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TITLE: A phase 2 trial of pembrolizumab plus bevacizumab in patients with metastatic melanoma or non-small cell lung cancer with untreated brain metastases.

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1.0 TRIAL SUMMARY

Abbreviated Title	Pembrolizumab and bevacizumab in untreated brain metastases
Trial Phase	II
Clinical Indication	Untreated brain metastases from melanoma or non-small cell lung cancer
Trial Type	Open label
Type of control	None
Route of administration	IV
Trial Blinding	NA
Treatment Groups	Two cohort phase II trial, one for melanoma patients and one for non-small cell lung cancer patients
Number of trial subjects	53
Estimated enrollment period	January 1, 2016 – December 31, 2022
Estimated duration of trial	96 months
Duration of Participation	36 months

2.0 TRIAL SYNOPSIS

Concept and Rationale: Important progress has recently been made in treating metastatic melanoma and non-small cell lung cancer (NSCLC) using pembrolizumab, an inhibitor of PD-1, a negative immune check-point regulator found on cytotoxic T-cells _ENREF_1. Approximately 34% of patients with melanoma and 25% with NSCLC have partial or complete responses to this drug at optimal doses, and additional patients have prolonged stable disease. An ongoing study of pembrolizumab in patients with brain metastases from melanoma or NSCLC has shown that while the drug has substantial activity in brain metastases, peri-lesional edema can result in significant morbidity. Bevacizumab, an antibody to VEGF has been shown in a number of prior clinical studies to reduce peri-lesional edema in the CNS. Moreover, in preclinical models the addition of VEGF inhibitors to PD-1 inhibitors has been shown to enhance anti-tumor immunity, providing further rationale for combining pembrolizumab and bevacizumab in this patient population.

Every year there are almost 50,000 patients from these diseases who will develop brain metastases. Melanoma in particular tends to metastasize to the brain frequently, and the incidence on autopsy is up to 70%. Brain metastases are present in 10% of patients with NSCLC at diagnosis, and another 30% develop CNS disease over the course of their disease. Multifocal disease is common in both of these diseases, with about half of patients with CNS disease presenting with more than one brain lesion.

Many effective drugs currently in development have not been well studied for CNS penetration, and patients with brain metastases are excluded from most clinical trials. With recent advances in delivery of local therapies, particularly stereotactic radiosurgery (SRS), local control of brain metastases has improved. However, SRS still has limitations and complications such as radiation necrosis, cerebral edema, and delayed tumor hemorrhage.

Beyond these local therapies, effective systemic therapies that are active in the CNS may be of significant benefit to patients.

The purpose of this trial is to study the activity of pembrolizumab in combination with bevacizumab in patients with untreated brain metastases from melanoma or NSCLC to determine activity and safety of the drug combination. Furthermore, in patients who undergo resection of biopsy of a brain metastasis, we will evaluate biomarkers predictive of treatment benefit, and will also conduct correlative biomarker studies on extra-cerebral specimens in all patients in whom a systemic biopsy is feasible or in archival tumor tissue when available.

Confirmed, objective CNS responses were seen in 11 of the first 20 melanoma patients, for a brain metastasis response rate of 55%. The primary endpoint of the study for the melanoma cohort has been met. We will validate the findings in the melanoma cohort in the next 20 melanoma patients. The NSCLC component is a pilot study

Primary Objectives/Endpoints:

(1) To determine the brain metastasis response rate in patients with melanoma or NSCLC with untreated brain metastases, treated systemically with pembrolizumab plus bevacizumab.

Secondary Objectives/Endpoints:

1) To determine the incidence of steroid use for control of cerebral edema during treatment with pembrolizumab plus bevacizumab in patients with metastatic melanoma or NSCLC to the brain compared to previous study of patients treated with pembrolizumab alone.
2) To determine the best overall response rate (ORR) in patients with brain metastases from melanoma or NSCLC treated with combination pembrolizumab plus bevacizumab.
3) To evaluate median progression free survival (PFS) and overall survival (OS) in patients with melanoma or NSCLC metastatic to the brain, treated with pembrolizumab plus bevacizumab.
4) To determine the safety of pembrolizumab in combination with bevacizumab in patients with untreated brain metastases from melanoma or NSCLC

<u>Exploratory objective:</u>
To study PD-L1 expression and other potential predictive biomarkers in CNS, tumors and blood, and correlate results with response to pembrolizumab plus bevacizumab.

Study Design: This is a phase II study with the 2 tumor types treated in separate cohorts. Key eligibility criteria include advanced melanoma or non-squamous NSCLC with at least 1 brain metastasis that is at least 5mm but smaller than 2cm in size, and no requirement for systemic steroids to control peri-lesional CNS edema. Patients who have had prior resection of a CNS lesion will be required to provide a tumor block for correlative studies and all patients will be required to provide an extra-cerebral specimen when feasible

Pembrolizumab will be administered on day 1 of every cycle until disease progression or withdrawal from study. Bevacizumab will be administered in addition to pembrolizumab on day 1 of cycles 1, 2, 3, and 4 (or alternative cycles if bevacizumab is held during these cycles). Three weeks constitutes one cycle.

Efficacy evaluation will be performed every 6 weeks for the first 3 months of treatment, and every 9 weeks thereafter, including MRI of the brain and CT or PET scans of systemic disease. Patients will continue on study until they have disease progression, toxicities that preclude continuing the study drug, withdrawal from study, development of other severe illness, neurologic or systemic complications following local therapy to any lesion, termination of study, or death. Dose reductions for severe immune-related toxicities will not be allowed, however, discontinuation of one of the drugs will be allowed for toxicity if the investigator determines that the other drug is providing clinical benefit. Patients may remain on study after disease progression if they are deemed by the investigator to be deriving clinical benefit from treatment.

Number of Patients:

A total of 40 melanoma patients and 13 NSCLC patients will be enrolled for a total of 53 patients. The study will accrue for approximately 24 additional months and will be open for approximately 12 additional months after the last patient is accrued as patients on study are being followed.

Statistical Methods

The primary goal of this study is to determine the efficacy and safety of pembrolizumab plus bevacizumab in patients with untreated brain metastases from melanoma or NSCLC. The primary endpoint is the brain metastasis response rate (BMRR). A drug with minimal activity would be expected to have a BMRR of 10%. The study was initially designed so that this combination would be considered worthy of further study in patients with untreated brain metastases if its true BMRR is similar to the systemic response rate with pembrolizumab (Melanoma - 34%, NSCLC with PD-L1 positive tumors - 25%).As of February 2019, 20 patients had been accrued to the melanoma cohort of the trial and brain metastasis responses were obser**ved in 55%, 95% CI: 0.32-0.77)** Therefore, the statistical plan was amended to include an additional 20 patients to the melanoma cohort to validate the findings in the initial cohort. The initial pre-defined criterion that the combination of pembrolizumab and bevacizumab would not be worthy of further study if the BMRR was <10% was **modified** to 30%, which is the lower bound of the 95% confidence interval from the first 20 melanoma patients. To account for the uncertainty of the historical response rate, we will now follow the approach proposed by Thall and Simon (1994),[63] and after enrolling and evaluating the outcomes of the 20 patients in the confirmatory cohort, we will conclude the combination worthy of further study if there are at least 8 responses in these 20 patients.

A pilot study of 13 NSCLC will be conducted**.** See statistical analyses section for more details.

INVESTIGATOR SIGNATURE PAGE

Protocol Title: *A phase 2 trial of pembrolizumab plus bevacizumab in patients with metastatic melanoma or non-small cell lung cancer with untreated brain metastases*

Protocol Version: 12

By signing this agreement, I, the Site Investigator named above, acknowledge receipt of this protocol version. I have read this protocol in its entirety and agree to conduct this study according to this protocol, Good Clinical Practices and all applicable codes of federal regulations (CFRs) and guidelines.

Site Investigator's Printed Name: _____

Signature: _____

Date: _____

3.0 TRIAL DESIGN

3.1 Trial Design and duration

This is a phase II study for melanoma and a pilot study for NSCLC. Patients will be treated in separate cohorts. After establishing eligibility criteria, target lesions in the CNS will be chosen and will be followed by modified RECIST (mRECIST) criteria to determine best brain metastasis response. When feasible, after obtaining informed consent, patients may undergo local therapy (resection or laser interstitial thermocoagulation therapy - LITT) to the brain. Biopsy of tumor tissues from extra-cerebral sites will be studied for PD-L1 and other biomarkers (see section 11.0 below) as potential predictors of sensitivity to pembrolizumab and bevacizumab, although it should be noted that the presence of a biomarker is not necessary for melanoma patients to go on trial. Expression of PD-L1 determined by Central and/or local testing of PD-L1 IHC by any FDA-approved assay or an assay performed in a CLIA laboratorywill be required for eligibility for NSCLC patients. This will be followed by treatment with pembrolizumab 200mg IV every 3 weeks. Bevacizumab 7.5 mg/kg will be administered on Day 1 of cycles 1, 2, 3, and 4 (or alternative cycles if bevacizumab is held during these cycles). Day 1 of the study will be defined as the first day a patient receives pembrolizumab. Each cycle is 3 weeks.

Clinically Evaluable Lesion(s)**:** A clinically evaluable lesion is at least one asymptomatic, untreated brain metastasis that measures at least 5 mm in minimum cross-sectional diameter and < 20 mm in maximum cross-sectional diameter as determined by brain MRI at baseline, and is determined by the investigator to be safe to follow clinically without local therapy. If MRI slice thickness is >2.5mm, the minimum cross-sectional diameter must be at least twice the MRI slice thickness. An untreated brain metastasis is defined as a lesion not present at the time of whole brain radiation therapy or included in a stereotactic radiotherapy field (or within 2mm of a treated lesion), or any lesion that is new or unequivocally progressing since prior radiation therapy. At time of enrollment, the clinically evaluable lesion(s) will be defined.

Patients will proceed directly to therapy with bevacizumab and pembrolizumab. If, at any time while on study, the patient develops symptoms related to any cerebral metastases the patient may have brain imaging prior to the pre-defined imaging time points and proceed to local therapy of the lesion(s) if indicated.

Efficacy evaluation will be performed every 6 weeks for the first 3 months and every 9 weeks thereafter, including PET or CT scans of systemic disease. Brain metastasis response will be determined using modified RECIST (mRECIST) criteria. Patients will continue on study until they have overall disease progression in either their clinically evaluable CNS lesions (by mRECIST criteria) or in their systemic metastases (by RECIST 1.1 criteria), toxicities that preclude continuing the study drugs, withdrawal from study, development of other severe illness, neurologic or systemic complications following local therapy to any lesion, termination of study, or death. There will be no dose reduction for either pembrolizumab or bevacizumab but one drug may be held as outlined below. If a patient develops symptoms related to ANY lesion, local therapy may be administered to that lesion.

