Research Article

Phase I Trial of a Yeast-Based Therapeutic Cancer Vaccine (GI-6301) Targeting the Transcription Factor Brachyury

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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 carcinomas as well as in chordoma, a rare tumor for which there is no standard systemic therapy. Preclinical studies have shown that 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 was 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 carcinoembryonic antigen (CEA), remains on study for greater than 1 year with stable disease, evidence of decreased tumor density, and decreased serum CEA. This is the first-in-human study to demonstrate the safety and immunogenicity of this therapeutic cancer vaccine and provides the rationale for exploration in phase II studies. A randomized phase II chordoma study is now enrolling patients. *Cancer Immunol Res; 3(11); 1248–56. ©2015 AACR.*

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.

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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 radiotherapy. 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. Although 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 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 (DC) 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 after 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 antitumor activity (27). The studies reported here describe the first-in-human clinical trial of a vaccine directed against the transcription factor brachyury.

Materials and 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 to 1, and had a negative result on a yeast allergy skin test. Any prior chemotherapy, radiotherapy, 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 shown 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 (NCT01519817).

Vaccine administration

A yeast-brachyury (GI-6301) vaccine composed of heat-killed recombinant *S. cerevisiae* expressing the human brachyury protein was supplied by GlobeImmune, Inc., 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 nonhematologic 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.

Patients 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 posttreatment 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, adrenocorticotropic hormone, 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 examination and review of symptoms. Antinuclear antibody 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 2 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 used for particular tumor types (e.g., 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, whereas radiographic responses were assessed by Response Evaluation Criteria in Solid Tumors Group (RECIST) 1.1.

Immunoassays

Peripheral blood mononuclear cells (PBMC) 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 HLA and CEFT (a mixture of peptides of cytomegalovirus, EBV, influenza, and tetanus toxin) served as negative and positive controls, respectively. Peptide mixes were purchased from JPT, 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% CO_2 in complete medium (Iscove's modified Dulbecco's medium supplemented with 10% Human AB, 2 mmol/L 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) 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); 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), anti–CD8-AF700 (clone OKT8; Ebioscience), 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). 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, was subtracted from those obtained after therapy. Values ≥250 were scored as positive if they were also at least 2-fold greater than that obtained with HLA.

Results

Thirty-four patients were enrolled on this trial. Four patients were enrolled at dose level 1 (4 YU), 3 at dose level 2 (16 YU), 16 at dose level 3 (40 YU), and 11 at dose level 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, 2 had prostate adenocarcinoma, 1 had urothelial carcinoma, and 1 had non–small cell lung cancer. 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 men (91%). The primary sites in the chordoma subgroup were clival (27%), sacral (55%), and spinal (18%). All of the chordoma patients had received both prior surgery and radiotherapy, and 45% had received prior chemotherapy or targeted therapy.

Toxicity

The treatment was well tolerated, with no DLTs. The most common adverse events attributed to the yeast-brachyury vaccine were injection site reactions (Table 2). No injection site reaction was higher 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 7 distinct patients and 2 events of decreased absolute lymphocyte count (ALC) in 2 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 after versus before treatment in any patient with regard to the thyroid, pituitary, B cells, CNS, or testicles based on extensive tissue-specific autoimmune evaluation.

