Abbreviated Title: Phase I MVA-Brachyury-TRICOM
CC Protocol #: 14-C-0142 OSP#: 1404-1301
Version Date: September 14, 2016 IBC#: RD-14-V-01
Amendment: C

Title: An open-label phase I study to evaluate the safety and tolerability of a modified vaccinia Ankara (MVA)-based vaccine modified to express brachyury and T-cell costimulatory molecules (MVA-brachyury-TRICOM)

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A. Obtain information by intervening or interacting with living individuals for research purposes
B. Obtaining identifiable private information about living individuals
C. Obtaining the voluntary informed consent of individuals to be subjects
D. Makes decisions about subject eligibility
E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
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G. Some/all research activities performed outside NIH

Investigational Agents:

Drug Name:	MVA-brachyury-TRICOM
IND Number:	15990
Sponsor:	Center for Cancer Research
Manufacturer:	Bavarian Nordic, Inc.

PRÉCIS

Background:

- MVA-brachyury-TRICOM is a novel recombinant vector-based therapeutic cancer vaccine designed to induce an enhanced immune response against brachyury, which is overexpressed in many solid tumor types, such as lung, breast, ovarian, chordoma, prostate, colorectal, and pancreatic adenocarcinoma.
- Modified vaccinia Ankara (MVA) is a replication-deficient, attenuated derivative of vaccinia. It is used in the smallpox vaccination and is now being developed as a recombinant viral vector to produce vaccines against infectious diseases and cancer.
- Many MVA vector-based trials conducted in patients with cancer have demonstrated its safety and the immunogenicity of its transgenes.
- Brachyury is a member of the T-box family of transcription factors. It is overexpressed in cancer cells compared with normal tissue and has been linked to cancer cell resistance and metastatic potential.
- Brachyury as a vaccine target has been demonstrated to be safe in an ongoing phase I study of recombinant yeast-brachyury and to generate brachyury-specific T-cell responses.
- Poxviral vaccines delivering a triad of three human T-cell costimulatory molecules designated TRICOM (B7.1, ICAM-1 and LFA-3) have been extensively studied in both preclinical and clinical studies and have demonstrated their ability to induce robust T-cell activation and provide evidence of clinical benefit.
- *In vitro*, MVA-brachyury-TRICOM is able to effectively expand brachyury-specific CD8+ and CD4+ T cells from peripheral blood mononuclear cells.
- Previous work indicates that MVA-brachyury-TRICOM will induce activation a distinct T-cell subpopulation from that seen with yeast-brachyury vaccine already in development.

Objectives:

- To determine the safety and tolerability of escalating doses of MVA-brachyury-TRICOM vaccine.

Eligibility:

- Patients must have histologically confirmed malignancy that is metastatic or unresectable locally advanced malignant solid tumor. In the case of chordoma, unresectable, locally recurrent, or metastatic tumors are acceptable for enrollment, given that this represents incurable disease. As much as possible, patients enrolled will have tumor types with known increased expression of brachyury (such as lung, breast, ovarian, prostate, colorectal, pancreatic, or chordoma).
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 at study entry
- Age ≥ 18 years.
- Prior Therapy: Completed, or disease progression on at least one prior line of disease-appropriate therapy for metastatic disease, or not a candidate for therapy of proven efficacy for their disease.

Design:

- This is an open-label, phase I trial with sequential cohorts of patients (3–6 patients per dose cohort) with dose escalation of MVA-brachyury-TRICOM vaccine.
- Three cohorts will receive MVA-brachyury-TRICOM vaccine administered subcutaneously as either 1, 2, or 4 injections of study drug (1 injection = 2 x 10^8 infectious units (IU) at monthly (28 days +/– 4 days) intervals for 3 months (treatment).
- Expansion cohorts of up to 10 patients may be enrolled at the two highest tolerated dose levels. These cohorts will allow certain standard, relatively non-toxic therapies to continue while patients receive vaccine.
- Up to 18 patients may be required to be enrolled in the 3 cohorts, plus an additional 10 at the MTD and at the dose level just below it. Thus, up to 38 patients may be theoretically required to complete this trial. If 3 patients per month can be accrued, the study is expected to require 1 year to complete the necessary enrollment.

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1.1.2.1 To evaluate CD8 and CD4 immunologic response as measured by an increase in brachyury-specific T cells.

1.1.2.2 To evaluate evidence of clinical benefit, such as progression-free survival, RECIST criteria, reduction in serum markers, and/or reduction in circulating tumor cells.

1.1.2.3 Parameters of general immune activation: frequency of immune cell subsets in peripheral blood and changes in serum levels of cytokines and antibodies to brachyury.

1.1.2.4 Correlation of brachyury expression in tissue samples with clinical outcomes.

1.2 BACKGROUND AND RATIONALE

MVA-brachyury-TRICOM is a novel recombinant vector-based therapeutic cancer vaccine developed by the LTIB in collaboration with our CRADA partner Bavarian Nordic, Inc. The vaccine is designed to enhance the immune response against brachyury, which is overexpressed in many solid tumor types, including lung, breast, ovarian, prostate, chordoma, colorectal, and pancreatic adenocarcinoma, as well as some hematologic malignancies.

1.2.1 Description and Identification of Brachyury

Using a computer-based differential display analysis tool to conduct global comparison of expressed sequence tag (EST) clusters in the Unigene database,[1,2] the gene encoding for the transcription factor brachyury was identified as highly represented in tumor-derived libraries and rarely observed in normal tissue-derived libraries.[3] Brachyury is a member of the T-box family of transcription factors, characterized by a highly conserved DNA-binding domain designated as T-domain.[4-7] Brachyury homologs have been reported to be involved in embryonic mesodermal development.[4,8-11]

1.2.2 Brachyury Function in Epithelial-to-Mesenchymal Transition (EMT)

Epithelial-to-mesenchymal transition (EMT) is a reversible process during which cells switch from a polarized, epithelial phenotype into a highly motile, mesenchymal phenotype.[12,13] At the biochemical level, the EMT program involves the downregulation of epithelial proteins such as E-cadherin and cytokeratins and the induction of mesenchymal proteins, including fibronectin, N-cadherin and vimentin.[14,15] Numerous observations support the concept that the EMT process plays a role in the progression of human carcinomas (Figure 1).[16]

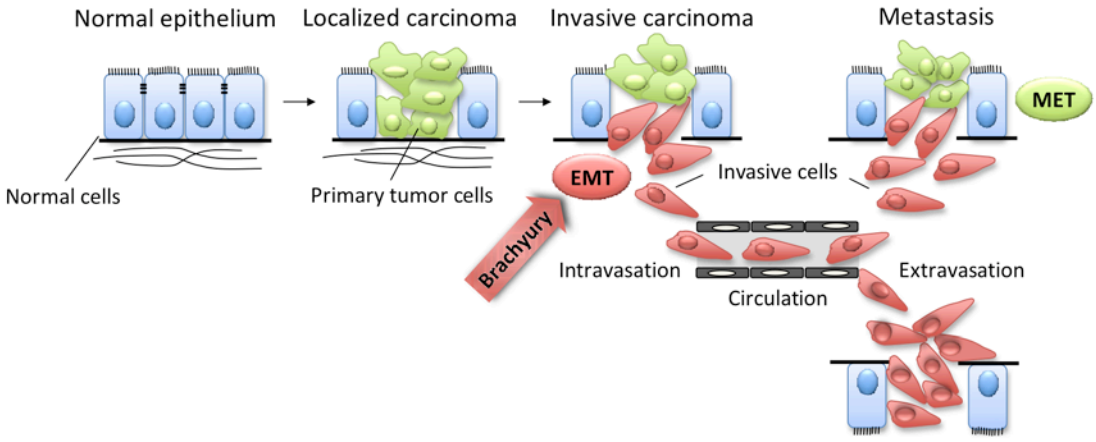


Figure1. Role of brachyury in epithelial-to-mesenchymal transition during tumor progression

We have demonstrated[17] that overexpression of brachyury in human carcinoma cell lines is able to drive a switch from an epithelial to a mesenchymal-like phenotype (i.e., EMT). For example, stable transfection of a pancreatic cancer cell line, PANC-1, with a plasmid encoding for full-length human brachyury resulted in cell spreading, decreased epithelial E-cadherin, and increased mesenchymal fibronectin expression (Figure 2). Western blot also confirmed the overexpression of other mesenchymal markers and downregulation of epithelial markers (Figure 2), providing evidence that brachyury may facilitate EMT.[17]

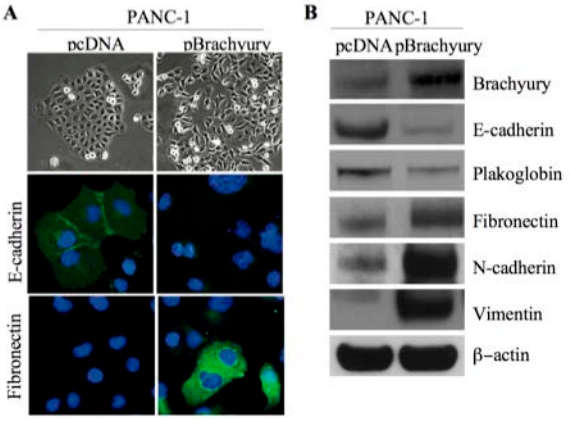


Figure 2. Brachyury alters morphology and EMT marker expression in human pancreatic cancer epithelial tumor cells. (A) Bright field images of PANC-1-pcDNA (control) and PANC-1-p/brachyury cells and immunofluorescent analysis of EMT markers; merged images with DAPI stained nuclei are shown. **(B)** Western blot analysis of brachyury-induced EMT markers. See (17) for details.

Overexpression of brachyury in epithelial tumor cells also resulted in a concomitant increase in tumor cell migration and extracellular matrix invasion (Figure 3A and B). Stable silencing of brachyury expression in brachyury-positive human carcinoma cells (lung H460 cells) resulted in downregulation of mesenchymal markers and upregulation of epithelial markers, with concomitant loss of cell migration and invasion (Figure 3C and D).[17]

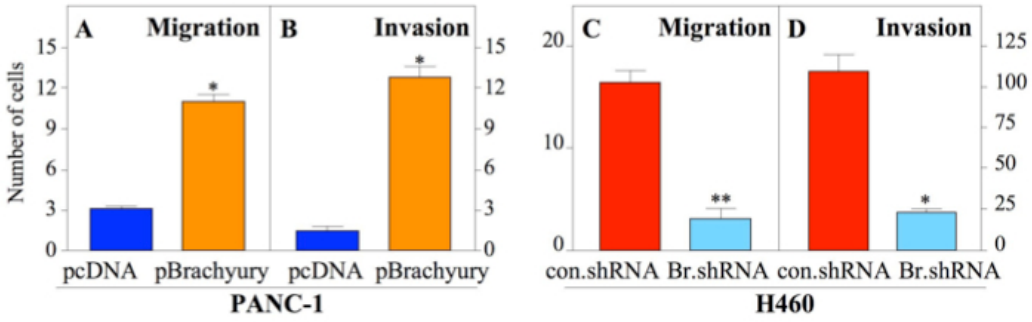


Figure 3. Brachyury induction of migration and invasion. (A and B) Brachyury induces a highly migratory and invasive EMT phenotype *in vitro* in human PANC-1 pancreatic carcinoma cells. **(C and D)** Silencing brachyury expression in H460 human lung carcinoma cells reduces both migratory and invasive capacity. See (17) for details.

Brachyury expression also negatively modulated tumor cell-cycle progression and correlated with low expression of cyclin D1, and carcinoma cells silenced for brachyury expression grew at a greater rate *in vitro* (Figure 4A). These brachyury-silenced cells grew at a comparable rate to control cells when implanted subcutaneously in athymic mice (Figure 4B); however, they had a diminished capacity to metastasize to the lungs from the primary, subcutaneous tumor, or to form experimental lung metastases when injected i.v. (Figure 4C).

