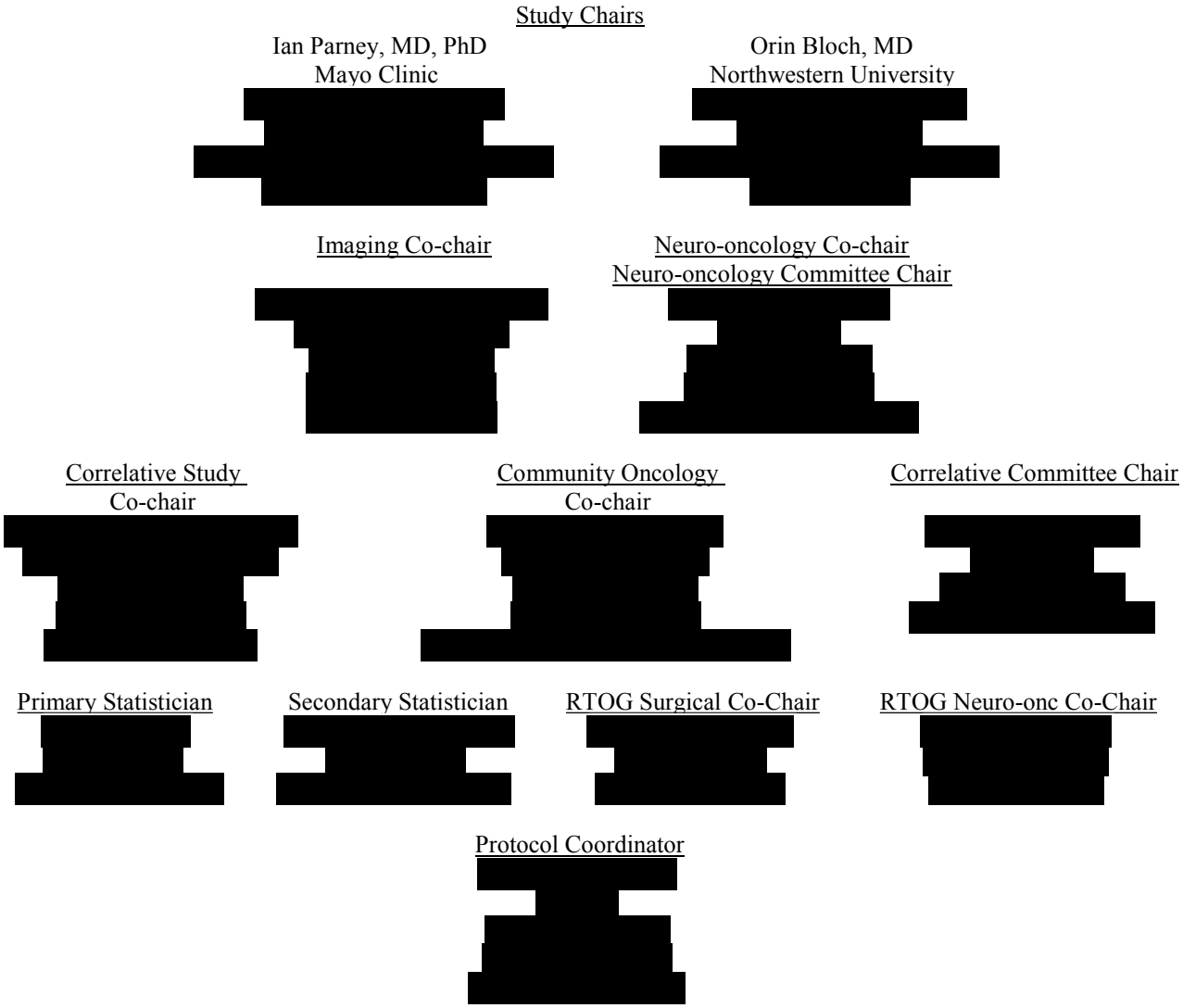
ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

ALLIANCE A071101

A PHASE II RANDOMIZED TRIAL COMPARING THE EFFICACY OF HEAT SHOCK PROTEIN-PEPTIDE COMPLEX-96 (HSPPC-96) (NSC #725085, ALLIANCE IND #15380) VACCINE GIVEN WITH BEVACIZUMAB VERSUS BEVACIZUMAB ALONE IN THE TREATMENT OF SURGICALLY RESECTABLE RECURRENT GLIOBLASTOMA MULTIFORME (GBM)

Investigational Agent: Autologous Heat Shock Protein Peptide Complex-96 (HSPPC-96)
(NSC #725085, Alliance IND# 15380) supplied and distributed by Agenus Inc.
Commercial Agent: Bevacizumab
ClinicalTrials.gov Identifier: NCT01814813



Participating Groups:
ALLIANCE / Alliance for Clinical Trials in Oncology (lead)
NRG / NRG Oncology

Alliance Central Protocol Operations Office
[redacted]

Alliance Statistical Data Center
[redacted]

Expedited Adverse Event Reporting
https://eapps-ctep.nci.nih.gov/ctepaers/

Medidata Rave® iMedidata portal
https://login.imedidata.com

OPEN (Oncology Patient Enrollment Network)
https://open.ctsu.org

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Non-paraffin biospecimens:
[redacted]

IROC
[redacted]

Protocol-related questions may be directed as follows:	
Questions	**Contact (via email)**
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair, Nursing Contact, Protocol Coordinator, or Data Manager
Questions related to data submission, RAVE or patient follow-up:	Data Manager
Questions regarding the protocol document and model informed consent:	Protocol Coordinator
Questions related to IRB review	Regulatory Affairs Manager: [redacted]
Questions regarding CTEP-AERS reporting:	Alliance Pharmacovigilance Inbox: [redacted]
For a copy of the Investigator Brochure (IB):	[redacted]

The pharmacogenomic component of this study is conducted as part of the NIH Pharmacogenomics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/pharmacogenomics/research_net.html) for details

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

CONTACT INFORMATION		
For regulatory requirements:	**For patient enrollments:**	**For study data submission:**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at [redacted] to receive further instruction and support. Contact the CTSU Regulatory Help Desk at [redacted] for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at [redacted].	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions. Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.		
For clinical questions (i.e., patient eligibility or treatment-related) see Protocol Contacts Page 2		
For non-clinical questions (i.e., questions unrelated to patient eligibility, treatment, or clinical data submission contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – [redacted], or [redacted]. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Web site is located at https://www.ctsu.org.		

A PHASE II RANDOMIZED TRIAL COMPARING THE EFFICACY OF HEAT SHOCK PROTEIN-PEPTIDE COMPLEX-96 (HSPPC-96) (NSC #725085, ALLIANCE IND# 15380) VACCINE GIVEN WITH BEVACIZUMAB VERSUS BEVACIZUMAB ALONE IN THE TREATMENT OF SURGICALLY RESECTABLE RECURRENT GLIOBLASTOMA MULTIFORME (GBM)

Patient Eligibility for Pre-registration:

Prior histologic diagnosis of GBM or gliosarcoma
1st or 2nd recurrence of GBM or gliosarcoma considered surgically resectable
No radiotherapy within 90 days
No prior treatment with any anti-angiogenic agent targeting the VEGF pathway
No prior treatment with HSPPC-96 or other investigational immunotherapy
Prior treatment with radiotherapy and temozolomide for histologically confirmed GBM at initial diagnosis
No tumor directed therapy for this recurrence of GBM
No prior Gliadel® wafers
No clinically significant cardiovascular disease
No significant bleeding within 6 months; no bleeding diathesis or coagulopathy
No abdominal fistula, GI perforation or intra-abdominal abscess within past 12 months
Age ≥ 18 years
No evidence of any systemic autoimmune disease or immunosuppressant therapy
Karnofsky functional status rating ≥70
No more than 16mg/day dexamethasone
Non-pregnant/non-nursing

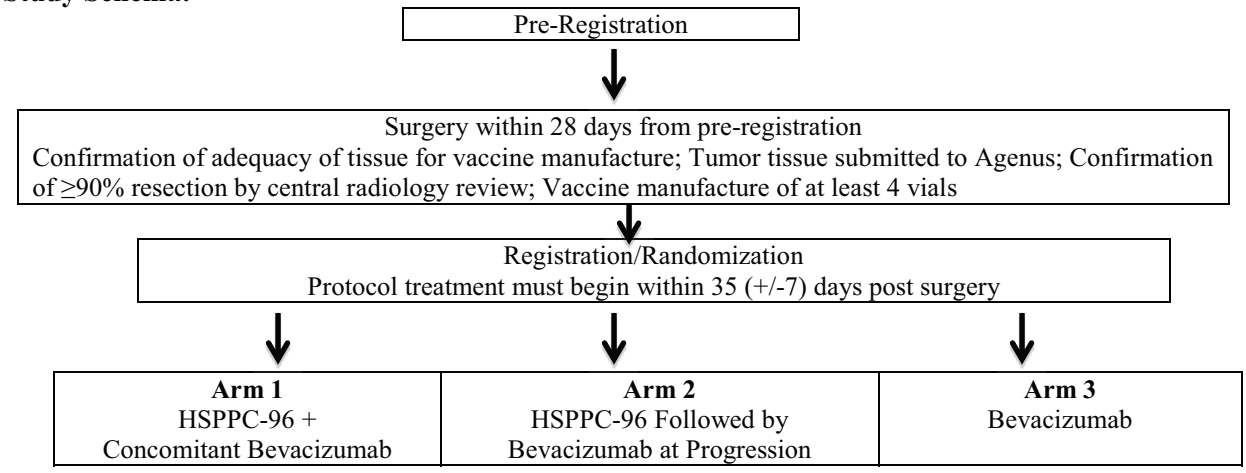
Required Initial Laboratory Values

Granulocytes ≥1,500/μL
Platelet count ≥100,000/μL
Total Bilirubin ≤ 2.0 x ULN
UPC ratio <1
Or
Urine protein ≤ 1+
Calculated creatinine clearance ≥45 ml/min
SGOT/SGPT (AST/ALT) ≤2.5 x ULN

Patient Eligibility for Registration:

Pre-registration criteria continue to be met
Confirmed histological diagnosis of recurrent GBM or gliosarcoma
Must have had a ≥90% surgical resection confirmed by central radiology review
At least 5 grams of resected tumor for vaccine manufacture
Availability of ≥ 4 clinical vials of HSPPC-96
No serious, non-healing wounds or ulcers
At least 7 days since any minor surgery such as port placement
No major surgical procedures, open biopsy or significant traumatic injury ≤28 days prior to registration or anticipation of need for elective or planned surgery during study. Core biopsy or minor surgical procedures ≤7 days prior to registration
No active or recent hemoptysis ≤30 days prior to registration
No new bleeding on D28 (+/-3) MRI (or CT if MRI is contraindicated)
No clinical deterioration at the time of registration/randomization
If a second surgery is needed for completion of resection, this should be within 30 days of the first surgery

Study Schema:



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1.0 INTRODUCTION

1.1 Rationale for Selected Approach and Trial Design

Primary malignant brain tumors are uniformly fatal, and the 5-year survival rate for the highest grade of malignant glial neoplasm, GBM, is now less than 4% [1]. Improvements in conventional treatment modalities have provided some progress, however median survival remains at just over one year from initial diagnosis for patients treated at tertiary care centers [2]. Currently approved therapy for a newly diagnosed GBM patient in the United States includes maximal surgical resection followed by radiation and temozolomide [1]. Upon recurrence there are few approved options and these include surgical implantation of chemotherapy bearing wafers [3] (polifeprosan 20 with carmustine implant, Gliadel® Wafer) and systemic administration of the anti-angiogenic agent bevacizumab, which has shown a partial response rate of 20% in one trial, and 26% in another [4, 5]. Each of these therapies has shown modest improvement in survival of recurrent GBM patients, with notable treatment related toxicities including wound breakdown after surgical resection [6].

There is an unmet medical need for highly specific and non-toxic adjuvant therapy to treat recurrent GBM patients undergoing surgical resection. Immunotherapy is an appealing method to specifically target tumor cells in glioma patients, while minimizing adverse treatment effects [7]. There is evidence of immune mediated processes involved in GBM: antigens in the CNS lead to production of cytotoxic T cells and antibody response. Also, antigen specific T cells are seen in CNS tumors, and microglia and macrophages act as antigen presenting cells in the CNS. CNS tumors themselves cause immunosuppression, as evidenced by low lymphocyte counts, low antigen and mitogen responses, and T cells with impaired function. Any immunotherapy undertaken must overcome this. One way to overcome this is to resect tumor, as this decreases steroid requirement and the decrease in tumor bulk helps to downregulate the immunosuppression caused by the tumor [7].

Several methods have been used successfully to evoke anti-tumor immunity in GBM patients, with evidence of peripheral and site-specific immune responses. Prins et al investigated the utility of immunotherapy for intracranial tumors in a mouse model. The anti-TRP vaccine successfully induced CD8+ T cell response, and this was demonstrated with bioluminescent imaging to occur within the CNS. The vaccine was also shown to decrease tumor size and increase survival. Wheeler et al treated newly diagnosed patients (after surgery and radiation) with either chemotherapy, autologous antigen pulsed dendritic cell vaccine, or vaccine and chemotherapy. The groups were not matched in that the chemotherapy only group had less full resections. The median overall survival was 15.9 months in chemotherapy, 17.9 months in vaccine, and 26 months in both. Follow-up work by this same group also found increased survival time in immune responders versus nonimmune responders, on the order of 1.8 versus 1.2 years. A single arm trial by Liau et al looked at 12 patients with newly diagnosed or recurrent GBM, treated with dendritic cell tumor peptide vaccine, and found a median overall survival of 2 years. A phase II trial with matched cohort by Sampson et al looked at a peptide vaccine to EGFRvIII. Patients had newly diagnosed disease and minimal residual disease (>95% resected). With the 18 vaccine patients, the median progression free survival was 14.2 months, and the matched cohort was 6.3 months. The median overall survival was 26 months in the vaccine cohort, and 15 months in the non-vaccine group, for a hazard ratio of 5.1. [8-12]. Overall, it can be said that to date there has been data showing potential benefit for the use of immunotherapy in GBM.

Heat Shock Protein-Peptide Complex-96 (HSPPC-96) consists of the heat shock protein glycoprotein-96 (HSP gp-96) and a wide array of chaperoned proteins, including autologous antigenic peptides. Heat shock proteins (HSP) are molecules that respond to cellular stress and

counteract abnormal protein folding. They are known to modulate immune responses, especially the HSP gp-96. In a stressful environment, such as a tumor, HSPs are upregulated and highly expressed on tumor cells. This protects the tumor and leads to resistance to therapy. HSP expression is associated with cellular proliferation, apoptosis evasion, tissue invasion, metastasis, and angiogenesis.

HSPPC-96 immunization works mechanistically by interacting with antigen presenting cells (APCs) via specific receptors, including CD91 [13,14]. The highly specific nature of the interaction between HSPPC-96 and APCs is a significant advantage over other cancer vaccine approaches; and has been shown to facilitate robust CD4+ and CD8+ T-cell immune responses. There are multiple mechanisms by which the vaccine works. The first is the following: The autologous antigens in the vaccine bind to the chaperone proteins (HSP) with noncovalent bonds. Macrophages transfer the antigens from HSP to antigen presenting cells, via receptor-mediated endocytosis (with receptors such as CD91). The antigens are then expressed via MHC class I on antigen presenting cells (APCs), interacting with naïve T cells, which are activated into specific CD8 T cells.

Another mechanism of the vaccine is an innate immunity pathway. In this mechanism, HSP-Ag is internalized into the APC, which activates the APC. This leads to the following:

- Activated APCs express co-stimulatory molecules (CD80, CD86, and CD40) and express MHC class II, which interacts with T cells and produces tumor specific CD4 cells.
- Activated APCs cause cytokine release (IL-1b, IL-12, IL-6, TNF-alph, GMCSF, chemokines, and nitric oxide). IL-12 stimulates NK cells, which enhance the antitumor activity of T cells.
- The interaction between HSP-antigen and APCs causes dendritic cell maturation, which stimulates NK cells.

A final mechanism of the vaccine is that exposure to Gp96 leads to translocation of NFkB into the APC nucleus, which then regulates cancer cell growth, progression, and apoptosis.

