Amendment

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Protocol Title: An Open Label Phase I Study to Evaluate the Safety and Tolerability of GI-6301 Vaccine Consisting of Whole, Heat-killed Recombinant Saccharomyces Cerevisiae (yeast) Genetically Modified to Express Brachyury Protein in Adults with Solid Tumors

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AM I

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: N/A

DEC Clearance Date: 12/10/2015

Protocol Version Date: 11/30/2015

Abbreviated Title: Ph 1 GI-6301 Yeast Brachyury Vaccine
NCI Protocol #: 12-C-0056 IBC#: RD-11-VIII-02
Version Date: November 30, 2015 Version I OBA#: 1107-1119

Title: An Open Label Phase I Study to Evaluate the Safety and Tolerability of GI-6301 a Vaccine Consisting of Whole, Heat-Killed Recombinant *Saccharomyces Cerevisiae* (Yeast) Genetically Modified to Express Brachyury Protein in Adults with Solid Tumors

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Investigational Agents:

Drug Name	GI-6301 Vaccine (Yeast-Brachyury)
IND Number	BB-IND # 14895
Sponsor	Center for Cancer Research, NCI
Manufacturer/Supplier	Celgene

Identifying words: vaccine therapy, dose limiting toxicity, maximum tolerated dose, clinical response, immune response.

PRÉCIS

Background:

- Vaccines based on recombinant heat inactivated yeast have been shown to be immunogenic and well tolerated in animals and humans.
- Using a computer-based differential display analysis tool to conduct global comparison of expressed sequence tag (EST) clusters in the Unigene database, the gene encoding for the transcription factor Brachyury was identified as highly represented in tumor-derived libraries and rarely observed in normal tissue-derived libraries. By using reverse-transcription followed by polymerase chain reaction (RT-PCR), investigators in the LTIB have identified the over-expression of Brachyury in gastrointestinal, bladder, kidney, ovary, uterus, and testicular carcinomas. Similar studies also found over-expression of Brachyury mRNA in cell lines of lung, colon and prostate cancers, but not in the majority of normal tissues tested, with the exception of expression in the testis, thyroid and low levels of expression in B cells pooled from multiple normal donors.
- Brachyury is a member of the T-box family of transcription factors, characterized by a highly conserved DNA-binding domain designated as T-domain. Data indicates that the transcription factor Brachyury confers on the tumor cells a mesenchymal phenotype, as well as migratory and invasive abilities and enhances tumor cell progression.
- A murine model of MC38 cells engineered to over express human Brachyury gene has demonstrated increased metastatic potential of Brachyury over-expressing MC38 cells.
- Brachyury specific T cells can lyse human cancer cells expressing Brachyury in an MHC restricted manner.
- GI-6301 (Yeast-Brachyury vaccine) has been tested *in vitro* and in the mouse model. These studies showed Brachyury-specific T cell responses and decreased metastasis in mice treated with vaccine.
- An ongoing study of a Hepatitis B vaccine (GS-4774) using the yeast platform (heat killed *Saccharomyces cerevisiae)* indicated safety of 80 YU dose (4 injection sites at 20 YU injections per site).

Objectives:

- The primary objectives are to:
    - Determine the safety and tolerability of escalating doses of GI-6301 (Yeast-Brachyury vaccine) a heat-killed yeast-based vaccine
    - Determine in an expanded cohort if a significant change in Brachyury specific T cells will be detectable post vaccine.

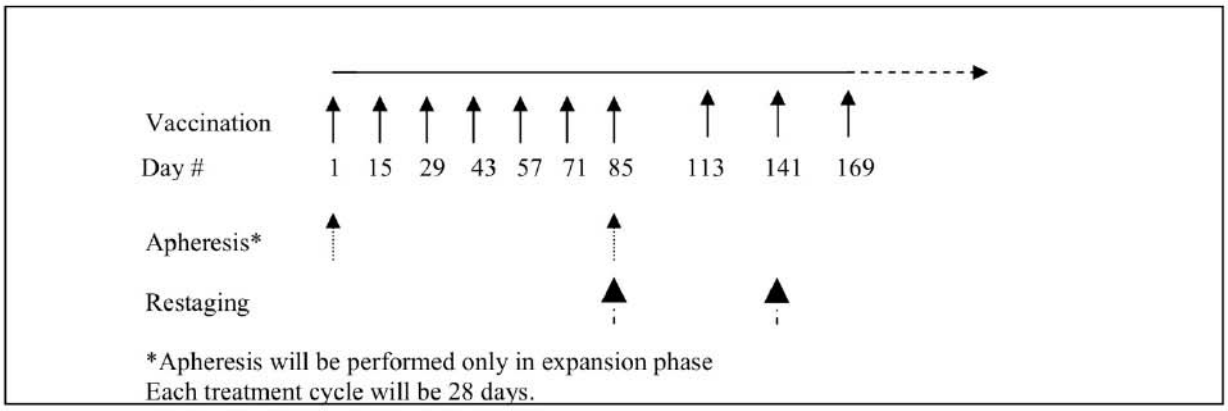
Eligibility:

- Adults with histologically proven metastatic or locally advanced solid tumors for which standard curative or palliative measures are no longer effective. Efforts will be made, as much as possible, to enroll patients with tumor types with known increased expression of Brachyury (such as lung, breast, ovarian, prostate, colorectal, or pancreatic).
- Adequate organ function as defined by liver, kidney, and hematologic laboratory testing.
- Patients with acquired immune defects, systemic autoimmune disease, concurrent use of steroids, pericardial mass > 1 cm, chronic infections, concurrent tricyclic antidepressant therapy, or allergy to yeast or yeast-based products will be excluded.

Design:

- This is an open label, phase I trial with sequential dose escalation cohorts of patients (3-6 patients per dose cohort) for 3 doses of GI-6301 (Yeast-Brachyury vaccine).
- GI-6301 (Yeast-Brachyury vaccine) will be administered subcutaneously at 4 sites biweekly for 7 visits (day 1, 15, 29, 43, 57, 71, 85), then monthly until patients meet off-study criteria (patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to receive vaccine once every 3 months instead of monthly).
- All patients on a given dose level will have completed 28 days on-study without DLT before enrollment can begin on the next dose level or on the expansion phase (see statistical analysis section).
- **Expansion Phase:** 10 additional patients will be enrolled on the MTD dose level (or the highest dose level explored in the event that a true MTD is not reached), receiving the same treatment regimen, to assess for immunologic responses and clinical responses.
- **Amended dose escalation:** 10 patients will be enrolled at an additional dose level (80 YU per dose, 4 injection sites at 20 YU per site) to determine the safety of this dose level.
- Up to 33 total patients may be required to complete enrollment of this study.

Treatment Schema



Dose Escalation Schema

Dose Level	Dose and Schedule
1 N = 4	1 Yeast Unit (1 YU = 10^7 yeast particles) per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until progression
2 N = 3	4 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression
3 N= 13	10 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression
4 N = 10	20 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 PRIMARY OBJECTIVE(S):

1.1.1.1 To determine the safety and tolerability of escalating doses of GI-6301 (hereafter referred to as Yeast-Brachyury vaccine), a heat-killed yeast-based vaccine that targets tumor cells that express Brachyury.

1.1.1.2 Determine in an expanded cohort if a significant change in T-cell precursors will be detectable as measured by an increase in Brachyury-specific T cells in ELISPOT assay and proliferation in response to Brachyury protein.

1.1.2 SECONDARY OBJECTIVE(S):

1.1.2.1 To evaluate evidence of clinical benefit, such as progression-free survival, clinical radiographic response (modified irRC and RECIST 1.1 as described in section 6.2 and Appendix A in section 12.1 and Appendix B in section 12.2; for patients with Chordoma, modified Choi criteria will be used, described in section 6.2 and Appendix F in section 0), reduction in serum markers, and/or reduction in circulating tumor cells.

1.1.2.2 To evaluate parameters of general immune activation: Frequency of immune cell subsets in peripheral blood (CD8 memory/effector cells, CD4 memory/effector cells, Tregs, NK cells, DCs) and changes in serum levels of cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, TGF-ß, etc.).

1.2 BACKGROUND AND RATIONALE:

1.2.1 DESCRIPTION AND IDENTIFICATION OF BRACHYURY

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

1.2.2 BRACHYURY FUNCTION IN EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT)

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

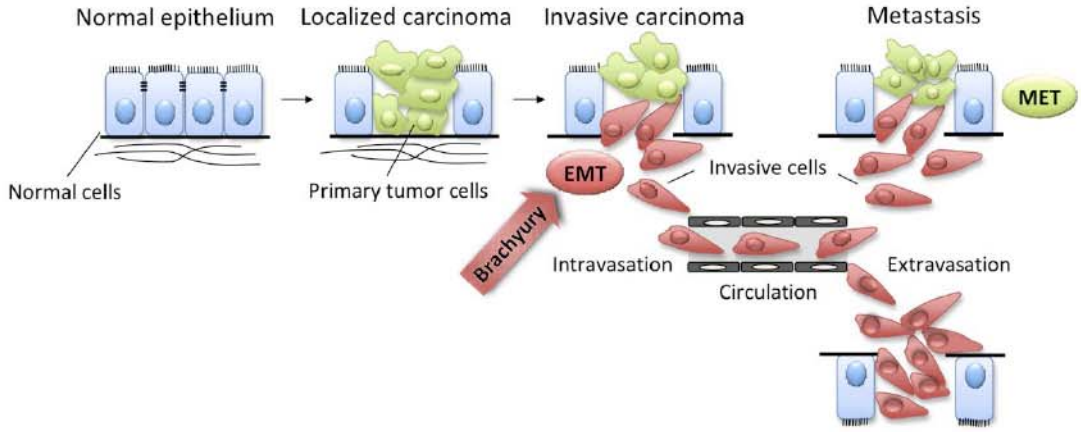


Figure 1. Role of Brachyury in epithelial to mesenchymal transition during tumor progression

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

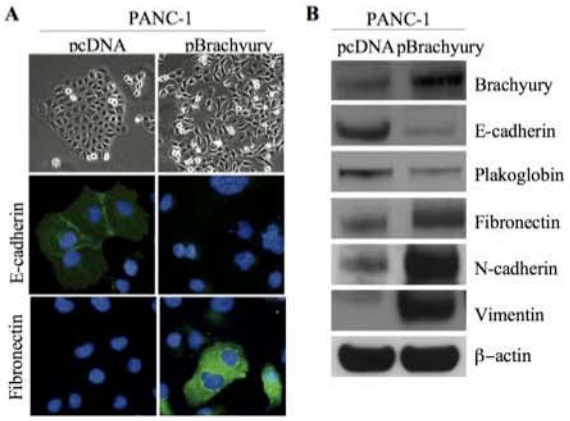


Figure 2. Brachyury alters morphology and EMT marker expression in human pancreatic cancer epithelial tumor cells. (A) Bright field images of PANC-1-pcDNA (control) and PANC-1-pBrachyury cells and immunofluorescent analysis of EMT markers; merged images with DAPI stained nuclei are shown. **(B)** Western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

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

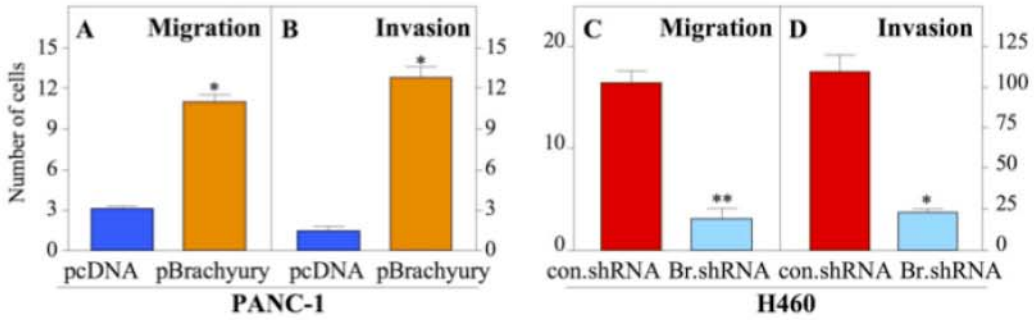


Figure 3. Brachyury induction of migration and invasion. (A and B) Brachyury induces a highly migratory and invasive EMT phenotype *in vitro* in human PANC-1 pancreatic carcinoma cells. **(C and D)** Silencing Brachyury expression in H460 human lung carcinoma cells reduces both migratory and invasive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

Brachyury expression also negatively modulated tumor cell cycle progression and correlated with low expression of cyclin D1, and carcinoma cells silenced for Brachyury expression grew at a greater rate *in vitro* (Fig. 4A). These Brachyury-silenced cells grew at a comparable rate to control cells when implanted subcutaneously in athymic mice (Fig. 4B); however, they had a diminished capacity to metastasize to the lungs from the primary, subcutaneous tumor, or to form experimental lung metastases when injected i.v. (Fig. 4C). Collectively, these results demonstrate that the transcription factor Brachyury confers on the tumor cells a mesenchymal phenotype as well as migratory and invasive abilities and enhances tumor progression[17].

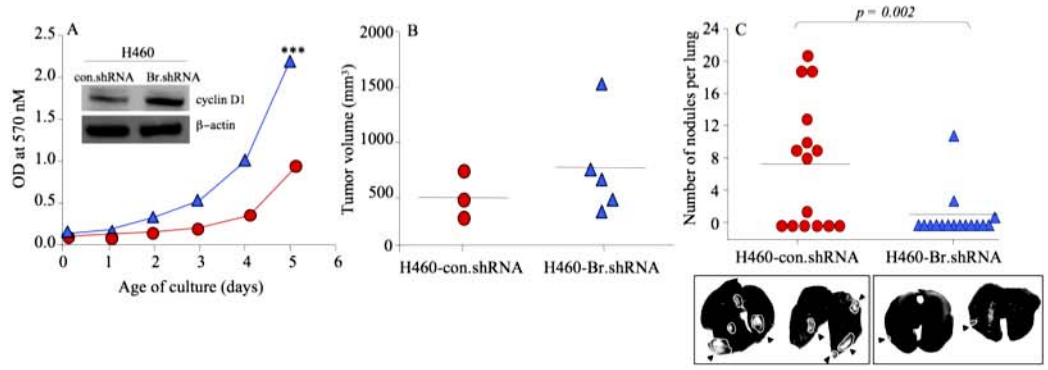


Figure 4. Brachyury inhibition with silencing RNA reduces tumor metastases. Compared to control (con.shRNA, circles), human H460 lung carcinoma cells silenced for Brachyury (Br.shRNA, triangles): **(A)** proliferate at a faster rate *in vitro* ($p = 0.003$); **(B)** grow at a similar rate when injected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

1.2.3 Analysis of Brachyury Expression in Human Tumors and Normal Tissues

RT-PCR: By using reverse-transcription followed by polymerase chain reaction (RT-PCR), investigators in the LTIB have identified the over-expression of Brachyury in gastrointestinal, bladder, kidney, ovary, uterus, and testicular carcinomas. Similar studies also found over expression of Brachyury mRNA in cell lines of lung, colon and prostate cancers, but not in the majority of normal tissues tested, with the exception of expression in the testis, thyroid and low levels of expression in B cells pooled from multiple normal donors (Table 2, see detailed analysis below) .

Immunohistochemistry (IHC): IHC analysis of Brachyury expression using an anti-Brachyury monoclonal Ab confirmed the tumor specificity of this transcription factor. Expression of Brachyury was found in approximately 40% of primary lung tumor tissues, including adenocarcinoma (48% positive), squamous carcinoma (25% positive) and others (50% positive) (Table 1, Fig. 5A-D). Over-expression of Brachyury was also observed by IHC analysis in breast primary tumor tissues and metastatic lesions. Brachyury was expressed by 16 of 20 primary tumor samples (80%) of infiltrating ductal adenocarcinomas. Moreover, Brachyury was highly expressed in 8 out of 8 metastatic lesions of breast cancer, obtained from lymph nodes (4), pleura (1), bone (2), and brain (1) (Fig. 5E).

