.1.1.6 Resolution of all toxic side effects of prior chemotherapy, radiotherapy, or surgical procedures to NCI CTCAE Grade ≤ 1.

2.1.1.7 Adequate hematologic function at screening, as follows:

- Absolute neutrophil count (ANC) ≥1.0 x 10^9/L
- Hemoglobin ≥ 9 g/dL
- Platelets ≥ 75,000/microliter.

- Prothrombin (PT)-international normalized ratio (INR) < 1.5.
- Partial thromboplastin time (PTT) < 1.5 x upper limit of normal (ULN).

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

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

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

  b) Male CrCl = [(140 - age in years) x weight in kg x1.00] / [72 x serum creatinine in mg/dL]

- Total bilirubin ≤ 1.5 x ULN OR in subjects with Gilbert's syndrome, a total bilirubin ≤ 3.0 x ULN
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN, unless liver metastases are present, then values must be ≤ 5 x ULN)

2.1.1.9 The effects of ETBX-051, ETBX-061 and ETBX-071 vaccines on the developing human fetus are unknown. For this reason subjects must agree to use a condom and acceptable contraceptive method with their partner during the study and for one month after the last dose of vaccines.

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

2.1.1.11 Castrate testosterone level (<50ng/dl or 1.7nmol /L)

2.1.1.12 Metastatic disease documented by at least one of the following:

- Metastatic bone disease on an imaging study, or
- Soft tissue disease documented by CT/MRI

2.1.1.13 Progressive disease at study entry defined as one or more of the following criteria occurring in the setting of castrate levels of testosterone:

- Radiographic progression defined as any new or enlarging bone lesions or growing lymph node disease, consistent with prostate cancer

OR

- PSA progression defined by sequence of rising values separated by >1 week (2 separate increasing values over a minimum of 2ng/ml (PCWG2 PSA eligibility criteria). If patients had been on flutamide, PSA progression is documented 4 weeks or more after withdrawal. For patients on bicalutamide or nilutamide disease progression is documented 6 or more weeks after withdrawal. The requirement for a 4-6 week withdrawal period following discontinuation of flutamide, nilutamide or bicalutamide only applies to patients who have been on these drugs for at least the prior 6 months. For all other patients, they must stop bicalutamide, nilutamide or flutamide the day prior to enrollment.

2.1.1.14 Patients must agree to continue to continuation of androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone analogue/antagonist or bilateral orchiectomy.

2.1.1.15 Prior treatment with immunotherapy, hormonal therapy, radium 223, chemotherapy

and/or other experimental therapy is allowed.

2.1.2 Exclusion Criteria

2.1.2.1 Treatment with an investigational drug study within 28 days of before starting on study treatment.

2.1.2.2 Subjects with concurrent cytotoxic chemotherapy or radiation therapy. There must be at least 28 days between any other prior chemotherapy (or radiotherapy) and study treatment. Prior antibody therapy must be discontinued 8 weeks prior to start of study treatment. Prior hormonal therapy can be discontinued 24 hours prior to start of study treatment.

2.1.2.3 Any prior PSA, MUC1, and/or brachyury-targeted immunotherapy (e.g., vaccine) must have been discontinued at least 12 weeks before initiation of study treatment. Subjects must have recovered from all acute toxicities from prior treatment prior to screening for this study.

2.1.2.4 Prior treatment with Adenovirus-Based vectors immunotherapy

2.1.2.5 Known active brain or central nervous system metastasis, or seizures requiring anticonvulsant treatment, cerebrovascular accident, or transient ischemic attack (< 6 months prior to enrollment).

2.1.2.6 Subjects with a history of autoimmune disease (active or past), such as but not restricted to inflammatory bowel disease, systemic lupus erythematosus, ankylosing spondylitis, scleroderma, or multiple sclerosis. Autoimmune-related thyroid disease, type I diabetes and vitiligo are permitted if the condition is well controlled.

2.1.2.7 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.

2.1.2.8 Subjects with a history of 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 prior to enrollment) of ventricular arrhythmia.

2.1.2.9 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.

2.1.2.10 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 steroid therapy (or other immunosuppressive, such as azathioprine or cyclosporin A) are excluded on the basis of potential immune suppression. Subjects must have had at least 6 weeks of discontinuation of any steroid therapy (except that used as premedication for chemotherapy or contrast-enhanced studies) prior to enrollment. Physiologic (replacement) doses od steroids as well as nasal, topical or inhaled steroids are allowed.

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.

2.1.2.15 Patients with second malignancy within 3 years of enrollment; Patients curatively treated non-melanoma skin cancers or carcinoma in situ of the bladder, are not excluded.

2.1.2.16 Use of herbal products that may decrease PSA levels (e.g. saw palmetto)

2.1.2.17 Patients who have received radiation therapy, radionuclide therapy or undergone surgery within certain duration (4 weeks) of enrollment

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

Confirmation of diagnosis by NCI Laboratory of Pathology or Walter Reed National Military Medical Center (at any time prior to enrollment). If no tissue is available, a pathologist's report documenting histological diagnosis of prostate cancer along with a clinical course consistent with the disease may be used.

Studies should be done within 28 days prior to enrollment unless otherwise noted below.

