TITLE: A Phase II Study of the Safety and Efficacy of SVN53-67/M57-KLH (SurVaxM) in Survivin-Positive Newly Diagnosed Glioblastoma

Roswell Park Cancer Institute

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SYNOPSIS

Title	A Phase II Study of the Safety and Efficacy of SVN53-67/M57-KLH (SurVaxM) in Survivin-Positive Newly Diagnosed Glioblastoma
Roswell Park Cancer Institute Study Number	I 259614
Roswell Park Cancer Institute Investigator	Robert Fenstermaker, M.D.
Sponsor	Roswell Park Cancer Institute
Study Drugs	• SVN53-67/M57-KLH • Montanide ISA 51 • Sargramostim (GM-CSF) • Temozolomide (Temodar®)
Objectives	Primary Objective: • To evaluate 6-month progression-free survival (PFS6) in patients with survivin positive newly diagnosed glioblastoma multiforme (GBM) treated with at least 4 doses of SurVaxM and standard of care temozolomide. PFS6 is defined as the percentage of patients without tumor progression or death from any cause 6 months after the date of diagnosis by biopsy. Secondary Objectives: • To determine the safety and tolerability of SurVaxM in patients receiving standard care adjuvant temozolomide. • To evaluate overall survival (OS) in patients with survivin positive newly diagnosed GBM treated with SurVaxM and adjuvant temozolomide. • To describe the immune response in patients treated with SurVaxM and predictors of response. • To evaluate objective tumor response rate (applicable only for patients with evaluable disease at study entry, as defined by RANO criteria) and predictors of response.
Study Design	This is a multi-center, open-label, single arm, phase II trial in adult patients with newly diagnosed GBM, evaluating the efficacy and safety of SurVaxM and adjuvant temozolomide after patients have received concurrent radiation and temozolomide.
Target Accrual and Study Duration	**Sample Size:** Approximately 50 eligible patients with survivin positive newly diagnosed GBM will be enrolled over a 25-month period. Subjects will be followed to determine progression free survival and overall survival. The study duration will be at least 37 months.

Statistical Analysis	**Efficacy Analysis:** The primary objective is to evaluate progression-free survival at 6 months (PFS6) in patients with newly diagnosed GBM treated with 4 priming doses of SurVaxM and standard-of-care adjuvant temozolomide as compared to historical control. Patients who are alive and disease free at the last study assessment will be treated as censored. Patients in this population defined by the eligibility criteria receive standard-of-care which consists of concurrent temozolomide and radiation followed by adjuvant temozolomide. Based on provided historical information, the 6 month PFS survival fraction is assumed to be 0.55. We hypothesize that treatment with SurVaxM and adjuvant temozolomide will increase the 6 month PFS survival fraction to 0.70. Therefore the PFS survival fraction will be measured after all patients have been followed for 6 months. The 6 month PFS survival fraction is estimated as standard binomial proportion. An exact one-sided binomial test will be used to test our primary hypothisis. The nominal significance level to be used for this test is 0.10. In addition, a Kaplan-Meier curve for PFS will be used calculated to generate summary descriptive statistics, e.g. median PFS. **Correlative Data Analysis:** Immunologic response to vaccine will be used as a secondary measure of vaccine activity. Survivin-specific CD8+ responses, CD4+ helper support, and anti-survivin antibody (humoral) responses will be measured individually by the Immunotherapy Immune Analysis Facility and Neuro-oncology Laboratory at Roswell Park Cancer Institute. To assess immunologic responses to SVN53-67/M57-KLH (SurVaxM), PBMC will be analyzed using multimer, IFNγ ELISPOT and qPCR assays of cytokine expression in CD4+ and CD8+ cells. Antibodies to survivin peptides and KLH in serum will also be measured by ELISA to assess humoral immune responses. HLA-A class I typing on blood samples will be performed at each study site as a part of screening to determine eligibility. Following entry, detailed HLA typing (MHC class I and MHC class II) will be performed in an exploratory fashion as part of the analysis of immunologic response to vaccination.

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: (Network sites use participant initials): _____

Medical Record No.: (Network sites use participant ID): _____

Title: A Phase II Study of the Safety and Efficacy of SVN53-67/M57-KLH (SurVaxM) in Survivin-Positive Newly Diagnosed Glioblastoma

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Age ≥ 18years of age.	
☐	☐	☐	2. Have a Karnofsky performance status ≥ 70 (i.e. the patient must be able to care for himself/herself with occasional help from others; refer to **Appendix B)**.	
☐	☐	☐	3. Documented survivin-positive tumor status.	
☐	☐	☐	4. Pathologically confirmed diagnosis of glioblastoma multiforme (GBM).or WHO grade IV[gliosarcoma]	
☐	☐	☐	5. Have the following clinical laboratory values obtained within 14 days prior to registration: a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L b. Platelets ≥ 100 x 10^9/L c. Hemoglobin (Hgb) > 9.0 g/dL d. Serum total bilirubin: ≤ 1.5 x ULN e. ALT and AST ≤ 4.0 x ULN	
☐	☐	☐	6. Patients on full-dose anticoagulants (e.g., warfarin or LMW heparin) must meet the following criteria: a. No active bleeding or pathological condition that carries a high risk of bleeding (e.g., tumor involving major vessels or known varices).	
☐	☐	☐	7. Adequate renal function, as defined below: • Creatinine ≤ 1.8 mg/dl	
☐	☐	☐	8. HLA-A*02, HLA-A*03, HLA-A*11 or HLA-A*24 positive patients.	
☐	☐	☐	9. MRI (Ideally completed within 96 hours after surgery) documenting gross total resection consisting of no gadolinium enhancement; or subtotal resection consisting of linear enhancement with (or without) nodular gadolinium enhancement measuring no greater than 1 cm x 1 cm x 1cm total volume or 100 mm^2 in cross sectional area.	
☐	☐	☐	10. No evidence of true progressive disease from the	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
			postoperative period to the post-chemoradiation period, based on changes in the neurologic exam, steroid use, or evident radiographic progression, according to RANO criteria (see **Appendix C**). Patients with increased or new gadolinium enhancement may continue on protocol if in the investigator's judgment that enhancement is likely due to pseuodoprogression. The use of correlative imaging studies (including PWI) or diffusion weighted imaging (DWI), and repeat imaging after an interval of 2-4 weeks is strongly encouraged to help distinguish between pseudoprogression and true progression.	
☐	☐	☐	11. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry, and have a negative pregnancy test prior to starting study treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	12. Dexamethasone dose less than or equal to 4 mg daily at time of study enrollment.	
☐	☐	☐	13. Patients must have completed initial radiation therapy (RT) and temozolomide (TMZ) for the treatment of their glioblastoma (i.e., completed 6-week course of RT and, completed ≥ 75% of 6-week course of induction TMZ chemotherapy).	
☐	☐	☐	14. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____ **Date:** _____

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: (Network sites use participant initials): _____

Medical Record No.: (Network sites use participant ID): _____

Title: A Phase II Study of the Safety and Efficacy of SVN53-67/M57-KLH (SurVaxM) in Survivin-Positive Newly Diagnosed Glioblastoma

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. The patient must not have received any immunotherapy for their brain tumor.	
☐	☐	☐	2. Patients with serious concurrent infection or medical illness, which in the treating physicians opinion would jeopardize the ability of the patient to receive the treatment outlined in this protocol with reasonable safety.	
☐	☐	☐	3. Patients who are pregnant or breast-feeding.	
☐	☐	☐	4. Patients receiving concurrent therapy for their tumor (i.e. chemotherapeutics or investigational agents) other than temozolomide.	
☐	☐	☐	5. Patients with a concurrent or prior malignancy are ineligible unless they are patients with curatively treated carcinoma-in-situ or basal cell carcinoma of the skin. Patients who have been free of disease (any prior malignancy) for at least 3 years are eligible for this study.	
☐	☐	☐	6. Patients who have had repeat craniotomy for tumor therapy after receiving RT and TMZ treatment.	
☐	☐	☐	7. Patients who received other chemotherapeutics or investigational agents in addition to their radiation therapy and concomitant temozolomide treatment.	
☐	☐	☐	8. Patients who have received Gliadel wafers or alternating electrical field therapy (ETTF) are not eligible for this study.	
☐	☐	☐	9. Known history of an autoimmune disorder.	
☐	☐	☐	10. Known human immunodeficiency virus (HIV) positivity or acquired immunodeficiency syndrome (AIDS) related illness or other serious medical illness.	
☐	☐	☐	11. Patients who have contraindication to MRI.	
☐	☐	☐	12. Unwilling or unable to follow protocol requirements.	
☐	☐	☐	13. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	14. Received an investigational agent within 30 days prior to registration.	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll participant in study.

Investigator Signature: _____ Date: _____

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1 BACKGROUND

1.1 Glioblastoma

Although the addition of temozolomide to radiation therapy for the treatment of glioblastoma multiforme (GBM) has resulted in improved outcomes, the estimated 2-year survival with maximal therapy remains only 27% [1]. In a randomized phase 3 multicenter trial[1], patients with newly diagnosed GBM received 60 Gy of radiation over 6 weeks with temozolomide 75mg/m2 for 42 days, followed by adjuvant temozolomide 150mg to 200mg/m^2 for 5 consecutive days each month for 6 months, vs. radiation alone. The median overall survival for the combined radiation and temozolomide group was 14.6 months compared to 12.1 months for radiation alone. Since this study was reported, no major improvements in therapy for GBM have been shown to be significantly better for the initial treatment of glioblastoma patients. Therefore new and improved treatment strategies are greatly needed.

1.2 Survivin Expression in Gliomas

Survivin is a 16.5 kDa intracellular protein that belongs to the inhibitor of apoptosis protein (IAP) family. It acts in concert with the mitotic spindle apparatus to regulate cell division and localizes to the spindle microtubule organizing center (MTOC) during the G2/M phase of cell cycle progression[2, 3]. Survivin has also been shown to modulate the function of a number of terminal effector cell death proteases (caspases) leading to an inhibition of apoptosis [4, 5]. Although expressed during fetal development, survivin is rarely detectable in the normal tissues of adult organisms [6]. Malignant gliomas express survivin at high levels; whereas, low grade gliomas and normal glial cells do not [7]. In fact, survivin is considered to be one of the most specific cancer molecules yet identified [3], despite the fact that it may also be seen in hematopoietic progenitor cells, some lymphocytes, neutrophils and vascular endothelial cells [8]. High-level survivin expression is associated with a poor prognosis [9, 10]. Survivin expression in tumors is also associated with a high rate of disease recurrence and resistance to therapy [11]. Consequently, survivin confers growth and survival advantages to cells that express it. A large number of human glioma specimens have been examined using several different techniques and survivin expression has been found in at least 90% of human malignant gliomas.

1.3 Immunization

Patients with cancers, including malignant gliomas, have immunologic responses to survivin. Antibodies to survivin and T cells that are specifically reactive to survivin epitopes have been detected in cancer patients [12-15]. Therefore, survivin is clearly immunogenic and stimulation of memory responses might be elicited by active specific vaccination to survivin epitopes. Since survivin is an intracellular protein, it is processed by the proteosome and can directly enter the MHC class I pathway. Peptide epitopes from intracellular proteins are presented at the cell surface by MHC class I molecules leading to CD8+ T-cell-mediated immune responses. There is substantial experimental evidence that survivin vaccination leads to anti-tumor immune responses in tumor-naive and tumor-bearing animals [16-20].

1.4 Study Drugs

1.4.1 SVN53-67/M57-KLH (SurVaxM)

1.4.1.1 Peptide selection for SurVaxM

The survivin peptide in SurVaxM is a defined, 15 amino acid antigenic peptide capable of binding several human MHC class I molecules, as well as murine H2-Kb molecules (as a model of the human response). SurVaxM contains a core epitope SVN56-64/M57 modified by substitution of methionine for cysteine at amino acid position 57, with flanking amino acids 53-56 and 65-67. This allows the core epitope in SurVaxM to be more immunogenic than the wild-type survivin peptide in humans since it binds HLA-A*02 molecules to a much greater extent [16]. While SurVaxM was designed to bind HLA-A*0201, computer algorithms predict its binding to numerous MHC class I molecules (including HLA-A*02, HLA-A*03, HLA-A*11 and HLA-A*24) that collectively represent a large patient population. Thus, SurVaxM is predicted to be effective at generating CTL in a diverse patient population with limited HLA restriction.

1.4.1.2 Immunization against intracellular proteins

Epitopes of intracellular proteins, if correctly presented on the surface of tumor cells with MHC class I expression, and recognizable by specific effector T cells, can serve as targets for cytotoxic antitumor responses. Thus, cell-surface expression of an entire tumor antigen is not required for effective anti-tumor immunologic responses. Instead, intracellular proteins like survivin are processed by the proteasome and their epitopes are presented by MHC class I molecules on the tumor cell's surface where they are recognizable by specific effector CTL.

The immune system of cancer patients is often primed to recognize survivin epitopes. Circulating anti-survivin antibodies have been detected in vaccine-naïve patients with cancer, but not in normal volunteers. Peptide epitopes from tumor-associated antigens (TAA), including survivin, can be recognized by cytotoxic T lymphocytes (CTL) in the context of MHC molecules. In a first-in-human phase I clinical study, vaccination with SurVaxM produced specific anti-survivin CD8+ T cells and anti-survivin antibodies in patients with recurrent malignant gliomas, primarily glioblastoma multiforme.

1.4.1.3 MHC class II ligands and CD4+ helper support for vaccines

To activate a CD4+ T-cell response, antigens must be presented to CD4+ T cells in conjunction with an MHC class II antigen [21]. Once CD4+ cells have been activated, they proliferate and produce cytokines (e.g. IFN-α, IL-2, and IL-4) that enhance the immune response [22, 23]. These cytokines are essential to provide a fully activated CD8+ antitumor CTL response [24]. The presence of MHC class II-restricted CD4+ T cells that are specific for tumor associated antigens has been recognized to be an important element for providing essential helper factors to elicit and sustain cytotoxic CD8+ responses against tumors [25, 26] . Vaccination with SurVaxM produces cytokine support as well as specific CTL responses and has the potential to stimulate more effective antitumor immunity than peptide vaccines that contain only class I epitopes.

1.4.1.4 Altered peptide antigens (molecular mimicry)

T cell clones with the capacity to be activated by self-proteins are frequently preserved following negative selection of higher affinity, self-recognizing clones in the thymus [27]. These potentially self-reactive cells remain tolerized under normal conditions [28]. Altered peptide ligands (mimics) can provide a way to break tolerance to the natural self-epitope. Altered peptide ligands generated by substituting single amino acids within a peptide epitope can markedly alter immune responses. This strategy may be used to increase the affinity of the peptide for MHC-I via alterations in the binding anchor residues [29, 30]. Effects of this manipulation can range from the induction of TCR antagonism, to T cell anergy, to enhancement of T cell responses [31]. A number of investigators have used altered immunogens (mimics) to enhance the immunogenicity of tumor-associated antigens. The cysteine-to-methionine substitution present in SurVaxM greatly enhances binding to HLA-A*0201 molecules leading to increased immunogenicity that is cross reactive to the wild type survivin molecule present in tumor cells. Thus, the design of SurVaxM incorporates several strategies to create an effective antitumor immunogen: 1) multiple epitopes, 2) peptide mimicry, and 3) antigen-specific cytokine support.

1.4.2 Montanide ISA 51

Montanide ISA 51, NSC 675756, is an oil-based adjuvant product similar to Incomplete Freund's Adjuvant, which when mixed with a water-based solution in 1:1 w/w ratio, forms a water-in-oil emulsion. It consists of highly purified oil (Drakol VR), and a surfactant, mannide oleate. Montanide ISA 51 is manufactured by Seppic, Inc., and is provided in amber glass ampoules containing 3 mL of the solution. It is non-irritating to the skin of rabbits and slightly irritating to the eye. Montanide ISA 51 will be provided through the RPCI Pharmacy. It is commonly used in human clinical vaccine protocols in the United States and is classified as an Investigational New Drug. Montanide ISA 51 acts to enhance immune responses to vaccines. Peptide-based vaccines in Montanide ISA-51 have been safely administered to more than 200 research participants and have induced T-cell responses against the immunizing peptides without major toxicities. Toxicities mostly commonly observed include local discomfort, induration, and erythema at the injection site. Addition of Montanide ISA 51 to various antigens induces both humoral and cellular immune responses. The precise mode of Montanides' action is unknown. Montanide ISA 51 is stored at room temperature.

1.4.3 Sargramostim

Sargramostim (Leukine®) is recombinant human GM-CSF, a glycoprotein produced by recombinant DNA technology in a yeast expression system. GM-CSF has many actions, including stimulating proliferation and differentiation of hematopoietic progenitor cells and as a dendritic cell (DC) attractant.

Please refer to the Physician Desk References and package insert for complete information.

SVN53-67/M57-KLH, Montanide ISA 51 and sargramostim will be provided at no cost to the patient or the patient's insurance carrier.

1.4.4 Temozolomide

Temozolomide (Temodar®) is an alkylating drug indicated for the treatment of adult patients with newly diagnosed glioblastoma multiforme (GBM) concomitantly with radiotherapy and then as maintenance treatment.

The most common adverse reactions (≥10% incidence) reported for temozolomide include: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia.

The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia.

Allergic reactions have also been reported.

Please refer to the Physician Desk References and package insert for complete information.

Temozolomide is part of the standard of care for glioblastoma and the patient and/or patient's insurance carrier will be responsible for charges related to its administration and for medications that may be needed to prevent or control side effects.

1.5 Pharmacodynamics of SVN53-67/M57

1.5.1 Peptide Binding Analysis

SurVaxM epitopes (**Figure 1-A**, blue) are capable of displacing HPV at low concentrations similar to that observed with other known MHC class I ligands gp100-209-217, and Flu-58-66. In contrast to the mimic peptides, the wild type peptides (red) could only displace the HPV peptide at almost 1,000-fold higher concentration. In addition, the core peptide of the mimic (SVN56-64/M57) had 74-fold higher binding to HLA-A*0201 than that of the wild type (**Figure 1-B**). Therefore, SVN53-67/M57 would be expected to lead to improved presentation of the MHC I binding epitopes to the human immune system. This leads to a longer association time between the mimic epitopes and the MHC I molecules. In turn, this should increase binding to lower affinity T cell receptors, which have not been deleted during development, can induce the proliferation of cross-reactive T cell clones that recognize wild type survivin epitopes.

