



Published in final edited form as:

Cancer Immunol Res. 2015 November ; 3(11): 1248–1256. doi:10.1158/2326-6066.CIR-15-0119.

Phase I trial of a yeast-based therapeutic cancer vaccine (GI-6301) targeting the transcription factor brachyury

Christopher R. Heery¹, B. Harpreet Singh², Myrna Rauckhorst², Jennifer L. Marté², Renee N. Donahue¹, Italia Grenga¹, Timothy C. Rodell³, William Dahut², Philip M. Arlen², Ravi A. Madan², Jeffrey Schlom^{1,a}, and James L. Gulley^{2,a}

¹Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. ²Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. ³GlobeImmune, Inc., Louisville, Colorado.

Abstract

The nuclear transcription factor brachyury has previously been shown to be a strong mediator of the epithelial-to-mesenchymal transition (EMT) in human carcinoma cells and a strong negative prognostic factor in several tumor types. Brachyury is overexpressed in a range of human carcinoma as well as in chordoma, a rare tumor for which there is no standard systemic therapy. Preclinical studies have shown a recombinant *Saccharomyces cerevisiae* (yeast) vaccine encoding brachyury (GI-6301) can activate human T cells in vitro. A Phase I dose escalation (3+3 design) trial enrolled 34 patients at 4 dose levels (3, 3, 16, and 11 patients, respectively, at 4, 16, 40, and 80 yeast units (YU)). Expansion cohorts were enrolled at 40 and 80 YU dose levels for analysis of immune response and clinical activity. We observed brachyury-specific T-cell immune responses in the majority of evaluable patients despite most having been heavily pretreated. No evidence of autoimmunity or other serious adverse events were observed. Two chordoma patients showed evidence of disease control (one mixed response and one partial response). A patient with colorectal carcinoma, who enrolled on study with a large progressing pelvic mass and rising CEA, remains on study for greater than 1 year with stable disease, evidence of decreased tumor density and decreased serum CEA. This study is the first-in-human to demonstrate the safety and

Corresponding Author: James L. Gulley, Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center, Drive, Room 12N226, Bethesda, MD 20892. Phone: 301-435-2956; gulleyj@mail.nih.gov.

^aBoth authors contributed equally.

Declaration of interests

Jeffrey Schlom is the Primary Investigator on a Cooperative Research and Development agreement with the sponsor, GlobeImmune. Timothy C. Rodell is president and CEO of GlobeImmune, Inc. All other authors declare no competing interests.

Contributors

Conception and Design: JG, CH, RM, JS

Acquisition of Data: CH, HS, JG, MR, WD, PA, RM, RD, IG, JS

Analysis and interpretation: CH, HS, JG, JM, RD, IG, JS

Writing, review revision: CH, HS, JG, TR, RM, RD, JS

Admin, technical material support: DW

Supervision: CH, JG (overall study), RD, JS (immune analysis)

Enrollment, recruitment, accrual and treatment of patients: CH, HS, JG, MR, RM, WD, PA

immunogenicity of this therapeutic cancer vaccine and provides rationale for exploration in Phase II studies. A randomized Phase II chordoma study is enrolling.

Keywords

Chordoma; Immunotherapy; Epithelial to mesenchymal transition (EMT); Tarmogen; Therapeutic vaccine

Introduction

The phenomenon of epithelial-to-mesenchymal transition (EMT) is being recognized as an important process in the metastatic potential of human carcinoma cells as well as in the emergence of tumor cell populations resistant to multiple therapeutic interventions (1, 2). There appears to be significant overlap between EMT and “stem-like cells” or “stemness” of carcinoma cells.(3) Previous studies in murine models and involving human cells in vitro have demonstrated that transcription factors such as Twist, Snail, Slug, and brachyury can mediate the EMT process (1, 2, 4–6). Twist, Snail, or Slug expression in tumors has been shown to be indicative of poor prognosis; their similar level of expression in human tumors and normal adult tissue, however, limits therapeutic interventions targeting these molecules (7).

Previous studies have shown that brachyury is overexpressed in a spectrum of human tumors with little or no expression in human adult tissues, with the exception of expression in testes, subsets of cells in thyroid biopsies, and in a minor subset of B cells (8). Overexpression of brachyury in a range of human epithelial tumor cells has previously been shown to confer the transition to the mesenchymal phenotype, greater invasive and metastatic potential, and greater resistance to therapeutics (8, 9). The silencing of brachyury expression in mesenchymal-like carcinoma cells, on the other hand, has been shown to confer the transition to the epithelial phenotype, decreased invasive and metastatic potential, and increased sensitivity to therapeutics. Overexpression of brachyury has also been shown to be a poor prognostic indicator in lung carcinoma, hepatocellular carcinoma, prostate cancer, head and neck carcinoma, and in breast carcinoma patients treated with tamoxifen (10–13). A recent study has also shown that brachyury mediates the most prominent pathway in distinguishing triple negative breast carcinoma from non-triple negative breast carcinomas (14).

Previous studies have shown that within biopsies of primary breast carcinomas a small subpopulation of cells is positive for brachyury, but the percentage of positive cells increases in invaded lymph nodes and metastatic sites (12). Brachyury expression has also been shown to increase with stage of lung carcinomas (9). These findings provide evidence that brachyury expression in epithelial tumors conveys an increased capacity of metastatic spread. Brachyury overexpression is also present in >95% of chordomas (15). The role of brachyury in chordoma, though, is related to the cell of origin from which the disease arises. Chordoma is a rare tumor with approximately 300 new cases per year diagnosed in the United States. Chordoma is thought to arise from residual notochord. Brachyury expression plays a critical role in the formation of the posterior mesoderm and notochord during human

embryogenesis (16). Tumors have a predilection for the axial skeleton, with the most common sites being the clivus or skull base (~25%), the sacrum (50%), and mobile spine (~25%). The primary treatment for chordomas involves surgery and/or radiation. When surgical resection is not possible, standard treatment is typically definitive radiotherapy to the tumor site. There is no approved therapy for advanced disease (17). Moreover, an extensive review of the literature and a series of personal communications with investigators at chordoma referral centers revealed that radiographic responses are exceptionally uncommon (i.e., <5% and perhaps more like 1%) and are not reported in most studies of patients treated with a range of therapeutic modalities (18–23).

Because of their location in the nucleus, and their lack of a hydrophobic groove for drug attachment, transcription factors are considered difficult to target. While the mode of action of transcription factors is in the nucleus, they are synthesized and degraded in the cytoplasm. This leads one to the possibility that a transcription factor such as brachyury could be degraded in such a manner that brachyury peptides are transported to the cell surface in the context of major histocompatibility complex (MHC)–peptide complexes. If this were the case, the potential would exist for T-cell receptor–mediated recognition of such complexes and subsequent lysis of such brachyury-expressing cells. We have indeed demonstrated that brachyury peptide-pulsed human dendritic cells (DCs) can activate human T cells, which in turn have the ability to selectively lyse brachyury expressing human tumor cells (8, 9, 24). We have also shown that cancer patients vaccinated with carcinoembryonic antigen (CEA) or prostate-specific antigen (PSA) vaccines will mount brachyury-specific T-cell responses post-vaccination, most likely due to cross-presentation of brachyury protein and/or peptides to immune cells as a consequence of tumor-cell destruction (25, 26). These findings support the concept of the immunogenicity of brachyury in humans and the potential for developing a vaccine to target brachyury.

