A Phase I Trial Using a Multitargeted Recombinant Adenovirus 5 (CEA/MUC1/Brachyury)-Based Immunotherapy Vaccine Regimen in Patients with Advanced Cancer

MARGARET E. GATTI-MAYS, JASON M. REDMAN, RENEE N. DONAHUE, CLAUDIA PALENA, RAVI A. MADAN, FATIMA KARZAI, MARUO BILUSIĆ, HOUSSEIN ABDUL SATER, JENNIFER L. MARTÉ, LISA M. CORDES, SHERI MCMAHON, SETH M. STEINBERG, ALANVIN ORPIA, ANDREA BURMEISTER, JEFFREY SCHLOM, JAMES L. GULLEY, JULIUS STRAUSS

Laboratory of Tumor Immunology and Biology and Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; Biostatistics and Data Management Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; Leidos Biomedical Research, Inc., Frederick, Maryland, USA

A novel adenovirus-based vaccine targeting three human tumor-associated antigens—CEA, MUC1, and brachyury—has demonstrated antitumor cytolytic T-cell responses in preclinical animal models of cancer.

Methods. This open-label, phase I trial evaluated concurrent administration of three therapeutic vaccines (ETBX-011 = CEA, ETBX-051 = MUC1 and ETBX-061 = brachyury). All three vaccines used the same modified adenovirus 5 (Ad5) vector backbone and were administered at a single dose level (DL) of $5 \times 10^{11}$ viral particles (VP) per vector. The vaccine regimen consisting of all three vaccines was given every 3 weeks for three doses then every 8 weeks for up to 1 year. Clinical and immune responses were evaluated.

Results. Ten patients enrolled on trial (DL1 = 6 with 4 in the DL1 expansion cohort). All treatment-related adverse events were temporary, self-limiting, grade 1/2 and included injection site reactions and flu-like symptoms. Antigen-specific T cells to MUC1, CEA, and/or brachyury were generated in all patients. There was no evidence of antigenic competition. The administration of the vaccine regimen produced stable disease as the best clinical response.

Conclusion. Concurrent ETBX-011, ETBX-051, and ETBX-061 can be safely administered to patients with advanced cancer. Further studies of the vaccine regimen in combination with other agents, including immune checkpoint blockade, are planned.

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and brachyury. In preclinical studies, TAV induced immune responses directed against TAAs with minimal to no "antigenic competition" [1]. A prior clinical trial in metastatic colorectal cancer showed that the CEA ETBX-011 vaccine was safe and had clinical benefit [2, 3]. The primary objectives of this trial were to assess the safety of TAV in advanced solid malignancies and to identify the recommended dose for future trials.

Ten patients enrolled on this open label, phase I trial from January 31, 2018, to April 24, 2018 (DL1, n = 6; expansion, n = 4). The data cutoff for final analysis was October 23, 2018. All patients were monitored for dose-limiting toxicities (DLTs) for 3 weeks after the first dose. Reported adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events v5.0. Computed tomography of the thorax, abdomen, and pelvis was performed at baseline, week 6, and then every 8 weeks.

Five patients were female. Median age was 51.7 years. Nine patients had colorectal cancer and one had cholangiocarcinoma. All patients were evaluable for clinical, safety, and immune responses. TAV was well tolerated with no DLTs. When given concurrently, the recommended phase II dose of TAV (ETBX-011, ETBX-051 and ETBX-061) is $5 \times 10^{11}$ VP per vaccine. There were no grade ≥3 AEs. All AEs attributed to TAV were temporary and self-limiting. Grade 1 or 2 injection site reactions occurred in all patients, with most reporting injection site pain (n = 9; 90%), erythema (n = 8; 80%), and induration (n = 7; 70%). These reactions generally occurred within 24 hours of administration and resolved within 7 days without intervention. Pyrexia (n = 5; 50%) and chills (n = 8; 80%) were common. Myalgias, nausea, and fatigue were also reported. The average time on treatment was 13.6 weeks (range 3–34 weeks). The best radiographic response was stable disease per RECIST v1.1.