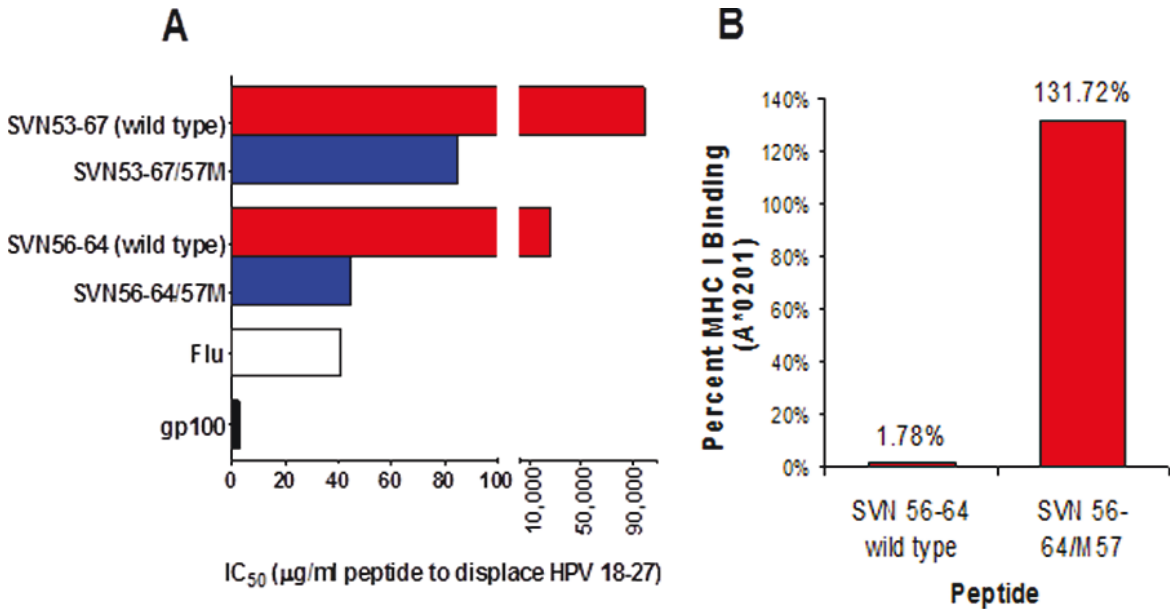


Figure 1: (A) MHC class I (HLA-A*0201) peptide competitive displacement assays. IC50 represents concentration of survivin peptide required to displace HPV 18-27 (known MHC I ligand) pre-loaded on human T2 cells. Flu and gp100 are known immunogenic MHC class I ligands. (B) REVEAL™ assays performed by ProImmune. Pentamer binding assay of SVN56-64 wild type and SVN56-64/M57 mimic peptides showing binding affinity of each for MHC class I.

1.5.2 Murine CTL studies of SurVaxM

A stronger ex vivo lytic response against murine glioma cells is observed with SurVaxM than with the corresponding wild type survivin peptide (**Figure 2**). This is associated with a concomitant increase in CD4+ T cell derived cytokine support (**Figure 3**). IFNα and IL-2 secretion was much greater in cultures stimulated with 15mer SVN53-67/M57 over that observed in cultures stimulated with the 9mer SVN56-64/M57 core peptide suggestive of CD4+ T cell help.

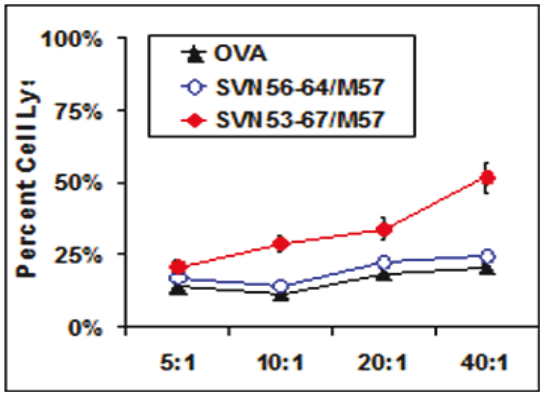


Figure 2: CTL responses against GL261 glioma cells using splenocytes of mice vaccinated with SVN53-67/M57-KLH. Data represent mean percent specific lysis ± S.E.M. of triplicate samples.

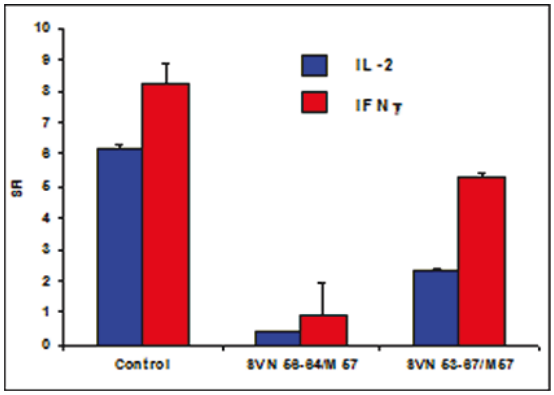


Figure 3: Intracellular IFN-γ and IL-2 production in vitro by CD4+ cells from SVN53-67/M57-KLH immunized mice restimulated ex vivo with SVN53-67/M57 overnight (with BD GolgiPlug). A standardized control (Mouse Cytokine Intracellular Control lymphocytes; eBioscience, San Diego, CA) were used as positive controls for IFNγ and IL-2 expression.

1.5.3 Pre-clinical Human CTL studies of SurVaxM

- Fresh PBMC from glioma patients were used to produce dendritic cells (DC) in vitro so that the ability of survivin peptides to stimulate CTL responses could be assessed against autologous human tumor cells ex vivo. It was demonstrated that the responses obtained are clearly linked to HLA status since an HLA mismatch of PBMC and tumor target cells led to an abrogation of cell-mediated cytotoxicity (**Figure 4-A**). HLA-A*0201, and HLA-A*0301 patients were able to be stimulated to lyse an allogeneic-matched and autologous glioma cells (**Figure 4-B, C, D**). In addition, this CTL activity was also found against autologous (HLA-A*2901/A*3002) primary CNS lymphoma (PCNSL) cells (Figure 4-E) and chronic lymphocytic leukemia (CLL) cells (**Figure 4-F**). As compared to the wild type peptide, SVN53-67/M57 elicits a 3- to 5-fold increase in CTL mediated killing against autologous human tumor cells (**Figure 4**). SVN53-67/M57 can elicit a cell-mediated immune response that is significantly greater than that induced by the wild

type peptide. Thus, SVN53-67/M57 may have broad applicability against cancers that express survivin.

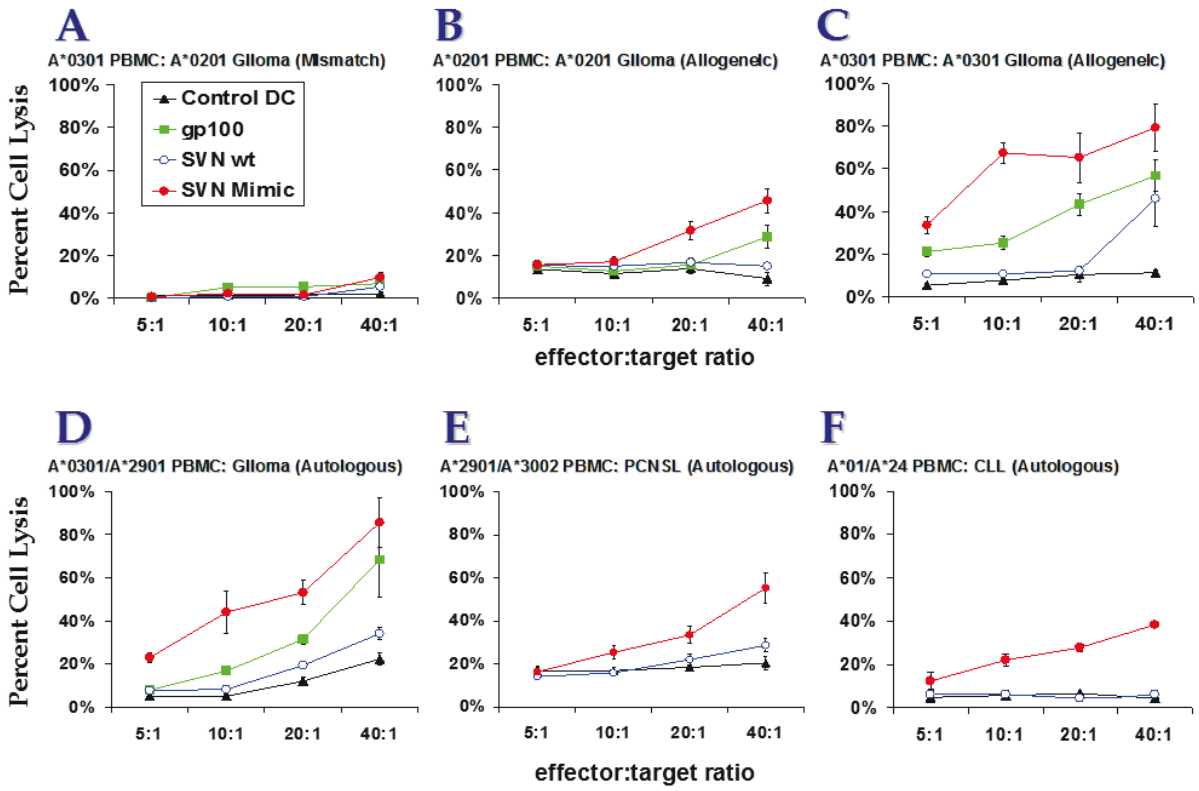


Figure 4: (A) HLA-A*0301 patient effector cells vs. allogeneic-mismatched HLA-A*0201 human U87 target glioma cells; (B) HLA-A*0201 patient effector cells vs. allogeneic-matched U87 glioma cells; (C) HLA-A*0301 patient effector cells vs. allogeneic-matched patient-derived tumor cells; (D) HLA-A*0301/HLA-A*2901 patient effector cells vs. autologous patient-derived glioma cells. (E) HLA-A*2901/HLA-A*3002 patient effector cells vs. autologous patient-derived PCNSL target cells; (F) HLA-A*01/HLA-A*24 patient effector cells vs. autologous patient-derived CLL target cells. Legend: Control DC = Flu/58-66; Positive control = gp100/209-217; SVN wt = SVN53-67; SVN Mimic = SVN53-67/M57.

- Multimers (ProImmune "pentamers") loaded with the core CTL epitope of SVN 53-67 (SVN 56-64) were used to show that significant T cell reactivity could be detected in HLA-A*0201 patient samples. Tetramer reactivity is used to measure T cell-derived immune responses. Antigen presenting cells were stimulated in cell culture with SurVaxM peptides to produce pentamer-reactive T cells which are cross reactive to the wild type survivin peptide (**Figure 5A**). These CTL are also capable of lysing autologous glioma target cells and lysis can be blocked with MHC I blocking antibody (**Figure 5B**).

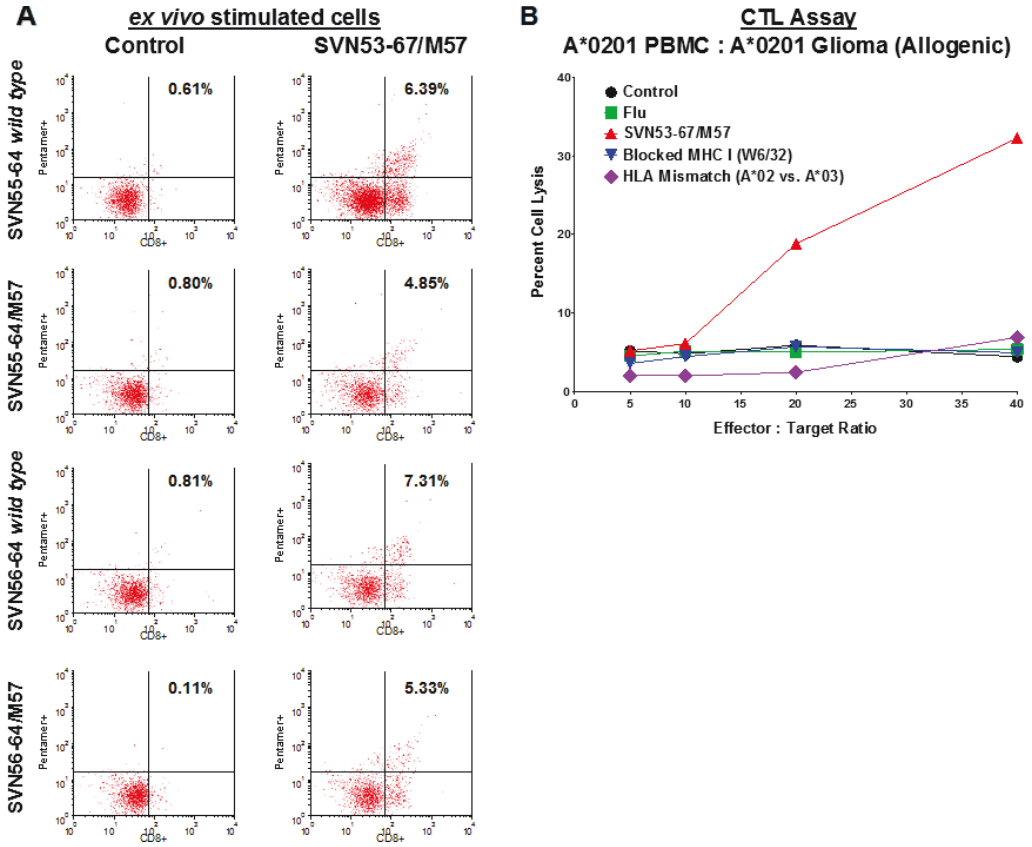


Figure 5: Pentamer binding assay using patient derived CTL. A) HLA-A*0201 pentamer binding of CD8+ T cells specific for SVN55-64 wild type peptide, SVN56-64 wild type peptide, SVN55-64/M57 peptide mimic or SVN56-64/M57 peptide mimic as a result of ex vivo SVN53-67/M57 stimulation in cell culture. Percentage shown in the upper right quadrant of each flow cytometry data panel represents double labeled pentamer+/CD8+ T cells. Control column are parallel ex vivo cell cultures that did not receive additional peptide. B) CTL Assay performed in parallel using patient cells shown in (A). Control cells are those not receiving additional peptide. Flu peptide represents a non-specific peptide stimulus. Blocked cells are target cells treated prior to T cell exposure with W6/32 anti-MHC I antibodies known to interfere with T cell receptor interaction. The HLA mismatched cells are shown to assess allogenic specificity against a second glioma cell line carrying the mismatched A*0301 MHC I allele.

1.5.4 Pre-clinical survivin reactive IgG antibodies in human serum

In a consecutive series of 6 vaccine-naïve glioma patients, 5 had moderate titers of survivin-reactive antibodies to whole recombinant survivin protein (see **Figure 6** below). Data indicate the possibility that previous immune exposure to survivin may naturally occur in GBM patients.

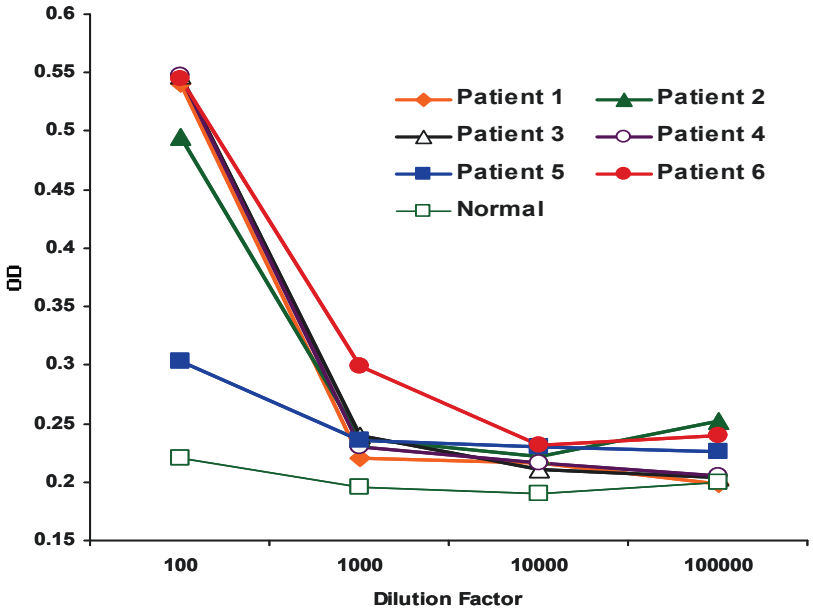


Figure 6: Serum ELISA for SVN53-67 reactive IgG antibodies in glioma patients who have not been vaccinated.

1.6 Safety Pharmacology

Peptides used for vaccination studies do not follow standard pharmacokinetics and such data are not likely to be useful to ascertain peptide safety. Previous studies of similar peptide designs have not shown any pharmacological effects upon vital functions. Consequently, pharmacological distribution studies of SurVaxM have not been performed.

Considering the route of administration (i.e., subcutaneous injection), it is very likely that SurVaxM will remain locally at the injection site for a long period and not be systemically available in any significant amount. Much of the peptide will be taken up by antigen presenting cells (APC) locally and then processed for presentation to the immune system. In addition, some fraction will be degraded locally by peptidases to produce clinically insignificant quantities of free amino acids and smaller peptides. Therefore, as with many other peptide vaccines tested to date, SurVaxM should not reach vital organs to directly affect them. Nonetheless every effort has been made to address the safety of SurVaxM through pre-clinical animal toxicity studies.

According to the study entitled "Subcutaneous Repeated Dose Toxicity Study of 012410-2 in C57BL/6 Mice with a Two-Week Recovery (Study No. 20003268)", subcutaneous administration of SurVaxM at a dose of 500 mcg in adjuvant Montanide ISA 51 VG injected with sargramostim every other week for 12 weeks was well tolerated by male and female mice throughout the dosage and recovery periods. Administration of SurVaxM did not cause mortality or adverse signs of toxicity as evaluated by clinical or skin reaction observations, changes in

body weights, ophthalmologic observations, necropsy observation, terminal body weights and organ weights, clinical pathology and histopathological observations.

1.7 Pharmacokinetics

The uptake of peptides used for vaccination studies is thought to be via phagocytosis by macrophages and dendritic cells, which do not follow established pharmacokinetic models. Absorption, distribution, metabolism and excretion of a 15 amino acid peptide and its composite naturally occurring amino acids cannot be confidently ascertained by standard methods. Upon administration, biological peptides essentially begin to be degraded to peptide fragments and amino acids masked by the systemic milieu. No formal pharmacokinetic studies have been conducted in either animals or humans since the drug is not systemically distributed upon administration.

1.8 Efficacy

1.8.1 Pre-clinical Data

1.8.1.1 SurVaxM in Syngeneic, Immunocompetent, Murine, Intracerebral Glioma Model

Beginning four days after tumor implantation, mice were immunized with either SVN53-67/M57 peptide-loaded dendritic cells or, by direct subcutaneous injection of 100 μg SVN53-67/M57-KLH peptide [in Incomplete Freund's Adjuvant (IFA)] plus 100 ng sargramostim. Vaccinations were repeated (boosted) every 7 days up to a total of 3 immunizations, and long-term survivors were confirmed to be tumor-free by MRI (**Figure 7**). SurVaxM produced enhanced survival in mice with GL261 cerebral gliomas with some survivors alive 1 year after implantation without detectable tumor. No significant difference was observed between survivin peptide-loaded DC or the peptide-KLH conjugate (SurVaxM), provided that sargramostim was used as a supporting adjuvant.