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

Table 1. Brachyury protein expression analyzed by IHC

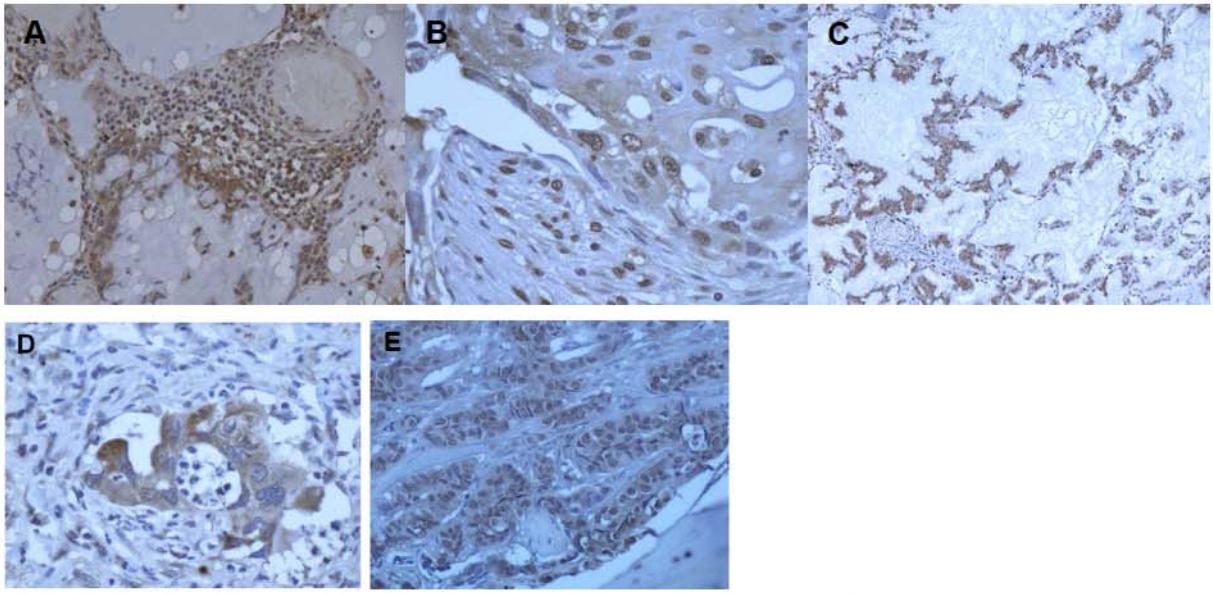


Figure 5. Tissue sections stained for Brachyury expression, corresponding to a lung adenocarcinoma (A); a squamous carcinoma (B); and a bronchioalveolar carcinoma (BAC), mucinous type (C). Lung tumor cells invading a blood vessel, positive for Brachyury expression (D). IHC staining for Brachyury in a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

IHC analysis of normal tissues obtained from non-cancer subjects demonstrated Brachyury expression in: 0/5 lung, 0/3 heart, 0/3 brain, 0/3 liver, 0/3 kidney, 0/3 spleen, 0/3 skeletal muscle, 0/1 adrenal gland, 0/1 skin, 4/6 thyroid, and 3/3 testis analyzed (Table 2).

Table 2. Brachyury protein expression analyzed by Brachyury-specific Monoclonal Antibody and/or PCR

Normal Human Tissues Negative for Brachyury Expression	
Adrenal	Liver
Blood Cells	Lung
Bone Marrow	Lymph Node
Breast	Ovary
Cerebellum	Pancreas
Cerebral Cortex	Placenta
Colon	Prostate
Endothelium	Skin
Gastrointestinal Tract	Spleen
Heart	Striated Muscle
Kidney (glomerulus, tubule)	Thymus

Normal Human Tissues Positive for Brachyury Expression
Testes (3/3)
Thyroid (4/6)

Detailed Analysis of Normal Human Tissues Expressing Brachyury: The expression of Brachyury mRNA in normal B cells was further evaluated in CD19+ B cell fractions isolated from various healthy donors; weak amplification was observed in 4/9 samples analyzed by RT-PCR. These results at the RNA level, however, contrasted with data obtained by immunohistochemistry analysis in normal spleens and lymph nodes, which resulted negative for the expression of Brachyury protein (Table 2). Moreover, the cytotoxic lysis of normal B cells was evaluated by using Brachyury-specific T cells as effectors; no lysis was observed with any of the normal B cells purified from the blood of five different healthy donors[3]. We have determined that EBV infection of human B cells enhances Brachyury expression; approximately 1 in 10^5-10^6 human B cells have latent EBV infection.

There was expression of Brachyury in testis (3 of 3 positive) (Table 2). However, due to the blood-testis barrier, a paucity of antigen presenting cells within the testis and a lack of MHC molecules on testicular cells, proteins expressed within the testis are considered immune privileged[18]. Cancer testis antigens form a class of proteins expressed on tumor cells and the testis and multiple vaccines have been generated against these antigens without immune related adverse events within the testis.

The expression of Brachyury protein was also detected in 3 of 4 thyroid tissue lysates evaluated by Western Blot. These results at the protein level contrasted with the expression of Brachyury at the mRNA level which was negative in 3/3 individual thyroid tissues tested by RT-PCR. Altogether, these results indicate Brachyury expression in 7/13 thyroid tissues analyzed. Brachyury expression, relative to GAPDH, is either less than or the same as both CEA and PAP in normal testis and thyroid tissue (Figure 6). Based on previous experience with vaccines directed against CEA and PAP (including the only FDA approved therapeutic cancer vaccine), which have been able to generate CEA and PAP specific T cells, and given the relatively lower expression of Brachyury in comparison, it seems unlikely that Brachyury presence in these tissues will lead to clinically significant auto-immune toxicity.

Testis, thyroid, pituitary, spinal cord showed low levels of mRNA Brachyury expression by RT-PCR. These RNA expression levels were far lower than that observed using probes for PAP, CEA and MUC1, which are antigens in widely evaluated vaccines with good safety profiles (**Figure 6**).

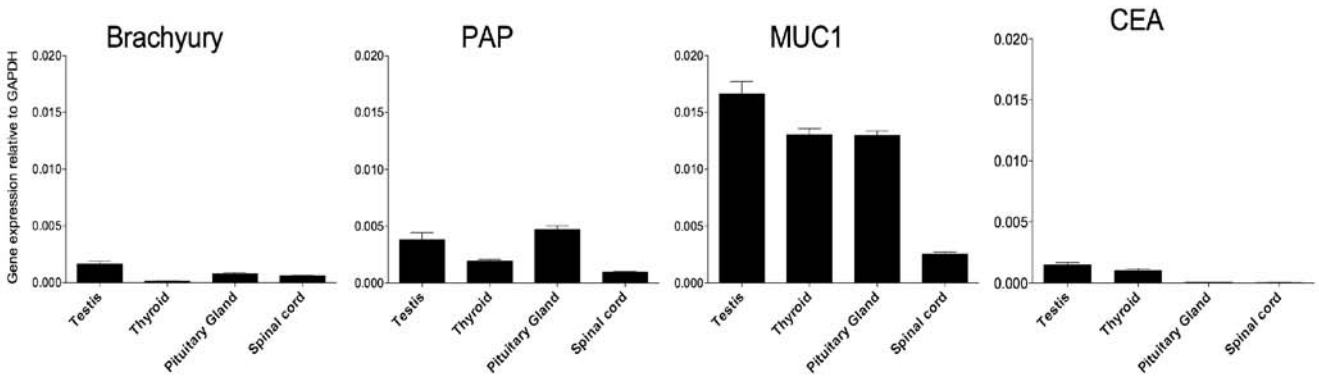


Figure 6. Level of Brachyury expression in comparison with other TAAs evaluated clinically, including PAP of Sipuleucel-T.

Brachyury Expression Associated with Stage and Grade of Tumor: Real-time PCR analysis of Brachyury mRNA expression in multiple human lung tumor tissues showed that the percentage of tumors positive for Brachyury expression increases with tumor stage; 30/48 (62.5%) of Stage II–IV lung cancer biopsies showed over-expression of Brachyury vs. 12/32 (37.5%) of Stage I lung cancers and 2/16 (12.5%) of histologically normal lung biopsies from lung cancer patients (Fig. 7A)[17]. Similar analysis conducted with primary tumor breast tissues demonstrated a significant difference in Brachyury mRNA expression between normal and primary tumor breast tissues (0.0% and 21.2% expression, respectively; p=0.003). Brachyury was expressed by a higher percentage of breast tumors of a poor grade of differentiation (Nottingham histological grade 3; 22.8%) compared to tumors of grade 1 or 2 (0% or 11.1%, respectively) (Fig. 7B).

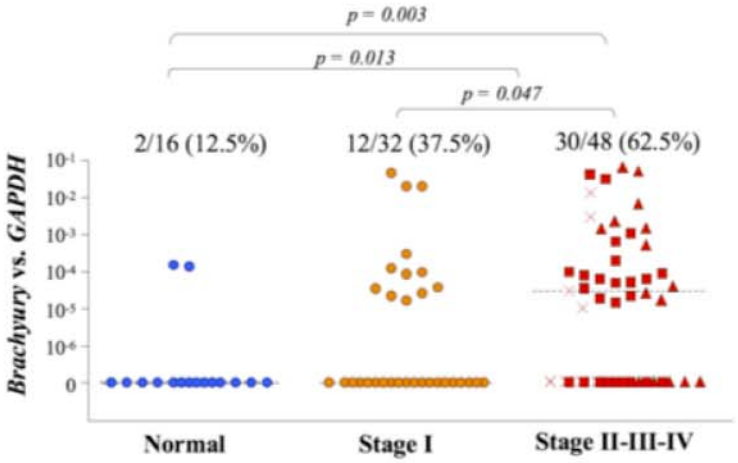


Figure 7A. Brachyury expression in human lung tumor tissues. Real-time PCR was performed for Brachyury on lung tumor tissue cDNA from 80 lung cancer patients of the indicated stages of disease. The Stage II, III, and IV cDNA samples are further represented by the symbols ■, ▲, and ✕, respectively. As controls, 16 samples of "normal" lung cDNA were analyzed, each obtained from a histologically normal section of lung from a lung cancer patient. See (17) for details.

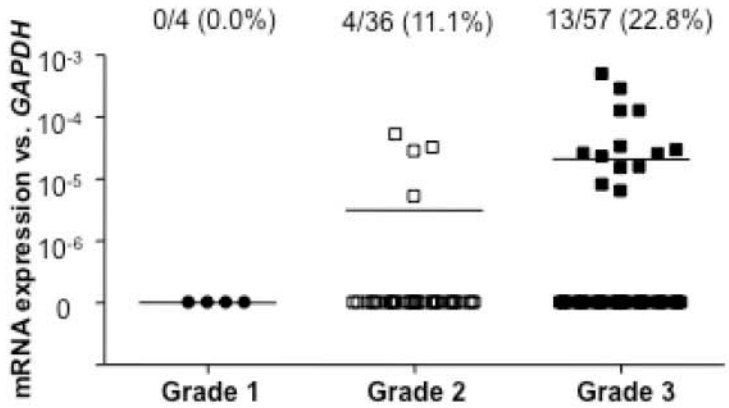


Figure 7B. Expression of Brachyury mRNA in breast tumor tissues. Brachyury mRNA expression, as determined by real-time PCR, is shown for breast primary tumor tissues grouped by histological tumor grade (Nottingham grading).

1.2.4 Brachyury Confers Chemotherapy Resistance

The LTIB has developed human lung carcinoma cell lines in which the expression of Brachyury has been knocked down (shBrachyury) and others transfected to stably over-express Brachyury (phBrachyury). All experiments contain control cells stably transfected with empty vector (for over-expression experiments) or a non-targeting vector for knock-down experiments. Matched pairs of lung cancer lines have recently been evaluated for their sensitivity to chemotherapy agents. Brachyury down-regulation significantly enhances sensitivity to chemotherapy, while stable over-expression of Brachyury in human lung cancer cells is associated with a significant decrease in cell death mediated by various chemotherapy agents, *in vitro* (Fig. 8)

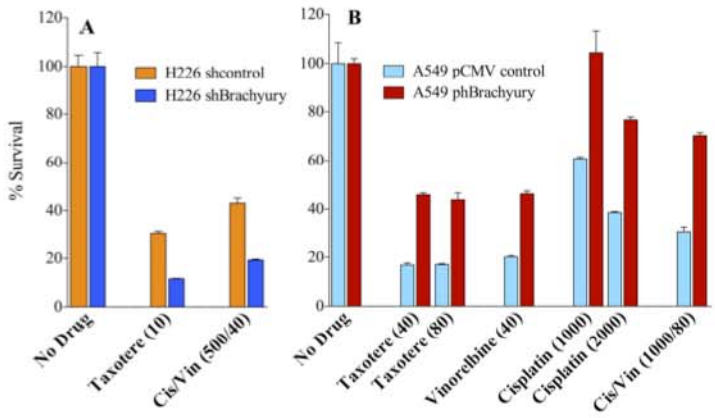


Figure 8. Expression of Brachyury in human carcinoma cells induces resistance to chemotherapy. (A) Silencing of Brachyury (shBrachyury) in H226 human lung carcinoma cells resulted in enhanced sensitivity to chemotherapy. **(B)** A549 lung cancer cells were stably transfected with a Brachyury vector (phBrachyury)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

1.2.5 Recombinant Yeast Vaccines

One of the main advantages of employing heat-killed yeast vaccines is their ease of development. We have thus been able to evaluate several novel yeast-TAA constructs immunologically quite rapidly and efficiently. Another advantage is safety; heat-killed yeast can be used in combination with chemotherapy and potentially in patients with preneoplastic lesions and/or in patients in the neoadjuvant or adjuvant setting. The excellent tolerability profile of yeast vaccines in humans has been observed in multicenter trials conducted by GlobeImmune with GI-5005, a yeast expressing hepatitis C proteins, in patients with hepatitis C[19,20] and in pancreatic cancer patients with GI-4000, a yeast expressing mutated Ras proteins[21], and in our trials with GI-6207, a yeast expressing carcinoembryonic antigen (CEA). Heat-killed yeast can also be used to vaccinate repeatedly without any host neutralizing activity[22]. The recombinant yeast is simply a protein delivery vehicle that is efficiently taken up by DCs, resulting in the release of protein in the cytoplasm for processing and MHC loading[23,24].

The appropriate maturation of DCs is important for any vaccine delivery vehicle[25-27]. Recombinant heat-killed yeast can mature murine DCs, and yeast-CEA can efficiently activate murine T cells *in vitro*[28]. We have also recently shown[22] that vaccination of CEA tumor-bearing CEA-Tg mice with heat-killed yeast- CEA vaccine resulted in CEA-specific T-cell responses and anti-tumor activity. Studies in the LTIB have shown[29] that control yeast or yeast containing protein for a TAA (yeast-CEA) can efficiently activate human DCs, resulting in increases in surface expression of numerous costimulatory molecules and MHC class I and II, and increased production by DCs of a range of cytokines and chemokines such as IL-12p70, TNF-γ, IFN-γ, and IL-8. We also showed that human DCs treated with yeast-CEA can efficiently generate and activate CEA-specific T-cell lines capable of lysing CEA+ human tumor cells. Gene expression profiles of human DCs treated with yeast-CEA show[29] increased expression of numerous genes involved in the production of chemokines and cytokines and their receptors, and genes related to antigen uptake, antigen presentation, and signal transduction.

A phase I study using the heat-killed yeast vaccine platform is ongoing at the NCI. A dose escalation study of yeast-CEA vaccine has completed enrollment and found no dose-limiting toxicity. Five patients have had stable or decreased serum CEA levels and stable disease by

RECIST criteria (unpublished data). Only one patient, with pleural and pericardial metastases, has had a grade 3 toxicity (see section 1.2.10).