After vaccination, all patients developed CD4+ and/or CD8+ T-cell responses [4] to at least one TAA encoded by the vaccine; 5/6 (83%) developed MUC1-specific T cells, 4/6 (67%) developed CEA-specific T cells, and 3/6 (50%) developed brachyury-specific T cells (Table 1). Two patients developed responses to all TAAs in the vaccines. Induction of antigen-specific T cells was rapid, with most occurring by week 6. Polyfunctional T cells (i.e., T cells positive for two or more of the following: interferon gamma, tumor necrosis factor, interleukin-2, or CD107a) specific for MUC1, CEA, or brachyury were generated in 50%, 33%, and 17% of patients, respectively. The presence of Ad5-neutralizing antibodies did not prevent the generation of TAA-specific T cells.

Although TAV does not appear to have single-agent activity, it has a manageable safety profile and generates TAA-specific T-cell responses in patients with cancer (Table 1). Future immuno-oncology trials aimed at enhancing synergistic antitumor mechanisms with TAV are planned.

### Table 1. Tumor-associated antigen T-cell responses developed after treatment with the TriAdeno vaccine regimen

| Immune responses to MUC1 | Immune responses to CEA | Immune responses to brachyury |
|--------------------------|-------------------------|------------------------------|
| Patient | Pre vac. no. | Post vac. no. | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ | CD4+ | CD8+ |
| P13 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Immune responses reported in this table are calculated by comparing the absolute number of CD4+ or CD8+ T cells producing cytokine (IFN, IL-2, TNFα) or positive for CD107a per $1 \times 10^{6}$ PBMCs plated at the start of the in vitro stimulation at the specified time points after vaccine. Background (obtained with the negative control peptide pool, human leukocyte antigen [HLA]) and any response prior to vaccine are subtracted: [TAA after vaccine – HLA after vaccine] – [TAA before vaccine – HLA before vaccine]. Positive immune responses are defined as ≥250 (highlighted).

Abbreviations: IFNg, interferon gamma; IL-2, interleukin-2; PT, patient; TNF, tumor necrosis factor.

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## Drug Information

### Drug 1
- **Generic/Working Name**: ETBX-011
- **Trade Name**: None
- **Company Name**: Etubics (a wholly owned subsidiary of ImmunityBio)
- **Drug Type**: Vaccine
- **Drug Class**: Immune therapy
- **Dose**: $5 \times 10^{11}$ viral particles per flat dose
- **Route**: Other; subcutaneous
- **Schedule of Administration**: ETBX-011 = CEA
  - Every 3 weeks for three doses, then every 8 weeks for 1 year

### Drug 2
- **Generic/Working Name**: ETBX-051
- **Trade Name**: None
- **Company Name**: Etubics (a wholly owned subsidiary of ImmunityBio)
- **Drug Type**: Vaccine
- **Drug Class**: Immune therapy
- **Dose**: $5 \times 10^{11}$ viral particles per flat dose
- **Route**: Other; subcutaneous
- **Schedule of Administration**: ETBX-061 = MUC1
  - Every 3 weeks for three doses, then every 8 weeks for 1 year

### Drug 3
- **Generic/Working Name**: ETBX-061
- **Trade Name**: None
- **Company Name**: Etubics (a wholly owned subsidiary of ImmunityBio)
- **Drug Type**: Vaccine
- **Drug Class**: Immune therapy
- **Dose**: $5 \times 10^{11}$ viral particles per flat dose
- **Route**: Other; subcutaneous
- **Schedule of Administration**: ETBX-051 = brachyury
  - Every 3 weeks for three doses, then every 8 weeks for 1 year

## Dose Escalation Table

| Dose level | Dose of drug: ETBX-011 | Dose of drug: ETBX-051 | Dose of drug: ETBX-061 | No. enrolled | No. evaluable for toxicity |
|------------|------------------------|------------------------|------------------------|--------------|---------------------------|
| 1          | $5 \times 10^{11}$ VP  | $5 \times 10^{11}$ VP  | $5 \times 10^{13}$ VP  | 10           | 10                        |
| −1         | $1 \times 10^{11}$ VP  | $1 \times 10^{11}$ VP  | $1 \times 10^{13}$ VP  | 0            |                           |

Abbreviation: VP, viral particles.