Collectively, these results demonstrate that the transcription factor brachyury confers on the tumor cells a mesenchymal phenotype as well as migratory and invasive capabilities and enhances tumor cell progression.[17]

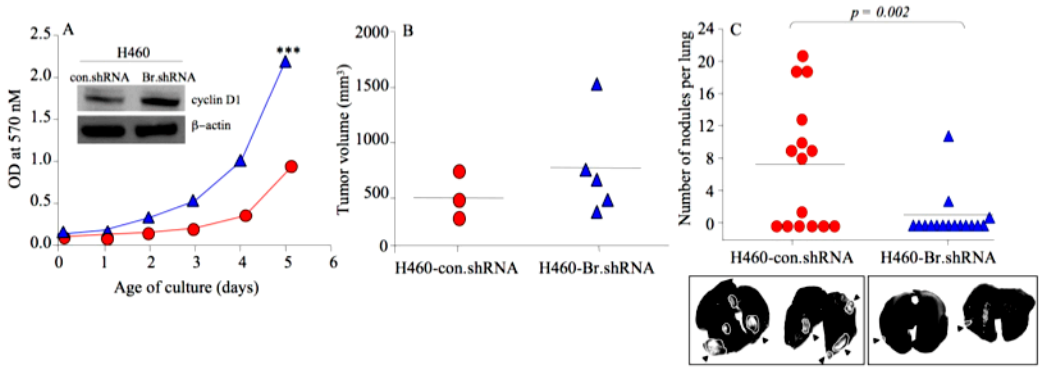


Figure 4. Brachyury inhibition with silencing RNA reduces tumor metastases. Compared to control (con.shRNA, circles), human H460 lung carcinoma cells silenced for brachyury (Br.shRNA, triangles) **(A)** proliferate at a faster rate *in vitro* ($p = 0.003$); **(B)** grow at a similar rate when injected s.c. in athymic mice; and **(C)** form less experimental metastases when injected intravenously ($p = 0.002$). See (17) for details.

1.2.3 Analysis of Brachyury Expression in Human Tumors and Normal Tissues

By using reverse transcription followed by polymerase chain reaction (RT-PCR), investigators in the LTIB have identified the overexpression of brachyury in gastrointestinal,

bladder, kidney, ovary, uterus, and testicular carcinomas. Similar studies also found overexpression of brachyury mRNA in cell lines of lung, colon and prostate cancers, but not in the majority of normal tissues tested, with the exception of expression in the testis and low levels of expression in B cells pooled from multiple normal donors. The expression of brachyury mRNA in normal B cells was further evaluated in CD19+ B-cell fractions isolated from various healthy donors; weak amplification was observed in 4/9 samples analyzed by RT-PCR. These results at the RNA level, however, contrasted with data obtained by immunohistochemistry analysis in normal spleens and lymph nodes, which were negative for the expression of brachyury protein. Moreover, the cytotoxic lysis of normal B cells was evaluated by using brachyury-specific T cells as effectors; no lysis was observed with any of the normal B cells purified from the blood of 5 different healthy donors.[3] We have determined that EBV infection of human B cells enhances brachyury expression; approximately 1 in 10^5–10^6 human B cells have latent EBV infection. Immunohistochemistry (IHC) analysis of brachyury expression using an anti-brachyury monoclonal Ab confirmed the selective expression in tumors of this transcription factor. No expression of brachyury was observed in the majority of normal tissues tested, with the exception of testis (3/3 positive) and thyroid (4/6 positive). The expression of brachyury protein was also detected in 3 of 4 thyroid tissue lysates evaluated by Western blot. These results at the protein level contrasted with the expression of brachyury at the mRNA level, which was negative in 3/3 individual thyroid tissues tested by RT-PCR. Altogether, these results indicate brachyury expression in 7/13 thyroid tissues analyzed. Immunohistochemistry analysis of normal tissues obtained from non-cancer subjects demonstrated brachyury expression in 0/5 lung, 0/3 heart, 0/3 brain, 0/3 liver, 0/3 kidney, 0/3 spleen, 0/3 skeletal muscle, 0/1 adrenal gland, 0/1 skin, 4/6 thyroid, and 3/3 testis samples analyzed.

Quantification of brachyury expression has been performed in multiple carcinoma tissues by real-time PCR analysis of brachyury mRNA levels. These studies have been performed, for example, with lung[17,18] and breast carcinomas.[19] In the same studies and in Hamilton et al,[20] PCR analysis of brachyury mRNA expression in multiple normal tissues has also been performed. Unlike with carcinomas, the expression of brachyury mRNA was undetectable or very low in the majority of normal tissues evaluated. The only normal tissue that showed brachyury mRNA levels comparable to those found in some carcinoma tissues was the normal testis. Analysis of brachyury protein expression was also conducted by immunohistochemistry.[18,19] Expression of brachyury protein was demonstrated in lung carcinomas and invasive ductal breast carcinomas, both primary and metastatic lesions. Unlike with carcinomas, expression of brachyury protein was negative among the normal tissues analyzed, with the exception of testis and some cases of normal thyroid.

The transcription factor brachyury is a driver of EMT. This phenomenon, which is responsible for the acquisition of tumor invasiveness and tumor resistance to multiple therapeutics, is a manifestation of tumor plasticity and therefore is expected to take place in a transient fashion along tumor progression. A few tumor cells in a primary tumor mass might express brachyury at a certain time under the influence of environmental factors such as TGF-β, IL-8, and others, and then disseminate from the primary tumor into circulation and secondary metastatic sites. The

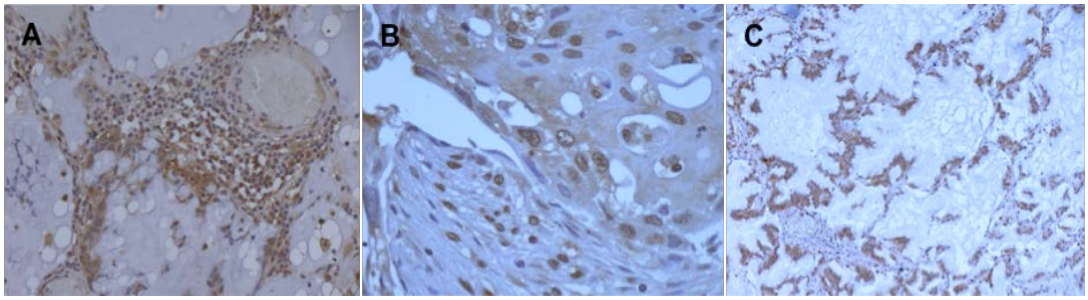
analysis of a primary tumor biopsy or surgical sample might, therefore, be negative for brachyury expression due to the transient nature of the phenomenon, while disseminated tumor cells might still be positive for expression of the target brachyury. Thus, when analyzing a primary tumor by IHC or PCR, it is difficult to determine whether results reflect the state of individual tumor cells at one point in time, or whether a subpopulation of cells had previously undergone the EMT process via brachyury expression and had already metastasized. Additionally, our IHC studies with matched primary/metastatic tumors demonstrated that primary tumors with focal brachyury expression corresponded to metastatic sites highly positive for the expression of brachyury. These observations constitute the rationale for not including the level of expression of brachyury in primary tissue samples as a criterion for the inclusion of patients in the study.

1.2.4 Brachyury Expression in Lung Carcinomas

Expression of brachyury was found in approximately 40% of primary lung tumors (Table 1, Figure 5A-D). Real-time PCR analysis of brachyury mRNA expression in multiple human lung tumor tissues showed that the percentage of tumors positive for brachyury expression increases with tumor stage: 30/48 (62.5%) of stage II–IV lung cancer biopsies showed overexpression of brachyury vs. 12/32 (37.5%) of stage I lung cancers and 2/16 (12.5%) of histologically normal lung biopsies from lung cancer patients (Figure 6).[17]

Table 1. Brachyury protein expression analyzed by IHC

Lung tumor tissues	Brachyury-positive
Adenocarcinoma	**10/21 (48%)**
Squamous carcinoma	**3/12 (25%)**
Undifferentiated carcinoma	**2/4 (50%)**
Bronchioalveolar carcinoma	**1/1 (100%)**
Small cell lung cancer	**0/1 (0%)**
Total	**16/39 (41%)**



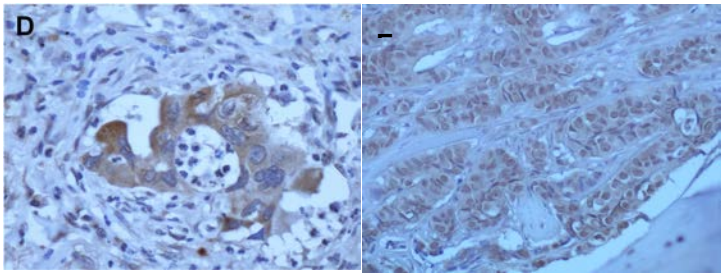


Figure 5. Tissue sections stained for brachyury expression, corresponding to a lung adenocarcinoma **(A)**; a squamous carcinoma **(B)**; and a bronchioalveolar carcinoma (BAC), mucinous type **(C)**. Lung tumor cells invading a blood vessel, positive for brachyury expression **(D)**. IHC staining for brachyury in a bone metastasis of breast cancer **(E)**.

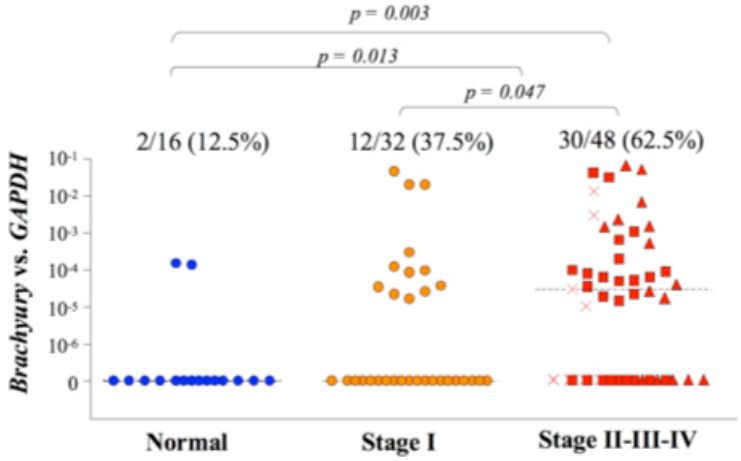


Figure 6A. Brachyury expression in human lung tumor tissues. Real-time PCR was performed for brachyury on lung tumor tissue cDNA from 80 lung cancer patients of the indicated stages of disease. The stage II, III, and IV cDNA samples are further represented by the symbols ■, ▲, and ✕, respectively. As controls, 16 samples of "normal" lung cDNA were analyzed, each obtained from a histologically normal section of lung from a lung cancer patient. See (17) for details.

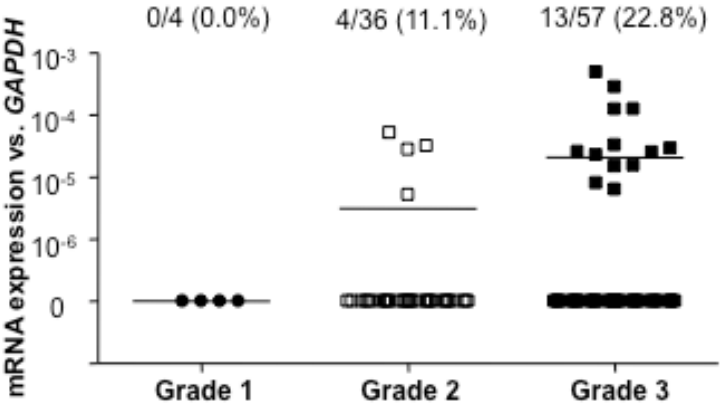


Figure 6B. Expression of brachyury mRNA in breast tumor tissues. Brachyury mRNA expression, as determined by real-time PCR, is shown for breast primary tumor tissues grouped by histological tumor grade (Nottingham grading).

1.2.5 Expression of Brachyury Protein in Breast Tumor Samples

The expression of brachyury was further analyzed in breast tissues by IHC with a murine anti-brachyury monoclonal Ab. Twenty-seven of 30 primary breast tumors (90%) were positive for brachyury expression. Sixteen benign breast tissues were negative for brachyury, with the exception of 2 cases of fibroadenoma where focal expression of brachyury protein was detected. Expression of brachyury was further evaluated in matched primary carcinoma and metastatic lymph nodes from 2 patients. As shown in Table 2, brachyury was expressed at higher levels in tumor-positive lymph nodes compared to the primary tumor, while the tumor-negative lymph node was negative for brachyury expression in both cases. Additional metastatic lesions (pleura, bone, and brain) also exhibited high levels of brachyury expression (Table 2, Figure 5E).