The advantage of the HSPPC-96 vaccine over others is the following:

- Multifactorial mechanism: as described above, the vaccine works by multiple pathways to exert its effect, rather than 1 pathway.
- The vaccine is individualized, and does not require identification of immunogenic antigens required by other vaccines.
- Multiple antigens are used, which decreases the chance of immune evasion that occurs with a single antigen vaccine.

The downside of the HSPCC-96 vaccine is that:

- A large amount of tissue is required
- High purity criteria
- There is difficulty designing immunomonitoring due to the fact that there is not a typical antigenic characteristic of GBM.

- Autoimmune reactions are possible due to the non-specificity of the vaccine (although these have not been noted in trials to date) [15,16].

Clinical grade HSPPC-96 can be easily purified from solid tumor and has been safely tested in hundreds of patients with melanoma and renal cell cancer. The toxicity encountered in renal cell carcinoma in prior trials is the following: injection site erythema (50%), injection site induration (48%), back pain (12%), headache (12%), fatigue (10%), nasopharyngitis (9%), incision site complication (9%), arthralgia (9%), hypertension (9%), urinary tract infection (8%), nausea (7%), diarrhea (6%), asthenia (6%), influenza (6%), constipation (5%), pyrexia (5%), and dizziness (5%). In melanoma, toxicity seen was pyrexia (8%), fatigue (6%), and nausea (5%). Between the trials there were 3 serious adverse events, 2 were thyroid dysfunction, and 1 was cellulitis [17-20].

A single center single arm Phase 1 clinical trial and a multicenter single arm phase 2 clinical trial for recurrent glioblastoma patients treated with HSPPC-96 have been completed. Collectively these studies have shown that: 1) HSPPC-96 vaccine made from recurrent GBM tumor is safe and well tolerated, 2) HSPPC-96 vaccine extends survival in recurrent GBM patients over historical controls, and 3) HSPPC-96 is immunogenic in recurrent GBM patients, inducing both an innate and adaptive immune response. Specifically, in the phase I trial, testing was performed pre and post vaccine on all patients. Eleven of 12 patients were shown to have a significant immune response, which was measured by testing peripheral blood lymphocytes (PBLs). PPBLs were tested for phenotype, reactivity to vaccine, IFN gamma production, and proliferation. Vaccine was well tolerated with no serious adverse events and the only toxicity noted was injection site erythema or induration. The median survival in responders was 47 weeks, compared to 16 weeks in the non-responder. In the phase II trial, 33 patients were treated. Again, there were no serious adverse events attributed to vaccine. Toxicities reported were injection site reaction in 12 patients, and fatigue in 9 patients. Eleven patients were still alive at time of analysis, with median survival of 333 days for the evaluable population. Also, all patients studied showed immune response, based on CD8 IFN gamma production [22, 33].

Based on these positive preliminary findings, a large randomized trial, incorporating the current best available treatment (bevacizumab), in recurrent GBM is warranted. Furthermore, as described below, there is a theoretical scientific basis for potential synergies between bevacizumab and a specific active immunotherapy such as HSPPC-96.

There are at least two mechanisms by which bevacizumab and HSPPC-96 may work synergistically. The first is based on the transient normalization of tumor vasculature achieved with a variety of anti-angiogenic agents like bevacizumab, which in turn facilitates infiltration of lymphocytes into the tumor. Lymphocytes activated by HSPPC-96 vaccination are thus expected to more readily target tumor cells. The second mechanism of potential synergy is based on the observation that excessive vascular endothelial growth factor (VEGF) can inhibit the differentiation and maturation of dendritic cells (DCs). Such immature DCs are less potent stimulators of T cells [24]. Thus, VEGF neutralization by bevacizumab is expected to restore the capacity of DCs to optimally process and present HSP associated antigens to T cells.

Published data from both human (sipuleucel-T) and animal studies

One publication reports the combination of bevacizumab and a cancer vaccine in the clinical setting. The vaccine was sipuleucel-T, which was tested in a Phase 1 study with recurrent prostate cancer. The agents were administered concurrently. Peripheral blood mononuclear cell (PBMC) proliferation and IFN ELISPOT assays were conducted for evaluation of immune

response before and at various times after therapy. In all patients evaluated for immune response, an increase in response over baseline was detected. Whether the responses were indicative of an additive or synergistic effect of bevacizumab and sipuleucel-T could not be determined since patients receiving single agent therapy were not included in the study [25]. In animal models several studies have reported a synergistic effect of anti-angiogenic agents targeting VEGF or VEGF receptors in combination with cancer vaccines. The tumor types tested include melanoma, colon cancer, squamous cell carcinoma, breast cancer and fibrosarcoma. Several positive effects of the combined therapy were reported, including: (a) prolonged survival, (b) tumor shrinkage, (c) reduction in tumor infiltrating regulatory T cells, (d) reduction in tumor infiltrating immature DCs and increase in infiltrating mature DCs, (e) increased tumor infiltration of CD4+ and CD8+ effector lymphocytes as well as NK cells and (f) increase in cytotoxic T-lymphocyte (CTL) response to tumor antigen. It is of note that enhanced tumor lymphocyte infiltration was observed despite a reduction in density of tumor vasculature [26, 27].

Study Design

In the present study, patients must have ≥90% resection to be included, which is consistent with populations studied in previous trials of HSPPC-96 in recurrent GBM. It is believed that the optimal setting for studying cancer vaccine efficacy is in patients who have the least amount of residual tumor burden at the time of vaccination. HSPPC-96 can be easily purified from human tissue through a series of biochemical steps. Surgically resected tumor is homogenized and then run through columns that purify the HSPPC-96, eventually concentrating it in sufficient quantities to facilitate biological efficacy. After purification, HSPPC-96 undergoes a series of quality tests, which includes confirmation of biological activity through an in vitro antigen presentation assay, prior to shipping for clinical use.

1.2 Study Rationale

This trial is important because there are currently no approved adjuvant treatments in recurrent GBM that significantly extend survival. This trial is designed to provide sound evidence towards determining whether an autologous active immunotherapy, HSPPC-96, used as an adjuvant treatment to surgery and in combination (either concomitantly post-surgery or serially at the point of progression) with the best available and approved therapy, bevacizumab, in recurrent GBM can extend overall survival. Since this trial includes an arm of bevacizumab alone, this affords the opportunity to also better characterize the effect of bevacizumab on overall survival in a randomized, controlled setting, which remains an important open clinical question.

Beyond the primary goal of demonstrating an impact on overall survival, this trial will also advance the biological understanding of a vital area of cancer research. The use of cancer vaccines in combination with other immune-based, targeted agents has been an area of increasing focus but clinical efforts to undertake combination trials have been limited to date. As such, this trial provides the opportunity to advance the understanding of cancer vaccines and combination therapy in a meaningful clinical setting.

In addition, positive findings in recurrent GBM would likely have implications for utility of HSPPC-96 in surgically resectable newly diagnosed GBM. From a biological perspective, positive findings could also open additional avenues of research with HSPPC-96 and bevacizumab in other cancer indications.

1.3 Relevant Data

The relevant data, which supports the use of the experimental and control arms in the setting of recurrent GBM are provided in the summary table below. Please note that the first 2 studies are discussed above and full detail is provided therein.

Experimental Arm: HSPPC-96			
Population	**Study Design**	**Key Results**	**Supporting publications/ abstracts/posters**
Resectable, recurrent GBM	Phase 1, single-arm, single center trial to evaluate safety and immunogenicity	12 evaluable patients enrolled No toxicities attributable to HSPPC-96 92% of patients showed a peripheral immune response Median OS of 42 weeks	Crane et. al., Clinical Cancer Research, 2012 Aug 7. [Epub ahead of print]http://www.ncbi.nlm.nih.gov/pubmed/22872572 [22]
Resectable recurrent GBM	Phase 2, single-arm, multi-center trial to evaluate 6-month survival rate, overall survival and immunogenicity	30 evaluable patients enrolled 6-month survival of 93% Median OS of 47.6 weeks All patients showed a peripheral immune response	Parsa et al., J Clin Oncol 29: 2011 (suppl; abstr 2565) http://www.asco.org/ASCOv2/Meetings/Abstracts?&vmview=abst_detail_view&confID=102&abstractID=85010 [23]
Resectable, newly diagnosed GBM	Phase 2, single-arm, multi-center trial to evaluate overall survival and safety	100% enrolled, 46 patients treated, 11 deaths reported thus far; 3 patients to date have reached end of study (2 years) without progression	Abstract accepted for oral presentation at the 2013 AANS annual meeting
Control Arm: bevacizumab			
Recurrent GBM	Phase 2, randomized, multi center to evaluate 6-month PFS and OR rates	25.9% median response rate (per label) Duration of response of 4.2 months (per label) 6-month PFS of 29% (per Friedman et al) Median OS of 31 weeks (per Friedman et al)	Approved labeling http://www.gene.com/gene/products/information/pdf/avastin-prescribing.pdf Friedman et al., J Clin Oncol 27:4733-4740 http://www.ncbi.nlm.nih.gov/pubmed/19720927

Importantly, both the phase I and phase II trials used entry criteria of > 90% resection to generate a minimum of 7 grams of tissue to generate 4 more doses of 25 micrograms of HSPPC-96 vaccine. In the phase I rGBM trial, 12 patients received a median of 5.5 doses with a range of 4-22, with 3 patients (25%) receiving only 4 doses [46]. Importantly, 11/12 patients generated an immune response to HSPPC-96, confirming the immunologic effect of 4 doses. In the phase II rGBM trial of 41 patients, the median number of vaccines administered was 6, with a range of 1-15 [47]. Based on prior phase I and II trial designs and for consistency, a 4 dose minimum for trial entry is required, with the expectation that most patients will have more than that. The 4 dose minimum was also used in a ND GBM trial as a criterion for treatment [48].

A 4 dose minimum will increase the number of eligible patients as the number of vials required for quality assurance purposes increases with the increased number of vials required for entry. While seven grams is felt to be optimal, patients where at least 7 grams of tissue resected could be sufficient for vaccine generation (depending on the quality of the tissue), but both clinician and patient would need to be advised that vaccine generation might not occur.

1.4 Inclusion of Women and Minorities

This study will be available to all eligible patients regardless of race, gender, or ethnic group. There is no information currently available regarding differential agent effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analyses will, as always, look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for such subset analyses. A total of 165 patients may be enrolled. Based on prior studies involving similar disease sites, we expect about 13% of patients will be classified as minorities by race and about 40% of patients to be women. Expected sizes of racial by gender subsets are shown in the following table:

DOMESTIC PLANNED ENROLLMENT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	1	2			3
Asian	2	2			4
Native Hawaiian or Other Pacific Islander					
Black or African American	6	8			14
White	49	74	9	12	144
More Than One Race					
Total	58	86	9	12	165

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether there is an overall survival advantage of HSPPC-96 administered with bevacizumab, given concomitantly or at the point of progression, in comparison with bevacizumab alone in patients with surgically resectable recurrent GBM.

2.2 Secondary Objectives

2.2.1 To evaluate the safety and tolerability of HSPPC-96 with bevacizumab.

2.2.2 To evaluate the progression free survival of HSPPC-96 with bevacizumab, given concomitantly or at the point of progression.

2.3 Correlative Science Objectives

2.3.1 To evaluate whether patients who demonstrate an immune response to HSPPC-96 will have an improved survival outcome in comparison to those patients who do not show immune response.

2.3.2 To explore the expression of B7-H1 protein expression and PI3K pathway activation (which are associated with immunoresistance) at the tissue level before treatment and correlate with therapeutic response.

2.3.3 To explore whether T-cell infiltrate of tumor at baseline correlates with response to vaccine.

3.0 ON-STUDY GUIDELINES

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. Although they will not be considered formal eligibility (exclusion) criteria, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness, which would prevent the patient from giving informed consent.
- Medical condition such as uncontrolled infection (including HIV), which in the opinion of the treating physician would make this protocol unreasonably hazardous for the patient.
- Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and are free of disease for ≥ 3 years.
- Women and men of reproductive potential should agree to use an appropriate method of birth control during treatment and for 6 months after the last treatment in this study due to the teratogenic potential of the therapy utilized in this trial. Appropriate methods of birth control include abstinence, oral contraceptives, implantable hormonal contraceptives or double barrier method (diaphragm plus condom)
- Life expectancy of <8 weeks.

4.0 ELIGIBILITY CRITERIA

All questions regarding eligibility criteria should be directed to the Alliance Study Chair. Please note that the Study Chair cannot grant waivers to eligibility requirements.

4.1 Pre-registration (Pre-Surgery) Eligibility Criteria

4.1.1 Histologic documentation: Prior histologic diagnosis of GBM at first occurrence.

4.1.2 Stage: First or second recurrence of GBM or gliosarcoma considered to be surgically resectable.

4.1.3 Prior Treatment

- No radiotherapy within 90 days prior to pre-registration.
- No prior treatment with any anti-angiogenic agent targeting the VEGF pathway including but not limited to bevacizumab, cediranib, vandetanib, sunitinib, pazopanib, aflibercept or sorafenib.
- No prior treatment with HSPPC-96 or other investigational immunotherapy.
- Must have received prior treatment with radiotherapy and temozolomide for histologically confirmed GBM at initial diagnosis.
- No tumor directed therapy for most recent progression.
- No prior Gliadel® wafers.

4.1.4 No clinically significant cardiovascular disease

- Patients with a history of hypertension must be well controlled (<150/90) on a regimen of antihypertensive therapy.
- History of arterial thrombotic events within the past 6 months, including transient ischemic attack (TIA), cerebrovascular accident (CVA), peripheral arterial thrombus, unstable angina or angina requiring surgical or medial intervention in the past 6 months, or myocardial infarction (MI). Patients with clinically significant peripheral artery disease (i.e., claudication on less than one block), significant vascular disease (i.e., aortic aneurysm, history of aortic dissection) are not eligible.
- Patients who have had a deep vein thrombosis or pulmonary embolus within the past 6 months are eligible if they are on stable therapeutic anticoagulation
- No current New York Heart Association classification II, III or IV congestive heart failure

4.1.5 No significant bleeding within the past 6 months; no bleeding diathesis or coagulopathy

4.1.6 No history of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within past 12 months

4.1.7 No evidence of any systemic autoimmune disease (e.g. Hashimoto's thyroiditis) and/or any history of primary or secondary immunodeficiency, and no

immunosuppressant therapy (with the exception of dexamethasone as noted below) for any reason

4.1.8 Age ≥ 18 years of age

4.1.9 Karnofsky functional status rating ≥70

4.1.10 No more than 16 mg dexamethasone (or equivalent) per day

4.1.11 Non-pregnant and non-nursing

4.2 Registration (Post-Surgery) Eligibility Criteria

4.2.1 Pre-registration eligibility criteria continue to be met

4.2.2 Histologic documentation: confirmed histological diagnosis of recurrent GBM or gliosarcoma.

4.2.3 ≥ 90% surgical resection of recurrent GBM confirmed by central radiology review by MRI with or without gadolinium per institutional guidelines. A CT scan is allowable in place of MRI only in situations where an MRI is contraindicated (e.g., patient has a heart pacemaker, metallic devices in the eye, brain or spine, severe claustrophobia).

4.2.4 Five grams of resected tumor available for vaccine manufacture as determined by institutional pathologist. Seven grams of tumor is preferred (see Section 6.5, footnote 1).

4.2.5 Availability of ≥ 4 clinical vials of HSPPC-96

4.2.6 Required Initial Laboratory Values:

Granulocytes	≥1,500/μL
Platelet count	≥100,000/μL
Total Bilirubin	≤ 2.0 x ULN
UPC ratio	<1
OR	
Urine protein	≤ 1+
Calculated creatinine clearance	≥45 ml/min
SGOT/SGPT(AST/ALT)	**≤2.5 x ULN**

4.2.7 No serious, non-healing wounds or ulcers

4.2.8 At least 7 days since any minor surgery such as port placement

4.2.9 No major surgical procedures, open biopsy or significant traumatic injury ≤28 days prior to registration or anticipation of need for elective or planned major surgical

procedure during the study. Core biopsy or other minor surgical procedures ≤7 days prior to registration.