1.2.6 MOUSE MODEL FOR YEAST-BRACHYURY

Development of a murine model for treatments directed against Brachyury has been difficult due to the limited expression of Brachyury in murine normal tissues and cancer cell lines, with the exception of the embryonic cancer cell line, P19, which is significantly positive for Brachyury expression. To overcome this issue, we sought to generate a murine cell line that over-expressed Brachyury. Since human and murine Brachyury proteins share 91% identical amino acids, the colon tumor MC38 cell line was stably transfected with a plasmid encoding for full length human Brachyury (phBrachyury) or an empty vector (pcDNA). Brachyury-overexpressing MC38 cells exhibited enhanced migration and invasion, and had a diminished growth rate *in vitro* (Fig. 9A). *In vivo* however, MC38-pBrachyury cells formed significantly more experimental lung metastases than MC38-pcDNA control cells, when administered i.v. (Fig. 9B).

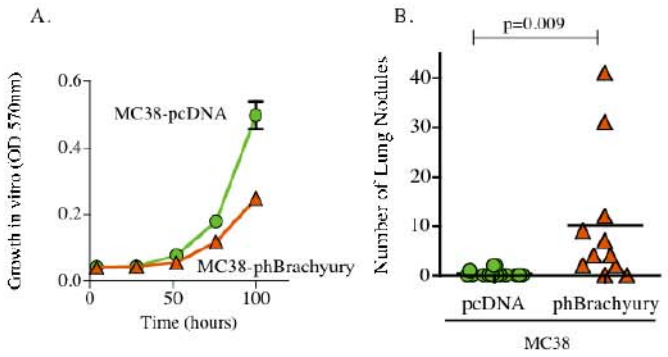
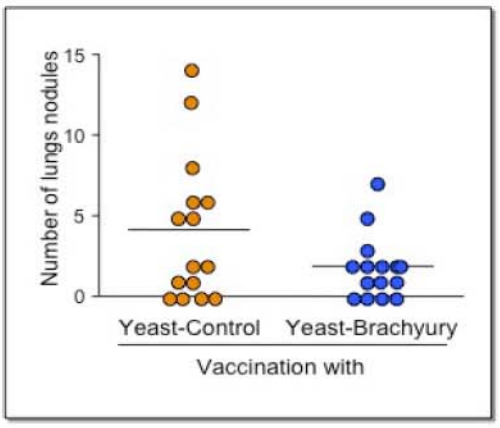


Figure 9. MC38 colon carcinoma cells were transfected with control plasmid (pcDNA) or plasmid encoding Brachyury (phBrachyury). (A) MC38 (Brachyury) tumor cells grow at a slower rate than control MC8 *in vitro*. **(B)** When injected i.v., MC38 (Brachyury) tumor cells resulted in significantly greater (p=0.009) number of lung nodules on day 40 than plasmid control MC38.

MC38 cells over-expressing human Brachyury were subsequently used to evaluate the effectiveness of the Yeast-Brachyury vaccine, *in vivo*. Mice were injected i.v. with 1 x 10^6 MC38-phBrachyury cells via tail vein on day 0, and vaccinated on day 4 with Yeast-Brachyury or Yeast-Control (1 YU x 4 sites, weekly boosters). At sacrifice (day 36-48), lungs were inflated with India ink, weighed, and the number of metastasis evaluated. There was a trend towards reduction of number of lung metastasis among animals vaccinated with Yeast-Brachyury vs. Yeast-control (Fig. 9), with 13.3% and 46.7% of mice treated with Yeast-Brachyury or Yeast-control, respectively, having ≥ 5 lung metastasis (Fig. 10).



Vaccine treatment	Animals bearing ≥ 5 lung nodules (%)
Yeast-Control	7/15 **(46.7%)**
Yeast-Brachyury	2/15 **(13.3%)**

Figure 10. MC38 colon carcinoma cells overexpressing Brachyury were inoculated i.v. via the tail vein on day 0. On day 4, mice were vaccinated with yeast-Brachyury or yeast-Control and boosted 4x at weekly intervals. Mice were sacrificed 1 week after the last vaccination. Number of lung nodules was evaluated.

We have also shown that vaccination of C57 BL mice with Yeast-Brachyury vaccine induces Brachyury-specific T-cell responses, *in vivo*. Mice were vaccinated at 4-weekly intervals with 1YU x 4 sites of Yeast-Brachyury or Yeast-Control for a total of 3 vaccinations. A Brachyury-specific CD4+ T-cell response was observed in animals vaccinated with Yeast-Brachyury vector (Fig. 11).

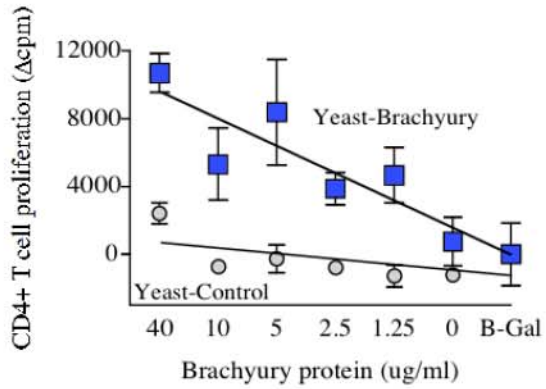


Figure 11. Ability of yeast-Brachyury to induce Brachyury-specific CD4+ T-cell responses. C57 BL mice were vaccinated at 4 weekly intervals with yeast- Brachyury or control yeast. Proliferation assays were carried out with purified Brachyury protein (from insect cells) and β-gal protein as control.

1.2.7 TOXICOLOGY STUDY FOR YEAST-BRACHYURY

There is 91% homology at the amino acid level between the human and murine Brachyury proteins. Toxicology studies were conducted in Balb/c mice given multiple vaccinations with yeast-Brachyury. Mice were vaccinated weekly with yeast-Brachyury, Yeast-WT (wild type, negative control) or saline (PBS, vehicle control) for 12 weeks (5 mice per group). During the course of vaccination no mice exhibited any signs of morbidity or respiratory distress, or had significant weight decreases. One week following the final vaccination, mice were sacrificed and toxicology assessed under GLP conditions by an outside contractor. The tests included CBC and differential (20 blood profile tests), 7 serum/liver enzyme assays, 5 assays for autoimmune

antibodies, and pathologic evaluation of brain, skin, kidneys, heart, liver, femur, bone marrow, colon, cartilage, and thyroid tissues.

No yeast-Brachyury related changes in clinical pathology data were noted. Blood cell populations from mice vaccinated with yeast-Brachyury did not differ significantly from those of mice vaccinated with yeast-WT or PBS, i.e., all were in the normal range. There were no significant differences in serum/liver enzymes between groups. There were no detectable autoimmune antibodies in sera samples from mice that received the yeast-Brachyury vaccinations or in the mice receiving control vaccinations. No gross findings were noted at necropsy; all collected tissues were essentially normal.

We have also evaluated tissue expression in non-human primates. Expression of Brachyury mRNA in Rhesus monkey adult brain and small intestine as well as testis were significantly elevated whereas there is no expression in human GI tract or brain. Thus, this model is not appropriate for screening for potential toxicity in humans.

1.2.8 Induction of Brachyury-specific Human T-cell Responses

By using a MHC-peptide binding prediction algorithm, several human HLA-A2 binding Brachyury peptides have been identified[3]. One of these peptides was successfully employed to generate human Brachyury-specific CD8+ T-cell lines from PBMC of both normal donors and cancer patients. These T cells could efficiently lyse Brachyury peptide-pulsed targets (Fig. 12A), and lyse human carcinoma cell lines endogenously expressing Brachyury in an MHC-restricted manner (Fig. 12B). The level of lysis was associated with the level of expression of Brachyury (Fig. 12C).

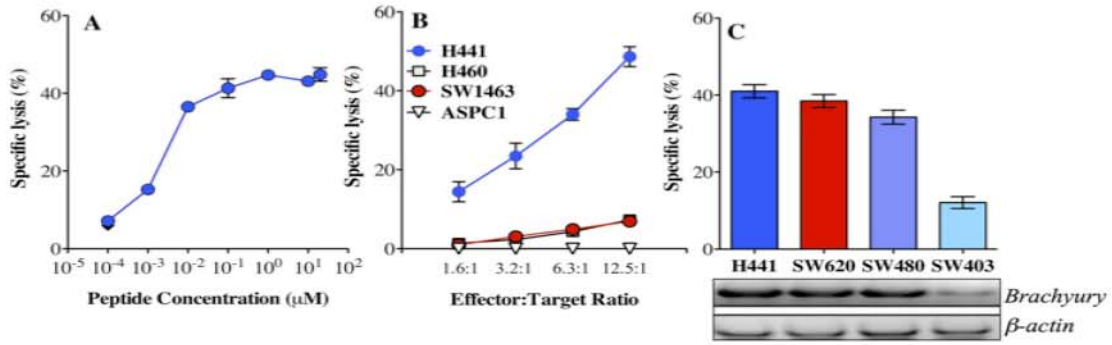


Figure 12. Cytolytic activity of human Brachyury CD8+ T cells. CD8+ T cells were generated from PBMC of a healthy donor using autologous DCs pulsed with a 9-mer Brachyury peptide. **(A)** Ability of T cells to lyse human T2 targets pulsed with various concentrations of Brachyury peptide. **(B)** Brachyury CTLs lyse H441 lung cancer cells (Brachyury+/HLA-A2+), and do not lyse lung cancer H460 (Brachyury+/A2–), colorectal cancer SW1463 (Brachyury–/A2+), or pancreatic cancer AsPC-1 (Brachyurylow/A2–). **(C)** Brachyury-specific T cells, derived from a carcinoma patient, lyse various Brachyury+/HLA-A2+ carcinoma cell lines. See (35) for details.

1.2.9 Induction of Human T cells by Yeast-Brachyury

We previously showed that human Brachyury-specific CD8+ T-cell lines could be generated from the blood of healthy individuals and cancer patients by using DCs pulsed with a 9-mer peptide epitope of Brachyury[3]. In order to investigate the ability of Yeast-Brachyury to expand Brachyury-specific T cells from the blood of normal donors, dendritic cells (DCs) were prepared

from peripheral blood mononuclear cells (PBMCs) by culture for 5 days in the presence of GM-CSF and IL-4, and subsequently incubated with Yeast-Brachyury or Yeast-Control. The DCs were used as APCs for stimulation of autologous T cells. At the end of the *in vitro* stimulation, T cells were stained with a control tetramer or a tetramer specific for a Brachyury peptide (Table 3).

Table 3. Expansion of Brachyury-specific T cells with Yeast-Brachyury vector treated DCs

Donor	Stimulation	Control Tetramer	Brachyury Tetramer
1	Brachyury Yeast	0.01	**1.24**
2	Brachyury Yeast	0.02	**0.36**
3	Brachyury Yeast	0.10	**2.57**
4	Brachyury Yeast	0.05	**0.33**

These results indicate that the Yeast-Brachyury vector is able to effectively expand CD8+ Brachyury-specific T cells from the blood of normal donors, as compared to the Yeast-Control vector. Subsequent studies demonstrated that isolated CD8+ T cell fractions were able to lyse Brachyury-positive tumor cells *in vitro* (Fig. 13).

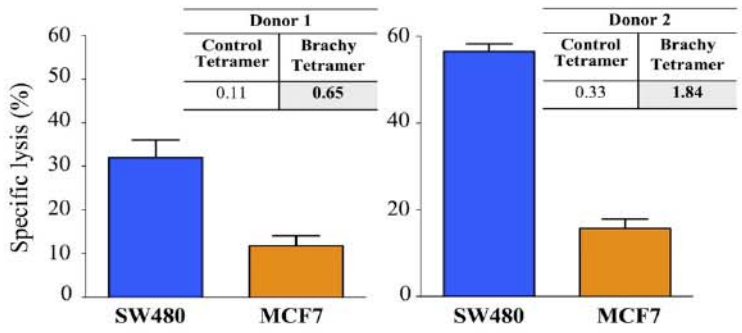


Figure 13. T cells expanded with Yeast-Brachyury lyse Brachyury-positive tumors. DCs from PBMC of two healthy donors were treated with yeast-Brachyury vector (vs. control yeast), and then exposed to DCs pulsed with Brachyury 9-mer peptide. Resulting T cells bound selectively to Brachyury tetramer, and were capable of lysis of SW480 colon carcinoma cells (A2+/Brach^{high}), with minimal lysis of MCF7 breast carcinoma (A2+/Brach^{low}).

Similar experiments were conducted with blood of two breast cancer patients post-vaccination with rV-, rF-CEA-MUC1-TRICOM vectors. As shown in Table 4, Yeast-Brachyury treated DCs were able to expand Brachyury-specific T cells that have the ability to lyse Brachyury-positive tumor cells.

Table 4. Tetramer staining of CD8$^+$ T cells from breast cancer patients following stimulation with Brachyury-yeast DCs and Brachyury-peptide-pulsed DCs.

Patient	Stimulation with:	Control Tetramer	Brachyury Tetramer
Pt 01	Brachyury Yeast DC/Tp2	0.18	**0.89**
Pt 10	Brachyury Yeast DC/Tp2	0.11	**0.36**

In a previous clinical study combining anti-CTLA4 monoclonal antibody (ipilimumab) with PSA-TRICOM, two patients developed Brachyury specific T-cell responses (measured by ELISPOT) through antigen cascade. Moreover, two patients in the yeast-CEA clinical trial had developed Brachyury specific T-cell responses post-vaccination, as determined by ELISPOT. In all of these cases, there was no evidence of immune-mediated thyroid or testicular toxicity and no evidence of B-cell disorders.

1.2.10 Prior Human Experience with Yeast Vaccine Therapy

Six studies using yeast based vaccines were conducted by GlobeImmune with enrollment of at least 325 subjects with varied malignancies, including pancreatic and colorectal cancer, NSCLC and metastatic tumors over-expressing CEA. Although no DLTs were observed in phase 1 trials, there have been up to 9 DLT type events (3%) (grade 3-4 events that are at least possibly related for which treatment was stopped) (see Table 5). Four of the 9 DLT-type events mentioned above occurred in a blinded study, and it is not yet known if these cases occurred in subjects in the placebo group or treatment group. However, this trial is monitored in an unblinded manner by an independent DSMB which has indicated adequate safety parameters for this study to continue.

Table 5. Dose-limiting toxicities in yeast-based vaccine trials.

Trial	Population	# Treated with Vaccine	# of Dose Limiting Toxicities
GI-4000-01	Pancreatic, colorectal, NSCLC	33	0
GI-4000-02	Pancreatic cancer	88	4 DLT type events (grade 3-4 events that are at least possibly related and for which treatment was stopped) This includes all subjects treated with blinded therapy (169 subjects), some may have received placebo.
GI-4000-03	NSCLC	24	1 (injection site pain grade 3)
GI-4000-05	Colorectal cancer	2	0
GI-5005	HCV	154	Phase I no DLT, Phase II, 4 DLT type events (grade 3-4 events that are at least possibly related and for which treatment was stopped)
GI-6207	Metastatic tumors over-expressing CEA	25	0

1.2.11 Yeast Vaccine Therapy is Well Tolerated at 80 YU Doses

The previous Sponsor, Globeimmune, reported on a Phase I study using GS-4774, a yeast based HBV-specific therapeutic vaccine. Doses of 10 Yeast Units (YU), 40 YU or 80 YU/dose were administered to 60 healthy volunteers. GS-4774 was tolerated with no serious adverse events.

Twenty patients received the 80 YU dose in total. Ten patients received the dose weekly for 5 doses followed by one monthly dose at day 57 while the other 10 received 3 monthly doses (days 1, 29, and 57). There was one treatment discontinuation due to skin reaction in the 80 YU cohort. The 80 YU dose level was otherwise well tolerated with a similar level of side effects as compared to the 10 YU and 40 YU dose levels.