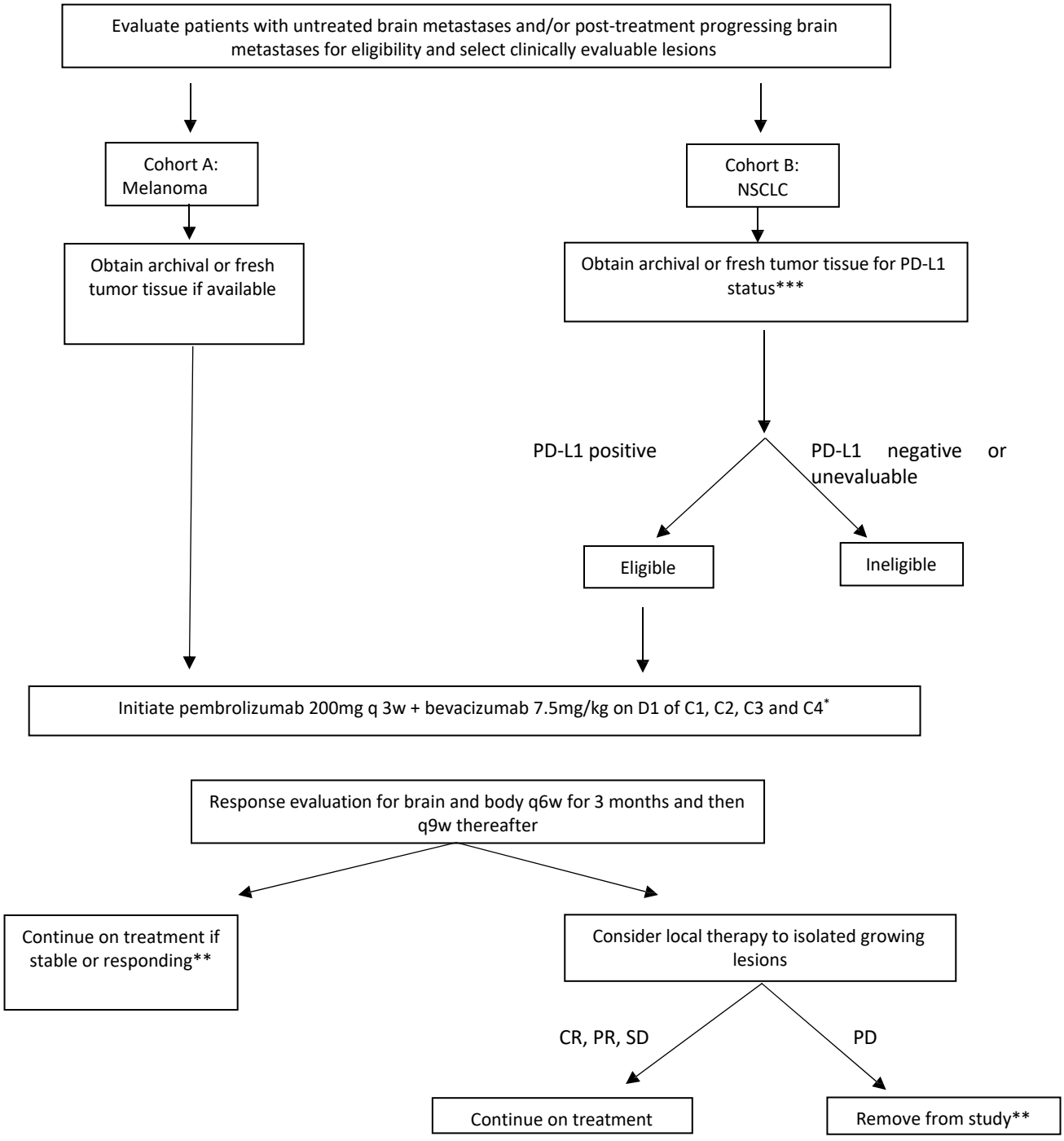
Patients with new symptoms from CNS lesions, or progressing lesions may be treated with local therapy to the brain metastases. These patients can continue therapy with both drugs or one of the drugs if the patient is otherwise deemed to be deriving clinical benefit. If patients develop clear intralesional or perilesional hemorrhage they can stay on study with cessation of bevacizumab.

Treatment beyond progression: If the treating physician determines that a patient is deriving clinical benefit from therapy, treatment beyond progression (as defined by mRECIST for CNS lesions and RECIST 1.1 for extracerebral lesions) will be allowed.

Discontinuation of bevacizumab OR pembrolizumab and continuation of monotherapy: Patients who develop toxicity clearly attributable to one drug who are felt to be benefitting from therapy will be allowed to remain on study. Examples include proteinuria, or thromboembolic events attributed to bevacizumab and severe autoimmune toxicity not controllable with steroids from pembrolizumab.

The plan is to enroll an additional 40 melanoma patients and 13 NSCLC patients in a pilot study for 53 patients total.

3.2 Trial Diagram



*Bevacizumab may be administered during later cycles if held during cycles 1-4, with the goal of administering a total of 4 cycles of combination pembrolizumab and bevacizumab

** Subjects who experience PD may continue on study if they are determined to be deriving clinical benefit from the study drugs and they continue to meet all other criteria for treatment

*** Subjects with PD-L1 test results from outside the study are not required to repeat testing for determination of eligibility as long as no systemic therapy was administered after the test results were obtained. If patients do not undergo PD-L1 testing for eligibility, fresh or archival tissue is still required at study entry for correlative studies.

3.3 OBJECTIVE(S) & HYPOTHESIS(ES)

3.3.1 Primary Objective(s) & Hypothesis(es)

1) **Primary Objective:** To determine the brain metastasis response rate in patients with melanoma or NSCLC treated with pembrolizumab plus bevacizumab.

   Hypothesis: The combination of pembrolizumab and bevacizumab will result in enhanced anti-tumor activity.

3.3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To determine the incidence of steroid use for control of cerebral edema during treatment with pembrolizumab and bevacizumab in combination in patients with metastatic melanoma or NSCLC.

Hypothesis: The combination of pembrolizumab and bevacizumab will result in reduced steroid use to control CNS edema compared to a historical cohort of patients treated with pembrolizumab alone.

(2) **Objective**: To determine the best overall response rate (ORR) in patients with brain metastases from melanoma or NSCLC treated with combination pembrolizumab and bevacizumab.

Hypothesis: The combination of pembrolizumab and bevacizumab will result in enhanced anti-tumor activity compared to a historical cohort of patients with pembrolizumab alone.

(3) **Objective**: To evaluate median progression free survival (PFS) and overall survival (OS) in patients with melanoma or NSCLC metastatic to the brain, treated with pembrolizumab plus bevacizumab.

Hypothesis: The combination of pembrolizumab and bevacizumab will result in longer PFS and OS compared to a historical cohort of patients with pembrolizumab alone.

(4) **Objective:** To determine the safety and toxicity of pembrolizumab in combination with bevacizumab in patients with untreated brain metastases

Hypothesis: The combination will result in an improved central nervous system toxicity profile and less perilesional edema than pembrolizumab monotherapy

3.3.3 Exploratory Objective

(1) Objective: To study PD-L1 expression and other potential predictive biomarkers in CNS, tumors and blood, and correlate results with response to pembrolizumab plus bevacizumab.

Hypothesis: Patients with high PD-L1 tumor content AND high TIL content, particularly increased CD-8 TILs, will be more likely to respond to the combination of pembrolizumab and bevacizumab.

4.0 BACKGROUND & RATIONALE

4.1 Background

Although immune-modulating antibodies are becoming the standard of care for treating melanoma and other malignancies, little is known regarding the effects of these agents on brain metastases. Ten to 40% of patients with metastatic melanoma develop brain metastases during their lifetime and > 75% have metastases at autopsy [1-5]. Among patients with non-small cell lung cancer (NSCLC), 10% have brain metastases at presentation and another 30% develop them over the course of their disease. Survival after the development of brain metastases is as dismal in those with NSCLC as it is for melanoma. Multifocal disease is common in both of these malignancies, with about half of patients with CNS disease presenting with more than one brain lesion [6]. Typical local management involves surgery or stereotactic radiosurgery (SRS), depending on the size and number of lesions, while whole brain radiation (WBRT) is used for patients with multiple lesions, or large lesions [7,8]. However, SRS has limitations and can cause complications such as radiation necrosis, cerebral edema and delayed tumor hemorrhage [9]. With the advent of active systemic therapies in these diseases, it is possible that brain metastases might be controlled with these newer drugs, similar to control of extra-cerebral metastases. The ability of systemic therapies to cross the leaky blood brain barrier found in tumors is the subject of ongoing investigations [3,10,11]. To date, most systemic therapy trials exclude patients with active brain metastases because of poor prognosis and unknown CNS penetration, and their activity in CNS metastases is typically understudied [3,12].

4.1.1 Pharmaceutical and Therapeutic Background

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 CD3ζ, PKCθ 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 Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

4.1.2 Preclinical and Clinical Trial Data for pembrolizumab

Please refer to the Investigator's Brochure for additional preclinical and clinical data.

4.2 Rationale

4.2.1 Rationale for combining pembrolizumab and bevacizumab

Pembrolizumab Rationale:
Antibodies blocking either PD-1 or its ligand PD-L1 are the most active of the immune modulating antibodies studied in clinical trials to date. Initial trials of antibody-mediated blockade of PD-1 alone showed promising results with durable tumor regression or prolonged disease stabilization in patients with advanced solid tumors including melanoma[13-15] and lung cancer [13]. Results from a phase 1b study of pembrolizumab, a monoclonal antibody against PD-1, in metastatic melanoma showed an overall response rate of 34% and a median PFS of 36 months [15]. Eighty percent of patients had a continued response to therapy. Based on this study, pembrolizumab, is now approved by the FDA for the treatment of metastatic melanoma [14]. A randomized phase III trial comparing pembrolizumab to ipilimumab in the frontline setting demonstrated a response rate of 34% in patients receiving pembrolizumab [16]. Recent data regarding activity of pembrolizumab in previously untreated NSCLC was presented at ASCO, 2014 and showed an overall response rate of 26% with 100% of patients having an ongoing response [17]. Additional studies have been conducted showing response rates that vary based on PD-L1 status and cut-points for positivity [18-21]. A phase III trial is underway comparing pembrolizumab to standard chemotherapy in NSCLC. In almost all trials conducted to date, clinical response to PD-1/PD-L1 pathway antagonists has been strongly associated with pre-treatment tumor cell or tumor infiltrating lymphocyte (TIL) surface expression of PD-L1. Correlative data also suggest that in many patients, pre-treatment tumor PD-L1 expression is induced by adjacent cytokine-producing T-cells; however, a subset of tumors can express PD-L1 in the absence of T-cell infiltration, presumably as a result of aberrant cell signaling [22]. Tumors expressing PD-L1 but lacking TIL, and conversely tumors containing non-cytokine producing TIL and absent PD-L1 expression, are less likely to respond to PD-1/PD-L1 pathway antagonists.