- History and physical exam including ECOG performance status, height, weight and vital signs
- 12-lead EKG
- Scans
  - CT CAP (chest abdomen and pelvis) with oral and IV contrast (MRI abdomen/pelvis with contrast + chest CT without contrast if IV contrast is contraindicated or CT CAP is inadequate)
  - Bone scan
- Clinical laboratory tests (within 16 days prior to enrollment)
  - Hepatic Panel (Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin)
  - Acute Care Panel (Sodium [NA], Potassium [K], Chloride [CL] Total CO2 [Bicarbonate], Creatinine, Glucose, Urea nitrogen, eGFR)
  - CBC with differential
  - Urinalysis (24-hour collection will be done as needed)
  - Serum PSA
  - Testosterone level
  - PT/INR, PTT

- 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.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@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.

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	ETBX-071; adenoviral PSA vaccine + ETBX-061; adenoviral MUC1 vaccine + ETBX-051; adenoviral brachyury vaccine dose de-escalation
2	Dose Expansion	ETBX-071; adenoviral PSA vaccine + ETBX-061; adenoviral MUC1 vaccine + ETBX-051; adenoviral brachyury vaccine

Stratifications
None

Randomization and Arm Assignment
Not a randomized study. Subjects in Cohort 1 will be assigned to Arm 1; subjects in Cohort 2 will be 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 weight, ECOG status and vital signs
- Hepatic Panel (Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin)
- Acute Care Panel (Sodium [NA], Potassium [K], Chloride [CL] Total CO2 [Bicarbonate], Creatinine, Glucose, Urea nitrogen, eGFR)
- CBC with differential
- PSA and testosterone

If these assessments have been previously performed within 24 hours prior to the first dosing, a second assessment at baseline/week 0 can be omitted.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase I trial in subjects to test the safety of 3 different adenovirus based vaccines (ETBX-051, ETBX-61, ETBX-71) administered to patients with metastatic castration resistant prostate cancer at the same time. All vaccines utilize the same second generation E1(-), E2 (-) vector.

ETBX-071 is a PSA-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector containing a PSA gene insert. The investigational product ETBX-061 is a MUC1-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector containing a modified MUC1 (MUC1c) gene insert. The investigational product ETBX-051 is a brachyury-targeting vaccine that comprises the Ad5 [E1-, E2b-] vector containing a modified brachyury gene insert.

Table 2: Investigational Products

Table 2a

Product Name(s):	ETBX-071 (Ad5 [E1-, E2b-]-PSA Vaccine)
Dosage Form:	Suspension for injection
Dose	5×10^{11} VP (standard dose), 1×10^{11} VP (DL-1), or 5×10^{10} VP (DL-2).
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 5×10^{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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

Table 2b

Product Name(s):	ETBX-061 (Ad5 [E1-, E2b-]-MUC1 Vaccine)
Dosage Form:	Suspension for injection
Dose	5 x 10^{11} VP (standard dose), 1 x 10^{11} VP (DL-1), or 5 x 10^{10} VP (DL-2).
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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

Table 2c

Product Name(s):	ETBX-051 (Ad5 [E1-, E2b-]-Brachyury Vaccine)
Dosage Form:	Suspension for injection
Dose	5 x 10^{11} VP (standard dose), 1 x 10^{11} VP (DL-1), or 5 x 10^{10} VP (DL-2).
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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

The vaccines will be administered subcutaneously (SC) in 3 separate injections every 3 weeks for 3 immunizations (there are no booster injections in dose escalation cohort). It is preferred to administer the injections in the anterolateral section of the same thigh separated from each other by a minimum of 5 cm. It is also preferred to administer ETBX-071 most proximal, ETBX-061 in the middle, and ETBX-051 most distal and the injected thigh should be alternated each treatment visit when possible. In the dose expansion cohort, ETBX-051, ETBX-61, ETBX-71 vaccines will be administered on Weeks 0, 3, and 6 for a total of three injections followed by booster injections every 8 weeks for 1 year.

3.1.1 Dose Limiting Toxicity

A dose limiting toxicity (DLT) is defined as occurring within 28 days after the first vaccine administration and meeting any of the below criteria:

- Any Grade 3 or greater toxicity as defined by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5 that is possibly, probably, or definitely related to the vacines 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, local skin reactions or rash, headache, nausea, emesis that resolves to Grade ≤ 1 or laboratory abnormalities that are not associated with organ pathology.
- Any Grade 2 or higher autoimmune reaction (except endocrine-related immune toxicity) or immediate hypersensitivity reaction. Any grade 3 autoimmune endocrine-related toxicity that has not resolved clinically within 7 days of initiating therapy will also be defined as a DLT.
- Generalized erythroderma or macular or papular rash lasting less than 7 days and not associated with desquamation will not be DLTs.
- For purposes of dose de-escalation the DLT evaluation period will be for 1 week 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, then initiation of the dose expansion phase will occur. If ≥2 of 3 or 6 experience DLT at the initial dose level, then dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-1) ($1x10^{11}$ 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 a further dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-2) ($5x10^{10}$ 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 dose level -2, then the study will be stopped.

In the second part, dose expansion will occur when the MTD has been determined. An additional 12 subjects will be enrolled in the dose expansion component of the trial, for a total of 18 subjects treated at the MTD.

A schematic overview of the study is shown in **Figure 16**.

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 7 days between enrolling successive subjects. DLTs will be monitored continuously.

Dose levels are shown in **Table 3**. No intra-patient dose escalations are permitted.