1.2.12 Summary of Rationale

1) Brachyury is a member of the T-box family of transcription factors that is over-expressed in cancer cells compared with normal tissue and has been linked to cancer cell resistance and metastatic potential.
2) A murine model has demonstrated increased metastatic potential of MC38 cells engineered to over-express human Brachyury gene when compared to unmodified MC38 cells.
3) Brachyury specific T cells can lyse human cancer cells expressing Brachyury in an MHC restricted manner.
4) Brachyury specific T cells have been identified in patients after treatment with a vaccine targeting a different antigen (PSA-TRICOM and Yeast CEA).
5) Yeast based vaccines:
    - are heat killed and have a good tolerability profile with Yeast-HCV (GI-5005), Yeast-Ras (GI-4000), and our study with Yeast-CEA (GI-6207) vaccines.
    - induce specific T cell responses against the specified antigen.
6) Yeast-Brachyury vaccine (GI-6301) has been tested *in vitro* and in the mouse model described, finding:
    - Brachyury-specific T cell responses
    - Decreased metastasis in mice treated with vaccine.
7) Based on more recent findings indicating the safety of 80 YU dose with a different target, with amendment D, an additional 10 patients will be enrolled at the 80 YU dose level to confirm safety and immunogenicity with this vaccine at the 80 YU dose.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

Participants must meet the following criteria for participation:

2.1.1.1 Diagnosis: Patients must have histologically confirmed malignancy by the Laboratory of Pathology, NCI, that is metastatic or unresectable locally advanced malignant solid tumor. In the case of Chordoma, unresectable, locally recurrent, or metastatic tumors are acceptable for enrollment, given that this represents incurable disease. Efforts will be made, as much as possible, to enroll patients with tumor types with known increased

expression of Brachyury (such as lung, breast, ovarian, prostate, colorectal, pancreatic, or chordoma).

2.1.1.2 Patients may have disease that is measurable or non-measurable but evaluable disease (See Appendix A and Appendix B).

2.1.1.3 Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 at study entry (Karnofsky ≥ 70) (See Appendix C).

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

2.1.1.5 Prior Therapy: Completed or had disease progression on at least one prior line of disease-appropriate therapy for metastatic disease, or not be a candidate for therapy of proven efficacy for their disease.

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

2.1.1.6.1 Serum creatinine ≤ 1.5 x upper limit of normal OR creatinine clearance on a 24-h urine collection of ≥ 60 mL/min.

2.1.1.6.2 ALT and AST ≤ 2.5 x the upper limits of normal.

2.1.1.6.3 Total bilirubin ≤ 1.5 x upper limit of normal OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0.

2.1.1.6.4 Hematological eligibility parameters (within16 days of starting therapy):

- ✓ Granulocyte count ≥ 1,500/mm^3
- ✓ Platelet count ≥100,000/mm^3

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

2.1.1.7 Recovered completely (Grade 1 or baseline) from any reversible toxicity associated with recent therapy. Typically this is 3–4 weeks for patients who most recently received cytotoxic therapy, except for the nitrosoureas and mitomycin C for which 6 weeks is needed for recovery.

2.1.1.8 There should be a minimum of 2 weeks from any prior chemotherapy, immunotherapy and/or radiation.

2.1.1.9 Prior immune therapy is allowed.

2.1.1.10 Men and women of child-bearing potential must agree to use effective birth control or abstinence during and for a period of 4 months after the last vaccination therapy.

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

2.1.1.12 Patients with ER+ breast cancer being treated with hormonal therapy (selective estrogen receptor modulator or aromatase inhibitor) who have rising tumor markers as evidence

of disease progression or metastatic disease on scans may continue on hormonal therapy while being treated with vaccine.

2.1.1.13 Patients must be negative for yeast allergy skin test (see Appendix D).

2.1.1.14 Ability to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

Patients with any of the following will not be eligible for participation in this study:

2.1.2.1 Patients should have no evidence of immune dysfunction as listed below.

2.1.2.1.1 Human immunodeficiency virus (HIV) positivity due to the potential for decreased immune response to the vaccine.

2.1.2.1.2 Active autoimmune diseases requiring treatment or a history of autoimmune disease that might be stimulated by vaccine treatment. This requirement is due to the potential risks of exacerbating autoimmunity. However, patients with vitiligo, diabetes mellitus, and hashimoto's thyroiditis on appropriate replacement therapy may be enrolled.

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

2.1.2.2 History of allergy or untoward reaction to yeast-based products (any hypersensitivity to yeast-based products will be excluded).

2.1.2.3 Pregnant or breast-feeding women, due to the unknown effects of the Yeast Brachyury vaccine on the fetus or infant.

2.1.2.4 Serious intercurrent medical illness which would interfere with the ability of the patient to carry out the treatment program, including, but not limited to, inflammatory bowel disease, Crohn's disease, ulcerative colitis, or active diverticulitis.

2.1.2.5 Untreated brain metastases (or local treatment of brain metastases within the last 6 months) and or spinal cord metastasis.

2.1.2.6 Patients with pericardial masses >1 cm will be excluded.

2.1.2.7 Concurrent chemotherapy. (However, the following anti-tumor therapies will be allowed: Trastuzumab for HER2+ breast cancer and hormonal therapy for breast (e.g., selective estrogen receptor modulators, aromatase inhibitors) and prostate cancer (e.g., GnRH antagonists/agonists or antagonists and androgen receptor antagonists).

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

2.1.2.9 Patients requiring continuous tricyclic antidepressant therapy should be excluded due to the interference with the yeast skin test in creating false negative test results.

2.1.2.10 Participation in another interventional clinical trial within 28 days before start of study treatment.

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

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

2.2 RECRUITMENT STRATEGIES

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

2.3 SCREENING EVALUATION

The screening evaluation will be conducted within 16 days before starting treatment unless otherwise specified:

2.3.1 CLINICAL EVALUATION

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

2.3.2 LABORATORY STUDIES

To be performed at any time before protocol enrollment:

- Confirmation of diagnosis by Laboratory of Pathology at the NIH Clinical Center.
- HLA Typing

To be performed within 8 weeks prior to protocol enrollment:

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

To be performed within 16 days prior to protocol enrollment:

- Complete blood count plus differential and platelet count
- Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, TSH)
- Beta-HCG for women of child-bearing age (repeated within 48 hours prior to treatment). Females of childbearing potential (FCBP): A female of childbearing potential is a sexually mature woman who:
    - Has not undergone a hysterectomy or bilateral oophorectomy

- Has not been naturally postmenopausal for at least 24 consecutive months (i.e. has had menses at any time in the preceding 24 consecutive months).

In addition, patients, both male and female, should be willing to practice effective birth control during the study and for four months following the last study treatment, unless they have had a prior hysterectomy or bilateral oopherectomy.

- Urinalysis
- ANA titer
- CD4:CD8 ratio, CD3, 4, 8, 19 subsets and NK markers (baseline, about day 29 and around day 85 prior to vaccination)

2.3.3 YEAST ALLERGY SKIN TEST (SEE APPENDIX D, SECTION 12.4)

2.3.4 ELECTROCARDIOGRAM (ECG) WITHIN 28 DAYS PRIOR TO PROTOCOL ENROLLMENT

2.3.5 SCANS AND X-RAYS:

To be performed within 28 days prior to the protocol enrollment (may include):

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

2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at the Central Registration Office, CRO staff will call the pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 OVERVIEW OF STUDY

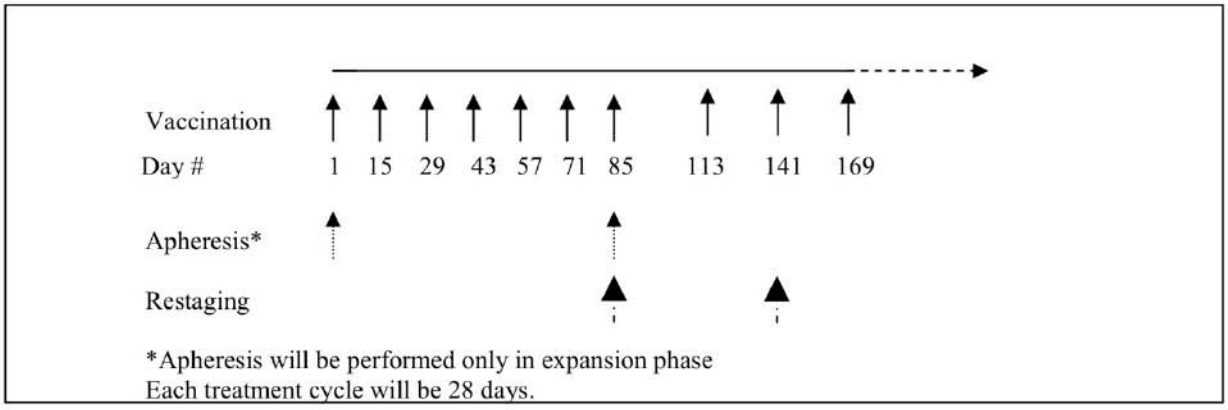
This is an open label, phase I trial with sequential cohorts of patients (3–6 patients per dose cohort) with dose escalation of a heat-killed Yeast-Brachyury vaccine. Yeast-Brachyury vaccine will be administered subcutaneously at 4 sites on 7 visits (Days 1, 15, 29, 43, 57, 71, 85 all +/- 2 days), then monthly until patients meet off-treatment criteria (patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to receive vaccine once every 3 months instead of monthly). The first 3 treatment cycles will consist of 2

vaccines and will last 28 days each; subsequent cycles will consist of one monthly vaccine treatment (or one vaccine every 3 months after 1 year as indicated above). Restaging of disease will be performed after the initial 3 cycles and then every 2 cycles thereafter (patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to be restaged every 3 cycles instead of 2 cycles).

For the first three patients on each dose level, there will be staggered enrollment of at least 2 weeks between patients.

Dose levels and responses to a vaccine are very different to drugs so we have no way of knowing a priori if a lower dose will result in more of a therapeutic anti-tumor immune response than a higher dose. Thus, up to 6 patients may be enrolled on each dose level in order to gain further information about safety and immunogenicity, providing that the maximally tolerated dose has not been exceeded.

Schema



The goals of the first phase of this study are to determine maximum tolerated dose (MTD) of Yeast-Brachyury vaccine on this schedule, and define the tolerability.

Toxicity in the 28 days following the first vaccination of Yeast-Brachyury vaccine of the last subject in a cohort (3^{rd} or 6^{th} subject, if applicable) in each dose level will be employed for decision-making (i.e., for dose-escalation and for maximum tolerated dose determination). Intra-patient dose escalation will not be allowed.

Once MTD is determined, the trial will enroll an additional cohort of subjects on the second phase of this study (up to 10) to further assess clinical and immunologic response to Yeast-Brachyury vaccine.

Based on new data, an additional dose cohort will be added (80YU dose) for safety and immunogenicity purposes.

3.1.2 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) will be defined as any one of the following:

- Any grade ≥ 4 hematologic toxicity,

- Any grade ≥ 3 non-hematologic toxicity that is possibly, probably or definitely related to Yeast-Brachyury vaccine, **except** transient (≤ 48 hour) grade 3 fatigue, local injection site reactions, flu like symptoms (i.e. myalgias, arthralgias, malaise), fever, headache, and laboratory abnormalities that are not associated with organ pathology,that is possibly, probably or definitely related to Yeast-Brachyury vaccine, occurring during the DLT evaluation period (28 days after administration of the first Yeast-Brachyury vaccine). Additional **exceptions include** generalized erythroderma or macular or papular rash lasting less than 7 days and not associated with desquamation.

All toxicity criteria are based on the NCI Common Terminology Criteria for Adverse Events, Version 4.0.

3.1.3 Study Stopping Rules

In addition, the study accrual will be halted, pending discussions with the IRB, study sponsor and FDA, if there is an occurrence of a grade 5 toxicity attributable to the treatment regimen, or if the MTD is exceeded in dose level 1.

Onset of delayed autoimmune toxicity that does not resolve to grade 1 or less with appropriate medical supportive measures within 7 days will also lead to halting accrual pending discussions with IRB, study sponsor and FDA.

3.1.4 Individual Patient Stopping Rules

Any ≥ grade 2 allergic and ≥ grade 2 autoimmune reaction(s) (**except** grade 2 or 3 thyroid or pituitary related immune toxicity that resolves clinically within 7 days of initiating supportive therapy).

Subjects who experience a DLT on study will not receive any further doses of Yeast-Brachyury vaccine.

If a subject experiences a clinically significant decrease in B cell number or function, pituitary function, thyroid function or neurologic toxicities attributable to vaccine, we would discontinue vaccine and treat symptomatically.

Possible toxicities include:

- Adrenal insufficiency as defined by clinical syndrome suggestive of adrenal insufficiency including hypotension, nausea, fatigue, depression, muscle weakness, vomiting, diarrhea, abdominal pain, weight loss, or renal insufficiency with corresponding laboratory changes including hyponatremia, hypoglycemia, decreased serum am cortisol and abnormal serum ACTH (should be low if adrenal insufficiency is secondary to pituitary dysfunction).
- Diabetes insipidus is clinically identified by production of large amounts of dilute urine, which is not improved by reduction of fluid intake and excessive thirst. Lab abnormalities consistent with central diabetes insipidus (secondary to

pituitary dysfunction) include hyponatremia, large volume dilute urine, and deficiency of vasopressin (AVP).

- Hypothyroid – clinically defined by fatigue, weight gain, confusion, lethargy. Lab abnormalities would include serum TSH level suppressed in combination with suppressed serum free T_4 (free thyroxine) and T_3 (triiodothyroxine). We would expect normal anti-Thyroid Peroxidase (TPO) antibody and anti-Thyroglobulin (TG) antibody as well, which would indicate a pituitary cause of hypothyroidism.

3.1.5 Dose Escalation

Three to six subjects will be enrolled to Dose level 1. Dose escalation will proceed as defined in Section 3.1.6.

Note: each dose listed is per injection site. Actual total dose per patient is 4 times the listed amount, i.e. 4 Yeast Units for dose level 1; 16 Yeast Units for dose level 2, 40 Yeast Units for dose level 3, and 80 Yeast Units for dose level 4.

The MTD dose level above (or the highest dose level explored in the event that a true MTD is not reached if sufficient DLTs were not found to declare the MTD), will be used as the dose and schedule in the expanded cohort.

Dose Level	Dose and Schedule
1 N = 4	1 Yeast Unit (1 YU = 10^7 yeast particles) per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until progression
2 N = 3	4 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression
3 N= 13	10 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression
4 N = 10	20 Yeast Units per site administered subcutaneously at 4 sites every 2 weeks x 7 courses, if no evidence of progression, then every 4 weeks until Progression

3.1.6 Definition of Maximum Tolerated Dose (MTD)

If unacceptable toxicity (DLT) is not observed in any subjects in a cohort (3 - 6 subjects per cohort) at the completion of the 28 days post 1st vaccine toxicity evaluation period, then subsequent cohorts will enroll 3 to 6 patients at the next dose level using the following standard 3+3 design; dose escalation will proceed within each cohort according to the following scheme.

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

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

3.1.7 Expansion Cohort

Ten additional patients will be enrolled on the MTD dose level (or the highest dose level explored in the event that a true MTD is not reached since sufficient DLTs were not found to declare the MTD), for a total of 16 patients at that dose level receiving the same treatment regimen, to assess for immunologic responses and clinical responses. At least 5 of these patients will be tested for $CD8^+$ responses by ELISPOT. All 10 patients will be tested for $CD4^+$ proliferation with Brachyury peptide. Alternatively, if the MTD is not found to be the optimal biologic dose (OBD) to induce the best immune response, the OBD will be used for the expansion cohort.

The inclusion and exclusion criteria will be modified for the expansion cohort to reflect the findings of safety identified in the dose escalation portion of the trial. For instance, because no toxicity of CNS, thyroid, pituitary, adrenal, and B cells was observed, the exclusion criteria related to the safety of those will be removed.

3.2 DRUG ADMINISTRATION

Yeast-Brachyury vaccine will be prepared and placed in syringes by Clinical Center Pharmacy personnel at the NIH. The total dose, based on assigned dose level, for each treatment course will be withdrawn into 4 separate syringes with a 25G (5/8") needle for subcutaneous administration at four separate injection sites. The 4 injections will be injected subcutaneously in the right and left inguinal areas and the right and left axillary chest walls (See Section 11.1.3 Preparation).

