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TITLE: A Phase 2 Study of MK-3475 in patients with metastatic melanoma and non-small cell lung cancer with untreated brain metastases

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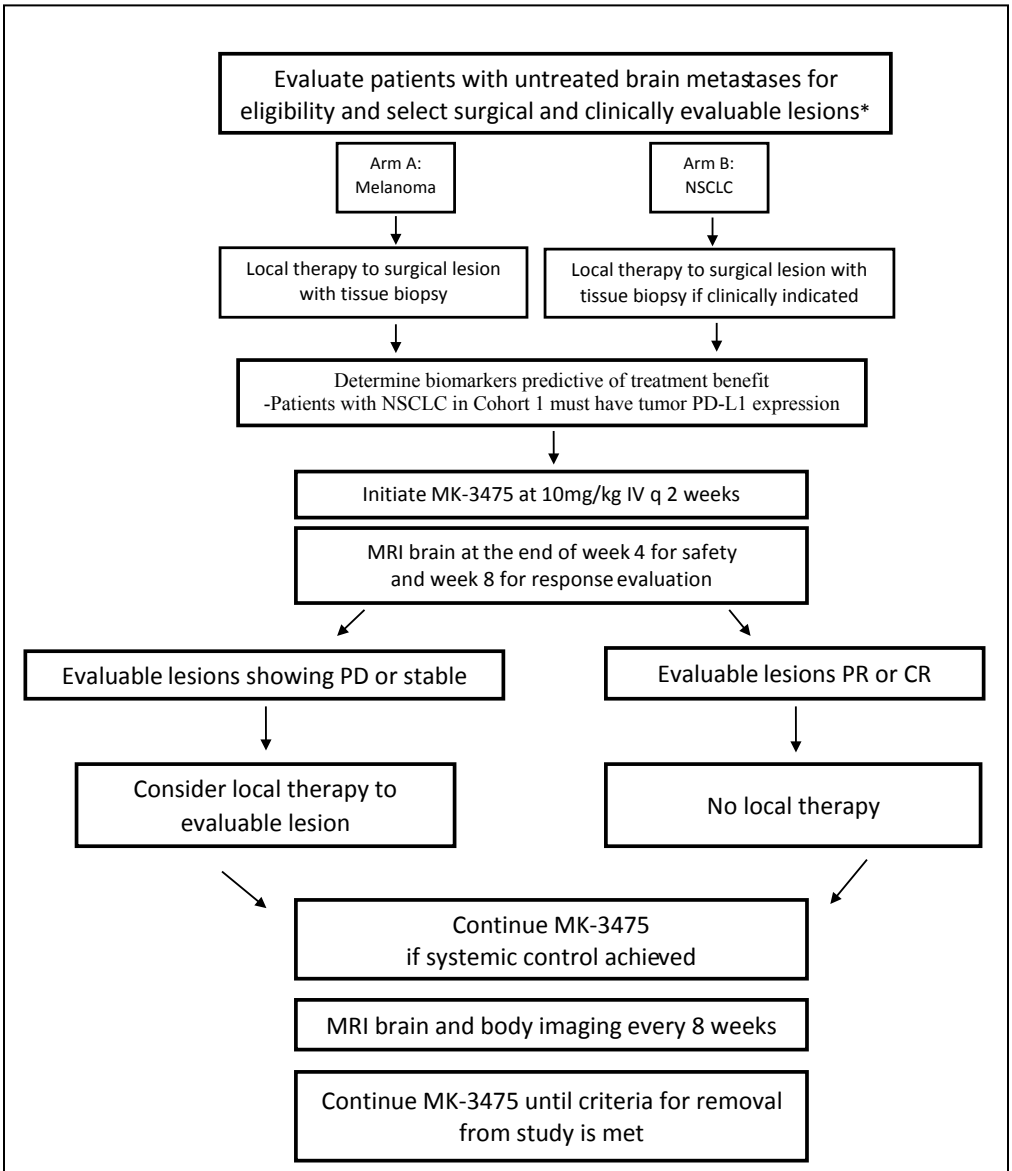

*Patients with NSCLC or those with prior resection of CNS metastases who have available tissue for analysis do not require identification or treatment of a surgical lesion. Patients with PD-L1 negative tumors or patients without accessible tumors will be eligible for Cohort 2 of the NSCLC arm.

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1. OBJECTIVES AND ENDPOINTS

The overall objective of this trial is to determine the efficacy of MK-3475 in patients with melanoma or non-small cell lung cancer (NSCLC) and untreated brain metastases. Efficacy will be determined by brain metastasis response rate, overall response rate, median progression free survival and overall survival. A secondary objective is to determine the safety of MK-3475 in patients with untreated brain metastases and to define the overall toxicity profile. An exploratory objective is to attempt to determine B7-H1 (PD-L1) expression and other potential predictive biomarkers in CNS and peripheral tumors and blood and their relationship to MK-3475.

1.1. Primary Endpoint

(1) To determine the brain metastasis response rate (BMRR) in patients with melanoma or NSCLC with untreated brain metastases treated systemically with MK-3475

1.2 Secondary Endpoints

(1) To determine the best overall response rate (ORR) in patients with untreated brain metastases from melanoma or NSCLC treated with MK-3475
(2) To study B7-H1 expression and other potential predictive biomarkers in CNS and peripheral tumors and blood, and their association with response to MK-3475
(3) To evaluate median progression-free survival (PFS) and overall survival (OS) in patients with melanoma or NSCLC metastatic to the brain, treated with MK-3475
(4) To determine the safety and toxicity of MK-3475 in patients with untreated brain metastases

2. BACKGROUND

2.1 Study Diseases

2.1.1 Melanoma

The incidence of melanoma has been rising faster than that of any other cancer in the United States, and the mortality rate has also been rising. In 2010, the projected incidence was 68,130 and the death rate from the disease was projected to rise to 8,700 [1]. This represents a sharp increase in recent years. For example, in 2006 the incidence was 62,190 and the death rate was 7,910, representing an increase of 10% in both incidence and death rate in a mere four years [2].

Therapeutic options are limited once melanoma metastasizes[3], although recent advances in development of novel therapies will likely expand the repertoire of active drugs for this disease. Older therapies, such as cytotoxic and cytokine agents, including dacarabazine, temozolomide, cisplatin, paclitaxel, interleukin-2 (IL-2), and interferon-alfa (IFNa), produce low objective responses in the range of 10-20%. None of these agents appear to have an impact on median survival, although responses can be associated with meaningful palliation of disease and 4-5% of patients treated with high-dose IL2 achieve long-term complete responses [4]. Various combinations of the active single-agents have been evaluated in phase II trials and in randomized phase III trials. Some of the regimens, such as cisplatin-vinblastine-dacarbazine (CVD), or CVD-IFNa-IL2 (biochemotherapy), produced high response rates exceeding 40% in single-arm trials.

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However, in prospective randomized studies, the combinations failed to demonstrate clinically meaningful improvements in median survival, and the toxicity was not negligible [5]. Until recently, dacarbazine has been the standard of care for treatment of metastatic melanoma, although median survival in patients treated with dacarbazine is less than 8 months [6-8]. For selected patients with good performance status and normal organ function, high-dose IL-2 remains a reasonable option, although treatment is associated with substantial acute toxicity and requires inpatient administration.

More recently, in a pivotal phase III study of ipilimumab, a monoclonal antibody against cytotoxic T lymphocyte associated antigen (CTLA-4), improved survival was observed[9]. This and other studies led to the FDA approval of ipilumumab for metastatic melanoma in March of 2011. Other recent clinical trials investigating targeted therapies in melanoma have shown promise. One novel agent, vemurafenib, an inhibitor of mutated B-raf, had a survival benefit when compared to dacarbazine[10]. Based on these studies, vemurafenib was recently FDA approved for metastatic melanoma.

2.1.2 Non-Small Cell Lung Cancer

NSCLC is the leading cause of cancer-related death in the United States and worldwide, and results in over 160,000 deaths in the United States per year[11]. In patients with advanced or metastatic disease, platinum-based chemotherapy had been the mainstay of management with a response rate and median overall survival of approximately 30% and 12 months, respectively[12]. In patients who are able to tolerate treatment beyond first line, responses are even lower and median survival is only minimally improved with chemotherapy. Targeted therapies, including agents targeting the epidermal growth factor receptor pathway have been shown to be effective as first-line therapies for select patients with response rates up to 70% and progression-free survival of about 1 year. However, none of these treatments has been shown to be curative in patients with metastatic disease, and despite the development of new and effective therapies, the five-year survival rate for patients with advanced NSCLC remains less than 5%.

Nivolumab, a PD-1 inhibitor, is the first immunotherapy agent to be approved for NSCLC with squamous cell carcinoma histology based on a randomized trial of nivolumab versus docetaxel in the 2nd line treatment setting. Nivolumab was approved by the FDA in all patients with squamous histology, without regards to PD-L1 status of the tumor. Further studies with nivolumab as well as other PD-1 and PD-L1 inhibitors are ongoing, and the role of PD-L1 as a potential biomarker continues to be explored.

2.2 MK-3475

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [13]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [14-29]. 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 solid malignancies such as ovarian, colorectal and pancreatic cancer, hepatocellular

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carcinoma, malignant melanoma (MEL) and renal cell carcinoma (RCC). TILs can be expanded ex vivo and re-infused, inducing durable objective tumor responses in cancers such as melanoma.

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) [25, 30]. The structure of murine PD-1 has been resolved [31-33]. 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 [30-32]. 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 [31, 32, 34, 35]. 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 [31, 32, 36]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [27]. 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 [37-40]. 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 [38]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various cancer types, including RCC, pancreatic carcinoma, hepatocellular carcinoma, and ovarian carcinoma [39, 41]. Furthermore, PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with MEL [41].