Table 2. Expression of brachyury protein in primary and metastatic breast carcinoma lesions by immunohistochemistry utilizing a murine monoclonal anti-brachyury Ab

Pt	Tissue	Brachyury	
		% Positivity	Intensity
6	Breast primary tumor	30	+
6	Met^+ lymph node (a)	90	+
6	Met^+ lymph node (b)	90	+
6	Non-met lymph node (c)	Neg	Neg
9	Breast primary tumor	Focal	+
9	Met^+ lymph node (a)	60	++
9	Met^+ lymph node (b)	60	++
9	Non-met lymph node (c)	Neg	Neg
31	Pleura	90	+
32	Bone	90	++
33	Bone	90	+
34	Brain	70	++

Matched breast primary tumor and metastatic lymph nodes from 2 patients and metastatic lesions from additional patients were analyzed for brachyury expression by IHC with a murine monoclonal anti-brachyury antibody. Two lymph nodes positive for metastasis from each patient (a, b) and one lymph node negative for metastasis from the same patient (c) were assayed.

1.2.6 Brachyury Expression and Prognosis

Breast tumor gene expression data (n = 4010) derived from 23 datasets from the Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI) was compiled. Each sample was assigned into low (first quartile, lowest 25%), intermediate (second quartile, intermediate 50%) and high (third quartile, highest 25%) subgroups according to brachyury mRNA levels. A Kaplan-Meier estimate of survival was used to evaluate differences in prognosis among the 3 subgroups. Among 357 breast cancer patients treated with tamoxifen monotherapy as adjuvant therapy for 5 years post-diagnosis, we found that high brachyury expression significantly correlated with higher risk of recurrence ($p = 0.0283$, $n = 357$, Figure 7A) and distant metastasis ($p = 0.0150$, $n = 332$, Figure 7B). To minimize the effect of clinical confounding factors such as tumor size, grade, nodal status, age, HER2, ER and PR status that might cause false positive association between gene expression and poor prognosis, a Cox Proportional-Hazards Regression (COXPH) survival analysis was conducted ($n = 270$). When mRNA expression signal was used as a continuous variable, increased expression of brachyury was significantly associated with higher risk of recurrence ($p = 2.77 \times 10^{-5}$, $n = 270$) and distant metastasis ($p = 0.0001$, $n = 270$).

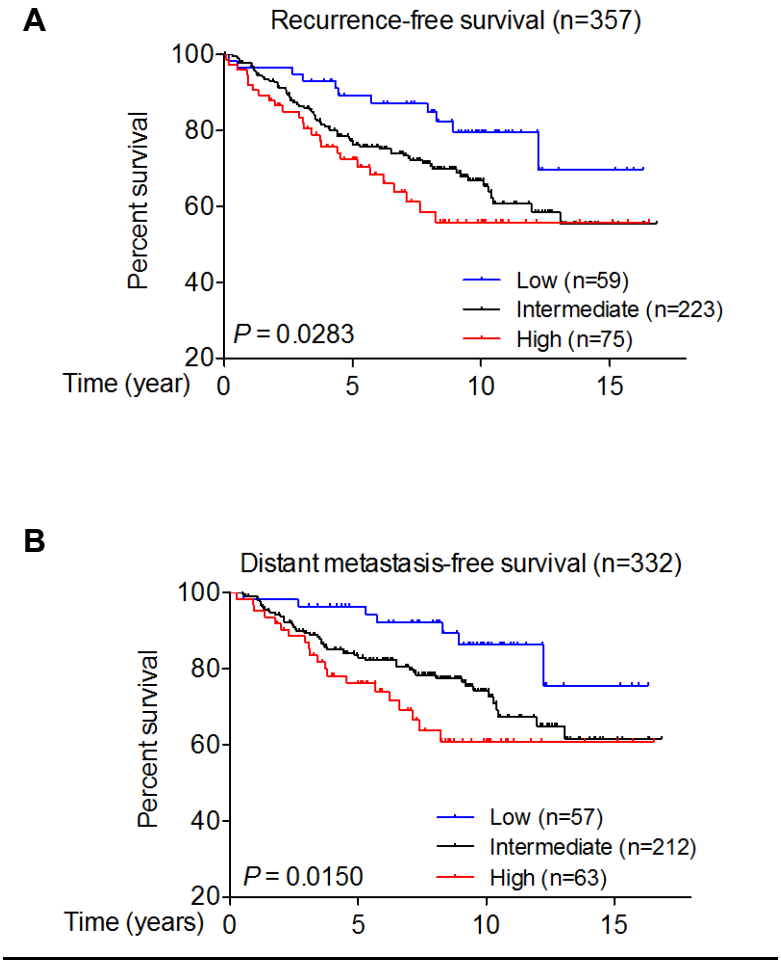


Figure 7. Brachyury expression and prognosis.

1.2.7 Brachyury Confers Chemotherapy Resistance

The LTIB has developed human lung carcinoma cell lines that have been either stably knocked down for expression of brachyury (sh/brachyury) or transfected to stably overexpress brachyury (ph/brachyury). Brachyury downregulation significantly enhances sensitivity to chemotherapy, while stable overexpression of brachyury in human lung cancer cells is associated with a significant decrease in cell death mediated by various chemotherapy agents *in vitro* (Figure 8).

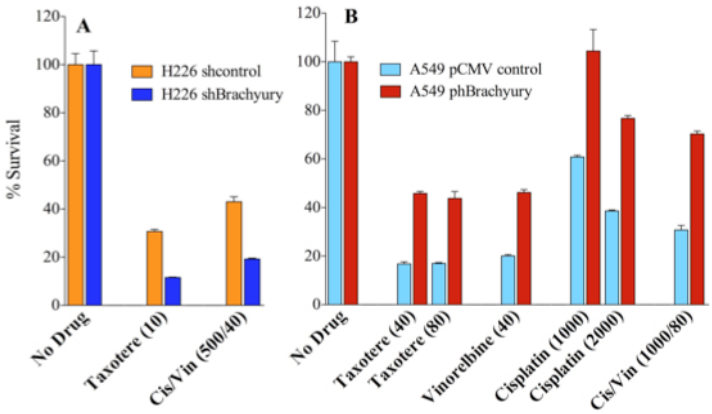


Figure 8. Expression of brachyury in human carcinoma cells induces resistance to chemotherapy. (A) Silencing of brachyury (sh/brachyury) in H226 human lung carcinoma cells resulted in enhanced sensitivity to chemotherapy. **(B)** A549 lung cancer cells stably transfected with a brachyury vector (ph/brachyury) had enhanced resistance to several chemotherapeutic agents.

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1.2.8 Immunogenicity of the Brachyury Protein

By using an MHC-peptide binding prediction algorithm, a 9-mer HLA-A2 binding epitope of the brachyury protein has been identified,[3] which was successfully used *in vitro* to expand human brachyury-specific CD8+ T-cell lines from peripheral blood of both normal donors and cancer patients. Functional assays demonstrated that these brachyury-specific T-cell lines are able to efficiently lyse, in an MHC-restricted manner, human carcinoma cell lines (breast, lung, colon) endogenously expressing the brachyury protein.

Utilizing the identified HLA-A2-binding epitope of brachyury, CD8+ brachyury-specific T-cell responses were detected in the blood of cancer patients following vaccinations with either CEA- or prostate-specific antigen (PSA)-based vaccines, likely due to the phenomenon of antigen cascade.[21] All together, these results demonstrated that brachyury is an immunogenic protein in humans.

1.2.9 Prior Human Experience with Brachyury Vaccine Therapy

A phase I study using escalating doses of yeast-brachyury vaccine (GI-6301) is ongoing at the NCI in 23 subjects with varied malignancies, including chordoma and colorectal cancer. The vaccine has been very well tolerated with no observed DLTs.

1.2.10 Poxviral Vectors

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. The virus actively replicates in human cells, resulting in the presentation of high levels of antigen to the immune system over a period of 1–2 weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the virus. As a result of its safety profile and ability to elicit both humoral and cell-mediated immunity in humans, the vaccinia virus was chosen as one of the vectors to deliver MUC-1, CEA, and TRICOM in previous NCI-sponsored trials.[22,23]

Immunization with live recombinant poxviral vectors that have been genetically engineered to express one or more antigens allows expression of tumor-associated antigens (TAAs) and subsequent co-presentation of antigenic peptides with host histocompatibility antigens, a strategy that favors the induction of cell-mediated immune responses. Recombinant poxviruses can infect antigen-presenting cells, including dendritic cells and macrophages, resulting in efficient expression of TAAs simultaneously with costimulatory molecules required to elicit T-cell responses. TAAs expressed by recombinant poxviruses are presented to the immune system together with highly immunogenic viral proteins, which may act as adjuvants to enhance immune responses to the TAAs. Thus, the use of recombinant poxviral vectors to present TAAs to the immune system results in the generation of killer T cells that specifically destroy the selected tumor with little incremental toxicity.

1.2.11 Safety of Poxviral Vectors

A recent report on safety data from NCI clinical trials with poxviral vaccines evaluated all vaccine injections from 215 patients in 8 clinical trials involving poxviral vector vaccines. Vaccines consisted of recombinant vaccinia and recombinant fowlpox encoded with 3 human costimulatory molecules (TRICOM), and prostate specific antigen (PSA), or carcinoembryonic antigen, and/or mucin-1. Vaccines were administered at doses between 1.2×10^8 to 2×10^9 pfu, subcutaneously, in all patients. 84 patients also received other concurrent treatment modalities, such as radiation, celecoxib, ipilimumab, samarium-153, or flutamide on 4 of these trials. All 8 clinical trials involved granulocyte-macrophage colony-stimulating factor (GM-CSF) 100 mcg, or recombinant fowlpox encoding GM-CSF at 1×10^8 pfu as an immune adjuvant. Grade 2 or higher adverse events at least possibly attributed to vaccine were reported (Table 3). A total of 1,348 poxviral injections were given in 215 patients. No contact transmission, inadvertent inoculation, or any serious adverse events (AEs) related to vaccinia were observed. Below is the summary of proportion of vaccine administrations associated with specific AEs. These data demonstrate a favorable safety profile of the poxviral vaccines at a broad range of doses, routes of administration, in combination with other treatments, and in various tumor types. Clinical trial information: **NCT00060528, NCT00096551, NCT00088413, NCT00081848, NCT00113984, NCT00450619, NCT00450463**.

Table 3. Safety of poxviral vaccines: NCI experience[24]

AEs	Grade 2	Grade 3	Grade 4	% of injections associate with an AE
Injection-site reaction	454	0	0	25.3
Injection-site cellulitis	0	2	0	0.1
Fever	22	2	0	1.3
General symptoms (fatigue, myalgia, rash, etc.)	53	7	0	3.3
Respiratory AEs (dyspnea, etc.)	4	2	0	0.3
Gastrointestinal AEs (nausea, etc.)	13	3	0	0.9
Neurologic AEs (headache, etc.)	9	2	0	0.6
Hypotension	3	0	0	0.2
Myocardial infarction	0	0	1*	0.1
Atrial fibrillation	1	0	0	0.1

AEs	Grade 2	Grade 3	Grade 4	% of injections associate with an AE
Cytopenia	6	1	0	0.4
TTP	0	0	1*	0.1
Lab abnormalities	7	0	0	0.4
Total	**572**	**19**	**2**	**33.1**

* One patient experienced multiple AEs associated with myocardial infarction and thrombotic thrombocytopenic purpura (TTP).

1.2.12 Modified vaccinia Ankara (MVA)

Despite the desirable features of poxviruses, replication-competent viruses like vaccinia should not be administered to severely immunocompromised patients. To address this problem, an attenuated vaccinia virus called modified vaccinia Ankara (MVA) was developed for high-risk individuals. MVA was generated by over 500 serial passages of a smallpox vaccine from Ankara, Turkey, in chicken embryo fibroblasts, resulting in over 15% loss of the vaccinia virus genome.[25] MVA can infect mammalian cells and express transgenes, but it cannot produce infective viral particles.

Bavarian Nordic has further generated a proprietary isolate of MVA designated MVA-BN. MVA-BN exhibits a severely restricted host range and fails to replicate in most mammalian cells, including primary human cells and most transformed cell lines.

1.2.13 Clinical Experience with MVA Vector-Based Trials

Several clinical trials in metastatic renal cell carcinoma have been conducted with the TroVax vaccine, which consists of a recombinant MVA expressing the 5T4 TAA. Initial trials demonstrated some objective clinical responses, stable disease, and both antibody and T-cell responses to 5T4. A phase III randomized, placebo-controlled study employed MVA 5T4 with and without cytokines and sunitinib in patients (n = 733) with metastatic renal cell cancer (Table 5). Treatment arms were well-balanced. There was, however, no significant difference in overall survival between the 2 treatment arms. The magnitude of the 5T4-specific antibody response post-vaccination was associated with increased patient survival, as seen in previous trials. A second vaccine has also been analyzed in patients with metastatic renal cell carcinoma. The TG4010 vaccine consists of MVA expressing recombinant MUC-1 and IL-2 transgenes. The vaccine was used either alone or in combination with exogenous IFN-α and IL-2. No objective responses were noted. Eighteen percent of patients had stable disease for >6 months with vaccine alone, and 30% had stable disease for >6 months with vaccine and cytokine. Median overall survival was 19.3 months for all patients and 22.4 months for patients receiving vaccine and cytokine.