Following reconstitution with sterile water for injection, each vial of Yeast-Brachyury vaccine yields a suspension of 20 YU/mL, except for dose level 1. Dose level 1 will be reconstituted to yield a suspension of 10 YU/mL. Based on the assigned dose level, prepare the following for each treatment course:

Dose Level 1 (4 YU total dose): Prepare 4 syringes, each containing 1 YU diluted to a final volume of 0.5 mL with 0.9% sodium chloride.

Dose Level 2 (16 YU total dose): Prepare 4 syringes, each containing 4 YU diluted to a final volume of 0.5 mL with 0.9% sodium chloride.

Dose Level 3 (40 YU total dose): Prepare 4 syringes, each containing 10 YU (undiluted.)

Dose Level 4 (80 YU total dose): Prepare 4 syringes, each containing 20 YU (undiluted.)

If the dose is not administered within two hours of withdrawal into the syringe, a small amount of air should be withdrawn into each syringe, the syringes rotated to ensure adequate dispersion of the yeast-cell suspension in the syringe, and the excess air expelled from the syringe, prior to injection.

3.2.1 PRECAUTIONS:

See Sections 11.1.8 and 11.1.9

3.3 DOSE MODIFICATIONS

No dose modifications will be allowed on this study.

Any patient who experiences a DLT or allergic or autoimmune toxicity as defined in Section 3.9.1 will not receive any additional vaccinations.

If a scheduled dose of the vaccine is missed, the vaccine may be given within 7 days of the appointed time (which resets the appointed date for further vaccinations) or be considered a missed dose. If the patient has a delay in vaccination not due to toxicity, the vaccine may be delayed for up to 42 days without removal of the patient from study.

Dosing Delay: Patients should have resolution to ≤ grade 1 or return to baseline of all toxicities prior to the start of the next injection of the vaccine. If > grade 2 toxicity attributed to vaccine persists for > 28 days, the patient will not receive further vaccine inoculations and will be removed from protocol therapy.

3.4 CRITERIA FOR STARTING A NEW CYCLE

Patients who have treatment-related toxicity must have recovered to ≤ grade 1 toxicity in the parameters used to assess levels of organ function required for eligibility (see section 2) after each vaccination in order to receive a subsequent vaccination. Follow-up will be performed during each vaccination and blood-draw interval for immunologic testing as described in Appendix E in Section 12.5. If patient has ≤ grade 2 toxicity not related to study drug and the patient is clinically stable, the patient may be retreated at the discretion of the investigator.

Subjects who meet the following criteria are eligible to receive an additional cycle of Yeast-Brachyury vaccine:

- ✓ No progressive disease (as defined in sections 6.2 and 12.1),
- ✓ No unacceptable toxicity (See Section 3.1.2 and 3.3), and
- ✓ ≤ grade 1 toxicity for the laboratory parameters used to assess levels of organ function as required for eligibility (See Section 2.1).

3.5 ON-STUDY EVALUATIONS (SEE STUDY CALENDAR, APPENDIX E, SECTION 12.5)

3.5.1 DURING STUDY TREATMENT

Medical assessment and performance status will be completed within 3 days prior to each of the first three vaccinations. Subsequently, medical assessments will only be required at monthly intervals. As a result, medical assessments will be performed on day 1, 15, 29, and then monthly, thereafter. Vaccination visits between monthly exams will require laboratory check prior to vaccination only. Patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to receive vaccine once every 3 months with visits only required on the day of vaccination and restaging scans required only every 3 months. Patients may also opt to continue the monthly dosing and visit schedule with scans every 2 or 3 months as they prefer. Medical assessments will include a complete neurologic examination including documentation of cranial nerve, motor, sensory, cerebellar, and deep tendon reflex examinations.

3.5.1.1 ECG at baseline and will be repeated about Day 85

3.5.1.2 Laboratory Assessments

- CBC/differential, with platelet count.
- Serum chemistries (Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH)
- ANA titer
- CD4:CD8 ratio, CD3, 4, 8, 19 subsets and NK markers (baseline, about day 29 and around day 85 prior to vaccination)
- Urinalysis

These laboratory tests will be drawn prior to each vaccination for all patients unless otherwise indicated. At a minimum CBC, serum creatinine, electrolytes, and a urinalysis will be obtained prior to each vaccination. Laboratory studies will

be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined to not be treatment-related.

3.5.1.3 Evaluation of Response

Appropriate imaging with computerized tomography (CT) of the chest/abdomen/pelvis to reassess tumor extent and to determine tumor measurements will be done around day 85 and then every 2 months. After patients have had stable disease or better (PR, CR) for at least 1 year, they may opt to have their restaging scans performed every 3 months or remain at every 2 month restaging, per patient preference. Other imaging techniques may be employed as well if appropriate for a particular tumor type (i.e. magnetic resonance imaging (MRI) or Tc-99 whole body scintigraphy (in prostate and breast cancer)).

3.5.2 AFTER TREATMENT COMPLETION

3.5.2.1 Post-treatment follow-up will be conducted 30 (+/- 2) days after last dose of treatment. This can be done as a clinical visit to the NIH Clinical Center or via phone call to the patient. A clinical visit to the NIH should include a history and physical exam, labs with CBC/differential and serum chemistries, and assessment of study drug AEs. Follow-up assessment via telephone should assess for ongoing events related to the study drug and/or any new adverse events that have developed since coming off-treatment that the Investigator deems related to the research. If patient had abnormal physical exam findings or abnormal lab values at the off treatment time point, then the patient should have follow-up visit with primary oncologist to focus on the abnormal finding(s). Results and report findings should be faxed to the NIH study team (301-480-1779).

3.5.2.2 Once the 30 day followup assessment is completed, the patient is then taken off-study.

3.5.2.3 Subjects removed from study for any reason must be followed for at least 30 days after the last dose of Yeast-Brachyury vaccine or until all toxicities have resolved to baseline or stabilized, whichever is later.

3.5.3 POST-STUDY EVALUATION

Patients will be offered enrollment in the 04-C-0274 "Follow-Up Study of Subjects Previously Enrolled in Immunotherapy Studies Utilizing Gene Transfer" once off study.

3.6 CONCURRENT THERAPIES

No concurrent investigational agents will be allowed during participation on this study. Requirement for concurrent anticancer treatment (chemotherapy, radiotherapy, immunotherapy, monoclonal antibodies including herceptin, and cytokine therapy except erythropoietin) or for concurrent systemic therapy with steroids or other immunosuppressive agents will require that subjects be taken off study. A specific exception is allowed, per section 3.8, for patients with chordoma, which would allow those patients to receive radiotherapy to one or more growing lesions when other areas of disease are stable or responding. Patients with prostate cancer must continue to receive GnRH agonist therapy (unless orchiectomy has been done). If a patient has

refused GnRH therapy, they may be enrolled on a dose level for which the safety has already been determined.

3.7 SURGICAL GUIDELINES

Not applicable

3.8 RADIATION THERAPY GUIDELINES

Patients with chordoma who are currently on protocol and receiving Yeast-brachury vaccine will be allowed to receive radiotherapy to one or more growing lesions when other areas of disease are stable or responding. During radiotherapy, if steroids are needed for symptom control per the radiation oncologist's discretion, they may be used intermittently. Ideally, steroids will not be used during the time of administration of the vaccine.

3.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.9.1 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY

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

3.9.1.1 Disease progression (as defined in sections 6.2 and 12.1),

3.9.1.2 Intercurrent illness or medical circumstances: if at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.

3.9.1.3 Unacceptable adverse event(s) as described in Section 3.1.2 (Definition of Dose Limiting Toxicity); or there is evidence of any ≥ grade 2 allergic and ≥ grade 2 autoimmune reaction(s) attributed to vaccine (**except** grade 2 or 3 thyroid related immune toxicity that resolves to grade 1 or less clinically within 7 days of initiating supportive therapy); or if > grade 2 toxicity attributed to vaccine persists for > 28 days, the patient will not receive further vaccine inoculations and will be removed from protocol therapy. Patients who come off treatment must be followed on study until resolution of toxicity and until disease progression.

3.9.1.4 Subject decides to withdraw from the study (In this event, the reasons for withdrawal will be documented), or

3.9.1.5 Patients will continue to receive vaccines approximately every 28 days for up to 2 years. (Patients that have not progressed after 1 year will be given the option to for vaccination every 3 months). If after 2 years, the vaccine remains available and there is clinical evidence that extended vaccination may be of clinical benefit, vaccinations may continue.

3.9.2 OFF-STUDY CRITERIA

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

3.9.2.1 Participant requests to be withdrawn from study; in this event, the reasons for withdrawal will be documented.

3.9.2.2 A patient who is noncompliant with protocol guidelines may be removed from the study at the discretion of the principal investigator.

3.9.2.3 Disease progression,

3.9.2.4 Death

The recombinant yeast is a protein delivery vehicle manufactured using gene therapy techniques and hence subject to the regulations of the Office of Biotechnology Activities. Patients will be offered enrollment in the 04-C-0274 "Follow-Up Study of Subjects Previously Enrolled in Immunotherapy Studies Utilizing Gene Transfer" once off study.

Authorized staff must notify the Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 CONCOMITANT MEDICATIONS/MEASURES

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

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

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

Patients with chordoma may be treated with radiation and remain on study as per sections 3.6 and 3.8. Additionally, steroids during the radiotherapy treatment for patients with chordoma are allowed if necessary as defined in section 3.8.

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

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

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 PARAMETERS OF IMMUNE ACTIVATION

Immunologic testing will include (Samples from baseline and about day 85):

- IFN-gamma ELISPOT assays for Brachyury-specific T lymphocytes using Brachyury-specific peptides

- A proliferation assay using Brachyury protein.
- CD3, CD4, CD8, and CD 19 subsets, NK markers and CD4:CD8 ratio (baseline, about day 29, and about day 85)
- Phenotypic and functional analysis of immune cell subsets (Natural Killer Cell and Regulatory T cell)
- Analysis for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, TGF-b, etc.), chemokines, antibodies (including yeast and brachyury), tumor-associated antigens and / or other markers.

More details on these tests can be found in section 6.3.

5.1.1.1 Collection of immunologic blood samples for research

Blood samples will be obtained via apheresis for patients who are HLA-A2+ in the Expansion phase of this study at baseline and around day 85. Apheresis will be performed only during the Expansion phase. In addition, all patients will have 6 (10ml) green top tube and 2 (7ml) SST drawn prior to all other vaccinations. Blood samples may be used for other research studies, which may include phenotypic and functional analysis of immune cell subsets, and analysis for cytokines, chemokines, antibodies, tumor-associated antigens and / or other markers.

5.1.1.2 The samples will be processed at

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

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

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

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

5.1.2 CIRCULATING TUMOR CELLS (CTC)

5.1.2.1 Rationale of investigation

5.1.2.1.1 Methods are in development for the purification and analysis of circulating tumor cells (CTC).

5.1.2.1.2 Novel microfilter and microfluidic chip-based CTC technologies are in development and validation at Dr. Liang Cao's lab for the isolating of live CTC cells. The technologies have two novel advances over the marker leader CellSearch technology

from Veridex of Johnson and Johnson: isolation of CTC independent of EpCAM and live cell retrieval.

5.1.2.1.3 Novel detection technologies have been developed for the quantitative analysis of the expression levels of the target antigen for vaccine, brachyury, in single live cancer cells.

5.1.2.1.4 The expression of brachyury will be normalized with that of EpCAM, a marker that is commonly expressed in prostate cancer cells, but is completely absent in PBMC cells.

5.1.2.1.5 Multiplex analysis technologies have been developed to examine the levels and activities of androgen receptor.

5.1.2.2 Blood collection

5.1.2.2.1 At the time of enrollment and at restaging visits, two tubes of 5 cc (2X5 cc) of blood in K2 EDTA tubes will be drawn. It is preferred that the CTC blood is drawn at the end of the blood draw. The tubes need to be inverted 8 times for optimal mixing to prevent coagulation. The CTC can remain at room temperature for up to 6 hrs, prior to transporting and processing.

5.1.2.2.2 CTC blood needs to be processed in the same day. Please contact Mr. Yunkai Yu at Genetics Branch for transferring the blood.

5.1.2.2.3 Yunkai Yu, tel: 301-443-2799; email: yuyun@mail.nih.gov

5.1.2.3 Sample processing and storage

5.1.2.3.1 The CTC blood will be processed in the same day to give to enriched CTC samples. The CTC samples will be labeled, immediately frozen, and stored at -80°C.

5.1.2.3.2 All specimens are stored in a secure, limited access facility at Bldg 37 in NIH with sufficient security, back up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research. Specimens are stored in accordance will applicable NIH Protection of Human Subjects Regulations.

5.2 Adult Patients: Blood Drawing Limits for Research Purposes

The amount of blood that may be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

5.3 Storage and Tracking of Collected Blood and Tissue Samples

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

5.3.1 Samples sent to Clinical Services Program (CSP)

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by Leidos couriers.

Samples will be tracked and managed by the Central Repository database. All samples will be stored in either a -80˚C freezer or liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

Fisher BioServices manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

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

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

Sample data is stored in the BioSpecimen Inventory (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.3.2 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above.

The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples providing they have an IRB approved protocol and patient consent.

The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss. The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification numbers. Data will be entered into the NCI C3D data base.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS and FDA regulations as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 CASE REPORT FORMS

All CRF pages are to be filled out completely by the examining personnel, then reviewed and electronically signed by the PI. The site staff will be required to enter available patient data from a visit no later than 10 business days after the visit is completed. The site staff will respond in a timely fashion to all queries made to the CRFs by the sponsor team and field monitors.

6.2 RESPONSE CRITERIA

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

Each patient will be assigned one of the following categories: 1) complete response (CR); 2) partial response (PR); 3) stable disease (SD); 4) progressive disease (PD); and 5) not evaluable (NE) (death prior to restaging from malignant disease, toxicity, or due to other causes; or insufficient data). For the purposes of this study, patients will be re-evaluated for response after 2 cycles.

Because of direct clinical observations of immune cell influx into tumor causing enlargement in some patients prior to sustained response, recently it has been suggested that clinical trials involving the use of immunotherapy use alternative guidelines, called immune related response criteria (irRC) to determine radiographic response or progression after therapy. These recommendations have been used in recent clinical trials. One study of 227 subjects with metastatic melanoma showed that the approximately 10% of patients who had PD by modified WHO criteria but either CR, PR or SD by irRC had a similar overall survival as those patients who had SD, PR or CR by both criteria. The irRC was created using bidimensional measurements (as previously widely used in the WHO criteria). We have taken the concepts of the irRC and combined them with the recently revised RECIST 1.1[30] [*Eur J Ca* 45:228-247, 2009] to come up with the modified irRC used in this protocol (See Appendix A in Section 12.1). Consistent with the irRC, the main changes from RECIST 1.1 are (a) a requirement for confirmation of both progression and response by imaging at least 4 weeks after initial imaging and (b) not automatically calling the appearance of new lesions progressive disease if the total measurable tumor burden has not met criteria for progressive disease.

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

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

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

Because subjects with non-measurable disease (also called evaluable only disease) are eligible for enrollment, those subjects will be evaluated with imaging studies for any evidence of disease

progression. In these cases, progression will be defined as new lesions found on imaging studies. In the spirit of the irRC, any new lesions in these subjects would then require confirmation of the new lesion on repeat imaging at least 4 weeks later to ensure that new lesions were not immune-related phenomena.

While we will use the irRC to determine disease progression for treatment purposes, we will also report the radiographic responses by RECIST 1.1.

For patients with Chordoma who enroll on the study, modified Choi criteria will be used to evaluate disease progression, stability, or response using MRI. Stacchioti, et al., demonstrated a better correlation with clinical outcomes in other soft tissues sarcomas using this technique[31]. Consensus is building from the Chordoma community, with whom we discussed endpoints for this population on multiple occasions, to use modified Choi criteria for radiographic evaluation.