Even in the subgroup of patients most likely to respond to PD-1/PD-L1 pathway antagonists (*i.e.,* tumors containing TIL and associated PD-L1 expression), combinations may be necessary to higher rates of durable responses. Particularly for patients with metastatic disease to the brain, the addition of a second agent in combination with pembrolizumab may allow for improved responses with decreased deleterious inflammatory effects, such as peri- lesional edema. Optimal approaches to such combinations are influenced by both preclinical studies and results from post-treatment biopsies conducted on patients in our institution with brain metastases treated with pembrolizumab, in which there can be peri-lesional edema with tumor regression or so-called pseudoprogression (necrosis and associated inflammation). Data from a phase II trial of pembrolizumab in patients with metastatic melanoma or non- small cell lung cancer with untreated brain metastases in our institution showed an acceptable safety profile; responses have also been seen, however, in some patients, early cerebral edema has required discontinuation of therapy and/or treatment with steroids to control inflammation. In a survival analysis of 23 melanoma patients enrolled on this trial, 48% of patients were alive at 24 months, demonstrating durable responses and responses were concordant between the brain and extracerebral sites. In the preliminary results from the

phase II portion of this trial combining pembrolizumab and bevacizumab, 11 out of 20 patients with melanoma had a response in brain metastases as of February 2019.

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

Bevacizumab Rationale:
Bevacizumab, a recombinant humanized monoclonal antibody blocking angiogenesis by vascular endothelial growth factor A (VEGF-A) inhibition, is a compelling combination partner for PD-1 pathway antagonists in patients with metastatic disease to the brain. VEGF decreases dendritic cell function and subsequently antigen presentation and T cell activation while promoting the development of myeloid-derived suppressor cells.[23,24] VEGF is commonly found in the tumor microenvironment where it functions in immune evasion by altering the tumor vasculature to reduce lymphocyte adhesion to vessel walls within the microenvironment, thus contributing to decreased trafficking across endothelium into tumor deposits.[25-27] Restoration of dendritic cell and T cell function may improve T cell response and result in overall improved clinical activity.

In a murine melanoma model, the combination of VEGF blockade and adoptive immunotherapy was synergistic, resulting in improved antitumor activity, prolonged survival and increased trafficking of T cells into tumors.[28] Other murine models have tested combined inhibition of PD-L1 and VEGF pathway in the syngeneic colorectal cancer model MC-38, showing synergism between the drugs. In addition, in the melanoma Cloudman model, bevacizumab was synergistic with PD-1 pathway blockade.[29,30]

Synergistic effects of immunotherapy and VEGF blockade have been observed clinically in patients with melanoma receiving anti-CTLA-4 and bevacizumab.[31] In a cohort of 10 patients with metastatic renal cell carcinoma treated with bevacizumab and MPDL3280A, McDermott et al.[32] reported partial response (PR) in 4 patients and an additional 5 patients achieved stable disease (SD). Treatment was well-tolerated with no unexpected toxicities. Responses were observed in a variety of other tumors.[33] Specific to metastatic melanoma, Phase 1 studies were recently initiated combining bevacizumab with MPDL3280A (anti-PD- L1) and a large randomized phase 2 trial is ongoing comparing MPDL3280A with bevacizumab versus MPDL3280A with sunitinib versus MPDL3280A alone in previously untreated patients with metastatic renal cell carcinoma.

For NSCLC, bevacizumab is an approved drug for patients with non-squamous histology in first-line therapy when added to chemotherapy. Initial studies showed a median survival of 12.3 months with the addition of bevacizumab to paclitaxel plus carboplatin compared to 10.3 months with chemotherapy alone.[34] As predicted, patients with brain metastases were excluded from this trial because of the risk of CNS hemorrhage. However, as early as 2009, bevacizumab was explored for the treatment of brain metastases in solid tumors. A phase 2 trial, AVF3752g (PASSPORT), specifically addressed safety in patients with NSCLC and previously treated brain metastases who underwent systemic therapy with bevacizumab.[35] CNS hemorrhage was rare. Subsequently, bevacizumab was retrospectively studied in a

small cohort of patients with NSCLC and active brain metastases who received bevacizumab alone and in combination with chemotherapy.[36] One of 6 patients had an asymptomatic grade 1 intra-tumoral hemorrhage which occurred in the setting of concurrent anticoagulation. Notably, these patients required less corticosteroids when given bevacizumab. In another study, 18 patients with untreated brain metastases from various malignancies were treated with bevacizumab based therapy; 60% had PRs and overall the edema was reduced.[37]

Bevacizumab has long been established as a safe and effective treatment of glioblastoma (GBM). Angiogenesis in brain tumors such as GBM leads to a highly permeable blood brain barrier which results in disruption of autoregulated cerebral blood flow.[38] Angiogenesis in the brain promotes intravascular fluid extravasation into the extracellular spaces, otherwise known as vasogenic edema. If severe, this may lead to ischemia in the brain and ultimately, deterioration of neurologic function. Initial studies of bevacizumab for the treatment of GBM showed clinical activity, decreased tumor size, increased progression-free survival (PFS) and a decreased steroid requirement for edema.[39,40] The risk of cerebral hemorrhage appears to be independent of bevacizumab therapy.[41] Many studies have investigated the effects of bevacizumab in the treatment radiation necrosis in a variety of brain tumors, and it has been shown to be highly effective.[42-45],[46] . These studies, while small, suggested that bevacizumab may have some inflammatory antitumor effects in addition to restoration of vascular permeability of the blood brain barrier, which might improve drug delivery.

4.2.2. Rationale for Trial and Selected Subject Population

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been established.[47] Antagonists of PD-1 and PD-L1 have been shown to produce significant clinical activity in patients with metastatic melanoma and NSCLC. Current research efforts with these agents are directed towards identifying patients who are most likely to benefit from treatment and also to develop combinations that increase clinical activity in both the 'responsive' and non-responsive populations. In almost all trials conducted to date, clinical response to PD-1/PD-L1 pathway antagonists has been strongly associated with pre-treatment tumor cell or tumor infiltrating lymphocyte (TIL) surface expression of PD-L1.[48,49] Correlative data also suggest that in many patients, pre-treatment tumor PD-L1 expression is induced by adjacent cytokine-producing T-cells; however, a subset of tumors can express PD-L1 in the absence of T-cell infiltration, presumably as a result of aberrant T-cell signaling[22]. Lack of PD-L1 expression suggests that TILs are either unable to produce cytokines or cannot recognize tumor antigens. Tumors expressing PD-L1 but lacking TIL, and conversely tumors containing non-cytokine producing TIL and absent PD-L1 expression, are unlikely to respond to PD-1/PD-L1 pathway antagonists.

Even in the subgroup of patients that are most likely to respond to PD-1/PD-L1 pathway antagonists (*i.e.,* with tumors containing TIL and associated PD-L1 expression), combination therapies may be necessary to achieve very high rates of durable complete responses. We know that pembrolizumab has activity in untreated brain metastases in a subset of patients, however, in our institutional experience from an ongoing phase II trial of pembrolizumab in

patients with melanoma or NSCLC and untreated brain metastases, some patients develop peri-lesional edema. This might limit the ability to administer pembrolizumab and often requires steroids. A number of lines of evidence support the synergistic effects of the combination of bevacizumab and pembrolizumab. We propose a phase 2 trial of these two agents in patients with metastatic melanoma and a pilot study of NSCLC patients with untreated brain metastases. We postulate that bevacizumab would substantially decrease the cerebral inflammatory effects of pembrolizumab and vasogenic edema, and may have some antitumor efficacy, as well. It is expected that the results of this trial will produce sufficient data to determine if a subsequent randomized trial is justified for further development of this combination in melanoma and NSCLC as well as other metastatic malignancies.

4.2.3 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) has been conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date.

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 pembrolizumab 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. Pembrolizumab 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 pembrolizumab 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. 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 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.

Rationale for administering pembrolizumab at a flat dose of 200mg every 3 weeks and bevacizumab at 7.5 mg/kg on day 1 of cycles 1, 2, 3 and 4:

A phase II trial of pembrolizumab in patients with melanoma or NSCLC and untreated brain metastases conducted at Yale is ongoing. Interim analysis shows good anti-tumor activity. However, a number of patients have had to hold doses or take steroids due to peri-lesional edema. Our ongoing trial is utilizing a dose of 10mg/kg every 2 weeks. Safety MRI of the brain, which has been an important component of the trial, is done at 4 weeks and complete staging studies are conducted every 8 weeks. Prior trials using bevacizumab every 2 weeks for treating perilesional CNS edema have determined the safety and efficacy of a regimen of 7.5mg/kg of bevacizumab every 2 weeks [50]. For simplicity and convenience, we will administer both drugs every 3 weeks for the first four doses (on Day 1 of cycles 1, 2, 3 and 4), while continuing pembrolizumab every 3 weeks.

4.2.4. Rationale for Endpoints

Efficacy Endpoints

The primary purpose of this trial is to determine the activity of pembrolizumab and bevacizumab in combination in patients with untreated brain metastases from melanoma or NSCLC. The primary endpoint is therefore brain metastasis response rate. In our previous experience there is a small number of patients that have discordant responses in the brain and the body, and extra-cerebral response as well as overall response are therefore secondary efficacy endpoints. PFS and OS is expected to be shorter in this patient population with very advanced disease than in stage IV patients with melanoma or NSCLC without brain metastases, and determination of OS and PFS are secondary endpoints. We note that patients might receive additional active antineoplastic agents after progression on these drugs, resulting in more prolonged OS than expected for an untreated population of patients with brain metastases.

Safety Endpoints

One of the secondary endpoints is to determine the safety of pembrolizumab in combination with bevacizumab. A previous trial with pembrolizumab monotherapy in this population showed that many of these patients develop peri-lesional edema, and we expect that the combination will be better tolerated and result in a decrease in steroid use.

Biomarker Research

Biomarker studies (Section 11) will be conducted retrospectively with the goal of improving patient selection for this regimen. If predictive biomarkers or a predictive biomarker panel is identified, it will be further studied prospectively in future trials. These studies are

considered exploratory at this juncture, and we note that the panel of biomarkers studied might change over time as the scientific field evolves.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1. Diagnosis/Condition for Entry into the Trial

Metastatic melanoma or non-squamous NSCLC with untreated brain metastases and/or post-treatment progressing brain metastases.

