University of Texas MD Anderson Cancer Center
Department of Neuro-oncology

TITLE: Pharmacodynamic Study of Pembrolizumab in Patients with Recurrent Glioblastoma

Product: Pembrolizumab

Support Provided By
Merck

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1.0 TRIAL SUMMARY

Abbreviated Title	Pharmacodynamic Study of Pembrolizumab in Patients With Recurrent Glioblastoma
Trial Phase	Phase 2
Clinical Indication	Recurrent glioblastoma
Trial Type	Pharmacodynamic Study
Type of control	N/A
Route of administration	200 mg , intravenously (IV) once every 3 weeks prior to surgery for two doses and then restarting 200 mg Q 3wk following surgical resection
Trial Blinding	N/A
Treatment Groups	open label, single arm
Number of trial subjects	20
Estimated duration of trial	8-9 months for trial enrollment
Duration of Participation	Each subject will participate in the trial from the time the subject signs the informed consent form through the final protocol specified contact. After a screening phase, eligible subjects will receive treatment with pembrolizumab every 3 weeks for 2 doses prior to surgical resection. Patients will have a 14-21 day recovery period from surgery and then start again to receive treatment with pembrolizumab study therapy every 3 weeks and will continue until documented confirmed disease progression, unacceptable adverse event(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, noncompliance with trial treatment or procedure requirements, subject receives 24 months of study medication, or is withdrawn for administrative reasons. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment). Subjects who discontinue for reasons other than disease progression will have post-treatment follow up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow up. All subjects will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study.

2.0 TRIAL DESIGN

2.1 Trial Design

This will be an open label, single arm exploratory biomarker-driven Phase 2 clinical trial in patients with glioblastoma at first or second recurrence. Patients must have received prior radiation and/or chemotherapy. Approximately 20 patients with recurrent glioblastoma who require reoperation for tumor progression will be treated with up to 2 doses of pembrolizumab prior to surgery (day -21 and day -1, prior to the surgery on day 0).

During the pre-surgery phase, if, after one dose of pembrolizumab , a patient experiences progressive clinical symptoms, then the second dose of pembrolizumab will be deferred and the patient will proceed to surgery to correlate imaging changes with immunologic effects within the tumor.

All patients enrolled on this clinical trial must have unstained slides from their original tumor surgery to assess PD-L1 expression. Although PD-L1 expression is not an inclusion criterion for enrollment, our preliminary data suggest that the majority of patients have >1% PDL-1 tumor expression indicating that all the patients enrolled on the trial will likely have PD-L1 expression in their archival tumor tissue specimen. In addition to the original tumor specimens, the recurrent surgical specimens will be sent for testing to compare the difference between PD-L1 expressions in recurrent and primary untreated tumor in each patient enrolled on the trial.

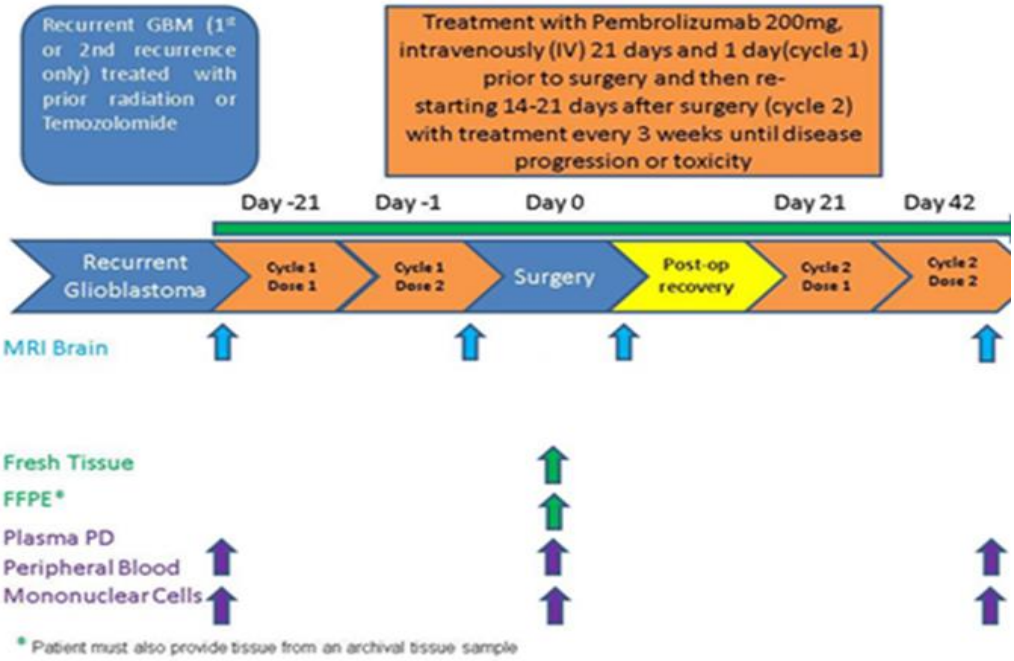
Tumor tissue from the surgical resection and peripheral blood will be evaluated longitudinally for pharmcodynamic effects including PD-L1 expression on tumor cells, changes in circulating Tregs, changes in CD3+/PD-1+ T-cells, and immune modulation. In addition, T-cells will be isolated *ex vivo* from the post-treatment surgical resection specimen and evaluated using flow cytometry for an increase in IL-2, TNF-α, IFN-γ, perforin and granzyme and other pro-inflammatory cytokines indicative of a reduction in immune suppression. Multiple imaging biomarkers will be assessed before and after pembrolizumab treatment and correlated with immunological changes in the tumor. The evaluation of tissue PD biomarkers will be correlated with circulating biomarker effects following drug exposure and PFS6 to determine if treatment of recurrent glioblastoma with pembrolizumab monotherapy will result in an increase of the polyfunctional effector T cells:Treg ratio and improve the anti-tumor immune response.

After recovery from surgery (approximately 2-3 weeks), patients will receive pembrolizumab until disease progression or the development of unacceptable toxicities. Treatment will be administered every 3 weeks. Cycles will be defined as every 42 days. Responses will be assessed by clinical examinations and MRI scans every 6 weeks. Treatment will continue until disease progression or completion of 24 months of treatment with pembrolizumab. The progression free survival at 6 months (PFS6), median duration of response, overall response rate (ORR), and overall survival (OS) will be determined.

2.2 Trial Diagram

Patients with glioblastoma at first or second recurrence who have received prior radiation and/or chemotherapy and require reoperation for tumor progression will be treated with up to 2 doses of pembrolizumab prior to surgery (21 days and 1 day prior to the surgery). During the presurgery phase, if after one dose of pembrolizumab, a patient experiences progressive clinical symptoms, then the second dose of pembrolizumab will be deferred and the patient will proceed to surgery to correlate imaging changes with immunologic effects within the tumor.

After recovery from surgery (approximately 2-3 weeks), patients will receive pembrolizumab until disease progression or the development of unacceptable toxicities. Treatment will be administered every 3 weeks. Cycles will be defined as every 42 days. Participants will remain on treatment until tumor progression, as long as there are no unacceptable toxicities or until completion of 24 months of treatment with pembrolizumab. Responses will be assessed by clinical examinations and MRI scans every 6 weeks. The MRI Brain following surgical resection will be considered the baseline MRI for evaluation of treatment response. The PFS6, median duration of response, ORR, OS will be determined.