The observed correlation of clinical prognosis with PD-L expression in multiple cancers 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.

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a mono-therapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a

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mono-therapy in models of squamous cell carcinoma, pancreatic carcinoma, MEL and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-g, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [42-47]. In addition, the combination of gemcitabine and anti-PD-L1 mAb demonstrated synergy in the rejection of pancreatic mouse tumors. In-house experiments have confirmed the in vivo efficacy of PD-1 blockade as a mono-therapy as well as in combination with chemotherapy in syngeneic mouse tumor models (see the IB).

2.3 Clinical Development, Efficacy and Safety of MK-3475

An open-label Phase I trial (Protocol 001) has been conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W), in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. Based on PK data showing a half-life of 21 days, the protocol was amended to change the dosing frequency in the expansion cohort to every 3 weeks (Q3W). Promising preliminary anti-cancer activity has been observed. Of further note, those patients with melanoma who were treated with MK-3475 10 mg/kg Q2W had a best objective response rate of 29/52 [56%; 95% CI 41%, 69%] , and those treated at 10 mg/kg Q3W had a best objective response rate of 16/45 [36%; 95% CI 22%, 51%] . Updated response rates reported at the annual meeting of the American Society of Clinical Oncology in 2014 indicated an overall response rate of 34% among 411 treated patients. Based on the promising response rates, MK-3475 (pembrolizumab) was approved by the FDA in 2014 for patients with unresectable melanoma whose disease had progressed after treatment with BRAF inhibitors (for patients with BRAF mutant melanoma) and ipilimumab. Of note, nivolumab, also an inhibitor of PD-1, was approved for use in the same setting in December 2014.

The most common AEs were fatigue, nausea, rash, diarrhea, cough, pruritus, arthralgia, headache, abdominal pain, increased AST, pyrexia, and decreased appetite. The most common drug-related AEs included fatigue, rash, pruritus, diarrhea, and arthralgia. The incidence of Grade 3-5 AEs was 27%. Potentially immune-related AEs have been observed, including pneumonitis in both the melanoma and NSCLC cohorts. Although most cases do not result in death, in one instance, a 96-year old man with melanoma who experienced Grade 2 pneumonia/pneumonitis suffered a fatal myocardial infarction while being treated for the pneumonia/pneumonitis. Please refer to the IB for additional clinical data.[48]

MK-3475 Protocol 001 (PN001) Part C enrolled 38 previously treated subjects with NSCLC to receive monotherapy MK-3475 (internal data). The preliminary objective response rate was 21% by RECIST 1.1 criteria. Clinical responses have been observed in subjects with both squamous and non-squamous histologies. The median duration of response was not yet reached at 62 weeks. The response rate among the patients with PD-L1 positive tumors was 57%, while response rate among those with PD-L1 negative tumors was 9%. Given these results,the use of PD-L1 as a predictive biomarker is being further explored in subsequent NSCLC studies. More patients with NSCLC are being enrolled to MK-3475 PN001 for single agent treatment with MK-3475. Additionally, a Phase II/III study of MK-3475 vs docetaxel is

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enrolling patients with NSCLC that has been previously-treated with a platinum-containing doublet.

2.4 Local Surgical Therapy with Laser Interstitial Thermocoagulation Therapy (LITT)

Surgical management of brain tumors traditionally involves craniotomy with surgical debulking or resection of large superficial surgically accessible lesions. For small or deep lesions, the risk of craniotomy in a patient with stage IV cancer has not been justifiable. More recently, LITT has been FDA approved for use in the treatment of brain tumors. Radiofrequency thermocoagulation has been used for over 3 decades in the neurosurgical treatment of movement disorders and has been shown to effectively coagulate brain tissue and ultimately result in tissue necrosis at temperatures of 80 degrees Celsius and above[49]. The limitation of prior treatments with thermocoagulation, however, was that lesion volume did not correlate well with radiofrequency dose, resulting in a lack of the ability to predict extent of heat diffusion and therefore size of the lesion created. In the treatment of functional disorders, since treatment success was based on functional improvement, lesion size was far less important. With tumors, however, volume of thermal injury is critical. Using intraoperative MR thermometry, the temperature of tissues and the direction in which the heat is delivered can be monitored in real time. This enables the surgeon to watch the temperature changes in the tissues by MRI as heat is being delivered, stop or change treatment to control the size of thermal injury and to protect adjacent critical neural structures. LITT does not always require the administration of general anesthesia and from a surgical standpoint is considered a minimally invasive procedure. The procedure only requires a small scalp incision, the creation of a single burrhole in the skull, and the stereotactic passage of the laser catheter into the lesion as would be done for lesion biopsy. Most lesions then only require up to one hour of thermocoagulation time and, overall, LITT has been reported to carry less risk than a standard brain biopsy. Many centers have now reported good results using LITT in the treatment of deep seated metastatic brain tumors of <30mm diameter where craniotomy is not justifiable with obliteration of tumor within the area of thermal injury. Both LITT and craniotomy are currently being used in the surgical management of melanoma brain metastases at our institution.

2.5 Study Rationale

While recent advances in the treatment of metastatic melanoma and NSCLC with agents targeting PD-1 are striking, there remains a significant need to develop therapies for patients with untreated brain metastases who were excluded from prior trials with MK-3475 and the majority of other studies in these diseases. The brain is a common site of disease spread in many solid tumors, most notably metastatic melanoma and NSCLC. 10-40% of patients with metastatic melanoma develop brain metastases during their lifetime and >75% have brain metastases at autopsy [50-54]. Overall, historical melanoma patient cohorts have reported a median survival of patients with brain metastases in the order of 2.5 – 4 months despite use of whole brain radiation therapy (WBRT) and surgery. One older patient series showed a median survival of < 4 months in melanoma patients with brain metastases and a neurological death rate of >30% despite the treatment of intracranial metastases with whole brain radiation therapy (WBRT)[55]. Among those with NSCLC, 10% have brain metastases at presentation and another 30% develop

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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 diseases, with about half of patients with CNS disease presenting with more than one brain lesion[56].

Patients with untreated brain metastases have been excluded from most clinical trials of systemic therapy for two reasons: (1) historically their prognosis has been poor (overall survival ≤ 4 months) and (2) experimental drugs are presumed to not penetrate the blood brain barrier (BBB) or BBB penetration is not well studied [52]. In melanoma, for example, one phase III study evaluating ipilimumab excluded patients with untreated brain metastases and another study evaluating ipilimumab and dacarbazine excluded all patients with a history of brain metastases, regardless of prior treatment[57]. A subsequent trial with ipilimumab for patients with untreated brain metastases indeed showed that the drug had some activity in treating CNS disease. In the initial vemurafenib studies, patients with progressing or unstable CNS metastases were excluded. The pivotal BRIM-3 trial excluded patients with brain metastases unless metastases had been definitively treated more than three months prior to trial enrollment [58]. Both temodar and sorafenib cross the BBB. Initial studies with sorafenib alone and in combination with chemotherapy excluded patients with active brain metastases [59, 60]. A combination study of temodar and sorafenib in patients with or without brain metastases showed modest activity in patients without a history of prior temodar, and was thought to be favorable in part due to local therapies[61]. Although a number of studies have been conducted for melanoma patients with untreated brain metastases using established therapies, most initial clinical trials with novel agents exclude these patients. A recent trial with dabrafenib, an inhibitor of mutated B-raf, is an exception to the long-standing paradigm. A phase I/II study of this agent included a subset of patients with untreated brain metastases [62]. At the 2010 meeting of the European Society for Medical Oncology, Long et al reported that nine of the ten patients with untreated cerebral metastases enrolled in this trial had shrinkage of their brain lesions [62]. This was the basis for a recent phase II trial of dabrafenib specific for patients with untreated brain metastases[63]. In NSCLC, a small number of trials have shown that combination chemotherapy regimens can induce a response in the CNS with untreated brain metastases with a median PFS up to 4 months[64, 65]. These studies demonstrate that asymptomatic brain metastases, similar to asymptomatic metastases in other sites, can be treated systemically on clinical trials, and that drug activity in the CNS is not necessarily different than in other metastatic locations.

Current standard of care for brain metastases that require immediate local intervention (based on symptoms, location, size, or other concerning features) is craniotomy with resection or radiation therapy. As an adjunct to standard craniotomy, LITT is emerging as a new, minimally invasive local therapy to treat previously surgically inaccessible brain metastases. Not only is cell death instantaneous, thus decreasing the risk of delayed intra-tumoral hemorrhage, but another theoretical advantage of using LITT as part of management of brain metastases is that the hyperthermia breaks down the blood brain barrier at the edge of the coagulation region thereby possibly increasing access of chemotherapeutic agents into the lesion [58]. In patients in whom either craniotomy or LITT are performed, biopsies of tumor and surrounding normal brain can also be obtained at the time of local therapy. The institutional standard of care at Yale is to perform a local therapy (craniotomy, LITT or stereotactic radiosurgery) to patients without

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overwhelming brain metastases instead of whole brain radiation therapy to minimize toxicity and improve disease control. Patients with overwhelming brain metastases based on number or size of lesions will not eligible for this protocol.