6.3 ASSAYS FOR IMMUNOLOGIC RESPONSE

Studies have demonstrated the ELISPOT assay for IFN-γ production to be quantitative and reproducible as a measure of human T-cell responses to vaccination[32,33]. The continued use of one reproducible assay has been instrumental in our ability to evaluate and compare patients' immune responses using different vaccines and vaccine strategies in the same institution, and among different cancer centers. We have used this ELISPOT assay, employing CEA peptides, to demonstrate that cancer patients can mount a T-cell response to CEA post-vaccination. We plan to use this assay to evaluate patients' T-cell responses to this Yeast Brachyury vaccine. We will be using peptides we have previously identified and tested that are HLA-A2 epitopes. We will also employ Brachyury protein to measure patients' CD4 response pre- and post-vaccination (this can be done in all patients). In order to obtain, for research purposes, sufficient numbers of samples to be tested, all patients in expansion phase will undergo leukapheresis. Leukapheresis will be conducted prior to the first vaccination (day 1), and after the third cycle (day 85). $5 \times 10^8 - 2 \times 10^9$ mononuclear cells will be obtained by a single-access (single venipuncture) "4-pass" mononuclear cell procedure on the Haemonetics V-50 instrument, during which 2.0 liters of whole blood will be processed at a flow rate of about 70–80 mL/min. The total duration of the procedure is 20 min per pass or about 80 min. Patients will have to have a minimum HCT of 28% and a platelet count of at least 75,000 to undergo a Haemonetics procedure.

The potential side effects of apheresis may be pain and bruising at needle sites, lightheadedness and rarely, fainting due to a vasovagal response as can be seen with any phlebotomy procedure. There is a very small chance of introducing infection at the site of the needle. Blood infections from contamination of the Haemonetics V-50 apheresis machine are a remote possibility; furthermore, this has not occurred at the NIH thus far. Peri-oral numbness at the mouth and tingling sensation around the fingers or toes, and mild muscle cramps may occasionally occur as a side effect from EDTA (blood thinner) used during the procedure. This transient hypocalcemia can be relieved by oral calcium carbonate (e.g., TUMS®). These symptoms are brief and may often be stopped by slowing the procedure. Patients who may need central line placement for venous access may experience major bleeding in neck, chest, or groin, depending on where the line is placed. Other risks include pneumothorax, infection and local tissue damage. Patients have the option to forego the apheresis procedure and still be enrolled on the study.

The following patients will be excluded from central catheter placement:

- Patients with altered anatomy (due to surgery for example)
- Patients with poor venous access
- Patients with history of bleeding disorder
- Patients with platelet count less than 75,000 (without the benefit of transfusion)
- Patients on anticoagulant
- Patients with history of any clot in the area of catheter placement
- Patients with cutaneous skin lesion in the area of line placement

6.3.1 Assay for Natural Killer (NK) Cells

The number and phenotype of NK cells will be determined by phenotypic analysis of PMBCs stained for CD56, CD3, CD8, and CD16.

6.3.2 Regulatory T cell (Treg) Assay

Regulatory T cells have been shown to inhibit the activation and function of T cells that participate in antigen-specific immune responses. Higher levels of Tregs have been reported in the PBMCs of patients with several types of tumors. The number and phenotype of Tregs in PBMCs from patients in this study will be determined by 7-color flow cytometry analysis. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine srum) and stained for CD4, CD25, CD127, FoxP3, CTLA-4, CD45RA, and CD8. The ratios between Treg and CD4 effector cells and the ratios between Treg and CD8 Effector cells will also be analyzed.

6.3.3 Humoral Responses

Serum samples will be assayed for the presence of anti-Brachyury antibodies.

6.3.4 Additional Assays

Blood samples may be used for additional research studies, which may include phenotypic and functional analysis of immune-cell subsets and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, TGF-b, etc.), chemokines, antibodies, TAAs, and/or other markers.

6.4 Toxicity Criteria

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

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 ADVERSE EVENT

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

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2**, **7.3**, **7.4**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 SUSPECTED ADVERSE REACTION

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 UNEXPECTED ADVERSE REACTION

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

pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 SERIOUS

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 SERIOUS ADVERSE EVENT (SAE)

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

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

7.1.6 DISABILITY

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

7.1.7 LIFE-THREATENING ADVERSE DRUG EXPERIENCE

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

7.1.8 PROTOCOL DEVIATION (NIH DEFINITION)

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

7.1.9 NON-COMPLIANCE (NIH DEFINITION)

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

7.1.10 UNANTICIPATED PROBLEM

Any incident, experience, or outcome that:

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

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

(b) the characteristics of the subject population being studied; **AND**

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

7.2 NCI-IRB REPORTING

7.2.1 NCI-IRB EXPEDITED REPORTING OF UNANTICIPATED PROBLEMS AND DEATHS

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

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

7.2.2 NCI-IRB REQUIREMENTS FOR PI REPORTING AT CONTINUING REVIEW

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are not in the consent form, but are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

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

7.2.3 NCI-IRB REPORTING OF IND SAFETY REPORTS

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

7.3 IND Sponsor Reporting Criteria

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

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

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

William Dahut, M.D.

Bldg 10, Room 3-2571 MSC 1206

Telephone: 301-435-8183

William.Dahut@nih.gov

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

7.4 FDA Reporting Criteria

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

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

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinical important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

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

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

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

7.4.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer

The Sponsor will notify the IND Manufacturer of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information. The Sponsor will also notify the IND Manufacturer of Serious Adverse Events meeting any of the criteria defined by the bulleted list in Section 7.4.1 no later than 7 calendar days after determining that the information qualifies for reporting. A copy of the draft MedWatch Form 3500a may be used to meet these reporting requirements.

Notifications will be sent to:

Alicia Mattson, RN, BSN
1450 Infinite Drive
Louisville, CO 80227
303-625-2800 direct
Cell: 720-235-9007
Fax: 303-845-9366

A cumulative report of all AEs (serious and non-serious) will be provided at the request of the manufacturer, in NCI preferred format (listings, tables, or excel spread sheet).

7.5 Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The Principal Investigator, lead associate investigator and the research nurse will meet weekly at each clinic to review all adverse events for each subject in this trial and to determine dose limiting toxicities and escalation rules. Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB) and sponsor/FDA as outlined above. If trends are noted and/or risks warrant it, accrual will be interrupted, dose levels expanded and/or the protocol and/or consent will be modified accordingly.

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

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients will be randomly selected and monitored at least biannually or as needed, based on accrual rate. The patients selected will have 100% source document

verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

7.5.3 Safety Monitoring Committee (SMC)

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

7.6 NIH Office of Biotechnology Activities (OBA)/Institutional Biosafety Committee (IBC) Reporting Criteria

7.6.1 Serious Adverse Event Reports to OBA/IBC

The Principal Investigator will notify OBA/IBC via email of any unexpected fatal or life-threatening experience associated with the use of the Yeast Brachyury Vaccine as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the Yeast Brachyury Vaccine, but are no fatal or life-threatening, much be reported to NIH OBA/IBC as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information. Adverse events may be reported by using the Adverse Event Reporting template available on the NIH OBA website at: http://oba.od.nih.gov/oba/rac/Adverse_Event_Template.pdf or by using the FDA Form 3500a.

7.6.2 Annual Reports to OBA/IBC

The Principal Investigator will submit annual reports to OBA. The study Principal Investigator will submit to IBC a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect unless the IND sponsor has been authorized to submit this report.

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

7.6.2.1 Clinical Trial Information

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

- the title and purpose of the trial

- clinical site
- the Principal Investigator
- clinical protocol identifiers, including the NIH IBC protocol number, NIH grant number(s) (if applicable), and the FDA IND application number;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.6.2.2 Progress Report and Data Summaries

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.6.2.3 A copy of the updated clinical protocol including a technical and non-technical abstract.

8 STATISTICAL CONSIDERATIONS

This study will be conducted as a standard phase I trial with an expanded cohort at the MTD. In the dose escalation portion, there are 3 dose levels of yeast units to be evaluated, and initially 3 patients will be enrolled in the lowest dose level. If 0/3 have a dose limiting toxicity (DLT) after 28 days from the third patient receiving the initial vaccine on study, then subsequent patients will be accrued to the next higher dose level, as described in Sections 3.1.2, 3.1.5 and 3.1.6. Toxicities will be reported in all patients enrolled who receive at least one dose of GI-6301 (Yeast Brachyury Vaccine). If a patient is not evaluable for the primary endpoint (safety during the first 28 days after vaccination), that patient may be replaced.

The MTD will initially consist of 6 patients as indicated, and the study plans to enroll a total of 10 additional patients at the MTD (total of 16 at the MTD) in order to determine if a significant change in T-cell precursors will be detectable. It is expected that all patients enrolled at the MTD or optimal biologic dose (OBD) will be evaluable by T cell proliferation assay against Brachyury peptide, which would provide 16 subjects for immunologic testing. With 10 patients, there would be 80% power to detect a change in T-cell precursors from baseline equal to 1.0 standard deviations of the difference (effect size = 1.0) using a 2-tailed 0.05 alpha level paired t-test; if 11-16 patients at MTD are evaluable for changes in T-cell precursors, there would be > 80% power to detect changes.

Although the MTD will have been declared after 6 patients at that dose level have been evaluated, monitoring for toxicity in the additional cohort of patients will continue. In the event that more than one third of all patients enrolled at the MTD experience a DLT, protocol modifications will be undertaken to reduce the likely level of toxicity in future patients prior to continuing to enroll additional subjects.

Clinical response evaluations will take place in all enrolled patients to determine eligibility for subsequent cycles, but clinical response reporting will only be performed on patients enrolled at the MTD and the expansion cohort. The results will be considered secondary and will be used to determine the parameters for a subsequent phase II trial should the preliminary findings from this trial warrant further investigation.

With amendment D, we will enroll 10 additional patients at a 4^{th} dose level based on new information indicating that 80 YU can be given safely with this yeast-vector vaccine platform. The same statistical plan can be applied to these 10 patients as is described above for the co-primary endpoints. 23 patients have been enrolled prior to the addition of this 4^{th} dose level, so the addition of 10 patients on dose level 4 will bring the total number of patients to 33. In the case of an inevaluable patient, that patient may be replaced, bringing the total possible enrolled number of patients to 34.

The accrual of 10 total patients on dose level 4 will be dependent on $\leq$1 of the first 6 patients experiencing a DLT, making 80 YU the MTD. If $\geq$2 of the first 6 patients experience a DLT, accrual will be discontinued and the 40 YU dose level will be established as the MTD.

As a hypothesis generating analysis, the changes in T-cell precursors from baseline to post-treatment will be compared between the 40 YU and 80 YU arms using a two-tailed Wilcoxon rank sum test. Since there are limited patients expected in the comparison (13 vs. 10 patients), this analysis would be done with limited power (62% power for a two-tailed 0.05 significance level test with a 1 SD effect size).

9 COLLABORATIVE AGREEMENT

This study will be conducted under a Collaborative Research and Development Agreement (CRADA) with the manufacturer of GI-6301 Yeast Brachyury, Celgene.

10 HUMAN SUBJECTS PROTECTIONS

10.1 Rationale For Subject Selection

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

10.1.1 Inclusion of Women and Minorities

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

10.1.2 Exclusions

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

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

10.2 Participation of Children

Because no dosing or adverse event data are currently available on the use of Yeast Brachyury Vaccine in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials.

10.3 Evaluation of Benefits and Risks/Discomforts

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

Preliminary results of studies using Yeast Brachyury Vaccine have shown promising early immunologic responses and indication of clinical benefit, although in this phase I study, true benefit is unknown. Given pre-clinical data, the side effects are thought to be minor and reversible. Efforts to minimize risk include administration via subcutaneous route, close clinical monitoring after dose administration, and treatment of any side effects.

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

Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

10.4 Consent and Assent Process and Documentation

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

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness--*someone other than the health care provider*--will sign and date the consent. The original informed consent document will be mailed, via the US Postal Service or FedEx, back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

11 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

11.1 GI-6301 (Yeast Brachyury Vaccine)

11.1.1.1 Product Description

GI-6301 is a heat-killed, recombinant yeast-based vaccine engineered to express the transcription factor, Brachyury. The Brachyury gene is used to transfect the parental yeast strain (*S. cerevisiae* W303 - a haploid strain with known mutations from wild-type yeast) to produce the final recombinant vaccine product.

11.1.1.2 Other Names:

Recombinant yeast-based (*Saccharomyces cerevisia*) vector vaccine

11.1.2 Source

GI-6301 is manufactured and supplied by Celgene, Inc. in 3 mL glass vials containing 24 YU (yeast units) of GI-6301 as a sterile, lyophilized white cake. Reconstitution of each vial with 1.2 mL of Sterile Water for Injection, USP yields an opaque white suspension of GI-6301 containing 20 YU/mL in a Lyophilization Formulation Buffer.

(Note: 1 YU is equivalent to 1 x 10^7 yeast cells)

11.1.3 PREPARATION:

Dose Level 1:

Reconstitute each 24 YU vial of GI-6301 with 2.4 mL of Sterile Water for Injection, USP to yield an opaque, white, buffered, injectable suspension of GI-6301 containing 10 YU/mL. Following reconstitution, invert the vial 15 times to achieve uniform suspension prior to dose withdrawal.

The total dose, based on assigned dose level, will be equally divided and withdrawn into four separate 1 mL syringes with a 25G (5/8") needle for subcutaneous administration at four separate injection sites. Each of the four syringes for each treatment will be prepared as follows:

Dose Level (Total Dose)	**Volume of GI-6301 (10 YU/mL) per syringe**	**Volume of 0.9% sodium chloride per syringe**	**Total Syringe Volume**
Level 1 (4 YU)	0.1 mL (1 YU)	0.4 mL	0.5 mL

** A higher dilution is used for the lower dose to decrease variability related to small volumes.*

Dose Level 2, 3 and 4:

Reconstitute each 24-YU vial of GI-6301 with 1.2 mL of Sterile Water for Injection, USP, to yield an opaque, white, buffered, injectable suspension of GI-6301 containing 20 YU/mL. Following reconstitution, invert the vial 15 times to achieve uniform suspension prior to dose withdrawal.

The total dose, based on assigned dose level, will be equally divided and withdrawn into four separate 1-mL syringes (dose levels 1, 2, and 3) or four separate 3-mL-capacity syringes (dose level 4) with a 25G (5/8") needle for subcutaneous administration at four separate injection sites. Each of the four syringes for each treatment will be prepared as follows:

Dose Level (Total Dose)	**Volume of GI-6301 (20 YU/mL) per syringe**	**Volume of 0.9% sodium chloride per syringe**	**Total Syringe Volume**
Level 2 (16 YU)	0.2 mL (4 YU)	0.3 mL	0.5 mL
Level 3 (40 YU)	0.5 mL (10 YU)	None	0.5 mL
Level 4 (80 YU)	1.0 mL (20 YU)	None	1.0 mL

11.1.4 TOXICITY

Yeast-Brachyury vaccine (GI-6301) has not been administered before and the potential risks are based on information from other similar yeast-based vaccines. Patients with atopic dermatitis commonly express antibodies to various yeast proteins and it is possible that exposure to the vaccine might make the condition worse; however, this does not appear to be likely.

Likely adverse events may include: Injection site reactions (including pain, pruritis, redness, firmness, swelling, skin thickening)

Less likely adverse events may include: Fever, chills, fatigue, abdominal pain, cough, testicular inflammation, thyroid dysfunction and inflammation, headache, anorexia, diarrhea, constipation, rash, nausea, dizziness, insomnia, ulceration at the injection site, chronic inflammation of the skin, lymphadenopathy, hypersensitivity reaction (chest tightness, shortness of breath), hypersensitivity pneumonitis and hyponatremia.