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypotheses

Objective: The co-primary objectives of this study are to 1) evaluate immune effector function in resected glioblastoma tissue after treatment with intravenously administered pembrolizumab monotherapy in the neoadjuvant setting in patients with recurrent glioblastoma and 2) to correlate the progression free survival at 6 months (PFS6) with

objective increases in the immune effector Tcell:Treg ratio in tumor tissue as measured by *ex vivo* T-cell-specific cytokines profiling.

Hypothesis: Treatment of recurrent glioblastoma with pembrolizumab monotherapy will result in an increase of the polyfunctional effector T cells:Treg ratio and improve the anti-tumor immune response. Treatment with pembrolizumab monotherapy will improve the PFS6; Improvement in PFS6 will correlate with enhancement of the polyfunctional effector T cells:Treg ratio.

3.2 Secondary Objective(s) & Hypothesis

Objective: The secondary objectives include comparison of time to progression of last prior therapy to time to progression on pembrolizumab, median duration of response, ORR, and OS, as well as and safety.

Hypothesis: Treatment with pembrolizumab monotherapy will improve the OS in patients with recurrent glioblastoma. Improvement in OS will correlate with enhancement of the polyfunctional effector T cells:Treg ratio.

3.3 Exploratory Objective

Objective: To identify imaging characteristics associated with immunological changes in tumor following treatment with pembrolizumab.

Patients will be imaged prior to treatment and approximately 24-28 days post-first dose of pembrolizumab (within 24-28 hours of surgical resection) for surgical navigation purposes and within 24-48 hours following surgical resection to assess the extent of tumor resection. This imaging will incorporate the conventional MRI (T1 pre and post-contrast images, T2-weighted images (T2WI), Fluid attenuating inversion recovery (FLAIR) images) and advanced MRI (MR perfusion [dynamic susceptibility contrast (DSC), dynamic contrast enhancement (DCE) and arterial spin labeling (ASL)], MR- diffusion, and MR spectroscopy), and chemical exchange saturation transfer (CEST) sequences. Imaging post-processing will include but not be limited to modified RANO criteria, advanced quantitative imaging and volume tumor metrics, and voxel-by-voxel (texture, heterogeneity, energy, etc) imaging analysis. Both pre- and post-operative image analyses will include non-invasive assessments of cell death (apoptosis), active tumor proliferation, tumor invasion, tumor density, vascularity, vascular permeability, pH (acidity) microvascular density and metabolite profiles. Exploratory analyses will utilize quantitative imaging tumor metrics and texture maps to assess gene signatures of tumor cell apoptosis, invasion and immune cell infiltration. CEST imaging is non-invasive MRI technique sensitive to mobile proteins and peptides, respectively, and their tissue concentration. Exploratory analysis using quantitative imaging tumor metrics and texture maps will also be used to assess pseudoprogression, progression and response. These analyses will be compared to a retrospective cohort of glioblastoma patients that have been dichotomously analyzed for lack of inflammatory response versus robust intratumoral inflammatory responses to prospectively validate the sensitivity and specificity of this approach.

Additionally, patients will have their imaging processed prior to surgery and immediately post-op with treatment assessment response maps (TRAMs). TRAMs are a recently developed MRI-based methodology providing high resolution maps with clear differentiation between tumor/non-tumor tissues in brain tumor patients, unattainable by current imaging methods. This system is based on delayed contrast extravasation MRI where two sets of MRIs are acquired, 3 and 75 min on average after a conventional injection of contrast agent.

In order to calculate the TRAMs, conventional MRI exams will be extended by adding the delayed MRI point 60-100 min post contrast injection. The MRI exams will include standard pre- and post-contrast T1, T2, FLAIR and perfusion-weighted MRI, as routinely performed in the hospital. The patient will then be let out of the MRI system and will be asked to return for an additional T1-weighted scan 60-100 min post contrast injection.

The TRAMs will be calculated by subtracting the first T1-MRIs, acquired soon after contrast injection from T1-MRIs acquired at the delayed time point. These maps depict spatial distribution of contrast accumulation/clearance.

The MRIs are then processed to provide treatment assessment response maps (TRAMs) clearly depicting active tumor regions in one color (blue in the maps) and non-tumor regions in a different color (red). Blue/tumor regions in the TRAMs represent efficient clearance of contrast from the tissue (the signal in the delayed MRIs < than that of the 3 min images, reflecting contrast clearance) while red/non-tumor regions in the TRAMs represent contrast accumulation (the delayed signal > than the early signal). It has been demonstrated that the common feature of vessels morphology in the blue regions was undamaged vessels lumens, while vessels in the red regions presented different stages of vessel necrosis. Therefore, one explanation for the difference between the two populations may be that vessels in blue/tumor regions provide efficient contrast clearance from the tissue, while the damaged lumens in the red/TEs regions are unable to clear the accumulating contrast, resulting in contrast accumulation.

	Baseline	Pre-Op	Post-Op	1st assessment
Conventional MRI scan	X	X	X	X
Advance brain tumor imaging (ABTI)		X	X	X
Treatment assessment response maps (TRAMs) - delayed contrast (90 minute) scan		X		X
Chemical exchange transfer imaging (CEST)	X	X	X	X

4.0 BACKGROUND & RATIONALE

4.1 Background

Tumors exhibit various mechanisms of avoiding or suppressing the immune response to prevent their destruction and promote tumor growth. Programmed death 1(PD-1) is one such strategic receptor on activated T-cells that mediates immunosuppression in cancer. PD-1 ligands PD-L1 and PD-L2, present on tumor or stromal cells, interact with the PD-1 receptor causing a down regulation of the T-cell response. Prior in vitro studies have shown that inhibition of this interaction can improve T cell responses and facilitate anti-tumor activity. In vitro treatment with PD-1 is also advantageous by providing preferential activation of T-cells with specificity for the tumor avoiding immune over activation and damage to normal tissues.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [1]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [2; 3; 4; 5; 6]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells:FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

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 Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [7; 8]. The structure of murine PD-1 has been resolved [9]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is 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 (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [7; 10; 11; 12]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [13; 14]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [18; 19; 20; 13]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on

various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [13]. Although healthy organs express little (if any) PD-L1, a variety of cancers have been shown to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma [21]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

In a Phase I study of 9 patients with advanced solid tumors including non–small cell lung cancer, rectal cancer, melanoma, sarcoma and carcinoid treated with pembrolizumab (anti-PD-1 monoclonal antibody), an advanced stage melanoma patient was on therapy for more than 6 months with a partial response, and tumor reduction was seen in 3 additional patients. No Grade 3 or Grade 4 drug-related adverse events were observed.
In another recent trial consisting of patients with non-small cell lung cancer, melanoma or renal cell cancer treated with BMS-936559 (anti-PD-1 monoclonal antibody), 20-25% of patients had objective responses with durability. Furthermore, 64% of patients with response to this therapy were found to have responses lasting greater than one year. This was seen in multiple cancer types including 47% of heavily pre-treated patients (at least three prior treatment regimens). Treatment was well tolerated with only 14% (41 of 296) of patients having Grade 3 or Grade 4 treatment-related adverse events. PD-L1 tumor expression appeared to play a role in response to therapy as 36% (9/25) of patients with PD-L1 positive tumors had objective responses whereas 0% (0/17) of patients with PD-L1 negative tumors responded to therapy.