Table 1. Patient baseline characteristics

All patients (*n* = 34)	
Gender	*N* (%)
Male	19 (56)
Female	15 (54)
Age, median (range)	58 (32–79)
ECOG performance status	
0	14
1	20
Carcinomas (*n* = 23)	
Gender	*N* (%)
Male	1 (4)
Female	22 (96)
Age, median (range)	52 (32–79)
Tumor type	*N* (%)
Colorectal	11 (32)
Chordoma	11 (32)
Breast	5 (15)
Pancreatic	3 (9)
Prostate	2 (6)
Urothelial	1 (3)
Lung	1 (3)
Disease at study entry	*N* (%)
Stable disease	1 (4)
Progressive disease	22 (96)
Prior cytotoxic regimens	
0	1
1	2
2	8
≥3	12
Chordoma (*n* = 11)	
Gender	*N* (%)
Male	10 (91)
Female	1 (9)
Age, median (range)	58.5 (32–66)
Tumor anatomical location	
Sacral	6 (55)
Clival	3 (27)
Spinal	2 (18)
Disease at study entry	*N* (%)
Stable disease	2 (18)
Progressive disease	9 (82)
Prior therapy	*N* (%)
Surgery	11 (100)
Radiotherapy	11 (100)
Systemic therapy	5 (45)

Immune analyses

Sufficient PBMCs were available before and after vaccination (approximately day 84, i.e., after six vaccinations) from 31 of 34 patients to analyze brachyury-specific CD4 and CD8 T-cell responses. The FACS-based assay for T cells expressing type I cytokines IFNγ, IL2, TNFα, and/or CD107a (a marker for lytic potential) is described in detail in Materials and Methods. All assays for a given patient's samples before and after vaccine were carried out at the same time. Including all dose levels, 17 of 31 (54%) patients developed brachyury-specific CD4 and/or CD8 T-cell responses after vaccination (Table 3). Thirteen (42%) patients had some level of brachyury-specific T cells prior to therapy. Of those, 8 of 13 (62%) developed enhanced brachyury-specific T-cell responses after vaccination. Of the remaining 18 patients who had no detectable level of brachyury-specific T cells prior to vaccination, 9 of 18 (50%) developed brachyury-specific T cells after vaccine.

As seen in Table 3, 1 of 3 carcinoma patients in the 4-YU and 16-YU cohorts showed development or enhancement of the level of brachyury-specific T cells after vaccination. This was observed in 4 of 9 (44%) patients receiving 40 YU and 5 of 6 (83%) patients receiving 80 YU. Of the chordoma patients, 4 of 7 in the 40 YU cohort and 2 of 3 in the 80 YU cohort developed or enhanced brachyury-specific CD4 and/or CD8 responses after vaccination. Although 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 (PR) and remained on study with a continued decrease in tumor size for 500+ days. PBMCs were thus obtained at subsequent time points after 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 after 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 after vaccination. Similar lack of the generation of antibody responses to the transgene-encoded recombinant protein was observed in murine models using yeast-brachyury and yeast-CEA vaccines and in a prior clinical trial using yeast-CEA.

Clinical outcomes

Of the 23 carcinoma patients enrolled, 21 were evaluable for objective response by RECIST 1.1. The other 2 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, 6 had stable disease at 3-month restaging and 15 had progressive disease. One

Table 2. Adverse events

	Grade 1		Grade 2	
	# events (% doses)	Patients (% of patients)	# events (% doses)	Patients (% of patients)
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)

NOTE: Calculation based on 266 administered doses. No events higher than grade 2 attributed to vaccine.

Table 3. Brachyury-specific T-cell responses after versus before vaccination with yeast-brachyury

| Carcinoma | Patient # | CD4 | | | | CD8 | | | | Dose | Number of responses[a] |
		CD107a	IFNγ	IL2	TNF	CD107a	IFNγ	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	**2,363**	0	**921**	0	0		
Breast	24	0	0	0	0	0	119	43	0		
Lung	**25**	0	63	227	114	**2,728**	167	0	**519**		
Breast	**27**	0	0	0	0	0	**3,014**	0	215	80 YU	5/6 (83%)
Ovarian	**28**	0	0	0	0	0	22	0	**446**		
Colon	**29**	0	**922**	**1,064**	**2,977**	**5,980**	**2,380**	167	**7,639**		
Colon	**32**	0	58	**337**	**1,557**	0	0	**822**	0		

