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TITLE: Phase II Trial of Pembrolizumab in Central Nervous System Metastases from Multiple Histologies

Coordinating Center: Massachusetts General Hospital

***Principal Investigator (PI):** Priscilla Brastianos
Massachusetts General Hospital
55 Fruit Street, Yawkey 9E
pbrastianos@mgh.harvard.edu

Other Investigators:

Daniel P. Cahill, MD, PhD
Massachusetts General Hospital
Boston, MA

Scott Carter, PhD
Broad Institute
Boston, MA

Elizabeth Gerstner, MD
Massachusetts General Hospital
Boston, MA

Kevin Oh, MD
Massachusetts General Hospital
Boston, MA

Eudocia Quant Lee, MD, MPH
Dana-Farber Cancer Institute
Boston, MA

Sandro Santagata, MD, PhD
Dana-Farber Cancer Institute
Boston, MA

Ryan Sullivan, MD
MGH Cancer Center
Boston, MA



Statistician:

Anita Giobbie-Hurder
Department of Biostatistics
& Computational Biology
Dana-Farber Cancer Institute

Agent(s): Pembrolizumab

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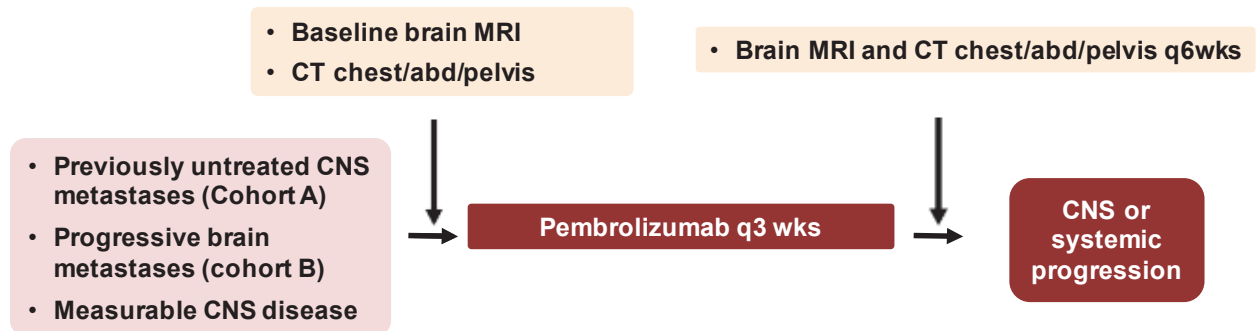
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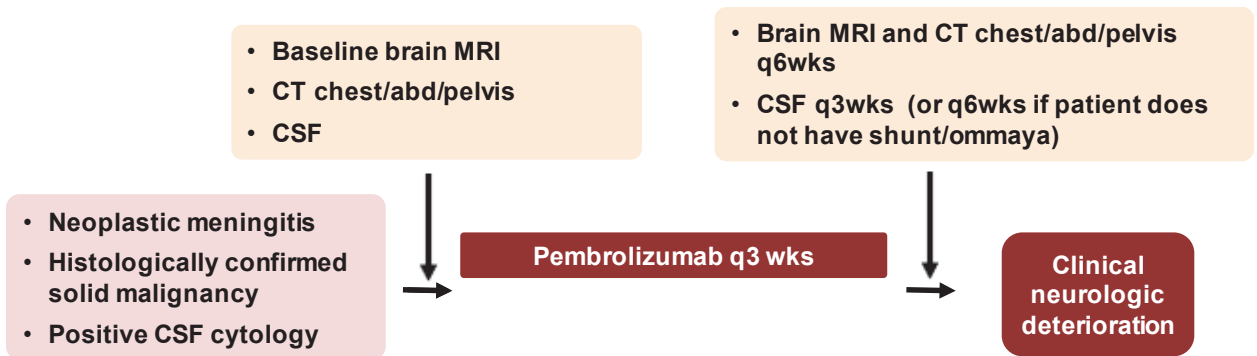
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SCHEMA

Cohorts A and B



Cohort C



Cohort D

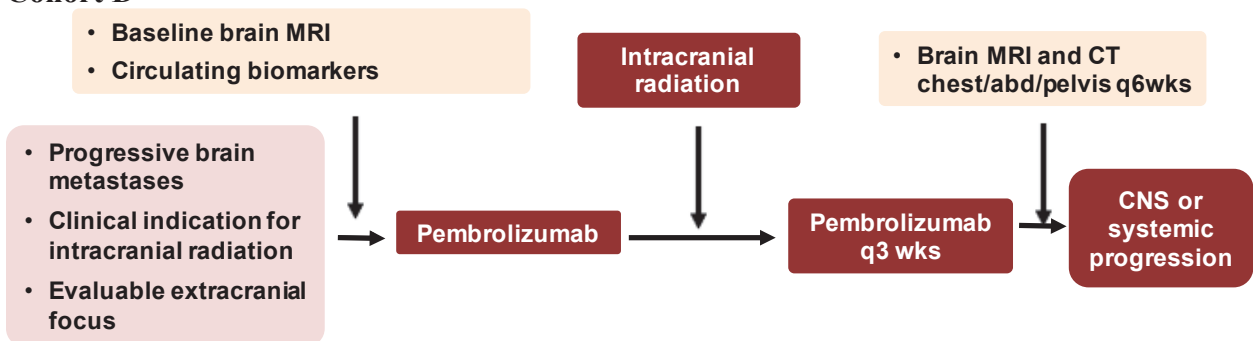


TABLE OF CONTENTS

SCHEMA	2
1. OBJECTIVES	2
1.1. Study Design	2
1.2. Primary Objectives	2
1.3. Secondary Objectives	2
1.4. Exploratory Objectives	2
2. BACKGROUND	2
2.1. Brain Metastases	2
2.2. Pembrolizumab (MK-3475)	2
2.3. Rationale	2
2.4. Correlative Studies Background	2
3. PARTICIPANT SELECTION	2
3.1. Eligibility Criteria	2
3.2. Exclusion Criteria	2
3.3. Inclusion of Women and Minorities	2
4. REGISTRATION PROCEDURES	2
4.1. General Guidelines for DF/HCC Institutions	2
4.2. Registration Process for DF/HCC Institutions	2
4.3. General Guidelines for Other Investigative Sites	2
4.4. Registration Process for Other Investigative Sites	2
5. TREATMENT PLAN	2
5.1. Treatment Regimen	2
5.2. Pre-Treatment Criteria	2
5.3. Agent Administration	2
5.4. General Concomitant Medication and Supportive Care Guidelines	2
5.5. Criteria for Taking a Participant Off Protocol Therapy	2
5.6. Duration of Follow Up	2
5.7. Criteria for Taking a Participant Off Study	2
6. DOSING DELAYS/DOSE MODIFICATIONS	2
7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS	2
7.1. Adverse Event Characteristics	2
7.2. Routine Adverse Event Reporting	2
7.3. Expedited Adverse Event Reporting	2
7.4. Events of Clinical Interest	2
8. PHARMACEUTICAL INFORMATION	2
8.1. Pembrolizumab	2

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.....	2
9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES	2
9.1. Exploratory Laboratory Correlative Studies: Whole exome sequencing, transcriptome sequencing, PD-L1 expression, tumor infiltrating lymphocytes and PD-1 expressing lymphocytes	2
9.2. Adaptive Biotechnologies immunoSEQ.....	2
9.3. Imaging Correlative Studies	2
10. STUDY CALENDAR	2
11. MEASUREMENT OF EFFECT	2
11.1. Antitumor Effect – Extracranial lesion assessments.....	2
11.2. Antitumor Effect – Intracranial Disease	2
11.3. Study Continuation Beyond Initial Progressive Disease	2
11.4. Response Review	2
11.5. Other Response Parameters	2
12. DATA REPORTING / REGULATORY REQUIREMENTS.....	2
12.1. Data Reporting.....	2
12.2. Data Safety Monitoring.....	2
12.3. Multicenter Guidelines.....	2
13. STATISTICAL CONSIDERATIONS.....	2
13.1. Study Design/Endpoints	2
13.2. Sample Size, Accrual Rate and Study Duration	2
13.3. Stratification Factors.....	2
13.4. Interim Monitoring Plan	2
13.5. Analysis of Primary Endpoints	2
13.6. Analysis of Secondary Endpoints	2
13.7. Analysis of Exploratory Endpoints.....	2
13.8. Reporting and Exclusions	2
14. PUBLICATION PLAN	2
REFERENCES	2
APPENDIX A PERFORMANCE STATUS CRITERIA	2

1. OBJECTIVES

1.1. Study Design

This is a Phase 2 study of pembrolizumab in patients with metastases in the central nervous system.

1.2. Primary Objectives

1. To estimate the clinical benefit rate (as defined by complete response, partial response or stable disease) of pembrolizumab in newly diagnosed or recurrent brain metastases (Cohorts A and B).
2. To estimate the overall survival of pembrolizumab in leptomeningeal metastases (Cohort C).
3. To estimate the systemic overall response rate to the combination of pembrolizumab and intracranial radiation in oligometastatic brain metastases (Cohort D).