The purpose of this trial is to study the activity of MK-3475 in untreated brain metastases from melanoma or NSCLC. Given the promising initial results of MK-3475 in these diseases but the lack of data in patients with untreated brain metastases thus far, this trial will study treatment in this patient population. Additionally, for patients with melanoma this trial requires local therapy with craniotomy or LITT to at least 1 brain lesion prior to systemic therapy, thereby allowing the acquisition of brain tumor tissue for correlative studies on biomarkers that may be predictive of clinical response in the CNS and systemically. We will also require a biopsy of an extra-cerebral metastasis when feasible, particularly when tissue from the brain lesion is not obtained in patients with NSCLC.

2.6 Correlative Studies Background

The phase I dose escalation studies of MK-3475 in patients with melanoma and NSCLC showed that response was not uniform, with response rates of 40% and 20, respectively. In the melanoma cohorts the association between response and expression of PD-L1 was unclear[66]. In NSCLC there appears to be an association between PD-L1 expression and response, although the numbers are small. Of the 22 patients with PD-L1 expression, six (27%) responded, while one of the 12 patients with expression below this threshold responded (8%). The association between PD-L1 expression and response to other PD-1 inhibitors is suggested by phase I trials in solid tumor patients, also with small numbers of patients[67],[68]. These studies need to be validated in additional patient cohorts. Moreover, not all patients with PD-L1 positive tumors responded to MK-3475. Some of the studies used whole tumor resections to determine PD-L1 expression, while others used cores from biopsies. In addition, a number of pre-clinical studies have now shown that PD-L1 expression is inducible with drugs such as interferon and vemurafenib[69]. Therefore, the timing of biopsies prior to treatment has to be reassessed, as inter-current therapies might effect PD-L1 expression. We propose here to treat NSCLC patients with any PD-L1 expression on biopsies done just prior to initiation of MK-3475. Based on our prior experience in the phase I trial, we anticipate that 65% of patients screened will be eligible. Given that the response rate in melanoma is higher, we will treat all melanoma patients that are otherwise eligible, regardless of PD-L1 status, and we will use their matched CNS and peripheral tumors to retrospectively study the association between PD-L1 status and other biomarkers and response to therapy, both in the CNS and in extra-cranial metastases.

Other studies conducted by Dr. Lieping Chen's group, that characterized the PD-L1/PD-1 axis a number of years ago, suggest that presence of CD4 and CD8 positive cells in tumor deposits might also be important for response to PD-1 targeting therapies. [70] They found that the majority of PD-L1 positive tumors were associated with CD4 and CD8 tumor infiltrating lymphocytes (TILs), while the minority of the PD-L1 negative tumors had associated TILs. Moreover, the staining pattern of PD-L1 on melanocytes differed based on proximity to TILs. Interferon-γ, a primary inducer of B7-H1 expression, can be detected at the interface of PD-L1 positive melanocytes and

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TILs, possibly reflecting activation of PD-1 on the TILs. This interaction, therefore, might be predictive of antitumor response to PD-1 blockade. Additional mediators of this pathway that might be associated with anti-tumor response, and will be studied on pre-treatment specimens from patients enrolled in this trial. Expression of other members of the B7 super-family of ligands, including PD-L2 and B7-H4 on tumor cells, might be associated with activation of PD-1 on tumor cells [71, 72]. Galectin-9 (the ligand to TIM-3), has been proposed as a mediator of PD-1 related T-cell exhaustion [73]. A more sophisticated and accurate predictive biomarker model might be built by incorporating markers on T cells, in addition to quantification of T cell tumor infiltrate. Specifically, co-expression of TIM-3 and PD-1 might be associated with sensitivity to PD-1 blockade [73]. LAG-3 (lymphocyte-activation gene 3) synergizes with PD-1 to inhibit T cell activation. [74] Co-expression of PD-1 and CTLA-4 on T cells is thought to be co-inhibitory, and co-inhibition of these molecules is the basis of ongoing clinical trials. [75] The T-cell stimulatory role of PD-1H (PD-1 Homologue) on T cells was recently described by Dr. Chen's laboratory; it's association with anti-tumor response in the setting of PD-1 blockade has yet to be determined. [76] Finally, the CNS environment might have a role in promoting anti-tumor immunity. Expression of the SALM family (synaptic-adhesion-like molecules) on neuronal cells might be associated with immune privilege (unpublished data) and will be studied as predictors of response to PD-1 inhibition.

3. PATIENT SELECTION

3.1 Inclusion Criteria

1. Biopsy proven metastatic melanoma or NSCLC as follows:
   a. Patients with metastatic melanoma must have untreated brain metastases including:
      -At least one cerebral metastasis that requires local intervention and is amenable to craniotomy or LITT therapy either due to symptoms, lesion size, location, edema or hemorrhage ("surgical lesion"). Alternatively, a patient may be eligible if a cerebral metastasis was resected or biopsied any time prior to enrollment and there is tumor tissue available for analysis.
      -At least one 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)").

      OR

   b. Patients with stage IV NSCLC with at least one 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)").
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 MK-3475 with the exception of bevacizumab which must have been administered at least 4 weeks prior to MK-3475. Patients are not required to have had prior systemic therapy. The exception to this is patients with NSCLC who test negative for PD-L1

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expression or are unevaluable for PD-L1 expression must have received prior platinum-based chemotherapy for entry into Cohort 2.
Note: Ipilimumab treatment should have been administered at least 4 weeks prior to the start of MK-3475.

5. Life expectancy of at least 3 months
6. A history of previously treated brain metastases is allowed, provided that at least 14 days have lapsed between radiation and initiation of MK-3475. 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 in tumor tissue from any site is required for patients with NSCLC for entry into Cohort 1. Tumor tissue must be obtained after the last systemic therapy. PD-L1 expression will be analyzed by a Merck assay. For NSCLC Cohort 2, patients may test PD-L1 negative or may be unevaluable for PD-L1 expression (i.e. insufficient tumor tissue). PD-L1 expression is not required for patients with melanoma, but melanoma patients are required to submit an extra-cerebral specimen for analysis, unless it is not feasible to obtain one.

8. 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
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

9. For women of childbearing potential, agreement to the use of two acceptable methods of contraception, including one barrier method, during the study and for 6 months after discontinuation of MK-3475.
10. For men with female partners of childbearing potential, agreement to use a latex condom, and to advise their female partner to use an additional method of contraception during the study and for 6 months after discontinuation of MK-3475.

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11. Negative serum or urine pregnancy test within 72 hours of commencement of treatment in premenopausal women.
12. Patients must have the ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

1. Symptomatic brain metastases. Symptoms may be present from the surgical lesion prior to resection or LITT but must have resolved by the time of administration of study drug.
2. Patients with brain metastases for whom complete surgical resection is clinically appropriate.
3. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy to the lung or brain within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent. Previous radiation to other sites may be completed at any time prior to initiation of MK-3475.
    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.
4. Has had prior treatment with any other anti-PD-1 or PD-L1 or PD-L2 agent or an antibody targeting other immune-regulatory receptors or mechanisms. Examples of such antibodies include (but are not limited to) antibodies against IDO, PD-L1, IL-2R, GITR. Prior ipilimumab, IL2, bevacizumab and adoptive cell therapy is allowed.
5. 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 2 weeks. Low-dose steroid use (≤10 mg of prednisone or equivalent) as corticosteroid replacement therapy is allowed
6. Has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
7. Presence of leptomeningeal disease
8. Has an active automimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of inhaled steroids or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.
9. 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 MK-3475, breastfeeding must be discontinued if the mother is treated with MK-3475.
10. 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.
11. Either a concurrent condition (including medical illness, such as active infection requiring treatment with intravenous antibiotics or the presence of laboratory abnormalities) or history

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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.

12. Concurrent, active malignancies in addition to those being studied (other than cutaneous squamous cell carcinoma or basal cell carcinoma)
13. 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.
14. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis
15. Has a known Human Immunodeficiency Virus (HIV), Hepatitis B (HBV), or Hepatitis C (HCV) infection.
16. Has received a live vaccine within 30 days prior to the first dose of trial treatment.
17. Evidence of interstitial lung disease

4. TREATMENT PLAN

4.1 Study Design

This is a 2-arm phase II study with each of the 2 tumor types (melanoma, NSCLC) will be treated on a different arm. After establishing eligibility criteria, for patients with melanoma the investigator will determine at least one lesion that requires local therapy (surgical resection or LITT) based on size, location, and/or risk of hemorrhage; this will be considered the "surgical lesion". All other eligible brain lesions will be considered "clinically evaluable lesions" and will be followed by modified RECIST (mRECIST) criteria to determine best response. Patients with NSCLC are not required to have a "surgical lesion" but must have at least one "clinically evaluable lesion" in the CNS. After obtaining informed consent, patients with melanoma will undergo local therapy to the study lesion (resection or LITT) and tumor tissue will be studied for B7-H1 (PD-L1) and other biomarkers as potential predictors of sensitivity to MK-3475, although it should be noted that the presence of a biomarker is not necessary for patients with melanoma to go on trial. Expression of PD-L1 will be determined both by Merck and by the Yale team. Patients on the NSCLC arm in Cohort 1 are required to have formalin-fixed, paraffin-embedded tumor tissue available for biomarker analysis, and those who do not have PD-L1 expression will be ineligible for treatment. Patients with NSCLC who test negative for PD-L1 expression or are unevaluable for PD-L1 expression will be eligible for Cohort 2. PD-L1 expression will be determined by the Merck assay. This will be followed by treatment with MK-34753 10mg/kg IV every 2 weeks. Each cycle consists of 2 weeks of treatment. Day 1 of the study will be defined as the first day a patient receives MK-3475.