5.1.2 Subject Inclusion Criteria

1. Biopsy proven metastatic melanoma or non-squamous NSCLC with at least one untreated cerebral metastasis that is at least 5 mm AND twice the MRI slice thickness, but less than 20 mm, that is asymptomatic and does not require local therapy at the time of enrollment ("clinically evaluable lesion(s)"). An untreated brain metastasis is defined as a lesion not present at the time of whole brain radiation therapy or included in a stereotactic radiotherapy field (or within 2mm of a treated lesion), or any lesion that is new or unequivocally progressing since prior radiation therapy. *All MRIs at screening will be reviewed centrally at Yale for sign off of eligibility.*
2. Age ≥18
3. ECOG performance status < 2
4. Any number of previous treatments with the **exception** of previous inhibitors of PD-1, PD-L1, or PD-L2. Other prior systemic therapies must have been administered at least 2 weeks before administration of pembrolizumab; the exception to this is ipilimumab which must have been administered at least 4 weeks prior to the start of pembrolizumab. Patients are not required to have had prior systemic therapy.
5. Life expectancy of at least 3 months
6. A history of previously treated brain metastases is allowed, provided that at least 7 days have lapsed between radiation and initiation of pembrolizumab. Any brain metastasis ≥20mm or causing symptoms must be treated with local therapy (i.e. radiation or surgical resection, as clinically appropriate) prior to study enrollment. Any lesion present at the time of WBRT or included in the stereotactic radiotherapy field (or within 2mm of the treated lesion) will NOT be considered evaluable unless it is new or documented to have progressed since treatment.
7. PD-L1 expression ≥1% in tumor tissue from any site is required for patients with NSCLC. Tumor tissue can be archival if no intercurrent systemic therapy was administered, however if no archival tissue is available or if intercurrent systemic therapy was administered, then a biopsy must be obtained for PD-L1 testing. PD-L1 expression must be obtained via central and/or local testing of PD-L1 IHC by any FDA-approved assay or an assay performed in a CLIA-certified laboratory. PD-L1 expression is not required for patients with melanoma.
8. All patients are required to submit a tumor specimen for analysis (brain or extra- cerebral). A formalin-fixed paraffin-embedded (FFPE) tissue block, or a 4mm punch from an

FFPE block must be submitted. If it is not possible to safely obtain a biopsy due

to anatomic location of tumors, and no prior tissue is available, this requirement may be waived upon discussion with the study PI or co-PI.

9. Patients must have normal organ and marrow function as defined below:

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥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 ≤ 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 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT) or Partial Thromboplastin Time (PTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

10. 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.

11. 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 5.6.2—Contraception). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

12. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.3 Subject Exclusion Criteria

1. Symptomatic brain metastases. Any neurologic symptoms present must have resolved with local therapy by the time of administration of study drug.
2. Patients with brain metastases for whom complete surgical resection is clinically appropriate.
3. Patients with lung cancer with squamous histology.

4. Has had prior chemotherapy or targeted small molecule therapy within 2 weeks prior to start of treatment or has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent. Previous radiation to extracranial sites may be completed at any time prior to initiation of pembrolizumab.
    a. Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
    b. Note: Toxicity that has not recovered to ≤ Grade 1 is allowed if it meets the inclusion requirements for laboratory parameters.
5. Has had prior treatment with any other anti-PD-1 or PD-L1 or PD-L2 agent.
6. The use of corticosteroids to control cerebral edema or treat neurologic symptoms will not be allowed, and patients who previously required corticosteroids for symptom control must be off steroids for at least 1 week prior to treatment on day 1 of cycle 1. Low-dose steroid use (≤10 mg of prednisone or equivalent) as corticosteroid replacement therapy is allowed
7. Has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
8. Presence of leptomeningeal disease
9. 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 (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
10. Pregnancy or breast feeding. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with pembrolizumab, breastfeeding must be discontinued if the mother is treated with pembrolizumab.
11. Patients may not be receiving any other investigational agents and may not have participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.
12. Either a concurrent condition (including medical illness, such as active infection requiring treatment with intravenous antibiotics or the presence of laboratory abnormalities) or history of a prior condition that places the patient at unacceptable risk if he/she were treated with the study drug or a medical condition that confounds the ability to interpret data from the study.
13. Concurrent, active malignancies in addition to those being studied (other than cutaneous squamous cell carcinoma or basal cell carcinoma)
14. Patients with active hemoptysis.
15. Any contraindication to MRI (i.e. patients with pacemakers or other metal implanted medical devices). An MRI safety questionnaire is required prior to MR imaging.
16. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
17. Has a known Human Immunodeficiency Virus (HIV), Hepatitis B (HBV), or Hepatitis C (HCV) infection.
18. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

19. Inadequately controlled hypertension (defined as systolic blood pressure >150 mmHg and/or diastolic blood pressure > 100 mmHg). Anti-hypertensive therapy to achieve these parameters is allowable.
20. History of myocardial infarction or unstable angina within 3 months prior to Cycle 1, Day 1
21. History of stroke or transient ischemic attack within 3 months prior to Cycle 1, Day 1
22. Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Cycle 1, Day 1
23. Evidence of bleeding diathesis or clinically significant coagulopathy (in the absence of therapeutic anticoagulation). Any history of significant bleeding or thrombosis should be discussed with the study PIs.
24. Current or recent (within 10 calendar days prior to Cycle 1, Day 1) use of dipyramidole, ticlopidine, clopidogrel, or cilostazol
25. Warfarin is not permitted. Prophylactic or therapeutic use of low molecular-weight heparin (e.g., enoxaparin), direct thrombin inhibitors or Factor Xa inhibitors (Direct or Indirect) are permitted.
26. History of abdominal or tracheoesophageal fistula or gastrointestinal perforation within 6 months prior to Cycle 1, Day 1
27. Serious, non-healing or dehiscing wound
28. Proteinuria > 2.0 g of protein in a 24-hour urine collection. All patients with 2 protein on dipstick urinalysis at baseline must undergo a 24-hour urine collection for protein.
29. Has a history of (non-infectious) pneumonitis that required steroids, current pneumonitis or evidence of interstitial lung disease.

5.2 Trial Treatments

The treatments to be used in this trial are pembrolizumab 200mg every 3 weeks and bevacizumab 7.5mg/kg on day 1 of cycle 1, 2, 3, and 4 (or alternative cycles if bevacizumab is held during these cycles). Each cycle is 3 weeks. Our intent is to treat with the combination of pembrolizumab and bevacizumab for four cycles and pembrolizumab monotherapy for up to 2 years.

5.2.1 Dose Selection/Modification

Dose Selection

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

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

APPROVED BY THE YALE UNIVERSITY IRB 6/25/2021

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

AEs associated with pembrolizumab exposure, including coadministration with additional compounds, may represent an immunologic aetiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab/combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in Table 1.

Attribution of Toxicity:

When study interventions are administered in combination, attribution of an adverse event to a single component is likely to be difficult. Therefore, while the investigator may attribute a toxicity event to the combination, to bevacizumab alone or to pembrolizumab alone, for adverse events listed in Table 1, both interventions must be held according to the criteria in Table 1 Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab.

Holding Study Interventions:

When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to recommended dose modifications.

Restarting Study Interventions:

Participants may not have any dose modifications (no change in dose or schedule) of pembrolizumab in this study, as described in Table 1.

- If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions.
- If the toxicities do resolve and conditions are aligned with what is defined in Table 1, the combination of Bevacizumab and pembrolizumab may be restarted at the discretion of the investigator. In these cases where the toxicity is attributed to the combination or to Bevacizumab alone, re-initiation of pembrolizumab as a monotherapy may be considered at the principal investigator's discretion.

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 PIs. The reason for interruption should be documented in the patient's study record.

Table 1: Dose Modification Guidelines for Drug-Related Adverse Events for Pembrolizumab monotherapy and IO Combinations

General instructions:

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

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 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 ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
				• Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic 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 [d]		
Hyperthyroidism	Grade 2	Continue		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	Grade 3 or 4	Withhold or permanently discontinue [d]	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 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		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	Grade 3	Withhold or discontinue based on the event [e]	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

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

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

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

Dose Selection/Modification of Bevacizumab

No reductions in bevacizumab dose are allowed in this study. Modification to bevacizumab administration should be done according to the label and per institutional guidelines. If adverse events occur that necessitate holding bevacizumab, the weight-based dose in mg/kg will remain unchanged after treatment resumes.

Patients who discontinue bevacizumab **transiently or permanently** for adverse events may continue on single-agent pembrolizumab until disease progression.

For non irAEs, temporary suspension of bevacizumab must occur if a patient experiences a serious adverse event or a Grade 3 or 4 non-serious adverse event assessed by the investigator as related to bevacizumab. If the event resolves to Grade ≤ 1, bevacizumab may be restarted at the same dose level.

The appropriate interval between the last dose of bevacizumab and major surgery is unknown. Because bevacizumab has a half-life of approximately 21 days, elective surgery should be delayed whenever possible. Re-initiation of bevacizumab should occur after wounds have healed. Re-initiation of bevacizumab after surgery requires approval of the principal investigators.

Infusion of bevacizumab should be interrupted in patients who develop dyspnea or clinically significant hypotension. Patients who experience an NCI CTCAE Grade 3 or 4 allergic reaction/hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from bevacizumab treatment. If possible, a sample for ATA assessment will be collected at the time of discontinuation.

Bevacizumab infusion should be slowed to ≤ 50% or interrupted for patients who experience any infusion-associated symptoms not specified above. If the infusion is interrupted, it may be resumed at ≤ 50% of the rate prior to the reaction after the patient's symptoms have adequately resolved and increased in 50% increments up to the full rate if well tolerated. Infusions may be restarted at the full rate during the next cycle.

Patients who develop Grade 4 toxicities (not immune related) related to bevacizumab for > 21 days should permanently discontinue bevacizumab.

If bevacizumab is held due to toxicity that resolves, it can be initiated during any subsequent cycle for a total number of four cycles of bevacizumab per patient.

Table 2: Dose Modification Guidelines for Non Immune related Drug-Related Adverse Events for Bevacizumab

Event	Action to Be Taken
Hypertension	
Grade 1 (asymptomatic, transient [< 24 hr] blood pressure increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits)	No bevacizumab dose modifications.
Grade 2 (recurrent or persistent [> 24 hr] or symptomatic increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits)	Hold bevacizumab. Start antihypertensive therapy per institutional guidelines. After blood pressure is < 150/100 mmHg, patient may continue bevacizumab therapy.
Grade 3	Requires more than one antihypertensive drug or more intensive therapy than previously: If not controlled to 150/100 mmHg with medication, discontinue bevacizumab.
Grade 4 (including hypertensive encephalopathy)	Discontinue bevacizumab.
Hemorrhage	
Grade 1 or 2 non-pulmonary or non-CNS events	No bevacizumab modifications.
Grade 3 non-pulmonary or non--brain or non-spinal cord hemorrhage	Hold bevacizumab until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Patients who experience a repeat Grade 3 hemorrhagic event will be discontinued from bevacizumab.
Grade 4 non-pulmonary or non-brain or non-spinal cord hemorrhage	Discontinue bevacizumab.