1.3. Secondary Objectives

1. To describe the toxicity of pembrolizumab in brain and leptomeningeal metastases (Cohorts A, B, C, D).
2. To estimate the overall survival in patients with brain metastases receiving pembrolizumab (Cohort A, B, D).
3. To estimate the central nervous system response rate of pembrolizumab in patients with leptomeningeal metastases (Cohort C).
4. To estimate the systemic response rate of pembrolizumab in patients with brain metastases (Cohorts A, B, C).
5. To estimate the systemic progression free-survival of patients receiving pembrolizumab (Cohorts A, B, C, D).
6. To estimate the intracranial progression free-survival of patients receiving pembrolizumab (Cohorts A, B, C, D).

1.4. Exploratory Objectives

1. To determine molecular biomarkers of response using next generation sequencing techniques.
2. To determine if changes in brain MRI parameters correlate with tumor response.

2. BACKGROUND

2.1. Brain Metastases

Brain metastases are a common and devastating complication of cancer. Median survival is limited after the diagnosis of a metastasis in the central nervous system despite having received standard therapies¹⁻³. Progression of intracranial disease is the cause of death in up to 50% with clinically significant brain metastases^{2,4,5}. Systemic therapy for brain metastases has a number of

challenges. Data supporting the use of systemic therapy in brain metastases is limited, partly because of the paucity of randomized studies focused on brain metastases, as well as the exclusion of brain metastases patients from most clinical trials in the US².

2.2. Pembrolizumab (MK-3475)

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8⁺ T-cells and the ratio of CD8⁺ effector T-cells / 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). The structure of murine PD-1 has been resolved. 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 CD3 ζ , PKC θ and ZAP70 which are involved in the CD3 T-cell signaling cascade. 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. 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. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. 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. 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. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated 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 (MEL). 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 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. Keytruda™ (pembrolizumab) has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. 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 MK-3475 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 MTD has been identified to date. 200mg IV Q3W will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). 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 indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

2.3. Rationale

Recently, great strides in immunotherapies have been made in the management of a number of malignancies namely the development of the immune checkpoint inhibitors targeting cytotoxic T-lymphocyte antigen 4 (CTLA4) and the programmed cell death (PD1) receptor-ligand (PDL1, PDL1). However, the role of these inhibitors in central nervous system metastases has not been adequately explored. Preclinical data demonstrates infiltration of activated regulated T cells, and PD-L1 expression in brain metastases of various histologies⁶.

We are thus proposing a prospective Phase 2 study of the PD-1 inhibitor, pembrolizumab in patients with metastases in the central nervous system across all histologies using a dose schedule that has demonstrated efficacy in systemic melanoma⁷. We propose to investigate the activity of pembrolizumab in untreated (Cohort A) and progressive treated brain metastases (Cohort B), as well as in leptomeningeal carcinomatosis (Cohort C). Additionally, we will investigate the abscopal effect, specifically, whether intracranial radiation augments the systemic response rate in oligometastatic melanoma (Cohort D). We also propose to incorporate advanced imaging, circulating tumor cells, tissue biopsies, and next generation sequencing to identify genetic and imaging predictors of response.

Cohorts A and B: Up to 25% of patients with cancer will develop brain metastases and median survival continues to be poor^{2,3,8,9}. Historically, the use of systemic therapies in brain metastases has been challenging because of resistance to conventional chemotherapy secondary to the blood brain barrier and an often heavily pre-treated patient population³. While whole brain radiation has been the standard treatment for multiple brain metastases, neurotoxicity occurs in a large proportion of patients¹⁰ and significantly impacts quality of life¹¹. Newer treatment approaches, including immunotherapy, need to be investigated in this setting. A recent study demonstrated that the anti-CTLA4 inhibitor, ipilimumab, has activity in patients with brain metastases from melanoma¹². However, the efficacy of pembrolizumab has not been systemically investigated in patients with brain metastases. An ongoing study by Goldberg and Kluger et al.^{13,14} showed that pembrolizumab is well tolerated and has promising activity in patients with asymptomatic brain metastases from melanoma and non-small cell lung cancer (NSCLC). Of 12 evaluable patients with melanoma brain metastases, partial responses were observed in 3 patients and stable disease in 2 patients¹⁴. Of 9 evaluable patients with brain metastases from NSCLC, partial responses were observed in 4 patients¹³. In our experience at Massachusetts General Hospital, we have treated over 20 patients with brain metastases with pembrolizumab and demonstrate that it is safe

and well-tolerated.

Cohort C: Approximately 5-8% of patients with cancer develop leptomeningeal carcinomatosis¹⁵. While virtually any malignancy can metastasize to the leptomeninges, this most commonly occurs with lung cancer, breast cancer and melanoma. As patients with cancer survive longer and diagnostic imaging techniques improve, leptomeningeal carcinomatosis is being diagnosed more frequently. Patients typically present with signs of increased intracranial pressure, cranial nerve palsies, seizures, stroke-like symptoms, encephalopathy, radiculopathies and cauda equina syndrome¹⁵. The median survival of patients with leptomeningeal metastases without treatment is approximately 4-6 weeks. Death usually results from rapid progressive neurologic deterioration. Treatment involves radiation to symptomatic sites of the neuraxis and to sites of bulk disease seen on imaging studies. Systemically administered chemotherapy has limited access to the CSF due to the blood-brain barrier. Intrathecal chemotherapy is designed to treat tumor cells floating in the cerebrospinal fluid (CSF), thereby preventing the development of new symptomatic sites of disease; however treatment-related toxicities of intrathecal chemotherapy are common, and include chemical meningitis, seizures and severe headaches¹⁶. Radiotherapy and intrathecal chemotherapy provide local control and may extend median survival of patients with leptomeningeal carcinomatosis to 3-6 months^{16,17}. Novel therapies are needed to improve outcomes in patients with leptomeningeal carcinomatosis. In our experience at Massachusetts General Hospital Cancer Center, we have observed 2 remarkable clinical responses in patients with leptomeningeal carcinomatosis from melanoma who received immune checkpoint inhibitors. The first one was a 24-year old with leptomeningeal melanoma treated with immune checkpoint blockade, who developed an abundant inflammatory reaction in the cerebrospinal spinal fluid in response to immune checkpoint blockade. The second patient was a 74 year old with metastatic melanoma treated who developed radiographic evidence of leptomeningeal carcinomatosis, followed by T2-hyperenhancement, and then complete resolution of his disease. He remains a complete responder 2 years later.

Cohort D: The term “abscopal effect” was coined in 1953¹⁸ and refers to the ability of radiation therapy to have effects at sites distant to the radiation field. The ability of radiation therapy to induce immune-mediated distant responses has been demonstrated in murine models of breast cancer and melanoma exposed to anti-CTLA4 antibodies^{19,20}. The proposed mechanism is that liberation of tumor antigens by radiation therapy functions as an *in situ* vaccine that increases antigen presentation and enhances T-lymphocyte mediated killing. There are a growing number of case reports in melanoma of radiation therapy eliciting an abscopal effect when used after immune checkpoint modulators²¹. Postow et al. reported a high-profile case report of a 33 year old woman with metastatic melanoma who failed the CTLA-4 antibody ipilimumab. Over 1 year later she enjoyed a systemic and durable response after receiving palliative radiation to a single-site followed by a rechallenge with ipilimumab²². Several other cases have been reported in melanoma²²⁻²⁶. In a phase I study, 12 patients with metastatic melanoma or renal cell carcinoma were treated with stereotactic body radiation therapy followed by high-dose interleukin-2 up to 6 cycles. The response rate in melanoma was higher than historical controls (71% experiencing partial or complete response)²⁷. A phase I trial of MK-3475 in ipilimumab-refractory advanced melanoma reported 26% overall response rate in both 2 and 10 mg/kg²⁸. The abscopal effect has also been reported in other tumors, including nonsmall cell lung cancer^{29,30}.

2.4. Correlative Studies Background

2.4.1. Molecular Correlatives

Our objectives are to use next generation sequencing technologies to identify genetic predictors of response to immune checkpoint inhibitor blockade using clinical samples collected as part of clinical care. Our group has extensive experience in genomic characterization of a number of brain tumors^{31,32}, including brain metastases. We recently performed whole-exome sequencing of 101 central nervous system metastases, primary tumors and normal tissue across all histologies^{33,34}. By performing an integrative analysis of somatic single nucleotide variants and somatic copy-number alterations, we elucidated the evolutionary patterns of these brain metastases and reconstructed phylogenetic trees relating all of the subclones in each pair. We identified genetic drivers, a number of which are known clinically actionable targets, in brain metastases, not detected in the primary tumor.

We propose to evaluate recurrent clinically relevant mutations, overall mutational burden and genetic heterogeneity in CNS metastases in an unbiased screen for point mutations, insertion-deletion events, and copy-number changes in all exons, using archived primary and metastatic tumors and paired normal tissue collected as part of clinical care. We will perform whole exome and transcriptome sequencing of archived metastatic sites. Furthermore, TILs/PD-L1 expression will be evaluated in intracranial and extracranial tissue biopsies to correlate with response, and with genetic biomarkers.

Our goal to use these techniques to identify genetic predictors of response to checkpoint inhibitors.