An MRI of the brain will be obtained after 2 cycles (4 weeks) of systemic therapy for patient safety (i.e., to confirm there is no imminent risk in the case of progressing lesion or new/worsening edema). Patients with new symptoms from CNS lesions, progressing lesions or worsening edema may be taken off study and treated with local therapy to the brain metastases. An efficacy evaluation will then be performed after 4 cycles (8 weeks) including MRI of the brain and CT scans of systemic disease. Brain metastasis response will be determined using modified RECIST (mRECIST) criteria. The next MRI of the brain will be obtained after 8 cycles (week 16) and then

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every 4 cycles (8 weeks), along with body CT, MRI or PET/CT scans. 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 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. If a patient develops symptoms related to ANY lesion, local therapy may be administered to that lesion.

The plan is to enroll 74 patients total: 20 with melanoma, 44 with NSCLC in Cohort 1, and 10 with NSCLC in Cohort 2. The first interim analysis will be after 8 patients on the melanoma arm, 18 with NSCLC on the NSCLC arm.

4.2 Local Therapy and Tissue Biopsy of Brain Metastases

Definition of surgical lesion(s): The surgical lesion is any lesion that is determined by the investigator to benefit from immediate local therapy via craniotomy/resection or LITT due to size, location, edema or hemorrhage.

Definition of 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.

At time of enrollment, the surgical lesion(s) and clinically evaluable lesion(s) will be defined. The exception to this is patients who have undergone surgical therapy to one or more lesion prior to enrollment on study and have tumor tissue available for analysis and patients with NSCLC who do not require surgical intervention. These patients will have clinically evaluable lesion(s) defined only.

In patients with melanoma, the choice of craniotomy versus LITT will be determined by the investigators and will depend on individual patient factors such as location of the brain metastasis, patient comorbidities, and patient preference. Only LITT or craniotomy may be considered for local therapy of the study lesion because only these two treatment modalities can result in tissue biopsy at time of therapy.

Two to seven days after local therapy is complete, depending on the extent of the surgical procedure and patient recovery, therapy with MK-3475 10mg/kg IV every 2 weeks will begin. Patients with NSCLC who will not undergo local therapy will proceed directly to therapy with MK-3475. 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.

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At the time of local therapy, tissue from the study lesion will be obtained for correlative endpoint studies. 1 cm x 2 mm tissue cores will be obtained at time of biopsy. Standard operating procedures for SNAP freezing of tumors in the operating room will be used. After frozen section confirmation of tumor pathology, tissue will be allocated for study as described in **Section 9**.

4.3 MK-3475 Formulation

Clinical Supplies will be provided by Merck & Co., Inc., as summarized in table 1 below.

Table 1. Product Description

Product Name & Potency	Dosage Form
MK-3475 50 mg	Lyophilized Powder for Injection
MK-3475 100 mg/ 4 mL	Solution for Injection

4.4 MK-3475 Storage

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.

4.5 MK-3475 Dosage and Administration

Trial Treatments

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
MK-3475	10 mg/kg	Q2W	IV infusion	Day 1 of each cycle

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical trial data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 2.

Table 2. Dose Modification Guidelines for Drug-Related Adverse Events

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General instructions:

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

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable

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	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that		

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		require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

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

NOTE:

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

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 Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the 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.** 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 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).

Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Subject is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

4.6 Dosing Delays

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record. See Tables 2 and 4 for details.

Table 4. Treatment Delays and Resumption of Therapy.

5	Treatment held on the days of any local therapy to brain lesions	One week later
6	Suspicion of pregnancy	Resume if pregnancy ruled out
7	Treatment held during any hospitalization for illness	Resume after discharge from hospital
8	Treatment held on day of any general surgery	Resume 1 week later (if outpatient surgery) or after discharge from hospital (if hospitalization is required for recovery or complications)

4.7 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:

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1	Disease progression (see Section 4.8)
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 MK-3475 (see Table 2)
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

4.8 Treatment Beyond Disease 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. Patients may continue treatment with MK-3475 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 medical monitor.

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

4.9 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.

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5 GENERAL CONCOMITANT THERAPY AND SUPPORTIVE CARE GUIDELINES

5.1 Concomitant Medication

Additional anti-tumor therapies are prohibited during study treatment. Additionally, the use of corticosteroids or other immunosuppressive medications will not be allowed during study therapy (unless used to treat a drug-related adverse event). Low-dose steroid use (≤10 mg of prednisone or equivalent) as corticosteroid replacement therapy is allowed.

5.2 Local Therapy

Local therapy for brain metastases:
Local surgical therapy in the form of craniotomy or LITT will be performed on the surgical lesion(s) prior to initiating systemic therapy. At any time point throughout the study, local therapy (surgery, gamma knife 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 MK-3475, provided that the patient is otherwise benefiting from therapy and has stable disease or disease shrinkage in other lesions by RECIST criteria. Approval from the study PI or co-PIs is required to continue treating the patient on MK-3475. 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 which will consider those patients who require local therapy while on study as having PD at the time of local therapy.

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 MK-3475 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.

5.3 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 AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Table 2. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection,

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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 of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to Table 2 for guidelines regarding dose modification and supportive care.

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

Management of Infusion Reactions: Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of MK-3475.

Table 4. 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.	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

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NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	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.**	
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 and Monitor Symptoms.** 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		

5.3.1 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 MK-3475 compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive

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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. Information on how to identify and evaluate irAEs has been developed and is included in the Immune-Related Adverse Event Guidance Document. 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 5.

Table 5 General Approach to Handling irAEs

irAEs	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold MK-3475	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold MK-3475 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.

5.3.2 Supportive Care Guidelines for Pneumonitis

Subjects with grade 2 pneumonitis (symptomatic pneumonitis requiring medical intervention) should immediately stop receiving MK-3475 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 6 and follow-up will be as routine for AEs associated with study drug including monitoring until full resolution of the AEs/pneumonitis.

Table 6. Recommended Approach to Handling Pneumonitis

Study drug associated pneumonitis	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1 (asymptomatic)	No action	Intervention not indicated
Grade 2	Withhold MK-3475, may return to treatment if	Systemic corticosteroids are indicated. Taper as indicated.

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	improves to Grade 1 or resolves within 12 weeks	
Grade 3 and Grade 4	Discontinue MK-3475	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 MK-3475 if upon rechallenge subject develops pneumonitis ≥ Grade 2
- Please consult the Events of Clinical Interest and Immune-Related Adverse Events Guidance Document for a more in-depth discussion of pneumonitis.

6 STUDY ASSESSMENTS AND PROCEDURES

Systemic treatment with MK-3475 is divided into 2-week cycles, with treatment occurring on Day 1. A window of +/- 3 days can be applied to scheduled visits if necessary for holidays, vacations, inclement weather, etc.

6.1 Screening Visit

Prior to initiation of therapy, the patient will require a screening visit. Scans must be done within 28 days prior to the start of systemic therapy; all other evaluations must be completed within 7 days prior to starting MK-3475. At the screening visit, the following will be addressed:

1) Informed consent
2) History and physical examination
3) Measurement of height, weight and vital signs (including blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature).
4) Performance status evaluation
5) CBC with differential, serum chemistry (Na, K, Cl, CO2, BUN, creatinine, glucose, calcium), magnesium, phosphate, liver function tests (albumin, total protein, alkaline phosphatase, total and direct bilirubin, ALT, AST), LDH, TSH, free T3, free T4
6) Plasma and pre-treatment tumor tissue for correlative studies. Melanoma patients are required to have cerebral and extracerebral tissue, unless it is not feasible to obtain the extra-cerebral tissue. Lung cancer patients are required to have extracerebral tissue, unless it is not feasible to obtain the extra-cerebral tissue. If lung cancer patients undergo a surgical resection or biopsy of a brain lesion, they are required to provide a portion of the tissue for research. Plasma can be obtained at screening or any time prior to Cycle 1 Day 1 dosing.
7) Pregnancy test (serum or urine) for women of childbearing potential. Sexually active women of childbearing potential must use an effective

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method of birth control during the course of the study, in a manner such that risk of failure is minimized.

8) Systemic tumor assessment with CT scan of the chest/abdomen/pelvis
9) CNS tumor assessment with Brain MRI. MRIs should be done with perfusion, if possible. For patients who will undergo local therapy on trial, investigators must identify "surgical lesion(s)" and "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, or those with NSCLC who are not eligible for surgical intervention, only identification of "clinically evaluable lesion(s)" is necessary.
10) NSCLC patients will be required to provide a specimen for determination of PD-L1 expression using the assay developed by Merck for entry into Cohort 1.
11) NSCLC patients will be required to have baseline pulmonary function tests.

6.2 On-Study Visits

Day 1 of each cycle (every 2 weeks):

1) History and physical examination
2) Measurement of height, weight and vital signs (including blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature).
3) Performance status evaluation
4) Review concomitant medications; initiation of medications for seizure prophylaxis in patients not on anti-convulsants.
5) Adverse events assessment
6) CBC with differential, serum chemistry (Na, K, Cl, CO2, BUN, creatinine, glucose, calcium), magnesium, phosphate, liver function tests (albumin, total protein, alkaline phosphatase, total and direct bilirubin, ALT, AST), LDH
7) Administration of MK-3475 10mg/kg IV

Day 1 of cycle 3 (after 4 weeks of therapy +/- 5 days)

1) Brain MRI to assess patient safety (i.e., to confirm there is no imminent risk in the case of progressing lesion or new/worsening edema). MRIs should be done with perfusion, if possible.

Day 1 every 8 weeks +/- 5 days

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1) TSH with reflex free T4 and free T3
2) Tumor response assessment with:
    a. CT scan of the chest/abdomen/pelvis
    b. Brain MRI; MRIs should be done with perfusion, if possible.
3) Plasma for correlative studies

6.3 Follow-up Visits

All patients who discontinue the study treatment will have a follow up visit at Day 28 ±7 days from the last study treatment.