| Chordoma | Patient # | CD4 | | | | CD8 | | | | Dose | Number of responses[a] |
		CD107a	IFNγ	IL2	TNF	CD107a	IFNγ	IL2	TNF		
Chordoma	15	0	0	0	0	0	0	0	0		
Chordoma	**16**	**1,148**	0	0	0	0	0	0	0		
Chordoma	**17**	**1,068**	**652**	226	**1,067**	**1,103**	**390**	**202**	**479**		
Chordoma	18	0	68	0	0	0	46	0	0	40 YU	4/7 (57%)
Chordoma	**19**	0	191	**445**	**1,178**	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**	**1,839**	**336**	0	0	**3,627**	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[b]	**18**	0	**679**	**348**	**402**	**1,928**	**624**	**346**	**624**	40 YU	

NOTE: Numbers in bold are those positive after versus before vaccination. Gray rows indicate a patient meeting defined immune response criteria.
Absolute # of CD4 of CD8 producing cytokine or CD107a$^+$/1 × 10^6 cells plated at start of *in vitro* stimulation.
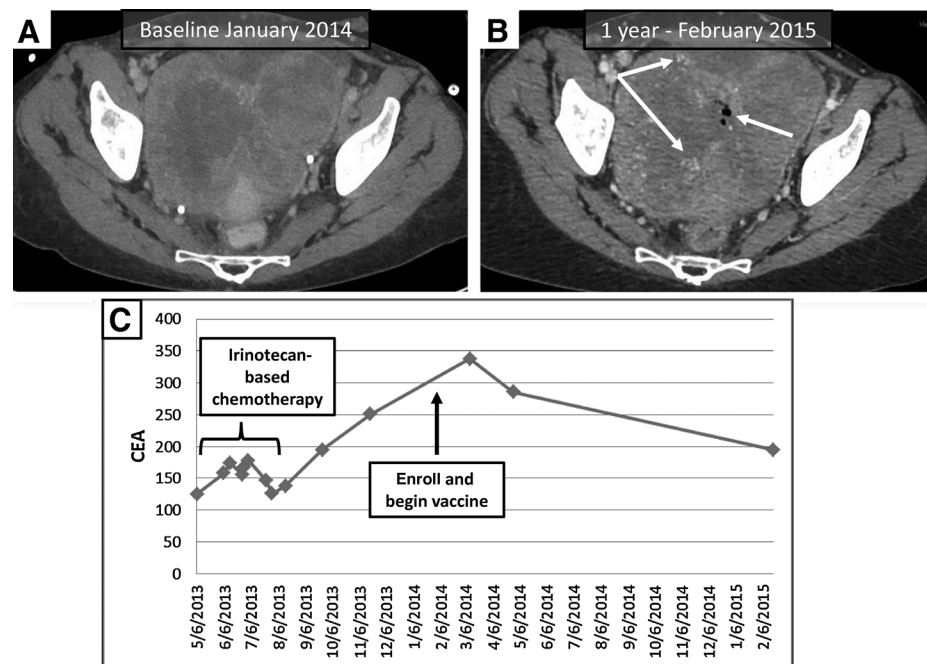[a]Cytokine or CD107a in CD4 or CD8.
[b]Analyzed on day 288.

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 10 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-month restaging, 8 patients had stable disease and 1 had progressive disease. At 5-month restaging, 7 of 10 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 the study. If we exclude those patients, 5 of 8 (62.5%) evaluable patients with evidence of disease progression had evidence of clinical benefit (PR or stable disease) at 5-month restaging. The median progression-free survival (PFS) in patients with chordoma who enrolled in the study, using the Kaplan–Meier analysis, was 253 days (about 8.3 months; range, 41–not reached at 600+ days; Supplementary Fig. S1). One chordoma

Figure 1.
A 48-year-old woman 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 shown 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).

patient had a mixed response and one had a confirmed PR; these 2 patients are discussed below (Cases 2 and 3).

Individual case reports

Case 1. A 48-year-old woman 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 (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 (Fig. 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 after vaccination of patients in this trial (patient #29; Table 3).