The TroVax vaccine has also been evaluated in 4 small single-arm trials in patients with metastatic colorectal cancer. Antibody and T-cell responses, as well as stable disease, were noted along with some complete responses and partial responses in the various trials. In a trial involving the combination of TroVax with FOLFIRI chemotherapy, 11/17 patients were considered evaluable for immunologic evaluation. Of these, 6 had complete or partial responses as well as T-cell or antibody responses to vaccine. These immune responses correlated with clinical benefit.

The TG4010 vaccine was also evaluated in prostate cancer patients (n = 40) with biochemical failure. Thirteen of 40 patients had a more than 2-fold improvement in PSA doubling time, and 10 patients had their PSA stabilized over 18 months. This vaccine has also been evaluated in patients (n = 65) with stage 3/4 non-small cell lung cancer. In this randomized phase II study, patients in arm 1 received vaccine in combination with chemotherapy; in arm 2 patients received vaccine monotherapy until disease progression, followed by vaccine plus the same chemotherapy. The median overall survival in arm 1 was 12.7 months versus 14.9 months for arm 2. One-year overall survival rate was 53% for arm 1 and 60% for arm 2.

1.2.14 Safety of Recombinant MVA Vectors

Safety data from the first clinical trials of recombinant MVA recorded no serious or severe adverse events.[26] MVA-METRAP (fusion between the string of multiple defined T-cell epitopes originally developed for the VLPs (known as ME), and a complete *P. falciparum* liver stage antigen, thrombospondin relative adhesive protein (TRAP)) was administered to 26 healthy volunteers aged 18–55 by intradermal injection at a dose of 3×10^7 pfu, in some cases following priming with a DNA vaccine also expressing METRAP administered either intramuscularly or by gene gun. Most adverse events were mild: flu-like illness, nausea, lethargy, or lymphadenopathy. Ten moderate adverse events were reported. In general, the vaccinations were well tolerated. Swabs were taken from the surface of the skin following injection and from a fluid-filled blister at one injection site and assessed for the presence of MVA-METRAP by PCR, which would have detected both live and dead virus, but none was found.

Several clinical trials have since been conducted in patients with varied malignancies, including renal, colorectal, NSCLC, prostate, and breast cancer.[27,28,29,30,31] MVA vaccines have been well tolerated in trials giving multiple doses, with a majority of adverse events reported as mild, transient, and following an expected pattern; most resolved within 48 hours of vaccination.

MVA-BN has been used to generate 2 active cancer immunotherapies: MVA-BN-HER2 and MVA-BN-PRO. MVA-BN-HER2 expresses a modified version of the HER2 protein and has been used to treat women with HER2-positive breast cancer. MVA-BN-PRO expresses both PSA and prostatic acid phosphatase and has been used to treat men with non-metastatic prostate cancer.

MVA-BN-HER2 was evaluated in 3 phase I studies, BNIT-BR-001, BNIT-BR-002, and BNIT-BR-003. In total, 45 patients were treated with single or multiple doses of vaccine and the drug was well-tolerated. No patients discontinued treatment due to a drug-related adverse event. In the BNIT-BR-003 study, one event of cellulitis in one patient was the only SAE reported and was considered possibly related. In the BNIT-BR-003 study, immunotherapy-induced HER-2 transgene-specific and MVA vector-specific T-cell and humoral responses were detected during and after treatment, indicating that MVA-BN-HER2 is immunologically active in the adjuvant setting of breast cancer.

MVA-BN-PRO was evaluated in prostate cancer patients enrolled in a single phase I clinical trial, BNIT-PR-001. The primary objective of this study was to evaluate the safety and tolerability of single and multiple injection regimens of MVA-BN-PRO for the treatment of androgen-insensitive prostate cancer. There were no dose-limiting toxicities identified and no drug-related serious adverse events noted in 24 patients treated.

1.2.15 TRICOM Costimulatory Molecules

Destruction of immunological targets requires T-cell lymphocyte recognition, via the T-cell receptor, of antigenic peptides presented in the context of MHC molecules on APCs. In addition to this antigen-specific signal, a second, antigen-independent signal is required for T-cell activation.[32,33] This second signal is provided by the interaction of specific ligands on the T-cell surface with costimulatory molecules expressed on APCs. The most extensively studied pathway of costimulation is that involving the interaction of the costimulatory molecule B7.1 expressed on APCs with CD28 and CTLA4 on the T cell.[34,35] A number of additional costimulatory molecules on APCs have been identified, including ICAM-1 and LFA-3, whose ligands are LFA-1 and CD2, respectively, on the surface of T cells.[36,37, 38]

Proper engagement of the T-cell receptor and costimulatory receptors requires the expression of both antigen and costimulatory molecules in the same cell. Therefore, coexpression of costimulatory molecules using a single recombinant vector presents the potential of cooperation among these proteins to enhance T-cell activation. Recombinant vectors coexpressing 3 costimulatory molecules, LFA-3, ICAM-1, and B7.1, designated TRICOM™, have been shown to have synergistic effects on antitumor responses compared to vectors expressing individual costimulatory molecules.[33] Mice immunized with a recombinant vaccinia virus coexpressing carcinoembryonic antigen (CEA) and murine TRICOM exhibited greater immune responses and antitumor responses than mice immunized with a recombinant vaccinia virus coexpressing CEA and murine B7.1. Enhanced antitumor immunity was also observed in mice that were transgenic (tolerant) for CEA.[39] MVA-brachyury-TRICOM, therefore, has been designed to simultaneously express brachyury together with B7.1, LFA-3, and ICAM-1.

1.2.16 MVA-TRICOM in CLL

We previously demonstrated that infection of CLL cells with MVA expressing the costimulatory molecules B7.1, ICAM-1, and LFA-3 (TRICOM) increased expression of these costimulatory

molecules on the surface of CLL cells and thus augmented their antigen-presenting capability. We further evaluated an alternative MVA vector platform encoding for human CD40L in comparison with MVA-TRICOM for their ability to enhance the immunogenicity of CLL cells, *in vitro*. Our results indicated that MVA-TRICOM-infected and MVA-CD40L-infected CLL cells are equally potent at inducing autologous T-cell proliferation.[40]

1.2.17 Infection of Human Dendritic Cells with MVA-Brachyury-TRICOM

In order to investigate the ability of MVA-brachyury-TRICOM to infect cells from the blood of normal donors, dendritic cells (DCs) were prepared from peripheral blood mononuclear cells (PBMCs) by culture for 6 days in the presence of GM-CSF and IL-4, and subsequently incubated with wild-type MVA (MVA-WT), MVA-TRICOM (expressing only the 3 costimulatory molecules), or MVA-brachyury-TRICOM.

Using MVA-WT alone, no increased expression of CD80, CD54, or CD58 was observed. MVA-TRICOM resulted in upregulation of each of the 3 costimulatory molecules. Addition of the target antigen, brachyury, did not interfere with upregulation of the costimulatory molecules, indicating that all 4 transgenes could be inserted into the vector and result in effective infection and gene expression (Table 4).

Table 4. *In vitro* infection of human DCs with MVA-brachyury-TRICOM

Infection with	CD80	CD54	CD58
MVA-WT			
5 MOI	15.6 (23)	86.6 (254)	89.0 (123)
10 MOI	17.3 (21)	81.2 (188)	82.9 (91)
MVA-TRICOM (Therion)			
5 MOI	71.2 (235)	96.2 (889)	96.8 (354)
10 MOI	**84.3 (523)**	**95.7 (1596)**	**97.0 (670)**
MVA-Brachyury-TRICOM			
5 MOI	**83.3 (490)**	**94.9 (847)**	**95.4 (394)**
10 MOI	**81.4 (587)**	**91.8 (792)**	**93.9 (381)**

Brachury expression was present when DCs were infected with MVA-brachyury-TRICOM, but was undetectable in uninfected or MVA-WT-infected DCs (Figure 9). Similarly, expression of brachyury in DCs infected with MVA-brachyury-TRICOM (MOI = 10) was evaluted by immunofluorescence using a monoclonal anti-brachyury Ab (Abcam) (Figure 10).

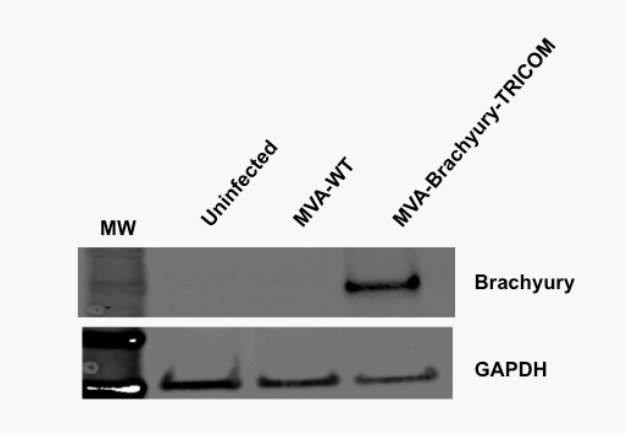


Figure 9. Detection of brachyury expression by Western blot analysis. Expression of brachyury was evaluated by Western blot analysis with a monoclonal rabbit anti-brachyury Ab (clone 54-1). GADPH expression is also shown. (MW = molecular weight marker).

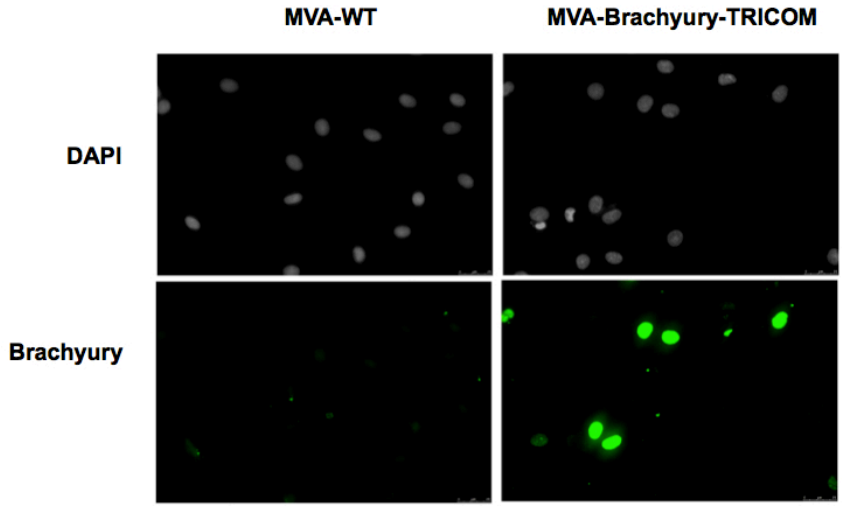


Figure 10. Detection of brachyury by immunofluorescence. Expression of brachyury was evaluated by immunofluorescence with a monoclonal anti-brachyury Ab (Abcam). (Green indicates brachyury expression, gray indicates DAPI-stained nuclei.)

These results indicate that the MVA-brachyury-TRICOM vector is able to infect human DCs, resulting in upregulation of brachyury as well as 3 costimulatory molecules (TRICOM).

1.2.18 Induction of Human T Cells by MVA-Brachyury-TRICOM

In order to investigate the ability of MVA-brachyury-TRICOM to expand brachyury-specific T cells from the blood of normal donors, DCs were prepared from PBMCs and infected with MVA-WT, MVA-TRICOM, or MVA-brachyury-TRICOM vectors. Brachyury-specific T cells were stimulated with irradiated DCs. Supernatants were collected and evaluated for IFN-gamma production by ELISA assay. Shown is the IFN-gamma production in response to MVA-brachyury-TRICOM vs. MVA-WT-infected DCs (Figure 11).

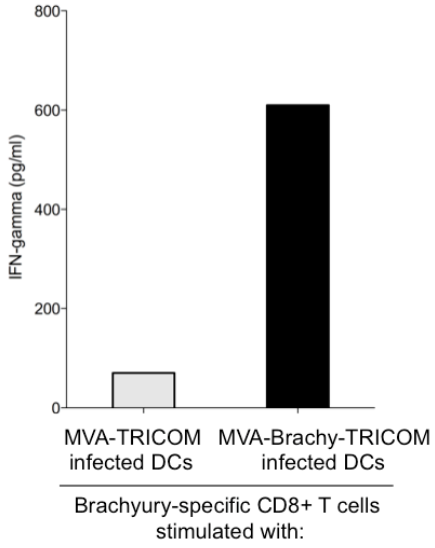


Figure 11. Brachyury-specific CD8+ T cell stimulation by MVA-brachyury-TRICOM-infected DCs compared with MVA-TRICOM-infected DCs.

