CASE WESTERN RESERVE UNIVERSITY | CASE COMPREHENSIVE CANCER CENTER

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

STUDY NUMBER: CASE 6318

ClinicalTrials.gov NCT #: NCT04013672

Protocol Date: 3-11-2019
Protocol Amendment 1: 7-22-2019
Protocol Amendment 2: 4-3-2020
Protocol Amendment 3: 5-28-2020
Protocol Amendment 4: 2-1-2021

STUDY TITLE: Phase II Study of Pembrolizumab plus SurVaxM for glioblastoma at first recurrence

PRINCIPAL INVESTIGATOR: David Peereboom M.D
Case Comprehensive Cancer Center
Cleveland Clinic
Taussig Cancer Center
9500 Euclid Avenue
Cleveland, OH 44195
[redacted]

STATISTICIAN: Wei, Wei (Auston)
Cleveland Clinic
9500 Euclid Avenue, CA-60
Cleveland, OH 44195
[redacted]

STUDY COORDINATOR: Marci Ciolfi
Case Comprehensive Cancer Center
Cleveland Clinic
9500 Euclid Avenue
Cleveland, Oh 44195
[redacted]

SPONSOR: Case Comprehensive Cancer Center

SUPPORT/FUNDING:	Merck Company
SUPPLIED AGENT:	Pembrolizumab
IND #:	18980
OTHER AGENT:	SurVaxM

SUMMARY OF CHANGES

Protocol Date	Section	Change
3/11/2019	Protocol Summary	Protocol Number/Title amended (administrative change on 5/8/19)
7/22/2019	Cover page	NCT # added
7/22/2019	Cover page	Protocol Amendment 1: 7/22/2019 added
7/22/2019	Appendices	Appendix 5 added-Contraceptive/Pregnancy Guidelines added
7/22/2019	Overdose	Definition of an Overdose and reporting of an overdose information added
7/22/2019	Page 25	Reporting of Pregnancy and Lactation information added
7/22/2019	Page 26	Events of Clinical Interest information added
7/22/2019	Inclusion Criteria	Inclusion Criteria #4 removed "of randomization". This is not a randomized study
7/22/2019	Inclusion Criteria	Inclusion Criteria #11 Removed "< x ULN for participants with liver metastases"
7/22/2019	Exclusion Criteria	Exclusion Criteria #19 amended from "through 120 days" to "through 180 days"
7/22/2019	Entire protocol	Deleted randomization from randomization/allocation
7/22/2019	2.1 Primary Objective	Hypothesis added
7/22/2019	2.2 Secondary Objective	Hypothesis added
7/22/2019	2.3 Exploratory Objective	Added bullet point 5-Outcomes in 10 patients with glioblastoma in first recurrence that have progressed on anti PD1 therapy (Arm B)
7/22/2019	3.0 Study Design	Trial Diagram/Schema added
7/22/2019	3.2	Additional information added regarding number of subjects
7/22/2019	6.3.1	Table 3 Dose modifications updated
4/3/2020	Cover Page	Protocol Amendment 2: 4/3/20 added
4/3/2020	Protocol Summary	Brief Background/Rationale amended: toxicity run in changed from 6 to 10 patients
4/3/2020	Section 3.2 Number of Subjects	Toxicity safety run in amended from 6 to 10. Additional language added---The probabilities of observing 3, 4, and 5 DLTs out of 10 patients with true toxicity rate of 33% is 23%, 26%, and 13%. If 4 or less DLTs observed in the 10 patients, the study proceed with more patients. If 5 or more DLTs observed, accrual will be suspended and toxicity profile we be fully evaluated. If toxicity threshold to stop will not be reached, an additional 31 patients will be enrolled for total of 41 patients.

Protocol Date	Section	Change
4/3/2020	13.0 Statistical Considerations	Additional language added: The probabilities of observing 3, 4, and 5 DLTs out of 10 patients with true toxicity rate of 33% is 23%, 26%, and 13%. If 4 or less DLTs observed in the 10 patients, the study proceed with more patients. If 5 or more DLTs observed, accrual will be suspended and toxicity profile we be fully evaluated. If toxicity threshold to stop will not be reached, an additional 31
5/28/2020	Cover Page	Statistician updated
5/28/2020	Section 4.2	Exclusion criteria #7 typo corrected. Exlude amended to Exclude
5/28/2020	Section 9.0	Cycle 4+ header changed from every 12 weeks to every 9 weeks
5/28/2020	Section 9.0	References to Diaz lab details amended to Lathia Lab details
5/28/2020	Section 9.0	FSH changed to TSH
5/28/2020	Section 9.0	Footnote 10 added-Day 1 Physical Exam and Laboratory testing not required if less than 7 days from screenin g
5/28/2020	Section 12.1.3	Diaz lab amended to Lathia Lab details
5/28/2020	Section 12.1.4	Diaz lab amended to Lathia Lab details
5/28/2020	Section 12.1.5	Diaz lab amended to Lathia Lab details
5/28/2020	Section 12.1.7	PBMC Isolation amended from Diaz Lab procedures to Lathia Lab procedures
2/1/2021	Section 9.1	Cycle 4+ amended from every 9 weeks to every 3 weeks
2/1/2021	Section 9.1	Footnotes 11 and 12 added to clarify Cycle 4+ vaccine schedule of every 12 weeks and Cycle 4+ MRI schedule of every 9 weeks
2/2/2021	Section 9.1	Footnote 13 added. Weight not required during week 4 rest period
2/1/2021	Cover page	Updated Sponsor-Investigator from Manmeet Ahluwalia M.D.to David Peereboom M.D.
2/1/2021	Section 12.1.2	Updated to David Peereboom M.D.
2/1/2021	Section 3.0	Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)
2/1/2021	Section 6.1	Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)
2/1/2021	Section 6.5	Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)
2/1/2021	Section 9.1	Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)

PROTOCOL SUMMARY

Protocol Number/Title	**Phase II Study of Pembrolizumab plus SurVaxM for glioblastoma at first recurrence**
Study Phase	Phase 2
Brief Background/Rationale	Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1.
	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. 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. Although expressed during fetal development, survivin is rarely detectable in the normal tissues of adult organisms. This will be a phase II clinical study with a 10 patient, toxicity run-in. The study follows upon the recently completed clinical study I-171010, entitled: Phase I study of safety, tolerability and immunological effects of SVN53-67/M57- KLH (SurVaxM) in patients with survivin-positive malignant gliomas. All patients will receive the study drug combination consisting of SurVaxM and pembrolizumab (PEM) with no randomization, stratification or dose escalation.
Agent	Pembrolizumab and SurVaxM.
Disease Sites/Conditions	Recurrent glioblastoma in the CNS.
Objectives	**Primary**: Assess clinical activity of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma using progression free survival at 6 months (PFS-6). **Secondary**: Assess safety and tolerability of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma. **Tertiary**: Measure both cellular and humoral immune responses during concurrent administration of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma.
Estimated Study Duration	3 years
Duration of Participation	3 years
Interventions-Experimental	Pembrolizumab 200 mg IV every 3 weeks SurVaxM (SVN53-67/M57-KLH) 500 mcg per dose, dosed

	every two weeks for 4 doses and then every 3 months Sargramostim (GM-CSF) 100 mcg per dose, dosed every two weeks for 4 doses and then every 3 months Montanide ISA 51, 1 ml per dose dosed every two weeks for 4 doses and then every 3 months

ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
DCRU	Dahm's Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals

TABLE OF CONTENTS

13.0 STATISTICAL CONSIDERATIONS

14.0 REFERENCES

APPENDICES

Appendix 1: ECOG Performance Status
Appendix 2: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)
Appendix 3: RANO Criteria
Appendix 4: Preparation of Peptide Vaccine Emulsion
Appendix 5: Contraceptive/Pregnancy Guidelines

1.0 INTRODUCTION

1.1 Background of Glioblastoma

Glioblastoma is the most common type of primary brain cancer [Venur, 2015]. The 5-year survival rate for patients with glioblastoma is 3.3% substantiating the great need for improved therapy. Following first recurrence, progression free survival with second-line therapy at six months (PFS6) is about 15% [Venur, 2015]. There is no therapy in recurrent glioblastoma that is associated with any survival benefit and there is an urgent need for better therapeutic options. Immunotherapy is one promising option for patients with cancer. This is being explored in glioblastoma and a number of forms of active specific vaccination and immune checkpoint based approaches have been devised and are being investigated in glioblastoma.

1.2 Pharmaceutical and Therapeutic Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure.

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [Disis, 2010]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells to FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as: ovarian, colorectal, and pancreatic; hepatocellular and renal cancers and malignant melanoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma [Dudley et al., 2005; Hunder et al., 2008].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [Greenwald et al., 2005; Okazaki et al., 2001].

The structure of murine PD-1 has been resolved [Zhang et al., 2004]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based

switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [Okazaki et al., 2001; Chemnitz et al., 2004; Sheppard et al., 2004; and Riley, 2009]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [Parry et al., 2005; Francisco, 2010]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in recurrent glioblastoma.

PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

For each individual trial, clinical supplies are to be stored in accordance with specific instructions on the label.

Chemical Properties

Pembrolizumab is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. Pembrolizumab binds to human PD-1 and blocks the interaction between PD-1 and its ligands. The theoretical molecular weight of the polypeptide is 146,288 daltons, and its theoretical isoelectric point is7.5. The parental murine antihuman PD-1 antibody (hPD-1.09Á) was produced by immunizing mice with hPD-1 deoxyribonucleic acid (DNA). The pembrolizumab antibody was generated by humanization of the parental antibody by the Medical Research Council (Cambridge, United Kingdom) using complementarity determining region grafting technology (US Patent No. 5,225,539). The gene segments encoding the variable heavy and light chains of pembrolizumab, as well as human IgG4, were codon-optimized, synthesized, and ligated into a vector.

A single expression plasmid, pAPD11V1-GA, was constructed for the expression of both the heavy and light antibody chains of pembrolizumab. The nucleotide sequences encoding the heavy and light chains, along with their respective promoters and poly A signal sequences, have been confirmed by DNA sequence analysis. The pAPD11V1-GA expression vectorwas subsequently used to transfect CHO-DXB-11 cells for the development of the pembrolizumab producing cell line.

The nomenclature of pembrolizumab drug substance is provided in Table below.

Nomenclature of Pembrolizumab Drug Substance

Code Name	MK-3475 (Anti–PD-1)
Other Code Name	MK3, 02P106, ORG 307448-0, SCH 900475 (Anti–PD-1)
Chemical Name	Humanized X PD-1-mAb (H409A11) IgG4
CAS Number	1374853-91-4
CAS Name	Anti-(human protein PDCD1 (programmed cell death 1) immunoglobulin G4 (human-Mus musculus monoclonal heavy chain) disulfide with human-Mus musculus monoclonal light chain, dimer
USAN and INN	pembrolizumab
Trade Name	KEYTRUDA®

Pembrolizumab is a highly selective humanized mAb designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is an IgG4/kappa isotype with a stabilizing sequence alteration in the Fc region. The theoretical molecular weights of the heavy and light chains derived from the amino acid sequences, excluding glycosylation, are 49.4 kiloDaltons (KDa) and 23.7 KDa, respectively. The antibody is heterogeneously glycosylated at asparagine 297 within the Fc domain of each heavy chain, yielding a molecular weight of approximately 149 KDa for intact pembrolizumab.