4.2.10 No active or recent hemoptysis (≥½ teaspoon of bright red blood per episode) ≤30 days prior to registration.

4.2.11 No new bleeding on D28 (+/-3) MRI (or CT if MRI is contraindicated).

4.2.12 No clinical deterioration at the time of registration/randomization.

4.2.13 If a second surgery is needed for completion of resection, this should be within 30 days from the first surgery.

5.0 PRE-REGISTRATION, REGISTRATION/RANDOMIZATION, AND STRATIFICATION

5.1 CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR Help Desk by email at [redacted].

5.2 CTSU Site Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients.

Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to:

- an active Federal Wide Assurance (FWA) number,
- an active roster affiliation with the Lead Network or a participating organization,
- a valid IRB approval, and
- compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intention to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in a given study.

5.2.1 Downloading Site Registration Materials

Site registration forms may be downloaded from the A071801 protocol page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or,
- Click on the By Lead Organization folder to expand.
- Click on the Alliance link to expand, then select trial protocol # A071801.

- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

5.2.2 Requirements for A071101 Site Registration

- IRB approval (local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted).

5.2.3 Checking Your Site's Registration Status

You can verify your site registration status on the members' section of the CTSU website. Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password.
- Click on the Regulatory tab
- Click on the Site Registration tab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

5.2.4 Submitting Regulatory Requirements

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission Portal

When applicable, original documents should be mailed to:

[redacted]

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at [redacted] in order to receive further instruction and support.

5.3 Pre-registration

5.3.1 Pre-registration Requirements

- Informed Consent: the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form is required.
- All patients must have signed a HIPAA authorization form if applicable.

5.3.2 Patient Pre-registration through OPEN

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the LPO or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at [redacted] or [redacted].

5.4 Registration/Randomization

5.4.1 Registration Procedures

- Confirmation must be sent from site to Agenus informing Agenus that tumor material for a patient is being shipped to attempt vaccine manufacture. The Tissue Procurement Form (TPF) must be completed and faxed to Agenus as per the instructions on the TPF. Sites can obtain the study-specific TPF from the CTSU website.
- Bulk tumor tissue must be sent from site to Agenus for vaccine manufacture per instructions in Appendix III. The completed Tissue Procurement Form (TPF) must be included with the tissue sent to Agenus.
- Notification must be sent to Agenus that ≥ 90% surgical resection of recurrent GBM has been confirmed by the Imaging and Radiation Oncology Core central radiology review.

5.4.2 Registration Requirements

Receipt by site of the designated Vaccine Production Notification Form from Agenus, which will inform the site of the status of the HSPPC-96 vaccine (i.e., successfully produced or not successfully produced) and, if successfully produced, the number of available vials that will be shipped along with the anticipated shipping date. The Vaccine Production Notification Form will be sent by Agenus to the site generally within 14 days of receiving the tumor material for processing. There have to be at least 4 clinical vials successfully produced for the patient to be considered eligible. If the vaccine was successfully produced, but the patient is not eligible to participate, the Vaccine Order Form MUST be faxed to Agenus. The site should check "No, please do not ship vaccine (Arm 3 or other reason) and identify in the comments section that the patient is a screen failure.

5.4.3 Patient Registration/Randomization through OPEN

Patients should be randomized 48 to 72 hours after the 28 (+/- 3) day scan with no bleed. After randomization, sites must complete the Vaccine Order Form and fax or email it to Agenus in order to have vaccine shipped (see Section 11.6 for ordering vaccine). Sites can obtain the study specific Vaccine Order Form from the CTSU website.

The site staff will enter the patient ID number obtained at pre-registration and the patient's age stratum (<55 vs. ≥55 years old) and KPS stratum (70 vs. 80-100) into the OPEN registration system to obtain a treatment assignment. Once the randomization is complete, note the patient's treatment assignment in your records.

OPEN Access Requirements

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the LPO or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at [redacted].

5.5 Stratification

Patients will be stratified by

- Age group: <55 vs. ≥55 years old
- KPS group: 70 vs. 80-100
- Vials produced: Four or 5 vials of HSPPC-96 vs. 6 or more vials of HSPPC-96

5.6 Registration to Sub-studies

Registration to the correlative science sub-study will be done at the same time as registration to the treatment study. **Registration to the treatment trial and the sub-study will not be completed if eligibility requirements are not met.**

There is one sub-study within A071101: **A071101-ST1**: Correlative Science in A071101. This study **must be offered to all patients** enrolled on A071101 (although patients may opt to not participate). This sub-study does not require separate IRB approval.

If a patient answers "yes" to model consent question #1, they have consented to participate in the sub-study described in Section 10.1. The patient should be registered to A071101-ST1 at the same time they are registered to the treatment trial (A071101). Samples should be submitted per Section 6.

5.7 Surgical Quality Assurance

It is recommended that the neurosurgeon has performed at least 20 brain tumor resections over the last 12 months.

Note: The neurosurgeon does not need to hold a Cooperative Group membership.

6.0 DATA AND SPECIMEN SUBMISSION AND CENTRAL RADIOLOGY REVIEW

Data collection for this study will be done exclusively through the Medidata Rave clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on either the LPO or participating organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at [redacted] or by e-mail at [redacted].

6.1 Data Submission

This study **will use** Medidata Rave for remote data capture (RDC) of all study data. Access the RAVE system through the iMedidata portal at https://login.imedidata.com.

For questions regarding forms completion and submission, please contact the Data Manager listed on the Protocol Resources page of the protocol.

6.2 Tumor Tissue Submission for Vaccine Manufacture

The manufacturer of HSPCC-96 may be available for site training. Institutions may contact the Alliance at [redacted] to express their interest in this optional training session with Agenus. Agenus will provide training for techniques in tissue procurement, tissue processing, packaging and shipping of tissue of vaccine manufacture.

See Appendix III for detailed instructions for handling, preparation, and submission of tumor tissue for HSPPC-96 vaccine manufacture.

The Tissue Procurement Form (TPF) must be included with the tissue sent to Agenus.

6.2.1 Kits for shipping tissue will be supplied by Agenus and should be ordered at the time of IRB submission because they will be needed at time of surgery. Kits are ordered by contacting Agenus via e-mail at: [redacted].

The dimensions of the kits are 17" x 15" x 15". Please order one or two kits at a time, based on your anticipated use, and please allow 3-4 days for shipping.

NOTE: Questions about the protocol and model consent MUST be directed to the Protocol Coordinator listed on the title page. For other questions, additional resources are identified on page 2.

6.3 Blood Submission for Immune Monitoring

For patients who consent to participate, blood samples will be used for the immune response analysis described in the correlative study section.

Only previously designated sites will process the blood at their institution and send PBMCs to Mayo BAP for banking. All other sites will send whole blood to the Mayo BAP for processing and banking.

Collection time points are described below according to treatment arm:

Arm 1 (HSPPC-96 + concomitant bevacizumab): blood will be drawn prior to first dose of vaccine /bevacizumab (can be drawn on same day of treatment or up to 14 days prior), at the vaccine dose #5/bevacizumab dose#3 prior to vaccine administration, at the vaccine dose #7/bevacizumab dose #5 prior to vaccine administration, 2 weeks following the final dose of vaccine, and 2 weeks following the final dose of bevacizumab.

Arm 2 (HSPPC-96 followed by bevacizumab at progression): blood will be drawn prior to first dose of vaccine (can be drawn on same day as treatment or up to 14 days prior) at the vaccine dose #5 prior to vaccine administration, at the vaccine dose #7 prior to vaccine administration, 2 weeks following the final dose of vaccine, and 2 weeks following the final dose of bevacizumab.

Arm 3 (bevacizumab alone): blood will be drawn prior to first dose of bevacizumab (can be drawn on same day as treatment or up to 14 days prior), prior to the bevacizumab at dose #3, prior to the bevacizumab at dose #5, and 2 weeks following the final dose of bevacizumab.

Approximately 50 ml of blood will be drawn at the above described time points.

6.3.1 Blood submission for previously designated sites sending PBMCs

This applies only to the following previously designated site that will be processing the blood at their institution and sending PBMCs to the Mayo BAP for banking: [redacted].

Blood collected for immunological studies will be processed according to the Appendix IV, Whole Blood PBMC Processing at Sites, "Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Human Blood Samples." These specimens will be batch shipped on dry ice to the Mayo BAP for banking.

Label samples with the following identification:

1) Procurement date **and time**

2) Alliance patient number

3) Alliance study number (A071101)

4) PBMC

6.3.2 Blood submission for sites sending whole blood

For sites collecting whole blood and shipping to the Mayo BAP for processing and banking:

Collect approximately 50 ml of peripheral venous blood divided evenly into five 10.0 ml green top (sodium heparin) tubes. The sodium heparin tubes should be inverted several times to mix and refrigerated immediately until shipped on cold pack by overnight mail to the Alliance BAP at Mayo. The samples should be shipped the same day that the blood is drawn.

Label samples with the following identification:

1) Procurement date **and time**

2) Alliance patient number

3) Alliance study number (A071101)

4) Whole blood

Note: Shipment must not involve weekends and holidays.

6.3.3 Shipping PBMCs on dry ice or whole blood on cold packs

For sites batch shipping PBMCs (see Appendix IV) samples should be sent overnight on dry ice. For sites shipping whole blood, samples should be sent overnight on cold packs (see Section 6.3.2).

Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary. Ship only on Monday-Thursday by overnight service to assure receipt. If shipping on Friday, FedEx must be used and the air bill must be marked "For Saturday delivery." Send the FedEx tracking number to the Biospecimen Resource Manager so arrangements can be made to stabilize the samples over the weekend. Do not ship specimens on Saturdays. Do not collect specimens the day before a holiday.

Ship samples to the following address:

[redacted]

For questions about blood submission contact:

[redacted]

[redacted]

6.4 Tissue Submission for Biomarker Analysis

Submit the following specimens and forms:

A representative formalin fixed, paraffin-embedded (FFPE) block of tissue from:

- prior histologic diagnosis of GBM at initial diagnosis
- total resection for recurrence
- if possible, from post-progression biopsy
- Specimen Submission: Tissue
- Surgical Pathology Reports from the initial diagnosis and recurrence
- BioMS shipping manifest

NOTE: If submission of diagnostic blocks is prohibitive and a non-diagnostic block of comparable tumor tissue is available, please prepare a second FFPE specimen to submit for biomarker analysis.

Tissue specimens should be sent to the Alliance Biorepository at Mayo Clinic Pathology Coordinator:

[redacted]

Use FedEx priority overnight delivery for all shipment. Extreme heat precautions should be taken when necessary. Ship only on Monday-Thursday overnight service to assure receipt.

6.4.1 Surgical guidelines

- Surgically remove a sample of **at least 5 mm^3**.
- Immerse fresh, surgically removed tumor tissue in 4-10% neutral buffered formalin.
- Immerse tissue as quickly as possible following surgical resection.
- Minimize or avoid necrotic tissue or non-tumor tissues.

6.4.2 Pathology processing guidelines

- Use a maximum fixation time of 24 hours (longer fixation times lead to over-fixation and more severe nucleic acid fragmentation).
- Thoroughly dehydrate samples prior to embedding.
- Embed in low melting temperature paraffin.

For questions pertaining to paraffin-embedded tissue pathology contact:

[redacted]

6.4.3 Tumor slide preparation guidelines

If due to institutional policy a block cannot be sent, utilize microfacing of the blocks to submit the following:

- 15-25 slides containing a minimum of 10 mm^2 primary tumor tissue in total, cut at a thickness of at least 10µm
- 12 slides cut at a thickness of at least 10µm from patient's tumor both at initial diagnosis and recurrent disease resection

When slides are sent, the following guidelines should be followed:

- Cut new sections from paraffin embedded blocks; do not send precut paraffin sections. Cut sections as close as possible to the shipment and processing date (RNA degrades significantly faster in the sections that in the block)
- Standard **uncharged** glass slides should be used to mount the sections. Examples standard glass slides: # 4430 Super UP-RITE frosted micro slides, Richard-Allen Scientific or equivalent.
- Cut 15-25 x 10-micron unstained tumor tissue sections mounted on regular slides for RNA isolation. Each slide must have as much tumor tissue as possible on the slide.
- For H&E staining/ tumor visualization, one 5-micron paraffin-embedded section must be cut from the same region of the tumor tissue block and mounted on a **charged** slide.
- Do not bake or place cover slips on the unstained slides
- Do not stick adhesive labels on the slides
- Label slides with patient ID number, accession number and order of section (i.e. 1-25), and thickness of section for 5 or 10 µm.

The goal of the Alliance Biorepository at Mayo Clinic is to provide investigators with quality histology sections for their research while maintaining the integrity of the tissue. All paraffin blocks that are to be stored at the Alliance Biorepository at Mayo Clinic will be stored at 4ºC to minimize degradation of cellular antigens. For these reasons it is preferred that the Alliance Biorepository at Mayo Clinic bank the block until the study investigator requests thin sections. Please contact the Alliance Biorepository at Mayo Clinic if additional assurances with your hospital pathology department are required.

6.5 Biospecimen Submission Summary

Samples	A071101 Sample Collection for Arm 1 and Arm 2							
	Pre-registration at Surgery	Pre-treatment (prior to vaccine dose #1)	Vaccine dose #5	Vaccine dose #7	2 weeks after final vaccine dose	2 weeks after final bevacizumab dose	Progression	Ship to:
Tumor tissue: 5-7 grams*	X							Agenus
Tumor block**		X					X+	Biorepository at Mayo
Whole blood (5 x 10ml green top tubes) or PBMCs (isolated from blood collected in (5) 10ml green top tubes)		X++	X++	X++	X	X		Mayo BAP

* **Mandatory** for vaccine manufacture. Successful manufacture of vaccine is dependent on receipt of viable tissue that is processed, packaged, and shipped correctly, and also dependent on sufficient quantity. Tissue must be non-necrotic, and must have viable tumor. Depending on the quality of the specimen shipped, it may be possible that 5 grams of tumor would be sufficient to manufacture the minimum 4 vials of vaccine that are required for treatment on this protocol. However, it is in the best interest of the patients care and potential for good outcomes, along with the science of the trial and correlative work on specimens, that maximum tissue is sent and good quality tissue is sent. Therefore, the goal with every patient is that the largest quantity of high quality tissue is sent for vaccine manufacture. The only tissue utilized at the site should be to confirm that the patient indeed has recurrent tumor, as every additional amount of tissue sent will make the difference as to whether the patient will be eligible to continue on the trial, and also how many vials of vaccine can be manufactured.

** Archival paraffin-embedded tissue from prior histologic diagnosis of GBM at initial diagnosis and paraffin block tissue from total resection for recurrence.

\+ From post-progression biopsy, if possible.

++ Blood drawn prior to vaccine administration.

Samples	A071101 Sample Collection for Arm 3						
	Pre-registration at Surgery	Pre-treatment (prior to bevacizumab dose #1)	Bevacizumab dose #3	Bevacizumab dose #5	2 weeks after final bevacizumab dose	Progression	Ship to:
Tumor tissue: 5-7grams*	X						Agenus
Tumor block**		X				X^{+}	Biorepository at Mayo
Whole blood (5x 10ml green top tubes) or PBMCs (isolated from blood collected in (5) 10ml green top tubes)		X	X^{++}	X^{++}	X		Mayo BAP

* **Mandatory** for vaccine manufacture. Successful manufacture of vaccine is dependent on receipt of viable tissue that is processed, packaged, and shipped correctly, and also dependent on sufficient quantity. Tissue must be non-necrotic, and must have viable tumor. Depending on the quality of the specimen shipped, it may be possible that 5 grams of tumor would be sufficient to manufacture the minimum 4 vials of vaccine that are required for treatment on this protocol. However, it is in the best interest of the patients care and potential for good outcomes, along with the science of the trial and correlative work on specimens, that maximum tissue is sent and good quality tissue is sent. Therefore, the goal with every patient is that the largest quantity of high quality tissue is sent for vaccine manufacture. The only tissue utilized at the site should be to confirm that the patient indeed has recurrent tumor, as every additional amount of tissue sent will make the difference as to whether the patient will be eligible to continue on the trial, and also how many vials of vaccine can be manufactured.