1.2.19 MVA-Brachyury-TRICOM-Infected Human DCs Stimulate Brachyury-Specific CD4+ T cells

CD4+ T cells from normal-donor PBMCs were exposed to either MVA-WT or MVA-brachyury-TRICOM-infected DCs and then exposed to a control (HSA) or brachyury protein. The CD4+ T cells exposed to MVA-WT did not proliferate in response to HSA or brachyury, but the CD4+ T cells exposed to MVA-brachyury-TRICOM DCs proliferated significantly better when exposed to brachyury protein compared with HSA (Figure 12). These results indicate MVA-brachyury-TRICOM is able to effectively expand CD4+ brachyury-specific T cells from the blood of normal donors, as compared to the MVA-WT vector.

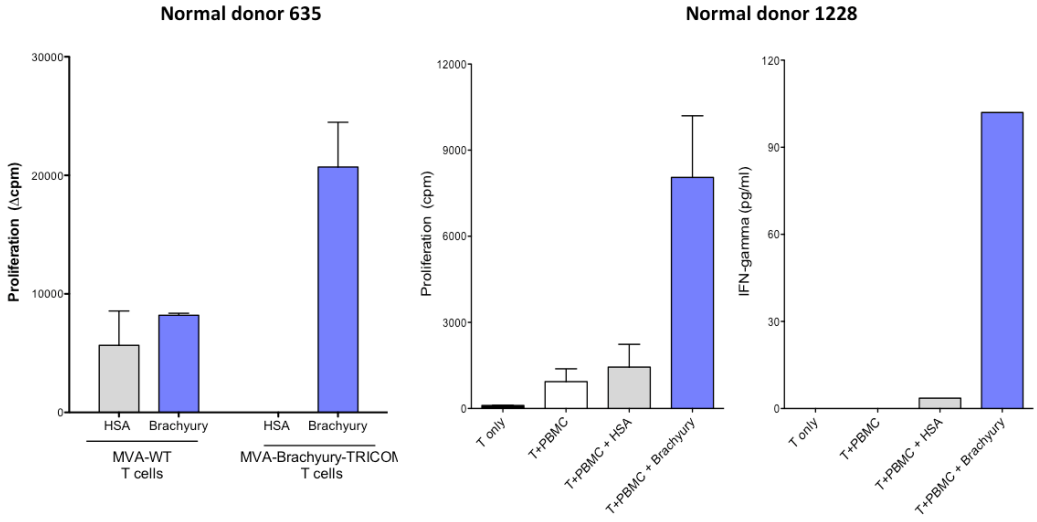


Figure 12. MVA-brachury-TRICOM (and not MVA-WT)-infected human DCs expand CD4+ T cells from PBMCs of normal donors that recognize purified brachyury protein.

1.2.20 MVA-Bavarian Nordic (MVA-BN)

MVA-BN effectively infects mammalian cells. Infection of mammalian cells results in transcription of the viral genes, but no MVA-BN is released from the cells due to a genetic block in the viral assembly and egress. The infected cells eventually undergo apoptosis (programmed cell death). MVA-BN replicates efficiently in CEF cells and probably also in certain other avian cell lines. Despite its high attenuation and reduced virulence, in preclinical studies MVA-BN has been shown to elicit both humoral and cellular immune responses to vaccinia and genes cloned into the MVA genome. MVA-BN is a potent inducer of type I interferon (IFN) in human cells. Like other MVA, MVA-BN expresses a soluble interleukin-1 receptor,[41] which has been implicated as an antivirulence factor for certain poxviruses.[42] MVA does not express soluble receptors for IFN-γ, IFN-α/β, tumor necrosis factor, or CC chemokines.[43]

MVA-BN is a further attenuated MVA strain that has lost its ability to replicate in most mammalian cell types, including almost all human cell lines, and is safe in severely immunocompromised animals (AGR129 mice). The hallmark of MVA-BN is the fact that it does not productively replicate in the human keratinocyte cell line HaCat, the human cervix adenocarcinoma cell line HeLa, the human embryo kidney cell line 293 (HEK293), and the human bone osteosarcoma cell line 143B. However, like other MVA strains, MVA-BN effectively infects mammalian cells.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria (All Subjects)

2.1.1.1 Patients must have a metastatic or unresectable locally advanced malignant solid tumor, histologically confirmed by the Laboratory of Pathology, NCI. In the case of chordoma, unresectable, locally recurrent, or metastatic tumors are acceptable for enrollment, given that this represents incurable disease. Efforts will be made, as much as possible, to enroll patients with tumor types with known increased expression of brachyury (such as lung, breast, ovarian, prostate, colorectal, pancreatic, or chordoma; other tumors may be included as data on the level of brachyury in those tumors becomes available).

2.1.1.2 Patients may have measurable or nonmeasurable but evaluable disease. See Section **6.2** for the evaluation of measurable disease. Patients with surgically resected metastatic disease at high risk of relapse are also eligible.

2.1.1.3 Prior therapy: Patients must have completed or had disease progression on at least one prior line of disease-appropriate therapy for metastatic disease, or not be candidates for therapy of proven efficacy for their disease.

2.1.1.4 There should be a minimum of 4 weeks from any prior chemotherapy, immunotherapy and/or radiation, with the exception of hormonal therapy for prostate and breast

cancers, HER2-directed therapy for HER2+ breast cancer (3+ IHC or FISH+), and erlotinib in EGFR-mutated lung cancer in the expansion cohort as detailed in section. There should be a minimum of 6 weeks from any prior antibody therapies, (such as ipilimumab or anti-PD1/PDL1) due to prolonged half-life.

2.1.1.5 Patients must have recovered (grade 1 or baseline) from any clinically significant toxicity associated with prior therapy. Typically, this is 3–4 weeks for patients who most recently received cytotoxic therapy, except for the nitrosoureas and mitomycin C, for which 6 weeks is needed for recovery.

2.1.1.6 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of MVA-brachyury-TRICOM vaccine in patients < 18 years of age, children are excluded from this study but will be eligible for future pediatric trials.

2.1.1.7 ECOG performance status ≤ 1 (Karnofsky ≥ 70%, see Appendix A, Section **13.1**).

2.1.1.8 Patients must have normal organ and marrow function as defined below:

- Serum creatinine ≤ 1.5 x upper limit of normal OR creatinine clearance on a 24-h urine collection of ≥ 50 mL/min.
- ALT and AST ≤ 3x the upper limits of normal.
- Total bilirubin ≤ 1.5 x upper limit of normal OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0.
- Hematological eligibility parameters (within 16 days of starting therapy):
    - Granulocyte count ≥ 1,500/mm^3
    - Platelet count ≥ 100,000/mm^3

2.1.1.9 Patients must have baseline pulse oximetry > 90% on room air.

2.1.1.10 The effects of MVA-brachyury-TRICOM on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and for a period of 4 months after the last vaccination therapy. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.11 Patients with prostate cancer must continue to receive GnRH agonist therapy (unless orchiectomy has been done). If a patient has refused GnRH therapy, they may be enrolled on a dose level for which the safety has already been determined.

Patients must be able to understand and be willing to sign a written informed consent document.

2.1.2 Inclusion Criteria (Expansion Phase Only)

The following inclusion criteria apply specifically to patients being considered for the expansion phase of the protocol, as described in Section **8**.

2.1.2.1 Subjects with EGFR-mutated lung cancer may continue erlotinib if they have been on the drug for ≥ 3 months with stable disease or a response. Erlotinib may also be continued in the case of a progressing tumor after prior response (or > 6 months stable disease).

2.1.2.2 Patients with ER+ breast cancer being treated with hormonal therapy (selective estrogen receptor modulator or aromatase inhibitor) who have rising tumor markers as evidence of disease progression or metastatic disease on scans may continue on hormonal therapy while being treated with vaccine.

2.1.2.3 Patients with Her2+ breast cancer receiving Her2-directed therapy (e.g. trastuzumab) may continue on that therapy when enrolling into a dose level for which safety has been established.

2.1.2.4 Subjects with mestastatic colorectal cancer may continue "maintenance" therapy with capecitabine and/or bevacizumab.

2.1.3 Exclusion Criteria

2.1.3.1 Concurrent treatment for cancer, with specific exceptions noted in inclusion criteria.

2.1.3.2 Chronic hepatitis B or C infection, because potential immune impairment caused by these disorders may diminish the effectiveness of this immunologic therapy.

2.1.3.3 Any significant disease that, in the opinion of the investigator, may impair the patient's tolerance of study treatment.

2.1.3.4 Significant dementia, altered mental status, or any psychiatric condition that would prohibit the understanding or rendering of informed consent.

2.1.3.5 Active autoimmune diseases requiring treatment or a history of autoimmune disease that might be stimulated by vaccine treatment. This requirement is due to the potential risks of exacerbating autoimmunity. However, patients with vitiligo or clinically stable autoimmune endocrine disease who are on appropriate replacement therapy (if such therapy is indicated) are eligible.

2.1.3.6 Concurrent use of systemic steroids, except for physiologic doses of systemic steroid replacement or local (topical, nasal, or inhaled) steroid use. Limited pharmacologic doses of systemic steroids (e.g., in patients with exacerbations of reactive airway disease or to prevent I.V. contrast allergic reaction or anaphylaxis in patients who have known contrast allergies) are allowed.

2.1.3.7 Patients who are receiving any other investigational agents within 28 days before start of study treatment.

2.1.3.8 Patients with untreated central nervous system metastases or local treatment of brain metastases within the last 6 months. Patients with stable brain metastasis for 6 months post-intervention are eligible. Subjects with chordoma will be eligible regardless of site of disease if other eligibility criteria are met.

2.1.3.9 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MVA-brachyury-TRICOM or other agents used in study.

2.1.3.10 Serious or uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that, in the opinion of the investigator, would limit compliance with study requirements.

2.1.3.11 Pregnant women are excluded from this study due to the unknown effects of the MVA-brachyury-TRICOM vaccine on the fetus or infant. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MVA-brachyury-TRICOM, breastfeeding should be discontinued if the mother is treated with MVA-brachyury-TRICOM. These potential risks may also apply to other agents used in this study.

2.1.3.12 HIV-positive patients are ineligible because of the potential for decreased immune response to the vaccine.

2.1.4 Recruitment Strategies

This study will be listed on available websites (www.clinicaltrials.gov, www.cancer.gov/clinicaltrials, http://clinicalstudies.info.nih.gov) and participants will be recruited from the current patient population at NIH.

2.2 SCREENING EVALUATION

Screening evaluation will be conducted within 16 days before starting treatment unless otherwise specified.

2.2.1 Clinical Evaluation

- History and physical examination including vital signs (blood pressure, pulse, respiratory rate, oxygen saturation)
- Height and weight
- Performance status determination (see Appendix A, Section **13.1**)

2.2.2 Laboratory studies

To be performed at any time before protocol enrollment:

- Confirmation of diagnosis by Laboratory of Pathology at the NIH Clinical Center.
- Request of a minimum of three unstained slides for expression of brachyury. Samples will be preferably from metastatic sites of disease as well as primary, if possible. This is optional and not required for enrollment.

To be performed within 8 weeks prior to protocol enrollment:

- Screening for HIV
- Screening for hepatitis B and C.