2.4.2. Imaging Correlatives

MRI is a very useful tool to non-invasively probe tumor response to treatment and is already part of the routine management of patients with brain metastases. The complicated relationship between tumor perfusion, vascular permeability, and tumor cellularity can be assessed by MRI. Dynamic susceptibility contrast (DSC) and dynamic contrast enhanced MRI can measure vascular parameters such as cerebral blood volume, blood flow, mean transit time, vessel size, and permeability - all of which are impacted by targeted therapy. We will perform exploratory analyses to see if changes in tumor vasculature/cellularity can shed light on the mechanism of action of palbociclib in brain metastases and measure the impact the drug has on the tumor microenvironment. These tools have been optimized for brain tumor imaging and we have extensive experience using advanced MRI in patients with brain tumors^{35,36}.

3. PARTICIPANT SELECTION

3.1. Eligibility Criteria

- 3.1.1. Participants must have histologically or cytologically confirmed disease from any solid tumor
- 3.1.2. Participants must have measurable disease in the CNS, defined as at least one lesion that can be accurately measured in at least one dimension as ≥ 5 mm (for cohorts A, B, D only).
- 3.1.3. Age ≥ 18 years.
- 3.1.4. ECOG performance status ≤ 2 (Karnofsky $\geq 60\%$, see Appendix A)
- 3.1.5. Life expectancy of greater than 6 weeks.
- 3.1.6. Participants must have normal organ and marrow function as defined in Table 1, all screening labs should be performed within 14 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	$\geq 1,500$ /mcL
Platelets	$\geq 100,000$ / mcL
Hemoglobin	≥ 9 g/dL or ≥ 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
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 $\leq$ 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
Albumin	≥ 2.5 g/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤ 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
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
^a Creatinine clearance should be calculated per institutional standard.	

- 3.1.7. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- 3.1.8. 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.
- 3.1.9. 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.
- 3.1.10. Ability to understand and the willingness to sign a written informed consent document.
- 3.1.11. Stable dose of dexamethasone 2mg or less for 7 days prior to initiation of treatment
- 3.1.12. Cohort Specific Eligibility Criteria
 - 3.1.12.1. Cohort A:**
 1. Measurable CNS disease (one intracranial lesion ≥ 5 mm)
 2. Previously untreated asymptomatic brain metastases
 3. Patients with newly diagnosed, previously untreated primary tumors that present with brain metastases should not forego available therapy that has demonstrated a definitive overall survival benefit as firstline therapy for metastatic disease; therefore, in cases of previously untreated systemic solid tumors only those patients for whom there is no available therapy with definitive overall survival benefit, those that have failed at least one line of prior therapy for their primary tumor, or those refusing standard therapy will be eligible for this study. Specifically, for patients with previously untreated primary tumors, the following diagnoses will be excluded: HER2-positive breast cancer; small cell lung cancer; NSCLC with targetable genomic tumor aberrations (e.g. EGFR, ALK).
 - Cohort B:**
 1. Measurable CNS disease (one intracranial lesion ≥ 5 mm)
 2. Progressive brain metastases after prior local CNS-directed therapy such as radiation or surgery as defined by:
 - a. Untreated measurable lesions in patients that have received surgery and/or SRS to one or more other lesions
 - b. Residual or progressive lesions after surgery if asymptomatic
 - c. Patients who have had prior WBRT and/or SRS and then whose lesions have progressed are eligible. Lesions treated with SRS may be eligible if there is unequivocal evidence of progression

3.1.12.2. Cohort C:

1. Carcinomatous meningitis, as defined by positive cytology

3.1.12.3. Cohort D:

4. Measurable CNS disease (one intracranial lesion ≥ 5 mm) from any solid tumor
5. No limit on number of brain metastases
6. Radiation therapy over 7Gy is indicated to any intracranial disease
7. Prior stereotactic radiosurgery or whole brain radiation is allowed

3.1.13. Patients may have progressive systemic disease

3.1.14. Patients with untreated spinal cord metastases are eligible if lesions are asymptomatic

3.1.15. Patients with untreated brainstem metastases are eligible if lesions are small and asymptomatic

3.2. Exclusion Criteria

3.2.1. Participants who have had chemotherapy, targeted small molecule therapy or study therapy within 14 days of protocol treatment, or those who have not recovered (i.e., $\leq$ Grade 1 or at baseline) from adverse events due to agents administered more than 2 weeks earlier. Subjects with $\leq$ Grade 2 neuropathy are an exception to this criterion and may qualify for the study. If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

3.2.2. Participants who are receiving any other investigational agents.

3.2.3. Has a diagnosis of immunodeficiency.

3.2.4. Requires treatment with high dose systemic corticosteroids defined as dexamethasone >2 mg/day or bioequivalent within 7 days of initiating therapy.

3.2.5. Has received systemic immunosuppressive treatments, aside from systemic corticosteroids as described in Section 3.2.4, within three months of start of study drug

3.2.6. Hypersensitivity to pembrolizumab or any of its excipients

3.2.7. Has a known history of active TB (Bacillus Tuberculosis)

- 3.2.8. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.2.9. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
- 3.2.10. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
- 3.2.11. HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with pembrolizumab. In addition, these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.
- 3.2.12. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
- 3.2.13. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
- 3.2.14. Has known history of, or any evidence of active, non-infectious pneumonitis.
- 3.2.15. Has an active infection requiring systemic therapy.
- 3.2.16. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
- 3.2.17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
- 3.2.18. Has received a live vaccine within 30 days of planned start of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
- 3.2.19. Unable to undergo brain MRI.

3.2.20. Participants who are receiving or will receive other concurrent chemotherapies or immunotherapies for their cancer (except for patients who will receive letrozole, anastrozole, exemestane, tamoxifen, fulvestrant, trastuzumab, bisphosphonates, denosumab or ovarian suppression therapy).

3.2.21. Will need immediate local surgery or radiation for their brain metastases

3.2.22. Acute symptomatic CNS hemorrhage

3.3. Inclusion of Women and Minorities

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

If there are any questions regarding the eligibility requirements, please reach out to our research nurse (contact information above) and patient may be presented at our multidisciplinary brain metastasis tumor board at Massachusetts General Hospital.

4. REGISTRATION PROCEDURES

4.1. General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations as soon as possible.

4.2. Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3. General Guidelines for Other Investigative Sites

N/A

4.4. Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1. Treatment Regimen

For all cohorts, pembrolizumab will be administered every 3 weeks, with 21 consecutive days defined as a treatment cycle. In Cohort D, cycle 1 and 2 of pembrolizumab will be administered 3 weeks apart and stereotactic radiosurgery will be administered between cycles. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

The treatment to be used in this trial is outlined below in the table below.

Table 2: Dose of Pembrolizumab

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Cycle Length
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	3 weeks

5.2. Pre-Treatment Criteria

Informed consent will be obtained after the study has been fully explained to the subject and before the conduct of any screening procedures or assessments. For C1D1 only, screening assessments may serve as day 1 assessments, except serum pregnancy (within 72 hours of drug) and except in the event that there are any indications that the participant's condition is deteriorating for which laboratory evaluations should be repeated within 48 hours prior to initiation. For all subsequent cycles, required assessments should be performed within 3 days of scheduled cycle day 1. Scans can be performed within 6 days of scheduled cycle day 1.

Pretreatment criteria will be assessed within 14 days of the first dose of study treatment to establish eligibility and baseline values. This will be considered the baseline clinical evaluation. Subsequent changes from screening physical exam (PE) findings that meet the definition of an AE will be recorded on the AE page of the eCRFs.

Demographic information and baseline characteristics will be collected at the Screening Visit. Standard demographic parameters include age, sex, and race/ethnicity (recorded in accordance with prevailing regulations). Baseline characteristics will include ECOG PS (Appendix A), disease status, medical histories, prior and concomitant medications, and PE findings. Relevant hormone receptor status for breast cancer patients will be collected.

Additional pre-treatment evaluations, also found in section 3.1.6, are CBC with differential, and

chemistries. Pregnancy testing for screening should be obtained in appropriate patients, as defined in Eligibility. Required results for initiation of protocol therapy include:

- Absolute neutrophil count > 1,500/ μ L
- Platelets > 100,000/ μ L
- Hemoglobin > 9g/dL
- Total bilirubin < institutional upper limit of normal (patients with documented Gilbert's disease are allowed total bilirubin up to 1.5X ULN)
- AST (SGOT)/ALT (SGPT) < 2.5 X institutional upper limit of normal
- Serum creatinine $\leq$ 1.5 X upper limit of normal (ULN) or creatinine clearance > 60 mL/min/1.73 m² for subjects with creatinine levels above institutional normal.
- Negative pregnancy test in appropriate patients

5.3. Agent Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed in the Study Calendar (Section 9.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. 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).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.4. General Concomitant Medication and Supportive Care Guidelines

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 investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

- **Diarrhea/Colitis:**
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).

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **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:** See Section 6.0 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids, regarding diagnosis and management of adverse experiences of a potential immunologic etiology.

Management of Infusion Reactions: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 3 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
<u>Grade 1</u> 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
<u>Grade 2</u> Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for <=24 hrs	Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the 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	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	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.	
<u>Grades 3 or 4</u> 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.		

5.4.1. Supportive Care Guidelines for Immune Related Adverse Events (irAEs)

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per the Table below. See Section 6.0 for supportive care guidelines, including use of corticosteroids.