The anti-tumor immune reactivity within glioblastoma has been extensively studied and although there are quantitative anti-tumor immune effector cells present within the glioblastoma microenvironment, these immune cells are anergic (non-reactive) (Hussain, Neuro-Oncology, 2006). The purpose of the study is to provide information on whether Pembrolizumab can specifically restore the qualitative anti-tumor effector responses (i.e. TNF-α, IFN-γ, granzyme B) in the glioblastoma microenvironment. If Pembrolizumab is not able to restore anti-tumor effector responses to any degree, then it is unlikely that radiation induced antigenic shedding would provide much additional benefit since the tumor-mediated immune suppression is not sufficiently reversed with this particular monotherapy.

It should be noted that the preclinical published study of a clonotypic glioma treated with radiation in combination with checkpoint inhibition demonstrates only a very modest

increase in survival (Zeng, Int. J of Rad. Oncol. Biol. Phys. 2013). This particular model system has well documented limitations for assessing the efficacy of immunotherapy and recapitulating the biology of human glioblastoma. More recent published studies have demonstrated that monotherapy targeting the PD-1/PD-L1 axis resulted in a 200% increase in survival with 60% animals cured in comparison to the Zeng study in which there was only a 8% increase in median survival. Furthermore, prior preclinical studies targeting another checkpoint, CTLA-4, demonstrated robust cures with >70% of mice harboring tumors surviving long-term (Fecci, CCR, 2007). If the submitted clinical trial were to demonstrate that Pembrolizumab were to enhance the functional anti-tumor immune responses, then we would possibly consider an efficacy clinical trial of the combination with radiation but preclinical indicates that there are other superior combinatorial approaches that are more compelling (Wainwright, CCR, 2014).

4.2.1 Rationale for the Trial and Selected Subject Population

The robust clinical responses seen in a diverse group of advanced stage tumors suggests that PD-1 antibody therapy in other tumors may also be beneficial. Glioblastoma is the most common primary brain tumor in adults and is notorious for high levels of immune suppressive chemokines and high numbers of infiltrating regulatory T-cells which mediate immune suppression. A recent study in an immune competent orthotopic mouse glioma model was done examining the combination of anti-PD-1 immunotherapy with radiation. Improved survival was seen in mice who received both radiation and anti-PD1 therapy with a median survival of 53 days in contrast to control mice, mice treated with anti-PD-1 antibody monotherapy, or mice treated with radiation monotherapy that had a median survival of 25, 27, and 28 days, respectively. Additionally, long term survival was also significantly improved in those mice that had received the combination with 20-40% alive at 6 months following treatment. These data suggest that PD-L1 expressing glioma tumors may benefit from anti-PD-1 targeted therapy.

Using both immunohistochemical (IHC) staining of a glioblastoma tumor microarray and ex vivo flow analysis cytometry, we have found that PD-L1 is frequently expressed in most glioblastomas. Using IHC, PDL-1 expression ranged from 1-99% whereas ex vivo flow cytometry of freshly isolated glioblastoma tumors demonstrated 4-7% of glioma cells expressing PD-L1.

Anti-PD-1 antibodies have demonstrated a significant treatment response in several advanced solid tumors in clinical trials as well as improved survival in mouse models of glioblastoma. Anti-PD-1 therapy appears to be a very promising treatment and one that deserves further examination in patients with glioblastoma. Specifically, the impact of immunotherapy on tumor biology is currently unexplored and a better understanding of the pharmacodynamics effect of immunotherapy on gliomas will greatly assist in the development of this therapeutic approach in patients with glioblastoma.

The new data has been obtained lately from glioblastoma tissue microarrays using different antibodies to detect expression and another group has published this as an under estimate. Specifically, PD-L1 expression (the ligand for PD-1) was observed in 103 of 117 (88.0%)

newly diagnosed and 13 of 18 (72.2%) recurrent glioblastoma specimens (Berghoff, Neuro-Oncology, 2014). In this latter study, the vast majority of glioma cells (>85%) were shown to express PD-L1. Regardless, we would like to emphasize that Pembrolizumab is not a direct cytotoxic molecular targeted therapeutic – the principals are different. The cut point of expression that is required that would induce significant tumor-induced immune suppression or the level of expression that correlates to a therapeutic response is unknown. For example, if 1% expression is sufficient to induce profound immune suppression, then this reversal would afford the immunological recognition and elimination of glioblastoma cells expressing tumor antigens (i.e. bystander effect). Glioblastoma are antigenically diverse and immunologically reactive (Doucette, CIR, 2013) commonly possessing tumor-specific antigens such as epidermal growth factor variant III (Heimberger, CCR, 2005) that have been robustly targeted for phase III registration clinical trials (Heimberger/Sampson, JCO, 2010; Sampson, NO, 2010). It should be noted that these clinical trials mandated gross total resections to eliminate/reduce tumor mediated immune suppression. As such, if the immune system were not restrained by immune suppressive mechanisms such as by immune checkpoints, available data indicates that the tumor would be eradicated/eliminated even if only a minor population is mediating that effect.

4.2.2 Rationale for Dose Selection/Regimen/Modification

The dose regimen of 200 mg Q3W of pembrolizumab is planned for all urothelial cancer trials. Available PK results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in PK exposures obtained at a given dose among tumor types. An open-label Phase 1 trial (PN001) in melanoma subjects is being conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No maximum tolerated dose (MTD) has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving pembrolizumab at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed. The clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive pembrolizumab at 2 mg/kg versus 10 mg/kg Q3W. The overall response rate (ORR) was 26% (21/81) in the 2mg/kg group and 26% (25/79) in the 10 mg/kg group (full analysis set (FAS)). The proportion of subjects with drug-related adverse events (AEs), grade 3-5 drug-related AEs, serious drug-related AEs, death or discontinuation due to an AE was comparable between groups or lower in the 10 mg/kg group.

Available pharmacokinetic results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in pharmacokinetic exposures obtained at a given dose among tumor types. Population PK analysis has been performed and has

confirmed the expectation that intrinsic factors do not affect exposure to pembrolizumab to a clinically meaningful extent. Taken together, these data support the use of lower doses (with similar exposure to 2 mg/kg Q3W) in all solid tumor indications. 2 mg/kg Q3W is being evaluated in NSCLC in PN001, Cohort F30 and PN010, and 200 mg Q3W is being evaluated in head and neck cancer in PN012, which are expected to provide additional data supporting the dose selection.

Selection of 200 mg as the appropriate dose for a switch to fixed dosing is based on simulation results indicating that 200 mg will provide exposures that are reasonably consistent with those obtained with 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. A population PK model, which characterized the influence of body weight and other patient covariates on exposure, has been developed using available data from 476 subjects from PN001. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose, with some tendency for individual values to range slightly higher with the 200 mg fixed dose. The slight increase in PK variability predicted for the fixed dose relative to weight-based dosing is not expected to be clinically important given that the range of individual exposures is well contained within the range of exposures shown in the melanoma studies of 2 and 10 mg/kg to provide similar efficacy and safety. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different tumor types and indication settings.

The company (MERCK) *mandated* that a starting dose of 200 mg IV every 3 weeks be used for this trial. This is the same dose being used in another trial in glioblastoma being started at Dana Farber Cancer Institute. This decision was made by the company based on their own internal efficacy data.

4.2.3 Rationale for Endpoints

Pharmacodynamic (PD) effects of intravenous administered pembrolizumab monotherapy in resected recurrent glioblastoma tissue have yet to be explored. This study will examine if improved PFS6 correlates with objective reversal of immunosuppression in tumor tissue as measured by ex vivo T-cell-specific cytokine profiling.