Event	Action to Be Taken
Grade 1 pulmonary or brain or spinal cord hemorrhage	Hold bevacizumab until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
Grade 2, 3, or 4 pulmonary hemorrhage Grade 2, 3 or 4 brain hemorrhage	Discontinue bevacizumab. Discontinue bevacizumab until all hemorrhagic lesions have undergone local therapy and upon discussion with the study PIs
Venous thromboembolic event	
Grade 1 or 2	No bevacizumab modifications.
Grade 3 or asymptomatic Grade 4	If the planned duration of full-dose anticoagulation is <2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed after 2 weeks of full-dose anticoagulation if all of the following criteria are met: • The patient must have an in-range INR (usually between 2 and 3) if on warfarin; LMWH, warfarin, or other anticoagulant dosing must be stable prior to restarting study treatment. • The patient must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation.
Symptomatic Grade 4	Discontinue bevacizumab.
Arterial thromboembolic event (new onset, worsening, or unstable angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)	
Any grade	Discontinue bevacizumab.
Congestive heart failure (left ventricular systolic dysfunction)	
Grade 1 or 2	No bevacizumab modifications.
Grade 3	Hold bevacizumab until resolution to Grade ≤ 1.
Grade 4	Discontinue bevacizumab.

Event	Action to Be Taken
Proteinuria	
Grade 1 (urine dipstick 1+ or urine collection 0.15 to 1.0 g/24 hr)	No bevacizumab modifications.
Grade 2 (urine dipstick 2+ to 3+ or urine collection >1.0 to 3.5 g/ 24 hr)	For 2+ dipstick, may administer bevacizumab and obtain 24-hour urine prior to next dose. For 3+ dipstick, obtain 24-hour urine prior to administration of bevacizumab. Hold bevacizumab for proteinuria >2 g/24 hr and resume when proteinuria is ≤ 2 g/24 hr.[a]
Grade 3 (urine dipstick 4+ or urine collection >3.5 g/24 hr)	Hold bevacizumab. Resume when proteinuria is ≤2 g/24 hr.[a]
Grade 4 (nephrotic syndrome)	Discontinue bevacizumab.
GI perforation	
Any grade	Discontinue bevacizumab.
Fistula	
Any grade tracheoesophageal fistula	Discontinue bevacizumab.
Grade 4 fistula (other than tracheoesophageal)	Discontinue bevacizumab.
Bowel obstruction	
Grade 1	Continue patient on study for partial obstruction not requiring medical intervention.
Grade ≥2	Discontinue bevacizumab.
Wound dehiscence	
Any grade (requiring medical or surgical therapy)	Discontinue bevacizumab.
Reversible posterior leukoencephalopathy	
Any grade (confirmed by MRI)	Discontinue bevacizumab.
CNS=central nervous system; GI=gastrointestinal; LMWH=low molecular-weight heparin. [a] All proteinuria values are from 24-hour urine collection.	

5.2.2 Timing of Dose Administration

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

5.2.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for up to 2 years or until one of the following criteria applies:

1	Disease progression and absence of clinical benefit
2	Symptomatic deterioration of health status without objective evidence of disease progression. Every effort should be made to document objective progression of disease in such cases.
3	Imminent risk in the case of progressing lesion or new/worsening edema seen on brain MRI
4	Unmanageable toxicity most probably attributed to pembrolizumab
5	Development of intercurrent illness limiting the ability to comply with study or the development of uncontrolled concurrent illness that prevents further administration of treatment or confound the ability to interpret data
6	General or specific changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the investigator
7	Patient request for discontinuation, patient withdrawal from study, or patient lost to follow up
8	Development of neurologic or systemic complications following local therapy to any lesion
9	Death
10	Study termination by sponsor

5.2.4 Treatment Beyond Progression

If radiologic imaging shows PD, tumor assessment should be repeated <4 weeks later in order to confirm PD with the option of continuing treatment while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms progressive disease, subject will be discontinued from study therapy unless the investigator believes that the patient is deriving benefit. Patients may continue treatment with pembrolizumab and/or bevacizumab beyond PD by RECIST 1.1 (in the body) or mRECIST in the brain, if they are determined by the investigator to be deriving clinical benefit from treatment and they continue to meet all other criteria for treatment. Patients who have rapid progression of disease or clinical deterioration will be removed from study. The decision to continue treatment beyond initial progression should be discussed with the study PIs.

Additionally, in cases where the majority of the disease is stable or responding, and progressing lesions can be controlled with local therapy (i.e. resection or radiotherapy), the patient may be continued on trial following local therapy.

5.2.5 Duration of Follow-up

Patients who discontinue early or who complete the study treatment period in full will be followed for survival after removal from study until death. See Trial Flow Chart (Section 6.0) for more details.

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

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

5.3.1 Acceptable Concomitant Medications

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

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

medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.6.3.1.

5.3.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial as listed below:

- Antineoplastic systemic chemotherapy or biological therapy < 2 weeks prior to treatment Day 1
- Immunotherapy not specified in this protocol < 2 weeks prior to treatment Day 1
- Investigational agents other than pembrolizumab or bevacizumab < 2 weeks prior to treatment Day 1
- Radiation therapy
    - Note: Radiation therapy to individual symptomatic solitary extra-cerebral lesions or to the brain may be allowed at the investigator's discretion. This should be discussed with the study PIs.
- 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 modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the PI.
- Anticoagulation other than low molecular weight heparin, direct thrombin inhibitors, or Factor Xa inhibitors (Direct or Indirect).

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 Local Therapy

Local therapy for brain metastases:

Local surgical therapy in the form of craniotomy or LITT (laser interstitial thermacoagulation therapy) will be performed when clinically appropriate prior to initiating systemic therapy. Patients will be required to provide tissue for research when available. At any time point

throughout the study, local therapy (surgery, radiotherapy or LITT) will be administered to a lesion that becomes clinically concerning if deemed necessary by the investigators. This can be followed by continuation on treatment, provided that the patient is otherwise benefiting from therapy. Approval from the study PI or co-PIs is required to continue treating the patient on pembrolizumab and bevacizumab. A brain metastasis that has been treated locally will not be considered evaluable for response and will not be included when calculating the sum of largest dimensions. If the treated lesion constitutes > 25% of the target lesions (for example, if one of three target lesions is treated locally), this will be considered progressive disease. If >75% of the baseline lesions are not treated with local therapy and evaluable by imaging, the patient will be considered evaluable, and response for the primary endpoint analysis will be assessed based on the remaining lesions. Additional response evaluation will be performed; those patients who require local therapy due to unequivocal growth while on study will be considered as having PD at the time of local therapy. Lesions requiring local therapy due to hemorrhage or edema will be considered unevaluable.

Local therapy for extra-cerebral metastases:

Local surgery or radiation therapy (if indicated for palliative measures only after discussion with the study PI or co-PIs) may be permitted and the patient can continue to receive pembrolizumab provided there is otherwise evidence of clinical benefit from treatment (i.e. stable disease or response in measurable lesions). The criteria applied for assessing brain metastasis response will be applied to extra-cerebral metastases as well.

5.5 Rescue Medications & Supportive Care

5.5.1 Supportive Care Guidelines

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 along with the dose modification guidelines in Table 1. 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. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance.).

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

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

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 3.

Table 3: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion 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 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.**	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).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

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

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

If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAEs. Subjects who develop a Grade 2 or higher irAEs should be discussed immediately with the Merck Clinical Monitor.

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

Table 4 General Approach to Handling irAEs

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

5.5.3. Supportive Care Guidelines for Pneumonitis

Subjects with grade 2 pneumonitis (symptomatic pneumonitis requiring medical intervention) should immediately stop receiving pembrolizumab and have an evaluation. The evaluation may include bronchoscopy and pulmonary function tests to rule out other causes such as infection. If the subject is determined to have study drug associated pneumonitis, the suggested treatment plan is detailed in Table 5 and follow-up will be as routine for AEs associated with study drug including monitoring until full resolution of the AEs/pneumonitis.

Table 5. Recommended Approach to Handling Pneumonitis

Study drug associated pneumonitis	**Withhold/Discontinue pembrolizumab?**	**Supportive Care**
Grade 1 (asymptomatic)	No action	Intervention not indicated
Grade 2	Withhold pembrolizumab, may return to treatment if improves to Grade 1 or resolves within 12 weeks	Systemic corticosteroids are indicated. Taper as indicated.
Grade 3 and Grade 4	Discontinue pembrolizumab	Systemic corticosteroids are indicated.

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

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

5.6 Diet/Activity/Other Considerations

5.6.1 Diet

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

5.6.2 Contraception

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

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.6.2—Reporting of Pregnancy and Lactation to the Sponsor 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.

5.6.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with bevacizumab and/ or 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 Sponsor and to Merck without delay and within 24 hours to the Sponsor 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 Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.6.2—Reporting of Pregnancy and Lactation to the Sponsor and to Merck.

5.6.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.7 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.4—Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression
  Note: For unconfirmed radiographic disease progression, please see Section 5.2.3—Duration of Therapy
  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 5.2.4—Treatment Beyond Progression
- Unacceptable adverse experiences as described in Section 5.2.1—Dose Selection/Modification
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.
  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 5.7.1—Discontinuation of Study Therapy after CR
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Trial Flow Chart) and Section 7.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.6.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the

end of the study, whichever occurs first.

5.7.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation

APPROVED BY THE YALE UNIVERSITY IRB 6/25/2021

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Screening evaluations are to be conducted within 28 days prior to start of systemic therapy. Scans must be done within 28 days prior to the start of systemic therapy and after other therapy not specified in this protocol is stopped. Screening labs must be done within 10 days of Cycle 1 Day 1. 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.

All assessments must be performed prior to administration of any study medication. All study assessments and medications should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Screening Visit	On-Study Visits				Follow-up Visit	Long Term Follow-up
	Day -28 to -1	Day 1 of each Cycle	Day 1 of Cycles 1, 2, 3, and 4	Day 1 Cycles 3 and 5	Day 1 Cycle 8, and every 9 weeks thereafter	Day 28 +/- 7 days from last study treatment[i]	Every 3 months +/- 14 days after the follow-up visit
Informed Consent	X						
History/Full Physical Exam	X	X				X	
Height/Weight/Vital Signs[A]	X	X				X	
Performance Status	X	X				X	
CBC, serum chemistry, liver function tests, LDH[B]	X	X				X	
INR or PT & aPTT or PTT	X						
Urinalysis	X		X			X	
TSH, free T3, free T4	X				X		
NSCLC patients: Determination of PD-L1 expression in tumor[N]	X						

	Screening Visit	On-Study Visits				Follow-up Visit	Long Term Follow-up
	Day -28 to -1	Day 1 of each Cycle	Day 1 of Cycles 1, 2, 3, and 4	Day 1 Cycles 3 and 5	Day 1 Cycle 8, and every 9 weeks thereafter	Day 28 +/- 7 days from last study treatment[I]	Every 3 months +/- 14 days after the follow-up visit
Plasma and PBMCs for correlative studies	X[H]			X	X[O]		
Obtain tumor specimen for correlative studies (Refer to Section 11.1)[J,M]	X						
Pregnancy Test[C]	X	X					
CT chest/abdomen (plus pelvis if disease is present)	X			X[D]	X[D]	X [E]	
Brain MRI	X [F]			X[G]	X[G]	X [E]	
Selection of "clinically evaluable lesion(s)"	X						
Review concomitant medications[L]	X	X				X	
Smoking Status	X						
Adverse events assessment		X				X	
Administration of pembrolizumab		X					
Administration of bevacizumab[K]			X				
Survival Follow-up							X

[A]Includes blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature. Height at screening only.