** Archival paraffin-embedded tissue from prior histologic diagnosis of GBM at initial diagnosis and paraffin-embedded tissue from total resection for recurrence

\+ From post-progression biopsy, if possible.

++ Blood drawn prior to bevacizumab administration.

6.6 Specimen Registration and Tracking

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org/ using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: [redacted]. For assistance in using the application or questions or problems related to specific specimen logging, please contact: [redacted].

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

6.7 MRI/CT Imaging

MRI (or CT only if MRI is contraindicated) imaging is to be completed on the following schedule (see Section 7.0):

- Within 28 days prior to surgery
- Within 48 hours post-surgery
- At 28 (+/- 3) days post-surgery
- Every 8 weeks (+/- 14 days) from registration until progression (all arms) and every 8 weeks (+/- 14 days) until 2nd progression (Arm 2)

Note: Scans that ***do not*** show progression (per sites' local reads) must be submitted to the IROC for banking and quality check (submit within 30 days of scan acquisition). Central review will not be performed on these images. Scans that ***do show*** progression should be submitted for real time review in addition to storage and QC/QA purposes. (Please see time frames below.)

For patients on Arms 1 and 3, protocol treatment should not be discontinued until central radiology review has confirmed progression. Patients on Arm 2 should not proceed to bevacizumab until their first progression has been confirmed by central radiology review or discontinue bevacizumab treatment until second progression is confirmed by central radiology review.

The 28 (+/-3) day scan must be submitted to the Imaging and Radiation Oncology Core in real time, but central review will not be performed.

6.7.1 Remote Central Review

MRI (or CT) imaging data sets of pre- and post-surgery will be concurrently reviewed by the A071101 central review panel **within no more than 72 hours after the post-surgery data receipt**. Confirmation of ≥90% resection for this protocol will be based on the centralized review results and NOT on local assessment.

MRI (or CT) imaging data sets providing evidence of radiographic disease progression as determined by the study site investigator will be concurrently reviewed by the A071101 central review panel **within no more than 72 hours after receipt of the scans.**

The Imaging and Radiation Oncology Core (IROC) will contact the A071101 central review panel, **within 24 hours (except weekends and holidays) after post-surgery images being received**, for scheduling a real-time remote review.

The IROC notifies the participating site, Alliance Data Manager, and Agenus of the central review result **within 24 hours after receiving the result from the central review panel**. If there are any uncertainties or the participating site disagrees with the first central review result, a second review by either another central reviewer or an adjudicator ([redacted]) will be performed. This process may take additional 24-48 hours turn-around time, and such review result will be used as the final decision for the outcome determinations. The Alliance A071101 trial committee renders final decisions of A071101 patient inclusion or exclusion. Progression is defined in Section 13.

For any such related questions, participating sites may directly contact the IROC instead of the central reviewer(s), via either the trial email at [redacted] or call at [redacted]

6.7.2 Data Archiving, Storage, and Submission

MRI (or CT only if MRI is contraindicated) MUST be completed within 28 days pre-surgery and within 48 hours post-surgery. The complete imaging data sets must be

submitted to the Imaging and Radiation Oncology Core (IROC) in digital DICOM format, within no more than **5 business days (pre-surgery)** and **within 24-48 hours (post-surgery)** upon the image acquisition is completed. **BMP files, JPEG files, or hard copies (films) are not acceptable.**

The **Adjunctive Data Form** and the **Central Review form—Resection or Central Review Form--Progression** must be submitted to theIROC together with the entire MRI DICOM data sets. Sites can obtain the study specific forms from the CTSU website.

De-identify the patient data using institutional procedures to remove patient name and medical record number while preserving the **patient ID number** and **protocol number** of the Alliance trial. The de-identified digital images may be burned to a CD or transferred to a PC based system for further electronic data transfer purposes.

Data should be **electronically** transferred to the Imaging and Radiation Oncology Core Lab by **1) Web Transfer or 2) FTP transfer**:

1) Web Transfer:

Any PCs with Internet access and web browser (e.g., Internet Explorer, Mozilla Firefox) can be used to transfer DICOM images and other required files to the IROC through website upload.imagingcorelab.com. The standard Web Transfer information will be provided separately through the specific trial e-mail [redacted] per the request by participating sites before their first data submission.

2) FTP Transfer:

Any FTP software can be used to initiate access to the secure FTP Server of the IROC The standard FTP access information will be provided separately through the specific trial e-mail [redacted] per the request by participating sites before their first data submission.

Send an e-mail notification to inform the Imaging and Radiation Oncology Core Lab at the specific trial email of the data submission **once the data transfer is completed**.

3) Shipment/Mail (not recommended):

If the above electronic data transfers cannot be achieved, the de-identified images in DICOM format can be burned to a CD, labeled with info of patient ID, study date, baseline/follow-ups on the CD cover, and mailed to the IROC via over-night shipment at:

Imaging and Radiation Oncology Core Department of Radiology

[redacted]

The IROC will notify site within 24 hours of the data receipt as well as within 72 hours of the quality check report via the specific trial email

[redacted]

Any questions or problems about the data transfer to the Imaging and Radiation Oncology Core Lab, email the IROC at the specific trial email, or call the IROC IT group at [redacted] for help.

7.0 REQUIRED DATA

Pre-Study Intervals

To be completed within 28 DAYS before surgery (pre-registration):

- History and physical including vital signs
- Neurological examination
- Performance status
- MRI (or CT only if MRI is contraindicated) of brain with or without gadolinium per institutional guidelines

To be completed DAY OF SURGERY:

Collection of tissue for vaccine manufacture
Collection of tissue for future biomarker analyses as available per institutional standards

To be completed within 48 HOURS following surgery:

MRI (or CT only if MRI is contraindicated) of brain with or without gadolinium per institutional guidelines to evaluate percentage of tumor resected

To be completed 28 (+/- 3) days POST SURGERY:

MRI (or CT only if MRI is contraindicated) of brain with or without gadolinium per institutional guidelines to evaluate localized bleeding prior to initiating treatment

To be completed within 16 days prior to registration:

Any baseline exams to evaluate eligibility
Blood work
Serum pregnancy test, if applicable

Tests & Observations	Pre-registration	Registration	Day 1 each cycle ***	Post-treatment follow up
History *	X	X	X	
Vital Signs[1]		X	X	
Weight[2] *		X	X	
Physical Examination*	X	X	X	
Neurological Examination*	X	X	X	
Performance Status	X		X	
Serum Pregnancy Test[3]	X	X		
Injection Site Assessment			X[5]	
Solicited Adverse Events/Abnormalities	X		X	
Laboratory Studies				
Clinical Lab Tests[4] **		X	X	
Urine protein		X	X[6]	
PT/PTT		X	X[7]	
Staging and Scans				
MRI	X[8]	X[8]	X[9]	
Histological Confirmation of recurrent GBM		X		
Survival/Disease Status				
Follow up/Event Monitoring				X[10]
Adverse Events: Other[11]			X	
Sub-study				
PBMC	See Sections 6.3 and 6.5			
Tissue Collection for Biomarkers	See Sections 6.4 and 6.5			

1 Vital signs to include pulse, temperature, blood pressure and respiratory rate to be completed before each dose of vaccine and 60 minutes (+/- 5 minutes) post each dose of vaccine. Vital signs to include pulse, temperature, blood pressure, and respiratory rate prior to every dose of bevacizumab.

2 It is not necessary to change the doses of bevacizumab due to changes in weight unless the calculated dose changes by ≥10%.

3 For women of childbearing potential only, to be completed at pre-registration and ≤ 16 days prior to beginning treatment

4 Includes:

- Albumin, alkaline phosphatase, aspartate amino transferase (AST), alanine amino transferase (ALT), bilirubin [total], glucose, creatinine,
- Hematology [red blood cell count, platelets, hematocrit, hemoglobin, white blood cell (WBC) count, plus WBC differential with absolute counts for neutrophils, eosinophils, basophils, lymphocytes, monocytes, and all labs must meet eligibility criteria.]
- Labs do not need to be repeated at the first administration if completed within 28 days prior to administration

5 30 to 60 minutes after vaccine administration

6 Urine protein within 48 hours of day 1 of bevacizumab cycles only.

7 PT/PTT required at registration for all patients; repeat required only in patients on therapeutic doses of warfarin

8 MRI (or CT only if MRI is contraindicated) completed within 28 days prior to surgery, within 48 hours post-surgery, and 28 (+/- 3) days post-surgery. The post-surgical scan at 28 (+/- 3) days is to evaluate the patient for their localized bleeding status prior to initiating treatment across all arms. See Section 6.7 for submission of images to IROC.

9 MRI (or CT only if MRI is contraindicated) completed every 8 weeks (4 cycles) from registration until progression (all arms) and every 8 weeks (4 cycles) until 2nd progression (Arm 2)

10 Every 8 weeks (+/- 14 days) until 18 months from surgery, then every 6 months (+/- 14 days) up to a maximum of 5 years. Event monitoring can include, but is not limited to 1) a phone call to the patient, 2) contact with the last known local medical doctor, or 3) medical record review from a recent clinic visit (within about 2 weeks of the event monitoring time point.

[11] Grade 1 and 2 with attribution of at least possibly related and all grade 3, 4, and 5 adverse events are to be reported.

* To be completed on the timepoints specified above or (-3) days.

** To be collected on the timepoints specified above or (-1) day.

*** For patients in Arm 2, this colum must be followed while receiving bevacizumab also.

8.0 TREATMENT PLAN

Note: Kits for shipping tissue will be supplied by Agenus and should be ordered at time of IRB submission because they will be needed at time of surgery

Surgery must be performed within 28 days from pre-registration.

NOTE: Sites will be followed for appropriate tissue handling.

Protocol treatment is to begin within 35 (+/-7) days post-surgery. **Questions regarding treatment should be directed to the Alliance Study Chair.**

Patients will be randomized 1:1:1 to one of three arms.

It is acceptable for individual therapy doses to be delivered ≤ 24 hour (business day) window before and after the protocol defined date for a scheduled dose. In addition, patients are permitted to have a new cycle of therapy delayed for up to 5 days for major life events without this being considered a protocol violation; documentation to justify this delay should be provided.

Although not required, patients may take acetaminophen prior to vaccine administration.

NOTE: Once HSPPC-96 is drawn up into the syringe, the vaccine is stable for up to two hours at ambient temperature **if immediate administration is not possible**.

8.1 Arm 1, HSPPC-96 + concomitant bevacizumab

(1 cycle=14 days)

HSPPC-96 0.4mL intradermal (see Section 11.6 for details on intradermal administration) on days 1 and 8 of cycles 1 and 2, then on day 1 of each cycle, up to a maximum of 12 doses (10 cycles), plus bevacizumab 10 mg/kg i.v. on day 1 of each cycle, until progression.

HSPPC-96 should be administered at least 60 minutes prior to starting bevacizumab infusion.

Note: If HSPPC-96 treatment has ended but there is no evidence of disease progression, the patient should continue to receive bevacizumab at the specified dose until progression.

8.2 Arm 2, HSPPC-96 with bevacizumab at progression

(1 cycle=14 days)

HSPPC-96 0.4mL intradermal (see Section 11.6 for details on intradermal administration) on days 1 and 8 of cycles 1 and 2, then on day 1 of each cycle, up to a maximum of 12 doses (10 cycles).

At progression: bevacizumab 10mg/kg i.v. on day 1 of each cycle, until further progression.

NOTE: It is possible that HSPPC-96 vaccination may end prior to evidence of progression. In this instance it is important to wait until there is confirmed evidence of progression before initiating treatment with bevacizumab.

Upon confirmation of progression the patient should initiate bevacizumab within 7 to 42 days from the last dose of vaccine.

8.3 Arm 3, Bevacizumab

(1 cycle = 14 days)

Bevacizumab 10mg/kg i.v. on day 1 of each cycle, until progression.

9.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

There are no dose reductions for bevacizumab. Bevacizumab may be delayed or discontinued for toxicity as described below.

9.1 Hypersensitivity and/or Infusion Reactions

Allergic or Infusion-Related Reactions

- The initial bevacizumab dose should be administered over a minimum of 90 minutes. If no hypersensitivity or infusion reactions occur, the second dose should be administered over a minimum of 60 minutes. If no hypersensitivity or infusion reactions occur with the second dose, the third and subsequent doses should be administered over a minimum of 30 minutes.
- For grade 1 or 2 hypersensitivity reactions during bevacizumab infusion, interrupt bevacizumab until symptoms resolve to < grade 1, then resume bevacizumab at the shortest infusion rate tolerated. Manage symptoms according to institutional procedures. Subsequent infusion rates should be titrated as stated above. Consider premedication with antihistamines for subsequent doses.
- Subjects who experience clinically evident bronchospasm (regardless of grade) should discontinue bevacizumab. Manage symptoms according to institutional procedures.

For grade 3 or 4 allergic reactions considered related to bevacizumab, discontinue bevacizumab. Manage symptoms according to institutional procedures. Continue HSPPC-96.

For grade 1 or 2 reactions considered related to HSPCC-96, manage symptoms according to institutional procedures. Continue on study.

For grade 3 or 4 allergic reactions considered related to HSPCC-96, discontinue HSPCC-96. Manage symptoms according to institutional procedures. Continue bevacizumab.

For grade 1 or 2 infusion-related reaction or cytokine release syndrome as characterized by back pain, headache, rigors, erythema, vasodilation, considered related to bevacizumab, decrease infusion rate to 50% of the previous rate. Manage symptoms according to institutional procedures. When symptoms improve to < grade 1, the infusion rate may be increased in 50% increments every 30 minutes. Subsequent infusions can be started at the planned rate.

For grade 3 or 4 infusion-related reaction or cytokine release syndrome considered related to bevacizumab, discontinue bevacizumab. Manage symptoms according to institutional procedures. Continue HSPPC-96.

For anaphylaxis considered related to bevacizumab, discontinue bevacizumab. Manage anaphylaxis according to institutional procedures. Continue HSPPC-96.

For anaphylaxis considered related to HSPPC-96, discontinue HSPPC-96. Manage anaphylaxis according to institutional procedures. Continue bevacizumab.

9.2 Injection Site Reactions

For grade 1 or 2 injection site reactions, continue on study; make sure to rotate injection sites as instructed in Administration section. Manage symptoms according to institutional procedures.

For grade 3 or 4 injection site reactions, discontinue HSPCC-96. Continue bevacizumab.

9.3 Hypertension

For grade 1 to 3 hypertension controlled with medication to <150/90 mmHg, continue bevacizumab.

For persistent or symptomatic hypertension despite antihypertensive therapy, delay bevacizumab for up to 6 weeks until BP <150/90 mmHg. Continue HSPPC-96. Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

For grade 4 hypertension, discontinue bevacizumab. Continue HSPCC-96. Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

There are no dose modifications for hypertension for HSPPC-96.

For signs and symptoms suggestive of reversible posterior leukoencephalopathy syndrome (RPLS) such as confusion, headache, seizures, and cortical blindness, hold bevacizumab for up to 6 weeks. Suspected RPLS should be investigated with MRI as described in Section 11.5. If diagnosis of RPLS is confirmed, bevacizumab should be permanently discontinued. If RPLS is ruled out via MRI, and signs and symptoms attributed to another cause, may resume bevacizumab. If bevacizumab is held or permanently discontinued for RPLS, continue HSPPC-96.

9.4 Hemorrhage

For grade 1 or 2 bleeding, restart bevacizumab once resolved to < grade 1. Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

For grade 3 or 4 bleeding: Discontinue bevacizumab. Continue HSPPC-96. Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

There are no dose modifications for hemorrhage (non-CNS) for HSPPC-96.

9.5 Venous Thromboembolic Events

Grade 1, 2, 3 or asymptomatic grade 4: Delay bevacizumab. If the planned duration of full-dose anticoagulation is <2 weeks, protocol treatment should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, protocol treatment may be resumed during the period of full-dose anticoagulation if all the following criteria are met:

- The patient must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) prior to restarting therapy;
- The patient must not have had a grade 3 or 4 hemorrhagic event while on study; and

For recurrent/worsening venous thromboembolic events after resumption of treatment: Discontinue bevacizumab.