Table 3 Dose Levels	
Dose level (DL)	**ETBX VP**
Standard (Dose level 1)	$5X10^{11}$
-1	$1X10^{11}$
-2	$5X10^{10}$

Standard dose DL 1 (5×10^{11} VP):

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

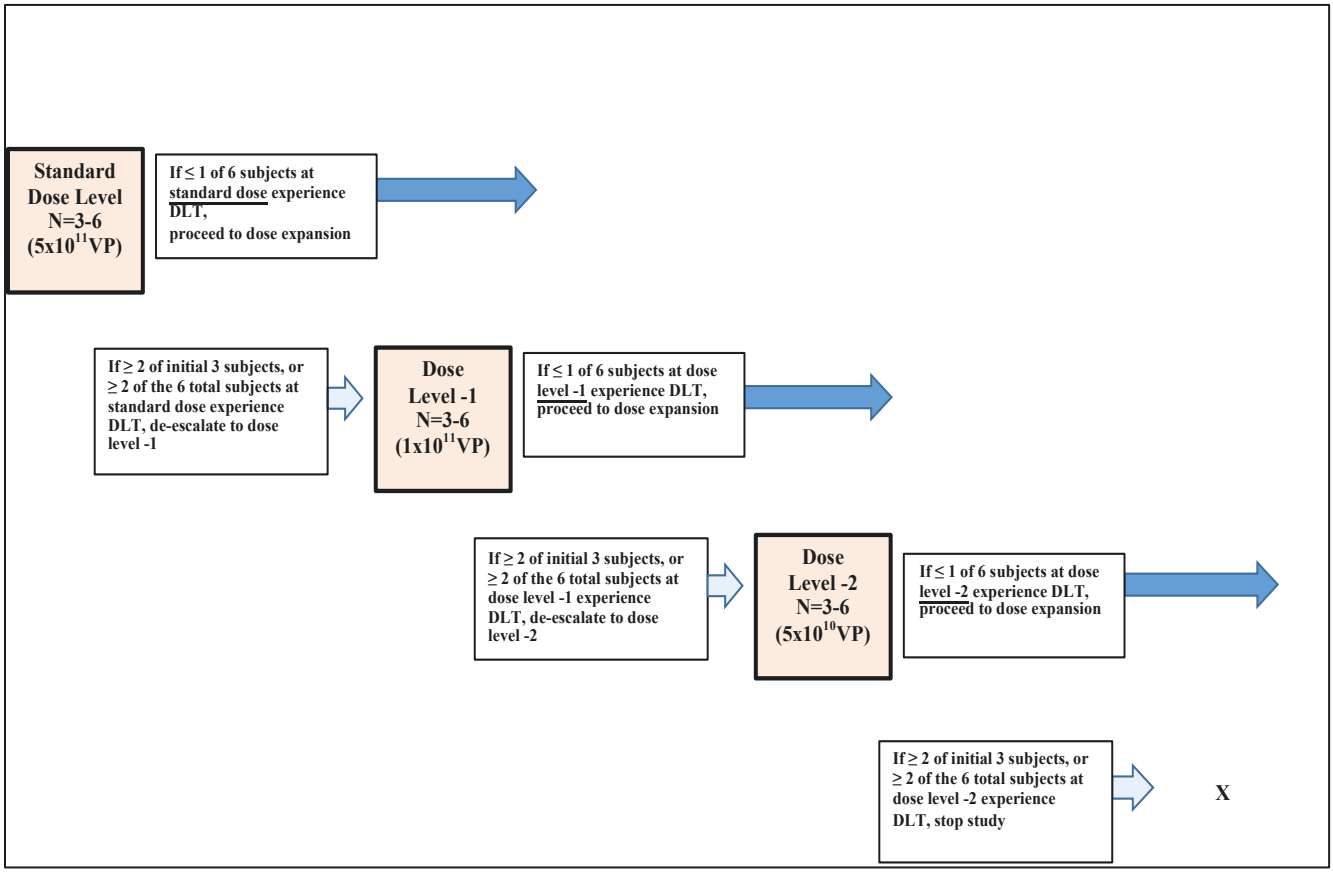
Dose de-escalation DL -1 (1×10^{11} VP):

- 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 ≥ 2 of the initial 3 subjects or ≥ 2 of the 6 total subjects experience a DLT, enrollment at DL -2 will commence.

Dose de-escalation DL-2 (5×10^{10} VP):

- If ≤ 1 of 6 subjects experience a DLT, this dose level will be defined as the MTD and dose expansion will commence.
- If ≥ 2 of the initial 3 subjects, or if ≥ 2 of a total 6 subjects at DL -2 experience a DLT, dosing will be suspended, and the study will be re-evaluated.

Figure 16: Study Design and Treatment Schema



3.2 ETBX VACCINE DOSE PREPARATION

The dose of each ETBX vaccine to be injected is 5 x 10^{11} VP per 1 mL. Dose levels of 1 x 10^{11} VP per mL (dose level -1), or 5 x 10^{10} VP per mL (dose level -2) are available for de-escalation. Prior to injection, the appropriate vial should be removed from the freezer and allowed to thaw at controlled room temperature (20–25°C, 68-77°F) for at least 20 minutes and not more than 30 minutes, after which it should be kept at 2-8°C (35–46°F).

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 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.

Note that storage of the vaccine in the vial at 2–8°C (35–46°F) must not exceed 8 hours. Also, once the vaccine has been thawed, it must not be refrozen, and, if not used, it should be disposed of as described above.