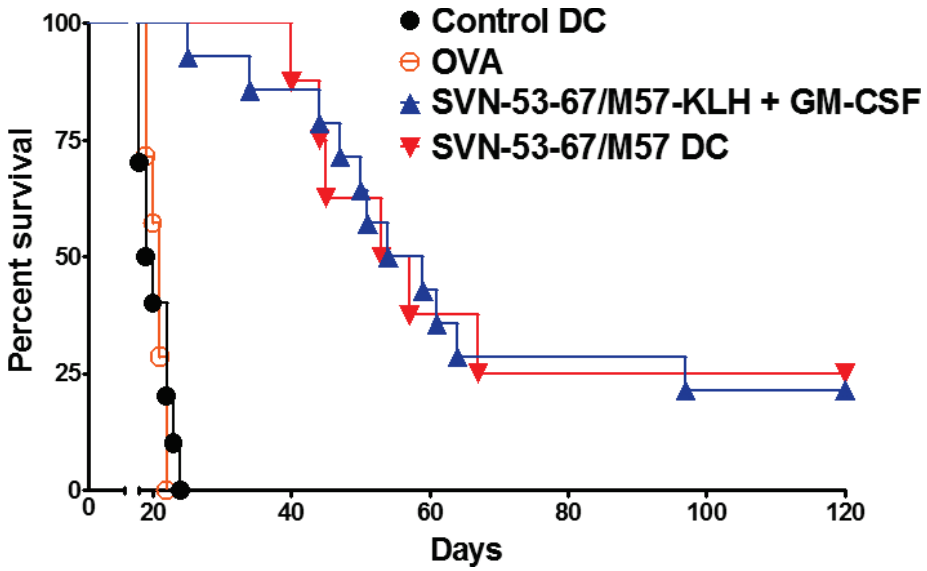


Figure 7: Survival of C57BL/6 mice bearing 1x10^5 GL261 intracerebral gliomas. 1) Control DC: n=10, survival range 18-24 days, with median survival 19.5 days; 2) OVA: n=7, survival range 19-22 days, with median survival 21 days; 3) SVN53-67/M57 DC: n=14, survival range 40-120+ days, with median survival 55 days; 4) SVN53-67/M57 KLH: n=8, survival range 25-120+ days, with median survival 56.5 days. SVN53-67/M57-KLH or SVN53-67/M57 DC vs. control DC both p>0.0001.

1.8.1.2 SurVaxM with Temozolomide in Glioma-bearing C57BL/6 Mice

Mice with intracranial GL261 murine gliomas were treated with temozolomide 5 mg/kg/day for 10 days following tumor implantation. SurVaxM was administered as 100 ug peptide in Montanide with 100 ng sargramostim on day 12. Median survival for mice receiving combination of temozolomide and SurVaxM was 46 days, compared to 32 days for mice receiving SurVaxM alone. Significant differences were observed in terms of median survival but not overall survival. This study demonstrated that: 1) the combination of temozolomide and SurVaxM while not synergistic, was not detrimental to immunization response and had minimal toxicity and, 2) treatment with SurVaxM at a late stage (12 days post-implantation to accommodate temozolomide administration) was still effective (compared to initiation of vaccinations 4 days post-implantation in earlier studies, with much smaller tumor mass).

1.8.2 Clinical Data

The literature provides several immunotherapy trials that incorporate wild type survivin peptides as target antigens and show the induction of specific T-cell activity in a limited number of patients with advanced solid tumors [32-35]. A phase II trial [36] in 61 HLA-A1/-A2/-B35-positive, metastatic melanoma patients, using survivin-targeted peptide vaccination demonstrated prolonged OS in 13/41 patients who exhibited survivin-specific T-cell reactivity (SSTR) (median 19.6 vs 8.6 months; p=0.0077). It also revealed that the induction of SSTR was associated with gender and disease stage, rather than age, HLA type, performance status or vaccination regimen. It was observed that the majority of treatment-related side effects were mild to moderate (CTC grade 1-2), including fever, chills on the day of vaccination and inflammatory reactions at the

injection sites. The occurrence of these post-vaccination inflammatory reactions was strongly associated with the presence of SSTR and a trend toward favorable survival.

SVN53-67/M57-KLH has been studied in a phase I clinical trial sponsored and conducted by Roswell Park Cancer Institute. In that trial, nine patients with recurrent, survivin-positive malignant gliomas who had failed standard therapy, received SVN53-67/M57-KLH [(500 mcg) in Montanide ISA 51 with sargramostim (100 mcg)] subcutaneously at two-week intervals, for four doses, until tumor progression. In addition, patients who survived six months or more without tumor progression, serious adverse events, or regimen limiting toxicity received maintenance phase dosing every three months. Toxicity attributable to SVN53-67/M57-KLH was mostly limited to mild injection site reactions. SVN53-67/M57-KLH-Montanide-sargramostim was well tolerated and the majority of AE were grade one. Six of nine patients experienced injection site reactions, all grade one, including localized areas of erythema related to vaccination. Three patients reported fatigue (grades 1 and 2), two patients experienced myalgias (grade 2). Lymphopenia was seen in 3 patients (all grade 1) and leukopenia (grades 1 and 2) occurred in three patients. The only grade 3 AE, a seizure, was not related to the vaccine. One SAE (renal failure) occurred in the maintenance phase in one patient but was unrelated to the study drug.

1.9 Risks and/or Benefits

1.9.1 SVN53-67/M57-KLH

Minor risks due to injection of SVN53-67/M57-KLH include:

- minor pain at the injection sites (common),
- local swelling of the subcutaneous tissues that may last several minutes to an hour (common)
- itching or redness at the injection site (common)
- infection is a possible risk of any injection (rare)

Major risks of SVN53-67/M57-KLH arise from the fact that although the survivin protein is rarely present in normal cells of the body, some normal cells could contain survivin making it possible that the vaccine could cause a reaction against normal tissues (autoimmunity). Patients will be observed for signs of such a reaction, using physical examinations and blood studies. A second major risk of treatment with SVN53-67/M57-KLH is that of a severe allergic or anaphylactic reaction. In the initial phase I clinical study, no allergic reactions or autoimmune phenomena were seen. All patients in this phase II study will be followed carefully for signs and symptoms of autoimmunity and allergic reactions.

1.9.2 Risks Associated with Montanide

SurVaxM (SVN53-67/M57-KLH) will be given together as an injection with Montanide ISA 51. Together these two agents will be injected just under the skin. Montanide helps to stimulate the immune system and keep the SurVaxM confined to the injection site so that it can work locally on the immune system. Montanide ISA 51 has been used extensively in clinical trials of vaccine

treatments and its risks are minor. These risks include: pain at the injection site, which can be treated with pain medicine; local skin irritation (swelling, local skin redness and sometimes itching) at the injection site; flu-like symptoms including muscle and joint aches.

Rarely, necrotizing granulomatous panniculitis with scarring and possible skin ulceration may occur as a result of subcutaneous injection of vaccines containing Montanide ISA 51. In suchy lesions, deep nodularity can occur at injection sites. High frequency ultrasound may be used to evaluate the injection sites of patients who complain of persistent tenderness or induration lasting over 4 weeks after injection to aid in the diagnosis.

If panniculitis is superficial it may affect the vascular supply to the epidermis leading to superficial skin ulceration. The risk of this complication may be minimized by ensuring that vaccine injection is not given too superficially. Patients that experience skin necrosis or ulceration may be treated with intralesional injection of Kenalog (1 ml lof 5 mg/ml solution) at the vaccine injection site approximately 4 weeks after vaccination. The injection site will be re-assessed 4 weeks after Kenalog administration and further doses be modified based on clinically determined response. It is possible that intralesional Kenalog could attenuate the desired immunological response to vaccination. If skin ulceration occurs, secondary cellulitis in the area of ulceration is possible. In such cases, microbial culture and appropriate antibiotic therapy may be indicated.

1.9.3 Risks Associated with Sargramostim

Sargramostim is indicated for use in a limited number of clinical settings, including post hematopoietic stem cell transplantation; where the recommended dose (250 mcg/m^2 daily, for multiple days) far exceeds the dose used in the current trial of SurVaxM. Many of the side-effects of sargramostim have been seen when the drug has been injected into a vein, rather than subcutaneously. Overall, the risks of short-term treatment in our study with low dose sargramostim administered subcutaneously are expected to be low.

There is the possibility that patients vaccinated with SurVaxM and sargramostim could develop an immune response against the pharmaceutical sargramostim, potentially neutralizing it and rendering the patient unresponsive to future use of this drug. The risk of this occurrence is not defined, but there is very little risk of these patients subsequently developing a condition where sargramostim therapy would be appropriate since allogeneic stem cell transplantation is not a current standard therapy for patients with recurrent glioblastoma.

- Minor Risks:
    - Tiredness and Headache. Headache can be treated with pain medicine.
    - Bone, joint and muscle pain. The doses at which these occur are generally higher than the dose used in this study.
    - Skin rash and itching.
    - Local skin irritation (swelling, local skin redness and sometimes itching) may occur at the injection site.

- Localized and widespread skin reactions are uncommon.
- Serious and potentially life-threatening allergic reactions with sargramostim are rare.
- Fluid retention. This is usually reversible after sargramostim is stopped or drugs to reduce fluid retention are given.
- High or low white blood cell or platelet counts.

- Major Risks:
  - Pericarditis (irritation of the covering of the heart)
  - Pericardial effusion (fluid around the heart)
  - Hypotension (low blood pressure) may occur as part of a "first-dose" reaction to sargramostim. This is much less common when sargramostim is injected under the skin, rather than into a vein. This reaction is accompanied by some or all of the following symptoms:
  - Low blood pressure
  - Rapid heart beat
  - Fainting
  - Fever
  - Flushing
  - Nausea
  - Vomiting
  - Sweating
  - Back pain
  - Leg spasms
  - Shortness of breath

Patients who have first-dose reactions may need treatment with fluids by vein and oxygen. Acetaminophen (Tylenol), Ibuprofen or narcotics (Lortab, Morphine or Dilaudid) may be given for pain relief. This reaction usually does not happen again with further doses of sargramostim during the same course of treatment.

- Abnormal heart beat (rare - less than 1%). This is reversible when the medicine is stopped. It occurs more commonly in patients with a history of abnormal rapid heart beating.
- Poor kidney function has occurred in patients with previous kidney problems. Patients with kidney problems are not eligible for this study.

- Problems with liver function. While there have been no reports of severe liver problems, reversible increases in liver enzymes have occurred with sargramostim.

2 RATIONALE

Despite advances in surgery, radiation therapy and chemotherapy, including establishment of concurrent radiation and temozolomide followed by temozolomide as standard of care, glioblastoma (GBM) remains an incurable disease with a dismal median overall survival of less than 15 months[1].

This phase II study in patients with newly diagnosed glioblastoma follows a completed phase I study of patients with recurrent malignant glioma who had failed standard treatment, including temozolomide. In that study, all patients had received extensive treatment with temozolomide prior to receiving SVN53-67/M57-KLH with Montanide and GM-CSF.

There is a strong rationale for moving vaccine-based cancer therapy to the upfront setting in which a patient's immune system is best able to respond to the treatment. Numerous studies have shown that patients with glioblastoma have severe immunosuppression that improves significantly following surgical tumor resection. Tumor-mediated immunosuppression returns when the tumor recurs following failure of standard therapy. Therefore, vaccine-based therapy given soon after initial surgical resection stands the best chance of improving time to tumor progression and overall survival for GBM patients. In addition, studies of one other KLH-conjugated peptide vaccine (CDX-110; rindopepimut) to treat glioblastoma, Sampson et al.[37] showed that temozolomide-induced leukopenia is associated with an enhanced immunologic response to that vaccine. The mechanism for this is unclear, although it may involve differential susceptibility of certain immunomodulatory cells to temozolomide.

The rationale for proceeding directly to a phase II study is based on the following four points. First, the completed phase I trial of SVN53-67/M57-KLH showed very little toxicity with no grade III or IV adverse events and no serious adverse events attributable to the study drug. Second, there is no scientific rationale to posit additive or synergistic toxicity between the two drugs, given that temozolomide is an alkylating agent and SVN53-67/M57-KLH is a peptide vaccine. Third, in three separate GBM trials (ACTIVATE[38], ACT II[39] and ACT III[37]), rindopepimut vaccine given in combination with temozolomide showed no serious toxicity with only minor injection site reactions, similar to expectations for SVN53-67/M57-KLH. Fourth, in addition to evaluation of immune responses and PFS, the current phase II design includes rigorous evaluation of toxicity of each drug with CTCAEv4.0 (see **Sections 6.2.1** and **6.2.2**).

3 OBJECTIVES

3.1 Primary Objective

To evaluate 6-month progression-free survival (PFS6) in patients with survivin positive newly diagnosed glioblastoma multiforme (GBM) treated with at least 4 doses of SurVaxM and standard of care temozolomide. PFS6 is defined as the percentage of patients without tumor progression or death from any cause 6 months after the date of diagnosis by biopsy.

3.2 Secondary Objectives

- To determine the safety and tolerability of SurVaxM in patients receiving standard care adjuvant temozolomide.
- To evaluate overall survival (OS) in patients with survivin positive newly diagnosed GBM treated with SurVaxM and adjuvant temozolomide.
- To describe the immune response in patients treated with SurVaxM and predictors of response.
- To evaluate objective tumor response rate (applicable only for patients with evaluable disease at study entry, as defined per RANO criteria [40]) and predictors of response.

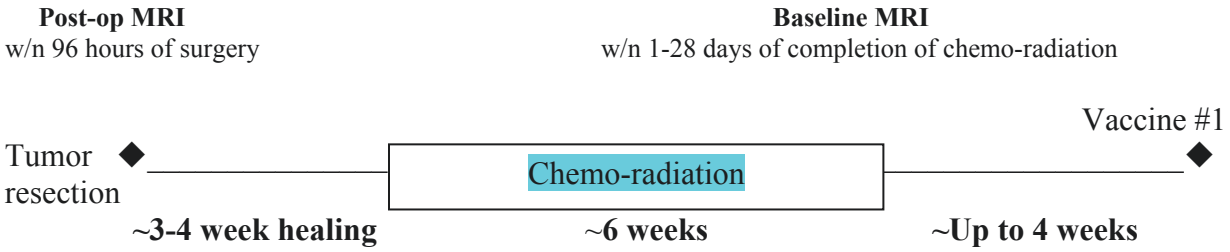
4 METHODOLOGY

4.1 Study Design

This is a multi-center, open label, single arm, phase II trial in adult patients with newly diagnosed GBM, evaluating the efficacy and safety of SurVaxM and adjuvant temozolomide after patients have received concurrent radiation and temozolomide.

Patients will receive a postoperative MRI, within 96 hours of surgical resection, followed by ~4 weeks recovery time. Then they will receive 6-week duration of radiation therapy with temozolomide. A post-chemoradiation MRI will be performed within 1-28 days to ensure no tumor recurrence or progression.

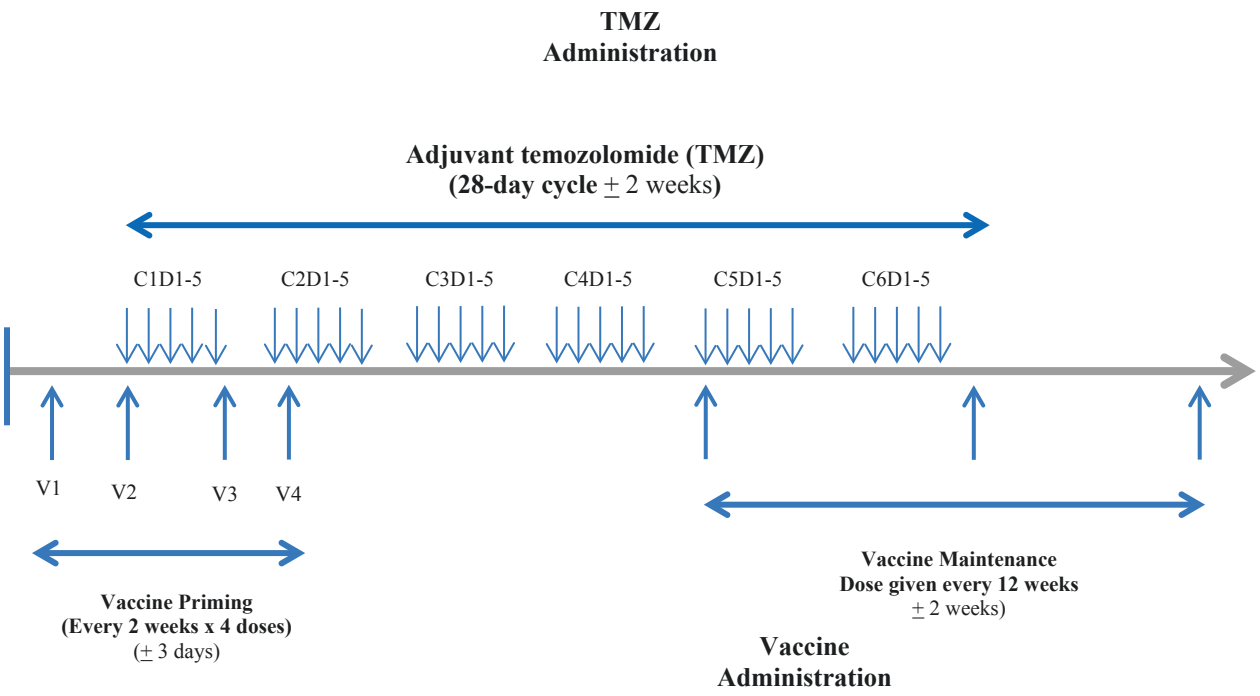
Figure 8 Pre-Study Treatment and Imaging Requirements



HLA tests and tumor testing for survivin status can be performed at any time prior to vaccine priming, while the rest of screening assessments should be performed after completion of chemo-radiation and prior to vaccine priming.

As shown in **Figure 8**, first priming administration of SurVaxM should occur within 7-28 days after completion of chemoradiation. Adjuvant temozolomide therapy (TMZ) will begin ≥ 28 days after completion of concomitant temozolomide radiation therapy and given for 6 cycles or more (at the discretion of the investigator), or until intolerance or tumor progression.

Figure 8 Treatment Schema



SurVaxM should be administered approximately every 2 weeks (± 3 days) during the priming phase and then approximately every 12 weeks (± 2 weeks) during the adjuvant phase. Following completion or discontinuation of TMZ in the absence of progression, SurVaxM may be continued approximately every 12 weeks during the maintenance phase until intolerance or disease progression.

All participants will sign an informed consent prior to study related tests. All participants will meet the inclusion and exclusion criteria summarized in **Section 5.1** and **Section 5.2** Participants will be treated on an outpatient basis.

4.2 Target Accrual and Study Duration

The target accrual for this phase II trial is 50 evaluable patients with survivin positive newly diagnosed GBM. Approximately 2 patients per month will be enrolled, with follow-up of at least 12 months after the last enrolled patient. Patients will be followed until death to determine overall survival. The participants will initially receive treatment with radiation and chemotherapy with temozolomide as per standard of care. Thereafter, patients will receive SurVaxM and 6

cycles of adjuvant temozolomide (or more at the discretion of the investigator), until intolerance or tumor progression. Patients are eligible for treatment with SurVaxM until disease progression or unacceptable toxicity.