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased.

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. Subjects who develop a Grade 2 or higher irAE should be discussed immediately with the overall Principal Investigator or designee. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

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

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 6.0 for dose modification.

- **Pneumonitis:**

- For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis:**

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).

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis**, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or $\geq$ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

- For **T1DM or Grade 3-4 Hyperglycemia**
 - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
 - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis:**

- For **Grade 2 events**, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism:**

Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

 - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
 - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
 - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
 - **Grade 3-4** hyperthyroidism
 - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic:**
 - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
 - Treat with IV or oral corticosteroids
 - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
 - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis:**
 - For **Grade 2** events, treat with corticosteroids.
 - For **Grade 3-4** events, treat with systemic corticosteroids.
 - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Cerebral Edema**

Due to the immunologic nature of pembrolizumab administration, cerebral edema could theoretically result as a consequence of pembrolizumab administration due to immune infiltration of the brain. Symptoms related to cerebral edema may include headache or neurologic deficit that is either new or worsened. Patients with any signs or symptoms of cerebral edema should be treated as clinically appropriate including initiation or increased systemic corticosteroid dosing, treatment with an osmotic diuretic or surgical decompression. Subsequent pembrolizumab dosing should be immediately interrupted if significant clinical symptoms attributable to cerebral edema develop. Treatment with

additional pembrolizumab doses may only be re-initiated if clinically significant symptoms attributable to cerebral edema have resolved to grade ≤ 1 or pre-treatment baseline. Patients who develop CTCAE 4.0 grade-4 cerebral edema attributable to pembrolizumab administration should not receive further pembrolizumab doses and should discontinue study therapy.

Recommendations to manage irAEs not detailed elsewhere in the protocol are detailed in Table 4.

Table 4 Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:	
1.	Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2.	For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3.	For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	- Monitor participants for signs and symptoms of pneumonitis - Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment - Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	- Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). - Participants with $\geq$ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. - Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 or 4	Permanently discontinue	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β -cell failure	Withhold	- Initiate insulin replacement therapy for participants with T1DM - Administer anti-hyperglycemic in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated.	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue ¹		
Hyperthyroidism	Grade 2	Continue	Treat with non-selective beta-blockers (eg, propranolol) or	Monitor for signs and symptoms of thyroid disorders.

	Grade 3 or 4	Withhold or permanently discontinue ¹	thionamides as appropriate	
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/persistent Grade 2	Withhold	Based on type and severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to $\leq$ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

5.4.2. 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 7.4.

5.4.3. 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:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
 - Note: Radiation therapy to a symptomatic single extracranial site and/or one intracranial site may be allowed at the investigator's discretion.
- 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, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to treat edema related to brain metastases or supportive post-operative management or to modulate symptoms from an event of suspected immunologic etiology. Intranasal, inhaled or topical corticosteroids (<5% of body surface area) for the treatment of mild/moderate asthma, allergies or dermatitis are permitted. The use of physiologic doses of corticosteroids may be approved after consultation with the Overall PI. Physiologic replacement of glucocorticoids as maintenance therapy for adrenal insufficiency are permitted. Standard doses of hydrocortisone for maintenance therapy are 10–20 mg/m²/day divided 2–4 times per day. For a subject with a body surface area (BSA) of 1.73 m², this translates to a total dose of 34.6 mg of hydrocortisone per day. The equivalent dose of dexamethasone is 1.2 mg per day. Some subjects may additionally receive mineralocorticoid- replacement maintenance therapy with fludrocortisone. The maintenance dose of fludrocortisone for this indication is 0.05–0.1 mg/day. Antihistamines and other non- steroidal anti-allergy medications are also permitted.

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.

5.4.4. Diet

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

5.4.5. 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-Reporting of Pregnancy and Lactation to the Overall PI and 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.

5.4.6. 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 the Overall PI and to Merck without delay and within 24 hours to the Overall PI and within 2 working days to Merck 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).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Overall PI. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Overall PI and to Merck and followed as described above and in Section 7.2.

5.4.7. 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.

5.5. Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with trial regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- The subject has a confirmed positive serum pregnancy test
- Subject is lost to follow-up

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Priscilla Brastianos at 617-643-1938.

5.6. Duration of Follow Up

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 7.3). 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.

5.7. Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A ODQ Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

6. DOSING DELAYS/DOSE MODIFICATIONS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity $\geq$ Grade 3 including laboratory abnormalities despite appropriate replacement therapies when appropriate, and severe or life-threatening AEs as per Table 5.

Table 5 Dose modification guidelines for drug-related adverse events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below) ^a	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2 ^b	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity ^c	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

^a For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

^b 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; Refer to

Table – Infusion Treatment Guidelines for further management details.

^c Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the PI of the study. The reason for interruption should be documented in the patient's study record.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs and the characteristics of an observed AE (Section 7.1) will determine whether the event requires expedited reporting **in addition** to routine reporting.

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. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

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

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

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time patient of registration and treatment allocation 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 7.3

7.1. Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- **For expedited reporting purposes only:**
 - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
 - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.
- **Attribution of the AE:**
 - Definite – The AE *is clearly related* to the study treatment.
 - Probable – The AE *is likely related* to the study treatment.
 - Possible – The AE *may be related* to the study treatment.

- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.1.1. 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 6 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 mild 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 to Merck product (continued)	The following components are to be used to assess the relationship between the test drug and the AE: (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. Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

7.3. Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI, as study sponsor, any serious adverse event (SAE) that occurs after subject registration, during treatment, or within 90 days of the last dose of treatment on the local institutional SAE form.

7.3.1. DF/HCC Expedited Reporting Guidelines to the IRB

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3.2. Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA. The Overall PI must report to the FDA any suspected adverse reaction to study treatment (i.e., including active

comparators) that is both serious and unexpected.

7.3.3 Expedited Reporting of Adverse Events to Merck

A serious adverse event is any adverse event occurring from the time of subject registration and allocation to a treatment arm, through during any use of Merck's product, through 90 days after last dose that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Refer to Table 6 for additional details regarding each of the above criteria.

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 of subject registration 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 24 hours to the Overall PI and 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 the Overall PI and 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.

7.3.4. Definition of an Overdose for This Protocol and Expedited Reporting of Overdose to the Overall PI and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥ 5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, 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 24 hours to the Overall PI and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.3.5. Expedited Reporting of Pregnancy and Lactation to the Overall PI and 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, or 90 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 24 hours to the Overall PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.3.6. Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.4. 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 24 hours to the Overall PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Overall PI and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Overall PI and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Overall PI, 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.

8. PHARMACEUTICAL INFORMATION

8.1. Pembrolizumab

Pembrolizumab is a potent and highly selective humanized mAb designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 potently blocks binding to both ligands with half maximal inhibitory concentration (IC₅₀) values below 1 nM. MK-3475 enhances T cell responses in human donor blood cell cultures with an EC₅₀ of ~0.1 to 0.3 nM.

Additional detailed information on pembrolizumab is available in the Investigator's Brochure.

8.1.1. Description

Pembrolizumab (MK-3475) is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. MK-3475 binds to human PD-1 and blocks the interaction between PD-1 and its ligands. The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. The parental

murine anti-human PD-1 antibody (hPD-1.09A) was produced by immunizing mice with hPD-1 DNA. The MK-3475 antibody was generated by humanization of the parental antibody by the Medical Research Council (Cambridge, UK) using complementarily-determining region (CDR) grafting technology (U.S. Patent No. 5,225,539). The gene segments encoding the variable heavy and light chains of MK-3475, as well as human IgG4, were codon-optimized, synthesized, and ligated into a vector.

A single expression plasmid, pAPD11V1-GA was constructed for the expression of both the heavy and light antibody chains of MK-3475. The nucleotide sequences encoding the heavy and light chains, along with their respective promoters and poly A signal sequences have been confirmed by DNA sequence analysis. The pAPD11V1-GA expression vector was subsequently used to transfect CHO-DXB-11 cells for the development of the MK-3475-producing cell line.

The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. Pembrolizumab exhibits linear pharmacokinetics at dose levels of clinical relevance (1-10 mg/kg). It exhibits low clearance and limited volume of distribution that is typical for therapeutic antibodies. Mean estimated t_{1/2} values are 14.1-21.6 days.

The nomenclature of pembrolizumab is provided in the table below:

Code Name	MK 3475 (Anti-PD-1)
Other Code Name	SCH 900475 (Anti-PD-1)
Chemical Name	Humanized X PD-1_mAb (H409A11) IgG4
CAS Number	1374853-91-4
CAS Name	Anti-(human protein PDCD1 (programmed cell death 1)) immunoglobulin G4 (human-Mus musculus monoclonal heavy chain) disulfide with human-Mus musculus monoclonal light chain, dimer
Generic Name	Not available
Commercial Name	Pembrolizumab

8.1.2. Form

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.

Pembrolizumab is supplied as a clear to opalescent solution that is essentially free of extraneous particles and may contain proteinaceous particulates. One dosage form of pembrolizumab will be provided by Merck in Type I glass vials intended for single use only as summarized in the following table:

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

Pembrolizumab solution for infusion is a sterile, non-pyrogenic, aqueous, preservative-free solution. Pembrolizumab solution for infusion contains an excess fill of 6.25 mg (equivalent to 0.25 mL solution) to ensure the recovery of label claim of 100 mg pembrolizumab per vial (equivalent to 4.0 mL of solution).