1) History and physical examination
2) Measurement of height, weight and vital signs (including blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature).
3) Performance status evaluation
4) Review concomitant medications
5) Adverse events assessment
6) CBC with differential, serum chemistry (Na, K, Cl, CO2, BUN, creatinine, glucose, calcium), magnesium, phosphate, liver function tests (albumin, total protein, alkaline phosphatase, total and direct bilirubin, ALT, AST), LDH
7) Tumor assessment with CT scan of the chest/abdomen/pelvis and brain MRI with perfusion, if possible, should continue every 8 weeks (+/- 5 days) until progression, withdrawal of consent, death, lost to follow-up, or start of a subsequent anti-cancer therapy

All patients who discontinue early or who complete the study treatment period will be followed for survival. Survival follow-up information will be collected via telephone calls, patient medical records, and/ or clinic visits every 3 months +/- 14 days until death, loss to follow-up or study termination. All patients will be followed for survival unless the patient requests to be withdrawn from follow-up. If the patient withdraws from study treatment but not from follow-up, the study staff may use a public information source to obtain information about survival status.

6.4 STUDY CALENDAR (Table 7).

Screening evaluations are to be conducted within 7 days prior to start of systemic therapy. Scans must be done 28 days prior to the start of systemic therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

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 Cycle 3 (after 4 weeks +/- 5 days of therapy)	Day 1 Cycle 5, and every 8 weeks, thereafter	Day 28 +/- 7 days from last study treatment	Every 3 months +/- 14 days after the follow-up visit
Informed Consent	X					
History/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	
PT/INR (for patients on warfarin)	X	X			X	
Carbamazepine or Phenytoin level (for patients on drug)	X	X			x	
TSH, free T3, free T4	X			x		
NSCLC patients: Determination of PD-L1 expression in tumor (Cohort 1 only)	X					
Plasma for correlative studies	X[I]			x		
Obtain extracerebral tumor specimen for correlative studies (Refer to Section 6.1 and 9.2)	X					
Pregnancy Test[C]	x					
CT chest/abdomen/pelvis	x			X[D]	x[E]	
Brain MRI	x[F]		X[G]	X[H]	x[E]	
Selection of "surgical lesion(s)" and "clinically evaluable lesion(s)"	x					
Pulmonary Function Tests – NSCLC patients only	X					
Review concomitant medications		X			x	
Adverse events assessment		X			x	
Administration of MK-3475		X				
Survival Follow-up						X

[A]Includes blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature. Height at Visit 1 only.

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[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 and direct bilirubin, ALT, AST), LDH

[C]Pregnancy test (serum or urine) is required for women of childbearing potential only.

[D]CT scan of the chest/abdomen/pelvis should be performed every 8 weeks +/- 5 days of therapy. Response evaluation is performed using RECIST 1.1 and ir-RECIST

[E]CT scan of the chest/abdomen/pelvis and brain MRI should continue every 8 weeks (+/- 5 days) until progression, withdrawal of consent, death, lost to follow-up, or start of a subsequent anti-cancer therapy

[F]For patients who will undergo local therapy on trial, investigators must identify "surgical lesion(s)" and "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 on Day 1 of Cycle 3 to assess patient safety only; response assessment should not be performed. MRIs should be done with perfusion, if possible.

[H]Brain MRI should continue every 8 weeks (+/- 5 days) along with systemic evaluation. CNS response evaluation is performed using mRECIST and ir-mRECIST criteria. Perfusion will be done, if possible.

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

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Safety Analysis

Safety will be analyzed for patients treated in this study. All patients who receive any amount of study drug will be evaluable for toxicity.

At each visit, a brief focused history will be obtained and any indication of treatment- related toxicity will be evaluated by appropriate examination and/or laboratory/radiographic studies.

Safety analyses will include summaries of adverse event rates and changes in laboratory results, as well as number of CTCAE (v4.0) toxicity grades for both laboratory and non-laboratory data.

The evaluation period should extend from date of first treatment until 90 days from discontinuation of treatment or until resolution from all acute toxicities associated with the drug administration.

7.2 Definition of Adverse Event Terms

Adverse Event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). [NIH Guidelines, January 2001]

Serious Adverse Event (SAE): Any adverse drug experience occurring at any dose that results in any of the following outcomes:

1	Death
2	A life-threatening adverse drug experience

3	In-patient hospitalization or prolongation of existing hospitalization
4	Any persistent or significant disability/incapacity
5	A congenital anomaly/birth defect
6	Is a new cancer (that is not a condition of the study)
7	Is associated with an overdose
8	Is an other important medical event
9	Pregnancy

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. [21CFR312.32(a)].

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

"Serious" Versus "Severe" Adverse Events : There is a distinction between serious and severe AEs. Assessment of seriousness will be made solely by the serious criteria listed above. Severity of AEs will be graded according to the NCI Common Terminology Criteria for Adverse Events v4.0. Therefore, serious events will not be automatically considered severe. For example, a stroke that results in only a limited degree of disability may be considered a mild (not severe) stroke, but it would still meet serious criteria and thus, be captured as an SAE. Similarly, severe events may not always be serious. An example would be an episode of severe, transient nausea which persists for several hours. This would be classified as a "severe" episode of nausea, but if it did not require treatment, intervention, or somehow meet other serious criteria, it would not be considered an SAE.

Life-threatening Adverse Drug Experience : Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e. it does not include a reaction that, had it occurred in a more severe form, might have caused death. [21CFR312.32(a)]

Unexpected Adverse Drug Experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan. "Unexpected" as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.[21CFR312.32(a)]

Laboratory test abnormalities: Laboratory abnormalities that constitute an Adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded. Whenever

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possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event.

Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. Please note that a dose hold or medication for a lab abnormality may be required by the protocol in which case the lab abnormality would still, by definition, be an adverse event and must be reported as such.

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

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for ≥1000 mg of pembrolizumab. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, study treatment should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

If a dose of Sponsor's product or vaccine 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 by the investigator within 24 hours to the Sponsor either by electronic media or paper.

7.3 Toxicity Grading

Toxicities will be graded according to the current version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The full text of the NCI CTCAE is available online at: http://ctep.cancer.gov/forms/CTCAEv4.pdf

If a certain event or symptom is not described in the CTCAE grades, use the following grading scale:

Mild	Awareness of event, but easily tolerated
Moderate	Discomfort enough to cause some interference with usual activity
Severe	Inability to carry out usual activity
Very Severe	Debilitating, significantly incapacitates patient despite symptomatic

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	therapy

7.4 Toxicity Attribution

Assessment of attribution is made by consideration of all clinically relevant data prior to, during, and after occurrence of the event, including diagnostic tests to assess the cause of the event. Clinically relevant data include, but are not limited to; underlying disease, past and present medical history (all concurrent non-malignant disease), concurrent medications, and timing between event and drug administration. The mechanism of action and prior toxicology of the study drug should be considered.

An adverse event is *associated with the use of the drug* when there is a reasonable possibility that the experience may have been caused by the drug. [21CFR312.32(a)]

Attribution Standards per NCI – CTEP:

Unrelated	The Adverse Event is **clearly not** related to the investigational agent (s)
Unlikely	The Adverse Event is **doubtfully** related to the investigational agent(s)
Possible	The Adverse Event **may be** related to the investigational agent(s)
Probable	The Adverse Event **is likely** related to the investigational agent(s)
Definite	The Adverse Event **is clearly** related to the investigational agent(s)

7.5 Yale Principal Investigator Safety Reporting Requirements

7.5.1 Expedited Reporting of Unexpected SAEs

AEs classified as "serious" and "unexpected" that are possibly, probably, or definitely attributed to drug administration, or SAEs whose frequency exceeds expectations, require expeditious handling and reporting.

The PIs will promptly investigate all safety information related to an adverse experience. If the results of the PIs' investigation show an adverse drug experience not initially determined to be reportable (based on whether the event is serious, unexpected, and associated with drug administration) is so reportable, the PIs will report such experience. Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

7.5.2 Reporting to the Yale Human Investigation Committee

All SAEs, whether originating at Yale or a collaborating center, meeting the criteria for expedited reporting will be reported to the Yale University Human Investigation Committee (HIC) using HIC Form 710 FR 4: Unanticipated Problem Involving Risks to Subjects or Others (UPIRSOs), including Adverse Events (AEs) Reporting Form as per IRB Policy 710.
A copy of the IRB Policy 710 Reporting Unanticipated Problems Involving Risks to Subjects or Others, including Adverse Events is available at:
http://www.yale.edu/hrpp/policies/documents/NewIRBPolicy710_UPIRSOSAE_4.3.2014_withflowchart_vF.PDF

7.5.3 Reporting to the Food and Drug Administration

This study will be conducted under an IND (Investigational New Drug application) that will be held by Yale University. 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 MK-3475

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 MK-3475

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

7.5.4 Reporting to Merck

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 (Investigator-Sponsor) and within two (2) working days to Merck Global Safety (GS).

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

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any time outside of the time period specified in the previous paragraph also must be reported immediately to the Investigator-Sponsor and to Merck.

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

Additionally, investigators will report any pregnancy occurring in association with use of a Merck Product to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220).

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

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

7.5.5 Duration of Reporting of SAEs

From the administration of first treatment until 90 days (unless otherwise specified) subsequent to last treatment or withdrawal of subject, new onset adverse events will be captured. Follow-up and reporting of these events will follow the same procedure as for AEs observed during the study period. In addition, any unexpected Serious Adverse Event that occurs more than 90 days after drug administration but is possibly, probably or definitely attributed to drug administration will be recorded and reported.