[B]Includes CBC with differential, serum chemistry (Na, K, Cl, CO2, BUN, creatinine, glucose, calcium), magnesium, phosphate, liver function tests (albumin, total protein, alkaline phosphatase, total bilirubin, ALT, AST), LDH

[C]Pregnancy test (serum or urine) is required for women of childbearing potential only on Day 1 of each cycle prior to treatment administration.

[D]CT scan of the chest/abdomen/pelvis can be performed +/- 5 days. Response evaluation is performed using RECIST 1.1

[E]CT scan of the chest/abdomen/pelvis and brain MRI should continue every 9 weeks (+/- 5 days) until progression, withdrawal of consent, death, lost to follow-up, or start of a subsequent anti-cancer therapy

[F]All MRIs will be reviewed centrally by the investigators at Yale prior to confirmation of eligibility. For patients who will undergo local therapy on trial, investigators must identify a "clinically evaluable lesion(s)". Brain MRI must be done within 28 days of intervention and should be repeated if more than 28 days will have lapsed by

the start of systemic therapy. For patients who previously underwent local therapy to a brain lesion and have tumor tissue available for analysis, only identification of "clinically evaluable lesion(s)" is necessary.

[G]Brain MRI can be performed +/- 5 days. CNS response evaluation is performed using mRECIST criteria. MRIs should be done with perfusion, if possible.

[H] Can be done at screening or any time prior to Cycle 1 Day 1 dosing

[i]Follow-up Visit should be 28 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first.

[J]If a biopsy is done at any point during the subject's participation in the trial, a sample of the tumor tissue will be taken for research.

[K] If bevacizumab is held at any point it can be reinitiated when deemed safe for a total of four doses per patient

[L] At screening, medications taken in the past 28 days need to be recorded, including over-the-counter medications and herbal supplements. Information on concomitant therapy including radiation therapy should be collected as well.

[M] For all patients, archival tumor tissue will be requested. If archival tissue is not available patients on the NSCLC arm, or if intercurrent systemic therapy has been administered since the archival tissue was obtained, patients will need to have a fresh biopsy.

[N] Subjects with PD-L1 test results from outside the study are not required to undergo repeat testing for determination of eligibility as long as no systemic therapy was administered after the test results were obtained. Central and/or local testing of PD-L1 IHC by any FDA-approved assay or an assay performed in a CLIA laboratory is acceptable. If patients do not undergo PD-L1 testing to determine eligibility, fresh or archival tissue is still required at study entry for correlative studies.

[O] Plasma and PBMC are to be collected during the participant's end of treatment visit in addition to those previously outlined.

7.0 TRIAL PROCEDURES

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

7.1 Administrative Procedures

7.1.1 Informed Consent

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

7.1.1.2 General Informed Consent

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

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

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

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

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

7.1.2 Inclusion/Exclusion Criteria

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

7.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease

for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.4 Prior and Concomitant Medications Review

Prior Medications

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

Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.6.3.1—Serious Adverse Events.

7.1.5 Disease Details and Treatments

Disease Details

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

Prior Treatment Details

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

Subsequent Anti-Cancer Therapy Status

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

7.2 Clinical Procedures/Assessments

7.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see Section 13.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.6 for detailed information regarding the assessment and recording of AEs.

7.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening, on day 1 of each cycle, and at the follow-up visit.

7.2.3 Directed Physical Exam

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

7.2.4 Vital Signs

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

7.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 13.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.2.6 Tumor Imaging and Assessment of Disease

Tumor imaging and assessment of disease will be performed every 6 weeks for the first 3 months of treatment, and then every 9 weeks thereafter. See section 12.0—Measurement of Response—for details.

7.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Before initiation of systemic therapy, a core biopsy will be obtained. This can be bypassed if there is archival tissue available. For NSCLC patients, it is acceptable to use archived tumor tissue rather than obtain a new biopsy to assess for PD-L1 status only if no intercurrent therapy was administered. In patients undergoing fresh biopsy, a minimum of two cores is required, and these will be formalin fixed and embedded in paraffin. These tissues will be stored for correlative studies mentioned below. Please see the Procedures Manual for tissue requirements and handling of tissue.

The stored tissue specimens will be analyzed at Yale University. In the context of a non-randomized trial, lack of validated assays and inability to identify and isolate tumor antigen specific T-cells, all correlative studies are considered exploratory. These are exploratory analyses that will be studying the tumor microenvironment and PBMC. Within the tumor microenvironment we will be assessing $CD8^+/IFNg^+$, PD-L1 expression, the ratio of $CD8^+$/Treg and other assays (please see correlative section below). Within the PBMC, we will be studying $CD8^+/CD127^+/PD\text{-}1^+$ (or $PD\text{-}L1^+$)/$IFNg^+$ cells, and PD-1/PD-L1 expression on central central memory and effector memory $CD4^+$ subsets. Plasma will be stored for cytokine analyses.

See section 11.0—Biomarker Studies—for details.

7.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 6.

Table 6 Laboratory Tests

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

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.4 Other Procedures

7.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.6—Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 5.7.1—Discontinuation of Study Therapy after CR. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.5.1) and then proceed to the Follow-Up Period of the study (described in Section 7.5.2).

7.5 Visit Requirements

Visit requirements are outlined in Section 6.0—Trial Flow Chart. Specific procedure-related details are provided above in Section 7.0—Trial Procedures.

7.5.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 28 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0- 1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

7.5.2 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 9 weeks (± 7 days) by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated. Survival Follow-up.

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

7.6 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. 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 the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.6.3.1.

7.6.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor 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 Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.6.2 Reporting of Pregnancy and Lactation to the Sponsor 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 completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.6.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

Serious Adverse Events

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

- 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 another important medical event

Refer to Table 7 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 the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Non-serious Events of Interest will be forwarded to Merck GS 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 Sponsor 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.

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

7.6.4 Reporting to the Food and Drug Administration

This study will be conducted under an IND (Investigational New Drug application) that will be held by Harriet Kluger, M.D. The Principal Investigator will report in an expedited manner all SAEs meeting the criteria of "serious", "unexpected" and "related to study treatment". Written safety reports will use a MedWatch Form 3500A. A "fillable pdf"

version with instructions is available at: http://www.fda.gov/medwatch/safety/FDA-3500A_Fillable_08-16-2006.pdf

There are two types of expedited safety reports to the FDA:

1. **7-Calendar-Day FDA Telephone or Fax Report:** The sponsor-investigator will directly notify the FDA, within 7 calendar days after his initial receipt of the information, of any adverse event that is ALL of the following:

Death or immediately life-threatening
Unexpected
Associated with the use of study drug

Notification to the FDA will be made directly to the new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever was responsible for the review of the IND. [21CFR312.32(c)] A written report of the event is to follow within 15 calendar days.

2. **15-Calendar-Day FDA Written Report:** The sponsor-investigator will directly notify the FDA within 15 calendar days of any adverse event that is ALL of the following:

Serious (due to non-fatal and non-life threatening criteria)
Unexpected
Associated with the use of study drug

Note: Serious Adverse Events which do not meet the criteria for expedited reporting will be reported to the FDA in the IND Annual Report.

7.6.5 Reporting to the Yale Human Investigation Committee

Sites will report to their IRB per local policy

7.6.6 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 5.0. 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 7
Evaluating Adverse Events

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

V50 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship 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.)

7.6.7 Sponsor Responsibility for Reporting Adverse Events

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

7.6.8 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

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 Sponsor 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 30 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor, either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Events of clinical interest for this trial include:

- an overdose of Merck product, as defined in Section 7.6.1 – Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

- 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.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Design

This is a phase II study of patients with melanoma and a pilot study of NSCLC patients who have untreated brain metastases. Subjects must have at least 1 brain lesion that does not require immediate local therapy and can be assessed for response. **Criteria for response are defined in Section 12.0**.

The primary goal of this study is to determine the efficacy of pembrolizumab plus bevacizumab in patients with untreated brain metastases from melanoma or NSCLC. The primary endpoint is the brain metastasis response rate (BMRR). A drug with minimal activity would be expected to have a BMRR of 10%. The study was initially designed so that pembrolizumab and bevacizumab would be considered worthy of further study in patients with untreated brain metastases if its true BMRR is similar to the systemic response rate with pembrolizumab, which is 34% for melanoma and 25% for NSCLC. For this to occur, the study was originally planned to enroll a total of 53 eligible patients (20 with melanoma, 33 with NSCLC). In the first stage of a Simon's optimal two stage design, it was planned that 9 patients with melanoma and 16 patients with NSCLC would be enrolled. If 2 or more of 9 evaluable melanoma patients had a response in brain metastases, accrual would continue to enroll another 11 patients to that cohort. Similarly, in the NSCLC cohort, if a brain metastases response were to be seen in 2 more of the 16 eligible NSCLC patients, another 17 patients were planned to be accrued.

Prior to February 2019, 20 patients were accrued to the melanoma cohort of the trial. We observed 11 brain metastasis responses in 20 patients (ORR=0.55, 95% CI: 0.32-0.77). For the melanoma cohort, we aimed to generate evidence that the true response rate of pembrolizumab plus bevacizumab in patients with untreated brain metastases is more than 30% and we expanded the cohort by another 20 patients. For the NSCLC cohort, we revised the statistics to conduct a pilot study as described below.

In the study of the first 20 melanoma patients, we decided that the combination is not worthy of further investigation if the true response rate is <=10%. However, because we observed a BMRR of 55% in these patients, we raised this bar from 10% to 30%, which is the lower bound of the 95% CI from the first 20 melanoma patients who were enrolled on the study.

For the melanoma cohort, the new null hypothesis that the true response rate is 30%. was to be tested against a one-sided alternative, using Simon's optimal two stage design, enrolling 8 patients in the first stage and 12 in the second.

Among the first six patients enrolled in the second melanoma cohort, one had a modified RECIST-defined complete response, and two had progression of disease due to initial tumor growth at six weeks or appearance of new lesions (one each), with subsequent tumor regression, remaining on study beyond progression. Two patients were unevaluable for intra-cranial response.