4.2.3.1 Efficacy Endpoints

Tumor tissue from the surgical resection and peripheral blood will be evaluated longitudinally for pharmcodynamic effects including an increase of the polyfunctional effector T cells:Treg ratio and improvement in the anti-tumor immune response. The evaluation of tissue PD biomarkers will be correlated to PFS6 with objective increases in immune effector function (IFN-γ. IL-2, TNF-α, etc) in tumor tissue as measured by ex vivo T-cell-specific cytokines profiling. Improvement in PFS6 will be examined for correlation with enhancement of the polyfunctional effector T cells:Treg ratio.

The immune system can and does cross the blood brain barrier; however anti-tumor effects are mitigated by tumor mediated immune suppression. Pembrolizumab works by releasing

the break on the immune system (immune checkpoints). Since it is the immune system that mediates the direct tumor effects, the question the study is intending to address is whether the immune system remains activated with the glioblastoma microenvironment by using Pembrolizumab. Thus, the query regarding whether the drug gets past the BBB is inconsequential and needs to be reframed in the context of whether the drug can induce a functional anti-effector response within the CNS tumor microenvironment - which is being directly addressed by the study.

Progression-free survival, defined as the time from study enrollment until the time of first occurrence of disease progression, relapse, or death due to disease. Patients who are alive without progression or relapse will be censored at the time of last contact. The point estimate of 6-month progression-free survival (PFS6) will analyzed.

Additionally, secondary objectives include comparison of time to progression of last prior therapy to time to progression on pembrolizumab, median duration of response, ORR, and OS will be monitored to further help evaluate efficacy of treatment of recurrent glioblastoma withpembrolizumab.

4.2.3.2 Biomarker Research

Multiple imaging biomarkers will be assessed before and after pembrolizumab treatment and correlated with pharmacodynamic changes in tumor (see section 3.3).
Depending on funding and tissue availability, other studies to be performed will include mutation assessment, transcriptiome analysis (RNAseq) and copy number determination. Protein phosphorylation analysis using RPPA will be performed if fresh tissue is available.

5.0 METHODOLOGY

5.1 Entry Criteria

Patients must have pathology confirmed WHO grade 4 glioblastoma, at first or second recurrence who require reoperation for tumor progression and have received prior radiation and/or chemotherapy.

All patients enrolled on this clinical trial must have unstained slides from their original tumor surgery to assess PD-L1 expression.

A baseline brain MRI will be obtained no less than 14 days prior to study enrollment, on a stable dose of steroids no greater than 2 mg a day of dexamethasone for at least 5 days, and is required prior to entrance of a patient onto the study.

5.1.1 Diagnosis/Condition for Entry into the Trial

Recurrent glioblastoma, at first or second relapse, requiring reoperation for tumor progression and having already received prior radiation and/or chemotherapy.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Have histiologically confirmed World Health Organization Grade IV malignant glioma (glioblastoma or gliosarcoma). Participants will be eligible if the original histology was low-grade glioma and a subsequent histiological diagnosis of glioblastoma or variants is made.
4. Patients must be at first or second relapse and clinically require reoperation for tumor progression within 4 to 6 weeks.. **Note:** Relapse is defined as progression following initial therapy (i.e., radiation, chemotherapy, or radiation+ chemotherapy). If the participant had a surgical resection for relapsed disease and no antitumor therapy instituted for up to 12 weeks, this is considered one relapse. For participants who had prior therapy for a low grade glioma, the surgical diagnosis of a high grade glioma will be considered first relapse.
5. Have measurable disease consisting of a minimal volume of 1 cm^3.
6. Have provided tissue from an archival tissue sample or newly obtained core or excisional biopsy of a tumor lesion.
7. Have a performance status of ≥ 60 on the KPS.
8. Stable dose of steroids for 5 days, no more than 2 mg dexamethasone (or equivalent) total per day
9. Demonstrate adequate organ function as defined in

10. Table 1, all screening labs should be performed within 14 days prior to registration (Reference Table 1).

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

11. Female subject of childbearing potential should have a negative serum pregnancy test.
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12. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.
13. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Has been treated previously with bevacizumab
2. Has tumor localized primarily to the brainstem or spinal cord.
3. Has received prior interstitial brachytherapy, implanted chemotherapy, or therapeutics delivered by local injection or convection enhanced delivery.
4. Is currently participating in or has participated in a study of an investigational agent or using an investigational device 4 weeks since last dose of agent administration.
5. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy > 2 mg of dexamethasone total per day or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
6. Has had a prior monoclonal antibody within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
7. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Subjects with alopecia, ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.
9. Has known carcinomatous meningitis, extracranial diseaese, or multifocal disease.
10. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.
11. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.
12. Has an active infection requiring systemic therapy.
13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation

for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment.
16. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
17. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies). Testing not required.
18. Has known history of Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). Testing not required.
19. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2

Table 2 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	3 weeks	IV infusion	24 months	Experimental
The Pembrolizumab dosing interval may be increased due to toxicity as described in Section 5.2.1.2.					

Trial treatment should begin on the day of registration or as close as possible to the date on which treatment is allocated/assigned.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

The dose amount of Pembrolizumab will be 200 mg every 3 weeks.

5.2.1.2 Dose Modification

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs as per Table 3 below.

Table 3: Dose modification guidelines for drug-related adverse events.

Toxicity	**Grade**	**Hold Treatment (Y/N)**	**Timing for restarting treatment**	**Dose/Schedule for restarting treatment**	**Discontinue Subject**
Hematological Toxicity	1, 2	No	N/A	N/A	N/A

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
	3* *Excluding Grade 3 neutropenia, anemia, and thrombocytopenia	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	
Non-hematological toxicity Note: Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference Section 5.6.1.1.	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to Grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule (reference Section 5.6.1.2 for recommendations regarding pneumonitis) *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	3, 4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued. At the discretion of the treating physician, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of adverse events, see Section 5.6.1.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be discontinued from trial treatment.

5.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 5.2.1.2). 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 of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

This will be an open label, single arm exploratory biomarker-driven Phase 2 clinical trial and thus no randomization is necessary.

5.4 Stratification

As an open label, single arm exploratory biomarker-driven Phase 2 clinical, no stratification is necessary. Known prognostic factors such as age, Karnofsky performance status prior to treatment and extent of surgical resection may be retrospectively examined.

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

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 subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator and the subject.

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject’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 as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

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

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy

  Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

  Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. Patients must be on a stable dose of steroids no greater than 2 mg a day of dexamethasone for at least 5 days prior to entrance of a patient onto the study. Patients should be maintained on the lowest dose of steroids possible following surgery. The use of physiologic doses of corticosteroids may be approved after consultation with the supporting Sponsor (Merck).

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial.
Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator including but not limited to the items outlined below:

- Diarrhea: Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic subjects, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered.
  - In subjects with severe enterocolitis (Grade 3), pembrolizumab will be permanently discontinued and treatment with systemic corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month.
  - In subjects with moderate enterocolitis (Grade 2), pembrolizumab should be withheld and anti-diarrheal treatment should be started. If symptoms are

persistent for more than one week, systemic corticosteroids should be initiated (e.g., 0.5 mg/kg/day of prednisone or equivalent). When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month. Regarding guidelines for continuing treatment with pembrolizumab, see Section 5.2.

- All subjects who experience diarrhea 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.

- Nausea/vomiting: Nausea and vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Subjects should be strongly encouraged to maintain liberal oral fluid intake.
- Anti-infectives: Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice.
- Immune-related adverse events: Please see Section 5.6.1.1 below and the separate guidance document in the administrative binder regarding diagnosis and management of adverse experiences of a potential immunologic etiology.
- Management of Infusion Reactions: Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

Table 5 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab .

Table 5 Infusion Reaction 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 subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS,	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one 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 subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
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: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room 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		

5.6.1.1 Supportive Care Guidelines for Events of Clinical Interest and Immune-related Adverse Events (irAEs)

Events of clinical interest of a potential immunologic etiology (irECIs) may be defined as an adverse event of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. irAEs may be predicted based on the nature of the pembrolizumab compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment.

If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAE. Information on how to identify and evaluate irAEs has been developed and is included in the Event of Clinical Interest and Immune-Related Adverse Event Guidance Document located in the Administrative Binder.

Recommendations to managing irAEs not detailed elsewhere in the protocol are detailed in Table 6.

Table 6 General Approach to Handling irAEs

irAE	Withhold/Discontinue pembrolizumab ?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold pembrolizumab	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold pembrolizumab Discontinue if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 12 weeks of toxicity	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks.

5.6.1.2 Supportive Care Guidelines for Pneumonitis

Subjects with symptomatic pneumonitis should immediately stop receiving pembrolizumab and have an evaluation. The evaluation may include bronchoscopy and pulmonary function tests to rule out other causes such as infection. If the subject is determined to have study drug associated pneumonitis, the suggested treatment plan is detailed in Table 7.

Table 7 Recommended Approach to Handling Pneumonitis

Study drug associated pneumonitis	Withhold/Discontinue pembrolizumab ?	Supportive Care
Grade 1 (asymptomatic)	No action	Intervention not indicated
Grade 2	Withhold pembrolizumab , may return to treatment if improves to Grade 1 or resolves within 12 weeks	Systemic corticosteroids are indicated. Taper if necessary.
Grade 3 and Grade 4	Discontinue pembrolizumab	Systemic corticosteroids are indicated. The use of infliximab may be indicated as appropriate. Refer to the Event of Clinical Interest and Immune-related Adverse Event Guidance Document for additional recommendations.

For Grade 2 pneumonitis that improves to ≤ Grade 1 within 12 weeks, the following rules should apply:

- First episode of pneumonitis
  - May increase dosing interval by one week in subsequent cycles
- Second episode of pneumonitis – permanently discontinue pembrolizumab if upon rechallenge subject develops pneumonitis ≥ Grade 2

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to Merck and followed as described above and in Section 7.2.2.

5.7.4 Use in Nursing Women

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, subjects who are breast-feeding are not eligible for enrollment.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be

withdrawn by the investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression
  Note: For unconfirmed radiographic disease progression, please see Section 5.2.2
  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 7.1.2.7.1
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of treatment with pembrolizumab
  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.5.2.1.
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.9 Subject Replacement Strategy

Additional subjects may be enrolled to ensure that the required number of evaluable subjects is achieved. A subject that discontinues the trial for progressive disease or a drug related adverse event will not be replaced and will be counted in the evaluable population of subjects.

5.10 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 subjects
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 subject treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screen Phase**	**Treatment Phase**										**End of Treatment**	**Post-Treatment**	
							To be repeated beyond 4 cycles							
Treatment Cycle*/Title:	Main Study Screening	C1D1	C1D21	surgery	C2D1	C2D21	C3D1	C3D21	C4D1	C4D21		Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window* (Days):	≤ 14 days prior to registration	-21	-1	0								30 days post discon	Every 8 weeks post discon	Every 12 weeks
Informed Consent	x													
Inclusion/Exclusion Criteria	x													
Demographics and Medical History	x													
Prior and Concomitant Medication Review	x	x	x		x	x	x	x	x	x		x		
Prior therapy data obtained	x													
Trial Treatment Administration		x	x		x	x	x	x	x	x				
Post-study anticancer therapy status												x		
Survival Status		x	x		x	x	x	x	x	x		x		x
Review Adverse Events		x	x		x	x	x	x	x	x		x		
Full Physical Examination	x	x			x		x		x			x		
Directed Physical Examination			x			x		x		x				
Vital Signs and Weight	x	x	x		x	x	x	x	x	x		x		
Karnofsky Performance Status	x	x	x	x	x	x	x	x	x	x		x		
	***Treatment day can have a +3 day window, excluding C1D1. All other clinical procedures and assessments have a -3 day window of the scheduled infusion.**													
Pregnancy Test – Serum β-HCG	x	x												
PT/INR and aPTT	x	x												
CBC with Differential	x	x	x	x	x	x	x	x	x	x		x		
Comprehensive Serum Chemistry Panel (see Table 9)	x	x	x	x	x	x	x	x	x	x		x		
Urinalysis	x	x												
T3, FT4 and TSH	x	x												
Tumor Imaging	x		x**		x**		x		x				x****	

Trial Period:	**Screen Phase**	**Treatment Phase**										**End of Treatment**	**Post-Treatment**	
							To be repeated beyond 4 cycles							
Treatment Cycle*/Title:	Main Study Screening	C1D1	C1D21	surgery	C2D1	C2D21	C3D1	C3D21	C4D1	C4D21		Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window* (Days):	≤ 14 days prior to registration	-21	-1	0								30 days post discon	Every 8 weeks post discon	Every 12 weeks
Archival or Newly Obtained Tissue Collection	x			x										
Correlative Studies Blood Collection***	x			x			X***					X***		

** C1D21 - within 72 hours prior to surgery. C2D1 - within 72 hours post surgery.
*** Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at progression
**** Subjects other than disease progression will be assessed every 8 weeks

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. The treatment day can have a +3 day window, excluding C1D1. All other clinical procedures and assessments have a -3 day window of the scheduled infusion. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.
Laboratory assessments performed as part of the screening evaluations and within 72 hours of the first dose of study treatment, are not required to be repeated on the first dosing day.

Furthermore, additional evaluations/testing may be deemed necessary by Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.6 Assignment of Screening Number

7.1.1.7 Assignment of Randomization Number

This will be an open label, single arm exploratory biomarker-driven Phase 2 clinical trial and thus no randomization is necessary.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 12.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irAE). See Section 5.6.1.1 and the separate guidance document in the administrative binder regarding the identification, evaluation and management of AEs of a potential immunological etiology.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Karnofsky Performance Scale (KPS)

The investigator or qualified designee will assess KPS status (see Section 12.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

A baseline MRI brain obtained no less than 14 days prior to study enrollment, on a stable dose of steroids no greater than 2 mg daily for at least 5 days, is required prior to entrance of a patient onto the study.

An MRI Brain will also be obtained within 72 hours prior to and within 72 hours after surgical resection. After recovery from surgery (approximately 2-3 weeks), patients will receive pembrolizumab until disease progression, the development of unacceptable toxicities or completion of 24 months of treatment with pembrolizumab. Treatment will be administered every 3 weeks. Cycles will be 42 days. Participants will remain on treatment until tumor progression, as long as there are no unacceptable toxicities.

Imaging response criteria will be based on a modified version of the RANO scale (appendix 12.3). Tumor response will be assessed with MRI scans every 6 weeks following surgery using modified RANO criteria (appendix 12.3) as outlined. Clinicians may repeat response

assessment more frequently as clinically indicated. The MRI Brain following surgical resection will be considered the baseline MRI for evaluation of treatment response.

Measurement of effect

Anti-Tumor Effect Definitions

Evaluable for objective response. Only those participants who have measurable disease present at baseline scan and have received at least one dose of therapy will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

Measurable disease. Bi-dimensionally, contrast-enhancing, measurable lesions with clearly defined margins by MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial 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 before a participant is entered on study. 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, 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) Participants must be on no steroids or on physiologic replacement doses only.
e) 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

Participants with 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 enhancing 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) 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.