A therapeutic cancer vaccine has been constructed that consists of heat-killed recombinant *Saccharomyces cerevisiae* (yeast) expressing brachyury. Prior experimental studies have demonstrated that the yeast-brachyury construct can efficiently be taken up by and induce maturation of human DCs, which in turn can activate human brachyury-specific CD4 and CD8 T cells. An experimental murine model revealed that vaccination of mice with yeast-brachyury induces brachyury-specific CD4 and CD8 T cells, and anti-tumor activity (27). The studies reported here describe the first-in-human clinical trial of a vaccine directed against the transcription factor brachyury.

Methods

Patients

Eligible patients had metastatic or unresectable locally advanced malignant solid tumors, including chordoma, histologically confirmed by the Laboratory of Pathology, National Cancer Institute. All patients had completed prior therapies, or had disease progression on at least one prior therapy for metastatic cancer, or were not candidates for therapy of proven efficacy for their disease. Patients were ≥18 years of age, had Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and had a negative yeast allergy skin test. Any prior chemotherapy, radiation therapy, or surgeries must have been completed 4

weeks prior to starting on study. Patients with prostate cancer were able to continue to receive androgen deprivation therapy. Patients with ER+ breast cancer being treated with hormonal therapy (selective estrogen receptor modulator or aromatase inhibitor) who had rising tumor markers or progressive metastatic disease on scans were able to continue on hormonal therapy while being treated with vaccine. Patients could have no history of autoimmune disease. The study was approved by the National Cancer Institute's Institutional Review Board, and all patients gave written informed consent according to the institutional and federal guidelines. This study was registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT01519817).

Vaccine administration

A yeast-brachyury (GI-6301) vaccine composed of heat-killed recombinant *Saccharomyces cerevisiae* expressing the human brachyury protein was supplied by GlobeImmune, Inc. (Louisville, CO), under a Cooperative Research and Development Agreement with the Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute. The total dose, based on assigned level, was equally divided and administered subcutaneously at four injection sites: bilateral inguinal area and axillae. This strategy was based on preclinical data demonstrating that multiple-site vaccination more effectively induces T-cell immunity and antitumor responses than single-site vaccination (28). Yeast-brachyury vaccine was administered biweekly seven times (days 1, 15, 29, 43, 57, 71, and 85) and then monthly until evidence of disease progression (clinical or radiographic).

Assessment of toxicities

Toxicities were graded using the National Cancer Institute's cancer clinical trials common toxicity criteria (CTCAE 4.0). Toxicities were identified by medical history, physical examination, and review of laboratory studies. A dose limiting toxicity (DLT) was defined as any grade 3, 4, or 5 non-hematologic toxicity and any grade 4 or 5 hematologic toxicity that was definitely, probably, or possibly related to the administration of the vaccine. The DLT evaluation period to determine dose escalation was 28 days from the start of vaccine for each patient evaluated.

Subjects were evaluated for tissue-specific autoimmune toxicity in the tissues known to express brachyury (thyroid, pituitary, neurologic tissue, testicles, and B-cells). Thyroid evaluations included baseline and post-treatment ultrasound (repeated at off-treatment visit) and thyroid hormone panel (repeated at least monthly; TSH, free T3, free T4). Pituitary function was monitored clinically and with serum cortisol, ACTH, TSH, prolactin, serum osmolarity and urine osmolarity. Patients with active Epstein-Barr virus (EBV) infection (defined by symptomatic infection within 1 year, elevated serum EBV level by PCR, or early antigen titer 1:20) were excluded due to increased expression of brachyury in EBV infected B-cells. B-cell number was monitored at baseline and restaging. Neurologic and testicular adverse events were evaluated clinically with physical exam and review of symptoms. ANA titer was drawn at baseline and restaging.

Study design

This dose-escalation trial evaluated the maximum safely tolerated dose of heat-killed yeast-brachyury vaccine (GI-6301). Using a standard 3+3 dose escalation design, sequential cohorts (3–6 patients per dose cohort) were treated with vaccine. If a DLT was not observed in any subjects in a cohort, subsequent cohorts were then enrolled. Patients were given a total of 4 YU (1 YU = 10^7 yeast) at dose level 1, 16 YU at dose level 2, 40 YU at dose level 3, and 80 YU at dose level 4. After safety was established at a dose level, additional patients were enrolled into the two highest dose levels (3 and 4) to provide more data for immune analysis and evidence of clinical benefit. Tumors were assessed by CT scan of the chest, abdomen, and pelvis at baseline and 3 months, and then every 2 months until disease progression. Other imaging techniques were employed for particular tumor types (e.g., magnetic resonance imaging (MRI) for chordoma or Tc-99 whole body scintigraphy for prostate and breast cancer). Immune-related response criteria (irRC) were used to determine disease progression for treatment purposes, while radiographic responses were assessed by Response Evaluation Criteria in Solid Tumors Group (RECIST) 1.1.

Immunoassays

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque density gradient separation, washed three times, and preserved in 90% heat-inactivated human AB serum and 10% DMSO in liquid nitrogen at a concentration of 1×10^7 cells/mL until assayed. Analysis of antigen-specific responses following therapy was assessed by intracellular cytokine staining (ICS) following a period of in vitro stimulation (IVS) with overlapping 15-mer peptide pools encoding the tumor-associated antigen (TAA) brachyury. The TAA peptide pool was designed to contain a brachyury agonist epitope that had been previously identified (29); peptide pools encoding for human leukocyte antigen (HLA) and CEFT (a mixture of peptides of CMV, EBV, influenza, and tetanus toxin) served as negative and positive controls, respectively. Peptide mixes were purchased from JPT (Berlin, Germany), reconstituted in DMSO, and utilized immediately. Cryopreserved PBMCs from patients before therapy and at approximately day 85 (unless otherwise indicated) were thawed and rested overnight at 37°C, 5% CO₂ in complete medium (IMDM supplemented with 10% Human AB, 2mM glutamine, 100 units/mL penicillin, and 100 µg/ml streptomycin). The next day (day 0), PBMCs were seeded in 12 well plates (2.5×10^6 in 1 mL), and stimulated with peptide mixes (0.1 µg/mL per peptide). Cultures were supplemented on days 3 and 5 with cytokines (IL7 and IL15, 10 ng/mL, PeproTech, Rocky Hill, NJ) and fresh medium, and on day 7 were rested (with removal of cytokine and peptide). On day 11, 1×10^6 cells were restimulated for 24 hours in 96 well plates with peptide mixes in the presence of anti-CD107a-APC (clone H4A3, BD Biosciences, San Jose, CA); brefeldin A (1 µl/mL) and monensin (0.7 µl/mL) (BD Biosciences) were added to cultures 2 hours after the start of the restimulation and incubated for the final 22 hours. PBMCs were then stained with anti-CD4-PerCP-Cy5.5 (clone OKT4, Biolegend, San Diego, CA), anti-CD8-AF700 (clone OKT8, Ebioscience, San Diego, CA), and anti-TNF-PE (clone MAb11), anti-IFNγ-PE-Cy7 (clone 4SB3), and anti-IL-2-BV521 (clone 5344.111) (BD Biosciences). Values using the HLA control peptide pool were subtracted from brachyury peptide pool values for all assays.