Therefore, we are redesigning the analyses to remove the Simon's optimal two stage design due to the possibility of delayed response and emerging evidence regarding the intra-cranial response rate to anti-PD-1 monotherapy (Long et.al, Lancet Oncology, 2018 [64], in which 5 of 25 patients responded and Kluger et al, Journal of Clinical Oncology 2019 [65], in which 6 of 23 patients responded), indicating that the response rate to anti-PD-1 alone is likely between 20-26%. Long et al also reported a response rate to anti-PD-1 and anti-CTLA-4 combination therapy of 46%, and Tawbiet al reported a response rate to anti-PD-1 and anti-CTLA-4 of 56% (Tawbi et al, New England Journal of Medicine, 2018) [66].

The combination of pembrolizumab and bevacizumab, a significantly less toxic regimen, would be worthy of further study if the intracranial response rate was 46%. In the first melanoma cohort in this trial, the intracranial response rate was 55%, and the purpose of the second cohort was to verify this result.

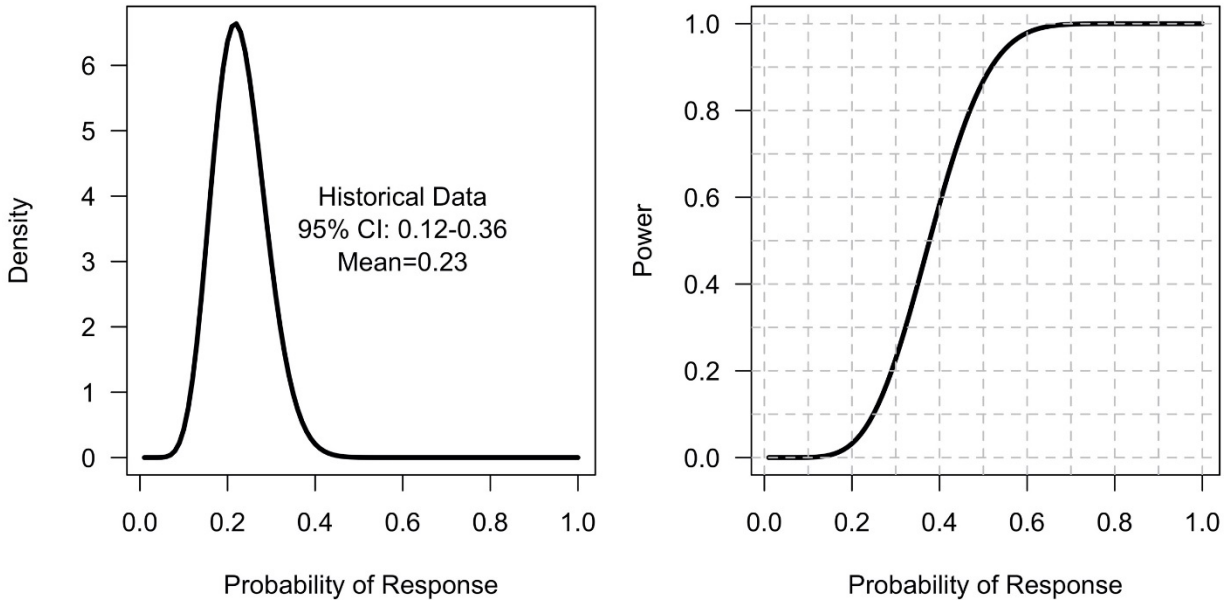
To account for the uncertainty of the historical response rate, we follow the approach proposed by Thall and Simon (1994) [63] and approximate the distribution of response rate to anti-PD-1 monotherapy using a beta distribution with a mean of 23% (95% CI: 0.12-0.36), assuming there are 11 responses (5+6) out of 48 (25+23) patients in historical controls. That is, we assume the historical response rate $\theta_H \sim Beta(\ n_H\ p_H,\ \ n_H(1-\ p_H)$, where $n_H = 48$ and $p_H = 0.23$. Assume the response rate of the combination has a uniform prior, that is, $\theta_E \sim Beta(1,1)$.

After enrolling and evaluating the outcomes of the 20 patients, we conclude the combination worthy of further study if

$$P(\theta_E > \theta_H | data) > 0.936,$$

i.e., we conclude the treatment is promising if there are at least 8 responses in 20 patients.

The probability of false positive is 0.032, 0.067 and 0.122 if the true probability of response is 0.20, 0.23 and 0.26, respectively. We achieve 78%, 87% and 94% power if the true probability of response is 0.46, 0.50 and 0.55, respectively.



The beta distribution modelling the response rate to monotherapy in historical controls (Left panel) and the power function of the design (right panel).

For the lung cohort, we will conduct a pilot study which will decrease the number of planned enrolled patients from 33 to 13 patients. Under the null hypothesis, the response rate corresponding to drug with minimal activity is 10%. We therefore consider a drug is worthy of further investigation if the true response rate is at least 25% for NSCLC.

Due to the pilot nature of this study, sample size calculation is not based on formal hypothesis testing. We provide the two-sided 90% confidence intervals for observing different number of responses. As shown by the table below, we can reject the null hypothesis if at least 4 responses are observed.

Number of Responses	Lower 90% CI	Upper 90% CI
3	0.07	0.49
4	0.11	0.57
5	0.17	0.65
6	0.22	0.71
7	0.29	0.78
8	0.35	0.83

Patients will be considered evaluable if they remain on treatment for the first 6-week scans or if they have unequivocal CNS progression prior to that.

8.2 Analytic Plan for Primary Objectives:

We will use mRECIST criteria to determine brain metastasis response rate (BMRR). The proportion of patients with a brain metastasis response in the clinically evaluable lesion(s) will be calculated along with a 95% confidence interval.

8.3 Analytic Plan for Secondary Objectives:

1) **Objective**: To determine the incidence of steroid use for control of cerebral edema during treatment with pembrolizumab and bevacizumab in combination in patients with metastatic melanoma or NSCLC.

The percentage of patients requiring corticosteroid use for control of perilesional edema will be compared that seen in a previous study with the same patient population using pembrolizumab alone. The proportion of patients using steroids for > 96 hours will be calculated with a 95% confidence interval.

2) **Objective**: To determine the best overall response rate (ORR) in patients with brain metastases from melanoma or NSCLC treated with combination pembrolizumab and bevacizumab.

   Best overall response rate (ORR) is defined as the percentage of patients who experience a CR or PR in any metastases (clinically evaluable cerebral or systemic) as determined by mRECIST criteria in the brain or RECIST criteria in the body. The proportion of patients with an ORR will be calculated with a 95% confidence interval. A similar analysis will be performed using ir-mRECIST and ir-RECIST.

3) **Objective**: To evaluate median progression free survival (PFS) and overall survival (OS) in patients with melanoma or NSCLC metastatic to the brain, treated with pembrolizumab plus bevacizumab.

   Progression-free survival will be calculated as the time from start of study drugs to progression (using mRECIST criteria for brain lesions and RECIST criteria for systemic disease) or death. Overall survival will be calculated as the time from start of pembrolizumab to death. Patients who do not meet the endpoint will be censored at the date of last follow-up. PFS and OS will be determined separate for each cohort and median survival will be estimated by using Kaplan-Meier methodology.

8.4 Exploratory Objective

(1) Objective: To study PD-L1 expression and other potential predictive biomarkers in CNS, tumors and blood, and correlate results with response to pembrolizumab plus bevacizumab.

PD-L1 expression and other potential biomarkers will be correlated to clinical endpoints. Exploratory, hypothesis-generating analyses will be performed. Details of the statistical plan for the biomarker studies are provided in Section 11.0.

(2) Objective: To determine the safety and toxicity of pembrolizumab in combination with bevacizumab in patients with untreated brain metastases

Safety and toxicity will be assessed using CTC v4.0 criteria. All participants who receive any amount of study drug will be evaluable for toxicity.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Clinical Supplies will be provided by Merck as summarized in Table 8.

Table 8 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

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

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Compliance with Trial Registration and Results Posting Requirements

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

10.2 Quality Management System

10.2.1 Monitoring

The study principal investigator and YCCI are responsible for monitoring the performance of all of the participating sites. This will be performed by conducting a study site initiation visit, as well as regularly scheduled monitoring visits and/or remote monitoring throughout the life of the protocol. At the end of the trial, the monitor will then perform a study site close-out visit at all participating sites.

YCCI will utilize their institution's initiation, monitoring and close-out visit reports. Following each site visit, a visit report will be generated containing information on site activities, and a summary of pertinent points and action items together with a copy of the follow-up letter will be sent to each investigative site.

During these monitoring visits, some of the items that will be reviewed are the following:

- Training of the sites
- Site personnel qualifications to participate in the trial
- That study related documents are current
- That regulatory compliance is accomplished
- That each subject has signed the informed consent
- That the current and approved protocol is complied with (including reporting and logging of all protocol deviations)
- That all SAEs and AEs have been reported to the local regulatory and Ethics/IRB Committees, YCCI, and Merck as appropriate
- That source documentation matches CRFs
- That required procedures for study drug accountability, distribution, and storage are followed.

YCCI will document the required study monitoring activities in a Study Monitoring Plan.

10.2.2 Audit and inspection

To ensure compliance with GCP and regulatory requirements, the YCCI Office of Quality Assurance and Training will audit the trial at least annually or as determined by the Yale Cancer Center DSMC. The overall principal investigator, study coordinator and/or data manager may request access to all source documents and other study documentation for on-site or remote monitoring, audit or inspection.

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the Principal Investigator or Yale. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor immediately of any such inspection.
The Sponsor-Investigator/institution and participating site investigators/institutions agree to allow the auditor or inspector direct access to all relevant documents and allocate their time and the time of their staff to the auditor/inspector to discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study.

10.2.3 Data Safety Monitoring Committee (DSMC)

The Yale Cancer Center DSMC will serve at the DSMC of record. The Yale DSMC will review and monitor compliance, toxicity and deviations 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 Principal Investigator.

The DSMC will review this protocol at a minimum of once every six months. Information to be provided to the committee includes: a study narrative by the PI, a summary DSMC report produced by OnCore (which includes participant accrual, response, trial status history, SAEs, Adverse Events, Deviations and survival); audit results, and monitoring reports as applicable. Other information (e.g. scans, laboratory values) will be provided upon request.

11.0 BIOMARKER STUDIES

11.1 Tissue-based correlative studies:

Correlative studies will be conducted on the pre-treatment tissue specimens. When available we will include both cerebral and extra-cerebral samples. PD-L1 staining will also be determined by c entral and/or local testing of PD-L1 IHC by any FDA-approved assay or an assay performed in a CLIA laboratory on FFPE tissue. A number of additional biomarkers will be studied for their potential predictive value in pre- treatment specimens. These exploratory studies include (but are not limited to):

1) Markers to be studied on tumor cells: PDL1, PDL2, B7-H2, B7-H3, B7-H4, Galactin-9, CEACAM-1, CD-70, HVEM, IDO, MHC class I antigens.
2) Percent of tumor sample that constitutes CD3, CD4 and CD8 cells and macrophages (CD68)
3) Markers on immune infiltrating cells: LAG-3 on CD4 cells, PD1-H (PD-1 homologue), CTLA4, TIM3, PD-1, IDO, BTLA
4) Vessel density will be determined by the area of CD34 staining in the sample. Tumor and vessel VEGF-receptor expression will be determined as previously described [52].