Case 2. A 61-year-old man with a sacral chordoma underwent surgery, radiotherapy, 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 after 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." An amendment to the protocol allowed radi-
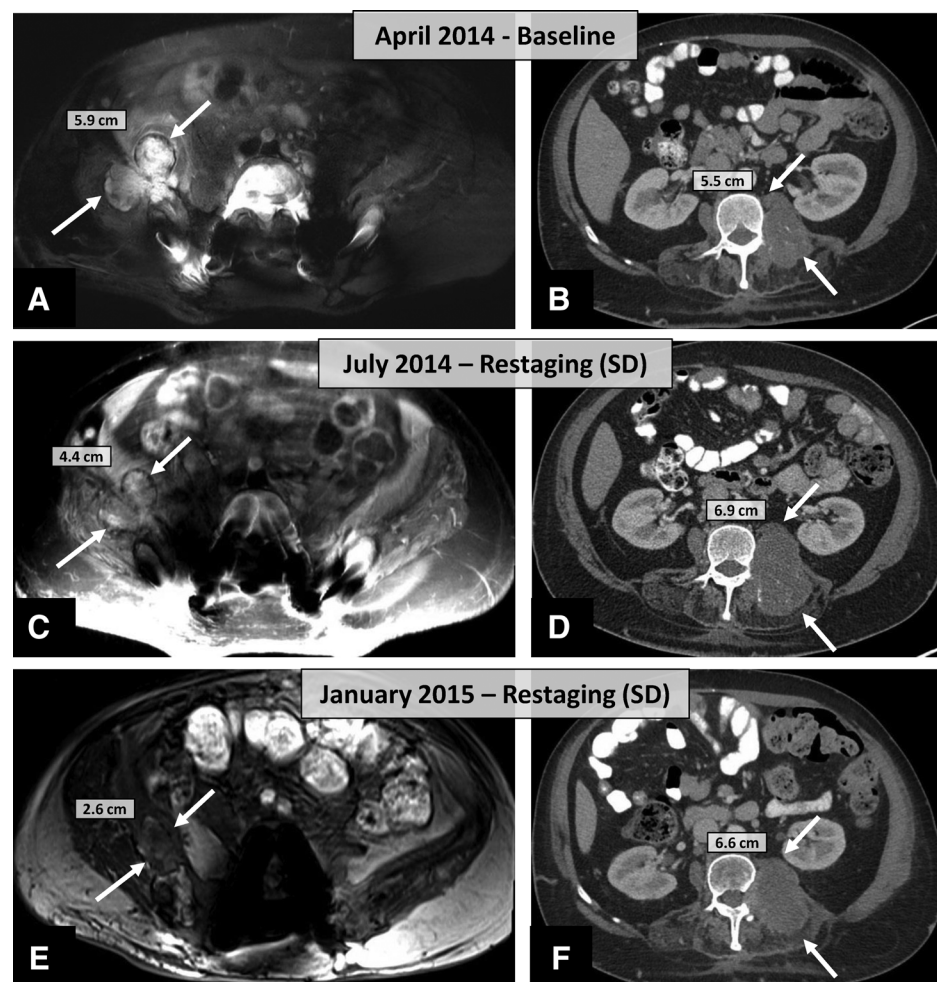
ation to the growing site of disease, which arrested growth. He remains on study with stable disease 12+ months after initiation of vaccine with continued reduction of tumor size at the primary lesion (Fig. 2E) and now shrinkage of the paraspinal mass after it was irradiated on study (Fig. 2F). For the purposes of PFS analysis, this patient was censored at the time of radiation to his paraspinal mass.