To be performed within 16 days prior to protocol enrollment:

- Complete blood count plus differential and platelet count
- Serum chemistries (Na+, K+, Cl–, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, TSH)
- Urinalysis

- Beta-HCG for women of child-bearing age (repeated within 48 hours prior to treatment). A female of child-bearing potential (FCBP) is a sexually mature woman who:
    - Has not undergone a hysterectomy or bilateral oophorectomy
    - Has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

2.3 BASELINE EVALUATION

2.3.1 Laboratory studies

To be performed at any time before protocol enrollment:

- HLA typing

To be performed within 16 days prior to protocol enrollment:

- Screening tests noted above will be used as baseline

Additional baseline labs to be performed

- ANA titer
- CD4:CD8 ratio, CD3, 4, 8, 19 subsets and NK markers (baseline, about day 29 prior to vaccination and around day 85, and then every 85 days thereafter while patients remain on study for follow-up)

2.3.2 Electrocardiogram (ECG) within 28 days prior to protocol enrollment

2.3.3 Scans and X-rays:

To be performed within 28 days prior to the protocol enrollment (may include based on site of disease):

- Computerized tomography (CT) of the chest/abdomen/pelvis
- Brain MRI
- PET scan
- Bone scan

2.4 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 (HOIS) 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.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open-label phase I trial with sequential cohorts of patients (3–6 patients per dose cohort to establish safety) with dose escalation of MVA-brachyury-TRICOM vaccine (see Section **8**). Three cohorts will receive MVA-brachyury-TRICOM vaccine administered subcutaneously as either 1, 2, or 4 injections of study drug (1 injection = 2 x 10^8 IU) at monthly (28 days– 4 days up to +14 days) intervals for 3 months (treatment).[44] A dose will be considered missed if not given within 14 days of the original schedule and the patient will resume treatment with the next scheduled dose. The time of patient monitoring post injection would be 2 hour observation after first dose and 30 minutes after subsequent doses.

Toxicity in the 28 days following the first vaccination after the 3rd subject (or 6th subject, if applicable) in dose levels 1 through 3 will be employed for decision-making (i.e., for dose-escalation and for maximum tolerable dose estimation). Intrapatient dose escalation will not be allowed. In addition, patients may be enrolled as an expansion cohort at the MTD and at the dose level just below the MTD after either 0 of 3 or no more than 1 of 6 patients have been treated at that dose level and have not experienced a DLT for at least 28 days. Cohorts 2 and 3 will accrue up to an additional 10 evaluable patients each (up to 16 patients total in each) for safety and correlative analysis.
Patients will be sequentially enrolled and monitored for safety as described at dose level 2 and dose level 3. If 0 of 3 or no more than 1 of 6 patients treated at each dose level have experienced a DLT for at least 28 days, we will enroll patients on the expansion phases.

Patients may be concurrently enrolled in the expansion cohort of dose level 2 while enrolling patients on dose level 3 for safety evaluation. Any patient who is eligible will be prioritized into a dose escalation slot. Once all dose escalation slots are filled, patients will be enrolled sequentially onto the expansion phase 2, followed by phase 3. Expansion slots will only be filled by subjects who are not eligible (due to concurrent therapy) for escalation slots until safety is established in both dose level 2 and 3. There will not be randomization.

Patients who come off study for any reason other than IND-related toxicity (defined as at least possibly related) prior to completion of the first restaging evaluation (about day 85) may be replaced. If a patient did not experience DLT and did not complete the DLT evaluation period (28 days), he or she will not be evaluable for toxicity and will be replaced in the dose level as outlined in Statistical Design (Section **8**).

- If 0/3 patients develop DLT on a dose level, then the dose may be escalated. For purposes of obtaining additional safety and efficacy information, cohorts up to 6 patients/cohort may be enrolled.
- If 1/3 patients develops DLT on a dose level, then up to 3 more patients will be enrolled.
- If no more than 1/6 patients develops DLT, then the dose will be escalated.

- If 2 or more patients experience DLT on a dose level, then the MTD has been exceeded and dose escalation has been completed. Three patients will be enrolled on the dose level below that level, provided that not more than 3 patients were treated on that dose level.

(The MTD will be the dose level at which no greater than 1/6 patients has a DLT, and the next higher dose level has at least 2 patients with a DLT.)

3.1.1 Dose-Limiting Toxicity

Dose-limiting toxicity will be defined as any one of the following: Any grade ≥ 3 hematologic toxicity or grade ≥ 3 non-hematologic toxicity that is possibly, probably, or definitely related to study drug, except transient (≤ 48 hour) grade 3 fatigue, local reactions, flu-like symptoms, fever, headache, and laboratory abnormalities that are not associated with organ pathology. Also any ≥ grade 2 allergic and ≥ grade 2 autoimmune reaction(s) (except endocrine-related immune toxicity) will be defined as a DLT. 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.

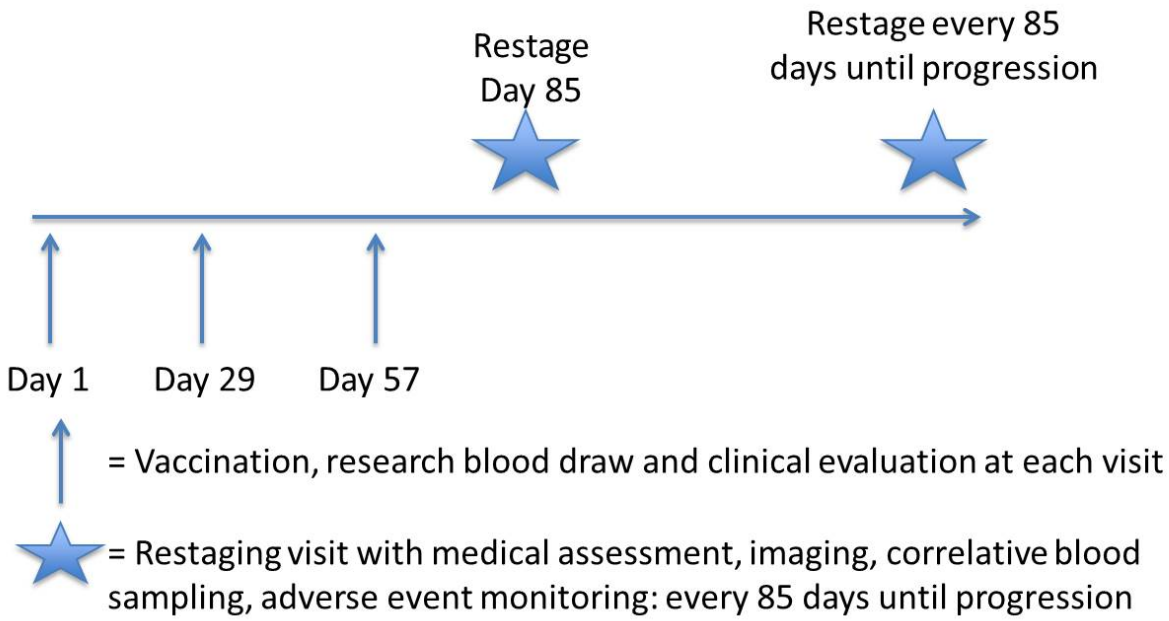
Generalized erythroderma or macular or papular rash lasting less than 7 days and not associated with desquamation will not be DLTs.

In addition, study accrual will be halted pending discussions with the IRB and the study sponsor if there is an occurrence of a grade 5 toxicity by the NCI-CTCAE (Version 4) attributable to the treatment regimen or if the maximum tolerated dose (MTD) is exceeded in dose level 1.

3.1.2 DLT Evaluation Period

The evaluation period for DLTs will be 28 days following the first injection of vaccine.

3.1.3 Schema



Dose Level	Dose and Schedule
1 N = 3 to 6	1 site of injection at 2 x 10^8 IU given every 28 days for 3 doses.
2 N = 10-16	2 sites of injection at 2 x 10^8 IU given every 28 days for 3 doses.
3 N = 10-16	4 sites of injection at 2 x 10^8 IU given every 28 days for 3 doses.

Dose escalation will proceed in cohorts of 3–6 patients. The MTD is the dose level at which no more than 1 of up to 6 patients have DLT during the first 28 days of treatment, and the dose below that at which at least 2 (of ≤ 6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not complete the DLT evaluation period (28 days), he or she will not be evaluable for toxicity and will be replaced in the dose level as outlined in the Statistical Design (Section **8**).

Dose escalation will follow the rules outlined in the table below.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 of 3	Enter up to 3 patients at the next dose level
$\geq$ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
$\leq$ 1 of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase II dose. At least 6 patients must be entered at the recommended phase II dose.

3.2 DRUG ADMINISTRATION

MVA-brachyury-TRICOM is supplied frozen in single-use vials. The vial must be thawed at room temperature for approximately 10-15 minutes prior to preparation, and should not be re-frozen after thawing. The thawed suspension will appear milky and may contain clumps or aggregates. To ensure homogeneity, the vial should be swirled vigorously, but not shaken, for approximately 10 – 20 seconds immediately prior to use. The thawed drug product is to be drawn into a syringe with an appropriately sized safety shielded needle suited to patient comfort (e.g. 22- to 28-gauge). MVA-brachyury-TRICOM is to be administered via subcutaneous injection in the upper arm and/or outer thigh per the schedule described in Section **3.1.3**.

3.3 STUDY CALENDAR

	Baseline[1]	Day 1	Day 29	Day 57	Restaging Day 85	Day 169 and every 85 days thereafter until progression
History and	X					

physical[1]						
Medical assessments[2]	X		X[8]	X[8]	X[8]	X
Imaging	X[3]				X	X
Urinalysis	X		X	X	X	X
Serum HIV antibody[4]	X					
Serum hepatitis B & C[5]	X					
CBC with differential, platelet count	X[1]		X	X	X	X
Chemistry[6]	X[1]		X	X	X	X
EKG	X[1]				X	X
Correlative / Immunology (blood)[10]	X		X	X	X	X
ANA titer	X				X	X
CD3, 4, 8, 19 subsets, NK markers and CD4:CD8 ratio	X		X		X	X
HLA typing	X					
Serum beta-HCG[7]	X					
Pulse oximetry	X					
MVA-brachyury-TRICOM vaccine[9]		X	X	X		
Adverse events			X	X	X	X
Concomitant medications	X		X	X	X	X

[1]Baseline: H & P and laboratory studies should be completed within 16 days of initiating treatment. Baseline radiographic, EKG, and immunologic studies should be obtained within 28 days of initiating treatment.

[2] Medical assessments: interim history (since last visit), vital signs, physical examination and ECOG performance status. To be performed at baseline, within 3 days of each dose of vaccine and with each restaging visit (about every 85 days on study).

[3] Radiologic studies consisting of CT chest/abdomen/pelvis (and MRI or bone scan when appropriate for tumor type) will be performed within 28 days prior to initiating treatment, on about day 85, and about every 85 days until progression.

[4] Serum HIV antibody should be completed within 8 weeks of initiating treatment.

[5]Serum hepatitis B & C antibody should be completed within 8 weeks of initiating treatment.

[6] Chemistry panel: Na+, K+, Cl–, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, TSH, and LDH.

[7]In females of child-bearing age, beta-HCG to be done at baseline within 48 hours prior to day 1.

[8] Within 3 days prior to each vaccination.

[9]Administered subcutaneously at 1, 2, or 4 sites (days 1, 29, 57 all - 4 days up to +14 days. In the event a dose is delayed, all subsequent doses will be scheduled about 28 days from that dose.)

[10] Correlative / Immunology blood: 6 (10mL) green top sodium heparin tubes for PBMC; 2 (8mL) SST tubes for serum samples, and 2 (10mL) green top sodium heparin tubes for circulating tumor cell assay.

3.4 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

Prior to documenting removal from protocol therapy or off study (if applicable), effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.4.1 Criteria for Removal from Protocol Therapy

Subjects will be removed from further vaccine therapy if any one of the following occurs:

- Disease progression (as defined by irRC in Appendix B, Section **13.2**).
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.
- Unacceptable adverse event(s) as described in Section **3.1.1** (Definition of Dose-Limiting Toxicity); or there is evidence of any ≥ grade 2 allergic and ≥ grade 2 autoimmune reaction(s) attributed to vaccine (**except** grade 2 endocrine-related immune toxicity or grade 3 endocrine-related immune toxicity that resolves to grade ≤ 1 clinically within 7 days of initiating supportive therapy); or if > grade 2 toxicity attributed to vaccine persists for > 28 days, the patient will not receive further vaccine inoculations and will be removed from protocol therapy. Patients who come off treatment must be followed on study until resolution of toxicity and until disease progression.
- Subject decides to withdraw from the study therapy. (In this event, the reasons for withdrawal will be documented.)
- Positive pregnancy test

3.4.2 Off-Study Criteria

Subjects will be removed from study for any one of the following criteria:

- Participant requests to be withdrawn from study; in this event, the reasons for withdrawal will be documented.
- A patient who is noncompliant with protocol guidelines may be removed from the study at the discretion of the principal investigator.
- Disease progression, defined by irRC in Appendix B (Section **13.2**).
- Death.

3.4.3 Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study 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 (HOIS) ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Anti-emetics and antidiarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically. Selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens should not include dexamethasone or other steroids.

Hormonal therapy in breast and prostate cancer, HER2-directed therapy in breast cancer, erlotinib in lung cancer, and "maintenance" capecitabine and/or bevacizumab in colorectal cancer are allowed only as per the eligibility criteria (Section **2.1**).