5 PARTICIPANT SELECTION

5.1 Inclusion Criteria

To be included in this study, participants must meet the following criteria:

1. Age ≥ 18 years of age.
2. Have a Karnofsky performance status ≥ 70 (i.e. the patient must be able to care for himself/ herself with occasional help from others; refer to **Appendix B**).
3. Documented survivin-positive tumor status.
4. Pathologically confirmed diagnosis of glioblastoma multiforme (GBM). Or WHO grade IV [gliosarcoma]
5. Have the following clinical laboratory values obtained within 14 days prior to registration:
   - Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
   - Platelets ≥ 100 x 10^9/L
   - Hemoglobin (Hgb) > 9.0 g/dL
   - Serum total bilirubin: ≤ 1.5 x ULN
   - ALT and AST ≤ 4.0 x ULN
6. Patients on full-dose anticoagulants (e.g., warfarin or LMW heparin) must meet the following criteria:
   - No active bleeding or pathological condition that carries a high risk of bleeding (e.g., tumor involving major vessels or known varices)
7. Adequate renal function, as defined below:
   - Creatinine ≤ 1.8 mg/dl
8. HLA-A*02, HLA-A*03, HLA-A*11 or HLA-A*24 positive patients.
9. MRI (completed within 96 hours after surgery) documenting gross total resection consisting of no gadolinium enhancement; or subtotal resection consisting of linear enhancement with (or without) nodular gadolinium enhancement measuring no greater than 1 cm x 1 cm x 1 cm total volume or 100 mm^2 in cross sectional area.
10. No evidence of true progressive disease from the postoperative period to the post-chemoradiation period, based on changes in the neurologic exam, steroid use, or evident radiographic progression, according to RANO criteria (see **Appendix C**). Patients with increased or new gadolinium enhancement may continue on protocol if in the investigator's judgment that enhancement is likely due to pseudoprogression. The use of correlative imaging studies (including PWI) or diffusion weighted imaging (DWI), and

repeat imaging after an interval of 2-4 weeks is strongly encouraged to help distinguish between pseudoprogression and true progression.

11. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry and, have a negative pregnancy test prior to starting study treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
12. Dexamethasone dose less than or equal to 4 mg daily at time of study enrollment.
13. Patients must have completed initial radiation therapy (RT) and temozolomide (TMZ) for the treatment of their glioblastoma (i.e., completed 6-week course of RT and, completed ≥ 75% of 6-week course of induction TMZ chemotherapy).
14. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

5.2 Exclusion Criteria

Participants will be excluded from this study for the following:

1. The patient must not have received any immunotherapy for their brain tumor.
2. Patients with serious concurrent infection or medical illness, which in the treating physicians opinion would jeopardize the ability of the patient to receive the treatment outlined in this protocol with reasonable safety.
3. Patients who are pregnant or breast-feeding.
4. Patients receiving concurrent therapy for their tumor (i.e. chemotherapeutics or investigational agents) other than temozolomide.
5. Patients with a concurrent or prior malignancy are ineligible unless they are patients with curatively treated carcinoma-in-situ or basal cell carcinoma of the skin. Patients who have been free of disease (any prior malignancy) for at least 3 years are eligible for this study.
6. Patients who have had repeat craniotomy for tumor therapy after receiving RT and TMZ treatment.
7. Patients who received other chemotherapeutics or investigational agents in addition to their radiation therapy and concomitant temozolomide treatment.
8. Patients who have received Gliadel wafers or alternating electrical field therapy are not eligible for this study.
9. Known history of an autoimmune disorder.
10. Known human immunodeficiency virus (HIV) positivity or acquired immunodeficiency syndrome (AIDS) related illness or other serious medical illness.
11. Patients who have contraindication to MRI.

12. Unwilling or unable to follow protocol requirements.
13. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.
14. Received an investigational agent within 30 days prior to registration.

5.3 Inclusion of Women and Minorities

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

6 TREATMENT PLAN

6.1 Dosing and Administration

Treatment will be administered on an outpatient basis. Reported adverse events (AEs) and potential risks are described in **Section 1.9**. Appropriate dose modifications are described in **Section 6.2**.

Vaccine Priming Phase

Beginning 7-28 days after completion of standard chemoradiation therapy, patients will receive the first of four priming doses of SurVaxM in emulsion with Montanide with Sargramostim. Subsequent priming doses are given every other week (on priming Days 15, 29 and 43 ± 3 days) via subcutaneous injection. Patients will receive approximately 500 mcg SurVaxM in 50/50 volume emulsion with Montanide ISA 51 (1 cc total) and a second separate injection of 100 mcg sargramostim in close proximity (1-3 cm) to the first injection.

Adjuvant Temozolomide (TMZ) / Vaccine Phase

TMZ will begin no sooner than 28 days after completion of concomitant temozolomide radiation therapy and only when the absolute neutrophil count (ANC) is ≥ 1500/μL and the platelet count is ≥ 100,000/μL.

TMZ will be dosed on days 1-5 of repeated 28-day cycles. A dose of 150 mg/m^2 body surface area per day will be given for the first cycle of TMZ. This dose will increase to 200 mg/m^2 body surface area per day in subsequent TMZ cycles, in the absence of Grade 3 and Grade 4 hematological toxicity and with adequate count recovery after the first cycle. Any change in weight of more than 10% will require re-calculation of the administered TMZ dose; otherwise, dosing may be based on the baseline weight. The total dose of TMZ shall be rounded to the nearest 10 mg increment. TMZ should continue for six cycles, or until intolerance or progression in accordance with RANO criteria.

Vaccine Maintenance Phase

Following completion or discontinuation of TMZ for reasons other than tumor progression, SurVaxM should be administered every 12 weeks (± 2 weeks) after the priming phase until intolerance or tumor progression.

6.1.1 Administration of SurVaxM

SurVaxM must be administered at the clinical trial site by appropriately trained staff.

SurVaxM injections must be performed using an appropriate needle for subcutaneous administration. A 23-gauge needle is recommended. Each dose of SurVaxM vaccine in emulsion with Montanide ISA 51 will be given in a total volume of 1.0 mL. Each dose will contain 500 mcg SurVaxM and will be administered subcutaneously. The location of each injection will be recorded. In addition, 100 mcg sargramostim will be administered subcutaneously as a second injection given in close proximity (1-3 cm) to the SurVaxM injection. The injection site should be administered on the opposite side of the body from the previous injection.

Injections over the deltoid muscle are preferred, and the site of administration should alternate between left and right arms for each successive treatment. Alternative sites, including the anterior thigh, may be used if necessary. SurVaxM injections should not be given to areas of skin with dermatologic conditions (such as persistent injection site reactions, infection, edema, or scarring) that will not allow easy access for study drug administration or evaluation of localized adverse events. If such conditions or other circumstances contraindicate injections as outlined above for an individual patient, alternate sites can be used, but this should be discussed with the Study Principal Investigator.

Following administration of SurVaxM, patients must remain in clinic for observation a minimum of one hour following each vaccination to evaluate and treat any potential immediate hypersensitivity reactions.

Measurement of Local Reaction: Measurement of any local reaction that may occur once the patient leaves the clinic will be performed by the patient or caregiver. At the time of injection, study staff should mark the outline of the injection site on the skin. Patients/caregiver will then be instructed by the study staff to measure the area of local reaction at its perceived maximum (using the diameter of a U.S. quarter – approximately 1 inch – as a reference standard), between 24-48 hours of administration of SurVaxM. Patients (or caregivers) will be asked to record the approximate dimensions of the reaction (if any) and the date it was measured. The study coordinator will contact the patient (or caregiver) within 3 to 5 days hr after the injection to obtain and record the patient's local reaction measurement.

6.1.2 Administration of Temozolomide

Temozolomide (TMZ) will be orally self-administered, or administered IV in the clinic, around the same time each day (in the evening) on days 1 through 5 of each cycle during the adjuvant phase. A dose of 150 mg/m^2 body surface area per day will be given for Cycle 1 of TMZ. This dose will increase to 200 mg/m^2 body surface area per day in subsequent TMZ cycles pending hematologic count recovery. Any change in weight of more than 10% will require re-calculation of the administered temozolomide dose; otherwise, dosing may be based on the baseline weight. The total dose of TMZ shall be rounded to the nearest 10 mg increment. Dosing should occur according to instructions in the product label and per standard practice, with consideration to the guidance outlined below (see **Section 6.2.2**). Patients who want to get their Temozolomide

locally and closer to home may do so by their oncologists. However, patients would still get their vaccine doses and disease assessments done at their enrollment site.

6.2 Dose Modifications

Every effort should be made to administer trial treatment on the planned schedule. In the event that individual patients experience treatment related toxicity, subsequent trial treatment may be delayed, omitted and/or discontinued as described below

6.2.1 SurVaxM Dose Omission or Discontinuation

Since all toxicities related to SurVaxM are expected to be immunologically mediated, no adjustment to the administered dose will be allowed.

If any unexpected significant toxicity (e.g., CTCAEv4 Grade 3) is observed, and there is a reasonable likelihood that the event may be associated with administration of SurVaxM and/or sargramostim, the next immunization for that patient should be withheld until the toxicity improves to mild severity or less. The immunization may be held for up to 2 weeks following the withheld dose. If significant toxicity is observed again or is not reversible, all further immunizations should be discontinued.

If vaccinations are discontinued for any reason, temozolomide administration may continue at the Investigator's discretion

6.2.2 Temozolomide Dose Adjustments

Temozolomide dose adjustments should be prescribed as clinically appropriate, as per the Investigator's discretion with consideration to the product label recommendations (which may be more conservative than the guidance below).

Initiation of TMZ should not begin until the patient's peripheral blood counts have recovered from chemoradiation such that absolute neutrophil count (ANC) is ≥ 1500/μL and platelet count is ≥ 100,000/μL. Delays in the initiation of temozolomide will be recorded in the CRF.

During temozolomide cycles, up to a three-week dose delay in temozolomide administration may be considered until the patient's blood count returns to an acceptable level (ANC ≥ 1500/μL and platelet count ≥ 100,000/μL).

Deviations from the above-outlined treatment schedule are allowed and will not represent protocol violations, when appropriate as per standard of care. However, such deviations must be discussed with the Study Principal Investigator in advance to ensure appropriate visit scheduling.

Table 1 Temozolomide Dose Levels for Maintenance Treatment

Dose	Level Dose (mg/m2/day)	Remarks
-1	100	Reduction for prior toxicity
0	150	Dose during Cycle 1

1	200	Dose during Cycles ≥ 2 in absence of Grade 3 or higher toxicity

Table 2 Temozolomide Dose Levels for Maintenance Treatment

Toxicity * Discontinue TMZ	Reduce TMZ by 1 Dose Level	Discontinue TMZ
Absolute Neutrophil Count	less than 1.0 x 10^9/L	See footnote†
Platelet Count	less than 50 x 10^9/L	See footnote†
CTC Non-Hematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 3	CTC Grade 4†

*TMZ dose levels are listed in **Table 1**.

†TMZ is to be discontinued if dose reduction to less than 100 mg/m^2 is required or if the same Grade 3 non-hematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction. TMZ=temozolomide; CTC=Common Toxicity Criteria.

If temozolomide is discontinued for any reason other than tumor progression, SVN53-67/M57-KLH vaccinations may continue at the Investigator's discretion

6.3 General Concomitant Medication and Supportive Care

The use of all standard supportive medication is permitted, although concurrent treatment with immunosuppressive or immunomodulatory agents is strongly discouraged. Concomitant systemic corticosteroids are to be avoided if at all possible. If used, doses of steroids should be the minimum necessary for appropriate clinical management. If any new or increased dose of steroids is necessary due to signs or symptoms, a rigorous evaluation for recurrent glioblastoma should be conducted and, in the absence of progressive disease, every effort should be taken to taper the patient off steroids as quickly as clinically feasible. If clinically necessary, topical or systemic non-steroidal anti-inflammatory or antihistamines may be used as alternatives.

When clinically appropriate, patients should strictly follow the study-prescribed treatment regimen. Therefore, patients should not receive additional investigational agents or anti-cancer therapies, unless progression of disease warrants discontinuation of study treatment and commencement of alternate therapies. Patients who begin new anti-cancer therapy, including investigational therapy, chemotherapy, cytokine therapy, immunotherapy (including other vaccines), or alternating electrical field therapy must discontinue SurVaxM, and will be followed for survival. Patients who undergo surgical resection or biopsy in the absence of progression may, upon consultation and agreement with Roswell Park Cancer Institute, be eligible to continue to receive study treatment with follow up to document persistent disease growth.

All concomitant medication will be documented in the CRF if taken within 30 days prior to Priming Phase through (whichever occurs first) either, a) 30 calendar days after the last

administration of study treatment or, b) initiation of alternate anticancer therapy. All concomitant medications required to treat study-drug related toxicity should be reported regardless of the timeframe relative to study drug dosing. All anti-cancer medications taken throughout the duration of study follow-up should also be recorded.

Participants may be pretreated for nausea and vomiting with appropriate anti-emetics.

6.3.1 Guidance for Management of Expected Toxicity

6.3.1.1 Local Injection Site Reaction

Based on the experience with SurVaxM and other peptide vaccines, local injection site reactions are likely to be the most common event associated with the administration of SurVaxM. However, not all patients may experience an injection site reaction with the first few administrations of vaccines, and not all patients who experience injection site reactions may experience a reaction with every injection of vaccine. Erythema and pruritus occurred most frequently, but localized swelling, pain, rash, induration, bruising and urticaria are also possible, and may be relatively common. Some injection site reactions may extend for multiple centimeters into the surrounding dermis and take up to several days to resolve.

The clinical experience to date suggests that nearly all injection site reactions are tolerable and resolve without the need for intervention. Topical corticosteroids are permitted to treat injection site reactions at the discretion of the investigator. Nevertheless, systemic corticosteroids should be reserved for severe injection site reactions only since they may also compromise the immune response to the vaccine and could negate its effect. Topical or systemic non-steroidal anti-inflammatory agents (e.g. ibuprofen, naproxen, aspirin) or antihistamines (e.g. Claritin®, Allegra®, Benadryl) may be used as alternatives; however, routine use to prevent injection site reactions should be avoided, and doses should be the minimum necessary for appropriate clinical management.

SurVaxM injection sites should be rotated in accordance with the applicable study protocol; injections should not be given to areas with evidence of persistent local reaction.

Injection site reactions are considered adverse events and should be reported as such, using an appropriate descriptive term. (For example, “injection site erythema” should be used rather than the less descriptive term “injection site reaction”.) **Section 10.1.2.1** provides guidance for the severity ratings of possible injection site reactions.

6.3.1.2 Hypersensitivity Reactions

Hypersensitivity-like reactions could occur. Precautions should be taken to prepare for the possibility of anaphylactic/hypersensitivity reactions after SurVaxM/Montanide/sargramostim administration. Patients should be observed as per institutional standards, but for a minimum of 1 hour following each vaccination to evaluate and treat any potential immediate hypersensitivity reactions. The use of all routine supportive medication is permitted for hypersensitivity reactions, although routine use of prophylactic antihistamines, nonsteroidal anti-inflammatory agents and concomitant systemic corticosteroids are to be avoided if at all possible. If used, doses of steroids should be the minimum necessary for appropriate clinical management. If any new

administration of or increased dose of systemic steroids is necessary, every effort should be taken to taper, and preferably discontinue treatment with them, as quickly as clinically feasible.

For pain or fever, acetaminophen (325 mg tabs, 1 or 2 p.o. every 4 hours p.r.n.) will be utilized preferentially. Pretreatment of participants with acetaminophen may be instituted as warranted for a temperature of 38°C. Fevers lasting more than 8 hours after treatment will be evaluated in terms of potential infection. Aspirin, naproxen and ibuprofen are acceptable alternatives to acetaminophen for pain or fever, but are not preferred.

For mild to moderate local injection site pain that is unresponsive to acetaminophen, oral opiates will be used (oxycodone, 5–10 mg p.o. every 4 hours, p.r.n.). Pain that is of more than mild to moderate grade will be investigated for sources other than the therapy, and managed accordingly.

Anticonvulsant, antihypertensive and antidiabetic medications should be used as indicated. Antiemetics may be administered at the discretion of the treating physician.

For delayed hypersensitivity reactions, investigators should treat for symptomatic relief but avoid corticosteroid use whenever possible.

6.3.1.3 Non-Injection Site Rashes

Investigators should treat for symptomatic relief but should avoid systemic corticosteroid use if at all possible.

6.4 Duration of Treatment

Participants may remain on study and continue to receive treatment in the absence of: disease progression, unacceptable toxicity, intercurrent illness that prevents further administration of treatment, participant demonstrates an inability/refusal to comply with oral medication regime, or participant withdraws from study.

6.5 Duration of Follow-Up

All patients treated on study will be followed until death or loss of follow-up.

6.6 Treatment Discontinuation

Upon treatment discontinuation, all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate eCRF.

A patient should be withdrawn from study treatment if, in the opinion of the Investigator, it is medically necessary. In addition, patients will be withdrawn from treatment in the case of:

1. RANO criteria defined disease progression. In cases where RANO criteria cannot be applied, progression should be based on unequivocal evidence of progressive disease sufficient to require a change in therapy.
2. A need for surgery, radiation, or for other anticancer therapy not specified in the protocol.

3. Lost to follow-up or noncompliant.
4. Pregnancy. Pregnant patients should be followed for the duration of the pregnancy and the outcome of the pregnancy should be documented.
5. Death
6. Participant voluntary withdrawal
    a. A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
7. Investigator decision.

7 INVESTIGATIONAL PRODUCTS

7.1 SVN53-67/M57-KLH (SurVaxM)

The synthetic peptide in SurVaxM (SVN53-67/M57) spans amino acids 53 through 67 of the mature human (and mouse) survivin protein sequence and contains a substitution of methionine (M) for cysteine (C) at position 57 which leads to enhanced MHC class I binding. The peptide is conjugated to Keyhole Limpet Hemocyanin (KLH). SVN53-67/M57-KLH contains a peptide mimic that is immunogenic in humans and in C57BL/6 mice. The planned investigational study is based on extensive experimental observation concerning the ability of SurVaxM to elicit potent and specific immune responses that are capable of inhibiting the growth of cerebral gliomas in mice and killing human glioma cells ex vivo via cytotoxic T lymphocyte (CTL) activity.

7.1.1 Pharmacological Class

This peptide is to be used as a vaccine and is a biological drug by class.

7.1.2 Structural Formula

SVN53-67/M57-KLH is 15-amino acids in length with the amino acid sequence: DLAQMFFCFKELEGW. It is conjugated to Keyhole Limpet Hemocyanin (KLH), which serves as a carrier protein and immune adjuvant.

7.1.3 All Active Ingredients

SVN53-67/M57-KLH is conjugated to Keyhole Limpet Hemocyanin (KLH). SVN53-67/M57-KLH is administered emulsified in Montanide ISA 51 VG, (Cross-Referenced Drug Master File 10870, Seppic, Inc. France) and is co-administered with a separate local injection of sargramostim; Leukine®).

7.1.4 Drug Shipment

SVN53-67/M57-KLH will be shipped to all participating sites from The University of Iowa Pharmaceuticals as individual vials containing 1 mg sterile lyophilized powder in 2 mL clear

borosilicate glass crimped rubber stopper vials. Labeled boxes of peptide holding 80 vials each are supplied and shipped at -20°C in a Styrofoam insulated cardboard carton.

The date of receipt, condition of the shipment, and the amount of drug received will be documented. Drug shipment records will be retained by the investigational pharmacist or designee.