For analysis of immune responses, at least 3×10^5 events in the live gate were acquired with a BD LSR-II flow cytometer equipped with a UV, violet, blue, and red laser. FCS files were analyzed with FlowJo V.9.7 for Macintosh (TreeStar, Ashland, OR). Fluorescence minus one (FMO) controls were used for gating, and nonviable cells excluded. The absolute number of CD4+ or CD8+ lymphocytes producing cytokine or positive for CD107a was calculated per 1×10^6 cells plated at the start of the IVS. The background signal (obtained with the HLA peptide pool), and values obtained prior to therapy, were subtracted from those obtained post-therapy. Values ≥ 250 were scored as positive if they were also at least 2-fold greater than that obtained with HLA.

Role of the funding source

Funding for this study was provided through the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health. The sponsor of the study, GlobeImmune, Inc., approved the study design, but had no role in data collection, data analysis, data interpretation, or writing of the report. The sponsor provided data monitoring data supplied by NCI investigators via intermittent data transfer. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Thirty-four patients were enrolled on this trial. Four patients were enrolled at dose level (DL) 1 (4 YU), three at DL 2 (16 YU), 16 at DL 3 (40 YU), and 11 at DL 4 (80 YU). Dose levels 3 and 4 enrolled additional patients in an expansion phase in order to better assess immune response and evidence of clinical benefit. Demographic data of patients are summarized in Table 1. Of the 23 patients enrolled with carcinomas, the most common primary diagnosis was colon adenocarcinoma ($n = 11$, 48%), followed by breast adenocarcinoma ($n = 5$, 22%). Three patients had pancreatic adenocarcinoma, two had prostate adenocarcinoma, one had urothelial carcinoma, and one had non-small cell lung cancer (NSCLC). Most patients had progressive treatment–refractory disease and had undergone a median of three prior chemotherapy regimens (range 0–6). Eleven patients with chordoma were enrolled, with a median age of 58.5 years, and a high percentage of males (91%). The primary sites in the chordoma subgroup were clival (27%), sacral (55%) and spinal (18%). All of the chordoma subjects had received both prior surgery and radiation and 45% had received prior chemotherapy or targeted therapy.

Toxicity

The treatment was well tolerated, with no DLTs. The most common adverse events (AEs) attributed to the yeast-brachyury vaccine were injection site reactions (Table 2). No injection site reaction was greater than grade 2 and none caused discontinuation of vaccination. Twenty-four events in 14 patients were classified as severe; of these, all were considered unrelated or unlikely related to vaccine and were related either to intercurrent infection or to symptoms of disease. Grade 2 adverse events related to vaccine included 8 injection site reactions in seven distinct patients and two events of decreased absolute lymphocyte count (ALC) in two patients. None of these events were considered serious. All injection site

reactions resolved without intervention. Both grade 2 ALC events occurred in patients with grade 1 ALC at baseline and were ongoing when patients went on to subsequent standard therapy for progressive disease.

There was no evidence of autoimmunity post- vs. pre-treatment in any patient with regard to the thyroid, pituitary, B cells, CNS, or testicles based on extensive tissue specific autoimmune evaluation.

Immune analyses

Sufficient PBMCs were available pre- and post-vaccination (approximately day 84, i.e., post-six vaccinations) from 31/34 patients to analyze brachyury-specific CD4 and CD8 T-cell responses. The FACS-based assay for T cells expressing type I cytokines IFN- γ , IL-2, TNF- α , and/or CD107a (a marker for lytic potential) is described in detail in the Methods section. All assays for a given patient's samples pre- and post-vaccine were carried out at the same time. Including all dose levels, 17/31 (54%) of patients developed brachyury-specific CD4 and/or CD8 T-cell responses post-vaccination (Table 3). Thirteen (42%) patients had some level of brachyury-specific T cells prior to therapy. Of those, 8/13 (62%) developed enhanced brachyury-specific T-cell responses post-vaccination. Of the remaining 18 patients who had no detectable level of brachyury-specific T cells prior to vaccination, 9/18 (50%) developed brachyury-specific T cells post-vaccine.

As seen in Table 3, 1/3 of carcinoma patients in the 4 YU and 16 YU cohorts developed or enhanced the level of brachyury-specific T cells post-vaccination. This was observed in 4/9 (44%) patients receiving 40 YU and 5/6 (83%) of patients receiving 80 YU. Of the chordoma patients, 4/7 in the 40 YU cohort and 2/3 in the 80 YU cohort developed or enhanced brachyury-specific CD4 and/or CD8 responses post-vaccination. While there were too few patients in each cohort to make any definitive statement concerning dose-related responses, there was a slight trend of more brachyury-specific T-cell responses at the higher doses. It should be pointed out that patients in this Phase I trial had received multiple prior therapies and were thus not optimal candidates for the generation of vaccine-mediated T-cell responses.

Chordoma patient #18 had no detectable brachyury-specific T-cell responses when assayed at day 84. As described below (see Case 3), this patient had a partial response and remained on study with a continued decrease in tumor size for 500+ days. PBMCs were thus obtained at subsequent time points post day 85 vaccination. While day 113 PBMCs were negative, days 197 and 288 displayed brachyury-specific T-cell responses (Table 3). PBMCs at day 372 post-vaccination showed no brachyury-specific T-cell responses. The reason for this fluctuation in T-cell responses seen here and in other trials by us and others is unknown at this time. Two patients had preexisting antibodies to brachyury and none of the vaccinated patients developed anti-brachyury antibodies post-vaccination. Similar lack of the generation of antibody responses to the transgene-encoded recombinant protein was observed in murine models employing yeast-brachyury and yeast-CEA vaccines and a prior clinical trial employing yeast-CEA.

Clinical outcomes

Of the 23 carcinoma patients enrolled, 21 were evaluable for objective response by RECIST 1.1. The other two patients were not evaluable for objective response due to withdrawal from the study or lack of measurable disease at baseline (this latter patient had only serum marker evidence of disease – PSA rising prostate cancer). Of the 21 evaluable carcinoma patients, six had stable disease at 3 months restaging and 15 had progressive disease. One patient with metastatic colorectal cancer remained on study for 16 months with stable disease before opting to come off study to start another therapy. Another patient with colorectal cancer remains on study with stable disease for 12+ months (see Case 1 below).

Eleven patients with chordoma were enrolled, of whom ten were evaluable for objective response by RECIST 1.1. One patient was not evaluable, coming off study due to infection prior to restaging. At 3 months restaging, eight patients had stable disease and one had progressive disease. At 5 months restaging, seven of ten evaluable patients had no evidence of disease progression, giving a clinical benefit rate of 70% at 5 months. Two of the patients with chordoma had radiographically stable disease for at least 6 months prior to study. If we exclude those patients, five of eight (62.5%) evaluable patients with evidence of disease progression had evidence of clinical benefit (partial response (PR) or stable disease (SD)) at 5 months restaging. The median progression-free survival in patients with chordoma who enrolled the study, using Kaplan-Meier analysis, was 253 days (about 8.3 months, range 41 - not reached at 600+ days, Supplemental Figure S1). One chordoma patient had a mixed response (MR) and one had confirmed PR; these two patients will be discussed below (Cases 2 and 3).