Participants 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 enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids
b) Any new enhancing measurable lesion
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

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.

Methods for Evaluation of Measurable Disease
All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed within 14 days prior to registration.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

<u>Evaluation of Best Response</u>
The best overall response is the best response recorded from the start of the treatment until disease progression (taking as reference for progressive disease the smallest measurements

recorded since the treatment started). If a response recorded at one scheduled MRI does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, i.e. still present on the subsequent MRI at least four weeks later, it will be recorded as a sustained response, lasting until the time of tumor progression. Participants without measurable disease may only achieve SD or PD as their best "response."

Study Continuation Beyond Initial Progressive Disease

RANO will be adapted to account for the potential tumor response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RANO may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab.

Therefore, the following adaptations of the RANO criteria will be used to assess response for patients treated on this study (Table 1):

• If radiologic imaging shows initial PD, tumor assessment should be repeated 4 weeks later in order to confirm PD with the option of continuing treatment as described below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued / resumed. If repeat imaging confirms progressive disease, defined as a 25% increase from baseline or best response, then the date of disease progression will be the first date the subject met criteria for progression and subjects will be discontinued from study therapy. Subjects who have confirmed disease progression will discontinue study medication and enter the follow up/survival phase of the study. In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions.

• Immune based therapies are expected to be associated with inflammatory changes that may include edema. RANO expanded the previously utilized Macdonald criteria to include the development of "significantly" increased T2 or FLAIR abnormality in the definition of progressive disease because such changes can be a major component defining radiographic progression following therapeutic use of VEGF/VEGFR-targeting therapeutics which are known to elicit potent anti-permeability changes that limit contrast uptake. Our study will define radiographic progressive disease by assessment of enhancing tumor burden and will not incorporate assessment of T2 or FLAIR changes as outlined in RANO because:
(1) there is no expectation that immunotherapy agents will falsely diminish enhancing tumor burden as has been noted with anti-angiogenic therapies; and
(2) immune based therapies may be associated with increased edema and associated T2/FLAIR changes which may inaccurately be interpreted to represent tumor progression (i.e. pseudoprogression).

In subjects who have initial evidence of radiographic PD, it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained a

minimum of 4 weeks later. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive study treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:
• The subject is believed to demonstrate clinical benefit from the study regimen as determined by the treating physician;
• The subject is adequately tolerating study therapy.

When feasible, subjects should not be discontinued until radiographic progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are exhibiting significant neurologic decline are not required to have repeat imaging for confirmation of progressive disease.

Table.1 Imaging and Treatment after 1st Radiologic Evidence of PD

	No Significant Neurologic Decline		Significant Neurologic Decline	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD	Repeat imaging >4 weeks later to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging >4 weeks later to confirm PD	Discontinue Treatment
Repeat scan confirms PD	No additional imaging required	Discontinue Treatment	No additional imaging required	Not applicable
Repeat scan confirms SD,PR, or CR	Continue regularly scheduled imaging assessments every 6 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 6 weeks	May restart study treatment if condition has improved and/or clinically stable per investigators discretion

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

All patients enrolled on this clinical trial must have unstained slides from their original tumor surgery to assess PD-L1 expression. In addition to the original tumor specimens, the recurrent surgical specimens will be sent for testing to compare the difference between PD-L1 expressions in recurrent and primary untreated tumor in each patient enrolled on the trial. Pembrolizumab pharmacokinetics from the blood will be correlated with other tumor pharmacodynamics markers. Tumor tissue from the surgical resection and peripheral blood will be evaluated longitudinally for pharmcodynamic effects. The evaluation of tissue PD

biomarkers will be correlated with circulating biomarker effects following drug exposure and PFS6 to determine if treatment of recurrent glioblastoma with pembrolizumab monotherapy will result in an increase of the polyfunctional effector T cells:Treg ratio and improve the anti-tumor immune response.

Biomarker Collection			
Specimen Type	*Lab Test*	*Time of collection*	*Lab Performing Test*
Blood			
	Serum cytokines profile: CSF-1, IL-6, TGF-β, VEGF, IL-10, IL-23 (20mL - Heparin Tubes)	Pre-dose Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at progression	MDACC Core Immunotherapy lab
	Th1 vs Th2 phenotyping: IL-2, IL4, IL-10, IL-13, INF-γ, TNF-α (20mL - Heparin Tubes)	Pre-dose Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at progression	MDACC Core Immunotherapy lab
	T-cell subtypes (CD3, CD4, CD8, FoxP3, CD25, etc.) including their functional status based on intracellular cytokines; effector to suppressor ratios (CD4:Treg; CD8:Treg) (20mL - Heparin Tubes)	Pre-dose Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at progression	MDACC Core Immunotherapy lab
	CD8+ CMV pp65 specific tetramer positive cells (30mL - Heparin Tubes)	Pre-dose Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at progression	MDACC Amy Heimberger lab
	Future correlative analyses 20mLs – Heparin Tubes	Pre-dose Prior to cycle 1 (baseline), at the time of surgery, prior to cycle 3, 6, and 9 and at	MDACC Amy Heimberger lab

		progression	
Tissue			
Fresh	T-cell subtypes (CD3, CD4, CD8) including their functional status via intracellular cytokine profiling (IL-2, IFN-γ, TNF-α, perforin, granzyme)	Day of Surgery	MDACC Core Immunotherapy lab or Amy Heimberger Lab
	PD-L1 expression on tumor cells and immune cell subtypes (either using a commercial antibody or a proprietary antibody provided by Merck, as available)	Day of Surgery	MDACC Amy Heimberger Lab or Core Immunotherapy lab
Formalin-fixed paraffin embedded tissue; 30 slides	PD-L1 (CD 274) and PD-L2 (CD 273	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab (PD-L2) QualTek Molecular Laboratories (PD-L1)
	Macrophages (CD68 and CSF1R)	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab
	Microvascular density (CD31)	Day of Surgery	MDACC Amy Heimberger Lab
	M2-skewed macrophage markers (CD163)	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab
	Hypoxia (carbonic anhydrase-9; CA9 and HIF1-α)	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab
	Tregs (FoxP3+)	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab
	CMV viral antigen pp65 expression	Day of Surgery	MDACC Amy Heimberger Lab or Wistuba Lab
	Additional CMV	Day of Surgery	MDACC Amy

	antigens may be assayed (i.e. gB, IE1) as well as non-viral related glioblastoma-specific and tumor-associated antigens (i.e. EGFR, EGFRvIII, survivin, IL-13R)		Heimberger Lab or Wistuba Lab

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

7.1.3.1 Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 9.

Table 9 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
	(CO_2 or biocarbonate)		Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only.

Laboratory tests for screening should be performed within 14 days prior to registration. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.2.1. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.4.2 Blinding/Unblinding

This is an open-label trial; therefore, the investigator and subject will know the treatment administered thus no blinding/unblinding is necessary.

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening

7.1.5.1.1 Screening Period

Any patient with recurrent glioblastoma at first or second relapse that is being considered for surgical resection will be eligible for screening. Screening will include vital signs, physical exam, and determination of availability of archival tumor tissue for PD-L1 staining, and laboratory evaluations described in Table 1. The window for screening will consist of 14 days prior to registration.