7.1.5 Preparation

Each vial of SVN53-67/M57-KLH peptide-conjugate, as delivered by The University of Iowa Pharmaceuticals, contains 1 milligram of lyophilized drug per vial. The peptide conjugate is reconstituted in the Investigational Drug Service Pharmacy with 1 mL sterile bacteriostatic saline injected into the primary 2 mL stock vial to yield a 1 mg/mL solution. The reconstituted solution will either be used for vaccine preparation or discarded.

7.1.6 Storage and Stability

Upon arrival, SVN53-67/M57-KLH is stored at -20°C in a locked, temperature alarmed and remotely monitored freezer under the control of the Investigational Drug Service Pharmacy. Stability testing will be performed on a regular schedule in accordance with applicable FDA regulations and the IND for SVN53-67/M57-KLH.

7.1.7 Handling and Disposal

The Investigator or designee will be responsible for prescribing all investigational drug provided by RPCI exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken per the institution's environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

All products dispensed will be recorded on a product accountability record. Records of product lot numbers and dates received will be entered on a product accountability form. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Used vials (excess drug) will be destroyed according to standard practices after properly accounting for the dispensing. Partially used vials of study drug will not be re-used for other participants.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

7.2 Vaccine Preparation: SVN53-67/M57-KLH, Montanide® ISA 51 and Sargramostim

For each vaccination, SVN53-67/M57-KLH will be mixed with Montanide® ISA-51 VG to create an emulsion using the standard operating procedures established in **Appendix D**. Sargramostim (Leukine) will be delivered locally as a separate injection.

Leukine (Sargramostim), NDC Product Code 50419, is an approved drug.

7.2.1 Interaction with Other Medicinal Products and Other Forms of Interaction

There have been no unexpected interactions observed between SurVaxM and other drugs as of the data cut-off date.

7.2.2 Overdose

No specific antidotes exist for the treatment of SurVaxM overdose.

7.2.3 Pregnancy, Lactation, and Pediatric Use

Fertility and teratology studies with SurVaxM have not been conducted. Safety for women of childbearing capacity cannot be implied from the existing data. Patients who are pregnant or are breast feeding are excluded from all SurVaxM clinical trials. Female patients must be surgically sterile or be postmenopausal, or must agree to use effective contraception during the period of treatment. All female patients with reproductive potential must have a negative pregnancy test prior to treatment with SurVaxM. Male patients must be surgically sterile or must agree to use effective contraception during the period of treatment. The safety of SurVaxM in pediatric patients has not been evaluated and, therefore, SurVaxM should not be administered to patients <18 years of age.

7.3 Adjuvant Temozolomide

Temozolomide will not be provided for this study and will be paid for by the patient's insurance carrier as part of the standard-of-care treatment of newly diagnosed glioblastoma.

8 STUDY PROCEDURES

Informed consent ***MUST*** be completed prior to receiving any study related procedures.

Unless otherwise defined in the written protocol text, all procedures/assessments will be conducted in accordance with RPCI Clinical Research Services Standard Operating Procedures.

8.1 Schedule of Investigations and Data Collection

The study is divided into phases with associated evaluations and procedures that must be performed at specific time points, as described in the following sections. The study Schedule of Procedures and Observations (**Table 3**) summarizes the frequency and timing of various activities, safety, and other measurements.

Every effort should be made to ensure that the protocol required tests and procedures are completed as described. However it is anticipated that from time to time there may be circumstances outside of the control of the investigator that may make it unfeasible to perform the test. In these cases the investigator will take all steps necessary to ensure the safety and well-being of the patient. When a protocol required test cannot be performed, the study investigator will document the reason for this and any corrective and preventive actions that have been taken to ensure that normal processes are adhered to as soon as possible. The study team will be informed of these incidents in a timely fashion.

8.1.1 Screening Period

Prior to the performance of any study-specific procedures, the patient will have the nature of the study explained to them, and will be asked to give written informed consent. Informed consent must be obtained prior to any study-specific procedures that do not form a part of the patient's normal care. However, assessments performed according to standard of care prior to receipt of informed consent may be utilized to fulfill the screening requirement, if completed within the required window for screening.

The assessments outlined for the Screening Visit in the study Schedule of Procedures and Observations (**Table 3**) will be completed for each patient prior to inclusion in the study, and results will be evaluated to verify entry criteria prior to study entry.

8.1.2 Treatment Phase

The patient should begin protocol treatment with Priming Vaccine Day 1 within 7-28 days after completion of standard chemoradiation. The study Principal Investigator is to be contacted for any extenuating circumstances that could prevent timely initiation of protocol treatment.

The on-study period will be divided into three components, to include the vaccine priming phase (four doses of SurVaxM), adjuvant temozolomide with SurVaxM (the adjuvant phase) and the SurVaxM maintenance period (the maintenance or boosting phase). Specific procedures to be performed throughout the treatment phases are illustrated in the Schedule of Procedures and Observations (**Table 3**).

8.1.3 Treatment Completion/Discontinuation

The End of Treatment Visit should be performed within 30 days (± 3 days) after last study drug dosing, or prior to initiation of alternate therapies (whichever occurs sooner). Follow-up and documentation of adverse events and concomitant medications should continue through day 30. Specific procedures to be performed at this visit are illustrated in the Schedule of Procedures and Observations (**Table 3**).

8.1.4 Post-Treatment Follow-up Phase

Patients who discontinue study treatment should be followed for survival with visits or phone contact every 12 weeks (± 4 weeks) until death or lost to follow-up.

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Table 3 Schedule of Procedures and Observations

Protocol Activity	Screen	Vaccine Priming Phase[1]				Adjuvant Temozolomide[2]	Vaccine Maintenance Phase[3]	Disease Assessment	End of Treatment[4]	Survival Follow-up[5]
		V1	V2	V3	V4	Day 1 of each 28 day cycle	Every 12-weeks (± 2 weeks)	Every 8 weeks (± 4 weeks)		Every 12 weeks (± 4 weeks)
Tumor tissue	X									
Medical history	X									
Physical examination	X	X	X	X	X	X	X	X		
Neurological examination/ MMSE[14]										
Vital Signs(to include weight)	X	X	X	X	X	X	X	X		
Karnofsky Performance Status	X	X	X	X	X	X	X	X		
Hematology[6]	X	X	X	X	X	X	X			
Chemistry[7]	X	X	X	X	X	X	X			
Urinalysis	X	X	X	X	X					
Pregnancy Test (serum)[8]	X	X	X	X	X	X				
HLA typing[13]	X									
Humoral and Cellular Immune Reponses[9]		X						X	X	
Brain MRI[10]	X							X	X	
Study Vaccine		X	X	X	X		X			

Protocol Activity	Screen	Vaccine Priming Phase[1]				Adjuvant Temozolomide[2]	Vaccine Maintenance Phase[3]	Disease Assessment	End of Treatment[4]	Survival Follow-up[5]
		V1	V2	V3	V4	Day 1 of each 28 day cycle	Every 12-weeks (± 2 weeks)	Every 8 weeks (± 4 weeks)		Every 12 weeks (± 4 weeks)
Measurement of local reaction[15]		X	X	X	X		X			
Adjuvant Temozolomide						X				
Concomitant Medications	X	X	X	X	X	X	X	X	X	X[11]
Adverse Events Assessment[12]		X	X	X	X	X	X	X	X	
Contact Information for survival follow-up										X

Footnotes for Schedule of Procedures and Observations:

Note: *Unless otherwise noted, study assessments should be completed prior to dosing at each treatment visit.*

1. V1 within 7-28 days after completion of chemoradiation and then every two weeks (14 days ± 3 days) for a total of 4 doses.
2. Adjuvant temozolomide therapy (TMZ) will begin ≥42 days after completion of concomitant temozolomide radiation therapy and given for 6 cycles or more (at the discretion of the investigator), or until intolerance or tumor progression. Temozolomide to be taken on Day 1 through Day 5 of each 28-day cycle ± one week window for tests and visit.
3. Maintenance Vaccine Phase: SurVaxM should be administered every 12 weeks (± 2 weeks) after the priming phase until intolerance or tumor progression (12-week cycle ± one week window for tests and visit).
4. The End of Treatment visit should be performed within 30 days (± 3 days) after last study drug dosing, or prior to initiation of alternate therapies (whichever occurs sooner). Follow-up and documentation of adverse events and concomitant medications should continue through day 30 (± 3 days), or until lost to death or follow-up.
5. Patients who discontinue study treatment should be followed on study for survival, with visits or phone contact every 12 weeks (±4 weeks) until death or lost to follow-up.

6. Hematology: CBC with differentials, platelets.
7. Chemistry: (CMP) defined as: chloride, CO_2, potassium, sodium, BUN, glucose, calcium, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, A/G ratio, BUN/creatinine ratio, osmol (Calc), anion gap.
8. Pregnancy test is required for women of child bearing potential only. A serum pregnancy test must be performed during screening. If the serum pregnancy test is not performed within 7 days of dosing on Priming Phase V1-Day 1, a urine pregnancy test must be performed on Priming Phase V1-Day 1 with results reviewed prior to dosing. Testing may be repeated throughout the study as indicated.
9. Blood: six 10 mL green top tubes and one 3.5 mL gold top tube to be collected for in vitro immunological assays (see **Section 8.2**).
10. Brain MRIs must be obtained from the postoperative period (ideally with 72 hours) and post-chemoradiation period (within 1-14 days of completion of chemoradiation).
11. Subsequent to progression, medication review is limited to new anti-cancer regimens.
12. Documentation of adverse events occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever occurs first. Any serious adverse event occurring any time after the reporting period must be promptly reported if a causal relationship to study treatment is suspected.
13. HLA –A low resolution panel
14. MMSE: Mini Mental State Exam will be done at the discretion of the PI. [41]
15. Patient to be instructed to check injection site for 24 to 48 hr post injection: staff to call within 3 to 5 days to document any reaction (see **Section 6.1.1**).

8.2 Blood Draws for Humoral and Cellular Immune Response

Whole blood samples for humoral and cellular immune response analysis will be collected via venipuncture using six 10 mL green top tubes and one 3.5 mL gold top tube.

Sample collection: Immunological analyses will be conducted on blood samples obtained prior to the patient's scheduled vaccination dosing (i.e., predosing), and at all study follow-up visits as indicated on **Table 3**. (to be drawn on day of priming vaccine one (V 1), with disease assessment every 8 weeks (± 4 weeks), and at progression or end of treatment). On each occasion, approximately 50 mL of blood will be obtained from a peripheral venipuncture and collected in six green top (heparinized) tubes and one 3.5 mL gold top tube. Blood tubes will be kept at room temperature until pickup for processing.

Additional End of Treatment blood draw (optional):

All collected blood samples from within RPCI will be sent from Phlebotomy directly to the RPCI Immunomonitoring Facility for PBMC separations and freezing.

Center for Immunotherapy
Immune Analysis Facility Shared Resource
Cancer Cell Center (CCC), C-416

Tel: 716-845-8459 (office)
Tel: 716-845-8952 (lab)

Junko.Matsuzaki@RoswellPark.org

Serum will be separated from whole blood gold top tube and frozen at -70°C. Whole blood will be processed to obtain PBMC and aliquoted into 2-10 cryovials per time-point containing 1 million cells per vial (number of vials is dependent upon cell number recovered: see **Appendix E)**. Screw cap polypropylene cryogenic tubes will be labeled with the participant's MR number, participant's initials, participant's study number, clinical study number, protocol time point, dose, and protocol day. The samples will immediately be frozen at -70ºC or below until analyzed. Samples collected after hours will be maintained at room temperature and processed within 24 hours in the Immune Analysis Facility (IAF).

A blood intake and inventory log will be maintained in the Immune Analysis Facility (IAF).

In vitro immunological assays will be performed at RPCI as a central immunomonitoring site. Pre-immune serum for baseline anti-survivin and anti-KLH antibody titers and T cell multimer assays will be performed in the Neuro-Oncology lab; cytokine analysis and ELISPOT assays will be performed in the RPCI Immune Analysis Facility (IAF). As noted above six 10 mL green top tubes and one 3.5 mL gold top tube will be processed for these assays. Serum from one 3.5 ml gold top tube will be directed to antibody assessments; PBMC collected from green top tubes (approximately equal to that collected from three tubes) will be designated for multimer studies. Remaining PBMC from green top tubes will be used for cytokine analysis and ELISPOT as well as repeat assays or multimer assay if there is a shortfall of PBMC.

Results will be collected at multiple time points throughout the study as groups of patient samples become available for complete analysis. Data will be reported in spreadsheet (Excel) format to be provided to CRS data management for entry.

NETWORK SITES: Follow directions above for blood sample collection and processing (PBMC isolation procedure: see **Appendix E**). PBMC tubes will be labeled with the Subject ID # (unique to Network patients), initials, the participant's study number, clinical study number, protocol time point, dose, and protocol day. PBMC samples from outside RPCI will be shipped frozen on dry ice via Federal Express Overnight in styrofoam-insulated containers, with delivery on Mon-Fri. NO SATURDAY OR SUNDAY DELIVERY. All samples should be shipped, along with the sponsor-provided shipping log, to the address below with e-mail notification to the Basic Science Co-investigator (Michael.Ciesielski@RoswellPark.org):

Roswell Park Cancer Institute
Cancer Cell Center (CCC), C-416
Attn: Immune Analysis Facility – I 259614
Elm & Carlton Streets
Buffalo, New York 14263

Tel: 716-845-8459 (office)
Fax: 716-845-1595

For additional information regarding the handling of samples please contact RPCI's Immune Analysis Facility Shared Resource at 716-845-1574 or 716-845-6555.

NOTE: In the event that a network site cannot process PBMC samples, whole blood may be shipped at ambient temperature overnight to the RPCI Immune Analysis Facility (IAF). Samples will be maintained at room temperature and processed within 24 hours.

8.3.1 Pathology

Survivin Expression Status

Over 85% of glioblastomas have survivin protein expression that is detectable by immunohistochemistry. A determination of survivin positivity by central pathology review is required for eligibility. All patients will have unstained slides or tissue blocks submitted to the central pathology core (Roswell Park Cancer Institute) for survivin testing.

A. Patients from Roswell Park Cancer Institute:

1. Unstained Slides:
   - Send all slides within 6 weeks of cutting
   - At least 24 unstained slides or one tissue block
   - Precut slides from paraffin blocks in 5 micron sections
   - Mount on Poly-L-Lysine-coated or plus (+) slides
   - Air dry. Do not oven dry

- Store specimen at room temperature

B. <u>Patients from Network Sites:</u>

1. <u>Unstained Slides:</u>

- Send all slides within 6 weeks of cutting
- At least 24 unstained slides or one tissue block
- Precut slides from paraffin blocks in 5 micron sections
- Mount on Poly-L-Lysine-coated or plus (+) slides
- Air dry. Do not oven dry
- Store specimen at room temperature

While unstained slides are preferred, tissue blocks are acceptable. If tissue blocks are being sent instead of unstained slides, please follow the procedure below for processing and shipping.

<u>Fixed Paraffin Block with Corresponding H&E:</u>

- Tissue should be well-fixed in formalin. If alternative fixative is used, it should be noted on the requisition.
- 0.2 cm x 0.2 cm x 0.2 cm tissue
- Store specimen at room temperature.
- Use cold pack for transport. Be sure cold pack is not in direct contact with specimen during transport.

For Network Sites: Send samples, along with the sponsor-provided shipping log, using study-specific subject ID number and tissue accession number to RPCI Pathology Network Shared Resource (Attn: Protocol Lab Team). The shipping label should read as follows:

Roswell Park Cancer Institute
Correlative Sciences Pathology Office, S-636
Attn: Protocol Lab Team – I 259614 Samples
Elm & Carlton Streets
Buffalo, NY 14263
(716) 845-8917
Email: CRSLabPathTeam@RoswellPark.org

Samples will be analyzed in RPCI's Pathology Network Shared Resource by Dr. Jingxin Qiu (Tel: 716-845-3457, Fax: 716-845-3427).

Tissue will also be tested for Survivan, MGMT methylation status, IDH-1, and PDL-1 expression. MGMT and IDH-1 tests require 6 unstained slides for each test. Survivan and PD-L1 immunohistochemistry stains on tumor tissue each require 3 unstained slides. For evaluable patients only, OmniSeq Comprehensive and OmniSeq Immune Advance will also be tested. 3 unstained slides are required for each of these.

Unstained slides will be sent for the following tests:

Test	Slides required
Survivin	3 unstained slides
PDL-1	3 unstained slides
Omniseq Comprehensive	3 unstained slides
Omniseq Immune Advance	3 unstained slides
IDH-1	6 unstained slides
MGMT methylation	6 unstained slides

9 EFFICACY EVALUATIONS

9.1 Objective Tumor Response

All protocol-defined imaging studies must be performed at the investigative site or sponsor-approved facility using protocol-defined parameters. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. The Modified Response Assessment in Neuro-Oncology (RANO) Response Criteria (**Appendix C)** will be used to define tumor response and progression [40].

It is important to use the same method of assessment from one scan to the subsequent scans.

Rarely, patients undergoing vaccine therapy for tumor can develop an inflammatory response in the tumor bed that mimics either true tumor recurrence or pseudoprogression. If a patient who has completed the 4-dose vaccine priming phase of treatment undergoes either biopsy or re-resection of new contrast-enhancing tissue and no tumor is determined by the local neuropathologist to be present on histologic exam, the patient may continue on treatment under

protocol with approval of the lead principal investigator. If any amount of tumor is found to be present at re-biopsy, the patient must be declared to have progressed as of the date of the MRI scan that first showed the new enhancement. Patients with new enhancement during the priming phase that is not suspected to be pseudoprogression are not eligible to continue treatment on protocol.

9.2 Evaluation of Response

The largest cross-sectional area on the T1-weighted contrast-enhanced images will be selected and measured in 2 dimensions with linear measures on the baseline MRI axial sequence. In addition, the largest cross-sectional area of a contiguous hyperintense lesion on FLAIR sequences will be measured on the baseline MRI axial sequence. If there is no enhancement on the baseline MRI done with 72 hours post-op, this should be noted as no evidence of disease (NED). All subsequent scans will be compared against these baseline measures (for both CE and FLAIR). New foci of FLAIR signal abnormality will be recorded on each subsequent evaluation. Response will be scored based on a combination of imaging and clinical features as defined by the modified RANO criteria [(40)]. Objective response rate is defined as the sum of partial responses plus complete responses.

For the purpose of treatment decisions, progression will be assessed by the treating investigator. 20% to 30% of patients who receive chemoradiotherapy for newly diagnosed glioblastoma can exhibit transient increase in tumor enhancement that eventually subsides without change in therapy (pseudoprogression) [(40)]. The protocol allows for investigators to confirm progression before removal of patients from study treatment, thus ensuring radiographic follow-up to a valid disease progression time point. In every case when there is doubt about progression versus "pseudoprogression", use of perfusion and diffusion weighted imaging is encouraged. Patients may continue on treatment and remain under close observation and evaluation at four-week intervals if there is uncertainty regarding whether apparent radiographic progression may represent "pseudoprogression" and the patient is doing well clinically or is clinically stable. If subsequent evaluations indicate that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised.

9.2.1 Survival

Duration of survival will be defined as the time from date of diagnosis to death due to any cause.

In all cases, progression-free survival will be assessed using the RANO response criteria for GBM which considers radiologic imaging, neurological status, and steroid dosing. According to these criteria, progression of disease will be assumed if any of the following criteria are met:

- $\geq$ 25% increase in the sum of the products of perpendicular diameters of enhancing lesions compared with baseline (post-chemoradiation) or best response after initiation of therapy.