Toxicities thought to be autoimmune-related and attributable to vaccine will lead to discontinuation of vaccine and symptomatic treatment. This may also include the use of immunosuppressive treatments such as glucocorticoids and replacement hormones if indicated.

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

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

Other investigational therapies will not be allowed while a subject participates in this study.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Parameters of Immune Activation

5.1.1.1 Blood samples to be collected:

Correlative / Immunology blood: 6 (10mL) green top sodium heparin tubes for PBMC; 2 (8mL) SST tubes for serum samples, 2 (10mL) green top sodium heparin tubes for circulating tumor cell assay.

5.1.1.2 Immunologic /correlative testing will include:

- Specific T-cell responses, evaluated using flow cytometry intracellular staining (ICS) of CD4 and CD8 T lymphocytes for cytokines including IFN-γ, tumor necrosis factor, and IL-2. Additional markers of specific T-cell activation may be included based on evolving results with this assay.
- CD3, CD4, CD8, and CD19 subsets, NK markers, and CD4:CD8 ratio (baseline, about day 29, and about day 85).
- Analysis for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, TGF-ß, etc.), chemokines, antibodies (including yeast and brachyury), TAAs and/or other markers.
- If sufficient samples are available pre- and post-vaccination, PBMCs will be evaluated as follows:
    a. IFN-γ ELISPOT assays for brachyury-specific T lymphocytes using a brachyury-specific peptide in selected HLA-A2 peptides.
    b. A proliferation assay using brachyury protein in selected patients.
    c. Phenotypic and functional analysis of immune cell subsets (NK cells and Tregs).
    d. For use in a circulating tumor cell analysis in collaboration with Epic Sciences, Inc. (10975 N. Torrey Pines Rd, La Jolla, CA 92037, 858-356-6610).

5.1.1.3 Sample Processing

The samples will be processed through:
Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick, MD 21702
301-846-1000

On days samples are drawn, Jen Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens.

The research samples will contain labels on the blood tubes that have the patient's initials, date of birth, the assigned protocol, and the date the sample was drawn. The transmittal forms accompanying the samples also contain the same information.

Once a patient's treatment schedule has been determined, it should be faxed to Sandra Doren at the Laboratory of Tumor Immunology and Biology/ NIH (Fax: [301] 496-2756; phone: [301] 496-9573) for planning purposes.

5.1.2 Brachyury expression in tissue samples

- Upon enrollment, a minimum of 3-4 unstained slides, preferably from a metastatic site of disease, will be requested for analysis of brachyury expression. Unstained slides from the primary will also be requested, if available. This is not required for enrollment and will be analyzed retrospectively.
- An optional on-study biopsy will also be presented as a means to obtain pathologic samples for correlation of clinical outcomes with Brachyury expression.

5.2 SAMPLE STORAGE, TRACKING, AND DISPOSITION

Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.2.1 Samples Sent to Clinical Services Program (CSP)

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

Fisher BioServices manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc., Frederick. NCI Frederick Central Repositories 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.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with Fisher BioServices Federal-Wide Assurance. Fisher BioServices' role is limited to clinical research databases and repositories containing patient specimens. Fisher BioServices 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. The Fisher BioServices IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

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

Sample data is stored in the BioSpecimen Inventory (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens and to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to system functions. BSI provides audit tracking for processes 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 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.

5.2.2 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 so long as sample or data analysis continues. Samples from consenting subjects 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.

The PI will report any loss or destruction of samples to the NCI 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, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be entered into the NCI C3D data base.

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

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 breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Case Report Forms

The site staff will be required to enter in C3D available patient data from a visit no later than 10 business days after the visit is completed. The site staff will respond in a timely fashion to all queries made to the CRFs by the sponsor team and monitors.

6.2 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained between 4 and 8 weeks following initial documentation of objective response or concern of progression, per irRC (per Appendix B, Section **13.2**).

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).[45] Changes in the largest diameter (unidimensional measurement) of tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

All patients in this study must be assessed for response to treatment, even if there are major treatment deviations.

Each patient will be assigned one of the following categories: 1) complete response (CR); 2) partial response (PR); 3) stable disease (SD); 4) progressive disease (PD); or 5) not evaluable (NE) (death prior to restaging from malignant disease, toxicity, or due to other causes; or insufficient data). For the purposes of this study, patients will be re-evaluated for response after every 3 planned dosesBecause of direct clinical observation of immune-cell influx into tumor causing tumor enlargement in some patients prior to sustained response, it has recently been suggested that clinical trials involving the use of immunotherapy use alternative guidelines, called immune-related response criteria (irRC), to determine radiographic response or progression after therapy. These recommendations have been used in recent clinical trials. One study of 227 subjects with metastatic melanoma showed that the approximately 10% of patients who had PD by modified WHO criteria but either CR, PR, or SD by irRC had overall survival similar to those patients who had SD, PR, or CR by both criteria. The irRC were created using bidimensional measurements (as previously widely used in the WHO criteria). We have taken the concepts of the irRC and combined them with the recently revised RECIST 1.1[45] to come up with the modified irRC used in this protocol (see Appendix B in Section **13.2**). Consistent with the irRC, the main changes from RECIST 1.1 are (a) a requirement for confirmation of both progression and response by imaging at least 4 weeks after initial imaging and (b) not automatically calling the appearance of new lesions progressive disease if the total measurable tumor burden has not met criteria for progressive disease.

For irRC, only index and measurable new lesions are taken into account. At baseline tumor assessment on this trial, target lesions will be measured along the longest axis and the measurements will be summed, called sum of largest diameter (SLD). These lesions must be a minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ system. At each subsequent tumor assessment, the unidimensional measurement of target lesions and of new measureable lesions is added together to provide the total tumor burden. As per the modified definitions in Appendix B (Section **13.2**), all responses and progression except SD require confirmation on a consecutive scan at least 4 weeks from the initial observation.

Patients who experience rapid disease progression mandating discontinuation of therapy prior to completing 12 weeks of therapy (prior to first restaging) will be considered treatment failures. When possible, the same imaging studies used to define the extent of tumor at baseline upon study entry will be used for restaging. Time to disease progression will be defined as from the first date of therapy until the first notation of clinical or radiographic progression.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not < 6–8 weeks) defined in the study protocol.

Because subjects with non-measurable disease (also called evaluable-only disease) are eligible for enrollment, those subjects will be evaluated with imaging studies for any evidence of disease progression. In these cases, progression will be defined as new lesions found on imaging studies. In the spirit of the irRC, any new lesions in these subjects would then require confirmation of the new lesion on repeat imaging at least 4 weeks later to ensure that new lesions were not immune-related phenomena.

While we will use the irRC to determine disease progression for treatment purposes, we will also report radiographic responses by RECIST 1.1 (Appendix C, Section **13.3**). For study patients with chordoma, modified Choi criteria will be used to evaluate disease progression, stability, or

response using MRI and/or CT imaging (Appendix D, Section **13.4**) in addition to irRC (RECIST 1.1). Both techniques will be used, while irRC will be used to determine progression for study purposes.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used to report AEs.All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

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.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable

possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

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

7.1.4 Serious

An unanticipated problem or protocol deviation is serious if it meets the definition of a serious adverse event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

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

7.1.7 Life-Threatening Adverse Drug Experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 Non-compliance (NIH Definition)

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

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB REPORTING

7.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.

2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All serious events regardless of attribution.

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

7.2.3 NCI-IRB Reporting of IND Safety Reports

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

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to Dr. William Dahut, authorized representative for the IND Sponsor (CCR) at:

William Dahut, M.D.

Bldg. 10, Room 3-2571 MSC 1206

10 Center Drive

Bethesda, MD 20892

Telephone: 301-496-4251

William.Dahut@nih.gov

Copy all MedWatch forms to: nciprotocolsupportoffice@mail.nih.gov

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of

exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

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

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

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

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

The same timelines apply when outcome information is available.

7.3.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of vaccine.

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 4 months after the last dose should, if possible, be followed up and documented.

7.4 FDA REPORTING CRITERIA

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or *in vitro* testing that suggest a significant risk in humans exposed to the drug

- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

7.4.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer

The Sponsor will notify the IND Manufacturer of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 3 calendar days of initial receipt of the information. The Sponsor will also notify the IND Manufacturer of Serious Adverse Events meeting any of the criteria defined by the bulleted list in Section **7.4.1** no later than 7 calendar days after determining that the information qualifies for reporting. A copy of the draft MedWatch Form 3500a may be used to meet these reporting requirements. Notifications will be sent to the IND Manufacturer's Pharmacovigilance department either via email at pharmacovigilance@bavarian-nordic.com or via fax at (888) 465-1219.

A listing of all other Adverse Events will be provided to the IND Manufacturer annually, e.g. via provision of a copy of the IND annual report which includes a safety summary and Adverse Event Listings. The safety database for this study also will be shared with the IND manufacturer at the end of the study.

7.5 NIH Office of Science Policy (OSP)/Institutional Biosafety Committee (IBC) Reporting Criteria

7.5.1 Serious Adverse Event Reports to OSP/IBC

The Principal Investigator will notify OSP via email (HGTprotocols@mail.nih.gov) and IBC of any unexpected fatal or life-threatening experience associated with the use of MVA-brachyury-TRICOM 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 MVA-brachyury-TRICOM, but are not fatal or life-threatening, much be reported to NIH OSP/IBC as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information. Adverse events may be reported by using the Adverse Event Reporting template available on the NIH OSP website at: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/gemcris or by using the FDA Form 3500a.

7.5.2 Annual Reports to OSP/IBC

The study Principal Investigator will submit to OSP via email (HGTprotocols@mail.nih.gov) and IBC a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect unless the IND sponsor has been authorized to submit this report.

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the FDA annual report can be sent to OSP/IBC in lieu of a separate report. Please include the OSP/IBC protocol number on the annual report, and the updated clinical protocol.

7.5.2.1 Clinical Trial Information

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

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers, including the NIH OSP/IBC protocol number, NIH grant number(s) (if applicable), and the FDA IND application number;
- 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

7.5.2.3 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.

7.5.2.4 A copy of the updated clinical protocol including a technical abstract.

7.6 DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (at least every other week) when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

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

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

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. 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.

At least 25% of enrolled patients will be randomly selected and monitored at least biannually or as needed, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

7.6.3 Safety Monitoring Committee (SMC)

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

8 STATISTICAL CONSIDERATIONS

This study will be conducted as a standard phase I trial with an expanded cohort at the MTD. There are 3 dose levels of vaccine to be evaluated, and initially 3 patients will be enrolled in the lowest dose level. If 0/3 have a dose limiting toxicity (DLT) after 28 days from the third patient receiving the initial vaccine on study, then subsequent patients will be accrued to the next higher dose level. If 1/3 has a DLT, then 3 additional patients will be enrolled at that dose level. If 1/6 has a DLT, escalation may proceed, while if 2+/6 have a DLT, then the MTD will be considered to have been exceeded, and up to 3 additional patients will be enrolled at the next lower dose level if 6 patients have not previously been enrolled at that dose. The MTD will be the dose level at which no greater than 1/6 patients has a DLT, and the next higher dose level has at least 2 patients with a DLT.

The MTD will initially consist of 6 patients as indicated, and the study plans to enroll a total of 10 additional patients each at the MTD and the dose level below MTD (total of 16 at the MTD and up to 16 at the dose level just below the MTD) in in an effort to identify significant changes in T-cell precursors will be detectable. Potential evaluation techniques include flow-cytometry based intracellular cytokine staining after bBrachyury 15-mer stimulation and ELISPOT. Evaluations will take place both with respect to absolute change as well as whether a positive response is identified.

Because we will be using new evaluation techniques with a first in human study, we will enroll 10 patients in an expansion cohort, resulting in 16 potential evaluable patients in the two highest tolerated dose level cohorts, which will provide 80% power to detect a change in T-cell precursors from baseline equal to 1.0 standard deviations of the difference (effect size = 1.0) using a 2-tailed 0.05 alpha level paired t-test. All 10-16 patients in each dose level with evaluable T-cell precursor data at this dose level will be included in the analysis, with 10 patients providing an illustration of 80% power based on that minimal number. The actual power could be greater than 80% if 11-16 patients are evaluable for this determination. In practice, a nonparametric Wilcoxon signed rank test will be used. A positive response using the flow based intracellular cytokine staining assay will be defined as the frequency of T lymphocytes positive for a cytokine or CD107a post-vaccine being increased greater than two-fold compared to both (1) pre-vaccine values and (2) HLA control post-vaccine; the fraction of the patients who have a positive response out of those evaluable for this determination will be noted and reported along with a corresponding 95% confidence interval.