Physio-Chemical Properties

Pembrolizumab drug substance (DS) is produced at several locations to yield: a partially formulated aqueous solution stored under refrigerated (2°C to 8°C) conditions at a concentration of 40.0 to 50.0 mg/mL in 10 millimolar (mM) histidine buffer, pH 5.2 to 5.8, and a fully formulated aqueous solution stored frozen (–40°C°±5°C) at a concentration of 22.5 to 27.5 mg/mL in 10 mM histidine buffer, pH 5.2 to 5.8, containing 7% sucrose and 0.02% polysorbate 80. The drug substance solution from both sources is a clear to opalescent liquid.

The manufacturing process for pembrolizumab is a suspension cell culture process that uses commercially available animal component-free medium. The contents of the production bioreactor are depth filtered to remove intact cells and cell debris and then 0.2 micron (µm) filtered into pre-sterilized bags.

The purification process for pembrolizumab drug substance consists of the following steps: 3 chromatography steps, 1 viral inactivation step, 1 viral filtration step, 1 ultrafiltration/diafiltration step, 1 formulation step (only for the fully formulated DS stored frozen), and 1 final 0.2 µm filtration step.

Pharmaceutical Formulation

Two drug product (DP) dosage forms are available for pembrolizumab: a white to off-white lyophilized powder, 50 mg/vial, and a liquid, 100 mg/vial, both in Type I glass vials intended for single use only. The drug products are manufactured using facilities and practices under Good Manufacturing Practice (GMP) requirements.

- Pembrolizumab Powder for Solution for Infusion, 50 mg/vial is a lyophilized powder that is reconstituted with sterile water for injection prior to use. It is manufactured using either the fully formulated DS or the partially formulated DS. The fully formulated DS uses L-histidine as a buffering agent, polysorbate 80 as surfactant, and sucrose as stabilizer/tonicity modifier. Pembrolizumab DP using the partially formulated DS is formulated with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier, and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (if necessary).
- Pembrolizumab Solution for Infusion 100 mg/vial is a liquid DP (manufactured using the fully formulated DS with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier).

Both DP dosage forms are stored under refrigerated conditions (2°C to 8°C).

The lyophilized DP after reconstitution with sterile water for injection and the liquid DP are clear to opalescent solutions, essentially free of visible particles. The reconstituted lyophilized product and the liquid product are intended for IV administration. The reconstituted DP solution or the liquid DP can be further diluted with normal saline or 5% dextrose in the concentration range of 1 to 10 mg/mL in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be used immediately to prepare the infusion solution in the IV bag, and the infusion solution should be administered immediately. Diluted pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. In addition, IV bags can be stored at 2°C to 8°C for up to a cumulative time of 20 hours. This recommendation is based on up to 24 hours of room temperature and up to 24 hours of refrigerated stability data of diluted pembrolizumab solutions in the IV bags.

EFFECTS IN HUMANS

Efficacy data for the Merck-sponsored clinical trials were used to support approvals in the US for the following indications: melanoma, NSCLC, HNSCC, cHL, UC, and MSI-H tumors.

Pharmacokinetics Studies

Pembrolizumab PK samples have been obtained from multiple trials for various tumor types including melanoma, NSCLC, HNSCC, UC and microsatellite instability high (MSI-H) tumors. The doses tested in these tumor types include one or more of the following doses: 2 mg/kg Q3W, 10 mg/kg Q3W, 10 mg/kg every 2 weeks (Q2W), and 200 mg Q3W.

Absorption

Pembrolizumab is administered intravenously and is therefore immediately and completely bioavailable.

Distribution

Consistent with a limited extravascular distribution, the volume of distribution of pembrolizumab at steady state is small (6.0 L; coefficient of variation [CV%]: 20%). As expected for an antibody, pembrolizumab does not bind to plasma proteins in a specific manner.

Metabolism

Pembrolizumab is catabolized through non-specific pathways; metabolism does not contribute to its CL.

Elimination

Pembrolizumab CL is approximately 23% lower (geometric mean, 195 mL/day [CV%: 40%]) after achieving maximal change at steady state compared with the first dose (252 mL/day [CV%: 37%]); this decrease in CL with time is not considered clinically meaningful. The geometric mean value (CV%) for the terminal half-life is 22 days (32%) at steady-state.

Summary of Overall Adverse Events Reported in the Reference Safety Dataset

This section presents an overall AE summary from the RSD. All event rows in the AE summary table (including serious adverse event [SAE] rows), are based on all AEs. After the end of study treatment, each subject was followed for 30 days for AE monitoring. Serious AEs were collected for 90 days after the end of treatment or for 30 days after the end of treatment if the subject initiated new anticancer therapy, whichever was earlier if the subject completed study treatment.

Table below presents an overall AE summary in pembrolizumab-treated participants from the RSD. The majority of participants, 2727 or 97.4%, experienced 1 or more AEs, and 2062 (73.7%) experienced 1 or more AEs reported as drug-related by the investigator. The percentage of participants who experienced SAEs was lower; 1041 (37.2%) of participants experienced 1 or more SAEs; 334 (11.9%) participants discontinued due to an AE, and 281 (10.0%) participants experienced a drug-related SAE, as determined by the investigator.

Adverse Event Summary in Subjects Treated With Pembrolizumab (ASaT Population)

	Reference Safety Dataset for Pembrolizumab[a]	
	n	
Subjects in population	2799	
with one or more adverse events	2727	(97.4)
with no adverse event	72	(2.6)
with drug-related† adverse events	2062	(73.7)
with toxicity grade 3-5 adverse events	1273	(45.5)
with toxicity grade 3-5 drug-related adverse events	386	(13.8)
with non-serious adverse events	2671	(95.4)
with serious adverse events	1041	(37.2)
with serious drug-related adverse events	281	(10.0)
with dose modification§ due to an adverse event	884	(31.6)
who died	110	(3.9)
who died due to a drug-related adverse event	10	(0.4)
discontinued‡ due to an adverse event	334	(11.9)
discontinued due to a drug-related adverse event	146	(5.2)
discontinued due to a serious adverse event	253	(9.0)
discontinued due t	101	(3.6)

† Determined by the investigator to be related to the drug.
‡ Study medication withdrawn.
§ Defined as overall action taken of dose reduced, drug interrupted or drug withdrawn.
MedDRA preferred terms "Neoplasm Progression", "Malignant Neoplasm Progression" and "Disease Progression" not related to the drug are excluded.
MedDRA version used is 18.1.
[a] Includes all subjects who received at least one dose of MK-3475 in KN001 Part B1, B2, B3, D, C, F1, F2, F3; KN002 (original phase), KN006, and KN010.
(KN001 Database Cutoff Date for Melanoma: 18APR2014). (KN001 Database Cutoff Date for Lung Cancer: 23JAN2015). (KN002 Database Cutoff Date: 28FEB2015).
(KN006 Database Cutoff Date: 03MAR2015).
(KN010 Database Cutoff Date: 30SEP2015).

Table below presents the most frequently reported (≥10%) AEs by decreasing frequency from the RSD. The 5 most frequently reported AEs were: fatigue (37.3%), nausea (24.5%), decreased appetite (22.5%), diarrhea (22.3%), and cough (22%).

Table Most Frequently Reported (≥10%) Adverse Events Presented by Decreasing Frequency in Subjects Treated with Pembrolizumab (ASaT Population)

Preferred Term	Reference Safety Dataset for Pembrolizumab[a]	
	n	
Subjects in population	279	
Fatigue	104	(37.3)
Nausea	685	(24.5)
Decreased appetite	630	(22.5)
Diarrhea	625	(22.3)
Cough	615	(22.0)
Pruritus	562	(20.1)
Dyspnea	534	(19.1)
Arthralgia	504	(18.0)
Rash	499	(17.8)
Constipation	497	(17.8)
Headache	400	(14.3)
Vomiting	387	(13.8)
Asthenia	362	(12.9)
Pyrexia	357	(12.8)
Back pain	349	(12.5)
Anemia	347	(12.4)
Oedema peripheral	285	(10.2)

Every subject is counted a single time for each applicable row and column. MedDRA version used is 18.1.
[a] Includes all subjects who received at least one dose of MK-3475 in KN001 Part B1, B2, B3, D, C, F1, F2, F3; KN002 (original phase), KN006, and KN010. (KN001 Database Cutoff Date for Melanoma: 18APR2014). (KN001 Database Cutoff Date for Lung Cancer: 23JAN2015). (KN002 Database Cutoff Date: 28FEB2015).

Table Most Frequently Reported (≥0.5%) Serious Adverse Events Presented by Decreasing Frequency in Subjects Treated with Pembrolizumab (ASaT Population)

Preferred Term	Reference Safety Dataset for Pembrolizumab[a]	
	n	
Subjects in population	279	
Pneumonia	85	(3.0)
Pleural effusion	48	(1.7)
Pneumonitis	46	(1.6)
Dyspnea	45	(1.6)
Pulmonary embolism	41	(1.5)
Pyrexia	35	(1.3)
Anemia	31	(1.1)
Colitis	31	(1.1)
Diarrhea	26	(0.9)
Dehydration	24	(0.9)
Abdominal pain	22	(0.8)
Acute kidney injury	22	(0.8)
Hyponatraemia	21	(0.8)
General physical health deterioration	19	(0.7)
Basal cell carcinoma	18	(0.6)
Nausea	18	(0.6)
Vomiting	18	(0.6)
Death	17	(0.6)
Chronic obstructive pulmonary disease	16	(0.6)
Pericardial effusion	16	(0.6)
Back pain	15	(0.5)
Cellulitis	15	(0.5)
Confusional state	15	(0.5)
Renal failure	15	(0.5)
Squamous cell carcinoma	15	(0.5)
Urinary tract infection	15	(0.5)

Respiratory failure	14	(0.5)
Sepsis	14	(0.5)
Every subject is counted a single time for each applicable row and column. MedDRA version used is 18.1. [a] Includes all subjects who received at least one dose of MK-3475 in KN001 Part B1, B2, B3, D, C, F1, F2, F3; KN002 (original phase), KN006, and KN010. (KN001 Database Cutoff Date for Melanoma: 18APR2014). (KN001 Database Cutoff Date for Lung Cancer: 23JAN2015).		

For additional safety information please refer to Investigators Brochure.

Dose of pembrolizumab for the proposed study:
The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg

Q3W as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.
Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.
Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

1.3 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.

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. In addition, the survivin molecule itself has recently been shown to be expressed on the outer surface of the tumor cell membrane where it is accessible to antibody-mediated immune attack (Clin Cancer Res. 2018 Jun 1;24(11):2642-2652. doi: 10.1158/1078-0432.CCR-17-2778. Epub 2018 Mar 14). There is substantial experimental evidence that survivin vaccination leads to anti-tumor immune responses in tumor-naive and tumor-bearing animals [16-20].

SVN53-67/M57-KLH (SurVaxM)

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 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.

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 the survivin immunogen SurVaxM produced specific anti-survivin CD8+ T cells and anti-survivin antibodies in patients with recurrent malignant gliomas, primarily glioblastoma.