NOTE: Due to the fact that little to no residual disease will be present at baseline in these patients, a 25% increase in area could easily reflect only imaging variance. Therefore, in order to be considered progressive disease, a > 5 mm increase in maximal diameter, as well as $\geq$ 25%

increase in the sum of the products of the progressing lesions will be required to assign a status of progressive disease.

- Significant increase in T2/FLAIR non-enhancing lesion compared with baseline (post-chemoradiation) or best response after initiation of therapy, not caused by comorbid events (e.g., radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).
- Clear progression of non-measurable disease.
- Any new lesion.

NOTE: Patients may continue on treatment and remain under close observation and evaluation at four-week intervals if there is uncertainty regarding whether apparent radiographic progression may represent "pseudoprogression". If subsequent evaluations suggest that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised. Similarly, stable disease may be assigned in cases of presumed "pseudoprogression" associated with decreased steroid use.

- Clear clinical deterioration not attributable to other causes apart from the tumor (e.g., seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose
- Failure to return for evaluation as a result of death or deteriorating condition

NOTE: Any cystic or surgical cavity should not be measured in determining response. At baseline, tumor around the surgical cavity should generally be considered nonmeasurable unless there is a nodular component measuring 10 mm in diameter.

10 SAFETY EVALUATION

10.1 Adverse Events

10.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

10.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

10.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

10.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

The following clinical laboratory tests will be performed during this study to assess safety (see Schedule of Procedures and Observations: **Table 3,** for specific tests):

- Hematology
- Serum Chemistries
- Urinalysis

The following evaluations will also be performed during the study to measure the safety and tolerability of study treatment:

- Vital sign measurements
- Physical examination
- Neurological examination
- Karnofsky Performance Status

10.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

10.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

10.1.2.1 Severity assessment of Injection Site Reaction

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Injection Site Reaction	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Acneiform or Papulopustular Rash	Papules and/or pustules covering <10% BSA, which may or may not be associated with symptoms of pruritus or tenderness	Papules and/or pustules covering 10-30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; associated with psychosocial impact; limiting instrumental ADL	Papules and/or pustules covering >30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; limiting self care ADL; associated with local superinfection with oral antibiotics indicated	Papules and/or pustules covering any % BSA, which may or may not be associated with symptoms of pruritus or tenderness and are associated with extensive superinfection with IV antibiotics indicated; life-threatening consequences	Death
Maculo-papular rash	Macules/papules covering <10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	Macules/papules covering 10-30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL	Macules/papules covering >30% BSA with or without associated symptoms; limiting self care ADL		
Pustular rash		Localized; local intervention indicated (e.g., topical antibiotic, antifungal, or antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or operative		
Pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL		
Induration	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin; limiting instrumental ADL	Severe induration, unable to slide or pinch skin; limiting joint movement or orifice (e.g., mouth, anus); limiting self care ADL	Generalized; associated with signs or symptoms of impaired breathing or feeding	Death

BSA = Body Surface Area

ADL = Activities of daily living.

10.1.3 Reporting Adverse Events

Table 4 Guidelines for Routine Adverse Event Reporting for Phase 2 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

10.2 Serious Adverse Events

10.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participant, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

10.2.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 10.4 for details on reporting Unanticipated Problems.

10.3 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.4 Unanticipated Problems

10.4.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:

a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.

b) The characteristics of the participant population being studied.

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 10.2**.

10.4.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Unanticipated Problem Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

10.5 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening;

Or, meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).

- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human participants including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org. **Network sites**: refer to **Appendix A**.

11 DATA AND SAFETY MONITORING

The RPCI Data and Safety Monitoring Board will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMB will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

12 STATISTICAL METHODOLOGY

12.1 Study Design

This is a multi-center, open-label; prospective one-arm phase II clinical trial evaluating the safety and efficacy of SurVaxM and adjuvant temozolomide in adult patients with newly diagnosed GBM. The statistical design is based on an assessment of the efficacy of the study treatment for patients as compared to an external standard, i.e., historical control. All enrolled patients will receive the study treatment and initiation of the treatment should take place as soon as possible following study enrollment. A total of up to 65 patients will be enrolled to meet our target of at least 50 evaluable patients. An evaluable subject is defined as a subject who meets eligibility requirements and who completes the initial four priming doses of the vaccine. If a patient signs an Informed Consent and does not receive all four priming injections or, the patient withdraws their consent before receiving the priming injections, the patient will be classified as unevaluable and will be replaced. Analyses will be performed with a focus on estimation of specific clinically important parameters for use in the planning of larger subsequent comparative trials designed to fully assess efficacy and safety.

All analyses will be performed using the SAS® statistical software system.

12.1.1 Missing Data

The amount and nature of missing data will be characterized and no method of imputation will be used for missing data. A summary of missing data will be provided according to the number of subjects, the time points where the data are missing, and clinical center. For each clinical center, the number and percent of subjects with no missing data will be presented in tabular form.

All analyses will be performed using the SAS® statistical software system.

12.2 Study Objectives

The primary objective of this study is:

- To evaluate 6 month progression-free survival (PFS) rates in patients with survivin positive newly diagnosed GBM treated with SurVaxM and adjuvant temozolomide.

The secondary objectives of this study are:

- To determine the safety and tolerability of SurVaxM in patients receiving standard of care temozolomide.
- To evaluate overall survival (OS) in patients with survivin positive newly diagnosed GBM treated with SurVaxM and adjuvant temozolomide.
- To describe the immune response in patients treated with SurVaxM and predictors of response.
- To evaluate objective tumor response rate (applicable only for patients with evaluable disease at study entry, as defined per RANO criteria) and predictors of response.

12.3 Study Endpoints

Primary Endpoint:

- PFS is defined as the time from date of diagnosis to the date of first observed disease progression or death due to any cause. All patients will be followed for a minimum of 6 months relative to evaluating the primary endpoint.

Secondary Endpoints:

- OS is defined as the time from date of diagnosis until date of death or the last date patient known alive (if death is not observed).
- Number of Grade 3 or 4 toxicities, according to National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAEs) Version 4
- Immune responses to SurVaxM and predictors of response

12.4 Sample Size Determination

The primary endpoint will be the 6 month PFS survival fraction. Let π represent the 6 month PFS survival fraction. A true rate of the 6 month PFS survival fraction greater than π_0=0.55 is considered acceptable and evidence such that we will the treatment worthy of further study. The null and alternative hypotheses to be tested are H_o:π= π_0 versus H_a:π>π_0. We hypothesize that patients treated with SurVaxM and adjuvant temozolomide will have a 6 month PFS survival fraction of 0.70 or larger. The sample size calculation is based on testing the hypotheses concerning the proportion of the population with a response to the treatment: H_o:π= π_0 versus H_a:π>π_0. For our calculations π_0=0.55,α =0.10, 1-β=0.80. Based on an exact one-sided test the sample size calculation yields n=50 patients.

Subjects will be accrued to the study on a first-come basis. The projected accrual is approximately 2 subjects per month, and therefore recruitment is expected to be complete at 25 months following the study starting point. Subjects will be followed to determine time to tumor progression (PFS) and until death to determine overall survival (OS).

12.5 Demographics and Baseline Characteristics: Descriptive Analyses

Measured outcome variables will be summarized overall and by relevant demographic and baseline variables. Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as the mean, standard deviation and range. A variety of graphical techniques will also be used to display data, ex. histograms, boxplots, scatterplots, etc.

12.6 Safety Analysis

Safety and tolerability will be assessed by incidence, severity, and changes from baseline of all relevant parameters including adverse events (AEs) (see **Section 12.8**), laboratory values and vital signs.

Vital sign results (systolic and diastolic blood pressure, pulse, respiration, and temperature) will be summarized descriptively for each scheduled and unscheduled protocol time point. Changes will be calculated relative to the assessments at baseline.

The changes in hematology, chemistry, and other laboratory values will be summarized descriptively for each scheduled and unscheduled protocol assessment time point. Changes will be calculated relative to the values collected at baseline. Data listings of all laboratory data collected during the study will be presented. Laboratory values outside normal limits will be identified in data listings and will include flags for high and low values.

12.7 Efficacy Analysis

12.7.1 Primary Analysis

The primary objective is to evaluate the 6 month PFS survival fraction in patients with newly diagnosed GBM treated with at least 4 priming doses of SurVaxM and standard-of-care adjuvant

temozolomide as compared to historical control. PFS is defined as the time from date of diagnosis to the date of first observed disease progression or death due to any cause. Patients who are alive and disease free at the last study assessment will be treated as censored.

Historical Control: Patients in this population defined by the eligibility criteria receive standard-of-care which consists of concurrent temozolomide and radiation followed by adjuvant temozolomide. Based on provided historical information, the 6 month PFS survival fraction is assumed to be 0.55. We hypothesize that treatment with SurVaxM and adjuvant temozolomide will increase the 6 month PFS survival fraction to 0.70. Therefore the PFS survival fraction will be measured after all patients have been followed for 6 months.

The 6 month PFS survival fraction is estimated as standard binomial proportion. An exact one-sided binomial test will be used to test our primary hypothisis. The nominal significance level to be used for this test is 0.10. In addition, a Kaplan-Meier curve for PFS will be used calculated to generate summary descriptive statistics, e.g. median PFS.

Secondary Analyses

The estimated distributions of overall survival (OS), defined as the time from date of diagnosis to death due to any cause, will be obtained using the Kaplan-Meier method. Estimates of quantities such as median survival will be obtained. Corresponding confidence intervals will be computed. It is assumed *a priori* that any drop out times will be non-informative in terms of the censoring mechanism. Groups defined by levels of categorical or dichotomized numeric demographic/baseline variables will be compared in regards to time-to-event distributions using the log-rank test. Cox proportional hazards model regression will be utilized for multivariate analyses.

A summary of anticancer therapies received following the discontinuation of protocol therapy will be provided. Such therapies will be classified as chemotherapy, biological therapy, radiation, surgery, or other.

Both objective tumor response rate (applicable only for patients with evaluable disease at study entry, as defined per RANO criteria) and toxicity rate (Grade 3 or Grade 4 toxicities, according to National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAEs) Version 4) will be estimated using simple relative frequencies. The corresponding 95% confidence intervals for the estimated probabilities will be computed using the method proposed in Clopper and Pearson, 1934 [(43)]. The relationship between binary outcomes and collected demographic and baseline variables will be statistically assessed using logistic regression in an exploratory fashion. Maximum likelihood estimation will be utilized in the model fitting procedures as implemented by SAS PROC LOGISTIC. Wald tests of the model effects will be performed to assess statistical significance; see SAS documentation for details. See Hosmer and Lemeshow, 2000 [(44)] for a discussion of such techniques. Alternative parametric models will be considered if model fit is found to be inadequate.

Objectives based on continuous outcomes such as immune response measured over time will be address in a similar fashion. A series of exploratory analyses will initially take place including individual subject-level profile plots and overall mean plots used to examining the mean structure. Formal statistical examination of longitudinal patterns will be done through the use of

a mixed model. Restricted maximum likelihood estimation will be utilized in the model fitting procedures as implemented by SAS PROC MIXED (version 9.2). Once the model is fit, specific linear contrasts based on the estimated model parameters will be constructed and used to test hypotheses concerning between time point comparisons. We acknowledge the possibility of informative missingness, that is, the probability of a particular observation being missing may be related to the health of the subject, and therefore analyses will be interpreted with caution.

All tests will be two-sided and tested at a 0.05 nominal significance level. Standard diagnostic plots will be used to assess model fit and transformations of variables may be considered in order to meet statistical assumptions.

12.8 Adverse Event

AEs will be coded using the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized for each treatment arm by the number and percentage of patients who experienced the event, according to system organ class and preferred term. Additional summaries will also be provided by severity grade and relationship to study drug, and for SAEs and events resulting in the permanent discontinuation of therapy. A patient reporting multiple cases of the same AE will be counted once within each system organ class and similarly counted once within each preferred term, and adverse events will be graded by worst severity grade. Unless specified otherwise, the denominator for these calculations will be based on the number of patients in each treatment arm who receive at least one dose of study drug, irrespective of the total number of doses administered. All participants who receive any study treatment will be considered evaluable for toxicity.

12.9 Replacement

Patients who meet either of the following conditions will be considered unevaluable for the primary end-point and will be replaced:

- Failure to receive all 4 priming doses of SurVaxM (making a patient unevaluable for the purposes of the primary end-point), or,
- Withdrawal of consent by patient.

Up to an additional 15 patients may be enrolled to replace unevaluable patients or patients who withdraw consent prior to receiving study treatment.

12.10 Interim Analysis and Criteria for Early Termination of the Study

There are no planned interim analyses (unless for safety reasons) or early termination criteria based on the following:

1. The completed phase I trial of SVN53-67/M57-KLH showed very little toxicity with no grade III or IV adverse events and no serious adverse events attributable to the study drug.
2. There is no scientific rationale to posit additive or synergistic toxicity between the two drugs, given that one is an alkylating agent and the other is a vaccine.

3. In three separate trials (ACTIVATE[38], ACT II[39] and ACT III[37]), rindopepimut (an immunotherapy for the treatment of EGFRvIII-expressing GBM) given in combination with temozolomide showed no serious toxicity with only minor injection site reactions, similar to SVN53-67/M57-KLH.
4. In addition to evaluation of immune responses and PFS, the current phase II design includes rigorous evaluation of toxicity of each drug with CTCAEv4.0 (see **Sections 6.2.1** and **6.2.2**).

13 CORRELATIVE DATA ANALYSIS

13.1 Immunological Assessments and HLA Typing

Immunologic response to vaccine will be used as a secondary measure of vaccine activity. Survivin-specific CD8+ responses, CD4+ helper support, and anti-survivin antibody (humoral) responses will be measured individually by the Immunotherapy Core Facility and Neuro-oncology Laboratory at Roswell Park Cancer Institute. PBMC and serum will be collected according to the schedule in **Table 3**. Samples from Network sites will be sent to the Immunotherapy Core Facility at Roswell Park Cancer Institute for processing and analysis. To assess immunologic responses to SVN53-67/M57-KLH (SurVaxM), PBMC will be analyzed using multimer, IFNγ ELISPOT and qPCR assays of cytokine expression in CD4+ and CD8+ cells. Antibodies to survivin peptides and KLH in serum will also be measured by ELISA to assess humoral immune responses. Each of these assay methods has unique advantages and currently, there is no uniform consensus as to which of them is the most critical indicator of an immunologic response to peptide vaccines. Flow cytometric analysis with multimers provides a relatively accurate estimation of the frequency of specific antigen-binding T cells, without requiring major in vitro manipulation of the patient's leukocytes. Multimer analysis can also be accomplished using a small blood sample. Therefore, multimer analysis will be used as the primary determinant of immunological response/endpoint. Positive reactivity is defined as survivin-specific CD8+ T cell levels 1% greater than registration baseline.

ELISPOT assays and qPCR analysis of cytokine expression indicate the functional status of the antigen-specific T cells and are important for assessing Type-1 T cell responses, but they require isolation of CD4+ and CD8+ populations by flow cytometry, and hence a larger blood sample than multimer assays. Therefore, IFNγ ELISPOT, qPCR and antibody assays will be used as secondary determinants of the immunological response/endpoint.

Immunological analyses will be conducted on blood samples obtained prior to vaccination and at all study follow-up visits. On each occasion, approximately 50 mL of blood will be obtained from a peripheral venipuncture and collected in six green top (heparinized) tubes and one 3.5 mL gold top tube. Blood tubes will be stored at room temperature until pickup for processing.

Simple HLA-A class I typing on blood samples will be performed at each study site as a part of screening to determine eligibility. Following entry, detailed HLA typing (MHC class I and MHC class II) will be performed in an exploratory fashion as part of the analysis of immunologic response to vaccination.

13.2 Survivin Expression Status

Over 85% of glioblastomas have survivin protein expression that is detectable by immunohistochemistry. All patients will have unstained slides or tissue blocks submitted to the central pathology core (Roswell Park Cancer Institute) for survivin testing to determine eligibility.

14 ETHICAL AND REGULATORY STANDARDS

14.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each participant (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the participant is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the participant log and participant records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining participant authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the participant is treated, in accordance with the Declaration of Helsinki, Good Clinical Practice, and according to the guidelines in this protocol, including attached appendices.

14.2 Informed Consent

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each participant or the participant's legally authorized representative in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator (or designee) shall provide a copy of the signed consent form to the participant and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options.

15 STUDY RESPONSIBILITIES

15.1 Data Collection

Data entry into the database is to be completed in a timely fashion (within 30 days) after the participant's clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Source Form, which is handled in an expedited fashion.

Data management activities will be performed using eClinical. eClinical is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the eClinical Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs (via the EXPeRT Module). eClinical is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

15.2 Maintenance of Study Documents

Essential documents will be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

16 ADMINISTRATIVE RULES

16.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

16.2 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

16.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

17 APPENDICES

Appendix A Instructions for Network Sites

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:

Roswell Park Cancer Institute
CRS Network Office
ASB K 104
Buffalo, New York 14263
Telephone:
Monday - Friday; 7: 00 AM to 4: 00 PM EST
716-845-3870
After hours, weekends, and holidays request the RPCI Investigator
716-845-2300
Fax: 716-845-8743

2. INFORMED CONSENT

- Informed consent must be obtained by the **site Investigator/designee** from any participants wishing to participate, **prior to any procedures or treatment**.
- An informed consent template is provided by RPCI and can be amended to reflect institutional requirements.
- All consent changes **must** be reviewed by RPCI Network Office prior to submission to the site IRB.
- The informed consent must be IRB approved.
- Always check that the most up to date version of the IRB approved consent is being used.
- Within 5 business days, notify the RPCI Network Office of all participant withdrawals or consent to limited study participation and appropriately document the discontinuation and the reason(s) why.

3. PARTICIPANT REGISTRATION

The participant completes the Gender, Race, and Ethnicity Form and this is placed in the study binder.

RPCI does not grant exceptions to eligibility criteria.

Phase 2 Protocol Registration Instructions

The Subject Screening and Enrollment Log must be faxed or emailed to the RPCI Network Office within 1 business day of the date the participant is consented. Once the Investigator has determined that eligibility has been met, complete the eligibility check list and fax or email it to the RPCI Network Monitor at 716-845-8743.

4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this must be reported to the RPCI Network, site IRB and any other regulatory authority involved in the study.
- All study deviation will be recorded on the **Study Deviation Log**.
- Participants inadvertently enrolled with significant deviation(s) from the study-specified criteria will be removed from the study, at the discretion of the Principle Investigator.
- Notify the RPCI Network Office of any early participant withdrawal and appropriately document the discontinuation and the reason why, within 5 business days.

5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The RPCI Network Monitor must be able to read what has been deleted.
- Do **NOT** use white-out, magic marker, scratch-outs.
- Do **NOT** erase entries.
- Use only black ink for documentation on the accountability form and any other study forms.
- It is the responsibility of RPCI to inform the Investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to RPCI upon written agreement between the Investigator and RPCI.

6. DRUG ACCOUNTABILITY

Drug accountability must be strictly maintained.