7.6 Yale Safety Reporting and Monitoring (DSMP)

The PIs and IND holders will monitor the clinical trial for safety. The PIs will assess all expedited adverse events and will periodically review all adverse events observed on the trial. Yale Cancer Center standard operating procedures (SOPs) for assessment and reporting of adverse events are followed which are in compliance with 21 CFR 312.32 and 312.22.

The clinical trial data consisting of all required observations, AEs, and laboratory data are entered into a computerized database in a timely manner. The accuracy and completeness of the database, timely submission of SAEs and compliance with the protocol, is assured by periodic auditing conducted by the Yale Cancer Center Office of Protocol Review and Monitoring, which reports to the Yale Data and Safety Monitoring Committee (DSMC) Committee. Safety data will be submitted to the DSMC at least once yearly or more often as required by the DSMP. On a regular interval basis, status reports of all laboratory parameters, AEs and SAEs are reviewed by the PI to view composite data across subjects. Regular meetings are held to discuss ongoing patient treatment and adverse events.

Expedited SAE reports submitted by the Investigator to FDA are also copied to the HIC and other relevant institutional safety committees within the timeframes required by Yale. These are also copied to Merck. The Principal Investigator will distribute manufacturer-provided safety reports and updated Toxicity Lists to the institution's HIC and all relevant personnel involved in the conduct of the study. The Toxicity List, in addition to the Investigator's Brochure, will be used as a reference for reporting any new SAE.

Possible actions taken by the PIs or the Yale DSMC if a new unexpected toxicity is identified from the above safety review, or if the periodic review of all adverse events and laboratory data indicates a pattern of incidence or severity of toxicity that raises a safety concern, can be to:
1. Revise consent form
2. Amend the protocol
3. Suspend the protocol

All AEs found to be expected or non-serious, will be included in the Annual Report.

7.7 Pregnancies

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.

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 Yale IRB, and within two (2) business days to Merck GS. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.8 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.

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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 7.2.1 – 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.

7.9 Warnings and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate, other than those noted in the provided Investigator Brochure. Additional safety information collected between IB updates will be communicated in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

8. PHARMACEUTICAL INFORMATION

Please see Investigators Brochure for details regarding the study drug.

8.1 MK-3475 Availability

MK-3475 is an investigational agent supplied to investigators by Merck.

8.2 Agent Accountability

Agent Inventory Records: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from Merck.

9. LABORATORY CORRELATIVE/SPECIAL STUDIES

9.1 Handling of Tissue

9.1.1 Collection and Shipment of Specimen(s):

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Before initiation of systemic therapy, local surgical therapy may be performed on the surgical lesion, and samples of brain tumor will be obtained. After tissue is obtained, it will be formalin fixed and embedded in paraffin. If sufficient tissue is available, a portion will be subjected to flash freezing in liquid nitrogen and SNAP frozen tissue will be stored for further studies. 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. A portion of the biopsy specimen will be used to conduct the studies mentioned in section 9.2. For patients with NSCLC 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.

9.1.2 Site(s) Performing Correlative Study:

The stored tissue specimens will be analyzed at Yale University. Please see **Section 11** on **Statistical Analysis** for information on how results will be analyzed. FFPE specimens will be analyzed for PD-L1 expression by a Merck assay for eligibility in the lung cohort.

9.2 Correlative Studies

Correlative studies will be conducted on the pre-treatment tissue specimen. PD-L1 positivity for study eligibility for the NSCLC cohort will be determined by Merck in a CLIA-certified laboratory on FFPE tissue. A number of additional biomarkers will be studied for their potential predictive value in pre-treatment CNS specimens taken at the time of surgery or LITT and/or on tumor tissue from a systemic site. These exploratory studies include (but are not limited to):

1) Markers to be studied on tumor cells: B7H1, PDL2, B7H4, Galactin-9.

2) Percent of tumor sample that constitutes CD4 and CD8 cells

3) Markers on T cells: LAG-3 on CD4 cells, PD1-H (PD-1 homologue) on T cells, CTLA4 on T cells TIM3 on T cells

4) SALM 5 on neuronal cells will also be studied.

Patients who undergo surgical procedures (either of the CNS or peripheral) while on systemic treatment will also be asked 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 [77]. 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[78-80]. The mask will by conjugated to Cy-2, and will be

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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 [58]. 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 will be conducted using standard methods. Associations between continuous AQUA scores and binarized 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 binarized biomarker levels and survival endpoints. Multivariable models will be generated to predict response.

Immune monitoring will be performed as a part of this study. Peripheral blood lymphocytes (PBLs) and plasma (approximately 40cc) will be collected before treatment, every 8 weeks on treatment, and when patients come off of study. Peripheral blood mononuclear cells will be separated and cryopreserved for T and B cell studies. Samples will be stored for future immunophenotyping studies and serological profiling to be conducted by Dr. Dhodapkar's laboratory, as well as cytokine profiling at the Yale Immune Monitoring Core Facility.

10. MEASUREMENT OF RESPONSE

Response will be evaluated after 8 weeks of systemic therapy and then every 8 weeks thereafter. The purpose of the 4 week scans is to determine safety. If symptoms develop or clinical deterioration occurs, patients may be imaged prior to the pre-specified time points for imaging. All responses must be confirmed by repeat imaging at least 4 weeks following initial documentation of objective response.

10.1 Brain Metastases 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 4 weeks with an equivalent or better response. Please refer to the original RECIST criteria if further

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reference is necessary. Please see section 5.2 for response assessment in patients requiring local therapy while on study.

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.

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.[81]

RECIST 1.1 limits the number of lesions measured to 5 in total, with 2 per organ. For our purposes, even 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.

Modified response criteria for brain metastases

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.

10.2 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)

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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.

10.3 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.

10.4 Duration of Response

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).

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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.

10.5 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.

10.6 Response Review

All responses will be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Sample Size Justification

This two-arm, phase II study will enroll patients with melanoma or NSCLC who have untreated brain metastases. Subjects with melanoma must have at least 1 brain lesion that requires immediate local therapy that is amenable to surgical resection or LITT, and at least 1 brain lesion that does not require immediate local therapy and can be assessed for response. In certain situations, one lesion can be biopsied and then assessed for response. Subjects with NSCLC are only required to have at least 1 brain lesion that does not require immediate local therapy and can be assessed for response. Subjects will undergo local therapy to 1 or more brain metastases (required for subjects with melanoma, optional for those with NSCLC), followed by treatment with MK-3475.

The primary goal of this study is to determine the efficacy of MK-3475 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%. Alternatively, MK-3475 will be considered worthy of further study in patients with untreated brain metastases if its true BMRR is similar to the systemic response rate with MK-3475 which is 38% for melanoma and 25% for NSCLC. [48] and internal data.

<u>Power and Sample Size</u>

Melanoma Arm

The melanoma arm will enroll a minimum of 8 subjects. If at least one of the first 8 has a brain metastasis response, a sequential monitoring procedure will be used to evaluate efficacy and futility simultaneously, based on the number of subjects with a confirmed or unconfirmed response according to the rules outlined in Figure 1, Table 8 and Table 9. Only patients who remain on treatment for at least 12 weeks will be considered evaluable for the futility analysis. Accrual will not be suspended during the interim evaluation. Subsequent decisions for pursuing future interim evaluations will be based on the boundaries identified by the sequential monitoring procedure. We will enroll an approximate maximum of 20 subjects in this arm.

With an approximate maximum of 20 subjects enrolled, we will have 86% power to demonstrate that the best brain metastasis response rate exceeds 10% at an overall one-sided 10% alpha-level, if the true best brain metastasis response rate is 38%. The calculation is based on the binomialSPRT function in the gsDesign package.

For the final analysis, the response assessment will be based on confirmed best brain metastasis response rate. The adjusted p-value and repeated confidence interval will be computed using sequential methods outlined by Jennison and Turnbull (2000). The minimum criterion for success is that the lower bound of the repeated CI > 10%. Given the null hypothesis underlying the true rate, this may occur when at least 6/20 subjects develop a confirmed brain metastasis response.

If the efficacy boundary is crossed at any time during the trial, enrollment may still continue to 20 subjects and subsequent trial planning may be initiated.

Operating characteristics are shown in Table 10.