8.1.3. Packaging and Labeling Information

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

8.1.4. Clinical Supplies Disclosure

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

8.1.5. Storage, Handling and Preparation Requirements

As specified in the Pharmacy Manual for pembrolizumab as provided by the Overall PI.

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

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

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

8.1.6. Administration

Pembrolizumab will be administered as a 30 minute IV infusion using an infusion pump (treatment cycle intervals may be increased due to toxicity as described in Section 6). 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). Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion. Maximum infusion rate should not exceed 6.7 mL/min through a peripheral or indwelling catheter. Use 30 mL normal saline to flush the infusion line at the end of the infusion if institutional guidelines allow.

Unused infusion solution should not be used for another infusion of the same participant or different participant.

DO NOT administer the product as an intravenous push or bolus.

DO NOT combine, dilute or administer it as an infusion with other medicinal products.

A central line is not required for Pembrolizumab administration, but may be used if available.

The following infusion set materials are compatible with Pembrolizumab

- PVC infusion set that is plasticized using Di-2-ethylhexyl Terephthalate DEHP
- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyethylene lined PVC infusion set
- Polyurethane
- Polybutadiene A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5 µm add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5 µm add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

8.1.7. Ordering

Participating Institutions will order their own investigational agent (Pembrolizumab) directly from Merck using the Drug Supply Request Form. Please allow for 3 weeks for drug to arrive after the order is submitted. The Participating Institution will ensure that the pharmacy will be able to receive and store the agent according to state and federal guidelines. The local IRB should be kept informed of who will supply the agent (i.e., Merck pharmaceuticals Inc.) so that any regulatory responsibilities can be met in a timely fashion.

8.1.8. Accountability

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.

8.1.9. Destruction and Return

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.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1. **Exploratory Laboratory Correlative Studies:** Whole exome sequencing, transcriptome sequencing, PD-L1 expression, tumor infiltrating lymphocytes and PD-1 expressing lymphocytes

9.1.1. **Background:** Comprehensive genomic characterization of samples collected as part of our proposed clinical trial will allow us to identify biomarkers of response and provide insight into mechanisms of resistance to immunotherapy. If available, archived primary and brain metastatic samples will be collected as part of this trial. Patients who progress on pembrolizumab will also undergo biopsies when clinically indicated. All samples will undergo whole exome, transcriptome and targeted sequencing. Analytic tools developed for our prior studies^{31,33,37,38} will be used to elucidate the genomic evolutionary profiles in matched primaries and metastases. Tumors will also be evaluated for PD-L1 expression, tumor infiltrating lymphocytes and PD-1 expressing lymphocytes. We will analyze the association between response and other demographic and clinical features and potential genetic biomarkers in the primary tumors and the matched brain metastases.

9.1.2. **Methodology:** An unbiased comprehensive genomic approach to determine the evolutionary profiles of patients may provide insight into biomarkers of response and mechanisms of resistance. Whole-exome sequencing will be performed at the Broad Institute using pipelines described in our recent studies^{31,33,37,38}. DNA will be subjected to solution-phase hybrid capture followed by Illumina sequencing to >150x mean coverage. Mutations and copy-number changes will be determined within the Firehose pipeline environment using a suite of existing tools, which include the published MuTect, Indelocator, GISTIC and MutSig algorithms^{31,37,39-43}. To understand inter- and intratumoral heterogeneity, we developed novel computational tools to perform an integrative analysis of somatic mutations and copy-number alterations. This analysis, termed Absolute^{33,34}, allows us to estimate the clonal architecture of tumor samples. We have refined these techniques to understand the clonal evolution across a large cohort of 101 brain metastases matched with their primary tumors³³.

RNA Sequencing from FFPE includes plating, poly-A selection and cDNA synthesis, library preparation, sequencing, sample identification QC, and data storage. Sequencing is performed using Illumina HiSeq 2000 or 2500 instruments with 76 Paired End read lengths. We will follow the analytic pipelines as previously described⁴⁴⁻⁴⁷. At the end of the pipeline, we will identify expression levels of genes and isoforms and detect differential expression between brain metastases and primary tumors.

Furthermore, PD-L1 expression will be measured from any remaining archival tissue and correlated with response. PD-L1 expression will be measured by the QualTek assay. To validate transcriptome results, we will evaluate PD-L1 in a pilot cohort of 10 matched primary and metastatic samples. Tissue will also be evaluated for tumor infiltrating lymphocytes and PD-1 expressing lymphocytes.

9.1.2.1. Collection, Processing, and Handling of Archived Specimens

For **each** prior resection and biopsy of both the primary cancer and all metastases, 1 FFPE block should be collected for molecular testing. If an institution is unable to provide a tissue block, cut five-micron sections from the FFPE block and mount on 15 charged glass slides. **Do not bake, stain, or place covers slips on the slide.** If less tissue is available than required by protocol, the available tissue should still be processed as per protocol. Slides for each case need to be cut with a new blade and using a fresh water bath to avoid contamination. Label the slides with patient ID number, accession number, and order of sections (*include thickness of section if applicable*). For samples containing less than 1 square cm of tumor tissue, multiple sections should be mounted onto each slide to ensure that the appropriate amount of tumor tissue is available.

If a patient undergoes a resection or biopsy during the study and there is fresh tissue available for research, 0.5cm³ tissue should be snapfrozen and shipped on dry ice to the address below. Label the container with patient ID number and accession number.

Brittany Bent

Massachusetts General Hospital Cancer Center
55 Fruit Street, Yawkey 9E
Boston, MA 02114

Shipping of Archived and Fresh Tissue Specimen(s): Archival tissue must be shipped to the below address within 60 days of patient registration. Shipment on Monday through Thursday by overnight service to assure receipt is encouraged. Do not ship specimens on Fridays or Saturdays. Ship samples with a copy of the pathology report to the below address:

Brittany Bent

Massachusetts General Hospital Cancer Center
55 Fruit Street, Yawkey 9E
Boston, MA 02114

9.1.2.2. Collection, Processing, and Handling of Blood Specimens

Collect two 10 mL tubes of venous blood at pre-registration and two 10 mL tubes every 6 weeks in EDTA anticoagulant (lavender top) vacutainer tubes. The tubes should be inverted approximately 8-10 times to mix the blood and EDTA. The sample should be clearly labeled with the below information:

- Patient's study ID and initials
- Collection date and time
- Study protocol number
- Sample type (e.g. blood)

Blood samples should be couriered immediately (within one hour of collection) to the address below. Do not delay sample shipment to include more than one sample type or samples from multiple patients. Place each blood sample in a biohazard bag, place samples on ice or on a cold pack (do not use dry ice), and ship according to IATA guidelines. When samples are shipped, page Nathaniel Goss and Brian Shaw at 2-3389 and 2-1928, respectively; for DFCI samples, call the MGH operator at 617-726-2000, ask to page 2-3389 and 2-1928, and leave a brief message indicating that the sample has been shipped. 2. For any questions regarding samples, please contact Nathaniel Goss and/or Brian Shaw.

Brastianos Lab
CPZN 3700
Simches Research Center
185 Cambridge Street
Boston, MA 02114

9.1.2.3. Collection, Processing and Handling of CSF for Cohort C

Collect 3-10cc of CSF at pre-registration and every 3 weeks (if patient has Ommaya) and every 6 weeks (if patient does not have an Ommaya) in a clear, glass CSF tube and clearly label with the below information:

- Patient's study ID and initials
- Collection date and time
- Study protocol number
- Sample type (e.g. CSF)

CSF samples should be couriered immediately (within one hour of collection) to the address below. Do not delay sample shipment to include more than one sample type or samples from multiple patients. The sample should be placed in a biohazard bag and shipped on ice or a cold pack according to IATA guidelines. When samples are shipped, page Mia Bertalan at 2-3389; for DFCI samples, call the MGH operator at 617-726-2000, ask to page 2-3389, and leave a brief message indicating that the sample has been shipped.

Brastianos Lab
CPZN 3700
Simches Research Center
185 Cambridge Street
Boston, MA 02114

9.2. Adaptive Biotechnologies immunoSEQ

FFPE specimens (or isolated tumor DNA) will also be shipped to Adaptive Biotechnologies.

Adaptive Biotechnologies profiles T-cell and B-cell receptors using high-throughput

sequencing. Samples sent to Adaptive will be handled in accordance with Adaptive SOPs. All arriving packages will be opened the same day they arrive and inspected thoroughly for correct labeling and packaging integrity. Adaptive will perform “survey level” sequencing on each tissue sample and “deep level” sequencing on each of the PBMC derived gDNA samples provided. As part of these analyses, Adaptive uses a proprietary assay for the amplification and massive parallel sequencing of millions of CDR3 regions in the T cell receptor beta gene (TCRB) using a multiplex PCR amplification across the VDJ junction of rearranged TCRB.

Using Adaptive’s unique capacity to perform next generation sequencing of T-cell receptors for this study, a large amount of data will be generated using tissue specimens from all collection time points. In addition to the immunoSEQ Analyzer software, which is available to all of Adaptive clients to aid in data analysis and visualization, Adaptive can directly involve its computational biology and biostatistics personnel in the data analysis.