- Responsibility rests solely with the Investigator but can be delegated as appropriate (e.g., to pharmacy personnel).
- A drug accountability record form (DARF) will record quantities of study drug received, dispensed to participants and wasted, lot number, date dispensed, participant ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.
- Study drug supply will only be used in accordance with the IRB approved study.
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.
- An inventory count must be performed with each transaction. Any discrepancies shall be documented and explained.
- Drug accountability forms must be stored with study related documents.

- Each medication provided for this study and each dosage form and strength must have its own DARF.
- Dispensing the wrong study supply is considered a **medication error**.
- **NEVER** replace investigational agents with commercial product.
- Do **NOT** "transfer", "borrow" or "replace" supplies between studies.

7. SERIOUS ADVERSE EVENT REPORTING

The site Investigator or designated research personnel will report all SAEs, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**. The site will notify the RPCI Network Monitor within 1 business day of being made aware of the SAE. A preliminary written report must follow within 1 business day of the first notification using the following forms:

- RPCI SAE Source form
- MedWatch 3500A

A complete follow-up report must be sent to the RPCI Network Monitor when new information becomes available.

8. UNANTICIPATED PROBLEM REPORTING

An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the criteria in **Section 10.4**.

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention, the participating physician or delegated research staff from each site will notify their local **IRB in accordance with their local institutional guidelines**. The site must also notify the RPCI Network Monitor within 1 business day of being made aware of the Unanticipated Problem by completing the **RPCI Unanticipated Problem Report Form** and faxing or emailing it to the RPCI Network Monitor.

Appendix B Karnofsky and ECOG Performance Status Scales

Status	Karnofsky	Grade	ECOG
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction.
Able to carry on normal activities. Minor signs or symptoms of disease	90	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
Normal activity with effort	80		
Care for self. Unable to carry on normal activity or to do active work	70	2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
Requires occasional assistance, but able to care for most of his needs	60		
Requires considerable assistance and frequent medical care	50	3	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
Disabled. Requires special care and assistance	40		
Severely disabled. Hospitalization indicated though death non-imminent	30	4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
Very sick. Hospitalization necessary. Active supportive treatment necessary	20		
Moribund	10		
Dead	0		

Appendix C Response Assessment in Neuro-Oncology (RANO)

Response	T1 Contrast Enhancement (CE)	FLAIR Images	Steroids	Neurologic Exam
Complete Response (CR)	No residual CE (complete disappearance of all enhancing measurable disease for at least 4 weeks; confirmatory MRI at 4 weeks is required to score as CR) and no new lesions.	Stable or reduced area of FLAIR signal abnormality	No steroids	Stable or improved from prior evaluation
Partial Response (PR)	>50% reduction in sum of products of the perpendicular diameters of all measureable enhancing lesions sustained for at least 4 weeks and no new lesions or progression of non-measurable lesions.	Stable or reduced area of FLAIR signal abnormality	Stable or reduced glucocorticoids from baseline MRI	Stable or improved from prior evaluation
Minor response (MR)	>25% reduction in sum of products of the perpendicular diameters of all measureable enhancing lesions and no new lesions (confirmatory MRI at 4 weeks is required to score as PR).	Stable or reduced area of FLAIR signal abnormality	Stable or reduced glucocorticoids from baseline MRI	Stable or improved from prior evaluation
Stable Disease (SD)	<25% reduction in area of CE maintained for at least 4 weeks duration. Does not qualify for CR, PR or progression.	Stable or reduced area of FLAIR signal abnormality	Stable or reduced glucocorticoids from baseline MRI	Stable or improved from prior evaluation
Progressive Disease	>25% in the sum of products of the perpendicular diameters of CE lesions; evidence of new lesion(s).	Measurable increase in the sum of products of the perpendicular diameters of FLAIR signal abnormality from the baseline scan or the scan representing the best response (if there was a response) following therapy and not attributable to other co-morbid events (seizure, radiation, injury, infection, ischemia, etc.) OR presence of a new focus of FLAIR signal abnormality that cannot be explained by any other pathologic process.	Stable or increased dose of glucocorticoids	Stable or worsening neurologic symptoms

Appendix D Preparation of Peptide Vaccine Emulsion

1. Required Materials

- 1 vial containing MONTANIDE ISA 51 VG sterile
- 1 vial containing SVN53-67/M57-KLH sterile
- 3 x 2ml NORM-JECT (latex/silicone-free) Luer lock syringes (Air-Tite Products 4020.00V0)
- 1 double sided female I-connector (Smiths Medical MX494 or equivalent)

2. Peptide Reconstitution (Aqueous phase)

Each vial of SVN53-67/M57-KLH contains 1.0 milligrams of the lyophilized peptide conjugate test agent. Immediately prior to use for vaccine production, lyophilized SVN53-67/M57-KLH is reconstituted by injecting 1.0 ml sterile bacteriostatic saline into the vial. Next, the vial is allowed to stand at room temperature for 5 minutes (step 1), followed by vortexing for 20 seconds (step 2). Steps 1 and 2 may be repeated up to twice if necessary. The reconstituted peptide solution is to be used within 2 hours for emulsion preparation.

3. Vaccine Preparation

Steps required to prepare 1.6 mL of vaccine emulsion in a 50/50 ratio (volume/volume) with Montanide ISA 51 VG:

Step 1: Loading Products

1.1. Using a sterile 2 ml NORM-JECT® Luer lock syringe equipped with a 20g sterile needle, withdraw 0.8 ml of saline-reconstituted SVN53-67/M57-KLH from the vial.

1.2. Discard the needle and remove the stopper from a female two-sided I-connector and connect the syringe to one end of the I-connector.

1.3. Remove all air from the system using light pressure on the syringe.

1.4. Place connected syringe and I-connector in sterile area.

1.5. Using another sterile 2 ml NORM-JECT® Luer lock syringe equipped with a 20g needle, withdraw 0.8 ml of Montanide ISA 51 VG from a single dose vial.

1.6. Discard the needle and remove air with light pressure on the syringe

1.7. Remove the stopper from the other end of the two-sided female I-connector and connect the syringe containing the Montanide to that end. Make sure that both connections are secure and leak-free.

1.8. An emulsion is created using a double-syringe technique via the interposed I-connector.

Step 2: Emulsification

2.1. The emulsification process is accomplished in 2 stages, including: A pre-emulsification stage, performed at low speed; and an emulsification stage, performed at high speed.

2.2. Hold the syringe-connector-syringe system firmly to maintain a secure connection. Each thumb will be used alternately to push the opposing plungers apart. To avoid leakage, never push with both thumbs simultaneously. Push on the plunger of one of the syringes in order to begin the mixing of both phases within a single syringe.

2.3. Begin emulsification by transferring the entire volume from one syringe to the other slowly and repetitively. One cycle corresponds to passage of the entire contents of one syringe to the other syringe and back again. Perform the first 20 cycles at a slow rate (i.e. about 2 seconds to transfer the pre-mix from one side to the other or about 4 seconds for each complete cycle). This "pre-emulsion" stage will take about 80-90 seconds

2.4. At the end of the first stage (20 cycles), the speed of mixing is increased. The following 40 cycles are made at high speed, or as fast as possible. When the emulsion starts to form, resistance can be felt when applying pressure to the syringe plunger. At this point, the mixture will take on a creamy appearance and will become viscous. A timer set to 40 seconds can be used to avoid losing count. The total process (pre-emulsion and emulsion) will take 60 cycles. The total process should take about 2.5 minutes.

Step 3: Testing of Emulsion

3.1 Place one droplet of the peptide emulsion into a beaker of distilled water. The droplet should retain its shape on the surface of the water. If it does not, vaccine preparation should be repeated from the beginning.

Step 4: Packaging

4.1. Transfer 1.0 ml of the emulsion into one of the two NORM-JECT syringes.

4.2. Disconnect this syringe from the mixing assembly and connect it to a sterile 23g needle.

4.3. Follow the procedure for patient vaccination using this syringe and needle.

*The Seppic emulsification insert is provided below for cross-reference.

Emulsifying Protocol
MONTANIDE ISA 51 VG and an I-CONNECTOR

1 - OBJECTIVE

The objective of this process is to manufacture homogenous Water in Oil emulsion using Montanide ISA 51 VG and an I-connector.

2 - MATERIAL

To facilitate this protocol, some dedicated materials and equipments will be needed:

- 1 vial containing MONTANIDE™ ISA 51 VG sterile
- 1 vial containing the aqueous phase
- 2 vial adapters
- 2 luer lock syringes
- 1 I-connector

Material references:

- ***Syringes:***

Latex + Silicon Oil Free and Rubber Tip Free Plunger

-OR 2 ml Injekt- (Ref: 46060701V) from B-Braun (Germany)
-OR 5 ml Injekt-F (Ref: 4606710VV) from B-Braun (Germany)
-OR 2 ml Norm-Ject (Ref: 4020.000V0) from Henke Sass Wolf GMBH (Germany)
-OR 5 ml Norm-Ject (Ref: 4050.000V0) from Henke Sass Wolf GMBH (Germany)

B-Braun sells its own brand in the US.
Henke Sass Wolf GmbH is distributed by Air Tite Products, Inc.:
http://www.air-tite-shop.com/p-15-norm-ject-luer-lock-syringe.aspx

The size of the syringe has to be adapted according to the volume of emulsion to be manufactured. In this protocol we manufacture emulsion with a total quantity of 2 ml.

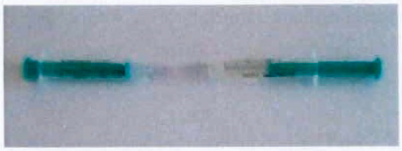
- ***Vial adapters:***

-13 mm or 20 mm vial adapter from West pharmaceutical devices:
http://www.westpharma.com/en/products/Pages/vialadapters.aspx

SEPPIC

Emulsifying Protocol
MONTANIDE ISA 51 VG and an I-CONNECTOR

8. Push the plunger very slowly in order to drain maximum of air from the system (this step can be also done with syringe N°1 instead of syringe N°2)
9. Remove syringe N°1 from the adapter and twist the syringe N°1 to the connector
10. The system is now ready for emulsification



Step 2: Emulsifying

The emulsification process will be set in 2 steps:
- A pre-emulsification at very low speed
- An emulsification part at high speed

Hold the syringe/connector/syringe system firmly to guarantee a constant connection. Thumbs will be used to push the plungers apart.
Do not push with both of the thumbs simultaneously to avoid any leak.

1. Push completely on the plunger of one of the syringe in order to get both phases in one syringe.
2. Start to emulsify by transferring alternatively the formulation from one syringe to the other very slowly.

One cycle corresponds to the passage of the entire formulation from one container to the other through the connector, and back.

The first 20 cycles are done at slow rhythm. It needs around 2 seconds to transfer the premix from one side to the other.
Then, a complete cycle requires an average of 4 seconds.
This first part will give a "pre-emulsion", and this full process should take 1 minute and half.

3. At the end of this first stage, the speed is dramatically increased. The 40 following cycles are made at high speed, as fast as possible. When the emulsion starts to form, a resistance can be felt when applying pressure to the syringe plunger. The mixture is getting a creamy viscous appearance at this time. A timer set on 40 seconds could be used to avoid loosing count.

The total process will need 60 cycles.

Total emulsification process should be around 2 minutes and half.

Emulsifying Protocol
MONTANIDE ISA 51 VG and an I-CONNECTOR

Nota
The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.

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Appendix E Isolation of Peripheral Blood Mononuclear Cells for Immuno-Monitoring

1. PURPOSE:
   1.1. To describe the general procedure for isolating and freezing PBMC/BM-MNC and serum from whole blood.
2. PRINCIPLE:

N/A

3. RESPONSIBILITY:
   3.1. Persons who process whole blood for the purpose of isolating PBMC/BM-MNC and or serum.
   3.2. The laboratory supervisor will oversee the process and ensure that this procedure is followed and properly documented.
4. SCOPE:
   4.1. This protocol is currently used when processing whole blood.
5. DEFINITIONS:
   5.1. DMSO: Dymethyl Sulphoxide
   5.2. FBS: Fetal Bovine Serum
   5.3. HBSS: Hank's Balanced Salt Solution
   5.4. LSM: Lymphocyte Separation Medium
   5.5. PBMC: Peripheral Blood Mononuclear Cells
   5.6. PBS: Phosphate Buffered Saline
   5.7. RBC: Red Blood Cells
   5.8. RPM: Revolutions Per Minute
   5.9. RT: Room Temperature
6. ATTACHMENTS:
   6.1. PBMC/serum Worksheet
   6.2. Freeze down medium Worksheet
7. REFERENCED DOCUMENTS:

N/A

8. MATERIALS/EQUIPMENT:
   8.1. Materials
      8.1.1. 5mL, 10mL, 25mL serological pipettes (Corning or equivalent)
      8.1.2. Pipet-Aid (Drummond, Cat#:4-000-220 or equivalent)

8.1.3. 125mL sterile bottle (Corning, Cat#:8388)

8.1.4. 50mL centrifuge tubes (Crystalgen, Cat#:23-2262 or equivalent)

8.1.5. 15mL centrifuge tubes (Crystalgen, Cat#:23-2265 or equivalent)

8.1.6. 1.7mL microcentrifuge tubes (Crystalgen, 23-2052 or equivalent)

8.1.7. 2.0mL cryovials (Simport, Cat#:T311-2 or equivalent)

8.1.8. 1000µL, 200µL, 20µL pipetman (Rainin or equivalent)

8.1.9. 1000µL, 200µL, 20µL tips for pipetman (Rainin or equivalent)

8.1.10. Cell counting slide (Bio-Rad, Cat#:145-0011)

8.1.11. Biohazard waste bags and boxes

8.1.12. Sharps container for glass tube waste

8.1.13. Nalgen cryo freezing container (Cat#:7-5100-0001)

8.2. Reagents & Solutions

8.2.1. LSM (Mediatech, Cat#:25-072-CV or equivalent)

8.2.2. HBSS (Mediatech, Cat#:21-023-CV or equivalent)

8.2.3. RedZ (Safetec, Cat#:41103)

8.2.4. CTL wash solution (Cat#: CTLW010)

8.2.5. 50mL of CTL Wash is diluted with 500mL of RPMI-1640 medium. Store diluted CTL Wash at 4 °C.

8.2.6. CTL freeze down medium (Cat#: CTLC-ABC)

8.2.7. RPMI-1640 cell culture medium (Mediatech, Cat#:10-040-CV or equivalent)

8.2.8. Trypan blue (Mediatech, Cat#:25-900-CI or equivalent)

8.2.9. PBS (Mediatech, Cat#:21-031-CV or equivalent)

8.2.10. Isopropanol (Avantor, Cat#:9084-05 or equivalent)

8.3. Instrumentation

8.3.1. Centrifuge (Beckman Allegra 25R centrifuge with TS5.1 rotor or equivalent)

8.3.2. Mini Centrifuge (Eppendorf Centrifuge 5147C or equivalent)

8.3.3. Biosafety cabinet

8.3.4. Bio-Rad TC20 cell counter (Bio-Rad, Cat#:145-0102)

8.3.5. -80°C freezer (Sanyo, Model:MDF-U76VC or equivalent)

8.3.6. Liquid nitrogen freezer (Custom BioGenic Systems, Model:V1500-AB or equivalent)

9. SPECIMEN REQUIREMENTS:

9.1. After receiving the specimen processing should begin as soon as possible.

10. SAFETY PRECAUTIONS:

10.1. Consider all specimens for handling potentially positive for infectious agents including HIV, HBV and the hepatitis C virus (HCV). Observe universal precautions; wear protective gloves and lab coat [PPE] during all steps of this method because of both infectious and chemical contamination hazards. Place all plastic and glassware contaminated with tissue in a plastic autoclave bag for disposal. Biosafety Level 2 containment procedures as described in CDC/NIH publication #88-8395 are to be used by those handling human specimens.

10.2. See MSDS on each particular chemical in the lab MSDS Appendix.

10.3. Appropriate safety precautions should be utilized for volatile solvents like isopropanol, including fume hood, gloves, gown and laminar flow hood.

11. PROCEDURE:

11.1. Receiving Blood Product

11.1.1. Record sample date and time received information on PBMC worksheet, attach label from sample to lower right corner of worksheet. Record protocol number and sample id number on worksheet.

11.1.2. If personnel have received a blood sample outside of recommended tolerances (e.g. low volume, blood contained in different tubes, unusual surface temperature of tubes), personnel will notify the IAF director.

<u>All steps from this point must be performed in a biosafety cabinet and sterile techniques should be used when handling samples.</u>

11.2. Serum Isolation

11.2.1. Place serum tube (gold or red top) in allegra centrifuge with a balance and spin at 600 x G for 10 minutes.

11.2.2. After centrifugation use a p1000 pipettor to collect as many aliquots as possible of serum (the upper phase) into sterile 1.7 mL microfuge tubes.

11.2.3. Centrifuge the microfuge tubes using the Eppendorf tabletop centrifuge at 3000 RPM for 3 minutes.

11.2.4. Transfer the supernatant into new 1.7 mL tubes to distribute aliquots 0.5 mL per tube.

11.2.5. Create a label and apply to worksheet. Place serum tubes in appropriate storage boxes in -20 °C freezer. Record the time when the tubes were placed in the freezer on the worksheet.

11.2.6. Update the electronic serum log.

11.3. PBMC/BM-MNCs Isolation with Green Top Tubes

11.3.1. Add 18mL of LSM to each of (4) 50mL conical tubes.

11.3.2. Pool blood from all green top tubes into one 125mL bottle using 10mL size pipettes (note volume from each tube and record the total volume on the worksheet).

11.3.3. Make up the volume to 96mL with HBSS, swirl gently to mix, i.e.: the volume of blood obtained was 46mL; 96-46= 50mL, therefore 50 mL of HBSS should be added to the bottle.

11.3.4. Using a 25mL pipette, layer 24mL of the blood/HBSS diluent to each of the (4) 50mL tubes with LSM. This is done by tilting the tube and placing the tip of the pipette on the top of the LSM. Then slowly layer the sample on top to avoid mixing of the sample with the LSM (Figure 1 left).

11.3.5. Place tubes in Beckman Allegra centrifuge and spin at 400 x G for 30 minutes, BE SURE TO TURN OFF THE BRAKE on the centrifuge.

11.3.6. While the samples are spinning prepare new 50mL tubes for wash steps.

11.3.6.1. Place (4) 50mL centrifuge tubes in a rack and add 5mL of HBSS to each, set aside until ready to use after spin.