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Figure 1. Sequential Monitoring Rules for Efficacy and Futility in Melanoma Arm

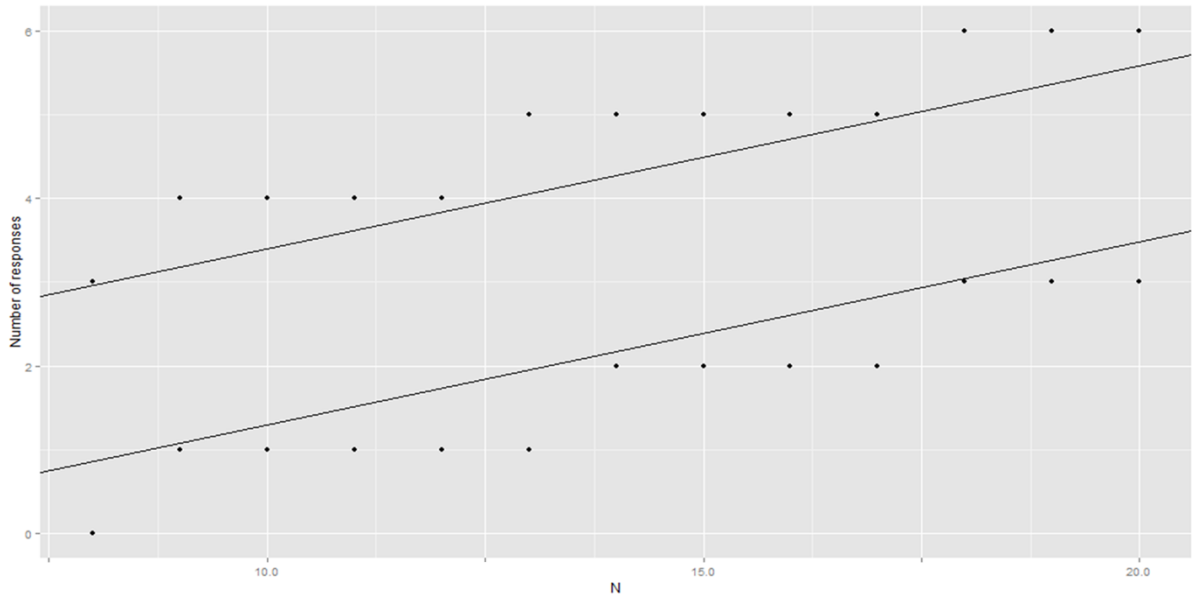

Table 8. Decision Rules based on Efficacy Bounds for Melanoma Arm

Monitoring Point (# subjects)	Minimum # Responders to Determine Efficacy
8	3
9-12	4
13-17	5
18-20	6

Table 9. Decision Rules based on Futility Bounds for Melanoma Arm

Monitoring Point (# subjects)	Maximum # subjects w/ response to declare Futility
8	0
9-13	1
14-17	2
18-20	3

Table 10. Operating Characteristics of Sequential Monitoring Approach for Melanoma Arm

True RR	Probability of Declaring Futility	Probability of Go at end of Study	Average Sample Size
10%	0.93	0.05	10.3
15%	0.79	0.16	12.3
20%	0.61	0.32	14.3
25%	0.43	0.50	16.1
30%	0.27	0.66	17.4
35%	0.16	0.80	18.4
40%	0.09	0.89	19.1
45%	0.05	0.94	19.5
50%	0.02	0.97	19.7

Non-Small Cell Lung Cancer Arm

Cohort 1:

The non-small cell lung cancer arm will enroll a minimum of 18 patients into Cohort 1. Following the time that the first 18 subjects have had at least 2 brain metastasis responders, a sequential monitoring procedure will be used to evaluate for efficacy and futility simultaneously, based on the number of subjects with a confirmed or unconfirmed brain metastasis response according to the rules outlined in Figure 2, Table 10, and Table 11. Only patients who remain on treatment for at least 12 weeks will be considered evaluable for the futility analysis. Accrual will not be suspended during the interim evaluation. Subsequent decisions for pausing enrollment and pursuing future evaluations will be based on the boundaries identified by the sequential monitoring procedure. A maximum of 44 subjects will be enrolled in this arm.

With a maximum of 44 patients enrolled in Cohort 1, we will have approximately 80% power to demonstrate that the best brain metastasis response rate exceeds 10% at an overall one-sided 10% alpha-level, if the true best brain metastasis response rate is 25%. The calculation is based on the binomialSPRT function in the gsDesign package.

For the final analysis, the response assessment will be based on confirmed best brain metastasis response rate. The adjusted p-value and repeated confidence interval will be computed using sequential methods outlined by Jennison and Turnbull (2000). The minimum criterion for success is that the lower bound of the repeated CI > 10%. Given the null hypothesis underlying the true rate, this may occur when at least 10/44 subjects develop a confirmed response.

If the efficacy boundary is crossed at any time during the trial, enrollment may still continue to 44 subjects and subsequent trial planning may be initiated.

Operating characteristics are shown in Table 13.

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Cohort 2:

A maximum of 10 patients with NSCLC will be enrolled in Cohort 2. These patients will meet the same eligibility requirements as other NSCLC patients, except for the following: they will be required to have had at least 1 prior platinum-based systemic therapy, and they must either be negative or unevaluable for PD-L1 expression. The response rate with MK-3475 in PD-L1 negative patients in a previous trial is 9%, however the efficacy in brain metastases is unknown. Therefore this cohort will be exploratory in nature.

Figure 2. Sequential Monitoring Rules for Efficacy and Futility in NSCLC Arm

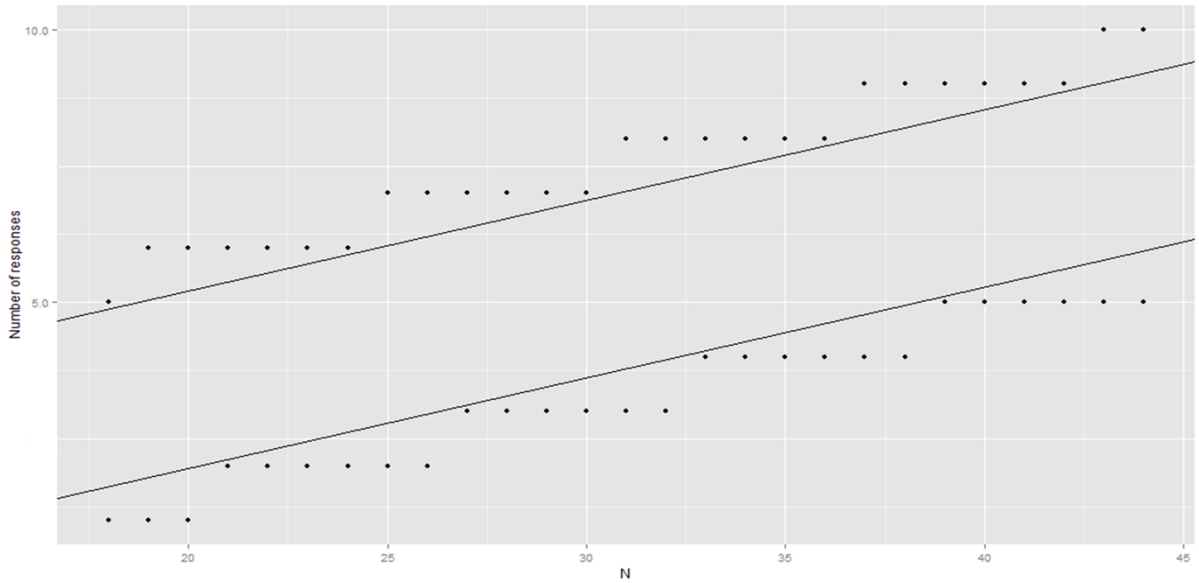

Table 11. Decision Rules based on Efficacy Bounds for NSCLC Arm

Monitoring Point (# subjects)	Minimum # Responders to Determine Efficacy
10-12	4
13-18	5
19-24	6
25-30	7
31-36	8
37-42	9
43-44	10

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Table 12. Decision Rules based on Futility Bounds for NSCLC Arm

Monitoring Point (# subjects)	Maximum # subjects w/ response to declare Futility
10-14	0
15-20	1
21-26	2
27-32	3
33-38	4
39-44	5

Table 13. Operating Characteristics of Sequential Monitoring Approach for NSCLC Arm

True RR	Probability of Declaring Futility	Probability of Go at end of Study	Average Sample Size
10%	0.87	0.05	24.9
15%	0.58	0.25	32.2
20%	0.29	0.55	38.1
25%	0.12	0.80	41.5
30%	0.04	0.93	43.1
35%	0.01	0.98	43.7

11.2 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.

11.3 Analytic Plan for Secondary Objectives:

(1) 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

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with an ORR will be calculated with a 95% confidence interval. A similar analysis will be performed using ir-mRECIST and ir-RECIST.

(2) B7-H1 (PD-L1) expression and other potential biomarkers will be correlated to clinical endpoints. Exploratory, hypothesis-generating analyses will be performed.

(3) Progression-free survival will be calculated as the time from start of MK-3475 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 MK-3475 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 arm and median survival will be estimated by using Kaplan-Meier methodology

(4) 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.

11.4 Reporting and Exclusions

11.4.1 **Evaluation of toxicity.** All patients will be evaluable for toxicity from the time of their first treatment with MK-3475.

11.4.2 **Evaluation of response.** All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Treatment response includes categories 1 and 2 (complete and partial response). Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

Conclusions will be based on all eligible patients. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals will also be provided.

11.5 Accrual Rate and Study Duration

The plan is to enroll 74 patients total: 20 with melanoma, 44 with NSCLC in Cohort 1, and 10 with NSCLC in Cohort 2. The first interim analysis will be after 8 patients on the melanoma arm, 18 with NSCLC on the NSCLC arm. Our estimated rate of accrual based on current volume and

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referral patterns is approximately 4 melanoma patients, 8 NSCLC patients with brain metastases at our institution monthly. Assuming a conservative 50% eligibility rate (based on the relatively high likelihood of screen failures) for the melanoma arm, the overall anticipated time of accrual is approximately 18 months. The eligibility rate for the NSCLC arm will be approximately 20%; accrual will take approximately 36 months.

APPENDIX A
Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
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.

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106-30.
3. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004;351:998-1012.
4. Atkins MB, Kunkel L, Sznol M, Rosenberg SA. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. Cancer J Sci Am 2000;6 Suppl 1:S11-4.
5. Jilaveanu LB, Aziz SA, Kluger HM. Chemotherapy and biologic therapies for melanoma: do they work? Clin Dermatol 2009;27:614-25.
6. Bedikian AY, Millward M, Pehamberger H, et al. Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol 2006;24:4738-45.