9.3. Imaging Correlative Studies

MR scans will be performed at the Martinos Center for Biomedical Imaging and include routine T1- and T2-weighted images, fluid attenuated inversion recovery (FLAIR), contrast agent enhanced T1-weighted permeability, diffusion tensor imaging (DTI), and T2/T2*-weighted perfusion scans. Resting state MRI and MR Spectroscopy may be performed if time allows. Each MRI will last 60-75 minutes versus 45 minutes for standard brain MRIs.

Gadolinium-diethylenetriaminepentaacetic acid (or gadopentetate dimeglumine, Gd DTPA) will be used as a contrast agent and injected intravenously twice in each scan session. The maximum contrast dose that could be given is 0.3 mmol/kg per visit, in line with the FDA-approved dosing for this class of contrast agents.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to the start of therapy. In the event that the participant’s condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted. Scans should be conducted within 6 days prior to initiation of the next cycle of therapy.

Patients in Cohort C will have CSF collected at the timepoints specified in the study calendar. Patients with Ommaya/shunts will have CSF collected at every cycle (every 3 weeks). Patients who will be getting lumbar punctures for CSF collection will only be getting CSF drawn at screening, and odd numbered cycles starting C3D1 and every 6 weeks afterword with imaging visits.

Trial Period:	Screening Phase ^a	Treatment Cycles (21 day cycles) ^{b,c}								End of Treatment ^d	Post-Treatment		
Treatment Cycle/Title:	Main Study Screening (Visit 1)	1	2	3	4	To be repeated beyond 8 cycles				Discon	Safety Follow-up ^e	Active Followup ^f	Longterm Followup ^g
						5	6	7	8				
Scheduling Window (Days):	-14 to 0		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks	Every 3 months
Administrative Procedures													
Informed Consent ^h	X												
Inclusion/Exclusion Criteria ⁱ	X												
Demographics and Medical History ^j	X												
Prior and Concomitant Medication Review ^k	X	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab Administration		X	X	X	X	X	X	X	X				
Post-study anticancer therapy status ^f										X	X	X	X
Survival Status ^g												X	X
Clinical Procedures/Assessments													
Review Adverse Events ^l	X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination ^m	X	X	X	X	X	X	X	X	X	X	X		
Vital Signs and Weight ⁿ	X	X	X	X	X	X	X	X	X	X	X		
ECOG Performance Status ^o	X	X	X	X	X	X	X	X	X	X	X		
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Pregnancy Test – Urine or Serum β-HCG ^p	X												
PT/INR and aPTT ^q	X									X			
CBC with Differential ^r	X	X	X	X	X	X	X	X	X	X			
Comprehensive Serum Chemistry Panel ^s	X	X	X	X	X	X	X	X	X	X			
TSH ^t	X	X	X		X		X		X	X			
Efficacy Measurements													
Tumor Imaging ^u	X	X		X		X		X					
Cerebrospinal fluid (Cohort C)	X ^v	X ^w	X ^w	X ^x	X	X	X	X	X				

Trial Period:	Screening Phase ^a	Treatment Cycles (21 day cycles) ^{b,c}								End of Treatment ^d	Post-Treatment		
Treatment Cycle/Title:	Main Study Screening (Visit 1)	1	2	3	4	To be repeated beyond 8 cycles				Discon	Safety Follow-up ^e	Active Followup ^f	Longterm Followup ^g
						5	6	7	8				
Scheduling Window (Days):	-14 to 0		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks	Every 3 months

Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood												
Archival Tissue ^y												
Blood ^z	X	X	X	X	X	X	X	X	X	X		

^aAll screening procedures to be performed within 14 days of start of study treatment, except scans, x-rays and informed consent may occur up to 28 days prior to start of study treatment

^bAll screening procedures to be performed within 14 days of start of study treatment, except informed consent may occur up to 28 days prior to start of study treatment. C1D1 and Day 1 of subsequent cycles: For C1D1 only, screening assessments may serve as day 1 assessments, except serum pregnancy (within 72 hours of drug) and except in the event that there are any indications that the participant's condition is deteriorating for which laboratory evaluations should be repeated within 48 hours prior to initiation. For all subsequent cycles, required assessments should be performed within 3 days of scheduled cycle day 1 and staging scans should be performed within 6 days of scheduled cycle's day 1.

^cStudy drug therapy administration windows include +/- 3 days for each agent.

^dEnd of Tx: End of treatment assessments to be performed within 7 days after last day drug administration or within 7 days after decision to end treatment. Assessments may continue for ongoing reportable adverse events.

^e30-Day Post Drug: A visit is to be performed at 30 days (+/- 5 days) after the last study drug is given. If the patient is unable to come for an in-clinic visit at 30 days, a phone call for adverse events and survival should be performed. All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug.

^fActive Follow Up: In participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by radiologic imaging every 6 weeks (+/- 7 days) until (1) the start of new anti-cancer treatment, (2) documented disease progression, (3) death or (4) the end of the study, whichever occurs first. Imaging results will be collected.

^gLong Term Follow Up: After 6 months of active followup, participants will be followed every 3 months (+/- 1 month) via contact or medical record review until death for post-treatment therapies, reason for stopping those therapies and survival.

^hInformed Consent: Informed Consent may be obtained within 28 days of start of study treatment

ⁱInclusion/Exclusion Criteria: source documentation providing investigator's confirmation that the participant had met all eligibility criteria must be available prior to registration.

^jDemographics and Medical History: to include review of treatment history for cancer, any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.

^kConcomitant medications and reason for administration should be documented in the case history from within 28 days before starting study treatment up to the 30-Day Post Drug Visit.

^lAdverse event assessment: adverse events and SAEs and ECIs experienced by participants will be collected and recorded from the first dose of study treatment up to the 30-Day Post Drug Visit of the last dose of study medication (+/- 5 days depending on when 30-Day Post Drug visit/contact occurs).

^mFull Physical Exam (including neurologic exam): to be completed by the investigator or qualified designee at screening, C1D1 and start of all subsequent cycles.

ⁿVital signs: weight, heart rate, blood pressure, respiration rate, temperature. Vital signs must be performed prior to administration of treatment on treatment days. Height required only at screening.

^oPerformance Status: See Appendix A for ECOG scale.

^pPregnancy Test: For women of child bearing potential, a urine pregnancy test should be performed within 72 hours of first dose of study treatment. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

^qCoagulation – PT/INR, PT, PTT required at screening and at end of treatment

^rHematology: erythrocytes (RBC), hemoglobin, hematocrit, platelets, total WBC plus differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

^sSerum Chemistry: albumin, alkaline phosphatase (ALP), bicarbonate (HCO₃), BUN, calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH) magnesium, phosphorous, potassium, SGOT (AST), SGPT (ALT), sodium, total protein, total bilirubin (if total bilirubin is greater than the upper limit of normal, direct and indirect bilirubin should be performed), and uric acid.

^tTSH will be performed at baseline, week 1 and then every 2 cycles. TSH does not need to be back prior to dosing

^uBrain MRI and CT of chest/abd/pelvis will be performed every 6 weeks within +/- 6 days of the protocol-specified date. If there is a delay in initiation of a cycle, scans can be delayed to align with the next odd numbered cycle (instead of a mid cycle scan).

^vCSF will be collected every cycle in Cohort C to assess disease status. At screening collect CSF cytology, protein and glucose and cell count.

Trial Period:	Screening Phase ^a	Treatment Cycles (21 day cycles) ^{b,c}								End of Treatment ^d	Post-Treatment		
Treatment Cycle/Title:	Main Study Screening (Visit 1)	1	2	3	4	To be repeated beyond 8 cycles				Discon	Safety Follow-up ^e	Active Followup ^f	Longterm Followup ^g
						5	6	7	8				
Scheduling Window (Days):	-14 to 0		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks	Every 3 months

^w C1D1 and all other even numbered cycles starting C2D1 collect research CSF and CSF cell count. This applies only to patients with shunts.

^x Odd numbered cycles (with restaging) starting C3D1 collect CSF cytology, protein and glucose and cell count and research if possible

^y Tissue collected as part of clinical care (e.g. from prior biopsy, craniotomy) will be collected. See Section 9 for specimen collection requirements

^z Blood will be collected at screening and every 3 weeks (two 10 mL EDTA/lavender top tube) and at the end of treatment

11. MEASUREMENT OF EFFECT

Response in the CNS and response in non-CNS sites will be evaluated and recorded separately in this trial.

Response in the CNS and response in non-CNS sites will be evaluated and recorded separately in this trial.

Patient with clearly progressive extracranial disease on study will be taken off study

11.1. Antitumor Effect – Extracranial lesion assessments

For the purposes of this study, participants should be re-evaluated for extracranial response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1. Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. For Cohort C, only those participants that have received at least one cycle of therapy will be considered evaluable for the overall survival primary endpoint. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2. Disease Parameters

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

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

the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

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

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

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

11.1.3. Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥ 5 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- (a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- (b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- (c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent

fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4. Response Criteria

11.1.4.1. Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of

target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2. Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3. Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment

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

For Participants with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** Only for non-randomized trials with response as primary endpoint. *** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.				
Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “<i>symptomatic deterioration</i>.” Every effort should be made to document the objective progression even after discontinuation of treatment.				