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.

Altered peptide antigens

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 T cell epitopes, 2) peptide mimicry, 3) antigen-specific cytokine support, and 4) anti-survivin antibody production.

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. 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 administered to 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 Montanide's action is unknown.

Sargramostim

Sargramostim (Leukine®) is recombinant human granulocyte macrophage colony stimulating factor (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.

Name/description of 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.

Pharmacological Class

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

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.

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®).

Drug Shipment

SVN53-67/M57-KLH will be shipped to all participating sites from The University of Iowa Pharmaceuticals (https://uip.pharmacy.uiowa.edu/) in 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 -20ºC 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.

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.

Handling and Disposal

The Investigator or designee will be responsible for prescribing all investigational drug exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken in accordance with 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. 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, except as approved by both the IRB and the lead study principal investigator.

Reconstitution of Lyophilized SurVaxM

Each vial of SVN53-67/M57-KLH peptide-conjugate 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.

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. Sargramostim (Leukine) will be delivered locally as a separate injection. Leukine (Sargramostim), NDC Product Code 50419, is an approved drug.

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.

Overdose

No specific antidotes exist for the treatment of SurVaxM overdose.

Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.
If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.
If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

Pregnancy, Lactation, and Pediatric Use

Fertility and teratology studies with SurVaxM have not been conducted. Safety for women of childbearing capacity cannot be inferred 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.

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck within 2 working days if the outcome is a serious adverse experience (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to

the mother or newborn). The study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck. If a male participant impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to Merck and followed as described in Section 7.2.2.
It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breast-feeding are not eligible for enrollment.

Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and infant exposure during breast feeding are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a participant (spontaneously reported to them) that occurs during the study.

Pregnancies and infant exposures during breastfeeding that occur after the consent form is signed but before treatment allocation

must be reported by the investigator if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and infant exposures during breastfeeding that occur from the time of treatment through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).
For the time period beginning when the consent form is signed until treatment allocation, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:
1. an overdose of Merck product - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

Peptide Binding Analysis

SurVaxM epitopes (**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 (**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.

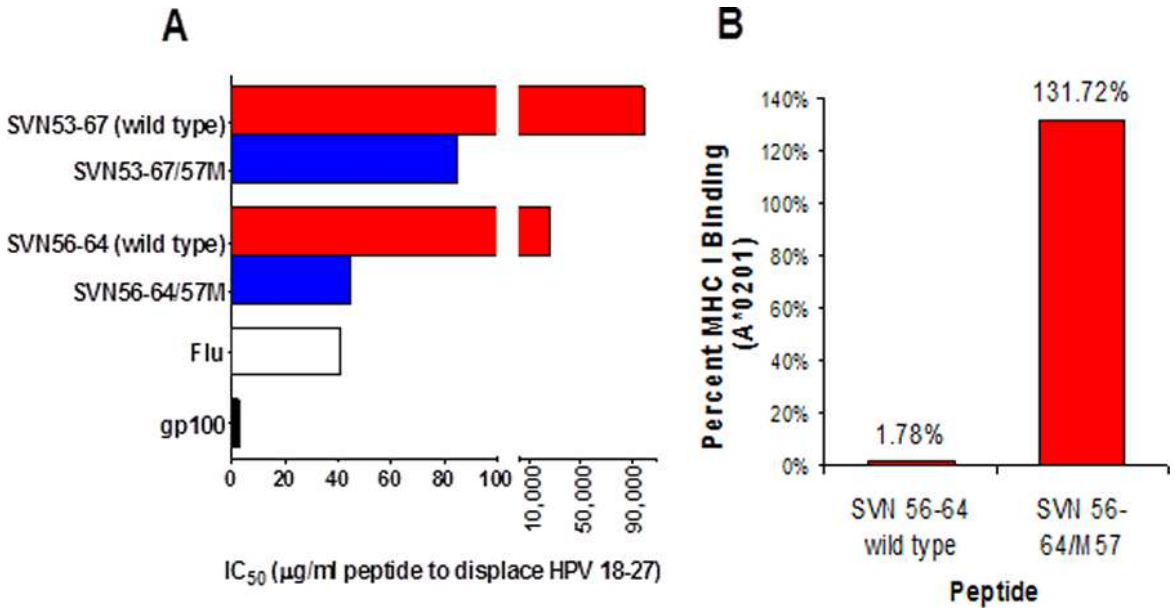


Figure 1: (A) MHC class I (HLA-A*0201) peptide competitive displacement assays. IC50 represents concentration of survivin peptide required to displace HPV 18-27 (known MHC I ligand) pre-loaded on human T2 cells. Flu and gp100 are known immunogenic MHC class I ligands. (B) REVEAL™ assays performed by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

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. This is associated with a concomitant increase in CD4+ T cell derived cytokine support). 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.

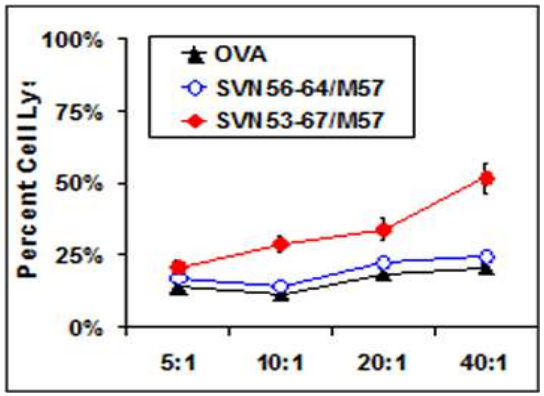


Figure 2: CTL responses against GL261 glioma cells using splenocytes of mice vaccinated with SVN53-67/M57-KLH. Data represent mean percent specific lysis ± S.E.M. of triplicate samples.

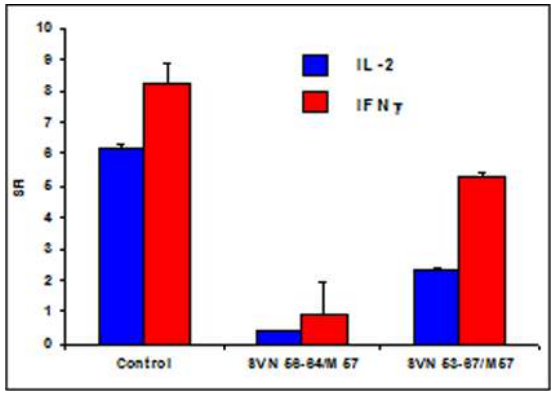


Figure 3: Intracellular IFN-γ and IL-2 production in vitro by CD4+ cells from SVN53-67/M57-KLH immunized mice restimulated ex vivo with SVN53-67/M57 overnight (with BD GolgiPlug). A standardized control (Mouse Cytokine Intracellular Control lymphocytes; eBioscience, San Diego, CA) were used as positive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

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 (**-A**). HLA-A*0201, and HLA-A*0301 patients were able to be stimulated to lyse an allogeneic-matched and autologous glioma cells (**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 (**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. 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.

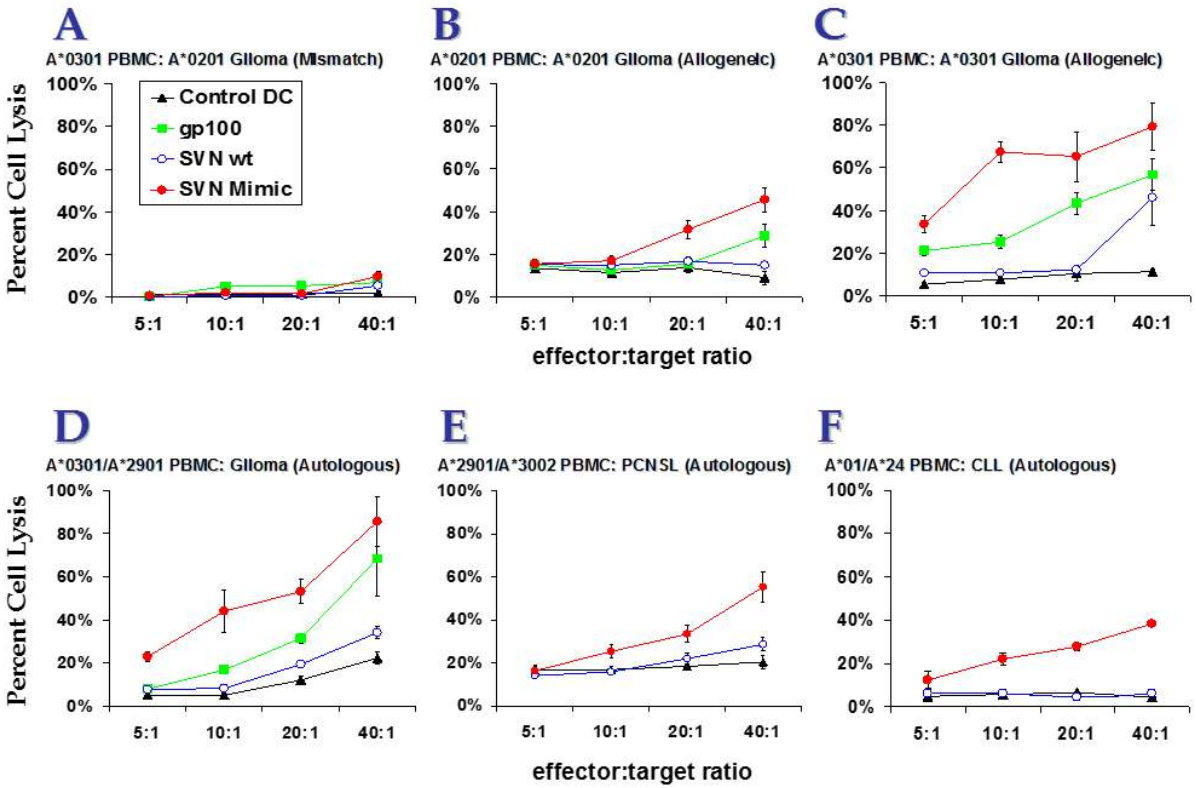


Figure 4: (A) HLA-A*0301 patient effector cells vs. allogeneic-mismatched HLA-A*0201 human U87 target glioma cells; (B) HLA-A*0201 patient effector cells vs. allogeneic-matched U87 glioma cells; (C) HLA-A*0301 patient effector cells vs. allogeneic-matched patient-derived tumor cells; (D) HLA-A*0301/HLA-A*2901 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 (**A**). These CTL are also capable of lysing autologous glioma target cells and lysis can be blocked with MHC I blocking antibody (**B**).