3.2.1 Instructions for Dose Preparation – 5 x 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 in the thigh without any further manipulation (refer to the vial label for a description of the vialed ETBX vaccine concentration).

3.2.2 Instructions for Dose Preparation – 1 x 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 vialed ETBX vaccine concentration).

3.2.3 Instructions for Dose Preparation – 5 x 10^{10} Virus Particles (For Dose De-escalation)

From a 5.0-mL vial of 0.9% sodium chloride for injection, remove 0.5 mL of fluid using a 1.0 mL tuberculin syringe, leaving 4.50 mL. Then, using another 1.0 mL tuberculin syringe, remove 0.5 mL from the vial labeled ETBX-071, ETBX-061, or ETBX-051, respectively, and deliver this volume into the 4.50 mL of sterile saline remaining in the 5-mL sterile saline vial. Mix the contents by inverting the 5 mL of diluted ETBX vaccine. Then withdraw 1 mL of the diluted ETBX vaccine, prepare the injection site with alcohol, and administer to the subject by SC injection in the thigh (refer to the vial label for a description of the vialed ETBX vaccine concentration). Alternatively, 4.5 mL of 0.9% sodium chloride for injection may be added to an appropriately sized sterile empty vial (e.g. 10 mL) using an appropriately sized syringe. Then, using a new syringe, remove 0.5 mL from the vial labeled ETBX-071, ETBX-061, or ETBX-051, respectively and deliver this volume into the sterile vial containing the 4.5 mL 0.9% sodium chloride for injection. Mix the contents by

inverting the 5 mL of diluted ETBX vaccine. Then withdraw 1 mL of the diluted ETBX vaccine, prepare the injection site with alcohol, and administer to the subject by subcutaneous injection in the thigh (refer to the vial label for a description of the vialed ETBX vaccine concentration).

3.2.4 Administration

ETBX-051, ETBX-61 and ETBX-71 vaccines will be administered on Weeks 0, 3, and 6 for a total of three injections. In dose expansion, ETBX-051, ETBX-61 and ETBX-71 vaccines will be administered on Weeks 0, 3, and 6 for a total of three injections followed by booster injections at 8-week intervals for up to 1 year. All study drug administration treatments should occur within ± 7 days of the planned visit date. 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.

The vaccines will be administered subcutaneously as 3 separate injections preferably in the anterolateral section of the same thigh separated from each other by a minimum of 5 cm. It is also preferred to administer ETBX-071 most proximal, ETBX-061 in the middle, and ETBX-051 most distal and the injected thigh should be alternated each treatment visit when possible.

3.2.5 Other Considerations

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, we recommend that it be handled under Biosafety Level-2 conditions. Any vialed 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.

3.3 Dose Modifications and Delays

3.3.1 Dosing Delay (applies to events occurring outside of the DLT evaluation period)

Patients must have recovered to ≤ grade 2 for injection site reaction or grade ≤ 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 (see Sections **2.1.1.7** and **2.1.1.8**).

If ≥ 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 grade 3 injection site reactions will have their vaccines held until injection site reaction resolves to grade 2 or less. Patients will receive a diary card to record injections site reactions (see **Appendix B**).

Patients who develop ≥ a grade 2 allergic or autoimmune disease that threatens vital organ function or any ≥ 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.

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.

If a scheduled vaccine dose is missed due to scheduling or logistical issues, the vaccine may be given within 7 days of the appointed time.

3.3.2 Dose Modifications

No dose modifications are allowed with these vaccines except for dose cohort de-escalation as described in section **3.1**.

3.4 STUDY CALENDAR

Assessment	**Screening**[l]	**Treatment (Every 3-Week Dosing)**[p]			**Bi-monthly Boosters (every 8 weeks – only expansion cohort)**[p]	**End of Treatment (within 90 days after the last vaccine)**[k]	**Post Study Therapy Follow Up**
Study Week	**Day -28 to -1**	**Baseline/ Wk-0**	**Wk-3**	**Wk-6**	**Week 14, 22, 30, 38, 46, 54**		
Clinic Visit	X	X	X	X	X	X	
Informed Consent[0]	X						
Inclusion/Exclusion	X						
Demographics	X						
Physical Examination, Height[a], Weight, ECOG	X	X[b]	X	X	X	X	
Medical History[c]	X	X					
Concomitant Medications	X	X	X	X	X	X	
Vital Signs[d]	X	X	X	X	X	X	
12-Lead ECG	X				X	X	
Confirm Contraceptive Measures[e]	X						
Study Drug Injection/ Injection Site Reaction Monitoring[f]		X	X	X	X		

Assessment	**Screening**[l]	**Treatment (Every 3-Week Dosing)** [p]			**Bi-monthly Boosters (every 8 weeks – only expansion cohort)** [p]	**End of Treatment (within 90 days after the last vaccine)** [k]	**Post Study Therapy Follow Up**
Study Week	**Day -28 to -1**	**Baseline/ Wk-0**	**Wk-3**	**Wk-6**	**Week 14, 22, 30, 38, 46, 54**		
Dispensation of Subject Diary Card[g]		X					
Review of Subject Diary Card[g]			X				
Tumor Imaging/CT scan and bone scan[h]	X				X		
Urinalysis	X[l]					X	
Chemistry Panel [m]	X[l]	X[b]	X	X	X	X	
CBC, Differential, Platelets	X[l]	X[b]	X	X	X	X	
Serum PSA and testosterone level	X[l]	X	X	X	X	X	
PT/INR, PTTT	X[l]					X	
Serum Virology (HIV, HBV, HCV)[i]	X						
Adverse Events		X	X	X	X	X	
Exploratory Immune Analysis		X		X	X (only week 14 and 30)	X	
Telephone Follow Up[j]							X