Individual case reports

Case 1—A 48-year-old female with metastatic colorectal cancer after disease progression through three prior lines of standard therapy enrolled on study with a rising carcinoembryonic antigen (CEA) in January 2014. Her main site of disease was a very large pelvic mass (Fig. 1A), which caused compression of the ureters and the rectum, resulting in the need for colostomy and bilateral nephrostomy tubes. She tolerated vaccine well with no adverse events other than injection site reaction and mild flu-like symptoms and fevers after doses, intermittently. Her tumor size remained very similar after 1 year on study, but there were interesting imaging findings, including increased contrast enhanced tumor perfusion and areas of central necrosis with gas pocket formation (Figure 1B). These findings were interpreted as a decrease in the tumor density. Also noteworthy, her previously rising CEA peaked about 1 month after enrollment on study and then fell and was declining at the time of this analysis (Fig. 1C). The imaging findings, consistent with a decreased tumor density and the CEA decline, suggest the potential of a slow destruction of tumor over a prolonged course of vaccination. It is of interest to note that this patient had the greatest level of brachyury-specific CD4 and CD8 T-cell responses post-vaccination of patients in this trial patient #29, Table 3).

Case 2—A 61-year-old male with a sacral chordoma underwent surgery, radiation and multiple systemic therapies with continued progressive disease. His largest sacral lesion was irradiated approximately 3.5 months prior to enrollment. Baseline scans are shown in Fig.

2A and B. At his 3-month restaging post-initiation of vaccine restaging, he had a clear reduction in tumor size at the site of previous radiation (Fig. 2C), but a clear growth in a paraspinal mass not in the radiation field (Fig. 2D). His tumor measurements totaled stable disease by RECIST criteria, which is considered a “mixed response” (MR). An amendment to the protocol allowed radiation to the growing site of disease, which arrested growth. He remains on study with stable disease 12+ months after initiation of vaccine with continued reduction of tumor size at the primary lesion (Fig. 2E) and now shrinkage the paraspinal mass after it was irradiated on study (Fig. 2F). For the purposes of PFS analysis, this patient was censored at the time of radiation to his paraspinal mass.

Case 3—A 47-year-old male was diagnosed in 2004 with a large sacral chordoma (12 cm). He underwent surgery followed by radiation with recurrent disease within 1 year. The patient subsequently underwent multiple surgical resections and further courses of radiation, with no response. Baseline MRI is shown in Fig. 3A and B. Approximately 3.5 months prior to enrollment on study the patient received radiation with 30 and 36 Gy, respectively, in 3 fractions (10 and 12 Gy fractions \times 3) to his recurrent pelvic-sacral tumors. There was no change in tumor size from pre-radiation to his enrollment on study. At the 3-month restaging after initiation of vaccine he had evidence of tumor size reduction, and then a $>30\%$ reduction in size at 5 months, which was confirmed as a PR 1 month later (Fig. 3C and D). He remains in a prolonged PR of 600+ days (Fig. 3E and F).

It should be noted that the responding lesions in Case 2 and Case 3, having been previously irradiated without clear progression of disease prior to enrollment, do not meet strict RECIST definitions, and are being described for the purposes of hypothesis generation.

Discussion

The trial reported here is the first-in-human targeting the transcription factor brachyury, in which safety was the primary endpoint. No grade 3 or greater toxicities attributed to vaccine were observed. The only grade 2 toxicities attributed to vaccine were injection site reactions in seven patients and decreased ALC in 2 patients with grade 1 ALC at baseline. Our previous immunohistochemistry (IHC) studies showed that subpopulations of cells in the thyroid and some B cells express brachyury. However, extensive clinical evaluation revealed no evidence of any autoimmune event at any dose level. This is also the first study to demonstrate that a vaccine designed to target brachyury can induce a T-cell response to brachyury in the majority of patients treated. These studies thus also demonstrate that one can generate both CD4 and CD8 T-cell responses to nuclear transcription factors such as brachyury. There appeared to be a trend of increasing T-cell responses with escalating dose of vaccine, with induction of brachyury-specific T cells in 2/6 patients at 4 or 16 YU, 8/15 patients receiving 40 YU and 7/9 patients receiving 80 YU. Since there was no additional toxicity at 80 YU, this dose will be chosen for further studies. Noting that the majority of patients in this study had advanced disease and were treated with a range of prior therapies, one might predict the generation of an even greater level of brachyury-specific T-cell responses in less advanced patient populations.

The induction of EMT by brachyury is quite dynamic and has been shown to be mediated by tumor microenvironmental factors such as TGF- β and IL-8 (8, 30). We hypothesize that vaccinating patients early in the disease course, including the adjuvant setting, may eliminate tumor cells with more mesenchymal and invasive potential. Vaccine-mediated targeting of brachyury in a more appropriate population (less heavily pretreated and less advanced disease), in Phase II studies could thus potentially reduce metastatic spread. Some studies have reported that when other transcription factors driving EMT result in metastatic lesions, a reversion to the mesenchymal-to-epithelial (MET) transition can occur. However, previous studies have shown greater numbers of persistent brachyury-expressing tumor cells in metastatic lesions as compared with primary tumors from the same patient, suggesting that brachyury may be an excellent target in treating and preventing metastatic disease. Targeting of brachyury-expressing cells may help control metastatic spread and serve a complementary function to cytotoxic therapies and other immunotherapies.

We and others have shown brachyury to be overexpressed in a range of human carcinomas, including lung, breast, colorectal, and hepatocellular. Brachyury is also expressed in virtually 100% of chordomas (15). In patients with recurrent chordoma following surgery, radiation is principally used for palliation, often halting growth, but rarely resulting in radiographic response.(20). In a series of prior trials (21, 22, 31) of chordoma patients (n=95) employing various agents, the response rate using RECIST criteria was approximately 2%: imatinib (1 PR (partial response)/50), lapatinib (0/19), and imatinib plus sirolimus (1 PR/9). There is thus a critical need for novel therapeutic interventions. It should be noted that of the two chordoma patients in this trial showing some evidence of disease control (one with a mixed response (MR) and one with a PR), both had radiation to their tumor approximately 3 months prior to enrolling on the vaccine study. Moreover, in the patient with the MR, the responding lesion to vaccine was the lesion that received prior radiation, while the non-responding lesion to vaccine did not. Overall, using Kaplan-Meier methodology, we found a median PFS of 253 days (range 41 – not reached at 600+ days, Supplemental Figure S1) in patients with chordoma on this study. This compares favorably with previous single-arm phase 2 studies with small-molecule therapies in similar a similar population of patients with chordoma (22), which may provide rationale for further exploration of this agent given the superior toxicity profile of this vaccine compared with those agents used commonly in clinical practice (32).

Several preclinical studies have shown that radiation to a tumor site can induce an inflammatory microenvironment leading to the influx of immune cells and subsequent anti-tumor effects. Studies have also shown in both murine *in vivo* models and employing human tumor cells *in vitro* that radiation can induce “immunogenic modulation” of tumor cells, i.e., changes in tumor cell phenotype to express higher levels of surface MHC-peptide complexes and death receptors, resulting in greater lysis by tumor-associated antigen-specific T cells (33). As a result of those studies, and the studies reported here, a randomized Phase II study is planned employing the yeast-brachyury vaccine in which chordoma patients will be randomized to receive radiation to tumor plus or minus vaccine (NCT02383498). Response rate will be the primary trial endpoint.