## Patient Characteristics

|                  |                  |
|------------------|------------------|
| **Number of Patients, Male** | 5 |
| **Number of Patients, Female**  | 5 |
| **Stage** | Advanced or metastatic solid tumor |
| **Age** | Median (range): 51.7 (36.1–65.6) |
| **Number of Prior Systemic Therapies** | Median (range): 2 (0–12) |

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Performance Status: ECOG

| Status       | Value |
|--------------|-------|
| 0 — 5        |       |
| 1 — 5        |       |
| 2 — 0        |       |
| 3 — 0        |       |
| Unknown      | 0     |

Other

Race: white, 7; Asian, 3

Cancer Types or Histologic Subtypes

Microsatellite stable colorectal cancer, 9; cholangiocarcinoma, 1

**Primary Assessment Method**

| Title                                      | Secondary objective: efficacy |
|-------------------------------------------|------------------------------|

| Number of Patients Screened               | 11                           |
| Number of Patients Enrolled               | 10                           |
| Number of Patients Evaluable for Toxicity | 10                           |
| Number of Patients Evaluated for Efficacy | 10                           |
| Evaluation Method                         | RECIST 1.1                   |
| Response Assessment CR                    | \( n = 0 \) (0%)              |
| Response Assessment PR                    | \( n = 0 \) (0%)              |
| Response Assessment SD                    | \( n = 6 \) (60%)             |
| Response Assessment PD                    | \( n = 4 \) (40%)             |
| Response Assessment OTHER                 | \( n = 0 \) (0%)              |
| (Median) Duration Assessments TTP         | 13.6 weeks                   |

**Outcome Notes**

Secondary objective

**Adverse Events**

| All Cycles | NC/NA | 1 | 2 | 3 | 4 | 5 | All grades |
|------------|-------|---|---|---|---|---|------------|
| Injection site reaction | 0% | 30% | 70% | 0% | 0% | 0% | 100% |
| Chills | 20% | 80% | 0% | 0% | 0% | 0% | 80% |
| Fever | 50% | 50% | 0% | 0% | 0% | 0% | 50% |
| Fatigue | 60% | 40% | 0% | 0% | 0% | 0% | 40% |
| Nausea | 90% | 10% | 0% | 0% | 0% | 0% | 10% |
| Myalgia | 90% | 10% | 0% | 0% | 0% | 0% | 10% |

Common Terminology Criteria for Adverse Events v5 used.

Grade 1 or 2 injection site reactions occurred in all patients, with most reporting injection site pain (\( n = 9 \); 90%), erythema (\( n = 8 \); 80%), and induration (\( n = 7 \); 70%). Some adverse events (AEs) were reported more than once by a single patient. There were two grade 3 AEs reported by two separate patients on the trial (anal pain in a previously radiated area and gram-negative rod bacteremia). Neither AE was attributed to the vaccines.

Abbreviation: NC/NA, no change from baseline/no adverse event.

**Dose-Limiting Toxicities**

| Dose level | No. enrolled | No. evaluable for toxicity | No. with a dose-limiting toxicity |
|------------|--------------|-----------------------------|----------------------------------|
| 1          | 10           | 10                          | 0                                |

**Assessment, Analysis, and Discussion**

Completion

Study completed

Investigator’s Assessment

Drug tolerable, efficacy indeterminant
This open-label, phase I trial demonstrated that the TriAdeno vaccine regimen (TAV) is safe and well tolerated. The recommended dose of TAV for use in future trials is $5 \times 10^{11}$ viral particles of each vaccine (ETBX-011, ETBX-051, and ETBX-061). The dosing schedule used in this study was every 3 weeks for three doses and then every 8 weeks; however, other studies using TAV are employing other dosing schedules. TAV induced antigen-specific immune responses directed against all three tumor-associated antigens (TAAs) with minimal to no “antigenic competition” [1]. Neutralizing antibodies to adenovirus 5 (Ad5) were measured as previously described with slight modification [2, 5–7]. At baseline, two of eight patients had neutralizing Ad5 antibodies. After one or two vaccinations, all eight patients analyzed developed neutralizing Ad5 antibodies. The presence of neutralizing antibodies to Ad5 did not prevent the generation of TAA-specific T cells.