Assessment	**Screening**[l]	**Treatment (Every 3-Week Dosing)** [p]			**Bi-monthly Boosters (every 8 weeks – only expansion cohort)** [p]	**End of Treatment (within 90 days after the last vaccine)** [k]	**Post Study Therapy Follow Up**
Study Week	**Day -28 to -1**	**Baseline/ Wk-0**	**Wk-3**	**Wk-6**	**Week 14, 22, 30, 38, 46, 54**		
Confirmation of diagnosis (path report) [n]	X						

[0]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

[a] Height will only be assessed at screening.

[b] If the assessment was performed within 24 hours prior to the first dosing, a second assessment at baseline/week 0 can be omitted.

[c] 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.

[d] 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.

[e] Contraceptive measures are described in **2.1.1.9**.

[f] Injection site reactions will be monitored.

[g] Subjects will be given a diary card for the self-evaluation and reporting of injection site reactions, see Section **Appendix B**. 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 after each dose 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 (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.

[h] 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 as described in Section **6.3**. Tumor imaging may be performed +/-5 days from the next treatment for logistical and scheduling purposes.

[i] 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) can be performed up to 12 weeks prior to enrollment.

[j] 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 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.
[k] 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.
[l] Should be performed within 16 days prior to enrollment.
[m] Chemistry include acute care panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, eGFR) and hepatic panel (alkaline phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin. Blood work may be obtained +/-5 days from the next treatment day for logistical and scheduling purposes
[n] Confirmation of diagnosis by NCI Laboratory of Pathology or Walter Reed National Military Medical Center can be done at any time prior to enrollment.
[p] Initial cohort: Treatment every 3 weeks (21 days: – 3 days up to + 3 days) intervals for 3 doses. Expansion cohort: Treatment every 3 weeks (21 days: – 3 days up to + 3 days) intervals for 3 doses then every 8 weeks (56 days: – 3 days up to + 8 days) intervals thereafter (treatment may be delayed up to 8 days for special circumstances like severe weather, travel delays or patient event/vacation).

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Regardless of reason for removal from study therapy, patients will be asked to have a 90 day follow up safety visit 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.

3.5.1 Criteria for removal from protocol therapy

- Completion of protocol therapy.
- Clinical or radiographic progression of disease (not including PSA progression). Patients with serological progression alone will remain on treatment at the discretion of the PI.
- Unacceptable Toxicity as listed in section **3.3.1**
- Any serious adverse event that is unexpected relative to the known safety profile of the investigational agents in the opinion of the investigator
- Participant requests to be withdrawn from active therapy
- Investigator discretion

3.5.2 Off-Study Criteria

- Completion of study follow up period
- 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)
- Lost to follow-up
- Death

3.5.3 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-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

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

For the administration of the 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 inhaled steroids, nasal sprays, and topical creams for small body areas is allowed. Use of physiological doses of steroids for replacement therapy (up to 10 mg prednisone/day or equivalent) is permitted. Use of steroids as premedication for imaging studies is also 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, 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 Immunological Studies

Samples from patients enrolled at the Clinical Center will be available for immunologic correlative studies as described below.

5.1.1.1 Peripheral blood

Five (10mL) green top sodium heparin tubes for PBMC isolation and 1 (8mL) red top SST tube for serum samples will be drawn at baseline (week 0), week 6, week 14, week 30 and at the end of study treatment at bi-monthly injection days, and at end of study as described in section **3.4**. The following assessments are planned in the Laboratory of Tumor Immunology and Biology (LTIB), CCR, NCI for the samples collected. Please see section **5.1.1.3** for sample processing information.

5.1.1.1.1 Analyses of PBMCs:

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 PSA, MUC-1, and Brachyury. Control peptide pools will involve the use of human leukocyte antigen (HLA) 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. The absolute number of $CD4^+$ or $CD8^+$ T lymphocytes producing cytokine or positive for CD107a will be calculated per 1×10^6 cells plated at the start of the IVS. The background signal (obtained with the negative control peptide pool) and any value obtained prior to vaccination, will be subtracted from those obtained after vaccination ([post-TAA − post-HLA] − [pre-TAA − pre-HLA]). An antigen specific immune response will be scored as positive if a patient has more than 250 $CD4^+$ or $CD8^+$ T cells that produce IFNg, TNF, IL-2, or are positive for CD107a at the end of the stimulation assay per $1x10^6$ cells that are plated at the start of the assay. A detailed description of this assay has been previously reported(55). If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens.

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(75), and for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs.

5.1.1.1.2 Analyses of antibodies and soluble factors:

Ad5 Neutralizing antibody titers will be determined as previously described(2, 3). Dilutions of heat-inactivated test sera will be mixed with Ad5 [E1-]-null viral particles and then added to HEK293 cells. An MTS tetrazolium bioreduction assay will be used to measure Ad5 neutralizing antibody titers.

Sera will be analyzed pre- and post-therapy for the following soluble factors: sCD27 and sCD40 ligand.