Case 3. A 47-year-old man was diagnosed in 2004 with a large sacral chordoma (12 cm). He underwent surgery followed by radiotherapy 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 × 3) to his recurrent pelvic-sacral tumors. There was no change in tumor size from preradiation 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 tumor 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 trial targeting the transcription factor brachyury, in which safety was the primary endpoint. No grade 3 or higher toxicities attributed to vaccine were observed. The only grade 2 toxicities attributed to vaccine were injection site reactions in 7 patients and decreased ALC in 2 patients with grade 1 ALC at baseline. Our previous

Figure 2.
Mixed response in a patient with chordoma. A 61-year-old man with a sacral chordoma underwent surgery, radiotherapy, 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 cm to 4.4 cm 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 radiotherapy (6.9 cm to 6.6 cm on follow-up scan) while patient remained on study. SD, stable disease.

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 of 6 patients at 4 YU or 16 YU, 8 of 15 patients receiving 40 YU, and 7 of 9 patients receiving 80 YU. Because 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.
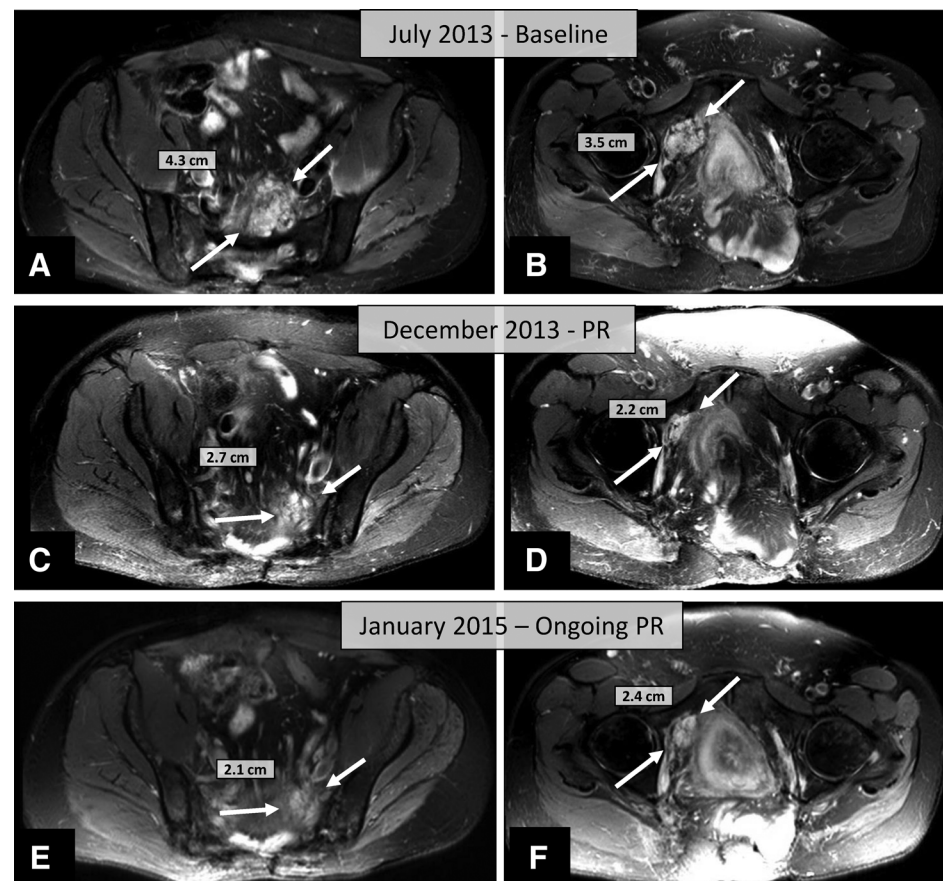
The induction of EMT by brachyury is quite dynamic and has been shown to be mediated by tumor microenvironmental factors, such as TGFβ and IL8 (8, 30). We hypothesize that vaccinating patients early in the disease course, including the adjuvant setting, may eliminate tumor cells with more mesenchymal 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 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 ($n = 95$) using various agents, the response rate using RECIST criteria was approximately 2%: imatinib (1 PR/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 2 chordoma patients in this trial showing