11.3.7. Obtain CTL/RPMI wash solution and the appropriate freeze down medium from the refrigerator.

11.3.7.1. If necessary, prepare freeze down medium that will be used.

11.3.7.1.1. Prepare CTL-ABC freeze down medium by thawing tube B and combine 1000µL of tube B into tube A, this is done by pipetting 100µL at a time into tube A and swirling to mix. Note following protocols require CTL-ABC freeze down media:

11.3.8. Record all reagent lot numbers, expiration dates and the date when the reagent was opened on the worksheet.

11.3.9. After centrifugation the sample is fractioned as shown in **Figure1** below. (PBMC can be seen as cloudy and white)

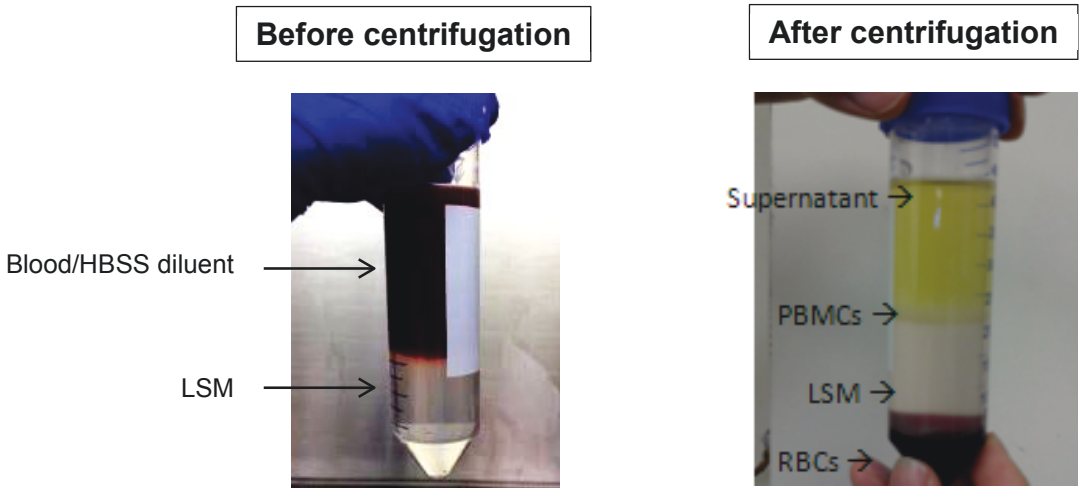


Figure 1. Before and after centrifugation

1.1.1. Using a 10mL pipette, harvest the lymphocyte layer (PBMC/BM-MNC) to ensure maximum recovery draw up as much of the lymphocyte layer as possible (Up to half of the LSM can be collected to ensure maximum recovery). Transfer layer to previously set up centrifuge tubes containing 5 mL of HBSS.

1.1.2. Bring up the volume of the lymphocyte/HBSS mixture to 30mL with HBSS. Gently invert the tubes 5-10 times to mix then centrifuge at 600 x G for 10 minutes to pellet the cells.

1.1.3. Discard supernatant in waste container and resuspend the pellet in 25mL of HBSS. Centrifuge the tubes at 500 x G for 5 minutes.

1.1.4. Discard supernatant and resuspend cells in 5mL of CTL/RPMI wash solution. Then pool the volumes from all 4 tubes into 1 tube for a final volume of 20mL.

1.2. Preparing PBMCs for counting and cryopreservation

1.2.1. Combine 20µL of the cell suspension with 20µL of 0.4% Trypan Blue, and perform a cell count using the Bio-Rad TC20 cell counter.

1.2.2. Centrifuge the pooled sample at 500 x G for 5 minutes.

1.2.3. Once the yield has been calculated, determine the number of tubes to freeze down based on the following criteria:

1.2.3.1. Freeze down around 10 million cells/vial, regardless of how low the yield.

1.2.3.2. Vial number ≤ 5: < 10 million cells/vial

1.2.3.3. Vial number > 5: > 10 million cells/vial

1.2.4. Prepare cryovials with labels using LIMS that include the sample id, the protocol number, the sample type, the date, and the cell number, attach one label on the worksheet as a record.

1.2.5. After the final spin, discard the supernatant and resuspend the cells in the appropriate freeze down medium.

1.2.5.1. If using CTL freeze down medium, resuspend PBMC/BM-MNC in "C" media at a concentration of 20 million cells/mL. Slowly over a 2 minute time period add an equal volume of CTL "AB" media to the cells.

1.2.5.2. If using 10%DMSO/90%FBS then pipette the appropriate volume of medium to the cells.

1.2.6. Aliquot 1.0 mL of the suspension into pre-labeled cryovials.

1.2.7. Place cells in Mr. Frosty cross off the number of uses on the container and place in -20 °C freezer. Record the time the cells were placed in the -60 to -80 °C on the worksheet. Discard 5th use of isopropanol down the sink followed by water. Replace isopropanol in Mr. Frosty for next use.

The next working day transfer PBMC to the liquid nitrogen storage tank and update electronic files and LIMS for serum and PBMC inventory as well as record the locations of the tubes in the tank on the worksheet.

2. Endpoints

2.1. When only 1-2 vials were obtained from 5-6 green top tubes, personnel will notify the IAF director.

2.2. The director or personnel will contact the PI and the CRS to determine if a redraw is required.

3. Endpoints Corrective Actions:

N/A

4. Data Management:

4.1. The Director will review the worksheet to ensure that the processing is appropriate. Any deviations should be noted on the worksheet.

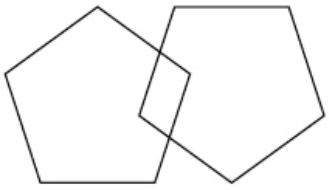
After review of the worksheet, the PBMC and serum electronic log of each protocol will be updated.

Appendix F. MMSE – Mini-Mental State Examination

The Mini-Mental State Exam

Patient_____ Examiner _____ Date_____

Maximum	Score	
		Orientation
5	( )	What is the (year) (season) (date) (day) (month)?
5	( )	Where are we (state) (country) (town) (hospital) (floor)?
		Registration
3	( )	Name 3 objects: 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. Count trials and record. Trials _____
		Attention and Calculation
5	( )	Serial 7's. 1 point for each correct answer. Stop after 5 answers. Alternatively spell "world" backward.
		Recall
3	( )	Ask for the 3 objects repeated above. Give 1 point for each correct answer.
		Language
2	( )	Name a pencil and watch.
1	( )	Repeat the following "No ifs, ands, or buts"
3	( )	Follow a 3-stage command: "Take a paper in your hand, fold it in half, and put it on the floor."
1	( )	Read and obey the following: CLOSE YOUR EYES
1	( )	Write a sentence.
1	( )	Copy the design shown.



_____ Total Score

ASSESS level of consciousness along a continuum _____

Alert Drowsy Stupor Coma

18 REFERENCES

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T, Radiotherapy G, National Cancer Institute of Canada Clinical Trials G. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96. doi: 10.1056/NEJMoa043330. PubMed PMID: 15758009.

2. Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature. 1998;396(6711):580-4. doi: 10.1038/25141. PubMed PMID: 9859993.

3. Rosa J, Canovas P, Islam A, Altieri DC, Doxsey SJ. Survivin modulates microtubule dynamics and nucleation throughout the cell cycle. Molecular biology of the cell. 2006;17(3):1483-93. doi: 10.1091/mbc.E05-08-0723. PubMed PMID: 16407408; PubMed Central PMCID: PMC1382334.

4. Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC, Hwang JI, Chung CW, Jung YK, Oh BH. An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry. 2001;40(4):1117-23. PubMed PMID: 11170436.

5. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res. 1998;58(23):5315-20. PubMed PMID: 9850056.

6. Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J, Altieri DC. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. The American journal of pathology. 1998;152(1):43-9. PubMed PMID: 9422522; PubMed Central PMCID: PMC1858132.

7. Chakravarti A, Noll E, Black PM, Finkelstein DF, Finkelstein DM, Dyson NJ, Loeffler JS. Quantitatively determined survivin expression levels are of prognostic value in human gliomas. J Clin Oncol. 2002;20(4):1063-8. PubMed PMID: 11844831.

8. Fukuda S, Pelus LM. Survivin, a cancer target with an emerging role in normal adult tissues. Molecular cancer therapeutics. 2006;5(5):1087-98. doi: 10.1158/1535-7163.MCT-05-0375. PubMed PMID: 16731740.

9. Islam A, Kageyama H, Takada N, Kawamoto T, Takayasu H, Isogai E, Ohira M, Hashizume K, Kobayashi H, Kaneko Y, Nakagawara A. High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. Oncogene. 2000;19(5):617-23. doi: 10.1038/sj.onc.1203358. PubMed PMID: 10698506.

10. Sasaki T, Lopes MB, Hankins GR, Helm GA. Expression of survivin, an inhibitor of apoptosis protein, in tumors of the nervous system. Acta neuropathologica. 2002;104(1):105-9. doi: 10.1007/s00401-002-0532-x. PubMed PMID: 12070671.

11. Zaffaroni N, Daidone MG. Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. 2002;5(2):65-72. PubMed PMID: 12135582.

12. Andersen MH, Pedersen LO, Becker JC, Straten PT. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res. 2001;61(3):869-72. PubMed PMID: 11221872.

13. Hadrup SR, Gehl J, Sorensen RB, Geertsen PF, Straten PT, Andersen MH. Persistence of survivin specific T cells for seven years in a melanoma patient during complete remission. Cancer biology & therapy. 2006;5(5):480-2. PubMed PMID: 16582593.

14. Kim CH, Woo SJ, Park JS, Kim HS, Park MY, Park SD, Hong YK, Kim TG. Enhanced antitumour immunity by combined use of temozolomide and TAT-survivin pulsed dendritic cells in a murine glioma. Immunology. 2007;122(4):615-22. doi: 10.1111/j.1365-2567.2007.02680.x. PubMed PMID: 17645496; PubMed Central PMCID: PMC2266048.

15. Rohayem J, Diestelkoetter P, Weigle B, Oehmichen A, Schmitz M, Mehlhorn J, Conrad K, Rieber EP. Antibody response to the tumor-associated inhibitor of apoptosis protein survivin in cancer patients. Cancer Res. 2000;60(7):1815-7. PubMed PMID: 10766164.

16. Ciesielski MJ, Ahluwalia MS, Munich SA, Orton M, Barone T, Chanan-Khan A, Fenstermaker RA. Antitumor cytotoxic T-cell response induced by a survivin peptide mimic.

Cancer Immunol Immunother. 2010;59(8):1211-21. doi: 10.1007/s00262-010-0845-x. PubMed PMID: 20422411.

17. Ciesielski MJ, Apfel L, Barone TA, Castro CA, Weiss TC, Fenstermaker RA. Antitumor effects of a xenogeneic survivin bone marrow derived dendritic cell vaccine against murine GL261 gliomas. Cancer Immunol Immunother. 2006;55(12):1491-503. doi: 10.1007/s00262-006-0138-6. PubMed PMID: 16485128.

18. Fenstermaker RA, Ciesielski MJ. Challenges in the development of a survivin vaccine (SurVaxM) for malignant glioma. Expert review of vaccines. 2014;13(3):377-85. doi: 10.1586/14760584.2014.881255. PubMed PMID: 24521310.

19. Wang YQ, Zhang HH, Liu CL, Wu H, Wang P, Xia Q, Zhang LX, Li B, Wu JX, Yu B, Gu TJ, Yu XH, Kong W. Enhancement of survivin-specific anti-tumor immunity by adenovirus prime protein-boost immunity strategy with DDA/MPL adjuvant in a murine melanoma model. International immunopharmacology. 2013;17(1):9-17. doi: 10.1016/j.intimp.2013.04.015. PubMed PMID: 23624214.

20. Xiang R, Mizutani N, Luo Y, Chiodoni C, Zhou H, Mizutani M, Ba Y, Becker JC, Reisfeld RA. A DNA vaccine targeting survivin combines apoptosis with suppression of angiogenesis in lung tumor eradication. Cancer Res. 2005;65(2):553-61. PubMed PMID: 15695399.

21. Baxevanis CN, Voutsas IF, Tsitsilonis OE, Gritzapis AD, Sotiriadou R, Papamichail M. Tumor-specific CD4+ T lymphocytes from cancer patients are required for optimal induction of cytotoxic T cells against the autologous tumor. J Immunol. 2000;164(7):3902-12. PubMed PMID: 10725753.

22. Moeller M, Kershaw MH, Cameron R, Westwood JA, Trapani JA, Smyth MJ, Darcy PK. Sustained antigen-specific antitumor recall response mediated by gene-modified CD4+ T helper-1 and CD8+ T cells. Cancer Res. 2007;67(23):11428-37. doi: 10.1158/0008-5472.CAN-07-1141. PubMed PMID: 18056471.

23. Yu J, Ren X, Cao S, Zhang W, Hao X. Th1 polarization and apoptosis-inducing activity of CD4+ T -cells in cytokine-induced killers might favor the antitumor cytotoxicity of cytokine-induced killers in vivo. Cancer biotherapy & radiopharmaceuticals. 2006;21(3):276-84. doi: 10.1089/cbr.2006.21.276. PubMed PMID: 16918305.

24. Surman DR, Dudley ME, Overwijk WW, Restifo NP. Cutting edge: CD4+ T cell control of CD8+ T cell reactivity to a model tumor antigen. J Immunol. 2000;164(2):562-5. PubMed PMID: 10623795; PubMed Central PMCID: PMC2239008.

25. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med. 1998;188(12):2357-68. PubMed PMID: 9858522; PubMed Central PMCID: PMC2212434.

26. Pardoll DM. Inducing autoimmune disease to treat cancer. Proc Natl Acad Sci U S A. 1999;96(10):5340-2. PubMed PMID: 10318881; PubMed Central PMCID: PMC33572.

27. Overwijk WW, Restifo NP. Autoimmunity and the immunotherapy of cancer: targeting the "self" to destroy the "other". Critical reviews in immunology. 2000;20(6):433-50. PubMed PMID: 11396680; PubMed Central PMCID: PMC2543120.

28. Lohr J, Knoechel B, Nagabhushanam V, Abbas AK. T-cell tolerance and autoimmunity to systemic and tissue-restricted self-antigens. Immunol Rev. 2005;204:116-27. doi: 10.1111/j.0105-2896.2005.00241.x. PubMed PMID: 15790354.

29. Parkhurst MR, Salgaller ML, Southwood S, Robbins PF, Sette A, Rosenberg SA, Kawakami Y. Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues. J Immunol. 1996;157(6):2539-48. PubMed PMID: 8805655.

30. Valmori D, Fonteneau JF, Lizana CM, Gervois N, Lienard D, Rimoldi D, Jongeneel V, Jotereau F, Cerottini JC, Romero P. Enhanced generation of specific tumor-reactive CTL in vitro by selected Melan-A/MART-1 immunodominant peptide analogues. J Immunol. 1998;160(4):1750-8. PubMed PMID: 9469433.

31. Evavold BD, Sloan-Lancaster J, Allen PM. Tickling the TCR: selective T-cell functions stimulated by altered peptide ligands. Immunology today. 1993;14(12):602-9. doi: 10.1016/0167-5699(93)90200-5. PubMed PMID: 8305133.

32. Lennerz V, Gross S, Gallerani E, Sessa C, Mach N, Boehm S, Hess D, von Boehmer L, Knuth A, Ochsenbein AF, Gnad-Vogt U, Zieschang J, Forssmann U, Woelfel T, Kaempgen E. Immunologic response to the survivin-derived multi-epitope vaccine EMD640744 in patients with advanced solid tumors. Cancer Immunol Immunother. 2014;63(4):381-94. doi: 10.1007/s00262-013-1516-5. PubMed PMID: 24487961.

33. Miyazaki A, Kobayashi J, Torigoe T, Hirohashi Y, Yamamoto T, Yamaguchi A, Asanuma H, Takahashi A, Michifuri Y, Nakamori K, Nagai I, Sato N, Hiratsuka H. Phase I clinical trial of survivin-derived peptide vaccine therapy for patients with advanced or recurrent oral cancer. Cancer science. 2011;102(2):324-9. doi: 10.1111/j.1349-7006.2010.01789.x. PubMed PMID: 21143701.

34. Rapoport AP, Aqui NA, Stadtmauer EA, Vogl DT, Fang HB, Cai L, Janofsky S, Chew A, Storek J, Akpek G, Badros A, Yanovich S, Tan MT, Veloso E, Pasetti MF, Cross A, Philip S, Murphy H, Bhagat R, Zheng Z, Milliron T, Cotte J, Cannon A, Levine BL, Vonderheide RH, June CH. Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination on the basis of hTERT and survivin after ASCT for myeloma. Blood. 2011;117(3):788-97. doi: 10.1182/blood-2010-08-299396. PubMed PMID: 21030558; PubMed Central PMCID: PMC3035073.

35. Tsuruma T, Hata F, Torigoe T, Furuhata T, Idenoue S, Kurotaki T, Yamamoto M, Yagihashi A, Ohmura T, Yamaguchi K, Katsuramaki T, Yasoshima T, Sasaki K, Mizushima Y, Minamida H, Kimura H, Akiyama M, Hirohashi Y, Asanuma H, Tamura Y, Shimozawa K, Sato N, Hirata K. Phase I clinical study of anti-apoptosis protein, survivin-derived peptide vaccine therapy for patients with advanced or recurrent colorectal cancer. J Transl Med. 2004;2(1):19. doi: 10.1186/1479-5876-2-19. PubMed PMID: 15193151; PubMed Central PMCID: PMC446218.

36. Becker JC, Andersen MH, Hofmeister-Muller V, Wobser M, Frey L, Sandig C, Walter S, Singh-Jasuja H, Kampgen E, Opitz A, Zapatka M, Brocker EB, Thor Straten P, Schrama D, Ugurel S. Survivin-specific T-cell reactivity correlates with tumor response and patient survival: a phase-II peptide vaccination trial in metastatic melanoma. Cancer Immunol Immunother. 2012;61(11):2091-103. doi: 10.1007/s00262-012-1266-9. PubMed PMID: 22565484; PubMed Central PMCID: PMC3493663.

37. Sampson JH, Aldape KD, Archer GE, Coan A, Desjardins A, Friedman AH, Friedman HS, Gilbert MR, Herndon JE, McLendon RE, Mitchell DA, Reardon DA, Sawaya R, Schmittling R, Shi W, Vredenburgh JJ, Bigner DD, Heimberger AB. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro-oncology. 2011;13(3):324-33. doi: 10.1093/neuonc/noq157. PubMed PMID: 21149254; PubMed Central PMCID: PMC3064599.

38. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, Gilbert MR, Herndon JE, McLendon RE, Mitchell DA, Reardon DA, Sawaya R, Schmittling RJ, Shi W, Vredenburgh JJ, Bigner DD. Immunologic Escape After Prolonged Progression-Free Survival With Epidermal Growth Factor Receptor Variant III Peptide Vaccination in Patients

With Newly Diagnosed Glioblastoma. Journal of Clinical Oncology. 2010;28(31):4722-9. doi: 10.1200/jco.2010.28.6963.

39. Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, Mrugala MM, Jensen R, Baehring JM, Sloan A, Archer GE, Bigner DD, Cruickshank S, Green JA, Keler T, Davis TA, Heimberger AB, Sampson JH. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro-oncology. 2015. doi: 10.1093/neuonc/nou348. PubMed PMID: 25586468.

40. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, Degroot J, Wick W, Gilbert MR, Lassman AB, Tsien C, Mikkelsen T, Wong ET, Chamberlain MC, Stupp R, Lamborn KR, Vogelbaum MA, van den Bent MJ, Chang SM. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010;28(11):1963-72. doi: 10.1200/JCO.2009.26.3541. PubMed PMID: 20231676.

41. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. Journal of Psychiatric Research.12(3):189-98. doi: 10.1016/0022-3956(75)90026-6.

43. Clopper C, Pearson E. The use of confidence or the use of confidence or fiducial limits illustrated in the case of the binomial. Biometrica. 1934;26(4):10. doi: 10.2307/2331986.

44. Hosmer D, Lemeshow S. Applied Logistic Regression. New York: Wiley and Sons; 2000.