Selected patients may be analyzed for cytokines and antibodies to human tumor antigens, including PSA, MUC-1, and Brachyury.

5.1.1.1.3 Additional assays

Blood samples may be used for additional research studies, which may include phenotypic and functional analysis of immune-cell subsets, T cell clonality, and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers. Auto-antigen proteomic arrays may also be explored

5.1.1.2 Analyses of Tumor Specimens

Analyses of Tumor Tissue (IHC) for expression of Target Antigens and Immune Related Markers

For patients with lesions amenable to biopsy (or in selected patients with archival tissue), study of expression of target antigens (including PSA, MUC-1, and Brachyury) will be performed (collaboration with Dr. Houssein Sater)

For patients with lesions amenable to biopsy, immune infiltration within the tumor microenvironment pre vs. post treatment may be performed to examine the frequency and localization of tumor infiltrated leukocytes (e.g. CD8, CD4 T-cells, Treg, NK cells, macrophage, M1/2 profile) by IHC (collaboration with Dr. Houssein Sater).

Level of PD-L1 expression may be assessed by immunohistochemistry staining (IHC). Of note, further techniques to evaluate the expression of PD-L1 and/or marker candidates impacting the targeting or contributing to improve its expression may be also investigated if needed.

Tissue from select patients may be tested for T cell clonality.

5.1.1.3 Sample Processing

5.1.1.3.1 Blood samples

Blood samples will be processed at:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick, MD 21702
301-846-1000

On the days that samples are drawn, Jennifer Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange courier delivery of the specimens to the processing lab. Couriers are assigned to CCR and this mechanism is in place.

The weekly patient lists of samples drawn will be emailed to Caroline Jochems at jochemscm@mail.nih.gov, Jen Bangh at jb478s@nih.gov and Theresa Burks (burkst@mail.nih.gov).

After processing, all non-FFPE samples will be transferred for labeling and tracking at:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick, MD 21702
301-846-1000

5.2 SAMPLE COLLECTION SUMMARY

Test/assay	**Sample/ volume blood**	**Type of tube**	**Collection point (+/- 48hrs)**	**Location of specimen analysis**
PSA-specific T cell response	15 mL blood	Two 10 mL Sodium heparin	Baseline, week 6, week 14, week 30 and end of treatment	Clinical Services Program, Frederick/ LTIB
PMBC subset correlation	15 mL blood	Two 10 mL Sodium heparin	Baseline, week 6, week 14, week 30 and end of treatment	Clinical Services Program, Frederick/ LTIB
T cell clonality	4 mL blood	One 10 mL Sodium heparin	Baseline, week 6, week 14, week 30 and end of treatment	Clinical Services Program, Frederick/ LTIB
Cytokine analysis	8 mL blood	One SST	Baseline, week 6, week 14, week 30 and end of treatment	Clinical Services Program, Frederick/ LTIB

5.3 SAMPLE STORAGE, TRACKING, DISPOSITION (CLINICAL SERVICES PROGRAM [CSP])

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 NIH without IRB notification and an executed MTA.

All data associated with the patient samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the NCI Frederick Central Repository by couriers.

Samples will be tracked and managed by the Central Repository database. All samples will be stored in 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, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

The subcontractor's role is limited to clinical research databases and repositories containing patient specimens. The subcontractor neither conducts nor has any vested interest in research on human subjects, but does provide services and supports the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only coded, linked samples and sample information. Protected information will not be sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as 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.

5.3.1 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 patient consent or an exemption from OHSRP.

The PI will report any loss or destruction of samples to the NIH IRB as soon as he/she is made aware of such loss. The PI 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 patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples, or other problems associated with samples will also be reported to the IRB and the NCI Clinical Director.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) 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.

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.

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 90 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be considered an AE 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.

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:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked 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.

6.2.2 Genomic Data Sharing Plan

No large scale genomic data are collected on this study. The GDS plan does not apply.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response at week 14 and every 8 weeks thereafter and at the of study treatment. In addition to a baseline CT and bone scan, confirmatory scans should also be obtained up to 6 (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1; Eisenhauer 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those 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 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 (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible 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 over and above the 5 target lesions should be identified as **non-target lesions** and should also 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.

6.3.2 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.

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 [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. 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 [*JNCI* 92:1534-1535, 2000].

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.

6.3.3 Response Criteria

6.3.3.1 Evaluation of Target Lesions

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 the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (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 at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

6.3.3.2 Evaluation of Non-Target Lesions

Complete Response (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).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.3.3 Evaluation of Best Overall Response

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

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

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

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

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

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

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

6.3.4 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.

6.3.5 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 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5. A copy of the CTCAE version 5 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc

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 reaction

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 docu-ments, 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 NIH IRB AND CLINICAL DIRECTOR (CD) REPORTING

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

The Protocol PI will report in the NIH Problem Form to the NIH 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 NIH IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NIH 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 NIH 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 NIH IRB.