Figure 3.
Partial response (PR) in a patient with chordoma. A 47-year-old man was diagnosed in 2004 with a large sacral chordoma (12 cm). He underwent surgery followed by radiotherapy with recurrent disease within 1 year. The patient subsequently underwent multiple surgical resections and further courses of radiotherapy, 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.

some evidence of disease control (one with a mixed response 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 mixed response, the responding lesion to vaccine was the lesion that received prior radiation, whereas the non-responding lesion to vaccine did not. Overall, using the Kaplan–Meier methodology, we found a median PFS of 253 days (range, 41–not reached at 600+ days; Supplementary Fig. S1) in patients with chordoma on this study. This compares favorably with the results of previous single-arm phase II studies with small-molecule therapies in a similar population of patients with chordoma (22), which may provide the 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 antitumor effects. Studies have also shown in both murine *in vivo* models and using 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, using the yeast-brachyury vaccine in which chordoma patients will be randomized to receive radi-

ation 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. Although her tumor mass remained similar in size at 1 year of vaccine, imaging findings showed 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.

Disclosure of Potential Conflicts of Interest

T.C. Rodell is CEO and acting CMO at, reports receiving commercial research support from, and has ownership interest (including patents) in GlobeImmune, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C.R. Heery, W. Dahut, P.M. Arlen, R.A. Madan, J. Schlom, J.L. Gulley
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References

1. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420–8.
2. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006;7:131–42.
3. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells - what challenges do they pose? Nat Rev Drug Discov 2014;13:497–512.
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;13:7003–11.
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;68:3645–54.
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;117:927–39.
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;39:358–66.
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;120:533–44.
9. Roselli M, Fernando RI, Guadagni F, Spila A, Alessandroni J, Palmirotta 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;18:3868–79.
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:S509–16.
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;47:1080–5.
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 2014;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; 20:4949–61.
14. Ben-Hamo R, Gidoni M, Efroni S. PhenoNet: identification of key networks associated with disease phenotype. Bioinformatics 2014;30:2399–405.
15. Miettinen M, Wang Z, Lasota J, Heery C, Schlom J, Palena C. Nuclear brachyury expression is consistent in chordoma, common in germ cell tumors and small cell carcinomas, and rare in other carcinomas and sarcomas: an immunohistochemical study of 5229 cases. Am J Surg Pathol 2015 Apr 4. [Epub ahead of print].
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;209:157–65.
17. Walcott BP, Nahed BV, Mohyeldin A, Coumans JV, Kahle KT, Ferreira MJ. Chordoma: current concepts, management, and future directions. Lancet Oncol 2012;13:e69–76.
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;32:403–16.
19. Casali PG, Messina A, Stacchiotti S, Tamborini E, Crippa F, Gronchi A, et al. Imatinib mesylate in chordoma. Cancer 2004;101:2086–97.
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: E930–6.
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;30:914–20.
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;24:1931–6.
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). In Proceedings of the 54th Annual Conference of the Particle Therapy Co-Operative Group (PTCOG), 2015 May 18–23; San Diego, CA.
24. Palena C, Polev DE, Tsang KY, Fernando RI, Litzinger M, Krukovskaya LL, et al. The human T-box mesodermal transcription factor Brachyury is a candidate target for T-cell-mediated cancer immunotherapy. Clin Cancer Res 2007;13:2471–8.
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 1 dose-escalation trial. Lancet Oncol 2012;13:501–8.
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;63:225–34.
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;4:1777–90.
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;14:4316–25.
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;63:1307–17.
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;71:5296–306.
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;20:1886–94.
32. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Bone Cancer [Internet]. [Accessed 2015 May]. Updated as version 1: 2014 Aug 8. Available from: 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.

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Phase I Trial of a Yeast-Based Therapeutic Cancer Vaccine (GI-6301) Targeting the Transcription Factor Brachyury

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