The findings in the colorectal cancer patient who remains on study at this writing merit discussion. This patient had disease progression through three prior standard therapies and went on study with rising CEA and a very large pelvic mass. While her tumor mass remained similar in size at 1 year of vaccine, imaging findings included increased contrast enhanced tumor perfusion and areas of potential necrosis, interpreted as decreased tumor density. Concomitant decreases in serum CEA were also observed. Recent IHC studies (unpublished data) have revealed increased levels of brachyury expression in colorectal severe dysplasia, and primary and metastatic colorectal cancer. The potential thus exists for vaccine-mediated brachyury T-cell targeting of colorectal cancer lesions among other brachyury-expressing cancer types as detailed above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the Chordoma Foundation for referral of patients to this clinical trial. The authors also thank Debra Weingarten for her editorial assistance in the preparation of this manuscript.

References

1. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest.* 2009; 119:1420–1428. [PubMed: 19487818]
2. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol.* 2006; 7:131–142. [PubMed: 16493418]
3. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells - what challenges do they pose? *Nat Rev Drug Discov.* 2014; 13:497–512. [PubMed: 24981363]
4. Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res.* 2007; 13:7003–7011. [PubMed: 18056176]
5. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res.* 2008; 68:3645–3654. [PubMed: 18483246]
6. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell.* 2004; 117:927–939. [PubMed: 15210113]
7. Hamilton DH, Litzinger MT, Fernando RI, Huang B, Palena C. Cancer vaccines targeting the epithelial-mesenchymal transition: tissue distribution of brachyury and other drivers of the mesenchymal-like phenotype of carcinomas. *Semin Oncol.* 2012; 39:358–366. [PubMed: 22595058]
8. Fernando RI, Litzinger M, Trono P, Hamilton DH, Schlom J, Palena C. The T-box transcription factor Brachyury promotes epithelial-mesenchymal transition in human tumor cells. *J Clin Invest.* 2010; 120:533–544. [PubMed: 20071775]
9. Roselli M, Fernando RI, Guadagni F, Spila A, Alessandrini J, Palmirota R, et al. Brachyury, a driver of the epithelial-mesenchymal transition, is overexpressed in human lung tumors: an opportunity for novel interventions against lung cancer. *Clin Cancer Res.* 2012; 18:3868–3879. [PubMed: 22611028]
10. Haro A, Yano T, Kohno M, Yoshida T, Koga T, Okamoto T, et al. Expression of Brachyury gene is a significant prognostic factor for primary lung carcinoma. *Ann Surg Oncol.* 2013; 20(Suppl 3):S509–S516. [PubMed: 23456319]

11. Kilic N, Feldhaus S, Kilic E, Tennstedt P, Wicklein D, Wasielewski R, et al. Brachyury expression predicts poor prognosis at early stages of colorectal cancer. *Eur J Cancer*. 2011; 47:1080–1085. [PubMed: 21220197]
12. Palena C, Roselli M, Litzinger MT, Ferroni P, Costarelli L, Spila A, et al. Overexpression of the EMT driver brachyury in breast carcinomas: association with poor prognosis. *J Natl Cancer Inst* epub. 2014 May 9.:106.
13. Pinto F, Pertega-Gomes N, Pereira MS, Vizcaino JR, Monteiro P, Henrique RM, et al. T-box transcription factor brachyury is associated with prostate cancer progression and aggressiveness. *Clin Cancer Res*. 2014; 20:4949–4961. [PubMed: 25009296]
14. Ben-Hamo R, Gidoni M, Efroni S. PhenoNet: identification of key networks associated with disease phenotype. *Bioinformatics*. 2014; 30:2399–2405. [PubMed: 24812342]
15. Meietinen M, Wang Z, Lasota J, Heery CR, Schlom J, Palena C. Nuclear brachyury expression is consistent in chordoma, common in germ cell tumors and small cell carcinomas and rare in other carcinomas and sarcomas. An immunohistochemical study of 5229 cases. *The American Journal of Surgical Pathology*. 2015 (in press).
16. Vujovic S, Henderson S, Presneau N, Odell E, Jacques TS, Tirabosco R, et al. Brachyury, a crucial regulator of notochordal development, is a novel biomarker for chordomas. *J Pathol*. 2006; 209:157–165. [PubMed: 16538613]
17. Walcott BP, Nahed BV, Mohyeldin A, Coumans JV, Kahle KT, Ferreira MJ. Chordoma: current concepts, management, and future directions. *Lancet Oncol*. 2012; 13:e69–e76. [PubMed: 22300861]
18. Amichetti M, Cianchetti M, Amelio D, Enrici RM, Minniti G. Proton therapy in chordoma of the base of the skull: a systematic review. *Neurosurg Rev*. 2009; 32:403–416. [PubMed: 19319583]
19. Casali PG, Messina A, Stacchiotti S, Tamborini E, Crippa F, Gronchi A, et al. Imatinib mesylate in chordoma. *Cancer*. 2004; 101:2086–2097. [PubMed: 15372471]
20. Chen YL, Liebsch N, Kobayashi W, Goldberg S, Kirsch D, Calkins G, et al. Definitive high-dose photon/proton radiotherapy for unresected mobile spine and sacral chordomas. *Spine (Phila Pa 1976)*. 2013; 38:E930–E936. [PubMed: 23609202]
21. Stacchiotti S, Longhi A, Ferraresi V, Grignani G, Comandone A, Stupp R, et al. Phase II study of imatinib in advanced chordoma. *J Clin Oncol*. 2012; 30:914–920. [PubMed: 22331945]
22. Stacchiotti S, Tamborini E, Lo Vullo S, Bozzi F, Messina A, Morosi C, et al. Phase II study on lapatinib in advanced EGFR-positive chordoma. *Ann Oncol*. 2013; 24:1931–1936. [PubMed: 23559153]
23. Kabolizadeh, P.; Chen, YL.; Liebsch, NJ.; Hornicek, FJ.; Schwab, JH.; Choy, E., et al. Analysis of tumor response in mobile spine and sacral chordoma treated with definitive high dose photon/proton radiotherapy (abstract). 54th Annual Conference of the Particle Therapy Co-Operative Group (PTCOG); May 18–23, 2015; San Diego, CA.
24. Palena C, Plev DE, Tsang KY, Fernando RI, Litzinger M, Krukovskaya LL, et al. The human T-box mesodermal transcription factor Brachyury is a candidate target for T-cell-mediated cancer immunotherapy. *Clin Cancer Res*. 2007; 13:2471–2478. [PubMed: 17438107]
25. Madan RA, Mohebtash M, Arlen PM, Vergati M, Rauckhorst M, Steinberg SM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase I dose-escalation trial. *Lancet Oncol*. 2012; 13:501–508. [PubMed: 22326924]
26. Bilusic M, Heery CR, Arlen PM, Rauckhorst M, Apelian D, Tsang KY, et al. Phase I trial of a recombinant yeast-CEA vaccine (GI-6207) in adults with metastatic CEA-expressing carcinoma. *Cancer Immunol Immunother*. 2014; 63:225–234. [PubMed: 24327292]
27. Hamilton DH, Litzinger MT, Jales A, Huang B, Fernando RI, Hodge JW, et al. Immunological targeting of tumor cells undergoing an epithelial-mesenchymal transition via a recombinant brachyury-yeast vaccine. *Oncotarget*. 2013; 4:1777–1790. [PubMed: 24125763]
28. Wansley EK, Chakraborty M, Hance KW, Bernstein MB, Boehm AL, Guo Z, et al. Vaccination with a recombinant *Saccharomyces cerevisiae* expressing a tumor antigen breaks immune tolerance and elicits therapeutic antitumor responses. *Clin Cancer Res*. 2008; 14:4316–4325. [PubMed: 18594015]