7.3 IND Sponsor Reporting Criteria

During the first 30 days after the subject receives investigational agent, the investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, 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 reporing 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 and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 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 study vaccines. 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 (ETBX-051, ETBX-061, ETBX-071)

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 immediately (i.e., within a maximum 2 business days after becoming aware of the event) 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-071, ETBX-061, and ETBX-051vaccines 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-071, ETBX-061, and ETBX-051vaccines, but are not fatal or life-threatening, much 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 regular 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 de-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. 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 NIH 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 CONSIDERATIONS

8.1 STATISTICAL HYPOTHESES

8.1.1 Primary Endpoints

- Safety and tolerability of the recommended phase 2 dose of PSA/MUC1/Brachyury vaccine as determined by the fraction of patients who experience a DLT.
- Dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD)
- Treatment-emergent adverse event (AEs) and serious adverse events (SAEs).
- Clinically significant changes in safety laboratory tests, physical examinations, electrocardiograms (ECGs), and vital signs.

8.1.2 Secondary Efficacy Endpoints

Preliminary assessments of:

- objective response rate (ORR) – percentage of subjects who experience partial or complete response at any time point during treatment period
- disease control rate (DCR) –percentage of subjects who experience partial response, complete response or stable disease lasting for at least 6 months
- duration of response – the time from when measurement criteria for PR or CR are met until disease recurrence or progression per dose cohort and overall
- progression-free survival (PFS) - the time from the date of first treatment to the date of disease progression or death (any cause) whichever occurs first per dose cohort
- overall survival (OS) - the time from the date of first treatment to the date of death (any cause) per dose cohort and overall
- PSA doubling time (PSADT) – week 14 and at the end of study treatment in subjects with advanced cancer treated with the ETBX-071, ETBX-061, and ETBX-051 vaccines

8.2 SAMPLE SIZE DETERMINATION

Up to six patients will be enrolled in an initial cohort. If ≤1 of 6 patients experience a DLT, then initiation of the dose expansion phase will occur. If ≥2 of 3 or 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) ($1x10^{11}$ 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 at dose level -1, then a further dose de-escalation will occur. Up to six patients will be enrolled at the lower dose level (-2) ($5x10^{10}$ 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 DLTat dose level -2, then the study will be stopped.

In the second part, dose expansion will occur when the MTD has been determined. An additional 12 subjects will be enrolled in the dose expansion component of the trial, for a total of 18 subjects treated at the MTD. Evaluation of T cell frequencies (CD4, CD8, NK and T Reg cells, etc.) will be performed and will result in exploratory comparisons between responders and non-responders. If approximately 25-30% of patients experience a clinical response, there should be approximately 3-4 responders and 8-9 non-responders out of 12 evaluable patients. In these patients, for a given measure, considered as an exploratory evaluation, a two-group t-test with a one-sided 0.10 significance level will have 80% power to detect a difference between the two groups with an

effect size of 1.5 (1.5 SDs of the measure of interest, assumed to be approximately the same in responders and non-responders), when there are 3 responders and 9 non-responders (total of 12 evaluable patients). With 4 responders and 8 non-responders, the same comparison would have 85% power. In practice, an exact Wilcoxon rank sum test will be used instead of a two-group t-test.

To accrue a total of 18 evaluable patients, it is expected that up to 2 years may be required. Since up to three dose levels may be explored, in addition to the 12 evaluable patients at the MTD, the accrual ceiling will be set at 30 patients.

8.3 Populations for Analysis

8.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with ETBX vaccines.

Evaluable for DLT: The first 6 - 18 subjects enrolled will be evaluable for DLT from the time of their first treatment with ETBX-vaccines until 28 days have passed. Subjects who do not complete the DLT observation period for reasons other than a DLT will be replaced.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one dose 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.

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 dose 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, along with comparison of parameters between responders and non-responders using a non-parametric test.

8.4.2 Analysis of the Primary 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 Analysis of the Secondary Efficacy Endpoints

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 cohort and overall. 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.

8.4.3.1 Duration of Response

The duration of overall response will be evaluated by dose cohort and overall. 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 PD the smallest measurements recorded since the treatment started).

8.4.3.2 Progression-Free Survival (PFS)

PFS will be evaluated by dose cohort 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.

8.4.3.3 Overall Survival

OS will be evaluated by dose cohort 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.

8.4.3.4 PSA doubling time will be determined using a standard formula.

8.4.4 Safety Analyses

DLTs will be evaluated continuously in a cohort. An overall assessment of whether to de-escalate to the next dose level will be made at least 1 weeks after the last subject in the previous cohort has received their first injection. Safety will be evaluated in 3 or 6 subjects at each dose level in the dose de-escalation component of the study. During the dose de-escalation phase of the study, 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). A DLT is defined in Section **3.1**. Safety will continue to be monitored among additional subjects treated at the MTD 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 4 weeks.

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

Overall safety will be assessed by descriptive analyses using tabulated frequencies of AEs by grade using CTCAE Version 5 within dose cohorts 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.

8.4.5 Baseline Descriptive Statistics

None will be provided.

8.4.6 Planned Interim Analyses and Halting Guidelines

8.4.6.1 Planned Interim Analyses

Safety data will be examined after 6 patients have been treated to ensure that enrolling the remaining patients is allowable.

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 Subgroup 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

The percentage of subjects with a positive immune response will be evaluated by dose cohorts and overall. A positive immune response is defined by CMI reactivity in ex vivo stimulation assays, with 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.(55)
Immune response will be assessed among the 18 subjects treated at the MTD (6 in dose escalation and 12 in dose expansion). The therapy will be considered of further interest if 9 of 18 subjects treated at the MTD exhibit an immune response as defined above. The magnitude of response will also be described. A subject will be considered evaluable for immune response if they receive at least three injections

T cell frequencies (CD4, CD8, NK, T Reg cells, etc.) will be determined post treatment, and compared among the 12 patients in the expansion cohort between responders and non-responders, using an exact Wilcoxon rank sum test, with a one-tailed p-value to determine if there is an increase in these values being reported, to demonstrate potential trends if identified in a very small number of patients. P-values will be presented without adjustment for multiple comparisons, but in the context of an exploratory analysis with limited numbers of subjects.