7.1.5.2 Treatment Period

Patients will be treated with up to 2 doses of pembrolizumab prior to surgery (day -21 and day -1 prior to the surgery).

During the presurgery phase, if, after one dose of pembrolizumab, a patient experiences progressive clinical symptoms, then the second dose of pembrolizumab will be deferred and the patient will proceed to surgery to correlate imaging changes with immunologic effects within the tumor.

After recovery from surgery (approximately 2-3 weeks), patients will receive pembrolizumab until disease progression, the development of unacceptable toxicities, or until completion of 24 months of treatment with pembrolizumab. Treatment will be administered every 3 weeks. Cycles are 42 days. Participants will remain on treatment until tumor progression, as long as there are no unacceptable toxicities until completion of 24 months of treatment with pembrolizumab.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial 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. Subjects 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-neoplastic 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.

7.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (56 ± 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-neoplastic therapy, disease progression, death, end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

7.1.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and will be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject 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. Lab abnormalities will be considered an abnormal event if clinically significant. Clinically significant will be defined if requiring a holding of the treatment, a dose reduction of the treatment or a new prescription is given. 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.
Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.
Abnormal laboratory values or test results occurring after informed consent constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study medication(s).

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and

symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

Adverse events will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, corresponding to grades 1 - 4, will be used. CTCAE grade 5 (death) will not be used in this study; rather, information about deaths will be collected though a Death form.

The occurrence of adverse events should be sought by non-directive questioning of the patient (subject) during the screening process after signing informed consent and at each visit during the study. Adverse events also may be detected when they are volunteered by the patient (subject) during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:
1. The severity grade (CTCAE grade 1-4)
2. Its duration (Start and end dates)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequalae, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in Section 7.2.3.

Surgical resection of recurrent GBM is standard of care in the study and is not required to be reported as an AE. However medical complications related to this surgery must be reported as AE.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The PI or physician designee is responsible for providing source documentation and assigning attribution for all AEs.
Adverse events will not be collected for subjects during the screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to Merck

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose,

pembrolizumab should be discontinued and the subject 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 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.2 Reporting of Pregnancy and Lactation to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant 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 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.3 Immediate Reporting of Adverse Events to Merck

Serious Adverse Events

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

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

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 outside of the time period specified in the previous paragraph also must be reported immediately to to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

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 215 993-1220) at the time of submission to FDA.

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

7.2.3.1 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to Merck, 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. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

3. In the event a subject develops any of the following AEs, a detailed narrative of the event should be reported as an ECI to to Merck Global Safety within 2 working days of the event:
   a. Grade ≥ 3 diarrhea
   b. Grade ≥ 3 colitis
   c. Grade ≥ 2 pneumonitis
   d. Grade ≥ 3 hypo- or hyperthyroidism

A separate guidance document has been provided entitled “event of Clinical Interest and Immune-Related Adverse Event Guidance Document.” This document provides guidance regarding identification, evaluation and management of ECIs and irAEs. Additional ECIs are identified in this guidance document and also need to be reported to Merck Global Safety within 2 working days of the event.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported within 24 hours to Merck Global Safety within 2 working days.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 10 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**

to Merck product (continued)	**Dechallenge**	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

This will be an open label, single arm exploratory biomarker-driven Phase 2 clinical trial in patients with glioblastoma at first or second recurrence. Patients must have received prior radiation and/or temozolomide therapy. A total of approximately 20 patients with recurrent glioblastoma who require reoperation for tumor progression will be treated and PFS6 will analyzed.

Progression-free survival will be defined as the time from study enrollment until the time of first occurrence of disease progression, relapse, or death due to disease. Patients who are alive without progression or relapse will be censored at the time of last contact. Time to progression and overall survival will be evaluated using the Kaplan-Meier product-limit survival curve methodology. PFS6 will be estimated using Kaplan-Meier estimates and associated two-sided 95% confidence intervals. Cox proportional hazard regression will be employed for multivariate analysis on time-to-event outcomes. In addition, summary statistics and linear regression will be used to analyze continuous variables. Frequency tables will be used to summarize categorical variables. Logistic regression will be utilized to assess the effect of patient prognostic factors on the response rate and the toxicity rate. Given the sample size of 20 patients and a significant level of 10%, we have 74% power to detect the improvement of the PFS6 from 15% (i.e., the historical PFS rate) to 35% in a two-sided test.

A Bayesian design will be used to conduct this single-arm phase II clinical trial, with an interim analysis performed after 10 patients are enrolled. Accrual will be halted, if needed, during the interim analysis. The interim analysis is based on co-primary endpoints: PFS6 and effector:Treg ratio measured at the time of surgery. We define effector:Treg ratio>=5% as a success (i.e., favorable outcome) and <5% as a failure (i.e., unfavorable outcome) and denote the success rate of Treg ratio as TRS. Historical data on similar patients show a PFS6 of 15% and TRS of 5% (Wong ET, 1999). Thus, we will early terminate the trial if, based on interim data, there is a high posterior probability that PFS6 <15% or/and TRS <10%. We assume that PFS6 and TRS marginally follow binomial distributions. Because our monitoring rule is based on the marginal distributions of PFS6 and TRS, we herein do not consider the joint distribution of PSF6 and TRS. As the sample size is small, we do not expect that this will cause noticeable loss of efficiency. We assign vague beta prior *Beta* (0.15, 0.85) to PFS6 and *Beta* (0.1, 0.9) to TRS. We will stop the trial if Pr (PFS6 <15%|Interim Data) >0.9 or/and Pr (TRS <10%|Interim Data)>0.9, which results in the following stopping boundary:

Stop the trial if 10 out of 10 patients have disease progression within 6 months or/and 10 out 10 patients have and effector:Treg ratio<5%.

The following table provides the operating characteristics of the design, generated by MultcLean software based on 10,000 simulations.

True TRS Rate	True PFS6 Rate	Prob(stop the trial early)
0.05	0.05	0.84
	0.15	0.68
	0.25	0.62
	0.35	0.60
0.10	0.05	0.74
	0.15	0.48
	0.25	0.39
	0.35	0.36
0.15	0.05	0.68
	0.15	0.36
	0.25	0.24
	0.35	0.21

We also monitor toxicity (TOX) using a Bayesian rule as follows: stop the trial if Pr (TOX > 30%|Data)>0.8, where TOX is defined as any grade 3 or higher adverse event that is possibly, probably, or definitely related to any therapy received on this protocol and occur within the first 6 weeks of therapy with one exception. Any grade 3 or higher adverse event that is potentially treatable with steroids will only count as a TOX if it does not improve to grade 1 or better within 2 weeks of steroid therapy. Additionally, a delay of greater than 3 months from the expected surgery date will count as a TOX. We monitor TOX based on cohort size of 5. Assuming a Beta prior *Beta* (0.3, 0.7) for the toxicity probability, this Bayesian rule translates into the following stopping boundary: stop the trial if the number of patients experienced TOX is 3-5/5, 5-10/10, or 7-15/15. The following table provides the operating characteristics of the design based on 1000 simulation.

	True TOX probability				
	0.1	0.2	0.3	0.4	0.5
Stopping probability	1%	7%	26.4%	54%	77.6%
Average sample size	19.9	19.0	16.8	13.3	10.3

Baseline characteristics will be assessed by the use of tables and/or graphs. No statistical hypothesis will be performed on these characteristics. The number and percentage of subjects screened, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variable (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by treatment either by descriptive statistics or categorical tables.
Longitudinal analyses of peripheral blood mononuclear cells immune response kinetics and circulating cytokines to pembrolizumab will be presented graphically and descriptively at each time point. Changes in the magnitude of the response relative to pretreatment after pembrolizumab therapy will be summarized descriptively. Changes in response between pre-

treatment and prior to initiation of cycle 3 of pembrolizumab, will be assessed using the Wilcoxon signed-rank test.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 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.