For Participants with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* ‘Non-CR/non-PD’ is preferred over ‘stable disease’ for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.5. Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6. Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7. Response Review

All brain MRI scans will be reviewed centrally by the DF/HCC Tumor Imaging Metrics Core (TIMC). CNS response will be assessed centrally using the RANO criteria outlined in Section 12.1.4.

All non-CNS scans will be reviewed centrally by the DF/HCC Tumor Imaging Metrics Core (TIMC).

11.2. **Antitumor Effect – Intracranial Disease**

For the purposes of this study, participants should be re-evaluated for intracranial response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained at 6 weeks following initial documentation of objective response. Response and progression will be evaluated in this study using the RANO Response Criteria, as specified below^{48,49}.

11.2.1. Definitions

Evaluable for objective response. Only those participants who have measurable CNS disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.) For Cohort C patients, participants who have received at least one cycle of therapy will be considered evaluable for the primary endpoint.

Evaluable non-measurable evaluable disease. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.2.2. Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥ 5 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

For multifocal intracranial disease, no more than 5 target measurable lesions (each ≥ 5 mm in diameter in both dimensions) should be selected for measurement. Target lesions should be selected on the basis of their size (lesions with longest diameter), be representative of other lesions and lend themselves to reproducible repeated measurements.

Non-measurable disease. Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 5 mm.

Target lesions. All measurable lesions up to a maximum of 5 lesions in total, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), and in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameter for all target lesions will be calculated and reported as the baseline sum diameters.

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

11.2.3. Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

11.2.4. Response Criteria

11.2.4.1. Evaluation of Intracranial Lesions

Complete Response (CR): Requires all of the following:

- complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks
- no new lesions
- stable or decreasing corticosteroids (the steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan)
- stable or improved clinically

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

Partial Response (PR): All of the following criteria must be met:

- Greater than or equal to 30% decrease compared to baseline in the sum of target 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.
- no progression of non-measurable disease.
- no new lesions
- stable or reduced corticosteroid dose (the steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan)
- stable or improved clinically

Progressive Disease (PD): At least one of the following must be true:

- $\geq 20\%$ increase in the sum of longest diameters of target lesions using the

- same techniques as baseline
 - clear worsening of any nonmeasurable disease*
 - appearance of any new enhancing lesion/site
 - clear clinical worsening (unless clearly unrelated to this cancer, e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection)
 - failure to return for evaluation due to death or deteriorating condition
- * Progression of non-measurable CNS lesions is defined as follows:
- a lesion initially at baseline ≤ 5 mm in diameter that increases to ≥ 10 mm in diameter, or
 - a lesion initially at baseline 6-9 mm in diameter that increases by at least 5 mm in diameter.

Stable Disease (SD): All of the following criteria must be met:

- Does not qualify for CR, PR or progression
- All measurable and non-measurable sites must be assessed using the same techniques as baseline
- Stable clinically.

A summary of the response criteria is in the below:

	Complete Response	Partial Response	Stable Disease	Progressive Disease
T1-Gd+	None	$\geq 30\%$ decrease in sum of longest diameters of target lesions	$<30\%$ decrease- $<25\%$ increase	$\geq 20\%$ increase in the sum of the longest diameters of target lesions*
New Lesion	None	None	None	Present*
Corticosteroids	Stable or decrease	Stable or decrease	Stable or decrease	Stable or increasing
Clinical Status	Stable or improved	Stable or improved	Stable or improved	Decrease*
Requirement for Response	All	All	All	Any*
NA=not applicable #: Progression occurs when any of the criteria with * is present Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration				

11.2.4.2. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best

response assignment will depend on the achievement of both measurement and confirmation criteria. 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.”

11.2.5. Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.3. **Study Continuation Beyond Initial Progressive Disease**

Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses which may manifest as initial worsening of enhancement and edema on MRI or CT scans (i.e. pseudo-progression). In addition, the response patterns seen with immunotherapeutics 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. For these reasons, the immune- related response criteria (irRC) have endorsed continuation of study therapy beyond initial radiographic evidence of progression for clinically stable patients undergoing immune based therapies.

Therefore, the following adaptations will be used to assess response for patients treated on this study:

Potential Pseudo-progression: If radiologic imaging of intracranial and/or extracranial disease shows initial PD, subjects who are not experiencing significant clinical decline, may be allowed to continue study treatment for up to 12 weeks. Patients who have radiographic evidence of further progression after up to 12 weeks, or who decline significantly at anytime, will be classified as progressive with the date of disease progression back-dated to the first date that the subject met criteria for progression and

such subjects will be discontinued from study therapy. Three months is the most common timeframe for pseudo-progression observed among patients with advanced melanoma or other solid tumors treated with PD-1/PD-L1 immune checkpoint blockade⁵⁰. Furthermore, recent data presented at ASCO 2017 with checkpoint inhibitors in brain metastases showed that the median time to response in brain metastases was 2.8 months with a range of 1-10 months (Tawbi et al. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients with Melanoma Metastatic to the Brain: Results of the Phase II Study CheckMate 204, ASCO 2017).

Among patients on this study with initial radiographic PD, tumor assessment should be repeated regularly 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 stabilization or reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued / resumed. If repeat imaging after up to three months confirms progressive disease, 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.

Tumor Enhancement to Define Progression: RANO expanded the previously utilized Macdonald criteria⁵¹ 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. However, immune based therapies are expected to be associated with inflammatory changes that may include edema. Therefore, our study will define radiographic progressive disease by assessment of enhancing tumor burden only and will not include assessment of T2 or FLAIR changes as outlined in RANO because:

- There is no expectation that immunotherapy agents including PD-1 inhibitors will falsely diminish enhancing tumor burden as has been noted with anti-angiogenic therapies; and
- Immune based therapies are expected to induce inflammatory responses which may be associated with increased edema and T2/FLAIR changes. Such radiographic finding 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 for up to 12 weeks pending confirmation of PD on follow-up imaging. 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 not experiencing significant clinical decline and if:

- 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, study therapy 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 response⁵². Subjects that are exhibiting significant neurologic decline are not required to have repeat imaging for confirmation of progressive disease.

Participants with progressive radiographic findings are encouraged to undergo surgical intervention in order to delineate pseudo-progression due to inflammation associated with PD-1 blockade from true tumor progression. Participants with histopathologic findings of significant immune infiltrate and evolving gliosis will be allowed to continue study therapy. In contrast, those with clear evidence of progressive tumor by histopathologic evaluation will be defined as progressive and discontinued from study therapy. For such patients, the date of tumor progression will be the first date the participant met radiographic criteria for PD.

11.4. Response Review

All brain MRI scans will be reviewed centrally by the DF/HCC Tumor Imaging Metrics Core (TIMC). CNS response will be assessed centrally using the modified RANO criteria outlined in Section 12.1.4.

All non-CNS scans will be reviewed centrally by the DF/HCC Tumor Imaging Metrics Core (TIMC).

11.5. Other Response Parameters

Cerebrospinal Fluid: Cerebrospinal fluid will be collected as part of clinical care in Cohort C of this study (leptomeningeal cohort). Because CSF cytology is not a reliable measure of LMD response, clinical decisions for continuing therapy should not be based on cytology alone.

Response	Cytologic	Neurologic	Radiographic
Complete Response	Reversion to (-) CSF cytology (or NA)	Disappearance of all disease related symptoms present prior to therapy (or NA)	Complete disappearance of all detectable radiographic EOD (or NA)
Partial Response	> 50% decrease in malignant CSF cytology *	Improvement in neurologic symptoms or no worsening of symptoms	Improvement in radiographic evidence of disease. No new sites of measurable or assessable CNS disease

Stable Disease	Not fulfilling the criteria for CR or PR, but not progressive disease	Not fulfilling the criteria for CR or PR, but not progressive disease	Not fulfilling the criteria for CR, or PR but is not progressive disease
Progressive Disease	> 50% increase in malignant CSF cytology	Development of new neurologic symptoms related to neoplastic meningitis	Increase in radiographic evidence of disease. Appearance of new sites of disease.

* CSF cytology will be estimated from the CSF analysis of cell count and cytology

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1. Data Reporting

12.1.1. Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2. Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with ODQ
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of treatment administration
Adverse Event Report Form	Within 10 days of AE assessment/notification
Response Assessment Form	Within 10 days of response assessment
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2. Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study

team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3. Multicenter Guidelines

N/A

13. STATISTICAL CONSIDERATIONS

This is a phase II trial to investigate the therapeutic effect of pembrolizumab in CNS metastases. The trial includes four patient cohorts: newly diagnosed brain metastases (Cohort A); recurrent brain metastases (Cohort B); leptomeningeal disease (Cohort C); and patients with 1-7 brain metastases undergoing SRS (Cohort D). Cohorts A and B will be combined for assessment of outcomes; Cohorts C and D will be analyzed separately. The patient cohorts will not be compared to each other and are independently designed as phase II studies for preliminary assessments of efficacy in comparison to historical control rates. Full details concerning analytic methods will be provided in a separate Statistical Analysis Plan (SAP).