Although the MTD will have been declared after 6 patients at that dose level have been evaluated, monitoring for toxicity in the additional cohort of patients will continue. In the event that more than one third of all patients enrolled at the MTD experience a DLT, protocol modifications will be undertaken to reduce the likely level of toxicity in future patients prior to continuing to enroll additional subjects.
Clinical response evaluation will take place in the patients enrolled at the MTD and the expansion cohort. The results will be considered secondary and will be used to determine the parameters for a subsequent phase II trial should the preliminary findings from this trial warrant further investigation.

Up to 18 patients may be required to be enrolled in the 3 cohorts, plus an additional 10 at the MTD and the next highest dose level. Thus, up to 38 patients may be theoretically required to complete this trial. If 3 patients per month can be accrued, the study is expected to require 1 year to complete the necessary enrollment.

9 COLLABORATIVE AGREEMENTS

9.1 AGREEMENT TYPE

The MVA-brachyury-TRICOM vaccine is being provided by Bavarian Nordic, Inc. under a Collaborative Research and Development Agreement (CRADA) with LTIB, CCR.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

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

10.1.1 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

10.1.2 Exclusions

Patients with active or chronic infections, including HIV, hepatitis B or C, innate immunodeficiency or autoimmune disease are excluded because this agent requires an intact immune system. In addition, these patients may be at increased risk of complications and side effects due to immunologic changes resulting from this agent.

The effects of yeast-brachyury vaccine on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method; abstinence) prior to study entry and for the duration of study participation.

10.2 PARTICIPATION OF CHILDREN

Because no dosing or AE data are currently available on the use of MVA-brachyury-TRICOM vaccine in patients < 18 years of age, children are excluded from this study but may be eligible for future pediatric trials.

10.3 Evaluation of Benefits and Risks/Discomforts

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve unforeseeable risks to patients. All patients will have blood tests, examinations, and CT scans of the chest/abdomen/pelvis or other imaging studies as described in the monitoring schedule.

Because this is a first-in-human study, nothing is known directly about the clinical benefit or risk of this vaccine. However, we have previously tested a vaccine targeting the same antigen (brachyury) with no significant toxicity. Additionally, the LTIB has a long history with viral-vector vaccines, including vaccinia, from which MVA is derived. MVA is considered to be a safer alternative to vaccinia. The toxicity profile of vaccinia as a vector for therapeutic cancer vaccines has been excellent.[46]

Given preclinical data, the side effects are expected to be minor and reversible. Efforts to minimize risk include subcutaneous administration, close clinical monitoring after dose administration, and treatment of any side effects.

If patients suffer any physical injury as a result of participation in this study, immediate medical treatment is available at the Warren Grant Magnuson Clinical Center, 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.

10.4 Consent and Assent Process and Documentation

The investigational nature and objectives of this trial, the procedures involved, and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be explained to the patient and a signed informed consent document obtained. Once the patient has reviewed the consent, an investigator on this study will discuss the specifics of the consent and request the patient sign once all questions are satisfactorily answered. Moreover, any experimental invasive procedure will require a separate consent form. All listed associate investigators (except those listed on the cover sheet as not being able to make clinical decisions) are permitted to obtain informed consent.

10.4.1 Telephone (Re-)Consent Procedure

Reconsent 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 and a copy of the informed consent document will be kept in the subject's research record.

10.4.2 Informed Consent of 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, OSHRP SOP 12, 45 CFR 46.117 (b) (2), 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 (using either the long translated form or the short form). 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 for non-English speaking subjects 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 MVA-BRACHYURY-TRICOM

11.1.1 Source

MVA-brachyury-TRICOM is an active cancer immunotherapy. This phase I study is being conducted under an IND held by the Center for Cancer Research (CCR), National Cancer Institute (NCI). Bavarian Nordic, Inc. is the manufacturer of MVA-brachyury-TRICOM and has transferred sufficient product to the NCI to complete this phase I study.

11.1.2 Toxicity

MVA-BN-derived vectors that encode heterologous (non-vaccinia virus) antigens are being developed for the treatment of cancer. In GLP studies, MVA-BN and MVA-BN-derived vectors have been administered to 5 different animal species, including primates. In addition, MVA-BN and MVA-BN-derived vectors have been administered in clinical trials to over 7800 human subjects, including immunodeficient individuals. No marked toxicity and no drug-related serious AEs have occurred in any of these studies.

11.1.3 Formulation and Preparation

MVA-Brachyury-TRICOM is supplied as a frozen aqueous suspension in 2 mL clear borosilicate glass vials. The closure is a sterile bromobutyl rubber stopper, crimped with an aluminum cap and covered with a polypropylene closure. Each vial contains one 0.5 mL dose of MVA-Brachyury-TRICOM, which is 2.0×10^8 Infectious Units (Inf. U.). The thawed product will be a slightly turbid to milky suspension which may contain clumps or aggregates.

MVA-brachyury-TRICOM is supplied frozen in single-use vials. Each vial must be thawed at room temperature for approximately 10–15 minutes prior to preparation, and should not be re-frozen after thawing. The thawed suspension will appear milky and may contain clumps or aggregates. To ensure homogeneity, the vial should be swirled vigorously, but not shaken, for approximately 10–20 seconds immediately (within 3 minutes) prior to use. The thawed drug product is to be drawn into a syringe with an appropriately sized safety-shielded needle suited to patient comfort (e.g., 22- to 28-gauge).

11.1.4 Stability and Storage

MVA-brachyury-TRICOM should be stored frozen at -20°C or below and remain frozen until use.

11.1.5 Administration Procedures

MVA-brachyury-TRICOM is to be administered via subcutaneous injection, using a syringe with an appropriately sized safety-shielded needle suited to patient comfort (e.g., 22- to 28-gauge), in the upper arm and/or outer thigh per the schedule described in Section **3.1.3**. The injection must be delivered within 9 hours of completion of preparation as indicated in Section **11.1.3** to ensure stability. When possible, each subsequent dose should be administered at the same injection site of the first dose. Feasibility will be determined at the discretion of the investigator based on the clinical findings at the time of administration.

11.1.6 Incompatibilities

There are no known drug interactions associated with the use of MVA-BN and MVA-BN-derived vectors. As stated in Section **11.1.2**, no marked toxicities or serious AEs have been noted in clinical trials conducted with MVA-BN and MVA-BN-derived vectors.

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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 > 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 > 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: IMMUNE-RELATED RESPONSE CRITERIA

Responses will be evaluated in all cohorts to determine eligibility for subsequent cycles, but only responses evaluated in the expanded cohort and in patients enrolled at the MTD will be used for statistical analysis. For the purposes of this study, patients will be re-evaluated for response after day 85 (after 3 doses of vaccine).

Because of direct clinical observations of immune-cell influx into tumor causing tumor enlargement in some patients prior to sustained response, it has recently been suggested that clinical trials involving the use of immunotherapy use alternative guidelines, called immune-related response criteria (irRC), to determine radiographic response or progression after therapy.[47] These recommendations have been used in recent clinical trials. One study of 227 subjects with metastatic melanoma showed that the approximately 10% of patients who had PD by modified WHO criteria but either CR, PR, or SD by irRC had overall survival similar to patients who had SD, PR, or CR by both criteria. The irRC were created using bidimensional measurements (as previously widely used in the WHO criteria). We have taken the concepts of the irRC and combined them with the recently revised RECIST 1.1[45] to come up with the modified irRC used in this protocol. Consistent with the irRC, the main changes from RECIST 1.1 are (a) a requirement for confirmation of both progression and response by imaging at least 4 weeks after initial imaging and (b) not automatically calling the appearance of new lesions progressive disease if the total measurable tumor burden has not met criteria for progressive disease.

For irRC, only index and measurable new lesions are taken into account. At baseline tumor assessment on this trial, target lesions will be measured along the longest axis and the measurements will be summed, called sum of largest diameter (SLD). These lesions must be a minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ system. At each subsequent tumor assessment, the unidimensional measurement of target lesions and of new measureable lesions is added together to provide the total tumor burden. As per the modified definitions below, all responses and progression except SD require confirmation on a consecutive scan at least 4 weeks from initial observation.

Definitions of irRC:

Response	**irRC**
New measurable lesions	**Incorporated into tumor burden**
New non-measurable lesions	**Do not define progression (but preclude irCR)**
Non-index lesions	Contribute to defining irCR (complete disappearance required)
Overall irCR	100% disappearance of all lesions, whether measurable or not, and no new lesions, in 2 consecutive observations not less than 4 weeks from the date first documented. Also, all measurable lymph nodes must have reduction in short axis to < 10 mm.
Overall irPR	≥ 30% decrease in SLD compared with baseline confirmed by a consecutive assessment at least 4 weeks after first documentation.

Overall irSD	Not meeting criteria for irCR or irPR, in absence of irPD: 30% decrease in SLD compared with baseline cannot be established nor 20% increase compared with nadir.
Overall irPD	At least 20% increase in SLD compared with nadir (minimum recorded tumor burden) and an increase of at least 5 mm over nadir, confirmed by repeat, consecutive observations at least 4 weeks from the date first documented.

Overall responses derived from changes in index, non-index and new lesions as demonstrated in the following table:

Measurable response	**Non-measurable response**		**Overall response using irRC**
Index and new, measurable lesions (tumor burden)* %	**Non-index lesions**	**New, non-measurable lesions**	
Decrease 100	Absent	Absent	irCR[&]
Decrease 100	Stable	Any	irPR[&]
Decrease 100	Unequivocal progression	Any	irPR[&]
Decrease ≥ 30	Absent/stable	Any	irPR[&]
Decrease ≥ 30	Unequivocal progression	Any	irPR[&]
Decrease < 30 to increase < 20	Absent/stable	Any	irSD
Decrease < 30 to increase < 20	Unequivocal progression	Any	irSD
Increase ≥ 20	Any	Any	irPD

* Decreases assessed relative to baseline

[&] Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart.

13.3 APPENDIX C: RECIST 1.1 CRITERIA

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

Target lesions	**Non-target lesions**	**New lesions**	**Overall response**	**Best overall response when confirmation is required***
CR	CR	No	CR	> 4 weeks confirmation**
CR	Non-CR/non-PD	No	PR	> 4 weeks 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 weeks from baseline**
PD	Any	Yes or no	PD	No prior SD, PR, or CR
Any	PD***	Yes or no	PD	
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.

a. 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.

b. In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesions be investigated (fine needle aspirate/biopsy) before confirming complete response status.

13.3.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met.

13.3.2 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.

13.3.3 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as > 20 mm by chest x-ray, as > 10 mm by CT scan, or > 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

13.3.4 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 follow-up, only the short axis will be measured and followed.

13.3.5 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 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.

13.3.6 Evaluable Disease

Disease that cannot be measured directly by the size of the tumor but can be evaluated by other methods.

13.3.7 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.

13.3.8 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.

13.4 APPENDIX D: MODIFIED CHOI CRITERIA

The Choi Criteria were introduced for radiographic evaluation of gastrointestinal stromal tumors treated with imatinib mesylate[48,49] and have been evaluated in other soft tissue sarcomas[50] and renal cell carcinoma.[51] After discussion with experts in the field of chordoma as well as the Chordoma Foundation, we have agreed that, at this point, there is a consensus to use a modified Choi Criteria, with MRI instead of CT, as the best imaging technique to determine radiographic response, stability, or progression in patients with chordoma.

The differences between RECIST and Choi Criteria are defined below:

Tumor Response according to RECIST and Choi Criteria

Response	RECIST Criteria	Choi Criteria
Complete response	Disappearance of all lesions	Disappearance of all lesions
	No new lesions	No new lesions
Partial response	≥30% decrease in the sum of greatest diameters	≥10% decrease in the greatest maximal diameter or a ≥15% decrease in tumor attenuation at CT or contrast enhancement at MR imaging
	No new lesions	No new lesions
Stable disease	Does not meet criteria for complete response, partial response, or progressive disease	Does not met criteria for complete response, partial response, or progressive disease
Progressive disease	≥20% increase in the sum of greatest diameters	≥10% increase in the greatest maximal diameter and does not meet criteria for partial response by using tumor attenuation at CT or contrast enhancement at MR imaging or ≥15% increase in tumor attenuation at CT or contrast enhancement at MR imaging and does not meet the criteria for partial response by using tumor size
	New lesion	New lesion
		New intratumoral nodule or increase in the size of existing intratumoral nodule