Patients who undergo surgical procedures (either of the CNS or peripheral) while on systemic treatment will also be required, when feasible, to provide a research specimen to determine changes in the above markers in the context of response or resistance to therapy.

Levels of all of the above markers will be measured by a method of Automated Quantitative Analysis (AQUA) of in situ protein levels in Dr. Kluger's laboratory at Yale University. This method has been validated for epithelial cancers and melanoma, and has shown to be more precise than pathologist-based scoring of 3,3′-diaminobenzidine stain. AQUA is highly reproducible and quantitative, as reviewed [53]. Tumors will be stained with a number of fluorophores: A tumor mask for melanoma will be made using a cocktail of anti-S100 and anti-Melan-A, and for NSCLC the mask will be generated by anti-cytokeratin antibodies. The methods for masking tumor have been described in the literature [52,54,55]. The mask will by conjugated to Cy-2, and will be differentiated from the target antigen, which will be conjugated to Cy-5. Lymphocytes will be identified by the appropriate antigen conjugated to Cy-7. After fluorescent staining is completed, images are taken at the different wavelengths, and the images will be analyzed using algorithms that have been extensively described. A monochromatic, high-resolution images of each histospot will be obtained using the 10× objective of an Olympus AX-51 epifluorescence microscope (Olympus) with automated microscope stage and digital image acquisition driven by a custom program and macrobased interfaces with IPLabs software (Scanalytics, Inc.). Tumor will be distinguished from stromal elements by the tumor mask signal. The signal intensity of the target biomarker will be scored on a scale of 0-255 (the AQUA score). This will provide quantitative results.

Biomarker analysis for individual markers will be conducted using standard statistical methods. Associations between continuous AQUA scores and dichotomized outcome variables (such as response) will be done by ANOVA. Cox univariate analysis will be used to study the associations between the survival endpoints and biomarker expression; Kaplan

Meier curves will be generated to depict the association between dichotomized biomarker levels and survival endpoints. Multivariable models will be generated to predict response. Biomarker selection will be done by determining area under the receiver operating characteristic (ROC) curves (AUCROC) at the time of each event in the testing set and we average these AUCROCs across all these events; this is a variant of the weighted average of time dependent ROC curve approaches [56-59].

11.2 Blood-based studies:

We will collect approximately 10 cc of blood from patients pre-treatment, after 6 weeks of therapy, 12 weeks of therapy, and every 9 weeks thereafter while on treatment, and at the end of treatment visit (5 cc of plasma and 5 cc of whole blood). We will immunophenotype cells from pre- and on- treatment blood samples to determine the array and activation status of different immune cell populations. We will do this using polychromatic flow cytometry which provides a powerful assessment of immune function based on differences in cell numbers, cell types and the expression of cell-associated surface and intracellular molecules related to immune perturbation [60]. Lyophilized antibody cocktails have been developed in the form of lyoplates to improve standardization between different labs and will be utilized here, with a different panel to examine multiple immune cells types (see Table below for details). Approximately 5ml of blood will be used for this assay.

We also will perform a multiplex cytokine analysis assay to determine the cytokines present pre-treatment and after 9 weeks on treatment with pembrolizumab [61]. We will use the Luminex assay which multiplexes 25 cytokines from plasma. The cytokines in the panel include: GM-CSF, IFN-γ, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-1β, IL-2, IL-21, IL-22, IL-23,IL-27, IL-28A, IL-31, IL-33, IL-4, IL-5, IL-6, IL-9, MIP-3α/CCL20, TNFα, and TNFβ .This assay requires about 100ul of plasma and utilizes a bead set coated with a capture antibody specific for each cytokine. A second biotinylated antibody and streptavidin-phycoerythrin is used to detect the captured cytokine. The Luminex 200 analyzer is a dual laser, flow-based, sorting and detection platform which can determine the specific cytokines present and the amount of cytokine bound. Both the immunophenotyping flow cytometry assay and the multiplex cytokine analysis are available through the Immune Monitor Core Facility at Yale under the direction of Dr. Lesley Devine, PhD.

Blood-based immunophenotyping and cytokine assays:

Antibody panels for immunophenotyping											
Lyoplate panel #	**FITC**	**PE**	**PE-Texas Red**	**PerCP-Cy5.5**	**PE-Cy7**	**APC**	**AlexaF-700**	**APC-H7**	**V450**	**V500**	**Purpose**
1	CD27	CCR7	Viability Dye	CD4	CD45RA	CD38	CD28	CD8	CD3	HLA-DR	**T cells**
2	CXCR5	CXCR3	Viability Dye	CCR4	CCR6	PD-1		CD8	CD3	CD4	**T_H1, T_H2, T_H17 cells**
3	CD39	CD25	Viability Dye	CD4	CCR4	CD127	FoxP3	CD45RO	CD3	HLA-DR	**T_{Reg} Cells**
4	CD21	CD24	Viability Dye	CD19	CD27	CD38	CD10	CD20	CD3	IgD	**B-cells**
5		CD56	Viability Dye	CD123	CD11c	CD16		CD3+19+20	CD14	HLA-DR	**DCs, monocytes and NK cells**

Please note that the profile of biomarkers is likely to change as scientific knowledge evolves.

11.3 Collection and Handling of Specimens

Before initiation of systemic therapy, local surgical therapy may be performed when clinically appropriate, and samples of brain or extracerebral tumor will be obtained. After tissue is obtained, it will be formalin fixed and embedded in paraffin. These tissues will be stored for correlative studies mentioned in the biomarker spectrum. If a patient has previously undergone local therapy to a brain lesion and tissue is available for analysis, paraffin-embedded tissue will be obtained from the sample. For patients who do not have tissue from a brain lesion available, tissue from a systemic lesion will be used to perform the planned studies. This tissue may include a FFPE specimen or SNAP frozen tissue. Please see the Procedures Manual for tissue requirements and handling of tissue

12.0 MEASUREMENT OF RESPONSE

Response will be evaluated with a CT chest/abdomen/pelvis and MRI brain every 6 weeks for the first 3 months of treatment and then every 9 weeks thereafter. If symptoms develop or clinical deterioration occurs, patients may be imaged prior to the pre-specified time points for imaging.

12.1 Brain Metastasis Response Assessment

RECIST criteria v1.1 will be modified to account for differences in measuring the response of clinically evaluable brain lesions as opposed to systemic lesions (modified RECIST, or mRECIST). Size is considered the tumor's largest diameter. Measurements from multiple lesions are summed to calculate the sum of the diameters (SD). The SD calculated on a baseline scan performed within 28 days of study drug initiation will be used as a reference to determine the objective response of the clinically evaluable lesions. All responses must be confirmed at ≥6 weeks with an equivalent or better response. Please refer to the original RECIST criteria if further reference is necessary.

12.2 Brain Metastasis Measurable disease

Specification of a minimal lesion diameter for measurable lesions reduces the potential for variation in the measurement of smaller lesions due to slice selection and volume averaging. The minimal lesion diameter should be greater than or equal to 2 times the section thickness and a minimum of 5mm. A previously irradiated lesion will not be measured as a target lesion unless it is documented to have progressed since treatment.

12.3 Brain Metastasis Nonmeasurable disease

Nonmeasurable lesions at baseline are important in clinical trials because tumor progression may occur at these sites. Nonmeasurable lesions include enhancing lesions that are less than the specified smallest measurable diameter (5mm when slice thickness is 2.5mm or twice the slice thickness), hemorrhagic or predominantly cystic or necrotic lesions that are difficult to accurately measure and track, and lesions that are indeterminate for metastatic disease. Intrinsic T1-hyperintensity is noted within hemorrhagic lesions that may be misinterpreted as enhancing tumor, and for this reason, the precontrast T1-weighted image must be examined at baseline to prevent this error. Nonmeasurable lesions should be briefly described on the imaging case report form for each study.[62]

RECIST 1.1 limits the number of lesions measured to 5 in total, with 2 per organ. For our purposes, if there are multiple measurable lesions in the brain, the sum of diameters of up to a maximum of 5 of the largest lesions will be considered for response assessment. assessment.

12.4 Modified Response Criteria for Brain Metastases

Up to 5 CNS lesions can be selected as target lesions for response evaluation

Complete Response (CR): Disappearance of all target lesions.

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

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum recorded since the treatment started or the appearance of one or more new lesions (new lesions must be greater than slice thickness).

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

Please refer to RECIST v1.1 for additional details regarding response criteria.

12.5 Systemic Disease Response Assessment

Response of all systemic, non-cerebral metastases will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee v1.1. Version 1.1 stipulates that the number of lesions required to assess tumor burden for response determination has been reduced to a maximum of five total lesions (two lesions maximum per organ).

Briefly, measurable lesions must have a minimum size of 10 mm by CT scan (CT scan slice thickness no greater than 5 mm) in at least one dimension. Lesions with a longest diameter of <10 mm are considered non-measurable lesions and will be tracked as non-target disease. Tumor lesions in a previously irradiated area, or in an area subject to other loco-regional therapy, are not considered measurable unless there has been demonstrated progression in the lesion. For complete definitions of measurable and non-measurable disease, please refer to the RECIST v1.1 criteria.

12.6 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. The best response will take into consideration both the clinically evaluable brain metastases and systemic metastases, and therefore will include measurements from both mRECIST criteria (in the brain) and RECIST criteria (in the body) as follows:

Complete Response (CR): Disappearance of all target lesions in the brain and systemic disease.

Partial Response (PR): Partial response in both the brain and the systemic disease, or if there is PR at one site and SD at the other, the sum of diameters from the brain and systemic disease will be added and the best overall response will be considered PR if there is at least a 30% decrease, taking as reference the baseline sum diameters.

Progressive Disease (PD): Progressive disease in either the brain or the systemic disease will qualify as PD as the best overall response.

Stable Disease (SD): Stable disease in both the brain and systemic disease, or if the sum of diameters from the brain and systemic disease does not qualify for PR or PD (at least a 20% increase in the sum of diameters of target lesions), taking as reference the smallest sum diameters since the treatment started.

Duration of overall response: The duration of overall response is measured from the time that 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).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented. Please note that objective documentation implies confirmation of response by imaging.

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.

Progression-Free Survival (PFS): PFS is defined as the time from initiation of study drug until the first documented, confirmed progression of clinically evaluable brain metastases based on mRECIST criteria, systemic disease based on RECIST criteria, or death. If there is progression of disease that is then confirmed on a follow up scan at least 4 weeks later, the initial date of documented progression should be used in the PFS analysis.

13.0 APPENDICES

13.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

13.2 Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting.

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