These safety and immunologic data are consistent with findings from a prior clinical trial in metastatic colorectal cancer that showed that the ETBX-011 vaccine (CEA) was safe and induced CEA-specific cytotoxic T-cell activity [2, 3]. There was also some evidence of clinical benefit, with half of patients who received ETBX-011 alive at 1 year after treatment and a little less than one third of patients alive at 18 months after treatment [8]. Currently, immune checkpoint blockade (ICB) benefits only a small percentage of patients, with response rates for Food and Drug Administration-approved agents around 20%–30% depending on the type of cancer. However, the addition of agents such as vaccines that generate tumor-specific immune responses and induce immunogenic cell death may be an important component to expand the benefit of ICB to more patients [9–13]. For example, antitumor activity exceeding what would be expected historically has been observed with vaccines plus ICB in small data sets [9, 10]. For several reasons, vaccines like TAV are promising candidates for generating immune responses when used in combination with other immuno-oncology (IO) agents.

The three TAAs (CEA, MUC1, and brachyury) encoded in TAV are associated with several common malignancies. CEA is an attractive target for immunotherapy because it is overexpressed in multiple adenocarcinomas. In addition, CEA is a good target for T-cell-mediated immunity because it contains known epitopes that are recognized via a major histocompatibility complex (MHC)—restricted fashion by human cytolytic T lymphocytes that bind to MHC loci human leukocyte antigens (HLA) A2, A3, and A24 [1, 14]. MUC1 is expressed on the majority of human adenocarcinomas, with high expression seen in colorectal cancer, breast cancer, non-small cell lung cancer, bladder cancer, and pancreatic cancer. Multiple enhanced agonist epitopes of MUC1 including the C-terminus of MUC1 act as oncogenes and can induce plasticity [15, 16]. Human T-cell lines generated using MUC1 agonist epitopes generated antigen-specific interferon gamma (IFNγ) and lysis of tumor cells that express the native MUC1 [16, 17]. Brachyury is an embryonic transcription factor of the T-box family that regulates cellular plasticity [18]. High brachyury expression is found in lung cancer, colorectal cancer, breast cancer, prostate cancer, and gastrointestinal stromal tumor [19]. Carcinoma cells that undergo a phenotypic transition exhibit enhanced motility and invasiveness in vitro and the propensity to metastasize in vivo [20]. Using a 9-mer peptide of the brachyury protein, brachyury-specific CD8+ T cells were expanded in vitro from the blood of patients with cancer and then used in cytotoxic assays for effective lysis of human tumor cells that endogenously express brachyury [21, 22]. Additionally, multiple studies have demonstrated that MUC1 and/or brachyury expression are markers of poor prognosis [23–27], treatment resistance [28–31], and tumor aggressiveness [32, 33]. In vitro and preclinical animal models with the TAAs demonstrated antitumor cytolytic T-cell responses. Multiple therapeutic cancer vaccine trials have been conducted using one or two of these TAAs, but this is the first trial to our knowledge that uses this triad of TAAs.

TAV vaccination generated CD4+ and/or CD8+ T-cell responses to at least one TAA encoded by the vaccine in all patients. Two patients developed responses after vaccination to all three TAAs in the vaccine. Furthermore, polyfunctional TAA-specific responses, defined as CD4+ or CD8+ T cells that express ≥2 of the following markers: IFNγ, tumor necrosis factor, interleukin-2, or CD107a, were measured before and after vaccination. Using the criteria of a >10-fold increase after versus before vaccination, or the presence of ≥1,000 polyfunctional cells after vaccination per 1 × 10⁶ peripheral blood mononuclear cells (if negative at prevaccination), polyfunctional T-cells specific for MUC1, CEA, or brachyury were generated in 50%, 33%, and 17% of patients, respectively. Although there is no overt clinical benefit seen in this small phase I trial, the generation of long-lasting polyfunctional T cells has been previously associated with improved overall survival [34]. In patients with melanoma, polyfunctional T cells can be detected as early as after one vaccination, and these T cells can persist for years after initial vaccination in responders [34].

In conclusion, this work adds to the existing literature [11, 35–38] that antitumor vaccines directed against CEA, MUC1, and brachyury are well tolerated and can generate antitumor immune responses. TAV generated antigen-specific T cells to one or more target antigens in all patients evaluated. Planned studies are aimed at interrogating the TAV regimen’s potential antitumor activity when used in combination with other IO agents such as ICB and immunocytokines.

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The authors indicated no financial relationships.
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