Rare, but serious adverse events may include: Severe hypersensitivity reaction (difficulty breathing, hypotension, wheezing) and renal thrombotic microangiopathy. Renal thrombotic microangiopathy was reported in 2 of more than 100 patients treated; however the attribution was determined by an independent data safety monitoring committee to be related to the diagnosis of pancreas cancer and not likely related to the yeast-based vaccine.

Review of the published literature relevant to safety

Please see section 1.2.10 for safety information on similar vaccines.

Bacterial Infection: Infection of the vaccination site, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.

11.1.5 STABILITY AND STORAGE

Store intact vials of GI-6301 refrigerated at 2-8°C.

Shelf-life stability studies of the intact vials are ongoing. Following reconstitution, vials of GI-6301 are stable for up to 24 hours at room temperature. Once withdrawn into a syringe for administration, GI-6301 is stable for up to 6 hours at room temperature.

CAUTION: The lyophilized dosage form contains no antibacterial preservatives. Therefore, discard the reconstituted product 8 hours after initial entry of the vial.

11.1.6 ADMINISTRATION PROCEDURES

GI-6301 is administered by subcutaneous injection. The total dose, based on assigned dose level, for each treatment will be equally divided and withdrawn into 4 separate 1 mL syringes with a 25G (5/8") needle for subcutaneous administration at four separate injection sites. The 4 injections will be administered subcutaneously in the right and left inguinal areas and the right and left axillary chest walls. If the dose is not administered within two hours of withdrawal into the syringe, a small amount of air should be withdrawn

into each syringe, the syringes rotated to ensure adequate dispersion of the yeast-cell suspension in the syringe, and the excess air expelled from the syringe, prior to injection.

11.1.7 SPECIAL HANDLING:

Handle GI-6301 as a Biosafety Level 1 (BSL1) agent. BSL1 agents are not known to cause disease in healthy human adults and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. Clinicians can use techniques generally acceptable for nonpathogenic material. The recombinant vaccine contains DNA sequences derived from the human genome. Handle the recombinant vaccine as a biohazardous substance using adequate personnel protective apparel and containment procedures and dispose of waste materials as biohazardous waste.

For more information about biohazard risk group classification and biohazard safety levels see:

- *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines): April, 2002 (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html)*
- *Biosafety in Microbiological and Biomedical Laboratories 5th Edition. U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Healt:, February, 2007 (http://www.cdc.gov/od/ohs/biosftv/bmbl5/bmbl5toc.htm).*

11.1.8 PRECAUTIONS FOR HEALTHCARE WORKERS:

Healthcare workers with a history of allergy or serious reaction to yeast-based products should use appropriate contact precautions when administering the vaccine.

11.1.9 PATIENT CARE IMPLICATIONS AND CONTRAINDICATIONS:

Patients with a history of allergy or serious reaction to yeast-based products should not receive the vaccine. All patients will have a screening hypersensitivity skin test for *S. cerevisiae* (with positive and negative controls) prior to study enrollment. A negative response to *S. cerevisiae* is required. Instruct patients to avoid becoming pregnant, fathering a child, or breast-feeding for at least 4 months following therapy completion with the recombinant vaccine

12 APPENDICES

12.1 APPENDIX A: IMMUNE RELATED RESPONSE CRITERIA

Responses will be evaluated in all cohorts to determine eligibility for subsequent cycles but only responses evaluated in the expanded cohort and in the patients enrolled at the MTD will be used for statistical analysis. For the purposes of this study, patients will be re-evaluated for response after day 85 (prior to 7th treatment with Yeast-Brachyury vaccine).

Because of direct clinical observations of immune cell influx into tumor causing enlargement in some patients prior to sustained response, recently it has been suggested that clinical trials involving the use of immunotherapy use alternative guidelines, called immune related response criteria (irRC) to determine radiographic response or progression after therapy[34]. These recommendations have been used in recent clinical trials. One study of 227 subjects with metastatic melanoma showed that the approximately 10% of patients who had PD by modified WHO criteria but either CR, PR or SD by irRC had a similar overall survival as those patients who had SD, PR or CR by both criteria. The irRC was created using bidimensional measurements (as previously widely used in the WHO criteria). We have taken the concepts of the irRC and combined them with the recently revised RECIST 1.1[30] [*Eur J Ca* 45:228-247, 2009] to come up with the modified irRC used in this protocol. Consistent with the irRC, the main changes from RECIST 1.1 are (a) a requirement for confirmation of both progression and response by imaging at least 4 weeks after initial imaging and (b) not automatically calling the appearance of new lesions progressive disease if the total measurable tumor burden has not met criteria for progressive disease.

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

Definitions of irRC:

Response	**irRC**
New measurable lesions	**Incorporated into tumor burden**
New non-measurable lesions	**Do not define progression (but precludes irCR)**
Non-index lesions	Contributes to defining irCR (complete disappearance required)
Overall irCR	100% disappearance of all lesions, whether measurable or not, and no new lesions, in two consecutive observations not less than 4 wks from the date first documented. All measurable lymph nodes also must have reduction in short axis to <10mm.

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

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

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

* Decreases assessed relative to baseline

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

12.2 APPENDIX B: RECIST 1.1 CRITERIA

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

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

See RECIST 11 manuscript for further details on what is evidence of a new lesion

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

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

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

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

12.2.1 CONFIRMATION

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

12.2.2 DURATION OF OVERALL RESPONSE

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

12.2.3 MEASURABLE DISEASE

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

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

12.2.4 MALIGNANT LYMPH NODES

To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

12.2.5 NON-MEASURABLE DISEASE

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.
Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

12.2.6 EVALUABLE DISEASE

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

12.2.7 TARGET LESIONS

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

12.2.8 NON-TARGET LESIONS

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.3 APPENDIX C: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

12.4 APPENDIX D: SKIN TESTING

All patients will have a hypersensitivity skin test for *S. cerevisiae* prior to enrollment in the trial.

Materials for performing the testing will include:

- Sterile disposable duotip applicators (Lincoln Diagnostics, Inc. Decatur, IL)
- Dipwell tray and protective lid
- 5ml vial of allergenic extract for S. cerevisiae. (see Allergenic extracts package insert for additional details Alk Abelló, Inc., Port Washington, NY)
- 5ml vial of negative control – glycerinated phenol saline control. (see Allergenic extracts package insert for additional details)
- 5ml vial of positive control – Histatrol 1mg/ml. (see Histratrol package insert for additional details – Alk Abelló, Inc., Roundrock, TX)

All solutions should be refrigerated at 2-8° C upon arrival at site. All solutions will have an expiration date on each vial. Testing will be performed at the **screening visit** to detect if the subject has a hypersensitivity to *S. cerevisiae*. **A negative response is required for entry into the trial. Prior to performing the test, ensure prohibited medications have been discontinued as specified below:**

- H2 antagonists for 24 hours.
- Phenothiazines for 72 hours.
- H1 antihistamines for 5 serum half lives (average approx. 2 days).
- Potent topical corticosteroids on areas where skin tests are to be administered (for up to 7 days).
- Leukotriene receptor antagonists the night before.
- Clonidine for 5 serum half lives (average approx. 2 days).
- MAOI and beta blockers for 5 serum half lives (average approx. 2 days).
- Tricyclic antidepressants for 2 weeks.

**Note: A subject may switch to an agent with a shorter half life.

Testing will be accomplished using the rotation technique described in the attachment "duotip-test II". Please see attachment for additional information if required. The test should be performed on the volar surface of the forearm. The tests should be set apart by approximately 2 inches. The rotation technique will be used to administer the test solutions. Hold the shaft of the immersed duotip between the index finger and thumb. Press points vertically against skin with enough pressure to slightly indent skin. Maintain pressure on skin while rotating the shaft clockwise or counter-clockwise 360 degrees. Repeat for the other solutions. Allow test solutions to remain over the test sites three to five minutes before wiping. Histamine result is to be read ten to fifteen minutes after administration. Results of the saline and the *Saccharomyces cerevisiae* are to be read fifteen to thirty minutes after administration (a positive reaction to *Saccharomyces cerevisiae* can occur up to thirty minutes post). The *Saccharomyces cerevisiae* site will be

graded against the positive and negative controls. Adults usually exhibit 7mm to 9mm wheals from properly administered histamine unless ability to react is impaired. Reactions that are similar to the saline reaction are negative regardless of size.

IF THE REACTION TO HISTAMINE AND *SACCHAROMYCES CEREVISIAE* ARE SIMILAR THEN THE TEST IS CONSIDERED POSITIVE AND THE SUBJECT IS EXCLUDED.

DUOTIP-TEST® II

Duotip-Test II is a sterile, disposable, plastic bifurcated needle used to administer skin test substances. When employed with allergenic extracts it provides a quick, convenient and standardized procedure that is well accepted by patients. Before using Duotip-Test II, or any testing device, the administrator must study carefully the package inserts accompanying allergenic extracts and control solutions.

Figure 1

The Duotip-Test II system consists of these components:

1. The sterile disposable applicator (Fig. 1).

2. The Dipwell Tray and its protective lid (Fig. 2).

Figure 2

Each tray consists of forty wells into which extracts and controls are placed. Points of individual Duotip-Test II units are immersed into test solutions and pick up test doses via capillary attraction. **See Dipwell Tray package insert for complete instructions on its use with Duotip-Test II.**

Directions for Use: Duotip-Test II will administer test solutions via modified prick procedure (Fig. 3) or by rotation technique (Fig. 4).

When using either approach, these steps must be followed after cleansing skin and loading the points:

Figure 3

1. Modified Prick - Hold gripping area of device so its shaft forms an approximate 45° angle with the skin plane. Prick and lift skin simultaneously **with one point**.

2. Rotation Technique - Hold gripping area between index finger and thumb. Press points vertically against skin with enough pressure to **slightly** indent skin. Maintain pressure on skin while rotating shaft clockwise or counter-clockwise.

Figure 4

With either procedure allow test solutions to remain over test sites three to five minutes before wiping.

Reading Results: Because patients differ in response to mechanical and chemical stimulation of the skin, interpretations of test results are most reliable when positive (glycerinated histamine) and negative (glycerosaline) controls are used. Response to histamine (1mg/ml) is considered 3+, while response to glycerosaline is considered negative.

12.5 APPENDIX E: TREATMENT AND MONITORING SCHEDULE: PRE, DURING AND POST THERAPY

	Baseline[1]	Day 15	Day 29	Day 43	Day 57	Day 71	Day 85	Monthly after Day 85 [12]
History and Physical[1]	X							
Medical Assessments[2]	X	X[13]	X[13]		X[13]		X[13]	X[10,13]
Imaging	X[3]						X	Every 2 months
Urinalysis	X	X	X	X	X	X	X	X[10]
Serum HIV antibody[4]	X							
Serum Hepatitis B & C[5]	X							
CBC with differential, platelet count	X[1]	X	X	X	X	X	X	X[10]
Chemistry[6]	X[1]	X	X	X	X	X	X	X[10]
EKG	X[1]						X	
Apheresis[7]	X						X	
Immunology (blood)[8]	X	X	X	X	X	X	X	X[10]
ANA titer	X						X	
CD3, 4, 8, 19 subsets, NK markers and CD4:CD8 ratio	X		X				X	
HLA typing	X							
Skin testing[11]	X							
Serum Beta-HCG[9]	X							
Pulse oximetry	X							
GI-6301 (Yeast-Brachyury Vaccine)[14]		X	X	X	X	X	X	X[14]
Adverse Events	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
Circulating Tumor Cell Assay	X						X	Every 2 months[15]

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

[2] Medical assessments: interim history (since last visit), vital signs, physical examination and ECOG performance status.

[3] Radiologic studies consisting of CT chest/abdomen/pelvis (and MRI or bone scan when appropriate for tumor type) will be performed within 28 days prior to initiating treatment, after the first three cycles, and every 2 months thereafter

(patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option of having these studies every 3 months instead of 2 months).

.

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

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

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

[7] Apheresis will be requested for immunologic testing from patients at baseline and approximately day 85. Apheresis will be performed on patients who are HLA-A2+ only during expansion phase.

[8] Blood will be obtained for immunologic assays including ELISPOT assay. Research blood will be drawn prior to each vaccination (at baseline and about days 15, 29, 43, 71) then every 28 days on the expansion phase. ANA titer will be drawn at baseline and about day 85. CD3, 4, 8, 19 subsets, NK markers and CD4:CD8 ratio will be drawn at baseline, about day 29 and about day 85.

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

[10] Medical assessment, clinical labs, and research labs will be performed on a monthly basis. Patients who have been on study for one year or more, have had stable disease or better (PR, CR) and opted to receive vaccine once every 3 months will have these evaluations every 3 months instead of monthly.

[11] This test is performed at the screening visit to detect if the subject has a hypersensitivity to *Saccharomyces cerevisiae*. **A negative response is required for entry into this trial.**

[12] Patients will continue to receive monthly vaccines for up to 2 years. Patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to receive vaccine once every 3 months instead. If after 2 years, the vaccine remains available and there is clinical evidence that extended vaccination may be of clinical benefit, vaccinations may continue.

[13] within 3 days prior to each vaccination.

[14] administered subcutaneously at 4 sites on 7 visits (Days 1, 15, 29, 43, 57, 71, 85 all +/- 2 days), then monthly until patients meet off-treatment criteria. Patients who have been on study for one year or more and have had stable disease or better (PR, CR) have the option to receive vaccine once every 3 months instead of monthly.

[15] cTC assay will be performed at baseline and at each restaging - day 85 and then every 2 months (patients who have been on study for one year or more, have had stable disease or better (PR, CR) and opted to receive vaccine once every 3 months will have this test every 3 months instead of 2 months).

Appendix F: Modified Choi Criteria

The Choi Criteria was introduced for radiographic evaluation of gastrointestinal stromal tumors treated with imatinib mesylate[35,36] and has been evaluated in other soft tissue sarcomas[31] and renal cell carcinoma[37]. After discussion with experts in the field of Chordoma as well as the Chordoma Foundation, we have agreed that, at this point, there is a consensus to use a modified Choi Criteria, with MRI instead of CT, as the best imaging technique to determine radiographic response, stability, or progression in patients with Chordoma.

The differences between RECIST and Choi Criteria are defined in the table below:

Tumor Response according to RECIST and Choi Criteria

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

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INSTITUTE: National Cancer Institute

STUDY NUMBER: 12-C-0056 PRINCIPAL INVESTIGATOR: James L. Gulley, M.D., Ph.D.

STUDY TITLE: An Open Label Phase I Study to Evaluate the Safety and Tolerability of GI-6301 Vaccine consisting of Whole, Heat-Killed Recombinant *Saccharomyces cerevisiae* (Yeast) Genetically Modified to Express Brachyury Protein in Adults with Solid Tumors

Continuing Review Approved by the IRB on 04/06/15
Amendment Approved by the IRB on 01/06/16 (I) Date Posted to the Web: 01/08/16
Standard

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Overview

This is a trial to determine the safety of a new cancer vaccine. The goal of cancer vaccines is to teach the immune system to target and then kill cancer cells. The target in this vaccine is a protein called Brachyury. Brachyury protein affects how tumor cells use their DNA, allowing those cells to spread to other sites. These metastatic cells also have been shown to contain Brachyury. Similar vaccines have been tested for safety using other proteins and have been very well tolerated.

Why is this study being done?

The NIH research team is studying an experimental vaccine made with yeast and designed to target cells expressing the Brachyury protein. Brachyury is a protein that controls when genes are switched on or off. Research shows that in some cancers (including colon, lung, breast, ovarian, pancreatic and prostate cancer) there is more Brachyury in the cells and this thought to play a role in cancer growth and metastasis. In our laboratory, mice treated with the Yeast Brachyury vaccine had less cancer metastases than mice that did not receive the vaccine.

The vaccine is made with baker's yeast (Saccaromyces cerevisiae). The yeast is genetically modified, made to express the Brachyury protein, and then is heat killed. The goal is to block the overexpressing Brachyury tumor cells, to help stop tumor cell growth and metastasis.