29. Tucker JA, Jochems C, Boyerinas B, Fallon J, Greiner JW, Palena C, et al. Identification and characterization of a cytotoxic T-lymphocyte agonist epitope of brachyury, a transcription factor involved in epithelial to mesenchymal transition and metastasis. *Cancer Immunol Immunother.* 2014; 63:1307–1317. [PubMed: 25186612]
30. Fernando RI, Castillo MD, Litzinger M, Hamilton DH, Palena C. IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells. *Cancer Res.* 2011; 71:5296–5306. [PubMed: 21653678]
31. Stacchiotti S, Marrari A, Tamborini E, Palassini E, Virdis E, Messina A, et al. Response to imatinib plus sirolimus in advanced chordoma. *Ann Oncol.* 2009; 20:1886–1894. [PubMed: 19570961]
32. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Bone Cancer. Version 1.2015. http://www.nccn.org/professionals/physician_gls/pdf/bone.pdf
33. Hodge JW, Kwilas A, Ardiani A, Gameiro SR. Attacking malignant cells that survive therapy: Exploiting immunogenic modulation. *Oncoimmunology.* 2013; 2:e26937. [PubMed: 24498561]

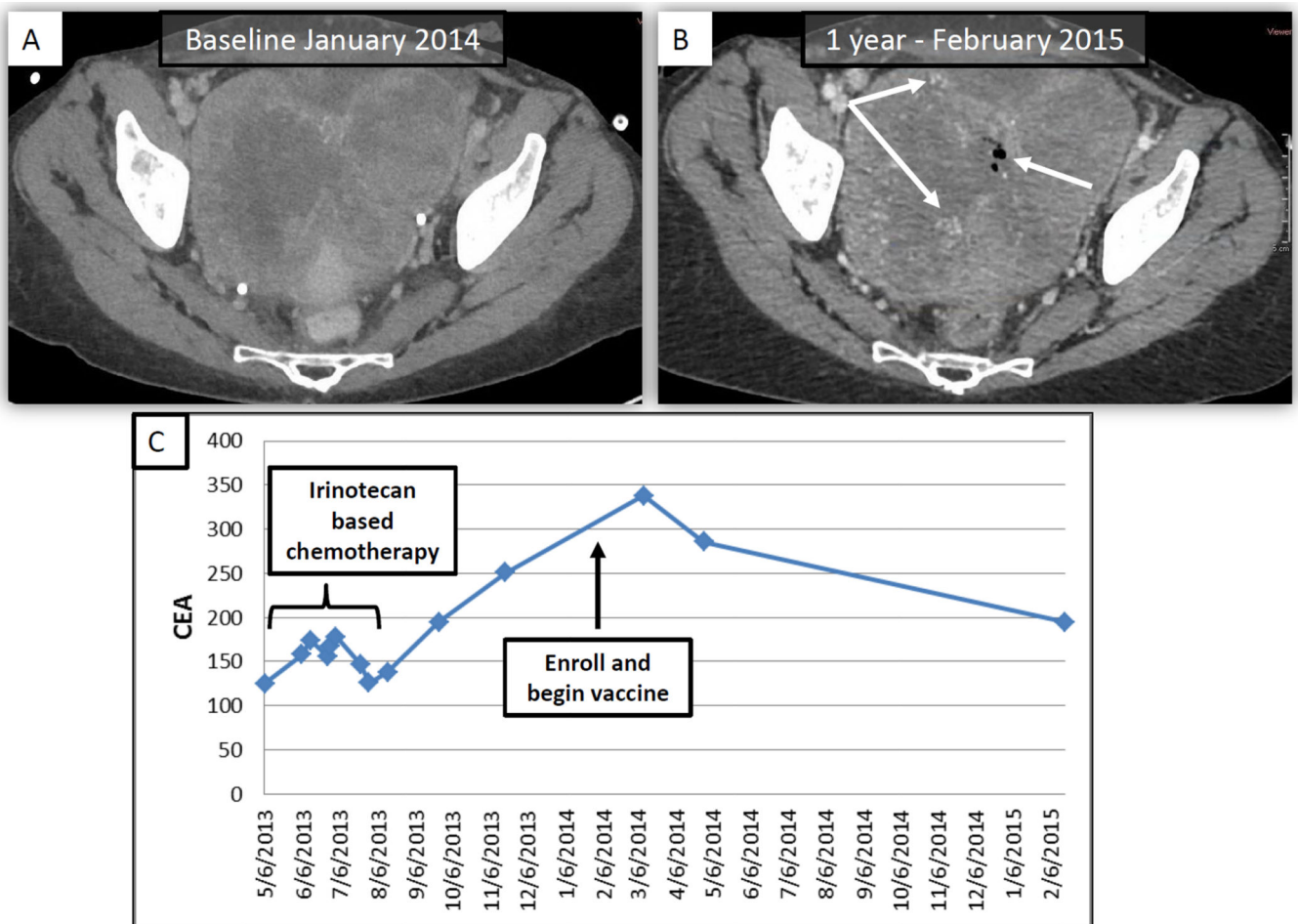


Figure 1.

A 48-year-old female with metastatic colorectal cancer after disease progression through three prior lines of standard therapy enrolled on study with a rising CEA in January 2014. Her main site of disease was a very large pelvic mass, which caused compression of the ureters and the rectum, resulting in the need for colostomy and bilateral nephrostomy tubes. **A**, Baseline CT scan of the chest, abdomen and pelvis demonstrating large (approximately 14 cm) pelvic mass with compression of ureters and rectum. Note the lack of contrast enhancing vasculature within the tumor and lack of central necrosis (gas). **B**, Restaging CT scan at 1-year follow-up demonstrating similar size pelvic mass with increased perfusion demonstrated by contrast enhanced vasculature (white arrows) and central gaseous necrosis (white arrow). **C**, CEA was rising prior to enrollment and declined after enrollment (arrow indicates time of enrollment and first vaccine).