For symptomatic grade 4: Discontinue bevacizumab.

Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

9.6 Arterial Thromboembolic Events

Arterial thrombosis may include angina, myocardial infarction, stroke (CNS cerebrovascular ischemia), or other arterial thrombotic event. In the event of new or worsening grade 2 or any grade 3, or 4 arterial thrombotic event, treatment with bevacizumab should be discontinued.

There are no dose modifications for arterial thromboembolic events for HSPPC-96.

9.7 Renal and Urinary Disorders: Proteinuria

See Appendix I for information regarding the calculation of UPC (urine protein to creatinine) ratio.

For proteinuria of ≥2+: Confirm total urine protein with a 24-hour urine collection or urine protein to creatinine (UPC) ratio. For 2+ proteinuria, give scheduled bevacizumab while awaiting the results of the 24-hour collection or UPC ratio. For >2+ proteinuria, delay bevacizumab while awaiting results of the 24-hour urine collection or UPC ratio. Continue HSPPC-96.

If monitoring urine protein with UPC, no confirmation is necessary.

For urine protein 2-3.4 g/24 hours or UPC ratio 2-3.4: Delay bevacizumab until urine protein improves to <2g/24 hours or UPC <2. If bevacizumab is delayed more than 6 weeks due to proteinuria, discontinue bevacizumab. Continue HSPPC-96.

For urine protein ≥ 3.5 g/24 hours or UPC ratio ≥3.5: Discontinue bevacizumab. Continue HSPPC-96.

There are no dose modifications for proteinuria for HSPCC-96.

9.8 Fistula, Perforation involving any Organ, Bowel Obstruction, and Wound Dehiscence

- **For any grade perforation of any organ, GI leak, or any fistula**: Discontinue bevacizumab. Continue HSPCC-96.
- **For any grade bowel obstruction requiring medical intervention:** Hold bevacizumab therapy until complete resolution. Continue HSPCC-96. If surgery is required, the patient may restart bevacizumab after full recovery from surgery and after consultation with the study chair.
- **For wound dehiscence requiring medical or surgical intervention**: Discontinue bevacizumab. Continue HSPCC-96.

Once bevacizumab is held 6 weeks, patient goes off protocol in Arms 2 and 3. In Arm 1, the patient continues the vaccine, stops the bevacizumab, and continues on trial.

9.9 Other Non-Hematologic Toxicity at Least Possibly Related to HSPCC-96

- **For other grade 1 or 2 non-hematologic toxicity,** continue on protocol once toxicity is resolved to < grade 1.
- **For other grade 3 or 4 non-hematologic toxicity considered at least possibly related to HSPCC-96,** discontinue HSPCC-96. Continue bevacizumab.

9.10 Dose Modifications for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by actual weight without any modification unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose

administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation. Physicians who are uncomfortable with calculating doses based on actual body weight should recognize that doing otherwise would be a protocol violation

10.0 CORRELATIVE SUB-STUDY

The correlative science study **must be offered** to patients enrolled on A071101.

A071101-ST1 will evaluate immune response and tissue markers in patients with resectable glioblastoma multiforme (GBM) receiving HSPPC-96.

10.1 Immune Response Study

10.1.1 Background

The impetus to treat GBM patients with immunotherapy has a clinical and scientific basis. Several groups have described a correlation between HIV-mediated immunosuppression and intracranial glial tumors [21, 22] [28, 29]. Immunosuppression in transplant recipients has also been implicated in the development of intracranial glioma [30]. In contrast, long-term remission of malignant brain tumors secondary to postoperative infection has generated the hypothesis that a heightened immune status can confer some protection against intracranial tumor [31, 32]. Consistent with this hypothesis is the epidemiological observation that a significant allergy history lowers an individual's lifetime risk for developing an intracranial glioma [33, 34]. Successful active immunotherapy for patients with glioma will require development of a specific peptide or polyvalent glioma vaccine. Serologic analysis of antigens using recombinant complementary DNA expression cloning (SEREX) has identified several tumor-associated antigens in other human cancers [35-42]. SEREX has also identified antigens capable of generating an immune response in patients with malignant glioma [43, 44]. An antibody response against one of these antigens, PHF3, is associated with an increase in survival. These results represent the first concrete evidence that immune responses generated against CNS tumor antigens are potentially protective. Moreover, recent phase I and II trials evaluating the immunogenicity of HSPPC-96 in vaccinated GBM patients have shown the increase of IFNg secreting T cells and NK cells post-vaccination. (Section 1.3)

10.1.2 Objectives

The objective is to demonstrate whether patients with an immune response to HSPPC-96 will have an improved survival outcome in comparison to those patients who do not.

10.1.3 Methods

A panel of assays will be performed to characterize the immune response generated to HSPPC-96 given in combination with bevacizumab. This includes the following tests: phenotyping of PBMC surface markers, intracellular cytokine staining to detect proinflammatory cytokines at the protein level, qPCR to detect cytokines at the mRNA level and CFSE dilution to assay T cell proliferation. Re-stimulation assays will be performed using autologous vaccine on blood isolated before and after vaccination. Recombinant gp96 will be used as a control [22]. The expectation is that patients who demonstrate a robust immune response will live longer than those who do not.

10.1.4 Statistical Design

This exploratory endpoint is to examine the relationship between immune response to HSPPC-96 and overall survival. The sample size for this correlative study is driven by the feasibility of obtaining high quality PBMCs for assessing immunological parameters.

Based on the prior clinical experience with HSPPC-96 in GBM it is likely that at least 50% of patients will be eligible based on the quality of the PBMCs obtained. We expect the relationship between immune response and survival will be similar between Arms 1 and 2, thus the analysis will be conducted with two arms pooled together. The expected accrual rate for the whole study is about 11 patients per month (Arms 1:2:3 = 1:1:1) according to Section 15.5. Therefore, it can be assumed that an average of 3-4 patients per month (50% of patients enrolled in Arms 1 and 2) will be enrolled into this correlative study. Survival curves will be estimated for immune responders vs. non-responders separately using Kaplan-Meier methods and be compared between the two groups using log-rank tests. We expect to observe about 100 events (death) by the time of analyzing the primary endpoint within Arms 1 and 2 according to Section 15.4. With a total of roughly 48 events, (assume patients enrolled in Arms 1 and 2 who participate in this correlative study provides 50% of expected events), we have 80% power in detecting a hazard ratio of 1.85 assuming a 1-sided type I error rate of 0.1.

10.2 Tissue Marker Study

10.2.1 Background

It has been published that over expression of B7H1 secondary to activation of PI3 kinase results in immunoresistant mechanisms at the local tumor site. It is therefore postulated that patients having low expression levels of B7H1 and PI3 kinase may have a better immune response and potentially a better overall response as indicated by improved overall survival outcomes versus those patients who have high levels of these markers

10.2.2 Objectives

The objective of this study is to explore the expression of B7-H1 protein expression and P13K pathway activation at the tissue level before treatment and at recurrence and correlate it with the extent of immune infiltration. We hope to determine that patients with lower expression of B7H1 and less PI3 kinase activation at baseline develop a stronger immune response post-therapy and show a longer survival. Tissue blocks obtained at the time of surgery will be prepared and stained for expression of B7-H1 protein as well as downstream markers of PI3K activation (i.e. p-Akt and p-S6K). The extent of staining will be graded and then compared to clinical endpoints. The expectation is that the extent of B7-H1 expression and extent of PI3K activation will correlate inversely with clinical outcome on the vaccine arms of the study, with no effect on the non- vaccine arm. [45] In addition tissue blocks will be stained for T-cell markers (CD3, CD8, CD4) and assessed for extent of infiltration, which will be and compared to clinical outcome. For those patients who undergo biopsy or resection after vaccination at time of progression, tissue will be collected and analyzed for T-cell infiltrate, and compared to originally tumor tissue that was resected at time of surgery for enrollment.

10.2.3 Methods

Immunohistochemistry will be performed using B7-H1 specific antibodies as well as antibodies specific for phospho-Akt and phosphor-S6Kinase. B7-H1 is an immunoresistant protein that is regulated post-transcriptionally by the PI(3)K pathway. Activation of PI3K can be measured by staining for 2 downstream targets phospho-Akt and phosphor-S6Kinase.

10.2.4 Statistical Design

There is no formal statistical design for this correlative study as it is highly exploratory. The endpoint is to explore a potential correlation between B7H1 and PI3 kinase expression

levels in baseline tumor tissue, corresponding immune response and overall survival. Although tumor tissue samples should be taken on all patients, exploration of this correlative measure requires that patients undergo immune response assessments. Therefore, approximately 50% of patients enrolled in Arms 1 and 2 (148*50%=74 patients) should constitute the sample size for this study. The monthly accrual rate is the same as noted for the immune response assessment. To explore the correlation between biomarkers expression levels at baseline with respect to immune response, the alteration in biomarkers throughout the study will be summarized descriptively and graphically to evaluate how these values changed after the vaccine. Baseline expression levels will be compared between responder vs. non-responders using two-sample t-tests (or Wilcoxon rank sum tests as needed) as well as side-by-side boxplots. Assume 40% of patients are responders, a sample size of 74 will provide us at least 80% power to detect an effect size of 0.67 with a two-sided type I error rate of 0.05. We will also explore dichotomized versions of the biomarkers and assess their relationship to response using Fisher exact test as well as bar graphs as appropriate. We will have at least 80% power to detect a difference of 35% (25% vs. 60%) assuming a response rate of 40%, and a two-sided type I error rate of 0.05. We will also explore the impact of these biomarkers in the univariate and multivariable setting on response using logistic regression model. Cox proportional hazard models will be used to explore the relationship between survival and baseline biomarker values. Power estimation will be similar to that specified in Section 10.1.4.

11.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

11.1 Qualified personnel

Personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

11.2 Unused agents

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

11.3 Bevacizumab dose rounding

The total administered dose of bevacizumab may be rounded up or down within a range of 5% of the actual calculated dose.

11.4 Weight changes

It is not necessary to change the doses of bevacizumab due to changes in weight unless the calculated dose changes by ≥10%.

11.5 Bevacizumab (rhuMAb VEGF, Avastin®) (NSC #704865)

Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody, consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-determining regions. Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.

Availability

Commercially available.

Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid for parenteral administration, in 4 mL and 16 mL vials, at a concentration of 25 mg/ml. Each vial also contains phosphate, trehalose, polysorbate 20, and Sterile Water for Injection USP.

Storage and Stability

Intact vials of bevacizumab should be stored in a refrigerator (2° to 8°C) and should remain refrigerated until just prior to use. Do not freeze. Do not shake. The sterile single use vials contain no antibacterial preservatives; therefore, vials should be discarded eight hours after initial entry. Solutions diluted for infusion may be stored in the refrigerator for up to 8 hours.

Preparation

Vials contain no preservative and are intended for single use only. The calculated dose should be diluted in 100 mL of 0.9% Sodium Chloride for Injection. Once diluted in 0.9% Sodium Chloride for Injection, the bevacizumab solution must be administered within 8 hours.

Administration

Bevacizumab is administered as an intravenous infusion. The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur after the initial dose, the second dose should be administered over a minimum of 60 minutes. If no adverse reactions occur after the second dose, all subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated.

Toxicities

Hypertension: Hypertension is commonly seen with bevacizumab and oral medications have been used to manage the hypertension when indicated. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS). RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances/ cortical blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and discontinuation of bevacizumab.

Reversible posterior leukoencephalopathy syndrome (RPLS) or similar leukoencephalopathy syndrome: RPLS or clinical syndromes related to vasogenic edema of the white matter have been recently reported in association with bevacizumab therapy. These syndromes have been seen in < 1% of patients to date. Clinical presentations are variable and may include altered mental status, seizure and cortical visual deficit. HTN is a common risk factor and was present in most (though not all) patients on bevacizumab who developed RPLS. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypointensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure or other CNS findings. RPLS is potentially reversible, but timely correction of the underlying causes, including control of BP and interruption of the offending drug, is important in order to prevent progression to irreversible tissue damage.

Neutropenia: When combined with chemotherapy, bevacizumab is reported to increase the risk of neutropenia over that of chemotherapy alone.

Proteinuria**:** Proteinuria ranging from asymptomatic abnormal urinalysis to nephrotic syndrome has been described in 10% or more of patients receiving bevacizumab. See Section 9.7 for dose modifications for proteinuria.

Thromboembolic events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer (CRC) trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials. In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events that was not statistically significant in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%). There was also a higher rate of arterial TE events (3% vs. 1%) such as myocardial infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina. A pooled analysis of the rate of arterial TE events from 5 randomized studies (1745 patients) showed that treatment with chemotherapy plus bevacizumab increased the risk of having an arterial TE event compared with chemotherapy alone (3.8% vs. 1.7%, respectively). Furthermore, subjects with certain baseline characteristics (age $\geq$ 65 years and/or a history of a prior arterial TE event) may be at higher risk of experiencing such an event.

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin $\leq$ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similar retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005. Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal perforation: Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to bevacizumab has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, and breast) and may be higher in incidence in some tumor types.

Wound healing complications: Wound-healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. The appropriate interval from discontinuation of bevacizumab to subsequent elective surgery required to reduce the risk of impaired wound healing has not been determined. Decision on such an interval should take into consideration the half-life of bevacizumab (approximately 20 days). It is generally recommended that bevacizumab should be discontinued at least 4-8 weeks prior to major elective surgery. In addition, bevacizumab should not be restarted until at least 4 weeks after major surgery provided that the wound has adequately healed.

Hemorrhage: Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types. The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-associated hemorrhage was observed in phase I and phase II bevacizumab studies. Six serious events, of which 4 had fatal outcome, were observed in a phase II trial of patients with non-small cell lung cancer receiving bevacizumab. These events occurred suddenly and presented as major or massive hemoptysis in patients with either squamous cell histology and/or tumors located in the center of the chest in close proximity to major blood vessels. In five of these cases, these hemorrhages were preceded by cavitation and/or necrosis of the tumor. Tumor-associated hemorrhage was also seen rarely in other tumor types and locations, including central nervous system (CNS) bleeding in a patient with hepatoma with occult CNS metastases and continuous oozing of blood from a thigh sarcoma with necrosis.

Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Congestive heart failure: CHF has been reported in bevacizumab clinical trials and may be increased in incidence in patients with prior exposure to anthracyclines or prior irradiation to the chest wall. In a phase III trial (AVF2119g) of capecitabine with or without bevacizumab for metastatic breast cancer, 7 subjects (3.1%) who received capecitabine plus bevacizumab developed clinically significant CHF compared with 2 subjects (0.9%) treated with capecitabine alone; of note, all subjects in this trial had had prior anthracycline treatment. In addition, 2 subjects had a left ventricular ejection fraction < 50% at baseline and 2 others had prior left chest wall irradiation. A recently published phase II study in subjects with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or decreases to <40% in left ventricular ejection fraction) of 48 subjects treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but one of these subjects had significant prior exposure to anthracyclines as well. Other studies are ongoing in this patient population.

Osteonecrosis of the jaw: There are several reports in the literature suggesting that bevacizumab increases the likelihood of osteonecrosis of the jaw in patients receiving bisphosphonates, and that bevacizumab may cause osteonecrosis of the jaw in patients not receiving bisphosphonates. This effect is thought to be related to inhibition of angiogenesis.

For a comprehensive list of adverse events and potential risks (CAEPR), see Section 16.3. See the most current version of the package insert for additional information.

11.6 Heat Shock Protein-Peptide Complex-96 Vaccine (HSPPC-96)(NSC #725085) (Alliance IND # 15380)

See the Investigator Brochure for additional information.

To obtain a copy of the IB, contact [redacted].

HSPPC-96 is an autologous tumor-derived vaccine under clinical investigation for the treatment of a variety of cancer types. It is composed of the 96-kDa heat shock protein gp96 in complex with autologous tumor-derived peptides. The gp96 in HSPPC-96 is a highly conserved, abundant, nonpolymorphic stress protein found in every cell type of the body. gp96 isolated from normal or tumor tissues is found in complex with a diverse repertoire of peptides, which are specific to the tissue of origin. Published studies in mouse tumor models have shown that HSPPC-96 confers protective immunity only to the tumor from which it is derived and not to antigenically distinct tumors. The specific immunogenicity of the HSP preparations can be attributed to the unique repertoire of antigenic peptides that exists in different cancers.