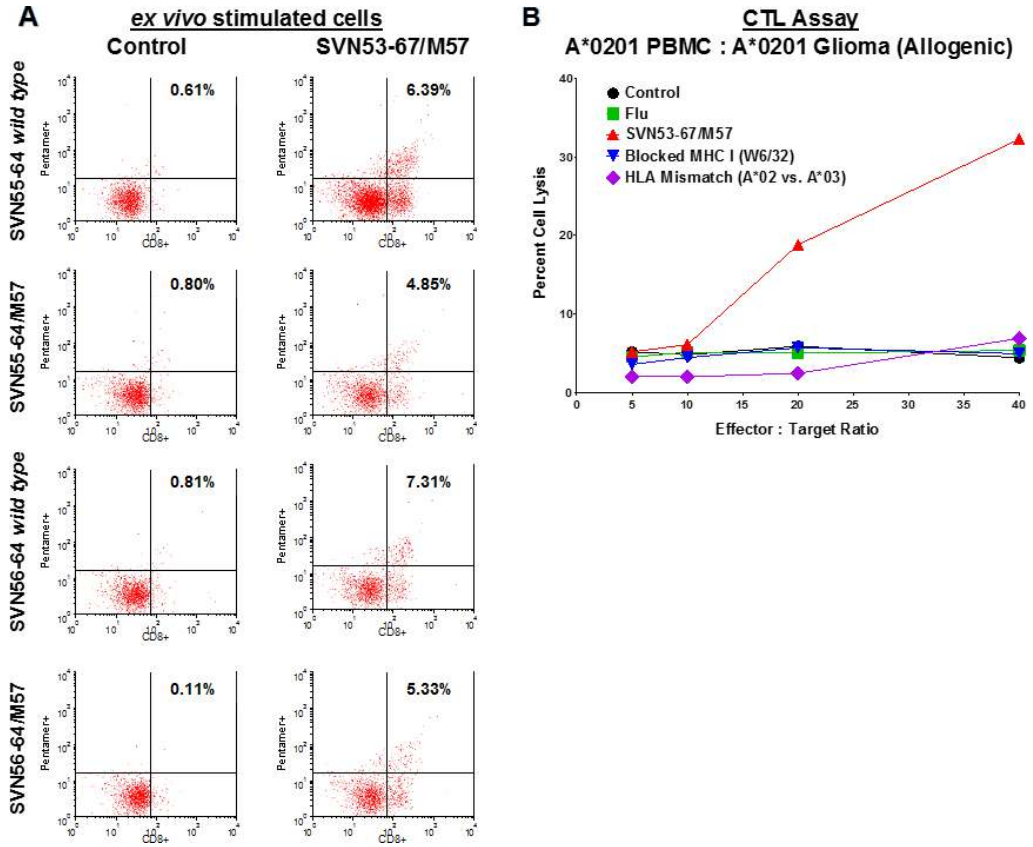


Figure 5: Pentamer binding assay using patient derived CTL. A) HLA-A*0201 pentamer binding of CD8+ T cells specific for SVN55-64 wild type peptide, SVN56-64 wild type peptide, SVN55-64/M57 peptide mimic or SVN56-64/M57 peptide mimic as a result of ex vivo SVN53-67/M57 stimulation in cell culture. Percentage 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.

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 below). Data indicate the possibility that previous immune exposure to survivin may naturally occur in GBM patients.

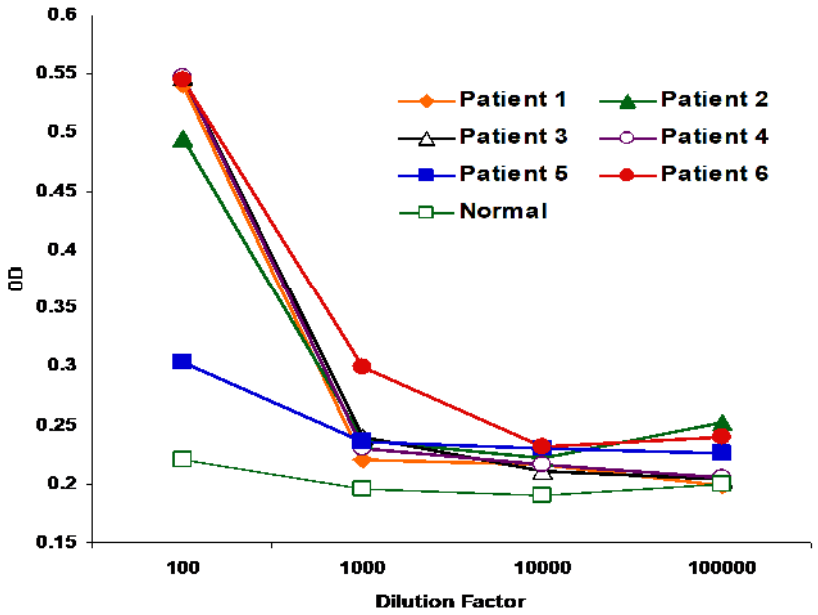


Figure 6: Serum ELISA for SVN53-67 reactive IgG antibodies in glioma patients who have not been vaccinated.

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.

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.

Efficacy

Pre-clinical Data

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). 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.

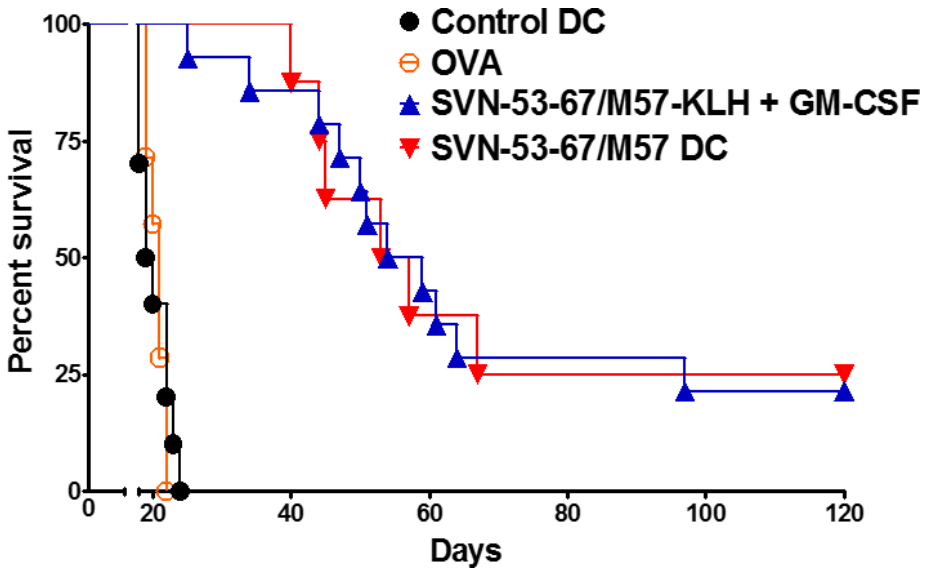


Figure 7: Survival of C57BL/6 mice bearing $1x10^5$ GL261 intracerebral gliomas. 1) Control DC: n=10, survival range 18-24 days, with median survival 19.5 days; 2) OVA: n=7, survival range 19-22 days, with median survival 21 days; 3) SVN53-67/M57 DC: n=14,

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.

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).

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 Comprehensive Cancer Center. 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. There is an

ongoing phase II trial of SVN53-67/M57-KLH-Montanide-sargramostim and temozolomide in newly diagnosed glioblastoma that has confirmed the safety of the combination of vaccine with temozolomide. Interim data (as of 2/2/2018) obtained from this phase II study has only reported two instances of grade 3 AE/SAE from separate patients at least possibly related to SVN53-67/M57-KLH from 63 total patients; one patient reported maculo-papular rash (grade 3 SAE) and one other patient experienced confusional state (grade 3 SAE). No higher grade AE or SAE has been observed. Based upon this data SVN53-67/M57-KLH continues to be safe in glioblastoma patients.

1.4 Rationale for use in recurrent glioblastoma

Approximately 22,850 people are diagnosed with primary cancer of the nervous system every year leading to 15,320 deaths (1). Glioblastoma is by far the most common type of primary brain cancer. The 5-year survival rate for patients with glioblastoma is 3.3% substantiating the great need for improved therapy (1). Following first recurrence, progression free survival with second-line therapy at six months (PFS6) is about 15%. Immunotherapy is one promising option for patients with glioblastoma and a number of forms of active specific vaccination have been devised and are being investigated this cancer. These include both cellular therapies and peptide conjugates such as rindopepimut (Rintega) (2). The latter provides active specific vaccination to EGFRvIII, which is expressed by only about 30% of glioblastomas (3).

Survivin is a common cancer-associated protein that is immunologically targetable (4). It is expressed by at least 90% of human glioblastomas and by many other non-CNS cancers as well (5). SVN53-67/M57-KLH (SurVaxM) is long survivin peptide mimic that has enhanced binding to HLA-A*02 molecules (4, 6). SurVaxM has been tested at a fixed dose of 500 µg in a first-in-man study to assess safety, tolerability and immunologic effects in patients with recurrent malignant glioma following failure of standard therapy. In that study, SurVaxM was given in emulsion with Montanide ISA 51 together with sargramostim (GM-CSF) to a total of 9 patients. The vaccine was well-tolerated. Mild (grade I) injection site reactions were common, but no serious adverse events due to the vaccine were observed. All evaluable patients developed survivin-specific CD8+ T cell responses and antibody titers to the survivin peptide and to the conjugated carrier protein KLH. Evidence of specific cytokine-mediated helper support was also observed. Median overall survival was 68 weeks from trial entry (7). This phase I study was followed by a multi-center phase II clinical trial of SurVaxM in combination with standard therapy (Stupp protocol) in patients with newly diagnosed glioblastoma being conducted at five institutions. An interim analysis of data from the first 55 patients was conducted. Patients ranged in age from 20-82 years (median = 60), male:female = 38:25 with survivin tumor expression of 1-40% (median = 12%) by immunohistochemistry. PFS-6 was 96.3% (n=55) measured from diagnosis and 62.8% (n=43) from first immunization. OS-12 was 90.9% (n=33) from diagnosis and 70.8% (n=24) from first immunization. As in the phase I study, the regimen was generally well tolerated and immunization-related adverse events were mild with no serious adverse

events directly attributable to SurVaxM. The vaccine was highly immunogenic and produced survivin-specific antibody (IgG) titers and CD8+ T-cells detectable by survivin dextramers.

Glioblastoma is known to be an immunosuppressive cancer and the microenvironment of these tumors is not highly permissive for CD8-mediated tumor cell killing (8). In addition, these tumors secrete substances that suppress systemic anti-tumoral immunity. Following surgical resection of bulk disease immune responses improve substantially. Therefore, immunotherapy is more likely to be effective in such patients following reduction of bulk disease by surgical resection. In one study of over 1,200 glioma patients, PD-1 expression on tumor-infiltrating lymphocytes was detected in 48% of gliomas and was higher in glioblastoma than in lower grade gliomas (54% vs. 30%, $p = 0.005$)(9). In addition, PD-L1 expression on tumor cells was seen in 27% and was more common in tumors with unmethylated MGMT promoters (36% vs. 18%, $p = 0.01$), which is the group of glioblastoma patients in greatest need of improved therapies (9). It is likely that PD-1 and PDL-1 expression are even higher in patients with recurrent malignant gliomas. Therefore, immune checkpoint blockade with anti-PD-1 could potentiate the effect of vaccines in such individuals.