APPROVED BY THE YALE UNIVERSITY IRB 2/22/2019

7. Middleton MR, Grob JJ, Aaronson N, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol 2000;18:158-66.
8. Chapman PB, Einhorn LH, Meyers ML, et al. Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol 1999;17:2745-51.
9. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. New England Journal of Medicine 2010;363:711-23.
10. Chapman PB, Hauschild A, Robert C, et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. New England Journal of Medicine 2011;364:2507-16.
11. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: a cancer journal for clinicians 2010;60:277-300.
12. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. The New England journal of medicine 2002;346:92-8.
13. Disis ML. Immune regulation of cancer. J Clin Oncol;28:4531-8.
14. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8:793-800.
15. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol 2002;2:116-26.
16. Brown JA, Dorfman DM, Ma FR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol 2003;170:1257-66.
17. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev;236:219-42.
18. Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 2007;13:1757-61.
19. Hino R, Kabashima K, Kato Y, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer;116:1757-66.
20. Nomi T, Sho M, Akahori T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 2007;13:2151-7.
21. Fourcade J, Kudela P, Sun Z, et al. PD-1 is a regulator of NY-ESO-1-specific CD8+ T cell expansion in melanoma patients. J Immunol 2009;182:5240-9.
22. Cai G, Karni A, Oliveira EM, Weiner HL, Hafler DA, Freeman GJ. PD-1 ligands, negative regulators for activation of naive, memory, and recently activated human CD4+ T cells. Cell Immunol 2004;230:89-98.
23. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 2007;56:739-45.
24. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A 2002;99:12293-7.
25. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med;366:2443-54.
26. Al-Shibli K, Al-Saad S, Andersen S, Donnem T, Bremnes RM, Busund LT. The prognostic value of intraepithelial and stromal CD3-, CD117- and CD138-positive cells in non-small cell lung carcinoma. APMIS;118:371-82.

APPROVED BY THE YALE UNIVERSITY IRB 2/22/2019

27. Hillen F, Baeten CI, van de Winkel A, et al. Leukocyte infiltration and tumor cell plasticity are parameters of aggressiveness in primary cutaneous melanoma. Cancer Immunol Immunother 2008;57:97-106.
28. Piersma SJ, Welters MJ, van der Burg SH. Tumor-specific regulatory T cells in cancer patients. Hum Immunol 2008;69:241-9.
29. Rao UN, Lee SJ, Luo W, Mihm MC, Jr., Kirkwood JM. Presence of tumor-infiltrating lymphocytes and a dominant nodule within primary melanoma are prognostic factors for relapse-free survival of patients with thick (t4) primary melanoma: pathologic analysis of the e1690 and e1694 intergroup trials. Am J Clin Pathol;133:646-53.
30. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev 2007;26:373-400.
31. Sznol M, Chen L. Antagonist Antibodies to PD-1 and B7-H1 (PD-L1) in the Treatment of Advanced Human Cancer--Response. Clin Cancer Res.
32. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res;19:1021-34.
33. Flies DB, Sandler BJ, Sznol M, Chen L. Blockade of the B7-H1/PD-1 pathway for cancer immunotherapy. Yale J Biol Med;84:409-21.
34. Thumar JR, Kluger HM. Ipilimumab: a promising immunotherapy for melanoma. Oncology (Williston Park);24:1280-8.
35. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med;363:711-23.
36. Hodi FS, Dranoff G. The biologic importance of tumor-infiltrating lymphocytes. J Cutan Pathol;37 Suppl 1:48-53.
37. Hiraoka N. Tumor-infiltrating lymphocytes and hepatocellular carcinoma: molecular biology. Int J Clin Oncol;15:544-51.
38. Lee HE, Chae SW, Lee YJ, et al. Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 2008;99:1704-11.
39. Leffers N, Gooden MJ, de Jong RA, et al. Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer. Cancer Immunol Immunother 2009;58:449-59.
40. Nishimura H, Honjo T, Minato N. Facilitation of beta selection and modification of positive selection in the thymus of PD-1-deficient mice. J Exp Med 2000;191:891-8.
41. Oble DA, Loewe R, Yu P, Mihm MC, Jr. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. Cancer Immun 2009;9:3.
42. Polcher M, Braun M, Friedrichs N, et al. Foxp3(+) cell infiltration and granzyme B(+)/Foxp3(+) cell ratio are associated with outcome in neoadjuvant chemotherapy-treated ovarian carcinoma. Cancer Immunol Immunother;59:909-19.
43. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005;23:2346-57.
44. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 2008;358:2698-703.
45. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A 2001;98:13866-71.
46. Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001;2:261-8.

APPROVED BY THE YALE UNIVERSITY IRB 2/22/2019

47. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515-48.
48. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med;369:134-44.
49. Aronow S. The use of radio-frequency power in making lesions in the brain. J Neurosurg 1960;17:431-8.
50. Sampson JH, Carter JH, Jr., Friedman AH, Seigler HF. Demographics, prognosis, and therapy in 702 patients with brain metastases from malignant melanoma. J Neurosurg 1998;88:11-20.
51. Retsas S, Gershuny AR. Central nervous system involvement in malignant melanoma. Cancer 1988;61:1926-34.
52. Sloan AE, Nock CJ, Einstein DB. Diagnosis and treatment of melanoma brain metastasis: a literature review. Cancer Control 2009;16:248-55.
53. de la Monte SM, Moore GW, Hutchins GM. Patterned distribution of metastases from malignant melanoma in humans. Cancer Res 1983;43:3427-33.
54. Amer MH, Al-Sarraf M, Baker LH, Vaitkevicius VK. Malignant melanoma and central nervous system metastases: incidence, diagnosis, treatment and survival. Cancer 1978;42:660-8.
55. Douglas JG, Margolin K. The treatment of brain metastases from malignant melanoma. Semin Oncol 2002;29:518-24.
56. Gavrilovic IT, Posner JB. Brain metastases: epidemiology and pathophysiology. Journal of neuro-oncology 2005;75:5-14.
57. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med;364:2517-26.
58. Chapman et al, New England Journal of Medicine, 2011
59. Eisen T, Ahmad T, Flaherty KT, et al. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. Br J Cancer 2006;95:581-6.
60. Lorigan P CP CD, et al. . Phase II trial of sorafenib combined with dacarbazine in metastatic melanoma patients. J Clin Oncol 2006;24:8012 (suppl).
61. Amaravadi RK, Schuchter LM, McDermott DF, et al. Phase II Trial of Temozolomide and Sorafenib in Advanced Melanoma Patients with or without Brain Metastases. Clin Cancer Res 2009;15:7711-8.
62. Kefford R AH, Brown MP, et al. . Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. J Clin Oncol 2010;28:suppl;abstr 8503.
63. Long GV, Trefzer U, Davies MA, et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:1087-95.
64. Barlesi F, Gervais R, Lena H, et al. Pemetrexed and cisplatin as first-line chemotherapy for advanced non-small-cell lung cancer (NSCLC) with asymptomatic inoperable brain metastases: a multicenter phase II trial (GFPC 07-01). Ann Oncol 2011;22:2466-70.
65. Lee DH, Han JY, Kim HT, et al. Primary chemotherapy for newly diagnosed nonsmall cell lung cancer patients with synchronous brain metastases compared with whole-brain radiotherapy administered first : result of a randomized pilot study. Cancer 2008;113:143-9.
66. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013;369:134-44.
67. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol;28:3167-75.

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68. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.
69. Jiang X, Zhou J, Giobbie-Hurder A, Wargo J, Hodi FS. The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. Clin Cancer Res 2013;19:598-609.
70. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med;4:127ra37.
71. Fauci JM, Straughn JM, Jr., Ferrone S, Buchsbaum DJ. A review of B7-H3 and B7-H4 immune molecules and their role in ovarian cancer. Gynecol Oncol.
72. Sun SQ, Jiang CG, Lin Y, Jin YL, Huang PL. Enhanced T cell immunity by B7-H4 downregulation in nonsmall-cell lung cancer cell lines. J Int Med Res;40:497-506.
73. Zhou Q, Munger ME, Veenstra RG, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. Blood;117:4501-10.
74. Mathieu D, Kondziolka D, Cooper PB, et al. Gamma knife radiosurgery for malignant melanoma brain metastases. Clin Neurosurg 2007;54:241-7.
75. Sznol M. Advances in the treatment of metastatic melanoma: new immunomodulatory agents. Semin Oncol;39:192-203.
76. Flies DB, Wang S, Xu H, Chen L. Cutting edge: A monoclonal antibody specific for the programmed death-1 homolog prevents graft-versus-host disease in mouse models. J Immunol;187:1537-41.
77. Jilaveanu L, Zito C, Lee SJ, et al. Expression of sorafenib targets in melanoma patients treated with carboplatin, paclitaxel and sorafenib. Clin Cancer Res 2009;15:1076-85.
78. Berger AJ, Camp RL, Divito KA, Kluger HM, Halaban R, Rimm DL. Automated quantitative analysis of HDM2 expression in malignant melanoma shows association with early-stage disease and improved outcome. Cancer Res 2004;64:8767-72.
79. Camp RL, Chung GG, Rimm DL. Automated subcellular localization and quantification of protein expression in tissue microarrays. Nat Med 2002;8:1323-7.
80. Kluger HM, Siddiqui SF, Angeletti C, et al. Classification of renal cell carcinoma based on expression of VEGF and VEGF receptors in both tumor cells and endothelial cells. Lab Invest 2008;88:962-72.
81. Henson JW, Ulmer S, Harris GJ. Brain tumor imaging in clinical trials. AJNR American journal of neuroradiology 2008;29:419-24.

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