This study will test the safety of giving the Yeast Brachyury vaccine to people with cancer. The vaccine is manufactured by Celgene and is known as GI-6301. In previous studies, similar yeast vaccines were found to be safe, well-tolerated, and had few side effects.

Why are you being asked to take part in this study?

You are being asked to participate in this study because standard therapies have not been effective for your type of cancer and we are testing possible new agents for safety and to see if they are effective. Your participation is entirely voluntary.

How many people will take part in this study?

Up to 33 patients will take part in this study. There will be three groups of patients in the first part of the study to see what the best, safe dose is. Between 3-6 patients will be enrolled in the first group. If no serious side effects occur, the next set of 3-6 patients will get a higher dose of the GI-6301 vaccine. If the second dose level is found to be safe, an additional 3-6 patients will receive the highest dose level, until all three dose levels are tested.

The dose you receive is not based on what the doctor believes is best for you, but rather your dose level is based on the order in which you are enrolled in the study and how previous participants have reacted. Ask your doctor what dose level you will receive.

After these 3 groups are filled, an additional 10 patients will receive vaccine at the highest dose found to be safe (called the expansion cohort).

An additional dose level above the initially designed highest dose level has been added to the study based on new information indicating that a higher dose may be safe. Up to 10 patients will be enrolled on the new highest dose level.

Description of Research Study

What will happen if you take part in this research study?

Before you begin the study:

You will need to have the following exams, tests and procedures if you can be in the study. These exams, tests and procedures are part of regular cancer care and may be done even if you do not join the study. If you have had some of them done recently, they may not need to be repeated. This will be up to the study team.

- A complete medical history, including your cancer history and prior cancer treatments, all drugs that you may be taking, including over the counter drugs, vitamins and herbal supplements.
- A complete physical examination, including assessing your ability to do physical activities and measuring your blood pressure, heart rate and breathing.
- Standard blood and urine tests to evaluate your organs' functioning (such as liver, kidneys, blood sugar and blood electrolytes), including tests of your immune system and presence of infections.
- Evaluation of your cancer, which may include a CT scan, a PET scan, Bone scan and/or a Brain MRI.
- A 12 lead ECG (electrocardiogram) to assess your heart.
- As part of this study, we will test you for infection with the human immunodeficiency virus (HIV), the virus that causes AIDS. If you are infected with HIV you will not be able to participate in this study because this cancer drug relies on the immune system to work. We will tell you what the results mean, how to find care, how to avoid infecting others, how we report newly diagnosed HIV infection, and the importance of informing your partners at possible risk because of your HIV infection.
- A pregnancy test will be performed in women who are able to have children. Women who are pregnant or breast-feeding will not be allowed to participate, as the effects of vaccine on a developing fetus or infant are not known.
- HLA typing: this blood test is done to see what protein is expressed by your blood cells, human leukocyte antigen (HLA).
- Skin test: you will be given a skin test to see if you have a yeast allergy. This will be read within 15-20 minutes after it is given. If you have a positive test, you will not be eligible for this study.

During the study

If the initial exams and tests show that you can participate in this study, you will come to the NIH about every two (2) weeks for GI-6301 vaccines, exams and tests, for 7 visits (days 1, 15, 29, 43, 57, 71, and 85). After the 7th visit, if your Day 85 restaging indicates your cancer has not gotten worse, you will begin coming monthly (or about every 28 days) to receive your GI-6301 vaccine, and have exams and tests (if you have been on study for one year or more and your disease has not worsened, you have the option of coming every 3 months instead of monthly).

The GI-6301 vaccine will be given as 4 small shots (injections) under the skin in the right and left chest area, below your armpit, and the right and left area of your upper thigh, around the pelvic region. These sites were chosen because these areas of your body drain into parts of your body that contain a large number of lymph nodes. Lymph nodes contain large numbers of immune cells which can potentially be activated (or turned on) by the vaccine to target cancer cells.

The following tests and procedures will be done during the time you are on this study. Many of them are part of regular cancer care; however, some tests and procedures may be done more often because you are on this study.

- **Clinic visit (physical exam):** we will ask you how you are feeling and do a physical examination. This exam will be done every 2 weeks initially and then monthly if you are not having any problems.
- **Vital signs:** we will measure your blood pressure, temperature, pulse, breathing and weight each time you come to the clinic.
- **Routine blood tests:** lab tests of your organ functions (liver, kidneys, blood clotting, red and white blood cells, platelets, blood sugar and blood electrolytes) will be tested each time you come to clinic. In addition, there are some Brachyury-expressing cells in the thyroid, so we will test your thyroid function at regular intervals to make sure the vaccine is not harming the thyroid gland
- **Urine test**: you will be asked to give a urine sample for testing each time you come for an outpatient visit.
- **CT scans or MRI** that detects your tumor will be done at the first re-staging visit (which is after 3 months of experimental vaccine) and then every 2 months while you are receiving GI-6301 vaccine (if you have been on study for one year or more and your disease has not worsened, you have the option of having scans every 3 months instead of 2 months). This is done so that any benefit of the vaccine can be determined or if your cancer is not responding to the vaccine, the study team can tell you and help you to seek other treatment options.
- **ECG**: this will be done to check your heart rhythm at 2 time points - once before starting vaccine and again after you have completed 3 months of vaccine.

Research Blood Samples: It is important to understand the effects of this experimental vaccine on your body, particularly the immune system. To do this, blood samples will be taken at specific time points during your participation in this study.

1) **Research blood samples** (8 tubes of blood or about 5 tablespoons) will be taken at each outpatient visit (day 1, 15, 29, 43, 57, 71, 85 and then every month), prior to getting the vaccine.

2) **Apheresis:** If you are in the expanded cohort and have a particular HLA blood type (HLA-A2), we will also ask you to have a procedure called apheresis on Day 1 and Day 85. Apheresis is a procedure during which you will have an IV inserted in your vein and attached to a machine. You will lie down in a bed while your blood will be filtered through the machine that takes out some of your white blood cells for research testing, and gives you back the rest of the blood. It will take about 90 minutes.

   If you have poor vein access in your lower arms, we may ask that a temporary central venous access line be placed in a vessel in your neck or in your groin in order to obtain immune cells during the apheresis procedure. You have the option to forego the apheresis procedure and still be enrolled on the study.

   The following patients will be excluded from central catheter placement:
   - Patients with altered anatomy (due to surgery for example)
   - Patients with history of bleeding disorder
   - Patients with platelet count less than 75,000 (without the benefit of transfusion)
   - Patients on anticoagulant medications
   - Patients with history of any clot in the area of catheter placement
   - Patients with skin lesion in the area of line placement

How long will I receive vaccines?

After receiving GI-6301 vaccines every 2 weeks for the first 3 months, you will continue to receive vaccines monthly (or every 28 days, which we call a course), as long as you do not have unacceptable side effects, your cancer does not get worse, and you are willing to continue participating. If you have been on study for one year or more and your disease has not worsened, you have the option of receiving vaccine every 3 months instead of monthly). If after 2 years there is evidence that your cancer is not getting worse, you are tolerating the vaccine and the GI-6301 vaccine remains available, you may continue to receive the vaccine. GI-6301 vaccine availability may depend on several factors including, but not limited to, the capacity for Celgene to continue producing it, the results of this study regarding benefit and tolerability. This possibility will be discussed with you by one of the clinical investigators at that time.

When you are finished getting the vaccine (treatment)

When you stop the GI-6301 vaccine, we will ask you to return to NIH for clinic visits until you recover from any side effects and/or your cancer worsens. At these visits you will have exams, tests and procedures that are part of regular cancer care, including physical exam, standard blood tests, scans and x-rays.

Study Chart

The study chart below shows what will happen to you during course 1 and future courses, after you sign the consent and start the study. The left-hand column shows the day in the course, and the right-hand column tells you what will happen on that day.

Day	What to do and what will happen to you
Before starting the vaccine	• Get routine blood and urine tests • Pregnancy test • Check-in at Outpatient clinic • Have a history taken of how you feel, undergo a physical examination by a health care provider • CT scan or MRI • HLA blood typing • Skin test to see if you have a yeast allergy • ECG to check your heart • Sign informed consent
Course 1, Day 1	• Get research blood drawn (have Apheresis procedure for those patients who are HLA-A2 in the expansion cohort) • Receive the first GI-6301 vaccine in the Outpatient Day Hospital
Day 15 (same for Days 29, 43*, 57, and 71*)	• Return to the NIH clinic • Have a history taken of how you feel and have a physical exam by health care provider • Get routine blood and urine tests • Get research blood drawn • Receive GI-6301 vaccine • *On days 43 and 71, no history and physical exam is required.
Day 1 of Subsequent courses (on or about Day 85), each course is about 28 days long	• Return to the NIH clinic • Have a history taken of how you feel and have a physical exam by health care provider • Get routine blood and urine tests • Get research blood drawn (have Apheresis procedure for those patients who are HLA-A2 in the expansion cohort) • ECG to check your heart rhythm will be done on day 85, will not be done thereafter

	• CT scan or MRI to determine how your cancer is being affected by the vaccines (will be done every 2 months from this point on while you are on the study). If you have been on study for one year or more and your disease has not worsened, you have the option of having scans every 3 months instead of 2 months. • Receive GI-6301 vaccine
After 2 years	• If after 2 years there is no evidence that your disease is getting worse and the GI-6301 vaccine remains available, vaccinations may continue if there is evidence that it is benefiting you.

Long Term Follow Up

At the end of this study, we would like to follow you for any late side effects, and see how you do on other treatments. We will request that at that time you enroll on our Long Term Follow-Up Study, 04-C-0274, which the study team will review with you.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Instead of being in this study, you have these options**:**

- Getting treatment or care for your cancer without being in a study
- Taking part in another study
- Getting no treatment
- Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, appetite problems and other problems caused by the cancer. It does not treat the cancer directly. Instead, it tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Please talk to your doctor about these and other options.

Risks or Discomforts of Participation

What side effects or risks can I expect from being in this study?

You may have side effects while on the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen when taking the GI-6301 vaccine, so it is important to report changes that you notice, even if your study team does not ask you specifically about them. It is very important that you tell a member of the research team before starting any new medications. We do not know what side effects could happen when other drugs are given with the GI-6301 vaccine.

Side effects to the vaccine may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away with those medicines, and others can go away soon after you stop receiving the vaccine. In some cases, side effects can be serious, long lasting, or may never go away, and may result in death. You should talk to your study team about all side effects that you have while taking part in the study.

The GI-6301 vaccine is made from a laboratory manufactured recombinant (or bringing together genetic material from different sources) yeast that serves as a carrier to take the Brachyury protein to the body's receptors, hence it is considered a type of gene therapy. The yeast is destroyed by heat so it cannot multiply in your body. This technology (heat-killed yeast vaccines) has been used successfully in several other large clinical trials.

GI-6301 vaccine has not been administered before and the potential risks are based on information from other similar yeast vaccines. Patients with a skin condition called atopic dermatitis commonly express antibodies to various yeast proteins and it is possible that exposure to the vaccine used in this study might make atopic dermatitis worse; however this does not appear to be likely.

Likely	Less Likely	Rare but Serious
• Injection site reaction (including pain or discomfort, itching, redness, firmness, swelling, skin thickening, or bumps)	• Acute fever • Please see reproductive risk below • Chills • Flu-like symptoms (including fatigue, soreness, general body pain, abdominal pain, cough, fever, headache) • Chest tightness • Shortness of breath • Leg pain and / or swelling • Headache • Loss of appetite • Weight loss • Diarrhea • Constipation • Rash • Nausea • Dizziness • Mild inflammation of the tissue lining the lung • Chronic inflammation of the skin • Insomnia • Open sores at the injection site	• Difficulty breathing • Low blood pressure • Wheezing • Clots in the lung • Clots in the leg • Kidney damage • Decreased oxygen levels in the blood • Fluid around the lining of the lungs • Fluid around the lining of the heart

	(ulceration) • Enlargement of the lymph nodes • Low blood levels of sodium • Inflammation of the testicles (in men) • Inflammation of thyroid tissue, causing changes in thyroid function test results	

Reproductive risks:

If you are a woman who is breast feeding or pregnant, you may not participate in the study because we don't know how this medicine would affect your baby or your unborn child. You should not become pregnant or father a child while receiving this vaccine. If you think that you or your partner is pregnant during this study, you should tell your study doctor or nurse at once. If you are a woman who can become pregnant, or are the male partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting the experimental vaccine and for 4 months after you finish the experimental treatment. There is a risk that this vaccine could impair your ability to reproduce. The target of this vaccine is found in large quantities on the developing fetus, and an immune reaction against that target may result in fetal abnormalities or miscarriage. Effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- hormonal [birth control pills, injections, or implants]
- tubal ligation
- hysterectomy
- vasectomy

OTHER RISKS AND SIDE EFFECTS RELATED TO RESEARCH PROCEDURES:

Blood Sampling: Bruising or bleeding at the needle site; rarely infection. This is treated with bandages, pressure and, if infection, antibiotic medicines.

Risks of blood draws: Blood samples will be drawn from a vein in your inner arm. The risk of a blood draw may include fainting or pain. There could be bruising or infection at the site where blood is drawn. The risk is no greater than if you were having blood drawn for routine and normal healthcare.

Risks from X-rays and / or Scans: The risks of the contrast material used with the CT scan include rare allergic reactions, nausea, flushing, low blood pressure, asthma, stroke and organ damage. If the contrast material is given via your vein, it may make you feel flushed and give you a sensation of "pins and needles" for a few seconds.

Risks of Apheresis: Only patients who have the HLA-A2+ tissue type and are part of the expansion cohort will have a procedure called apheresis. Those patients will have the apheresis procedure two times during the study. During this procedure blood will be withdrawn from a vein in your arm. It is similar to what happens when you donate blood. In the laboratory, white blood cells will be removed from your blood for further study. Then the remaining parts of your blood will be returned to your body. You may have pain or bruising where the needle is inserted. There is a very small chance of introducing infection at the needle. There is a slight chance of blood infections from contamination of the apheresis machine, but this has never occurred at the NIH. Some patients feel weak or dizzy during apheresis. Some have tingling or numbness in their lips, fingers or toes. These symptoms don't last long, and they often stop when the procedure is slowed down. If you need a central line placement for venous access, you may experience major bleeding in your neck, in your chest, or in your groin, depending on where the line is placed. Other central line placement risks include pneumothorax, infection and local tissue damage.

Potential Benefits of Participation

Are there benefits to taking part in this study?

The purpose of this study is to test the safety of the experimental vaccine, GI-6301 Yeast Brachyury, and to test whether it stimulates your immune system. Taking part in this study may or may not make your health better. We do not know if you will receive personal, medical benefit from taking part in this study. These potential benefits could include shrinking of your tumor or lessening of your symptoms, such as pain, that are caused by the cancer. Because there is not much information about the drug's effect on your cancer, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.

Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.

- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if your disease worsens during experimental vaccine treatment
- if you have side effects from the experimental vaccine that your doctor thinks are too severe
- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to Globimmune or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases cannot be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study are using a drug developed by Celgene through a joint study with your researchers and the company. The company also provides financial support for this study.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people
- National Cancer Institute Institutional Review Board
- Qualified representatives of Celgene, who produce the vaccine

Please see last page under "Confidentiality" for additional people that may have access to medical records.

Use of Specimens and Data for Future Research

We would like to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be identified by a number and not your name. Your specimens and data will be for research purposes only and will not benefit you. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you decide now that your specimens and data can be kept for research and shared, you can change your mind at any time. Just contact us and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research.

Please read the sentence below and think about your choice. After reading the sentence, circle and initial the answer that is right for you. No matter what you decide to do, it will not affect your care.

My specimens and data may be kept and shared for use in research to learn about, prevent, or treat cancer or other health problems.

Yes No Initials_____

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, James L. Gulley, M.D., Ph.D., Building 10, Room 13N208, Telephone: 301-435-2956. You may also call the Clinical Center Patient Representative at (301) 496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/ Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM APRIL 6, 2015 THROUGH APRIL 5, 2016.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____