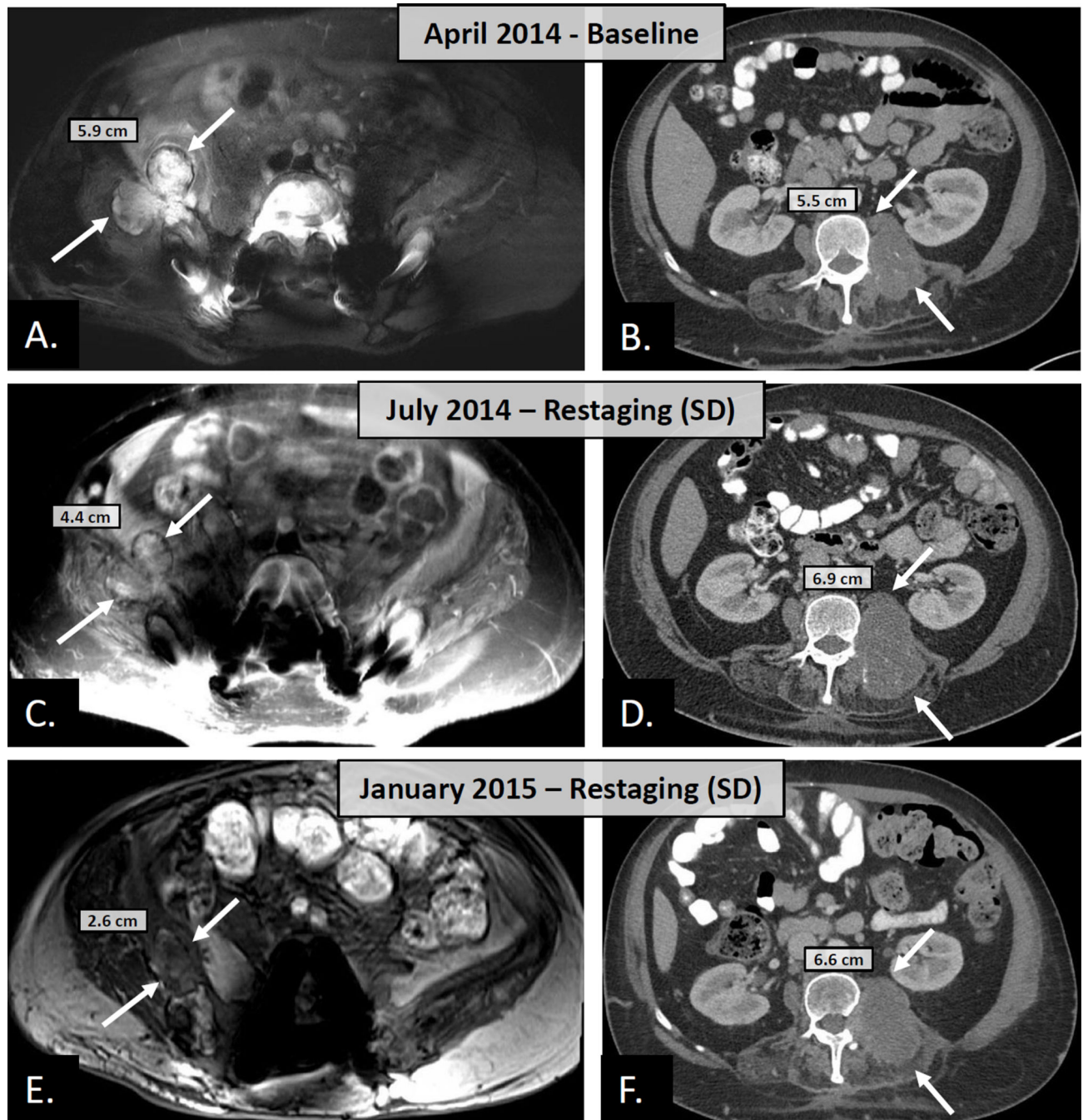


Figure 2. Mixed response in a patient with chordoma. A 61-year-old male with a sacral chordoma underwent surgery, radiation and multiple systemic therapies with continued progressive disease. His right iliac mass (**A**) was irradiated approximately 3.5 months prior to enrollment. **A, C, and E**, Baseline, first restaging, and most recent restaging MRI of the right iliac mass, which was previously irradiated. Mass decreased from 5.9 to 4.4 to 2.6 cm. **B, D, and F**, Baseline, first restaging, and most recent restaging CT scan of left paraspinal mass, which was not previously irradiated. This mass increased in size from 5.5 cm to 6.9

cm at first restaging, and growth was halted after radiation was given (6.9 to 6.6 cm on follow-up scan) while patient remained on study.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