Since there have been relatively few trials of immune checkpoint inhibitors in glioblastoma patients to date, particularly those in which PEM is combined with specific cancer vaccines, there are a number of unanswered question that the proposed study can begin to address. A fundamental question is whether PD-1 inhibitors will be more effective when combined with active specific vaccination against a common cancer antigen, such as survivin. First, it will be important to study the combination of SurVaxM and PEM to assess safety, tolerability and toxicity. Second, we plan to measure the immunologic effects of the vaccine when given together with PEM and compare those results to our first-in-man study of SurVaxM in patients with recurrent malignant glioma. Third, we plan to assess potential clinical activity of the drug combination to see if any radiologic responses are evident and to measure the effects on PFS, compared to reported historical patient cohorts with recurrent glioblastoma. Since there is ongoing study of Nivolumab in patients with newly diagnosed glioblastoma, this proposed study will allow patients who have failed previous anti-PD1 blockade to be treated with the combination of anti-PD1 and SurVaxM. These patients will be studied in a separate exploratory cohort.

2.0 OBJECTIVES

2.1 Primary **Objective:**

1. Assess clinical activity of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma using progression free survival at 6 months (PFS-6) as determined using RANO criteria

Hypothesis:
The combination of Pembrolizumab and SurVaxM improves PFS-6 in patients with recurrent glioblastoma compared to historical controls

2.2 Secondary **Objective(s)**:

1. Assess safety and tolerability of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma
2. Assess response rates of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma as determined using RANO criteria
3. Assess overall survival with the combination of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma
4. Assess PFS using Pembrolizumab and SurVaxM in patients with recurrent glioblastoma

Hypothesis:
1. The combination of Pembrolizumab and SurVaxM is safe in patients with recurrent glioblastoma
2. The combination of Pembrolizumab and SurVaxM produces responses in patients with recurrent glioblastoma
3. The combination of Pembrolizumab and SurVaxM improves overall survival in patients with recurrent glioblastoma
4. The combination of Pembrolizumab and SurVaxM improves PFS in patients with recurrent glioblastoma

2.3 Exploratory **Objective(s):**

1. Measure both cellular and humoral immune responses during concurrent administration of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma

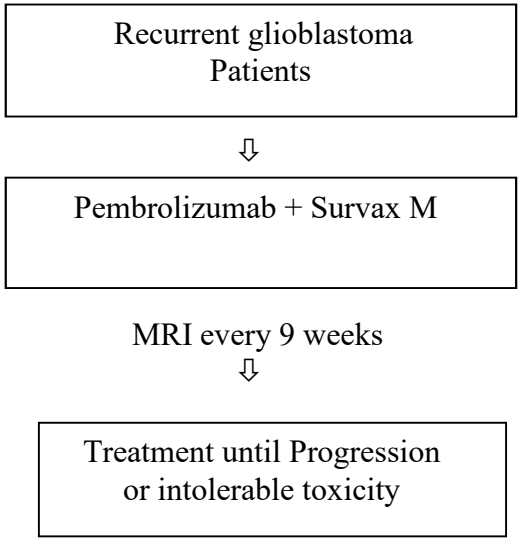
2. Correlate clinical responses with molecular markers measured on tumor cells (survivin, PDL-1, PDL-2, MGMT and IDH-1) and on peripheral blood mononuclear cells (PD-1)
3. Assess response rates of Pembrolizumab and SurVaxM in patients with recurrent glioblastoma as determined using iRANO criteria
4. Exploratory analysis of survivin expression and clinical outcome.
5. Outcomes in 10 patients with glioblastoma in first recurrence that have progressed on anti PD1 therapy (Arm B).

3.0 STUDY DESIGN

This is a Phase II study of two arms in patients with recurrent glioblastoma. Arm A is patients with first recurrence of glioblastoma who have failed prior chemotherapy and radiation but have not received any immunotherapy. Arm B is an exploratory arm of 10 patients who have failed prior anti-PD1 therapy.

This will be a phase II clinical study with a 10 patient, toxicity run-in. The study follows upon the recently completed clinical study I-171010, entitled: Phase I study of safety, tolerability and immunological effects of SVN53-67/M57- KLH (SurVaxM) in patients with survivin-positive malignant gliomas [1]. All patients will receive the study drug combination consisting of SurVaxM and pembrolizumab (PEM) with no randomization, stratification or dose escalation. The combination of PEM and SurVaxM will be tested in patients with recurrent or progressive glioblastoma following failure of standard therapy. Patients who have developed progressive or recurrent disease following treatment with surgery, fractionated external beam radiation therapy and chemotherapy with temozolomide, and who meet eligibility criteria will be enrolled. All patients must have histologic confirmation of glioblastoma and central neuropathology review of survivin expression by tumor cells. Patients will be followed on-study with regular neurologic and physical exams. While receiving PEM and SurVaxM, blood will be obtained regularly to assess hepatic, renal, electrolytes, adrenal and thyroid function. Immunologic assays will be performed prior to each dose of SurVaxM at induction and every 12 weeks thereafter, and at the off-study evaluation. Patients will be followed for up to 2 years, or until disease progression or death. Patients will be followed closely for regimen-limiting toxicity (RLT) using CTCAE v5.0 parameters. Brain MRI scans will be performed at entry and every 9 weeks thereafter (standard-of-care). Responses and tumor progression will be judged using RANO criteria and PFS6 will be recorded as the primary end-point.

Trial Diagram/Schema



3.1 Blinding/Unblinding
Non-applicable

3.2 Number of Subjects

This study consists of two arms to evaluate the anti-tumor activity of Pembrolizumab and SurVaxM and administered according to standard and reduced dosage schedules in subjects with recurrent glioblastoma.
This clinical trial will use a single stage involving a maximum accrual of 41 patients with glioblastoma at first recurrence (bevacizumab-naïve). This will include a 10-patient toxicity/safety run-in. Patients in the initial run-in will be included in the assessment of response. Patients in the run-in will be followed using CTCAE v 5.0 criteria and if the combination regimen is excessively toxic defined as dose limiting toxicity seen in more than 33% of the patients, the study will be stopped. The probabilities of observing 3, 4, and 5 DLTs out of 10 patients with true toxicity rate of 33% is 23%, 26%, and 13%. If 4 or less DLTs observed in the 10 patients, the study proceed with more patients. If 5 or more DLTs observed, accrual will be suspended and toxicity profile we be fully evaluated.
If toxicity threshold to stop will not be reached, an additional 31 patients will be enrolled for total of 41 patients.
There will an exploratory cohort of 10 patients who have failed prior PD1 blockade that will be treated with the combination of PEM + SurVaxM and results of this arm will be reported in a descriptive manner.
For this purpose, approximately 51 subjects will be enrolled in the two arms of the trial.

Week 1 of each cycle, the subject will receive both Pembrolizumab and the SurVaxM Vaccine.
On Week 3 of cycle 1, and Week 2 of cycle 2, the patient will receive the SurVaxM Vaccine alone.

Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)

4.0 SUBJECT SELECTION

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The checklist must be completed for each patient and must be signed and dated by the treating physician.

Patient's Initials _____

Patient ID_____

Research Nurse /
Study Coordinator Signature: _____ **Date** _____

Treating Physician [Print] _____

Treating Physician Signature: _____ **Date** _____

Gender:
Male and Female.

Age:
Patients must be at least 18 years of age.

Race:
Minorities will be actively recruited. No exclusion to this study will be based on race.

4.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Histologically confirmed diagnosis of World Health Organization Grade IV glioma (glioblastoma or gliosarcoma)
2. Age ≥ 18 years old
3. Previous first line treatment with at least radiotherapy with or without temozolomide
4. Documented first recurrence of GBM by diagnostic biopsy or contrast enhanced magnetic resonance imaging (MRI) performed within 21 days per RANO criteria.
5. If first recurrence of GBM is documented by MRI, an interval of at least 12 weeks after the end of prior radiation therapy is required unless there is either:
   i) histopathologic confirmation of recurrent tumor, or
   ii) new enhancement on MRI outside of the radiotherapy treatment field
6. Karnofsky performance status of 70 or higher or ECOG 0-2
7. Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to the start of study drug.
8. Previous treatment with anti PD1 will be allowed only in the exploratory arm
9. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.
10. Have provided archival tumor tissue sample or newly obtained core or excisional biopsy of a tumor lesion not previously irradiated. Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archive tissue
11. Screening/Baseline laboratory values must meet the following criteria

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.	

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

Male participants:

A male participant must agree to use a contraception as detailed in Appendix 5 of this protocol during the treatment period and for at least 180 days after the last dose of study treatment and refrain from donating sperm during this period.
For this study, male participants will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).
Female participants:
A female participant is eligible to participate if she is not pregnant (see Appendix 5), not breastfeeding, and at least one of the following conditions applies:
a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix 5
OR
b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix 5 during the treatment period and for at least 180 days after the last dose of study treatment.

4.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. A WOCBP who has a positive urine pregnancy test within 72 hours prior to If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
2. Has received prior therapy with an anti-PD-1 (except in the exploratory arm), anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX-40, CD137).
3. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks [could consider shorter interval for kinase inhibitors or other short half-life drugs] prior to allocation.

   Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible.

   Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.

4. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
5. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.
   Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.
6. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.

7. Patients that likely to have the potential risk of cerebral edema due to inflammation related to SurVaxM and pembrolizumab and will exclude patients with > 1 cm midline shift on imaging. Patients must not have cerebral edema requiring more than 10 mg daily of prednisone equivalent equivalent.

8. Has a known additional malignancy that is progressing or has required active treatment within the past 3 years. Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.

9. More than one recurrence of GBM
10. Presence of extracranial metastatic or leptomeningeal disease
11. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
12. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

13. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.

14.

- Has an active infection requiring systemic therapy

- Has a known history of Human Immunodeficiency Virus (HIV). No HIV testing is required.

15. Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority.

16. Has a known history of active TB (Bacillus Tuberculosis).
17. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

18. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

19. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 180days after the last dose of trial treatment.

5.0 REGISTRATION

All subjects who have been consented are to be registered in the OnCore® Database. The patients will be registered as per Case Comprehensive Cancer Center standard procedure. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All subjects will be registered through Cleveland Clinic and will be provided a study number by contacting the study coordinator listed on the cover page.

6.0 TREATMENT PLAN

Study drugs include both Non-investigational (NIMP) and Investigational Medicinal Products (IMP) and can consist of the following:

- All products, active or placebo, being tested or used as a comparator in a clinical trial.
- Study required pre-medication, and
- Other drugs administered as part of the study that are critical to claims of efficacy
- (e.g., background therapy, rescue medications)
- Diagnostic agents: (such as glucose for glucose challenge) given as part of the protocol requirements must also be included in the dosing data collection

6.1 Treatment Regimen Overview

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.

The investigational product should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

Vaccine Induction Phase

Patients will receive the first of four induction doses of SurVaxM in emulsion with Montanide and Sargramostim and given every other week on induction cycle 1 week 1, cycle 1 week 3, cycle 2 week 2, cycle 3 week 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.

Vaccine Maintenance Phase

SurVaxM should be administered every 12 weeks (± 2 weeks) after the induction phase until intolerance or tumor progression.

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 after the injection to obtain and record the patient's local reaction measurement.

Pembrolizumab Administration

Pembrolizumab may be administered 3 days before or after the scheduled day of each cycle due to administrative reasons (i.e., scheduling reasons). Pembrolizumab will be administered at a dose of 200 mg using a 30-minute IV infusion after all laboratory assessments have been completed and documented as reviewed and assessed as safe and acceptable by the Principal Investigator/medically-licensed and qualified designee. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes -5 min / +10 min).
Note: Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons (i.e. elective surgery, unrelated medical events, subject vacation, and holidays) not related to study therapy. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented. In the event of dosing interruptions and schedule changes, every effort should be made to revert to the original infusion schedule.