13.1. Study Design/Endpoints

Primary Endpoints:

1. Cohort A and B: Response in the central nervous system (intracranial benefit) as defined by complete response, partial response or stable disease by RANO criteria for brain metastases.
2. Cohort C: Overall survival.
3. Cohort D: Extracranial overall response (Section 10.1).

Secondary Endpoints:

1. Toxicity by CTCAE criteria (grade 3 or higher hematologic toxicity; grade 3 or higher neurologic toxicity) (Cohort A, B, C, D)
2. Overall survival in Cohorts A, B and D.
3. Intracranial response in patients with leptomeningeal metastases as defined in Section 10.5 (Cohort C).
4. Extracranial response as determined by RECIST and irRC (Cohort A, B, C, D)
5. Time to the first occurrence of extracranial disease progression, or death from any cause (Cohorts A, B, C, D)
6. Time to the first occurrence of intracranial disease progression, or death from any cause

(Cohorts A, B, C, D)

Exploratory Endpoints:

1. Genetic alterations
2. T2/FLAIR MRI Imaging Techniques

13.2. Sample Size, Accrual Rate and Study Duration

The total accrual for this phase II trial will be 101 patients divided among the four separate cohorts. Cohorts A and B will be combined in the analysis. Cohorts C and D will be analyzed separately.

Cohorts A and B: We will enroll a total of 58 patients to get 52 evaluable for the combined cohort.

The primary efficacy parameter for each cohort is objective response rate (CR+PR+SD) based on standard CNS response criteria (RANO criteria). We will compare a null hypothesis of 10% against an alternative of 24%. If at least 8 patients among total 52 patients have a response, treatment will be worthy of further study. This design has a type-I error of 10% and power of 89% (target type-II error 15%).

The estimated accrual rate for Cohorts A and B is based on our institutional patient volume of 14-18 patients with brain metastases seen in our multidisciplinary brain metastasis clinic each week. We estimate that 1-2 patients per month will be eligible in each cohort for this protocol. Thirty patients will be accrued within 14-17 months. Based on 6 months of follow-up (median survival of patients is approximately 6 months), the total study duration will be less than 24 months.

Cohort C: Accrual of 22 patients may be required to achieve 18 evaluable patients.

Patients will be considered evaluable for primary endpoint if they have received at least one cycle of therapy. The primary endpoint is the rate of overall survival at 3 months (OS3). Any patient whose vital status at 3 months cannot be determined will be counted as having died. A Simon two-stage design will be used to compare a null hypothesis OS3 of 18% against an alternative of 43%. Ten patients will be enrolled in the first stage. If 0 or 1 patients are alive at 3 months, the trial will be stopped early. If 2 or more patients are alive at 3 months, we will enroll an additional 8 patients. If at least 6 patients among the total of 18 patients are alive at three months, then the treatment would be promising in the cohort. This design has a type-I error of 9% (target 10%) and power of 85%. If the null hypothesis is true, then the probability is 0.45 of stopping at the end of the first stage.

The accrual rate in Cohort C is based on our institutional patient volume of 3-4 patients per month with leptomeningeal carcinomatosis seen in our multidisciplinary brain metastasis clinic. We estimate that 1-2 patients per month will be eligible for this protocol. Therefore, 22 patients will be accrued within 10-20 months. Based on four months of follow-up (median survival of patients is approximately 1-3 months), total study duration will be 24 months.

Cohort D: Accrual of up to 21 patients may be required to achieve 18 evaluable patients.

The primary endpoint in Cohort D is extracranial overall response rate (ORR). A Simon two-stage design will be used to compare a null ORR of 5% against an alternative of 25%. Eight patients will be enrolled in the first stage. If no responses are seen, then enrollment will stop. If at least one response occurs among these patients, then enroll 10 additional patients. If three or more patients have response out of the total of 18, then the therapy would be considered promising. This design has a type-I error of 5% (target 10%) and 82% power (target 80%). If the null hypothesis is true, the probability is 0.66 of stopping the trial at the end of the first stage.

The accrual rate in Cohort D is based on our institutional patient volume of 30-40 patients per month with brain metastases seen in our multidisciplinary brain metastasis clinic. We estimate that 1-2 patients per month will be eligible for this protocol. Therefore, 18 patients will be accrued within 12-18 months. Based on six months of follow-up (median survival of patients is approximately 1-3 months), total study duration will be 24 months.

13.3. Stratification Factors

NA

13.4. Interim Monitoring Plan

NA

13.5. Analysis of Primary Endpoints

Cohorts A and B: The primary endpoint is objective response rate (CR+PR+SD) based on standard CNS response criteria (RANO criteria). Contrast-enhanced cranial MRI will be performed every 6 weeks. The proportion of patients with a best response of CR or PR will be presented with a 90% confidence interval estimated using the method of Atkinson and Brown, which allows for the two-stage design. Based on a sample of 52 patients, the confidence interval will be no wider than 0.24.

Cohort C: The primary endpoint is the rate of overall survival at 3 months (OS3). Any patient whose vital status is unknown due to loss of follow-up will be classified as having died for purposes of estimating the primary endpoint. The proportion of patients alive at six months will be summarized with a 90% confidence interval estimated using the method of Atkinson and Brown, which allows for the two-stage design. Based on a sample of 18 patients, the confidence interval will be no wider than 0.42.

Cohort D: The primary endpoint is extracranial overall response rate (ORR). The proportion of patients with a best extracranial response of CR or PR will be presented with a 90% confidence interval estimated using the method of Atkinson and Brown, which allows for the two-stage design. Based on a sample of 18 patients, the confidence interval will be no wider than 0.42.

13.6. Analysis of Secondary Endpoints

Safety and Tolerability: All adverse events recorded during the trial will be summarized for all patients having received one or more doses of pembrolizumab. The data for each cohort will be summarized separately, although safety data may be combined, if appropriate. The proportions of patients with grade-3 or higher hematologic toxicities or grade-3 or higher neurologic toxicities will be presented with 90% exact binomial confidence intervals. The incidence of events that are new or worsening from the time of first dose of treatment will be summarized according to system organ class and/or preferred term, severity (based on CTCAE grade), type of adverse event, and relation to study treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, and type of adverse event.

Overall Survival: The distributions of overall survival will be summarized for Cohorts A and B (combined) and D using the method of Kaplan-Meier. Each cohort will be presented separately. The follow-up of patients who are alive at the time of analysis will be censored at the date of last assessment of vital status. Median OS will be presented and accompanied by 90% confidence intervals estimated using log(-log(survival)) methodology. Survival point estimates at 3 and 6 months will also be presented with confidence intervals.

Intracranial/Extracranial Response: The proportion of patients in Cohort C with intracranial response (CR or PR by RANO) will be presented with a two-sided, 90% exact binomial confidence interval. For a sample of 18 patients, the confidence interval will have maximum width of 0.42. Extracranial response rates (CR or PR) according to RECIST and irRC will be summarized for Cohorts A and B (combined), and C, and D, individually. Rates will be accompanied by two-sided 90% exact binomial confidence intervals. Cases where RECIST and irRC responses do not agree will be documented.

Extracranial PFS and Intracranial PFS: Extracranial PFS (EPFS) is defined as the time from first dose of pembrolizumab to documented extracranial progression (per RECIST or irRC) or death, whichever occurs first. Intracranial PFS (IPFS) is defined as the time from first dose of pembrolizumab to documented intracranial progression (RANO) or death, whichever occurs first. For both endpoints, the follow-up of patients who have neither died nor progressed at the time of analysis will be censored at the date of last adequate disease assessment. Each cohort will be presented separately. Median EPFS and IPFS will be presented and accompanied by 90% confidence intervals estimated using log(-log(survival)) methodology. Point estimates at 3 and 6 months will also be presented with confidence intervals.

13.7. Analysis of Exploratory Endpoints

Biomarkers of blood and tissue: Analyses of blood and tissue biomarkers will be based on descriptive statistics, as appropriate for the biomarker.

To examine response according to pre-treatment levels of biomarkers, the study sample will be divided retrospectively according to the primary endpoint of response or non-response. Pre-

treatment biomarker levels will be summarized descriptively for the two response groups and compared using Wilcoxon rank-sum tests for markers measured on a continuous scale, or Fisher's exact tests for those measured on a categorical scale. Where appropriate, visualization of the relationship between baseline marker levels and the distributions of PFS or OS will employ Kaplan-Meier estimates stratified by biomarker levels. Medians of the time-to-event endpoints will be shown with two-sided 90% confidence intervals; the distributions of PFS and OS will be compared across biomarker strata using the log-rank test.

Changes in biomarkers between pretreatment and progression or treatment discontinuation will be calculated (post-pre) for each patient and summarized descriptively. In addition, the correlation of changes in tissue biomarkers with changes in measures of peripheral blood markers will be explored graphically, or by appropriate statistical methods based on data availability, to assess associations.

13.8. Reporting and Exclusions

13.8.1. Evaluation of Toxicity

All participants who have received at least one dose of study medication will be evaluable for toxicity from the time of their first treatment.

13.8.2. Evaluation of the Primary Efficacy Endpoint

To be included in the assessment of the primary objective, patients need to receive at least one cycle. If systemic progression occurs prior to the 6 week CNS response timepoint, patient will still be included in the analysis of primary objective.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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