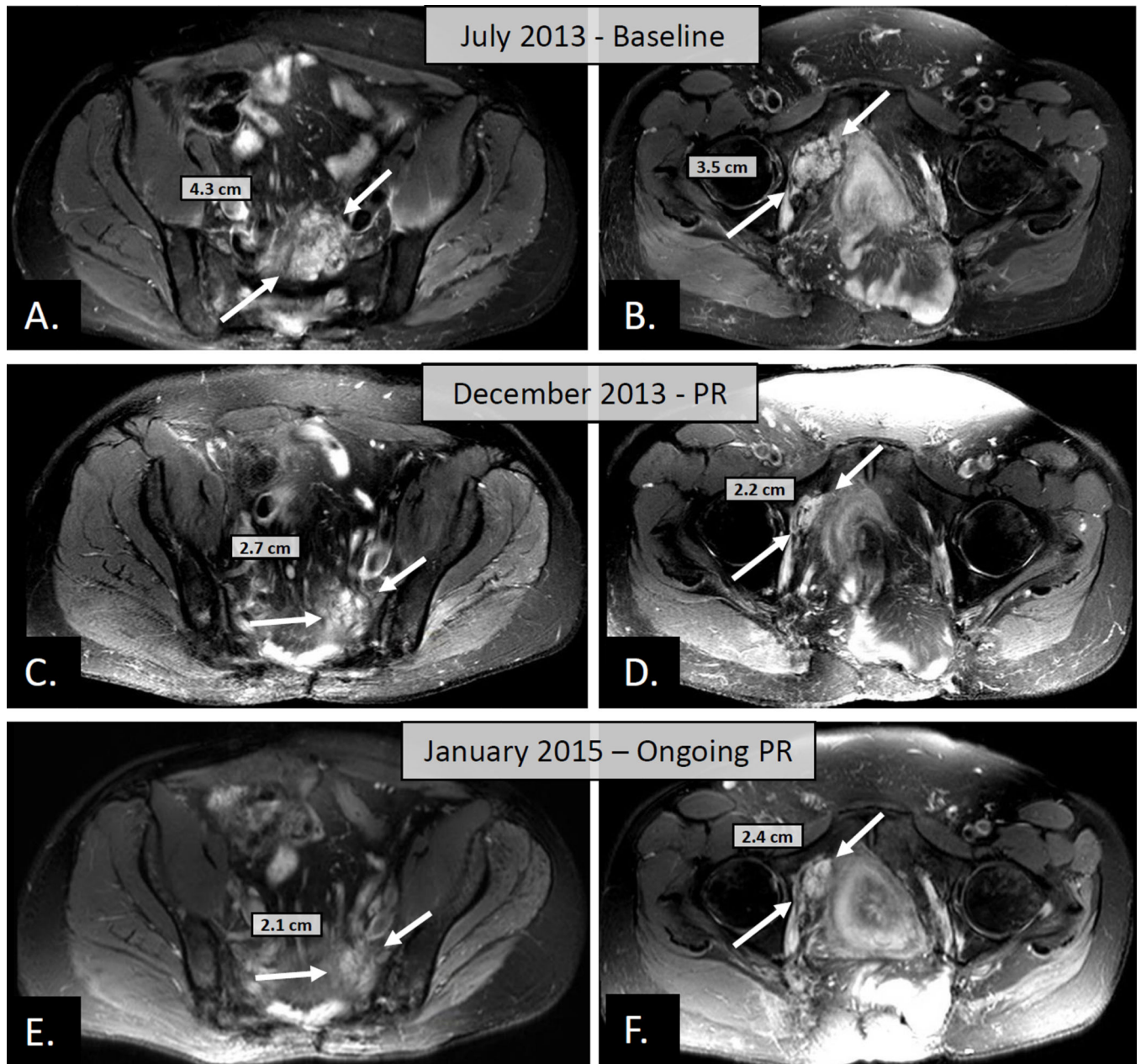


Figure 3. Partial response in a patient with chordoma. A 47-year-old male was diagnosed in 2004 with a large sacral chordoma (12 cm). He underwent surgery followed by radiation with recurrent disease within 1 year. The patient subsequently underwent multiple surgical resections and further courses of radiation, with no response. He was treated with 30 and 36 Gy (in 3 fractions; 10 and 12 Gy fractions), to the anterior and posterior pelvic masses, respectively, about 3.5 months prior to enrollment. **A and B**, Baseline MRI with right anterior pelvic mass, and left posterior pelvic mass. **C and D**, After 8 doses of vaccine, both masses decreased in size, constituting a partial response. **E and F**, The patient had a continued

response with a 42% decrease in overall size of tumors up to 9 months after the partial response.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Patient baseline characteristics

All patients (n=34)		Carcinomas (n=23)		Chordoma (n=11)	
Gender	# (%)	Gender	# (%)	Gender	# (%)
Male	19 (56)	Male	1 (4)	Male	10 (91)
Female	15 (54)	Female	22 (96)	Female	1 (9)
Age - Median (range)	58 (32–79)	Age - Median (range)	52 (32–79)	Age - Median (range)	58.5 (32–66)
ECOG Performance Status					
0	14	Tumor Anatomical			
1	20	Tumor Type	# (%)	Location	
ECOG Performance Status					
0	14	Colorectal	11 (32)	Sacral	6 (55)
1	20	Chordoma	11 (32)	Clival	3 (27)
ECOG Performance Status					
0	14	Breast	5 (15)	Spinal	2 (18)
1	20	Pancreatic	3 (9)		
ECOG Performance Status					
0	14	Prostate	2 (6)		
1	20	Urothelial	1(3)		
ECOG Performance Status					
0	14	Lung	1(3)		
1	20	Disease at Study Entry	# (%)	Disease at study entry	# (%)
ECOG Performance Status					
0	14	Stable Disease (SD)	1 (4)	Stable disease (SD)	2 (18)
1	20	Progressive Disease (PD)	22 (96)	Progressive disease (PD)	9 (82)
ECOG Performance Status					
0	14	Prior cytotoxic regimens	# (%)	Prior therapy	# (%)
1	20	0	1	Surgery	11 (100)
2	8	1	2	Radiation	11 (100)
3	12	2	8	Systemic therapy	5 (45)
ECOG Performance Status					
0	14	3	12		
1	20				
2	8				
3	12				

Table 2

Adverse events

	Grade 1		Grade 2	
	<u># events</u> <u>(% doses)</u>	<u># pts</u> <u>(% of pts)</u>	<u># events</u> <u>(% doses)</u>	<u># pts</u> <u>(% of pts)</u>
Likely/possibly related				
Injection site reaction	48 (18)	24 (71)	8 (2)	7 (21)
Fever	1 (0.4)	1 (2.9)	0 (0)	0 (0)
Flu-like symptoms	1 (0.4)	1 (2.9)	0 (0)	0 (0)
Lymphocyte count decreased	4 (1.5)	2 (6)	2 (0.8)	2 (6)
Joint effusion/joint swelling	1 (0.4)	1 (2.9)	0 (0)	0 (0)
Myalgias/body aches	1 (0.4)	1 (2.9)	0 (0)	0 (0)
Pruritus	1 (0.4)	1 (2.9)	0 (0)	0 (0)

Calculation based on 266 administered doses.

No events greater than Grade 2 attributed to vaccine.

Table 3
Brachyury-specific T-cell responses post- vs. pre-vaccination with yeast-brachyury

Carcinoma	Pt #	CD4				CD8				Dose	# of Responses ^d
		CD107a	IFNg	IL2	TNF	CD107a	IFNg	IL2	TNF		
Colon	1	0	0	0	0	0	0	0	0		
Colon	2	0	0	0	0	48	1	0	68	4 YU	1/3 (33%)
Colon	3	432	452	141	41	0	0	0	37		
Colon	4	22	0	115	220	0	15	0	20		
Colon	5	97	0	208	374	0	0	148	40	16 YU	1/3 (33%)
Colon	7	0	135	120	0	175	2	0	119		
Breast	8	0	25	0	0	0	0	0	0		
Pancreatic	9	146	540	345	897	100	0	0	0		
Urothelial	10	0	0	0	0	0	15	7	0		
Colon	11	226	25	0	0	0	0	0	0		
Pancreatic	12	328	144	19	229	490	0	29	80	40 YU	4/9 (44%)
Pancreatic	13	0	0	0	48	0	14	0	0		
Breast	14	0	85	356	606	636	420	202	475		
Breast	20	0	0	6	0	0	0	131	0		
Colon	23	0	0	222	2363	0	921	0	0		
Breast	24	0	0	0	0	0	119	43	0		
Lung	25	0	63	227	114	2728	167	0	519		
Breast	27	0	0	0	0	0	3014	0	215	80 YU	5/6 (83%)
Ovarian	28	0	0	0	0	0	22	0	446		
Colon	29	0	922	1064	2977	5980	2380	167	7639		
Colon	32	0	58	337	1557	0	0	822	0		
Chordoma	15	0	0	0	0	0	0	0	0		
Chordoma	16	1148	0	0	0	0	0	0	0		
Chordoma	17	1068	652	226	1067	1103	390	202	479		
Chordoma	18	0	68	0	0	0	46	0	0	40 YU	4/7 (57%)

Carcinoma	Pt #	CD4				CD8				Dose	# of Responses ^a
		CD107a	IFNg	IL2	TNF	CD107a	IFNg	IL2	TNF		
Chordoma	19	0	191	445	1178	0	0	0	0		
Chordoma	21	248	0	0	34	96	0	0	175		
Chordoma	22	288	321	40	279	120	0	12	186		
Chordoma	30	1839	336	0	0	3627	0	45	0		
Chordoma	31	0	0	180	0	0	0	0	0	80 YU	2/3 (66%)
Chordoma	33	0	47	251	0	823	0	0	0		
Chordoma*	18	0	679	348	402	1928	624	346	624	40 YU	

^a Cytokine or CD107a in CD4 or CD8.

* Analyzed on day 288.

Numbers in bold are those positive post- vs. pre-vaccination. Grey rows indicate a patient meeting defined immune response criteria. Absolute # of CD4 of CD8 producing cytokine or CD107a⁺ / 1×10⁶ cells plated at start of in vitro stimulation.