When injected into the host, HSPPC-96 interacts with antigen presenting cells (APCs) such as macrophages, dendritic cells or Langerhans cells, which take up HSPPCs via HSP receptors, including CD91. Once internalized by APCs, the peptides chaperoned by the HSPs are transferred to major histocompatibility complex (MHC) class I and II molecules in intracellular compartments and subsequently expressed at the cell surface. The cells then migrate to lymph nodes, where the complexes are processed so that the peptides chaperoned by the HSPs are re-presented to naive T cells. The T cells recognize the peptides and, as a result, are stimulated by them. Vaccination with HSPPCs thus elicits both a CD8+ and CD4+ T-cell response targeting potentially all relevant tumor antigens. The interaction of HSPs with receptors on APCs also leads to activation of various components of innate immunity, including cytokine and chemokine release by macrophage and dendritic cells, maturation of dendritic cells and activation of natural killer cells, as shown in both murine and human systems. Tumor immunity is largely mediated by T cells and the ability of HSPPC-96 to stimulate both T-cell arms to recognize a large variety of tumor antigens, coupled with the ability to activate innate immune responses, uniquely positions the product among other cancer vaccine strategies.

Availability

HSPPC-96 will be prepared by Agenus, Inc. and shipped directly to sites within approximately 2 weeks of Agenus' receipt of tumor tissue for patients randomized to Arms #1 and #2. HSPPC-96 vaccine, although produced for patients randomized to Arm #3, will not be shipped to sites. Vaccine produced for patients randomized to Arm #3 will be used for quality evaluation and will not be available for clinical use.

HSPPC-96 is supplied in a **single-use** vial as a clear, colorless solution. Each vial contains 25 µg of HSPPC-96 in a solution of 9% sucrose-potassium phosphate for intradermal (ID) injection. The total volume of each vial of HSPPC-96 is 0.47 mL. The total volume that should be administered is 0.4 mL.

Ordering HSPPC-96 Vaccine from Agenus

After randomization, the site must complete the Vaccine Order Form and send it back to Agenus either by fax (781-674-4260) or by email as a scanned copy to A071101@Agenusbio.com indicating whether or not vaccine should be shipped.

The site should check "Yes, please ship vaccine" for patients randomized to study Arms #1 or #2 and "No, please do not ship" for patients randomized to study Arm #3. **Additionally, in rare instances where vaccine has been produced, but the patient has failed other post-surgical eligibility criteria check "No, please do not ship" on the form and identify in the comments section that the patient is a screen failure.**

HSPPC-96 Vaccine Label

Each vial of vaccine vial is labeled with the batch number, patient study number, patient initials, and patient date of birth (DOB).

Storage and Stability

Following successful production of HSPPC-96 vaccine at Agenus, vials are shipped (for those patients randomized to Arms #1 and #2) to the clinical site on dry ice and must be stored at -80°C ± 20°C until administration to the patient. Keep the shipping kit until the patient has completed vaccine therapy, for return of any unused or expired vaccine.

Any unused or expired vaccine remaining after a patient has completed vaccine treatment should be returned to Agenus. See Appendix VI

NOTE: Once HSPPC-96 is drawn up into the syringe, the vaccine is stable for up to two hours at ambient temperature **if immediate administration is not possible**.

Preparation

Withdraw the vial from the freezer and roll gently between two fingers until it is completely thawed. Withdraw 0.4 mL, using a TB or insulin syringe.

Administration

The route of administration is intradermal injection. Using a 25 g needle, inject into 1 site or into 2 adjacent sites (0.2 mL each) a few centimeters apart. Appropriate sites for vaccination include the anterior deltoid regions, subclavicular region bilaterally, and medial inguinal regions of the upper thighs. Do not administer HSPPC-96 to areas distal to lymph node basins that have been resected or in areas just distal to a surgical scar. Rotate the injection sites so injections are not repeated at the same site at 2 consecutive administrations and utilize all potential sites for the patient before repeating injections at a previously used injection site. Syringes with slip-tip detachable needles or luer hubs with greater than 0.1 mL dead space should not be utilized. See instructions and diagram on the CTSU website.

Toxicities

Local injection-site reactions can occur and are usually of minimal extent and mild severity. If a local injection-site reaction requires medical attention, the investigator can provide local therapy as needed. Occasional systemic toxicities have been reported that have been mild in severity. The most frequently reported events include injection site reactions of erythema and induration, fatigue, nausea, pyrexia, headache, back pain, arthralgia, constipation, and asthenia.

For a complete list of events, refer to the IB.

12.0 ANCILLARY THERAPY

12.1 Supportive Care

Patients should receive full supportive care, including anti-seizure medications, transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on treatment form.

12.2 Hormones or Other Agents

Treatment with hormones or other chemotherapeutic agents may not be administered except for: no more than 16 mg dexamethasone (or equivalent) to control symptoms of cerebral edema and mass effect and should be discontinued as soon as possible; hormones administered for non-disease-related conditions (e.g., insulin for diabetes); and intermittent use of dexamethasone as an antiemetic.

12.3 Palliative RT

Palliative radiation therapy may not be administered.

12.4 Growth Factors

The following guidelines are applicable unless otherwise specified in the protocol:

12.4.1 Epoetin (EPO)

Use of epoetin in this protocol is prohibited.

12.4.2 Filgrastim (G-CSF) and sargramostim (GM-CSF)

Filgrastim (G-CSF), pegfilgrastim and sargramostim (GM-CSF) treatment may not be used.

12.5 Surgery

Patients who require surgery during protocol treatment may proceed as such, unless the surgery involves resection of GBM. Bevacizumab and/or HSPCC-96 should be held prior to and after surgery, at the discretion of the treating physician, for a maximum of 6 weeks.

If the patient requires an interruption of > 6 weeks, then they will be removed from protocol therapy.

13.0 CRITERIA FOR PROGRESSION

Patients are followed every 8 weeks until progression. Standard criteria for response do not apply as complete surgical resections of tumor tissue are being performed. Further, in considering the purported mechanism of action of HSPPC-96 it may be difficult to differentiate true progression from pseudoprogression (treatment effect); therefore, the definition of progression provided below takes this specific protocol setting and the investigational therapy being tested into account.

In Arm 2, these criteria for progression serve as a trigger to stop HSPPC-96 and start patients on bevacizumab. Determination of subsequent progression after initiation of bevacizumab post-progression on HSPPC-96 alone should follow the same set of criteria.

Progressive disease post-baseline is defined as one or more of the following:

1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids.

2. Increase by > 50% (modified from >25% according to published RANO criteria) enhancement from the first post-surgical scan, or a subsequent scan with smaller tumor size, and the scan 8 weeks or later on stable or increasing doses of corticosteroids.

3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment.

4. For patients receiving bevacizumab therapy, significant increase in T2/FLAIR non-enhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not a result of comorbid events (e.g., ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

14.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

14.1 Duration of Treatment

14.1.1 Free from Evidence of Disease:

Continue treatment at the protocol specified doses and regimens as directed based on the randomization arms until the appearance of disease progression per Section 13.

14.1.2 Disease Progression:

Evidence of disease progression as per Section 13 will be cause for either removal from protocol therapy or trigger a patient to stop one therapy and transition to another depending upon the randomization arm. See below for specific details. Document details including tumor measurements on the appropriate form.

Patients randomized to Arms 1 or 3 (HSPPC-96+ concomitant bevacizumab (Arm 1) or bevacizumab alone (Arm 3)) will be removed from protocol therapy at the point of disease progression.

Patients randomized to Arm 2 (HSPPC-96 followed by bevacizumab at progression) will be removed from protocol therapy with HSPPC-96, if they still had remaining vaccinations available at the point of progression, and will begin treatment with bevacizumab until further progression.

14.2 Extraordinary Medical Circumstances:

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy.
- Follow the patient for survival until death.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study overview

This study will be a randomized phase II trial in patients with surgically resectable recurrent GBM. The study includes three arms: Arm 1 - HSPPC-96 with concomitant bevacizumab, Arm 2 – HSPPC–96 followed by bevacizumab at progression, and Arm 3 – bevacizumab alone (the control arm). A dynamic allocation procedure will be used to allocate an equal number of patients to different arms (1:1:1). This procedure will balance the marginal distributions of the stratification factors between arms. The stratification factors that will be used are age (< 55 vs. ≥55 years old), KPS (70 vs. 80-100) and the number of HSPPC-96 vials produced for the patient (4 or 5 vs. 6 or more).

15.2 Objectives

15.2.1 Primary Objective

To assess whether HSPPC-96 administered in combination with bevacizumab (given either concomitantly with HSPPC-96 or at the point of progression) improves survival overall compared to bevacizumab alone in patients with surgically resectable recurrent GBM

15.2.2 Secondary Objectives

- To evaluate the safety and tolerability of HSPPC-96 when given in combination with bevacizumab
- To evaluate progression free survival of HSPPC-96 when given in combination with bevacizumab (given either concomitantly with HSPPC-96 or at the point of progression)
- To select an arm with better overall survival between two HSPPC-96 plus bevacizumab arms given the combined arm proved to extend the survival as compared to the bevacizumab alone arm.

15.3 Endpoints

15.3.1 Primary Endpoint

The primary endpoint is overall survival (OS), which is defined as the date from study registration to the date of death, due to any cause.

15.3.2 Secondary Endpoint

Time to progression free survival: which is defined as the date from study registration to the date of first observation of disease progression or death due to any cause (whichever comes first).

15.4 Sample Size Derivation

The primary objective of this study is to demonstrate that patients with recurrent GBM randomized to HSPPC-96 plus bevacizumab arms – either received concomitantly (Arm 1) or given at the time of progression (Arm 2) - have improved overall survival as compared to patients who were randomized to bevacizumab alone arm (Arm 3). In other words, Arms 1 and 2 will be combined and then compared to Arm 3 to explore the additive effect of HSPPC–96. Based on previous reports, the median OS for recurrent GMB patients treated on bevacizumab alone is about 9 months. A sample size of 165 patients (55 per arm) will yield an 85% power to detect a 36% decrease in the hazard rate using a one-sided logrank test with a false positive rate of 0.1. This is equivalent to a 56% increase in the median survival time from 9 months to 14 months. Sample size calculation is computed based on an accrual rate of 5 patients per month, and a minimum of 12 months follow up period. The required number of events will be 129.

15.5 Accrual and study duration

The study will be opened to all sites within The Alliance for Clinical Trials in Oncology (the Alliance) through the CTSU mechanism. We anticipate enrolling about 5 eligible recurrent GBM patients per month, and the accrual period for the study will be about 33 months and the total study duration will be about 45 months. We anticipate pre-registering 254 patients to reach a maximum accrual of 165 patients required for the analysis.

15.6 Interim analysis

One interim futility analysis will be applied when 50% of events (65deaths) have occurred (approximately 2 years after the start of the trial under null hypothesis). Rho family (rho = 2) β spending function was used to determine the boundary for the futility interim look. If the hazard ratio (experimental arm vs. control armassociated with the treatment covariate in the Cox proportional hazards model (adjusted for stratification factors) is> 1.026, the alternative hypothesis will be rejected and we will conclude there is little chance of demonstrating the combined arm is superior to the control arm and we may recommend terminating the trial.

15.7 Analysis Plan for Primary and Secondary Endpoints

15.7.1 Primary Endpoint

Efficacy analysis will be based on the intention-to-treat principle with all eligible patients belonging to the treatment arm to which they were randomized. Patients who have not died at the primary analysis time will be censored at the time of last known alive. The distribution of overall survival for combined and the control arms will be estimated using the Kaplan-Meier method. The hazard ratios and median survivals will be estimated with their 95% confidence intervals. The Cox proportional hazards model will be used to assess whether the distribution of overall survival times differ with respect to treatment regimen having adjusted for the stratification factors (age, KPS and number of vials produced). The corresponding p-value associated with the treatment covariate will be compared to the nominal p-value to make the conclusion regarding the primary efficacy comparison.

If the primary efficacy analysis indicates that the combined arm extend the overall survival significantly as compared to the bevacizumab alone arm, two HSPPC-96 plus bevacizumab arms will be compared to each other to select an arm for following up phase III study using

the pick-the-winner method. By the time the primary analyses were completed, we expect to observe about 82 events (deaths) between the two experimental arms, which will provide about 80% likelihood of selecting the better arm if one arm is superior to the other by 60% in median OS (11 vs. 17.6 months), at one-sided significance level of 0.1.

15.7.2 Secondary Endpoints

- **Progression free survival:** progression free survival (PFS) will be estimated using the Kaplan-Meier method. The hazard ratios and median PFSs will be estimated with their 95% confidence intervals, and the distribution of PFS will be compared between arms using Cox models.
- **Adverse events:** a secondary objective of the trial is to further characterize the safety and tolerability of HSPPC-96 in combination with bevacizumab. As per NCI CTCAE version 5.0, the phrase "treatment-related adverse event" is defined as an adverse event that is classified as either "possibly," "probably," or "definitely related" to study treatment. The maximum grade for each type of treatment-related adverse event will be recorded for each patient, and frequency tables for each arm will be reviewed to determine patterns. In addition, the number and percentage of patients in the treatment group who discontinued HSPPC-96 treatment due to an adverse event will be presented, together with the number and percentage of patients who died due to an adverse event. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either "unrelated" or "unlikely to be related" to study treatment in the event of an actual relationship developing. Adverse events and treatment-related adverse events will be evaluated using all patients who have received any study treatment as well as summarizing those who have been included in the efficacy analyses. Treatment-related adverse events will be tabulated for each arm and will be compared among arms using Chi-Square or Fisher's Exact tests.

15.7.3 Adverse Event Stopping Rule

The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation**.** With each arm, a specific interim toxicity analysis will be performed once at least 10 evaluable patients have been followed for 3 months (accrual will not stop during this three-month time period). Accrual will be temporarily suspended if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy either of the following:

- If 2 or more patients in the first 10 evaluable patients (or 20% or more after first 10 patients have been accrued) experience a grade 4 or higher non-hematologic adverse event.
- If 2 or more patients in the first 10 evaluable patients (or more than 20% after 10 patients have been accrued) experience a grade 3 or higher CNS bleed.

We note that we will review grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below. CTCAE v5.0 will be used to determine grading for these stopping rules.

15.7.4 Monitoring

15.7.4.1 This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. An abbreviated report containing cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

15.7.4.2 In addition to the regular and frequent assessment of the Adverse Event Stopping Rule, this study will also be reviewed by the study chair and study statistician to monitor the study for evidence of severe adverse effects and feasibility problems in conjunction with production of the annual Alliance Study Summary reports to identify any problems with accrual, toxicity, and endpoints. These study summary reports will be available to members at www.allianceforclinicaltrialsinoncology.org.

15.7.4.3 The principal investigator and the study statistician will review the study at least twice a year to identify accrual, adverse event/safety data, and any endpoint problems that might be developing. The Alliance Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the study statistician.

15.7.4.4 In addition to the above monitoring, there will be regularly scheduled (monthly) calls with the Study Chairs(s), Statistician(s) andAlliance Executive Officer to discuss toxicity reports.

15.8 Descriptive Factors

These factors relate to how any subset analysis will be performed.

15.8.1 Prior nitrosoureas: Yes vs. No

15.8.2 Prior temozolomide: Yes or No

15.8.3 Interval since end of RT (months)

15.8.4 Corticosteroid therapy at study entry: Yes vs. No

15.8.5 Extent of primary resection: None vs. biopsy vs. subtotal resection vs. gross total resection.

15.8.6 Side of primary resection: Right vs. left vs. midline vs. bilateral

15.8.7 Histologic type of primary tumor: oligodendroglioma vs. oligoastrocytoma vs. astrocytoma

15.8.8 Histologic grade of primary tumor: 2 vs. 3 vs. 4

15.8.9 Number of prior chemotherapy regimens: 0 vs. 1 vs. 2

15.8.10 Family history of brain tumor: Yes vs. No
If yes, check all that apply:
_____ Father
_____ Mother
_____ Brother/Sister

_____ ***Child***
_____ ***Other*** **(list: _____)**

16.0 ADVERSE EVENT REPORTING

16.1 Expedited Adverse Event Reporting

Investigators are required by Federal Regulations to report serious adverse events as defined below. Alliance investigators are required to notify the Alliance Protocol Operations Program, the Study Chair, and their Institutional Review Board if a patient has an adverse event requiring expedited reporting. All such events must be reported in an expedited manner using the CTEP Adverse Event Reporting System (CTEP-AERS). The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for expedited AE reporting beginning April 1, 2018. All treatment areas should have access to a copy of the CTEP version 5.0 of CTCAE. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. In the rare event when Internet connectivity is disrupted, the report should be submitted as soon as possible once connectivity is restored. An electronic report MUST be submitted upon re-establishment of Internet connection.