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-071 (adenoviral PSA 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 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 ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this study as this disease occurs only in men.

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 ≥ 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 (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E 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

Adult subjects, including those that have lost the capacity to consent

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.

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-071, ETBX-061, AND ETBX-051 VACCINE REGIMEN (IND# 17811)

11.1.1 Source

Table 4: Investigational Products

Table 4a

Product Name(s):	ETBX-071 (Ad5 [E1-, E2b-]-PSA Vaccine)
Dosage Form:	Suspension for injection
Unit Dose	5×10^{11} VP, 1×10^{11} VP, or 5×10^{10} VP.
Route of Administration	SC injection
Physical Description	Each ETBX vaccine is supplied as a sterile, clear solution in a 2-mL single-dose vial. The vaccine is provided at a concentration of 5×10^{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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

Table 4b

Product Name(s):	ETBX-061 (Ad5 [E1-, E2b-]-MUC1 Vaccine)
Dosage Form:	Suspension for injection
Unit Dose	5 x 10^{11} VP, 1 x 10^{11} VP, or 5 x 10^{10} VP.
Route of Administration	SC injection
Physical Description	Each 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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

Table 4c

Product Name(s):	ETBX-051 (Ad5 [E1-, E2b-]-brachyury Vaccine)
Dosage Form:	Suspension for injection
Unit Dose	5 x 10^{11} VP, 1 x 10^{11} VP, or 5 x 10^{10} VP.
Route of Administration	SC injection
Physical Description	Each 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 1.1 mL of the vaccine. The product should be stored at ≤ -20°C.
Manufacturer	Etubics

The ETBX-071, ETBX-061, and ETBX-051 vaccines will be supplied by the manufacturer, Etubics Corporation, through a Cooperative Research and Development Agreement (CRADA).

The manufacturing department of Etubics will supply the vaccine, which will be distributed to the sites by Etubics Corporation.

11.1.2 Toxicity

The safety of immunizations (injections) witha combination of three immunotherapeutic vaccines (ETBX-071, ETBX-061, and ETBX-051), 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 $\geq$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 $\geq$2 autoimmune events except for vitiligo or fever for less than 2 days and less than 101.5 ºF, Grade $\geq$2 allergic reactions (Grade 2 is defined as generalized urticaria as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE version 5), or Grade $\geq$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 $5x10^{11}$ 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: ≥ 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 ≥ 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 a clear colorless liquid filled in a 2-mL amber vial containing 1.0 mL of extractable vaccine. There are total of $5x10^{11}$ total virus particles (VP) in 1.0 mL of the product. 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 (<-80 °C) 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 temperature control of ≤-20 °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 Administration Procedures

Please see section **3.2.4**. Briefly, each ETBX-071, ETBX-061, and ETBX-051 vaccines will be administered on Weeks 0, 3, and 6 followed by booster vaccines every 8 weeks for up to a year in (only dose expansion cohor)t. All study drug administration treatments should occur within ± 7 days of the planned visit date.

Each of the three vaccines should be given by SC injection into a proximal limb, preferably in the thigh, after preparation of the site with alcohol. Preferably all three injections on a given day should be given to the anterolateral section of the same thigh and preferably the thigh chosen for injections should alternate between treatment days. Less preferably it is possible to give three injections to different limbs on a given day or to use the same thigh for consecutive treatment days. When the 3 injections are given on a given day to the same limb (e.g. thigh) the injections must be separated by at least 5 cm and it is preferred that ETBX-071 is administerd most proximal, ETBX-061 in the middle, and ETBX-051 most distal.

11.1.6 Incompatibilities

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

11.1.7 Other Considerations

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 vialed 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.

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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

To be complete by site:
Injection #: _____ Date of Injection: _____/_____/_____ Subject Number: _____
Month/Day/Yr

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

		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**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____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**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____mm**	**Yes / No** **_____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**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **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**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**

		Day 0 (Day of Injection)	**Day +1**	**Day +2**	**Day +3**	**Day +4**	**Day +5**	**Day +6**
5. Have you taken any medication for injection site pain?	**Circle: Yes / No** **Provide name and dose of medication**	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **Dose:** _____	**Yes / No** **Name:** _____ **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**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **Severe**	**Yes / No** **Mild** **Moderate** **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^{O}F for more than 24 hours, call your study doctor**	**Record Temp:** _____	**Record Temp:** _____	**Record Temp:** _____	**Record Temp:** _____	**Record Temp:** _____	**Record Temp:** _____	**Record Temp:** _____

Grading injection site pain:

Mild	**Noticeable, does not interfere with activity**
Moderate	**Interferes with activity, limiting instrumental activities of daily living**
Severe	**Limiting self-care activities of daily living, incapacitating**

Grading Chills:

Mild	**Mild sensation of cold, shivering, chattering of teeth**
Moderate	**Moderate tremor of entire body, narcotics indicated**
Severe	**Severe or prolonged, not responsive to narcotics**