Clinical Supplies will be provided by Merck as summarized in Table 11.

Table 11 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

- Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. **Pembrolizumab Solution for Infusion, 100 mg/vial**: Pembrolizumab Solution for Infusion vials should be stored at refrigerated conditions (2 – 8 °C) and protected from light.
  Note: vials should be stored in the original box to ensure the drug product is protected from light.

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.

9.5 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 subjects 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.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The investigator will assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation. Pursuant to this wording, patients will authorize the collection, use and disclosure of their study data by the Investigator and by those persons who need that information for the purposes of the study.

The use of the samples for research will be done in accordance with the guidelines defined by the FDA document "Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" (issued 25 April 2006). The subject's personal information will be removed before the research samples are used. The research samples will be de-identified by setting a study code /initials. If a subject requests destruction of their banked research tissue and blood samples, and the samples have not yet been de-identified, the investigator will destroy the samples as described in this FDA guidance.

10.2 Compliance with Law, Audit and Debarment

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB/EC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any amendments to the protocol after receipt of IRB/EC approval must be submitted by the Investigator to the IRB/EC for approval. The Investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB/EC prior to use.

10.3 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.4 Quality Management System

Quality assurance measures are provided by three mechanisms: ongoing in-house monitoring of protocol compliance, on-site audits, and response reviews. Data monitoring will begin at

the time of patient registration and will continue during protocol performance and completion. The Protocol Manager will perform the ongoing in-house protocol compliance monitoring with the support of the study investigators.

10.5 Data Management

All subjects will be registered in CORe and data will be entered in PDMS/CORe, the electronic CRF. Designated research staff will enter the data. All investigators will record information regarding on study, course (flowsheet), offstudy, survival, and toxicity data.

All data will be monitored on an ongoing basis by the PI(s), the research study nurses, and data managers Institutional Review Board (IRB) will be notified of any adverse events and provided data to permit a safety review of the study treatment. The IRB may request additional meetings or safety reports as deemed necessary. The IRB may also stop the trial following a review of results from such analysis.

A data set from a completed clinical trial (N=15) of GBM patients that includes age, sex, KPS, IDH1 mutational status, MGMT methylation status, p38 reactivity, and pERK status, extent of resection, treatment, survival and timing of the drug to surgery will be consolidated with a dataset of patients treated at Northwestern University to determine genetic biomarkers of response to anti-PD-1. Dr. Adam M Sonabend is our collaborator at Northwestern Medicine/Northwestern University.

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12.0 APPENDICES

12.1 Karnofsky Performance Status

KPS	**100**	Normal; no complaints; no evidence of disease
KPS	**90**	Able to carry on normal activity; minor signs or symptoms of disease
KPS	**80**	Normal activity with effort; some sign or symptoms of disease
KPS	**70**	Cares for self; unable to carry on normal activity or do active work
KPS	**60**	Requires occasional assistance, but is able to care for most personal needs
KPS	**50**	Requires considerable assistance and frequent medical care
KPS	**40**	Disabled; requires special care and assistance
KPS	**30**	Severely disabled; hospitalization is indicated, although death no imminent
KPS	**20**	Very sick; hospitalization necessary; active support treatment is necessary
KPS	**10**	Moribund; fatal processes progressing rapidly
KPS	**0**	Dead

12.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep**.cancer.gov/reporting/ctc.html)**

12.3 Response Assessment in Neuro-Oncology (RANO) Criteria for Evaluating Response

Definitions of Response (Based on RANO Criteria)

RANO Criteria:

Criterion	CR	PR	SD	PD***
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25%↑
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New Lesion	None	None	None	Present
Corticosteroids	None	Stable or ↓	Stable or ↓	NA**
Clinical Status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for Response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.

* Progression occurs when this criterion is present. To be considered progressive disease, increase in T2/FLAIR should occur on stable or increasing doses of corticosteroids compared to baseline scan or best response following initiation of therapy, and should not be due to co-morbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, post-operative changes, or other treatment effects).

**Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

*** this criteria only pertains to assessment of progression >12 weeks following completion of chemoradiation.

Progression is only diagnosed within the first 12 weeks following completion of chemoradiation in the context of new enhancement observed outside the radiation field and/or histological confirmation (biopsy-proven). (Wen, 2010)

Appendix 4: MD Anderson Form for reporting SAEs

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Internal SAE Report Form for Prompt Reporting
Institutional Review Board
Submit to: Office of Protocol Research, Unit 1437

Internal Adverse Events that are Serious, Unexpected and Related will require prompt reporting. MDACC IRB requires SAEs that meet the prompt reporting requirement to be submitted to OPR within 5 working days except Death. Deaths that are unexpected and definitely, probably or possibly related to study intervention that occur during and within 30 days after the last day of active study intervention will need to be reported within 24 hours.

Is adverse event serious ☐ Yes ☐ No Is adverse event unexpected ☐ Yes ☐ No Is adverse event related ☐ Yes ☐ No	Name of PI: _____ Department: _____ Unit #_____ PI's Phone: _____ PI Signature: _____ Signature Date: _____
Did the subject die? No ☐ Yes ☐ If Yes, provide date _____ & attribution/relation: _____	
Medical Record # _____ Accession # _____ Subject Initials _____ Gender _____	Protocol # _____ Protocol Title: _____
Name of person completing this form_____ Phones # _____	**Status:** Active ☐ Pending Activation ☐ Terminated ☐ CNPE (Pts on tx) ☐ CNPE (Pts off tx) ☐
Initial Report ☐ Follow-up Report ☐ Event/Reaction Onset Date _____ Follow up Date (if applicable) _____	Date Research Team became aware of SAE _____ Date IRB notified _____

SAE/Reaction (Use terms from NCI CTC Terminology)	Grade	Attribution:
_____	_____	Definite ☐ Probable ☐ Possible ☐
_____	_____	Definite ☐ Probable ☐ Possible ☐
_____	_____	Definite ☐ Probable ☐ Possible ☐
_____	_____	Definite ☐ Probable ☐ Possible ☐

Provide brief summary:

Are these events in the risk section of the ICD? No ☐ Yes ☐
Does the ICD need revision? No ☐ Yes ☐
Should current/previously enrolled subjects be notified of these SAEs? No ☐ Yes ☐
Is current condition being treated with Investigational agent? No ☐ Yes ☐
Other Relevant Medical History _____
List/Attach Relevant Tests, Notes and Lab Data _____
List/Attach all Concomitant Meds Subject is taking _____

Name of Agent or Device (list manufacturer and lot)	_____	Did reaction abate after stopping the drug? No ☐ Yes ☐ N/A☐ Date (if yes)_____	
		Did reaction reappear after reintroduction? No ☐ Yes ☐ N/A☐ Date (if yes) _____	
Dose (list frequency or schedule)	_____	route of administration	_____
		date(s) of administration	_____

Have you also reported this reaction to the manufacturer? No ☐ Yes ☐

OPR STAFF: _____ **Date returned to PI** _____ **for:** ☐ Incomplete form
☐ Signature missing ☐ Incorrect information ☐ Other ☐ Report during Continuing Review

Note: With this new form no need to attach the risk section of the ICD

January 2012