The Alliance requires investigators to route all expedited adverse event reports through the Alliance Protocol Operations Program for Alliance coordinated studies. Be sure to read this entire protocol section, as requirements are described in both the table and bullet points following the table. Note that the table and the Additional Instructions or Exclusions are protocol-specific, and in the case of a conflict, the Additional Instructions or Exclusions supersede the table. Most exclusions cover "expected" events that the sponsor would not be required to report to the FDA in an expedited manner.

16.2 Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND within 30 Days of the Last Treatment [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour; 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR, and in the bullets below.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last treatment require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs that are at least possibly related to treatment

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization and that are at least possibly related to treatment
- Grade 3 adverse events that are at least possibly related to treatment

Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for A071101:

- All adverse events reported via CTEP-AERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- A071101 uses a drug under an Alliance IND. The reporting requirements above should be followed for all agents (any arm) in this trial.
- Treatment expected adverse events include those listed in Sections 11.5 and 11.6, in the Investigator's Brochure for HSPCC-96, and in the CAEPR for bevacizumab. Note that the ASAEL column of the CAEPR has been replaced with the Specific Protocol Exceptions to Expedited Reporting (SPEER) column. The SPEER includes "expected" severity grades in addition to event terms. Events listed in the SPEER only require expedited reporting if the severity grade is above the grade noted in the SPEER.
    - Grade 3 hypertension, once controlled, does not require continued reporting via CTEP-AERS.
    - Grade 3 proteinuria does not require reporting via CTEP-AERS.
    - All new malignancies should be reported through CTEP-AERS whether or not they are thought to be related to either previous or current treatment. All new malignancies should be reported including solid tumors (including non-melanoma skin malignancies), hematologic malignancies, myelodysplastic syndrome (MDS)/acute myelogenous leukemia (AML), and *in situ* tumors. In CTCAE version 5.0, the new malignancies (both second and secondary) may be reported as one of the following (1) Leukemia secondary to oncology chemotherapy, (2) Myelodysplastic syndrome, (3) Treatment-related secondary malignancy, or (4) Neoplasm other, malignant (grade 3 or 4). Whenever possible, the CTEP-AERS reports for new malignancies should include, tumor pathology, history of prior tumors, prior treatment/current treatment including duration, any associated risk factors or evidence regarding how long the new malignancy may have been present, when and how the new malignancy was detected, molecular characterization or cytogenetics of the original tumor (if available) and of any new tumor, and new malignancy treatment and outcome, if available.
    - Patients who become pregnant on study risk intrauterine exposure of the fetus to agents, which may be teratogenic. For this reason, pregnancy occurring on study or within 6 months following the last dose of study therapy should be reported in an expedited manner via CTEP-AERS as "***Pregnancy, puerperium and perinatal conditions*** **- Other (*Pregnancy*)** under the ***Pregnancy, puerperium and perinatal conditions*** SOC and reported as Grade 3.
    - Pregnancy should be followed up until the outcome of the pregnancy is known at intervals deemed appropriate by the investigator and the outcome reported via CTEP-AERS.
    - All Adverse Events must be reported in routine study data submissions. AEs reported through CTEP-AERS must also be reported in routine study data submissions.

16.3 Comprehensive Adverse Event and Potential Risks List (CAEPR) for Bevacizumab (rhuMAb VEGF, NSC 704865)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3540 patients*. Below is the CAEPR for Bevacizumab (rhuMAb VEGF).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.5, May 2, 2018[1]

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 5.0 Term) [n= 3540]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
		Hemolytic uremic syndrome	
CARDIAC DISORDERS			
	Cardiac disorders - Other (supraventricular arrhythmias)[2]		***Cardiac disorders - Other (supraventricular arrhythmias)[2] (Gr 3)***
		Chest pain – cardiac[3]	
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction[3]	
		Ventricular arrhythmia	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Colitis		***Colitis (Gr 3)***
	Constipation		***Constipation (Gr 3)***
	Diarrhea		***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
		Gastrointestinal fistula[4]	
	Gastrointestinal hemorrhage[5]		***Gastrointestinal hemorrhage[5] (Gr 2)***
	Gastrointestinal obstruction[6]		
		Gastrointestinal perforation[7]	
		Gastrointestinal ulcer[8]	
	Ileus		

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 5.0 Term) [n= 3540]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Mucositis oral		***Mucositis oral (Gr 3)***
	Nausea		***Nausea (Gr 3)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 3)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 3)***
	Pain		***Pain (Gr 3)***
HEPATOBILIARY DISORDERS			
		Gallbladder perforation	
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction (Gr 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[9]		***Infection[9] (Gr 3)***
		Infections and infestations - Other (necrotizing fascitis)	
	Infections and infestations - Other (peri-rectal abscess)		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		***Infusion related reaction (Gr 2)***
		Injury, poisoning and procedural complications - Other (anastomotic leak)[10]	
	Wound complication		***Wound complication (Gr 2)***
	Wound dehiscence		***Wound dehiscence (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	Creatinine increased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 3)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 3)***
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Dehydration		***Dehydration (Gr 3)***
	Hyperglycemia		
	Hypokalemia		
	Hyponatremia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 3)***
		Avascular necrosis[11]	

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 5.0 Term) [n= 3540]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Generalized muscle weakness		
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia)[12]		
	Myalgia		***Myalgia (Gr 3)***
	Osteonecrosis of jaw[13]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 3)***
		Intracranial hemorrhage	
		Ischemia cerebrovascular	
	Peripheral sensory neuropathy[14]		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		***Hematuria (Gr 3)***
		Nephrotic syndrome	
	Proteinuria		***Proteinuria (Gr 2)***
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Reproductive system and breast disorders - Other (ovarian failure)[15]			
		Vaginal fistula	
	Vaginal hemorrhage		***Vaginal hemorrhage (Gr 3)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		***Allergic rhinitis (Gr 2)***
		Bronchopleural fistula	
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 3)***
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		***Epistaxis (Gr 3)***
	Hoarseness		***Hoarseness (Gr 3)***
		Pulmonary hypertension	
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Dry skin		
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus		***Pruritus (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 5.0 Term) [n= 3540]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Urticaria		***Urticaria (Gr 2)***
VASCULAR DISORDERS			
		Arterial thromboembolism[3, 16]	
Hypertension			***Hypertension (Gr 3)***
	Thromboembolic event		***Thromboembolic event (Gr 3)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Supraventricular arrhythmias may include supraventricular tachycardia, atrial fibrillation, and atrial flutter.

[3]The risks of arterial thrombosis such as cardiac or CNS ischemia are increased in elderly patients and in patients with a history of diabetes.

[4]Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[5]Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[6]Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[7]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation.

[8]Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[9]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[10]Anastomotic leak may include Gastric anastomotic leak; Gastrointestinal anastomotic leak; Large intestinal anastomotic leak; Rectal anastomotic leak; Small intestinal anastomotic leak; Urostomy leak; Vaginal anastomotic leak.

[11]There have been reports of non-mandibular osteonecrosis (avascular necrosis) in patients under the age of 18 treated with bevacizumab.

[12]Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

[13]Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

[14]Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

[15]Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.

[16]Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack, and stroke.

Adverse events reported on bevacizumab (rhuMAb VEGF) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that bevacizumab (rhuMAb VEGF) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Bone marrow hypocellular; Disseminated intravascular coagulation; Hemolysis; Thrombotic thrombocytopenic purpura
CARDIAC DISORDERS - Atrioventricular block complete; Atrioventricular block first degree; Cardiac arrest; Myocarditis; Pericardial effusion; Restrictive cardiomyopathy; Right ventricular dysfunction
EAR AND LABYRINTH DISORDERS - Ear and labyrinth disorders - Other (tympanic membrane perforation); Hearing impaired; Tinnitus; Vertigo
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (blindness); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (corneal epithelial defect); Eye disorders - Other (ischemic CRVO); Eye disorders - Other (macular pucker); Eye disorders - Other (transient increased IOP > or =30 mm Hg); Eye pain; Floaters; Keratitis; Optic nerve disorder; Photophobia; Retinal detachment; Retinal tear; Retinopathy; Vitreous hemorrhage; Watering eyes
GASTROINTESTINAL DISORDERS - Ascites; Cheilitis; Colonic stenosis; Dry mouth; Dysphagia; Enterocolitis; Esophageal pain; Esophageal stenosis; Flatulence; Gastrointestinal disorders - Other (peritonitis); Oral pain; Pancreatitis; Proctitis; Rectal mucositis; Rectal stenosis; Typhlitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Edema limbs; Edema trunk; Facial pain; Fever; Flu like symptoms; Gait disturbance; Injection site reaction; Localized edema; Multi-organ failure; Sudden death NOS
HEPATOBILIARY DISORDERS - Cholecystitis; Gallbladder necrosis; Gallbladder obstruction; Hepatic failure; Hepatic necrosis
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Burn; Dermatitis radiation; Fracture
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood antidiuretic hormone abnormal; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Electrocardiogram QT corrected interval prolonged; Forced expiratory volume decreased; GGT increased; INR increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypomagnesemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Fibrosis deep connective tissue; Head soft tissue necrosis; Joint effusion; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Neck pain; Osteonecrosis; Pain in extremity; Pelvic soft tissue necrosis; Soft tissue necrosis lower limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Arachnoiditis; Ataxia; Central nervous system necrosis; Cerebrospinal fluid leakage; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Encephalopathy; Extrapyramidal disorder; Facial nerve disorder; Hydrocephalus; Leukoencephalopathy; Memory impairment; Myasthenia gravis; Nervous system disorders - Other (increased intracranial pressure); Paresthesia; Peripheral motor neuropathy; Pyramidal tract syndrome; Seizure; Somnolence; Tremor; Vasovagal reaction
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Psychosis

RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Dysuria; Renal and urinary disorders - Other (ureterolithiasis); Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Erectile dysfunction; Irregular menstruation; Pelvic pain; Vaginal discharge
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Hypoxia; Nasal congestion; Pulmonary fibrosis; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (dry nares); Respiratory, thoracic and mediastinal disorders - Other (pulmonary infarction)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Hyperhidrosis; Nail loss; Pain of skin; Photosensitivity; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (diabetic foot ulcer); Skin and subcutaneous tissue disorders - Other (skin breakdown/ decubitus ulcer); Skin hyperpigmentation; Skin induration; Skin ulceration; Stevens-Johnson syndrome
VASCULAR DISORDERS - Flushing; Hot flashes; Hypotension; Lymphocele; Phlebitis; Vasculitis

Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

16.4 Routine adverse event reporting

Adverse event data collection and reporting, which are required as part of every clinical trial are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times according to the study calendar in Section 7.0.

Solicited Adverse Events: The following adverse events are considered "expected" and their presence/absence should be solicited, and severity graded, at baseline and for each cycle of treatment as noted below:

Allergic reaction (see CTCAE Immune system disorders; not at baseline)

Infusion related reaction (see CTCAE General disorders and administration site conditions; not at baseline)

Injection site reaction (see CTCAE General disorders and administration site conditions; not at baseline)

Hypertension (see CTCAE Vascular disorders)

Thromboembolic event, venous or arterial (see CTCAE Vascular disorders)

Proteinuria (see CTCAE Renal and urinary disorders)

Wound dehiscence (see CTCAE Injury, poisoning and procedural complications)

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APPENDIX I: UPC (URINE PROTEIN TO CREATININE) RATIO

The UPC (urine protein to creatinine) ratio directly correlates with the grams of protein found in a 24 hr urine. The UPC ratio can be used in the place of a 24-hour urine.

Procedure for Obtaining a Urine Protein/Creatinine Ratio:

1. Obtain at least 4 mL of a random urine sample in a sterile container (does not have to be a 24-hour urine sample).
2. Determine protein concentration (mg/dL).
3. Determine creatinine concentration (mg/dL).
4. Divide #2 by #3 above:

UPC Ratio =

$$\frac{\text{Protein Concentration (mg/dL)}}{\text{Creatinine Concentration (mg/dL)}}$$

APPENDIX II: KARNOFSKY PERFORMANCE STATUS

Performance status will be graded according to the following scale:

Karnofsky 100 Normal; no complaints; no evidence of disease

Karnofsky 90 Able to carry on normal activity; minor signs or symptoms of disease

Karnofsky 80 Normal activity with effort; some sign or symptoms of disease

Karnofsky 70 Cares for self; unable to carry on normal activity or do active work

Karnofsky 60 Requires occasional assistance, but is able to care for most personal needs

Karnofsky 50 Requires considerable assistance and frequent medical care

Karnofsky 40 Disabled; requires special care and assistance

Karnofsky 30 Severely disabled; hospitalization is indicated, although death not imminent

Karnofsky 20 Very sick; hospitalization necessary; active support treatment is necessary

Karnofsky 10 Moribund; fatal processes progressing rapidly

Karnofsky 0 Dead

APPENDIX III: VACCINE TISSUE PROCUREMENT AND SHIPPING INSTRUCTIONS

Tissue procurement kits provided by Agenus include:

50-mL nonpyrogenic tubes and patient information labels

Absorbent paper

Saf-T pouches

Pressure vessels (with foam insert)

Cardboard pieces (for box support and top of dry ice)

Original invoice letter (for international shipments only; must be reproduced on study center letterhead)

Shipping box and all shipping/address labels

Courier documents

Sites must provide the dry ice (8kg).

Specimens not shipped to Agenus immediately after procurement must be frozen and stored at -80°C ± 20°C. The specimen should not arrive on the weekend or a holiday unless this has been agreed to in advance by Agenus. Please call Agenus' Clinical Affairs at [redacted] with any questions.

If the tissue will not be shipped immediately, it should be placed into an -80° (+/- 20°) C freezer until ready to pack and ship. Do this within a maximum of 30 minutes of removal. Ship *as soon as possible*. Please be careful not to allow thawing when the tissue is packed.

For tumor tissue stored at sites prior to shipping to Agenus (i.e. overnight or for weekends/holidays), the freezer does not need to be sterile but it is recommended that it should be monitored for temperature (24/7) with alarms or other mechanism for identifying when temperature goes out of specification. The freezer or its location should have controlled access (locked with specified group members having access). The freezer should also be on a preventative maintenance program and ideally be hooked up to a back-up power system.

In the OR, wrap the tumor tissue in a sterile cloth or towel, and place in a basin, on ice (not dry ice). Ideally, this should be an en bloc resection. Aspiration contents from a cavitronic aspirator (e.g. CUSA) are not acceptable as vaccine manufacturing success with these materials is limited.

Transport the specimen immediately to the pathology/frozen section suite.

Share the following steps with the surgical pathologist, along with the tissue procurement kit:

a. Use basic sterile technique. Care should be taken to avoid any possible cross-contamination with tissues or fluids from other patients.

b. As small a section of tumor as possible should be kept for routine pathologic studies and diagnosis. Tissue for routine pathologic diagnosis should be processed according to institutional standards.

c. The pathologist should assess viability of the tumor.

d. When there is necrosis and/or cystic degeneration in the tumor specimen, the necrotic tissue and/or cystic component should be removed and should not be sent to Agenus.

e. **All** tumor tissue should be sent for HSPPC-96® vaccine preparation. A *minimum* of 5 grams of fresh, non-necrotic tumor tissue is sent (7 grams of tissue is preferable).