Administer infusion solution intravenously over 30 minutes (-5 to +10 minute window) through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2-micron to 5-micron in-line or add-on filter. **Do not co-administer other drugs through the same infusion line.**

Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)

LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

6.1.2 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Product Name & Potency Dosage Form

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection
SurVaxM (SVN53-67/M57-KLH) 500 mcg per dose	

6.1.3 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

6.1.4 Clinical Supplies Disclosure

This trial is open-label; therefore, the participant, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

6.1.5 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

6.1.6 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the participants and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

6.2 General Concomitant Medications and Supportive Care Guidelines

6.2.1. Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician.

6.2.2. Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs .

6.2.3 Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab and SurVaxM
- Radiation therapy

*Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.

- Live vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician. However, the decision to continue the participant on study treatment requires the mutual agreement of the investigator, the Sponsor and the participant.

6.3 Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

6.3.1 Table 3 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld, pembrolizumab may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold		• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus)

	Grade 4 or recurrent Grade 3	Permanently discontinue	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	• Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 - 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold		• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)

	Grade 3 or 4	Withhold or permanently discontinue [d]	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3, or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis and renal dysfunction: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 – 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Intolerable/persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e].		

	Grade 4 or recurrent Grade 3	Permanently discontinue		

[a] AST/ALT: >3.0 - 5.0 x ULN if baseline normal; >3.0 - 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 - 3.0 x ULN if baseline normal; >1.5 - 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 - 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 - 10.0 x ULN if baseline normal; >3.0 - 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM)

[e] Events that require discontinuation include but are not limited to: Guillain-Barre Syndrome, encephalitis, Stevens-Johnson Syndrome and toxic epidermal necrolysis.

Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4 Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of _____ with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration.
For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov

6.4. Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 6.3 [Table 3]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each is order, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab, and/or SurVaxM.
.
Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to [Table 3] in Section 6.3 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

6.5 Criteria for Removal from Study

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 8.0. – Other Procedures.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment.

- Confirmed radiographic disease progression

- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment

- Unacceptable adverse experiences as described in Section 6.3

- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, places the participant at unnecessary risk from continued administration of study treatment.

• The participant has a confirmed positive serum pregnancy test

• Noncompliance with study treatment or procedure requirements

• Recurrent Grade 2 pneumonitis

• The participant is lost to follow-up

Note: The number of treatments is calculated starting with the first dose.

• Administrative reasons
- Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years

6.5.1 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to participants

4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to participant treatment can be made.

6.6 Duration of Follow-Up

Post treatment follow up (F/U) visits will be done at 30 days post treatment. To capture all possibly delayed immune-related adverse events (irAEs), the safety follow-up period will be at least 90 days for all patients receiving, regardless of initiation of subsequent anticancer therapy.

6.6.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of study treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

6.6.2 Follow-up Visits

Participants who discontinue study treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (± 7 days) by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-cancer therapy, disease progression, death, end of the study or if the participant begins retreatment with pembrolizumab as detailed in Section 6.3.1. Information regarding post-study anti-cancer treatment will be collected if new treatment is initiated.

Participants who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 6.3.1.

6.6.3 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

7.0 DOSE DELAYS / DOSE MODIFICATIONS

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab.

8.0 ADVERSE EVENTS AND POTENTIAL RISKS

8.0.1 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency

and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment allocation must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

● All AEs from the time of treatment allocation through 30 days following cessation of study treatment must be reported by the investigator.

● All AEs meeting serious criteria, from the time of treatment allocation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.

● All pregnancies and exposure during breastfeeding, from the time of treatment allocation through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.

● Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify Merck.

8.2 Definitions

For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (75 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a nonserious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

8.3 Serious Adverse Event

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

For the time period beginning when the consent form is signed until treatment allocation, any serious adverse event, or follow up to a serious adverse event, including death due to any cause that occurs to any participant must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the

participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck Global Safety.

All participants with serious adverse events must be followed up for outcome.

8.4 Reporting Procedures for Serious Adverse Event

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

8.5 Serious Adverse Events and OnCore®

SAEs and OnCore

- All SAEs will be entered into OnCore.
- A copy of the SAE form(s) submitted to the sponsor-investigator is also uploaded into Oncore

8.5.1 FDA Reporting

The Cleveland Clinic Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. In accordance with 21 CFR 312.32, the Cleveland Clinic Principal Investigator is responsible for notifying the FDA of SAEs that are serious, unexpected (not listed in the Investigator Brochure) and judged to be related (i.e., possible, probable, definite) to the study

drug. Events meeting the following criteria need to be submitted to the FDA as Expedited IND Safety Reports.

7 Calendar Day IND Safety Report

Any unexpected fatal or life-threatening suspected adverse event represents especially important safety information and, therefore, must be reported more rapidly to FDA (21 CFR 312.32(c)(2)). Any unexpected fatal or life-threatening suspected adverse event must be reported to FDA no later than 7 calendar days after the Cleveland Clinic Investigator's initial receipt of the information (21 CFR 312.32(c)(2)). Cleveland Clinic Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission.

15 Calendar Day IND Safety Report

The timeframe for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the Cleveland Clinic Principal Investigator determines that the suspected adverse event or other information qualifies for reporting (21 CFR 312.32(c)(1)). This includes any serious, unexpected adverse events considered reasonably or possibly related to the investigational agent and that are not life-threatening or fatal. The Cleveland Clinic Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission. If FDA requests any additional data or information, the Cleveland Clinic Principal Investigator must submit it to FDA as soon as possible, but no later than 15 calendar days after receiving the request (21 CFR 312.32(c)(1)(v).

Follow-up IND Safety Report

Any relevant additional information that the Cleveland Clinic Principal Investigator obtains that pertains to a previously submitted IND safety report must be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)). The Cleveland Clinic Principal Investigator will maintain records of its efforts to obtain additional information.

Reporting Serious Problems to FDA
Medwatch Form FDA 3500A:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
Telephone: 1-800-332-1088
Fax: 301-796-9849

The fax cover sheet should note that this report will also be submitted formally in triplicate to the IND as an amendment per 21 CFR 312.32 (i.e. a formal paper submission to the Beltsville address).

Medwatch Form FDA 3500A:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
Telephone: 1-800-332-1088
Fax: 301-796-9849

The fax cover sheet should note that this report will also be submitted formally in triplicate to the IND as an amendment per 21 CFR 312.32 (i.e. a formal paper submission to the Beltsville address).

IND Annual Reports
A summary of all IND safety reports submitting during the previous year will be reported to the FDA in the annual report by the Cleveland Clinic principal investigator, as holder of the IND.A copy will be sent to Merck.

8.6 Data Safety and Toxicity Committee
It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the Case Comprehensive Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

The sponsor-investigator is responsible for submitting an annual report to the DSTC as per CCCC Data and Safety Monitoring Plan.

8.7 Data and Safety Monitoring Plan (DSMP)
This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

9.0 STUDY PARAMETERS AND CALENDAR

9.1 Calendar

	Screening -28 days to -1	**Cycle 1**			**Cycle 2**			**Cycle 3 (no vaccine at Wk 3)**			**Cycle 4+ (every 3 wks from C3 Wk 1**	**End of Treatment**	**Post-tx[1] Follow-up**
		Wk 1 D1 ± 3 days	Wk 2 ± 3 days	Wk 3 ± 3 days	Wk 1 D1 ± 3 days	Wk 2 ± 3 days	Wk 3 ± 3 days	Wk 1 D1 ± 3 days	Wk 2 ± 3 days	Wk 3 ± 3 days	Wk 1 D1 ± 3 days	At time of discontinuation	30 days post, then q. 8 wks
Treatment													
Pembrolizumab IV[1]		X			X			X			X		
SurVaxM Vaccine[2,11]		X induction		X induction		X induction		X induction			X (maintenance)		
Administrative Procedures													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics & Medical History	X												
Prior & Concomitant Meds	X												
Post-Study Anti-Cancer Therapy Status	X												
Clinical Procedures/Assessments													
Review of Adverse Events	X	X		X		X		X			X	X	
Physical Exam, including neuro[10]	X	X			X			X			X	X	
Vital Signs and Weight[3,10,13]	X	X	X	X	X	X	X	X	X	X	X	X	
ECOG Performance Status	X	X			X			X			X	X	
Laboratory Procedures/Assessments													

Pregnancy Test – urine or serum – HCG[4]	X											X	
PT/INR	X											X	
CBC with Differential[10]	X	X			X			X			X[9]	X	
CMP[5,10]	X	X			X			X			X[9]	X	
Urinalysis (micro as needed)	X	X			X			X			X	X	
T3, FT4 and TSH	X				X			X			X[9]	X	
HLA	X												
Efficacy Measurements													
Tumor Imaging[6,12]	X										X	X (if longer than 14 days from discontinuation)	
Archival Tissue or Newly Obtained Tissue Collection	X												
Survivin Status -Tissue Sample	X												
Correlative Studies Blood Collection[7]		X									X		
Correlative Studies Blood Collection for Lathia Lab for immune-phenotyping[8]		X			X			X			X		

[1] pembrolizumab is given every 3 weeks during the course of the study with window of ± 2 days in the first 3 cycles and then ± 5 days. Completion of pembrolizumab Q3W monotherapy consists of 35 treatments (approximately 2 years)
[2] The vaccine will be given before pembrolizumab. The vaccine is given every 2 weeks x 4 for induction and then every 12 weeks for maintenance. During induction vaccine can be given ± 2 days and then ± 14 days during maintenance.
[3] Including blood pressure, respiratory rate, heart rate, temperature, weight, height: height is required at baseline only.
[4] Serum pregnancy or urine test for women of child-bearing potential.
[5] CMP Including albumin, alkaline phosphatase, total bilirubin, calcium, creatinine, magnesium, phosphorus, potassium, SGOT, SGPT, sodium.
[6] MRI – Screening within 21 days; then every 9 weeks +/-2 weeks
[7] Humoral and Cellular Immune Response for Roswell Park Cancer Institute Lab -C1D1, and then every 9 weeks with MRI until progression
[8] Lathia Lab for immune-phenotyping Cleveland Clinic – C1D1, C2D1, C3D1 and then every 9 weeks with MRI until progression.
[9] CBC, CMP and thyroid function will be performed with every treatment of pembrolizumab during maintenance phase.
10 Day 1 Physical Exam and Laboratory testing not required if less the 7 days from screening
11 Cycle 4+ vaccine will be every 12 weeks

12 Cycle 4+ MRI will be every 9 weeks

13 Weight not required during rest period visit (week 4)

10.0 MEASUREMENT OF EFFECT

Tumor imaging will be obtained using contrast-enhanced magnetic resonance imaging (MRI). Modified RANO and iRANO criteria will be used in the protocol. The investigators can allow a subject to consider treatment despite radiologic PD if the subject is deriving clinical benefit as per principal investigator. The investigators decision to continue treatment should follow the RANO-defined radiological progression of disease (e.g., absence of clinical symptoms or signs indicating clinically significant disease progression; no decline in performance status; absence of rapid disease progression or threat to vital organs or critical anatomical sites requiring urgent alternative medical intervention; no significant, unacceptable or irreversible toxicities related to study treatment).

Imaging should continue to be performed until disease progression, the start of a new anticancer treatment, withdrawal of consent, death, or notification by the Sponsor, whichever occurs first. Disease progression may be confirmed 4 to 8 weeks after the first tumor imaging indicating PD, by the Investigator.

In participants who discontinue study treatment, tumor imaging should be performed at the time of treatment discontinuation (±4 week window). If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory. For participants who discontinue study treatment due to documented disease progression, this is the final required tumor imaging.

11.0 RECORDS TO BE KEPT/REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

11.1 Data Reporting

The Forte EDC™ and OnCore™ databases will be utilized, as required by the Case Comprehensive Cancer Center and Cleveland Clinic, to provide data collection for both accrual entry and trial data management. Forte EDC and OnCore™ are Clinical Trials Management Systems housed on secure servers. Access to data through Forte EDC and OnCore™ is restricted by user accounts and assigned roles. Once logged into the Forte EDC or OnCore™ system with a user ID and password, Forte EDC™ and OnCore™ define roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu for OnCore™ access, and taussigoncore@ccf.org for Forte EDC™ access.

Forte EDC™ is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. When properly utilized, Forte EDC™ is 21 CFR 11 compliant. This study will utilize electronic Case Report Form completion in the Forte EDC™ database. A calendar of events and required forms are available in Forte EDC™.

11.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

11.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject. Additionally, documentation of the consenting process should be located in the research chart.

11.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

11.2.3 Retention of records

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with local, national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

11.2.4 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. For multi-center studies, participating sites must inform the sponsor-investigator of pending audits.

12.0 CORRELATIVE / SPECIAL STUDIES

12.1 Peripheral blood and tumor tissue-based assays: Blood and tumor specimens (when available) will be collected from each patient.

12.1.2 Tissue:
Up to fifteen unstained slides of 5 microns thickness or a block of tissue will be required to be sent if tissue is available. If the tissue is not available then Principal investigator permissions is required for enrollment. If the patient undergoes recent biopsy or resection then the more recent tissue is preferred. If the patient didn't undergo any recent surgery then the tissue from diagnosis can be used. Whole exome sequencing, transcriptome analysis, tumor mutational burden. Additional markers for will be performed such as PDl- PD-1, PD-1L staining etc.

The tissue will be sent to
David Peereboom M.D.
Attn: Mary McGraw (Case) 6318
ND4-52 Lab,
Lerner Research Institute
9620 Carnegie Avenue, N Building, Cleveland, OH 44106

Survivin Expression Status will be established 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. 3 slides will be sent to central pathology core (Roswell Park Cancer Institute) for survivin testing.

The shipping label should read as follows:
Roswell Park Cancer Institute
Correlative Sciences Pathology Office, S-636
Attn: (Case) 6318
Samples
Elm & Carlton Streets
Buffalo, NY 14263
(716) 845-8917
Email: CRSLabPathTeam@RoswellPark.org

12.1.3 Blood: 1: Collect 4 green top tubes 10 ml for correlative work for Diaz Lab. Once the sample is collected it should be tubed to station 19, with a filled requisition.

Blood will be sent to the lab of Dr. Justin Lathia for analysis.
Lerner Research 2111 E. 96th St. NE4-216
Cleveland, OH 44106
Attention: Sadie Johnson

Provide advance notice by calling the lab at 216-445-7632 or emailing. Sadie Johnson at johnsos21@ccf.org.

12.1.4 Blood: 2: Collect 1 (one) green top tube 10 mL and one 3.5 mL gold top tube for humoral and cellular immune response, to be processed for PBMC and serum respectively by Dr. Justin Lathia
Blood will be sent to the lab of Dr. Justin Lathia for analysis.
Lathia Lab
Lerner Research 2111 E. 96th St.
NE3-214
Attention: Sadie Johnson
Provide advance notice by calling lab at 216-445-7632, paging Sadie at 82722 or emailing at johnsos21@ccf.org

Processed PBMC and Serum will be sent to RPCCC for analysis:
Roswell Park Comprehensive Cancer Center
Cancer Cell Center (CCC), C-416
Attn: Immune Analysis Facility
Elm & Carlton Streets
Buffalo, New York 14263
Tel: 716-845-8459
Fax: 716-845-1595
Provide advance notice by emailing Junko.Matsuzaki@RoswellPark.org; and Michael.Ciesielski@RoswellPark.org

12.1.5 Methods for Blood 1: Characterization of circulating immune cells and cytokine/chemokine profile:

Characterization of circulating immune cells: Frequencies of MDSCs, Tregs, $CD8^+$ T cells, $CD4^+$T and additional circulating immune cells will be determined by flow cytometry in both unfractionated blood and in purified PBMCs. PBMCs will be isolated from whole blood using the standard ficoll separation assay. Expression of immunomodulatory factors (PD-1, PD-1L, Lag3, Tim3, OX40, 41BB) on circulating immune cells will be also performed by flow cytometry. PBMCs will also be used for performing germline normal exome sequencing to identify somatic mutations in the tumor.

Cytokine/Chemokine profile: Plasma will be isolated from whole blood and analyzed for levels of cytokines/chemokines involved in Th1 and Th2 responses. A multiplex system that measures 50+ analytes will be used.

PLEASE DRAW:

(5) 10 ml Green top (Sodium Heparin) tubes and (1) gold top tube

Must fill tubes all the way
Mix/Invert 5-7 times after Draw
Send to Station 19
Attention Dr. Lathia Lab 216-445-7632(82722)

(DO NOT REFRIGERATE)
Send this requisition with the sample

Labeling instructions:

Blood tubes need to be labeled with the following information

1. Protocol Name
2. Subject Study Number
3. Subject Initials
4. Sample Date and Time

12.1.6 Methods for Blood 2: Blood Draws for Humoral and Cellular Immune Response

Sample Collection

Immunological analyses will be conducted on blood samples obtained prior to the patient's scheduled vaccination dosing (i.e., pre-dosing or pre-immune sample) and at study follow-up visits.

Samples will be drawn on the day of, but prior to:

- The first priming vaccine (V1),
- At disease assessment every 9 weeks (± 4 weeks), and
- At disease progression or end-of-treatment.

On each occasion, approximately 10 mL of blood will be obtained from a peripheral venipuncture or an appropriate existing venous access device and collected in one (1) green-top (heparinized) tube and one (1), 3.5 mL gold top tube. Blood tubes will be kept at room temperature until pickup for processing for shipping.

12.1.7 Processing and Shipping of Blood Samples for Immune Analysis

PBMC isolation:

Dilute blood 1:1 with RPMI in a sterile 50ml conical tube. Add 15ml ficoll to SepMate-50 tube. Holding SepMate tube at an angle, gently layer the ficoll with the diluted blood sample using a pipette. Gradually decrease angle as blood layer fills tube. Use up to 30mls of diluted blood per SepMate tube. NOTE: The pipette is touching the inside wall of the SepMate tube close to the ficoll, and the blood is allowed to gently steam onto the ficoll layer without making turbulence in the ficoll layer.

Spin for 15 minutes at 900g and 18ºC to 20ºC. Make sure the centrifuge brakes are turned off for ficoll spin

Quickly decant (pour) supernatant from SepMate into a new sterile 50ml conical tube containing 15ml RPMI for washing. Spin for 15 minutes at 400g

Resuspend the pellets in 1-2ml of 1x Red Blood Cell Lysis Buffer in water

Incubate at 37ºC for up to 5 minutes

Add PBS to increase the volume to 10ml

Centrifuge at 400 *g* for 5 minutes

Remove supernatant and resuspend in 10ml RPMI, count the cells.

Make Freezing Media (90%FBS, plus 10%DMSO, i.e. 3.6ml FBS + 400ul DMSO)

Spin PBMCs at 400 *g* for 5 minutes, remove supernatant and resuspend pellet in Freezing Media so that up to 1ml can be aliquoted per cryovial. Label tubes with all sample identification information and include cell number on tubes.

Place cryotubes in Corning CoolCell Container and place at -80C for at least 24 hours, but no longer than 1 week. Transfer cells to liquid nitrogen freezer and store until use.

Processing of Serum: Serum will be separated from whole blood in a gold top tube and frozen at -80°C.

PBMC and serum tubes will be labeled with the Subject ID # (unique to Net\work patients), initials, the participant's study number, clinical study number, protocol time point, dose number, and protocol day.

PBMC and serum samples from CCF (or network sites) 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) and Immune Analysis Facility Director : (Junko.Matsuzaki@RoswellPark.org).

Roswell Park Comprehensive Cancer Center

Cancer Cell Center (CCC), C-416

Attn: Immune Analysis Facility

Elm & Carlton Streets

Buffalo, New York 14263

Tel: 716-845-8459

Fax: 716-845-1595

Emails: Junko.Matsuzaki@RoswellPark.org; Michael.Ciesielski@RoswellPark.org

For additional information regarding the handling of samples please contact RPCCC'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 Roswell Park Immune Analysis Facility (IAF). Samples will be maintained at room temperature and processed within 24 hours.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens.

12.1.10 Immunological Analysis of Serum and PBMC

Immunological assays will be performed at Roswell Park. 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. As noted above one 10 mL green top tube 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 tube (approximately equal to that collected) will be designated for multimer studies.

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.

13.0 STATISTICAL CONSIDERATIONS

This clinical trial will use a single stage involving a maximum accrual of 41 patients with glioblastoma at first recurrence (bevacizumab-naïve). This will include a 10-patient toxicity/safety run-in. Patients in the initial run-in will be included in the assessment of response. Patients in the run-in will be followed using CTCAEv 5.0 criteria and if the combination regimen is excessively toxic defined as dose limiting toxicity seen in more than 33% of the patients, the study will be stopped. The probabilities of observing 3, 4, and 5 DLTs out of 10 patients with true toxicity rate of 33% is 23%, 26%, and 13%. If 4 or less DLTs observed in the 10 patients, the study proceed with more patients. If 5 or more DLTs observed, accrual will be suspended and toxicity profile we be fully evaluated.

If toxicity threshold to stop will not be reached, an additional 31 patients will be enrolled for total of 41 patients. PFS6 will be used as the primary end point. PFS6 = 15%; and 0.05 if PFS6 = 30%. This calculation will have type one error of 5% and type II error of 12%. If 12 or more patients are progression free at 6 months, the study would meet its primary endpoint.

Toxicities will be graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 5.

Dose-limiting toxicities (DLTs) will be defined as any of the following events occurring during the first 2 treatments with pembrolizumab and SurVaxM and within a 4 week period after that (total of 7 weeks). A DLT is defined as a clinically significant adverse event or abnormal laboratory value assessed as unrelated to disease progression, intercurrent illness, or concomitant medications and meets any of the criteria below.

Any death not clearly due to the underlying disease or extraneous cause

Hematological toxicities will be considered dose limiting if any of the following occur

- ANC of < 500/mm3.
- Platelets < 25,000/mm3.
- Febrile neutropenia lasting > one week (single episode)
- Grade 3 thrombocytopenia with clinically significant bleeding

Non-hematological toxicities will be considered dose limiting if any of the following occur:

- Grade 3 or higher non-hematologic adverse events with the allowed exceptions for
- Grade 3 nausea/vomiting or diarrhea < 72 hours with adequate antiemetic and other supportive care
- Grade 3 fatigue < 1 week
- ≥ Grade 3 electrolyte abnormality that lasts <24 to 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions
- ≥ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis
- Alopecia;
- Grade 3 hyperglycemia
- Grade 3 neurologic toxicity responding within two weeks to steroids, anticonvulsants, or electrolyte correction

A subject's first episode of deep venous thrombosis (DVT) or pulmonary embolism will not require dose modification.
There will an exploratory cohort of 10 patients who have failed prior PD1 blockade that will be treated with the combination of PEM + SurVaxM and results of this arm will be reported in a descriptive manner.

The primary and secondary goals of the trial are to evaluate the safety and efficacy of the two therapies and to obtain a preliminary assessment of whether or not they differ with respect to outcome.

The total sample size of 51 eligible and evaluable patients to the two therapies is recommended to provide adequate statistical power to describe the efficacy and toxicity profiles of the two treatment arms.

14.0 REFERENCES

[1] Venur VA, Peereboom DM, Ahluwalia MS. Current medical treatment of glioblastoma. Cancer Treat Res. 2015;163:103-15. doi: 10.1007/978-3-319-12048-5_7. Review

[2] Disis ML. Immune regulation of cancer. J Clin Oncol 2010;28(29):4531-8.

[3] Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005;23(10):2346-57.

[4] Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NYESO-1. N Engl J Med 2008;358(25):2698-703.

[5] Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515-48.

[6] Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine.Proc Natl Acad Sci U S A 2001;98(24):13866-71.

[7] Zhang X, Schwartz J-CD, Guo X, Bhatia S, Cao E, Chen L, et al. Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity 2004;20:337-47.

[8] Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol 2004;173:945-54.

[9] Sheppard K-A, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett. 2004;574:37-41.

[10] Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009;229:114-25.

[11] Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I,Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005;25(21):9543-53

[12] Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev 2010;236:219-42.

[13] Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al.iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e152. Epub 2017 Mar 2

[14] Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, et al.CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. Neuro-oncology. 2013 Nov;15 Suppl 2:ii1-56. PubMed PMID: 24137015. Pubmed Central PMCID: 3798196.

[15] Reardon DS, J.; Tran, D.; Fink, K.; Nabors, L.; Li, G.; Lukas, R.; Desjardins, A.; Ashby, L.; Duic, P.; Aneiro, L.; Hawthorne, T.; Green, J.; Yellin, M.; Davis, T.; Sampson, J. ReACT: A Phase II Study of Rindopepimut Vaccine (CDX-110) Plus Bevacizumab in Relapsed Glioblastoma. Neuro-oncology. 2014 November 2014;16(S5):116.

[16] Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, et al.A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro-oncology. 2015 Jan 13. PubMed PMID:25586468.

[17] Ciesielski MJ, Ahluwalia MS, Munich SA, Orton M, Barone T, Chanan-Khan A,et al. Antitumor cytotoxic T-cell response induced by a survivin peptide mimic.Cancer immunology, immunotherapy : CII. 2010 Aug;59(8):1211-21. PubMed PMID: 20422411.

[18] Katoh M, Wilmotte R, Belkouch MC, de Tribolet N, Pizzolato G, Dietrich PY. Survivin in brain tumors: an attractive target for immunotherapy. Journal of neuro-oncology. 2003 Aug-Sep;64(1-2):71-6. PubMed PMID: 12952288.

[19] Ciesielski MJ, Qiu J, Fenstermaker RA. Survivin as a Cancer Vaccine Target.Journal of Vaccines & Vaccination. 2014 May 24 2014;5(3):230.

[20] Fenstermaker RA, Ciesielski MJ, Qiu J, Yang N, Frank CL, Lee KP, et al. Clinical study of a survivin long peptide vaccine (SurVaxM) in patients with recurrent malignant glioma. Cancer immunology, immunotherapy : CII. 2016 Nov;65(11):1339-52. PubMed PMID: 27576783. Pubmed Central PMCID: 5069322.

[21] Fenstermaker RA, Ciesielski MJ. Immunotherapeutic strategies for malignant glioma. Cancer control : journal of the Moffitt Cancer Center. 2004 May- Jun;11(3):181-91. PubMed PMID: 15153842.

[22] Xiu J, Spetzler D, Bender R, Ghazalpour A, Gatalica Z, Reddy SK, et al. Tumor profiling on 1245 gliomas and paired tumor study on 19 high grade gliomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33:2058.

[23] Prasanna P,* Tiwari P*, Madabhushi A, "Co-occurrence of Local Anisotropic Gradient Orientations (CoLlAGe): A new radiomics descriptor", Nature Scientific Reports, 37241 (2016)

[24] Prasanna, P, Rose, A, Singh, G, Huang, R, Madabhushi, A and Tiwari, P, "Radiomic features from the necrotic region on post-treatment Gadolinium T1w MRI appear to differentiate pseudo-progression from true tumor progression in primary brain tumors", The International Society for Magnetic Resonance in Medicine (ISMRM) 24th Annual Meeting, May 2016.

APPENDICES

Appendix 1: ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix 2: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

APPENDIX 3 RANO Criteria:

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 leisions	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 measurable enhancing lesions sustained for at least 4 weeks and no new lesions or progression of no 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 measurable 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

Radiological tumor assessments will be performed at screening, as outlined in the Schedule of Assessments 6.1 and whenever disease progression is suspected. Another tumor assessment will be performed at the End of Study Visit if an assessment has not been performed within the prior 8 weeks. All patient files and radiological images must be available for CRF source verification.

IMMUNOTHERAPY RESPONSE ASSESSMENT IN NEURO-ONCOLOGY (IRANO) CRITERIA

Tumor response should be assessed every 8 weeks (+/- 1 week) for patients treated with immunotherapy using modified RANO criteria65 as outlined below. Clinicians may repeat response assessment more frequently as clinically indicated.

Anti-Tumor Effect Definitions

Evaluable for toxicity. All patients who receive at least one dose of immunotherapy treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those patients who have measurable disease present at baseline (recommend obtaining within 14 days of cycle 1, day 1) scan and have received at least one dose of immunotherapy will be considered evaluable for response.
These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

Measurable disease. For contrast-enhancing tumors, measurable disease is defined as the bidimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 slices which are at least 5 mm apart with 0 mm skip. For non-contrast-enhancing tumors, measurable disease is defined as the T2 or FLAIR lesions with a minimal diameter of 1 cm, and visible on 2 slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

Non-measurable evaluable disease. Unidimensionally measurable lesions, masses with margins not clearly defined, and/or lesions with maximal diameter < 1cm.

Response/Progression Categories

Complete response (CR). All of the following criteria must be met:
a) Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b) No new lesions.

c) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

d) Patients must be on no steroids or on physiologic replacement doses only.

e) For enhancing tumors: Stable or improved non-enhancing (T2/FLAIR) lesions

f) Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related disease

Patients with residual non-measurable disease cannot have a complete response. The best response possible is stable disease.

Partial response (PR). All of the following criteria must be met:
a) Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
b) No progression of non-measurable disease.

c) No new lesions.

d) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

e) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.

f) For enhancing tumors: Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.

g) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Patients with non-measurable disease cannot have a partial response. The best response possible is stable disease.

Progressive disease (PD). Any of the following criterion must be met:
a) > 25% increase in sum of the products of perpendicular diameters of measurable lesions (over best response [smallest tumor size] or baseline if no decrease) on stable or increasing doses of corticosteroids

b) Any new measurable lesion that when added to the change in initial tumor(s) exceeds a 25% increase in cross-sectional area.

c) Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.

d) Failure to return for evaluation due to death or deteriorating condition
Classification of progressive disease may be deferred for up to three months for patients with initial radiographic findings consistent with progressive disease (criteria a and b above) as detailed below. However, if follow-up imaging after three months confirms progression or if the patient experiences significant clinical decline at any time, the date of actual progression will be back-dated to the first date that the patient met criteria for progression and such patients should discontinue further immunotherapy.

Stable disease (SD). All of the following criteria must be met:
a) Does not qualify for CR, PR, or progression.

b) All measurable and non-measurable sites must be assessed using the same techniques as baseline.

c) Stable clinically.

Unknown response status. Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

Appendix 4

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.

SEPPIC

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.

SEPPIC

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.

Except as set forth above, SEPPIC* makes no warranties, whether express, implied or statutory, as to the product which is the subject of this document. Without limiting the generality of the foregoing, SEPPIC* makes no warranty of merchantability of the product or of the fitness of the product for any particular purpose. Buyer assumes all risk and liability resulting from the use or sale of the product, whether singly or in combination with other goods. The information set forth herein is furnished free of charge and is based on technical data that SEPPIC* believes to be reliable. It is intended for use by persons having technical skill and at their own discretion and risk. Since conditions of use are outside SEPPIC*'s control, SEPPIC* makes no warranties, express or implied, and assumes no liability in connection with any use of this information. Nothing herein is to be taken as a license to operate under or a recommendation to infringe any patents.

* SEPPIC being:

SEPPIC S.A.
22 Terrasse Bellini
92806 Puteaux
FRANCE
Tel.: +33 (0)1 42 91 40 00
info.seppic@airliquide.com

SEPPIC Inc.
Tel.: +1 973 882 5597
us.seppic@airliquide.com

SEPPIC Brasil
Tel.: +55 11 3242 3911
brasil.seppic@airliquide.com

SEPPIC Colombia SAS
Tel.: (571) 702 44 48
colombia.seppic@airliquide.com

Head Office
75, quai d'Orsay
75007 Paris
France

SEPPIC Japan Office
Tel.: 81 3 6414 6725
japan.seppic@airliquide.com

SEPPIC Asia Singapore
Tel.: +65 6278 6711
singapore.seppic@airliquide.com

SEPPIC Mumbai
Tel.: 91 22 42726464
india.seppic@airliquide.com

SEPPIC is a subsidiary of the AIR LIQUIDE Group

AIR LIQUIDE

SEPPIC Poland Office
Tel.: (48) 22 690 68 73
poland.seppic@airliquide.com

SEPPIC Dubaï Office
Tel.: +971 (4) 2395565
dubai.seppic@airliquide.com

SEPPIC China
Tel.: +86 (21) 64 66 01 49
china.seppic@airliquide.com

SEPPIC Italia Srl
Tel.: +39 02 38009110
italy.seppic@airliquide.com

SEPPIC Gmbh
Tel.: +49 (0) 2203-89830-20
germany.seppic@airliquide.com
www.seppic.com

www.seppic.com

Appendix 5: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)
A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)
Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:
Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 9 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.
  - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that has a low user dependency consistently and correctly as described in Table 9.

Table 9 Highly Effective Contraceptive Methods That Have Low User Dependency

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
• Progestogen- only contraceptive implant [a, b] • Intrauterine hormone-releasing system (IUS) [b] • Intrauterine device (IUD) • Bilateral tubal occlusion
• **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
• **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive implants are limited to those which inhibit ovulation. b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least [X days, corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential] after the last dose of study treatment.