The tumor procurement and shipping procedure is as follows:

1. The pathologist should section the tissue into 1-2 cm^2 sections. The sections should then be placed carefully, maintaining sterility, into the 50-cc nonpyrogenic tubes. Each tube should be filled no more than ½ to ⅔ full, to allow for expansion of tissue during freezing.
2. The tube(s) must be properly labeled, using only a regular ballpoint pen, with the study number (A071101), patient ID number, patient initials (last, first, middle), date of birth (e.g., 02/JAN/1901), date of resection, and name of the institution. A completed label must be affixed onto each tube. If there is no middle initial, a dash (-) should be included on the forms and specimen labels.

Tumor Shipping Checklist:

Each tube should be placed in a plastic Saf-T pouch, and the Saf-T pouch sealed closed.

Wrap the tubes in bubble wrap or place in the foam insert.

The piece of absorbent paper should be placed in the bottom of the orange screw-topped container (pressure vessel), and the bundle of tubes placed on top of the paper. Dry ice should NOT be placed inside the pressure vessel.

Place some dry ice in the bottom of the insulated shipper box.

The pressure vessel container should be placed above the dry ice, in the cardboard support, and more dry ice packed surrounding the container.

Shipping container MUST be filled with at least 8kg of dry ice. Dry ice should be added to about 10 cm from the top of the box, a second cardboard insert placed above the ice, and the foam plug placed into the box. If an insufficient amount of dry ice is placed in the box, and the tissue is not frozen on arrival, it cannot be processed and the patient must be withdrawn from the study.

Complete the Tissue Procurement Form. All the required information must be completed. The form should be faxed to Agenus at [redacted] so that the shipment is expected and can be traced by air bill number if it does not arrive. A copy of the form should also be kept for your records.

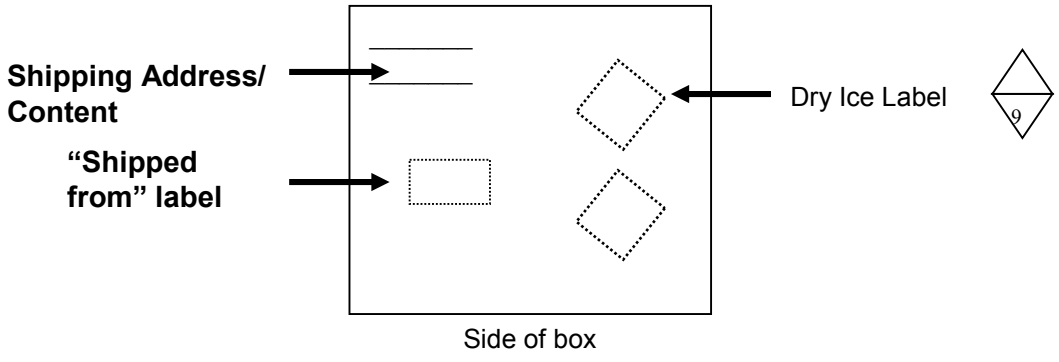
The original Tissue Procurement Form should be placed on top of the foam plug and the cardboard flaps closed and securely taped shut.

The following labels should be over the appropriate areas:

Dry ice (#9) with weight

Shipping address/content label

"Shipped from" label



Contact courier to arrange for a pick-up, if this was not done in advance.

Attach the courier air bill to the box. The shipment should be sent to:

[redacted]

Below is a list of some common issues that have been encountered with completing the Tissue Procurement Form (TPF). Please take great care in filing out this form correctly, as any such issues may lead to delays in vaccine production and potential rejection of the material if the correct identity cannot be confirmed.

- Patient study number does not match – tube labels vs. procurement form
- Patient date of birth does not match – tube labels vs. procurement form
- Patient initials do not match – tube labels vs. procurement form (i.e., ABC ≠ A-C)
- Patient study number, date of birth or patient initials are illegible on procurement form or tube labels
- Tube labels used are not the same as those issued by Agenus
- Tubes are not labeled at all, or information is recorded directly onto glass of tube and is then smeared upon receipt
- Pen or marker used to record information on tube label is not indelible ink, and information is then smeared upon receipt
- Tubes for more than 1 patient are placed into one pressure vessel
- Initials and date of correction are not recorded when cross-outs are made on tube labels or Tissue Procurement Form

APPENDIX IV: WHOLE BLOOD PBMC PROCESSING AT SITES*

*For previously designated sites only

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)
from Human Blood Samples

1.0 Suggested Equipment/Materials

Materials	Manufacturer/Distributor
Bench-top centrifuge	The Beckman Coulter Allegra 6R or equivalent[1] (Refrigerated and Room temperature)
Pipettes	Rainin or equivalent[1]
Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X)	GIBCO Cat# 14190-144 or equivalent[1]
Ficoll-Hypaque (Histopaque®-1077)	Sigma-Aldrich Cat# 10771-500ml or equivalent[1]
Fetal bovine serum (FBS)	
DMSO (Dimethyl Sulphoxide)	
-80 ºC freezer	REVCO or equivalent[1]
Liquid Nitrogen freezer	CRYOSAFE or equivalent[1]
Sterile 50 mL polypropylene conical tubes	Becton Dickinson Cat# 352070 or equivalent[1]
Sterile T-75 Costar Tissue Culture Flasks	Sigma-Aldrich Cat# 430641 or equivalent[1]
Cryotubes, polypropylene 2.0 ml	Sigma-Aldrich Cat# 430488 or equivalent[1]
Nalgene, Cryo 1°C "Mr. Frosty" Freezing Containers	VWR Cat# 55710-200 or equivalent[1]
Serological Pipettes 5 ml 10 ml 25 ml	Sigma-Aldrich Cat# CLS 4487 or equivalent[1] Sigma-Aldrich Cat# CLS 4488 or equivalent[1] Sigma-Aldrich Cat# CLS 4489 or equivalent[1]

[1]Equivalent means the same quality instrument, or the same quality or grade of material.

2.0 Blood Separation Procedure

It is suggested that these operations be performed in an ISO Class 5 Cabinet using standard aseptic technique.
Blood (green-top tubes) MUST be kept at room temperature (20-25^{o}C).
The laboratory must process the blood within 8 hours from the time it is drawn.

2.1 PBMC Separation

2.1.1 Whole Blood Separation from Green-top Tubes (Sodium Heparin)

Five 10 ml Green-top tubes will yield ~50 ml of blood.

1. Invert Green-top tubes 10 times to re-suspend blood cells in plasma.
2. Using a 10 ml pipette, pool the blood from all Green-top tubes into a T-75 sterile flask containing 65 ml of sterile D-PBS and gently mix the contents by swirling the flask.
3. Place 12 ml of Ficoll-Hypaque (at room temp) into each of **two** 50 ml sterile conical tubes.
4. Using a 25 ml pipette, carefully layer 37 ml of diluted blood on top of Ficoll into each 50 ml sterile conical tube.
5. Centrifuge tubes for 20 minutes at 2000 rpm (900 x g) at 25°C. **DO NOT** use the centrifuge brake.

If only one centrifuge is available, then the centrifuge needs to be refrigerated (4^{o}C) following this step for the rest of the procedure

6. With a 10 ml pipette, remove and discard the upper layer (plasma and platelets; yellowish in color).
7. Remove the PBMC interface (white in color) with 10 ml pipette and place each PBMC interface into a fresh 50 ml tube (total of 4 tubes).
8. Fill the tubes up to 50 ml with cold D-PBS and centrifuge for 8 min at 1500 rpm (500 x g) at 4°C. Use the centrifuge brake.
9. Discard the clear D-PBS on top of the cell pellet by gently inverting tubes.
10. Using a 5 ml pipette, re-suspend cell pellet in 5 ml cold D-PBS and gently pipette up and down 2-3 times to make a single cell suspension.
11. Pool the cell suspensions into **one** 50 ml sterile conical tubes.
12. Fill the tubes up to 50 ml with cold D-PBS and centrifuge for 8 min at 1300 rpm (400 x g) at 4°C. Use the centrifuge brake.
13. Discard the clear D-PBS on top of the cell pellet by gently inverting tubes.
14. Pool the cells into one tube.

2.2 Freezing PBMCs

At the time of use, FBS will be thawed in a water bath at 37^{o}C. Freezing solution will be prepared at the site by mixing 45 ml of FBS and 5 ml DMSO. It is suggested that the mixture be prepared at the time of blood processing, prior to the freezing step. Freezing solution should be placed on ice and kept cold up to the moment of use.

FBS and left over freezing solution can be stored at -20ºC, and DMSO stored at room temperature. The freezing solution can be frozen again after being thawed and remaining solution can be used for another time point or another patient's sample, whichever comes first.

1. Thaw/prepare required volume of freezing solution (FBS + 10% DMSO).
2. Label cryo-tubes (vials) with site ID, patient ID/number, and date. Always use a sharpie/permanent marker on the labels, not regular pens or other markers.
3. Keep freezing solution and the necessary vials on ice at all times during the procedure (in trays, placed in a laboratory bucket filled with ice).
4. Discard the clear D-PBS on top of the cell pellet by gently inverting tubes one at a time. Keep the rest of the tubes on ice.

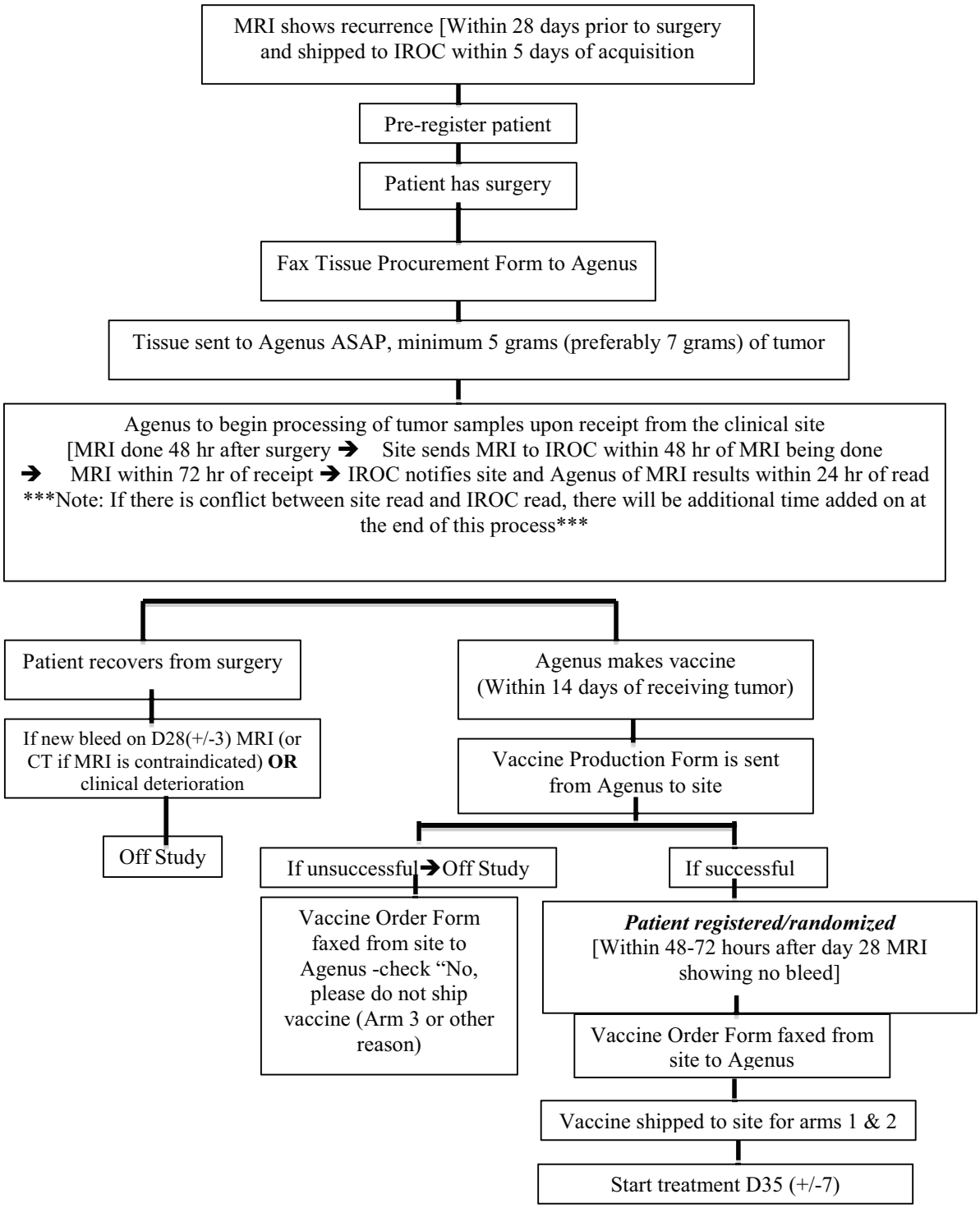
1. Re-suspend cell pellet with 8 ml of freezing solution.
2. Aliquot 1 ml per vial (8 vials).
3. Immediately place vials in -80°C freezer in a "Mr. Frosty" or equivalent slow-cooling container.
4. A) For samples that will be stored at the site and batch-shipped greater than 48 hours after collection, the frozen PBMC vials must be transferred from the -80° C freezer into a liquid nitrogen freezer or a -140° freezer 24-48 hours after they have been placed in the -80° C freezer. They cannot be allowed to stay at -80° C longer than 48 hours. Each patient should have a separate storage box in the liquid nitrogen freezer. When they are to be shipped, they should be transferred directly from the liquid nitrogen freezer or a -140° freezer to dry ice and shipped.

   B) For samples that are being shipped within 24-48 hours of being first placed in -80° freezer in a "Mr. Frosty" or equivalent slow-cooling container, they can be transferred from the -80° freezer to dry ice and shipped; at the site that receives them, they are to be transferred from dry ice to liquid nitrogen freezer or -140° freezer. They cannot be allowed to stay at -80° C longer than 48 hours.

Note for both 4A and 4B: Use dry ice to transfer vials if necessary to maintain the temperature of the frozen vials.

5. For sites which process and temporarily store PBMCs on site and batch ship, the freezer does not need to be sterile but it is recommended that it should be monitored for temperature (24/7) with alarms or other mechanism for identifying when temperature goes out of specification. The freezer or its location should have controlled access (locked with specified group members having access). The freezer should also be on a preventative maintenance program and ideally be hooked up to a back-up power system.

APPENDIX V: A071101 VACCINE SCHEMA



APPENDIX VI: VACCINE RETURN INSTRUCTIONS

I. Any unused or expired vaccine remaining after a patient has completed vaccine treatment, should be returned to Agenus according to the following instructions.

II. Please contact the Agenus Clinical Team in advance (i.e. minimum of 1 day) to inform Agenus when you plan to return unused or expired vaccine.

III. Agenus will provide a courier air-way bill for this shipment.

IV. Use the qualified HSPPC-96 shipping kit that was received with the original shipment of vaccine from Agenus. The shipping kit should have the following materials, which came as part of the vaccine shipment from Agenus:
- Outer cardboard shipping box
- Insulated liner and foam plug
- Pressure vessel container (with foam inserts)
- Cardboard pieces (2: 1 for upright positioning of base of pressure vessel and 1 for placement on top of pressure vessel surrounded with dry ice)
- Absorbent paper (on bottom of pressure vessel)

V. Place vials to be returned inside of the pressure vessel, inserted upright in the holes of the foam inserts. Screw the lid shut on the pressure vessel. Multiple patients' vaccines may be returned in one pressure vessel.

VI. Place a layer of dry ice on the bottom of the insulated liner that is within the outer cardboard shipping box. The pressure vessel container should then be placed above this layer of dry ice, with the base of the pressure vessel inserted into the cardboard support. Additional dry ice is then packed around the closed pressure vessel. The shipping container must be filled with at least 8kg of dry ice (i.e. level with the top of the pressure vessel). A second cardboard insert should be placed above the dry ice, and the foam plug placed into the box. The box is then sealed with packing tape.

VII. The following labels should be placed on the exterior of the outer cardboard shipping box:
- Dry ice (#9) with weight (8 kg)
- Shipping address/content label (Who the package is shipping to)
- Shipped from label (Where the package is shipping from)

VIII. Contact the courier to arrange for a pick-up, if a standard pick up time is not established for your site or if this was not done in advance.

IX. Attach the courier air bill to the top of the box. The shipment should be sent overnight to: