Phase 2 Study of MK-3475 in Patients with Microsatellite Unstable (MSI) Tumors

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TITLE: Phase 2 Study of MK-3475 in Patients with Microsatellite Unstable (MSI) Tumors

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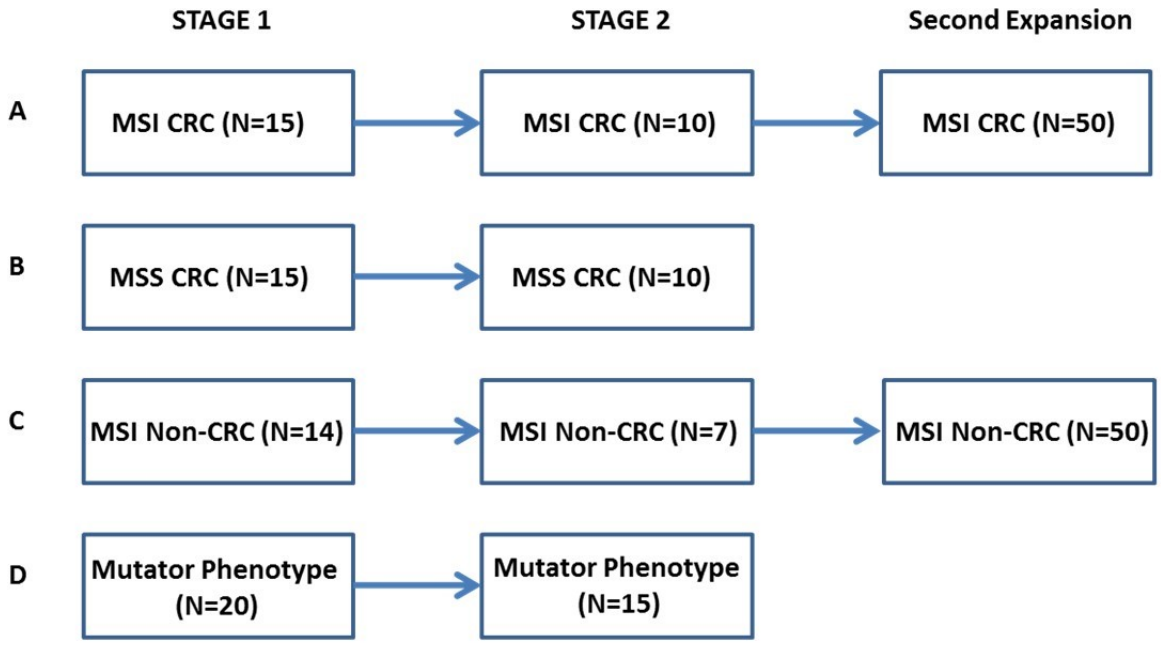


MSI = Microsatellite Instability; MSS = Microsatellite Stability; CRC = Colorectal Carcinoma

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Cohorts A and B: To determine the immune-related progression free survival (irPFS) rate at 20 weeks and objective response rate (irORR) in patients with MSI positive and negative colorectal adenocarcinoma treated with MK-3475 using immune related response criteria (irRC) during stages 1 and 2.

1.1.2 Cohort C: To determine the immune-related progression free survival (irPFS) rate in patients with MSI positive non-colorectal solid tumor malignancies treated with MK-3475 using immune related response criteria (irRC) at 20 weeks during stages 1 and 2.

1.1.3 Cohorts A and C: To determine the progression free survival (PFS) rate at 20 weeks and the objective response rate (ORR) in patients with MSI positive solid tumor malignancies treated with MK-3475 using RECIST 1.1 criteria during the second expansion.

1.1.4 Cohort D: To estimate objective response rate in patients with MSI-negative solid tumor malignancies with a mutator phenotype (i.e. high mutational burden) treated with MK-3475 using RECIST 1.1 criteria.

1.2 Secondary Objectives

1.2.1 To determine the overall survival of patients with MSI positive and negative tumors treated with MK-3475.

1.2.2 To estimate irPFS and PFS in patients with MSI positive and negative tumors treated with MK-3475 at 28 weeks using irRC and RECIST 1.1.

1.2.3 To estimate best overall response rate and disease control rate in patients with MSI positive and negative tumors treated with MK-3475.

1.2.4 To assess safety and characterize toxicities of MK-3475 in patients with MSI positive and negative tumors.

1.2.5 To evaluate MSI as a marker to predict treatment response.

1.3 Exploratory Objectives

1.3.1 To explore the association of MSI positive, PD-L1 positivity, and tumor infiltrating lymphocyte characteristics with clinical responses. Archived tumor tissue (when available) and tumor tissue obtained at baseline and during

treatment will be compared. In addition, we will compare differences in PD-L1 expression between the primary and secondary sites of cancer.

1.3.2 To assess tumor tissue for molecular determinants of response, progression and disease stability using next generation sequencing technology.

1.3.3 To assess tumor burden dynamics using both standard protein biomarkers such as CEA, CA19-9, and other exploratory circulating biomarkers in serial collections of sera and plasma at baseline and throughout treatment.

1.3.4 To assess the baseline characteristic of the subjects enrolled and to correlate these molecular and clinicopathologic criteria with treatment response and toxicity. DNA will be extracted from whole blood and used to evaluate for any germline mutations which may correlate with response or toxicity.

1.3.5 To collect peripheral blood lymphocytes to explore the association of MSI positive, PD-1 positivity, and lymphocyte activation markers with clinical responses.

1.3.6 To determine alternative markers of MSI status. This includes but is not limited to MLH 1, MSH 2, MSH 6, PMS2, BRAF pV600E, and TGFBR2.

1.4 Study Design

This is a multi-center, open label, two-stage, phase 2 study to evaluate the clinical activity of MK-3475 in MSI positive and MSI negative solid tumors. We will enroll four cohorts of patients to receive MK-3475: patients with MSI positive colorectal adenocarcinomas (Cohort A); patients with MSI negative colorectal adenocarcinomas (Cohort B); and patients with MSI positive solid tumor malignancies but not colorectal adenocarcinoma (Cohort C); and patients with MSI-negative solid tumor malignancies that have a mutator phenotype (Cohort D). MK-3475 will be administered at 10 mg/kg every 14 days for Cohorts A, B, and C. MK-3475 will be administered at 200 mg every 21 days for Cohort D. Archived tumor samples or newly obtained biopsies will be used for determining MSI, or a mutator phenotype.

MSI positive tumors will be defined using standard clinical criteria and require at least two affected loci[1, 2]. Patients in Cohorts A, B, and C will be evaluable if they receive one dose of MK-3475 and if MSI results are confirmed using the MSI Analysis System from Promega (**Section 3.4**). With amendment 9, the decision was made to not require retesting with Promega if MSI status is confirmed by IHC or other PCR.

Eligibility for Cohort D will be determined using a CLIA certified test measuring mutational burden. Patients in Cohort D will be evaluable if they receive one dose of MK-3475 and have a MSI-negative solid tumor malignancies with a documented mutation burden level measured at $\geq$ 20 mutations per megabase pairs (MB) using the

Foundation Medicine (FoundationOne.com) targeted sequencing panel that reports mutation burden.

The primary endpoints for Cohorts A and B are immune-related progression-free survival (irPFS) rate at 20 weeks and objective response rate (irORR) assessed using immune related response criteria (irRC); irPFS is defined as the proportion of subjects alive and free of disease progression at 20 weeks per irRC; and irORR is the proportion of subjects whose best overall response is a CR or PR. Each cohort will be assessed separately.

The primary endpoint for Cohort C is immune-related progression-free survival (irPFS) rate assessed using immune related response criteria (irRC) at 20 weeks, defined as the proportion of subjects alive and free of disease progression at 20 weeks per irRC.

The primary endpoint for Cohort D will be response rate, which is defined as the proportion of subjects who have response of CR or PR, using RECIST 1.1 criteria.

This design aims to evaluate treatment benefit and evaluate MSI as a predictive biomarker for treatment with MK-3475 simultaneously.

For Cohorts A and B, MK-3475 will be considered to be inactive and of no interest for further evaluation if the irPFS rate at 20 weeks is 5% (p_{10}) or less or irORR is 5% (p_{20}) or less, and considered active if the irPFS rate at 20 weeks is 25% or greater (p_{11}) and irORR is 21% or greater (p_{21}). The endpoints will be tested in the hierarchical order first with irPFS at 20 weeks. If the null hypothesis of 20-week irPFS being 5% is rejected (i.e. the result reaches statistical significance), we will proceed to test for irORR. If irPFS is not significant at the 0.05 level (one-sided test), the statistical testing for irORR will not occur.

A two-stage Green-Dahlberg design is used for Cohort A and B. An interim analysis will be conducted in order to assess futility after irPFS at 20 weeks are available for the first 15 evaluable patients. Based on Green-Dahlberg design, if 0 irPFS are observed, enrollment in that cohort will be terminated. If at least one patient is free of disease progression at 20 weeks per irRC, an additional 10 evaluable subjects will be enrolled into that cohort. If 3 or fewer patients are progression free at 20 weeks in the total of 25 evaluable patients enrolled in that cohort, then the regimen will be considered inactive in that cohort. If 4 or more patients are progression free at 20 weeks, we proceed to test for the endpoint of irORR in that cohort. If 4 or more responders (CR or PR) are observed, we conclude the regimen is promising with that cohort and warrants further study for that cohort.

For Cohort C, MK-3475 will be considered to be inactive and of no interest for further evaluation if the irPFS rate at 20 weeks is 5% (p_0) or less and considered active if the irPFS rate at 20 weeks is 25% or greater (p_1).

A two-stage Green-Dahlberg design is used for Cohort C. An interim analysis will be conducted in order to assess futility after irPFS at 20 weeks are available for the first 14 evaluable patients. If 0 irPFS are observed, enrollment in Cohort C will be terminated. If at least one patient is free of disease progression at 20 weeks per irRC, an additional 7 evaluable subjects will be enrolled into Cohort C. If 3 or fewer patients are progression free at 20 weeks in the total of 21 evaluable patients enrolled in Cohort C, then the regimen will be considered inactive in that cohort. If 4 or more patients are progression free at 20 weeks, we conclude the regimen is promising and warrants further study for this patient population.

As of 28Jan2015, four irPFS at 20 weeks and four PRs have been observed in each Cohort A and Cohort C. Since the emerging clinical data suggest promising clinical activity, up to an additional 50 patients will be enrolled in each of these cohorts under a second expansion in Amendment 7. Tumor assessments in the second expansion cohort will be evaluated by RECIST 1.1 criteria only, and ORR will be the primary endpoint for the expanded Cohort A and Cohort C.

For Cohort D, the regimen would be considered of insufficient activity for further study if the response rate is 5% or less, and the minimum required level of efficacy that would warrant further study with the proposed regimen is a 21% response rate. A two-stage Green-Dahlberg design is planned. A total of 20 subjects will be entered in the first stage. If zero response is observed, that cohort will be terminated and we will conclude the regimen is ineffective. If $\geq$1 subjects respond, then additional 15 subjects will be studied for that cohort. If $\leq$ 4 subjects respond in stage one and two combined, we consider this regimen ineffective. If $\geq$5 responses are observed, we conclude the regimen is promising and warrant further study. Each cohort could also be terminated as soon as 5 responses are observed before the total cohort is enrolled or evaluated in that cohort. The maximum sample size will be 35 for each cohort. This design provides 90% power to detect an absolute difference of 16% of response with a type I error of 0.05. There is a 36% chance to stop the trial early for futility under 5% response rate.

No intra-subject dose escalation or reduction is allowed. Patients who do not receive at least 1 dose of MK-3475 will be replaced.

Patient immune and clinical responses will be evaluated through the following methods at baseline and during treatment: 1. tumor biopsies, 2. peripheral blood lymphocytes, 3. serum and plasma, and 4. CT/MRI scans will be obtained for assessment of activity and correlative studies.

Patients who have a confirmed complete response by two scans $\geq$ 4 weeks apart and who have been on MK-3475 treatment for at least 6 months may discontinue MK3475 treatment at the discretion of the investigator after receiving at least two doses beyond the initial determination of CR. MK-3475 may be resumed upon disease recurrence in these patients. Treatment may also be resumed in patients with SD, PR or CR who stopped MK-3475 treatment after 24 months of study therapy for reasons other than

disease progression or intolerability. Criteria and procedures for retreatment are described in **Section 5.6** and **10.0**.

Patients with tumor progression by RECIST imaging or laboratory parameters prior to their 7 month evaluation but without rapid clinical deterioration or significant change in performance status who do not require additional immediate therapy, may continue to be treated with MK-3475 and clinically observed following the assigned imaging schedule to allow detection of a subsequent tumor response (see **Section 5.10.1** for further details).

All patients will be followed for at least 30 days after their last dose of study drug (serious adverse events will be collected for 90 days after the last infusion of MK3475) or until initiation of a new anti-cancer treatment, whichever occurs first.

Patients who are discontinued from the study due to an unacceptable drug-related AE will be followed until the resolution of the AE to Grade 0-1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first. Information of the new cancer therapy will be collected.

Patients who discontinued study therapy without documented disease progression should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue to be assessed every approximately every 12 weeks for 3 years (approximately every 6 months after 3 years) until, 1) start of a new antineoplastic therapy (information of the new cancer therapy will be collected), 2) documented disease progression, 3) study closure, or 4) until death, whichever occurs first.

Patients who discontinue from treatment should be contacted every three months to monitor overall survival. Information of other cancer therapies after discontinuation from the study treatment will be collected.

2. BACKGROUND

2.1 Study Disease

Microsatellite instability (MSI) is the accumulation of sequencing errors in microsatellites. This occurs in tumors with deficiency in DNA mismatch repair. MSI is present in Lynch Syndrome which is an inherited cancer syndrome that predisposes patients to colon, endometrial, gastric cancer, ovarian, small intestine, liver, hepatobiliary, upper urinary tract, brain, and prostate cancer. MSI is also present in 10-20% of sporadic colorectal [3], gastric [4], prostate [5], lung [6, 7], ampullary [8], and endometrial cancers [9]. Between 0.3% and 13% of pancreatic cancers are reported to be MSI as well [10]. The study is designed to test an immunotherapy in MSI colorectal adenocarcinomas with an additional exploratory cohort in other MSI disease types.

2.2 MK-3475

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [11]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [12-47]. 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 carcinoma, malignant MEL and RCC. TILs can be expanded *ex vivo* and re-infused, inducing durable objective tumor responses in cancers such as melanoma [48; 49].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control [11]. 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. The ligands for PD1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors [12-15]. 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 [50]. 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 renal cell carcinoma (RCC) [16], pancreatic carcinoma [17], hepatocellular carcinoma [18], ovarian carcinoma [19] and non-small cell lung cancer (NSCLC) [28]. Furthermore, PD-1 has been suggested to regulate tumor-specific Tcell expansion in patients with malignant MEL [20]. The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers suggests that the PD1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

2.3 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure [IB] for Preclinical and Clinical Data

2.4 Rationale

Blockade of immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA4) and programmed death-1 (PD-1) is showing promise in patients with cancer. CTLA-4 and PD-1 are upregulated on activated T cells and provide inhibitory signals to T cells undergoing activation. Inhibitory antibodies directed at these receptors have been shown to break immune tolerance and promote anti-tumor immunity. MK-3475 is a humanized monoclonal IgG4 antibody against PD-1 and is showing activity in multiple tumor types including melanoma and non-small cell lung cancer (NSCLC). Previously, activity of a different PD-1 blocking antibody, BMS-936558, a fully humanized monoclonal IgG4 antibody, also showed activity in melanoma, NSCLC, and a complete response in a single patient with colorectal cancer [28, 51].

As with all targeted therapies, we expect a subset of patients will respond to immunotherapy and there may be genetic determinants that define an 'immune responsive' phenotype. A cancer genomics perspective may provide insight as to why patients with melanoma and NSCLC, and perhaps other tumor types, respond to these therapies. The average number of somatic mutations in human cancer varies widely (**Table 1**) [52]. Three factors are known to be associated with high somatic mutation

burdens: exposure to environmental carcinogens (e.g. UV radiation and tobacco) [53, 54]; repair defects (e.g., mismatch repair gene inactivation) [55]; or exposures to mutagenic therapies (e.g., alkylating agents) [56]. Melanomas and NSCLC are known to have high burdens of somatic mutations. We therefore hypothesize that there will be a correlation between the number of somatic mutations in individual tumors and the response to immune checkpoint inhibitors since each protein-coding mutation has the potential to serve as a novel antigen for an immune response. If this hypothesis is correct, it would suggest that immune checkpoint inhibitors might work best in tumors with exceptionally high mutation burdens (>500 somatic mutations per exome). Furthermore, tumors deficient in mismatch repair, as well as other genetic alterations consistent with a mutator phenotype such as POLD1, and POLE mutations, may be particularly susceptible to immunotherapy as this phenotype results in ongoing accumulation of mutations at a high frequency.

Table 1. The average number of somatic mutations in a representative group of human cancers

Tumor	Average # of mutations per case
Tumors exposed to mutagenic therapies	>1000
Sun exposed melanomas	>1000
Mismatch repair deficient tumors	>600
Non-small cell lung cancers	540
Colorectal cancers	77
Pancreatic cancers	48
Glioblastomas	36
Meduloblastomas	8

MSI tumors are deficient in DNA mismatch repair which leads to a high rate of spontaneous mutations and the potential for the expression of neo-antigens. Furthermore, similar to melanoma, MSI positive colon cancers, there is often prominent lymphocyte infiltration [3, 4]. This is another clue that this subset of tumors may have baseline tumor infiltrating lymphocytes that can be harnessed with immune checkpoint blockade. Interestingly, the colorectal patient with the complete response is known to have an MSI positive tumor [57]. This study aims to evaluate MSI as a predictor of response to anti-PD-1 blockade with MK-3475.

Recent prospective studies looking at mutational burden (i.e. MSI) and PD-L1 staining have shown a positive correlation between these biomarkers and clinical response. Retrospectively, several candidate biomarkers appear promising including mutational burden in non-MSI tumors. Mechanistically, we believe that a tumor with high degree of 'foreignness' will trigger a potent anti-tumor immune response. A tumor will look foreign to the host immune system if they have a very high mutational burden or hypothetically (yet to be proven) if they harbor a virus or express an embryonic protein. The functional consequence of a highly foreign tumor is a lymphocytic infiltrate and

high IFN-γ levels. As a mechanism of immune evasion a number of immune checkpoints (i.e.PD-1) are upregulated. Since the functional consequence of tumor foreignness is lymphocytic infiltration of the tumor, we propose that this may be a highly sensitive predictive marker of response to checkpoint blockade.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Cohort A: Patients with histologically proven metastatic or locally advanced MSI colorectal adenocarcinoma.

3.1.2 Cohort B: Patients with histologically proven metastatic or locally advanced microsatellite stable (MSS) colorectal adenocarcinoma.

3.1.3 Cohort C: Patients with histologically proven metastatic or locally advanced non-colorectal MSI solid tumor malignancies.

3.1.4 Cohort D: Patients with histologically proven metastatic or locally advanced solid tumor malignancies that are microsatellite stable with a documented mutation burden level measured at ≥ 20 mutations per megabase pairs (MB).

3.1.5 Patients with the presence of at least one lesion with measurable disease as defined by 10mm in longest diameter for a soft tissue lesions or 15mm in short axis for a lymph node by RECIST 1.1 and irRC criteria for response assessment.

3.1.6 Patients must agree to have a biopsy at baseline and on treatment if the lesion can be biopsied with acceptable clinical risk (as judged by the investigator).

3.1.7 Patients with colon cancer (Cohort A and B) must have received at least 2 prior cancer therapy regimens. Patients with other cancer types (Cohort C) must have received at least 1 prior cancer therapy regimen. Patients in Cohort D must have received at least 1 prior cancer therapy regimen. Patients must have progressive disease on study entry.

3.1.8 Age ≥18 years.

3.1.9 ECOG performance status 0-1 (**Appendix A**).

3.1.10 Life expectancy of greater than 3 months.

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

- Absolute neutrophil count ≥1,000/mcL

□ Platelets	≥90 x 10^3 /uL
□ Hemoglobin	≥9.0 g/dL
□ Total bilirubin	≤1.5x ULN (patients with diagnosed Gilbert's Syndrome will not be excluded if their direct bilirubin is within normal institutional limits)
□ AST(SGOT)/ALT(SGPT)	≤3x ULN
□ Creatinine	□1.5 x ULN

3.1.12 Female patient of childbearing potential has a negative urine or serum pregnancy test. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. The serum pregnancy test must be negative for the patient to be eligible.

3.1.13 Female patients enrolled in the study, who are not free from menses for >2 years, post hysterectomy / oophorectomy, or surgically sterilized, must be willing to use either 2 adequate barrier methods *or* a barrier method plus a hormonal method of contraception to prevent pregnancy or to abstain from heterosexual activity throughout the study, starting with Visit 1 through 120 days after the last dose of study therapy. Approved contraceptive methods include for example; intra uterine device, diaphragm with spermicide, cervical cap with spermicide, male condoms, female condoms with spermicide, or oral contraceptives. Spermicides alone are not an acceptable method of contraception.

Male patients must agree to use an adequate method of contraception starting with the first dose of study drug through 120 days after the last dose of study therapy.

3.1.14 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 1 week prior to trial treatment. This is not applicable to patients with primary brain tumors.

3.2.2 Patient who has had chemotherapy, or biological cancer therapy within 2 weeks prior to the first dose of study drug. Patient who has had radiation within 2 weeks prior to the first dose of study drug.

3.2.3 Patient is currently participating or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of study drug.

3.2.4 Patient is expected to require any other form of systemic or localized antineoplastic therapy while on study.

3.2.5 Patients who have had surgery within 4 weeks of dosing of investigational agent, excluding minor procedures (dental work, skin biopsy, etc), celiac plexus block, and biliary stent placement.

3.2.6 Patients with a history of prior treatment with anti-PD-1, anti-PD-L1, anti-PDL2, anti-CD137, anti-OX-40, anti-CD40, or anti-CTLA-4 antibodies.

3.2.7 Patients who have received any of the following concomitant therapy: IL-2, interferon or other non-study immunotherapy regimens; immunosuppressive agents; other investigational therapies; or chronic use of systemic corticosteroids (used in the management of cancer or non-cancer-related illnesses) within 1 week prior to first dose. Note: Systemic steroid therapy allowed for subjects with primary brain tumors as long as ≤ dexamethasone 4 mg or its steroid equivalent.

3.2.8 Patients who have received a live vaccine within 4 weeks prior to or after any dose of MK-3475.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines, and are not allowed.

3.2.9 Patients receiving growth factors including, but not limited to, granulocytecolony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), erythropoietin, etc. within 2 weeks of study drug administration. Use of such agents while on study is also prohibited. Prior use of growth factors should be documented in the patient's medical history.

3.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.11 Has an active autoimmune disease that has required systemic treatment in past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.12 Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

3.2.13 Presence of any tissue or organ allograft, regardless of need for immunosuppression, including corneal allograft. Patients with a history of allogeneic hematopoeitic stem cell transplant will be excluded.

3.2.14 Patients with a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

3.2.15 Patients with a known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

3.2.16 Patients with evidence of interstitial lung disease.

3.2.17 Patients with a pulse oximetry of <92% on room air.

3.2.18 Patients on supplemental home oxygen.

3.2.19 Patient is, at the time of signing informed consent, a regular user (including "recreational use") of any illicit drugs or had a recent history (within the last year) of substance abuse (including alcohol).

3.2.20 Women who are pregnant or breastfeeding.

3.2.21 Women with a positive pregnancy test on enrollment or prior to investigational product administration.

3.2.22 Sexually active fertile men not using effective birth control if their partners are WOCBP.

3.3 Inclusion of Women and Minorities

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

3.4 MSI Testing

MSI testing is already standardized and performed in CLIA-certified laboratories without need for assay development. Archived tumor samples or newly obtained biopsies will be used for determining MSI. For patients who do not have sufficient normal tissue for MSI testing purposes, a buccal swab or purple top blood tube will be obtained. MSI testing in colorectal cancer is already clinically indicated. For other non-colorectal cancers where MSI testing is not currently clinically indicated, a prescreening consent will be obtained to collect tissue for MSI testing. The prescreening consent can be obtained over the phone. A copy of the consent will be faxed to the site and the original will be mailed. MSI status will be performed locally by CLIA certified immunohistochemistry (IHC) or PCR based tests for eligibility.

Evaluable patients will be confirmed using the MSI Analysis System from Promega. This test will determine MSI status through the insertion or deletion of repeating units in the five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24). At least 2 MSI loci are required to be evaluable in Cohorts A and C. Patients may be assigned to a new cohort and/or replaced based on the Promega test results. With amendment 9, the decision was made to not require retesting with Promega if MSI status is confirmed by IHC or other PCR.

The MSI Analysis System (Promega), Version 1.2, is a fluorescent multiplex PCRbased method used to detect microsatellite instability (MSI), a form of genomic instability. This instability is due to insertion or deletion of repeating units during DNA replication and failure of the mismatch repair system (MMR) to correct these errors. MSI analysis typically involves comparing allelic profiles of microsatellite markers generated by amplification from matching pairs of test samples, which may be MMR-deficient, and normal tissue samples. New alleles in the abnormal sample not found in the corresponding normal sample indicate the presence of MSI.

The MSI Analysis System, Version 1.2, includes fluorescently labeled primers (marker panel) for co-amplification of seven markers for analysis of the MSI-high (MSI-H) phenotype, including five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24) and two highly polymorphic pentanucleotide repeat markers (Penta C and Penta D). Amplified fragments are detected using an ABI PRISM® 310, 3100, 3100-Avant, 3130 or 3130xl Genetic Analyzer after spectral calibration. GeneMapper® 4.0 software will be used for data analysis and assignment of genotype.

For cohort D, any testing performed in a CLIA-certified laboratory for mismatch repair deficiency (MRD) or MSI using IHC, PCR or next generation sequencing (NGS) approach can be used to exclude the presence of MRD or MSI.

3.5 Testing for high mutational burden

We postulate that tumors that harbor a high number of somatic mutations will have a favorable response to checkpoint blockade. The best way to determine mutational

burden is through whole genome or exome sequencing. A surrogate to exome or whole genome sequencing is targeted sequencing of a large fraction of the exome to determine total mutational burden. Mutations can occur as silent mutations (synonymous) or missense mutations (non-synonymous). We would like to treat tumors that harbor $\geq$ 20 mutations per megabase pairs.

For the purposes of this study, measuring mutational burden will be performed locally by a CLIA certified test for eligibility. Evaluable patients will be confirmed using Foundation Medicine (http://foundationone.com/) targeted sequencing panel that reports mutation burden. Please see **Appendix B** for information regarding this test.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University by the Lead Study Coordinator. All sites should contact the Protocol Chair at [redacted] to verify ongoing study enrollment. The fax cover sheet, Registration Form, and Eligibility Worksheet will be supplied to each participating site.

If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.2 Registration Process

To register a patient, the following documents should be completed and sent to the Lead Study Coordinator at the Coordinating Center at [redacted]

- Fax cover sheet
- Registration Form
- Signed patient consent form
- HIPAA authorization form
- Eligibility Screening Checklist
- Copy of required screening tests and scans

The Research Nurse or Study Coordinator at the participating site will then call or email the Lead Study Coordinator at the Coordinating Center to verify eligibility. To complete the registration process, the Coordinating Center Lead Study Coordinator will:

- Assign a patient study number
- Register the patient on the study
- Fax or e-mail the patient study number to the participating site

- Call or e-mail the research nurse or data manager at the participating site and verbally confirm registration.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Appropriate dose modifications are described in **Section 6**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

REGIMEN DESCRIPTION					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
MK-3475	No prophylactic pre-medication will be given unless indicated by previous experience in an individual patient per **section 5.2.1**.	10 mg/kg (Cohorts A, B, and C) 200 mg (Cohort D)	IV over 30 minutes	Day 1	14 days (Cohorts A, B, and C) 21 days (Cohort D)

MK-3475 will be administered as a 30 minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The pharmacists will prepare the MK-3475 medication for administration. The Procedures Manual contains specific instructions for MK-3475 dose calculation, reconstitution, preparation of the infusion fluid, and administration. This document is available for reference by the pharmacist and study personnel.

5.2 General Concomitant Medication and Supportive Care Guidelines

MK-3475 is a humanized monoclonal Ab. Subjects should be closely monitored for potential adverse reactions during antibody infusion and potential adverse events throughout the study. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined in **Section 6.2**.

5.2.1 Infusion Reactions

MK-3475 infusion reactions may consist of fever, chills/rigor, nausea, pruritus, angioedema, hypotension, headache, bronchospasm, urticaria, rash, vomiting, myalgia, dizziness or hypertension. Severe reactions may include acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. Patients should be closely monitored for such reactions. Guidelines for patients who experience an infusion related or allergic reaction during or after infusion with MK-3475 are shown below.

Guidance on Infusion and Hypersensitivity Reactions:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
NCI CTCAE Grade	**Treatment**	**Premedication at subsequent dosing**
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.

5.2.2 Immune-Related Adverse Events (IRAEs)

Blocking PD-1 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/pruritis, diarrhea/colitis, pneumonitis, hepatitis, and hypothyroidism were drug-related, presumptive autoimmune events, now termed IRAEs, noted in previous BMS 936558 studies.

For the purposes of this study, an IRAE is defined as an AE of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE an IRAE. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Suspected IRAEs must be documented on an AE or SAE CRF. Identification and treatment of IRAEs can be found in **Section 6.2**.

Patients who experience a Grade 3 or higher irAE should be discussed with Dr. Le immediately. In addition, AEs listed in **Section 7.1.3** must be reported as an Event of Clinical Interest (ECI) within 24 hours to the IND Sponsor and Merck Global Safety even if no Serious Adverse Event Criteria are met.

5.2.3 Prohibited Medications

The following therapies are not permitted during the treatment period (if administered, the subject may be removed from the study):

- Any non-study anticancer chemotherapy or immunotherapy (approved or investigational)
- Filgrastim (Neupogen® or G-CSF) or sargramostim (Leukine® or GM-CSF)
- Live vaccines (examples of live vaccines include, but are not limited to: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid [oral] vaccine). Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the IND Sponsor.

  Note: Inhaled steroids for management of asthma are permitted.

  Note: Use of prophylactic corticosteroids to avoid allergic reactions (e.g., to IV contract dye) is permitted.

Note: Physiologic doses of corticosteroids for adrenal insufficiency are permitted.

5.3 Dosing Criteria

Dosing will be delayed for the following laboratory criteria:

- AST, ALT > 3 x ULN
- Total bilirubin >1.5 x ULN (patients with diagnosed Gilbert's Syndrome, direct bilirubin should be within normal institutional limits)
- Hemoglobin < 8 g/dL
- ANC < 1000/uL
- Platelets < 80 x 10^3/uL

5.4 Definition of an Overdose for this Protocol

Overdose is defined as:
The patient has taken (accidentally or intentionally) a dose exceeding the dose prescribed in the protocol by 20%. No specific information is available on the treatment of overdose of MK-3475. In the event of overdose, MK-3475 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 experience(s) is associated with ("results from") the overdose of test drug or vaccine, the adverse experience(s) is reported as a serious adverse experience, even if no other criteria for serious are met.

If a dose of test drug 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 experience must be reported within 24 hours to the Sponsor and Merck Global Safety. Merck Global Safety (GS) contact information can be found in Section 7.5.1.

5.5 Contraception, Use in Pregnancy, Use in Nursing

5.5.1 Contraception

MK-3475 may have adverse effects on a fetus *in utero*. Furthermore, it is not known if MK-3475 has transient adverse effects on the composition of sperm. Nonpregnant, non-breast-feeding women may be enrolled if they are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 2 years will be considered postmenopausal), or 3) amenorrheaic for <2 years

without a hysterectomy and oophorectomy and with a documented FSH value in the postmenopausal range, or 4) not heterosexually active for the duration of the study, or 5) heterosexually active and willing to use 2 methods of birth control (which is also required for the female partners of male patients). The 2 birth control methods can be 2 barrier methods *or* a barrier method plus a hormonal method to prevent pregnancy, used throughout the study starting with Visit 1through 120 days after the last dose of study medication. Male patients enrolled in this study must also agree to use an adequate method of contraception starting with Visit 1 through 120 days after the last dose of study drug.

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

Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study. If there is any question that a patient will not reliably comply with the requirements for contraception, that patient should not be entered into the study.

5.5.2 Use in Pregnancy

MK-3475 may have adverse effects on a fetus; therefore, women with a positive pregnancy test at screening will not be eligible for enrollment. If a patient inadvertently becomes pregnant while on treatment with MK-3475, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck Global Safety without delay. The outcome must be reported to the Sponsor within 24 hours and to Merck Global Safety if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or lifethreatening complication to the mother or newborn). If a male patient's partner becomes pregnant on study the pregnancy must be reported to the Sponsor and to Merck Global Safety as described in Section 7.5.1. The study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor and to Merck Global Safety.

5.5.3 Use in Nursing Women

It is unknown whether MK-3475 is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment.

5.6 Duration of Therapy

A subject must be discontinued from the trial for the following reason:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.

A subject must be taken off study (but may continue to be monitored in the posttreatment follow-up portion of the trial per **Section 5.9**) for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent for treatment
- Disease progression after 7 months of therapy. Approval for continuation from the IND sponsor may be obtained if the patient is thought to be clinically benefiting.
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (see **section 5.10.2**),
- Need for >2 dose delays due to the same toxicity as per the dose delay guidelines (see **Section 6.2**)
- Patient withdraws consent,
- If in the opinion of the Investigator, a change or temporal or permanent discontinuation of therapy would be in the best interest of the patient. The IND Sponsor and Merck should be included in this decision,
- Noncompliance with trial treatment or procedure requirements,
- Patient is lost to follow-up, or
- Patient becomes pregnant.

A subject must be taken off treatment (but continue to be monitored in the post-treatment follow-up portion of the trial per **Section 5.7.1**) for the following reason:

- Completed 24 months of treatment with MK-3475 (see **Section 5.7**)

Note: 24 months of study medication is calculated from the date of first dose.

5.7 Off Treatment Evaluation and Retreatment Criteria

5.7.1 Off Treatment Evaluation

If a patient is discontinued from MK-3475 after completion of 24 months of treatment (or the patient has discontinued MK-3475 after an investigator determined confirmed CR), a mandatory Off Treatment Evaluation should be performed 30 days (+/- 7 days) after the last infusion of MK-3475 as described in **Section 10**. The assessment of AEs and medications can be made either as part of a clinical visit or over the phone. Patients should continue to be monitored for disease status by radiologic imaging and tumor marker(s) every 12 weeks (+/- 2 weeks) for 1 year (and then every 24 weeks +/- 2 weeks after 1 year) while off treatment. These evaluations can be done locally if approved by the IND Sponsor. In addition, we will also continue to collect clinical labs, as well as peripheral blood, serum, and plasma research samples every 12 weeks (+/- 2 weeks) for 1 year starting from the last research blood draw as described in **Section 10**. After 1 year, clinical labs and research bloods should be collected every 24 weeks (+/- 2 weeks). Patients will also continue to be monitored for new drugrelated AEs and the resolution of ongoing drug-related AEs to ≤ Grade 1. SAEs that occur within 90 days of the last infusion of MK-3475 or before initiation of a new antineoplastic treatment, whichever occurs first, should also be followed and recorded.

5.7.2 Retreatment with MK-3475

Patients who stop MK-3475 with SD or better may be eligible for up to one year of additional MK-3475 therapy if they progress after stopping MK-3745 and they meet the following criteria:

EITHER

- Stopped initial treatment with MK-3475 after attaining an investigator determined confirmed CR according to RECIST 1.1
  - Was treated for at least 24 weeks with MK-3475 before discontinuing therapy
  - Received at least two treatments with MK-3475 beyond the date when the initial CR was declared

OR

- Subject had SD, PR or CR and stopped MK-3475 treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with MK-3475
- Did not receive any anti-cancer treatment since the last dose of MK-3475
- Have a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrate adequate organ function as detailed in **Section 5.3**
- Female subject of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (**Section 5.5.1**).
- Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment should resume at the same dose and cycle interval which they were receiving prior to discontinuation. Study visit requirements are outlined in the Study Calendar (**Section 10.0**).

5.8 Off Study/Safety Follow-up Visit

After a patient is discontinued from MK-3475, a mandatory Off Study/Safety FollowUp Visit should be performed approximately 30 days after the last infusion of study medication (or within 7 days prior to initiation of a new anti-cancer treatment, whichever comes first). Procedures and assessments performed at this visit and beyond should follow the respective guidelines described in **Section 10.0** as appropriate. The patient will be monitored for adverse events up to the mandatory Off Study/Safety Follow-Up Visit or to resolution of toxicity to Grade 0-1, whichever occurs later. SAEs that occur within 90 days of the last infusion of MK-3475 or before initiation of a new antineoplastic treatment should also be followed and recorded.

5.9 Duration of Follow Up

Patients who are discontinued from the study due to an unacceptable drug-related AE should continue to be followed for resolution of the AE to Grade 0-1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first.

Patients, who discontinued study therapy without documented disease progression, should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue to be assessed approximately every 12 weeks for 3 years (approximately every 6 months after 3 years) until, 1) start of a new antineoplastic therapy (information of the new cancer therapy will be collected), 2) documented disease progression, 3) study closure, or 4) until death, whichever occurs first.

Patients that have come off study to receive the FDA approved dose of pembrolizumab for microsatellite instability-high cancer should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue to be assessed approximately every 12 weeks for 3 years (approximately every 6 months after 3 years) until, 1) start of a new antineoplastic therapy (information of the new cancer therapy will be collected), 2) documented disease progression, 3) study closure, or 4) until death, whichever occurs first.

Patients who discontinue from treatment should be contacted every three months to monitor overall survival. Information of other cancer therapies after discontinuation from the study treatment will be collected.

SAEs that occur within 90 days of the last infusion of MK-3475 or before initiation of a new antineoplastic treatment should also be followed and recorded.

5.10 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in **Section 5.6** applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.10.1 Disease Progression

MK-3475 is expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months to be evident. Some patients may have objective volume increase of tumor lesions or other disease parameters within weeks following the start of MK-3475 dosing. Such patients may not have had sufficient time to develop the required immune activation or, in some patients, tumor volume or other disease parameter increases may represent infiltration of lymphocytes into the original tumor. In conventional studies, such tumor volume or relevant laboratory parameter increases during the first 2-4 months of the study would constitute PD and lead to discontinuation of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response.

Therefore, patients with tumor progression by RECIST imaging or laboratory parameters prior to their 7 month evaluation but without rapid clinical deterioration or change in PS who do not require additional immediate therapy, may continue to be treated with MK-3475 and clinically observed following the assigned imaging schedule to allow detection of a subsequent tumor response. Tumor assessments will be made using RECIST 1.1 and immune-related RECIST criteria (irRC). Subjects that meet the above criteria and continue on study therapy must discontinue MK-3475 if there are no signs of disease stabilization by 7 months using irRC (or by the radiologist if irRC is still pending). Tumor assessments in the second expansion cohort and Cohort D will be

evaluated by RECIST 1.1 criteria only. Approval for continuation from the IND sponsor may be obtained if the patient is thought to be clinically benefiting.

5.10.2 IRAEs

Permanent discontinuation of MK-3475 should be considered for any of the following:

1. Severe or life-threatening related adverse reactions, including, but not limited to, any of the following (IND Sponsor and Merck must be notified in the event of these AEs, and final decision regarding treatment will be made after a discussion):

    - Grade 3-4 toxicity (non-hematologic or hematologic). Grade 3 toxicities may be able to be re-dosed on a case by case basis after approval of the IND Sponsor and Merck.

    - Diarrhea with abdominal pain, fever, ileus, or peritoneal signs; increase in stool frequency (7 or more over baseline), stool incontinence, need for intravenous hydration for more than 24 hours, gastrointestinal hemorrhage, and gastrointestinal perforation

    - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >5 times upper limit of normal

    - Total serum bilirubin >3 times upper limit of normal

    - Steven-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration or necrotic, bullous or hemorrhagic manifestations

    - Severe (i.e., CTCAE Grade 3 or 4) motor or sensory neuropathy

    - Any grade Guillain-Barré syndrome, or myasthenia gravis or other neurologic symptoms that impact activity of daily living

    - Severe immune-mediated reactions involving any other organs (e.g., nephritis, pneumonitis, pancreatitis, non-infectious myocarditis)

    - Immune-mediated ocular disease that is unresponsive to topical immunosuppressive therapy

    - Grade 3 or 4 infusion reaction
2. Inability to reduce corticosteroid dose for immune-related adverse reactions to ≤10 mg prednisone or equivalent per day

    If any of the above events occur, the investigator should discuss with the IND Sponsor and Merck to make a decision on discontinuation of MK-3475 study treatment.

In case toxicity does not resolve or improve to ≤ Grade 1 within 12 weeks after last administration of MK-3475 study drug, study therapy discontinuation should be considered after discussion with the IND Sponsor and Merck. With IND Sponsor and Merck agreement, patients still at Grade 2 may continue in the study only if asymptomatic and controlled. Two dosing delays due to the same toxicity will be permitted. In the event of a third occurrence of the same toxicity which would require dosing delay, study therapy will be discontinued permanently.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Modifications

Dose reduction or dose increase of MK-3475 will not be permitted in individual patients.

6.2 Dosing Delays

MK-3475 will be withheld for the following drug-related toxicities and severe or lifethreatening AEs as per the table below:

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. The schedule for restarting treatment may be increased by 1 week for each occurrence if approved by the IND Sponsor. 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
	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 -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 betablockers (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[2]	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
All other immune-related AEs[2]	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

NOTES:

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

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

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued after consultation with the IND Sponsor. With IND Sponsor and Merck agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled.

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

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

This study will use the descriptions and grading scales found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for adverse event reporting that can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

All adverse events experienced by subjects will be collected and reported from the first dose of the investigational agent, throughout the study, and will only be followed for 30 days unless related to the investigational agent. All Serious adverse events will be collected for 90 days after the last infusion of MK-3475.

Subjects who have an ongoing adverse event related to the study procedures and/or medication(s) may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

Patients who experience a Grade 2 or higher irAE should be discussed with Dr. Le. In addition, AEs listed in **Section 7.1.3** must be reported as an Event of Clinical Interest (ECI) within 24 hours to the IND Sponsor and to Merck Global Safety even if no Serious Adverse Event Criteria are met.

Laboratory abnormalities: Laboratory abnormalities present at the screening visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as adverse events. A grade 1 or 2 clinical laboratory abnormality should be reported as an adverse event only if it is considered clinically significant by the investigator.

7.1 Definitions

7.1.1 Adverse Event (AE)

Adverse event is defined as any undesirable sign, symptom or medical condition occurring after starting the study drug (or therapy) even if the event is not considered to be related to the study. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). Medical conditions/diseases present before starting the study treatment are only considered adverse events if they worsen after starting the study treatment (any procedures specified in the protocol). Adverse events occurring before starting the study treatment but after signing the informed consent form will not be recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy.

7.1.2 Serious Adverse Event (SAE)

A serious adverse event is an undesirable sign, symptom or medical condition which:

- Results in death
- Is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form)
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose

Events **not** considered to be serious adverse events are hospitalizations for the:

- Admissions as per protocol for a planned medical/surgical procedure or to facilitate a procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases

- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

7.1.3 Adverse Events of Clinical Interest (ECI) for MK-3475

These selected non-serious related adverse experiences are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event of Clinical Interest Case Report Form found in **Appendix C**.

Events of clinical interest for this trial include:

- an overdose of Merck's product, as defined in **Section 5.4**, 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.

7.2 Relationship

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expectedness

Unexpected adverse event: An adverse event, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the Investigator's Brochure, package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

Expected (known) adverse event: An adverse event, which has been reported in the Investigator's Brochure. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

7.4 Handling of Expedited Safety Reports

In accordance with local regulations, the IND Sponsor (Dr. Le) will notify investigators of all SAEs that are unexpected (i.e., not previously described in the Investigator Brochure), and definitely, probably, or possibly related to MK-3475. This notification will be in the form of an expedited safety report (ESR) that is to be faxed to the investigators and the study coordinators. Upon receiving such notices, the investigator must review and retain the notice with the Investigator's Brochure and where required by local regulations, the investigator will submit the ESR to the appropriate IRB. The investigator and IRB will determine if the informed consent requires revision. The investigator should also comply with the IRB procedures for reporting any other safety information.

7.5 Reporting

7.5.1 General

All adverse events (both expected and unexpected) will be captured on the appropriate study-specific case report forms (CRFs).

In addition, all serious adverse events, regardless of causality to study drug and/or administration device, will be reported promptly to Dr. Dung Le (e-mail: [redacted]) within 24 hours of recognition of the adverse event using the form found in **Appendix D**. If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

All serious adverse events, regardless of causality to study drug and/or administration will be forwarded to Merck's Global Safety ("Merck GS") group within 24 hours of learning of the event.

After the initial SAE report, the investigator is required to proactively follow each subject and provide further information to the IND Sponsor and Merck in regards to the subject's condition.

All SAE(s) will be followed until:

- Resolution
- The condition stabilizes
- The event is otherwise explained
- The subject is lost to follow-up

As soon as relevant information is available, a follow-up SAE report will be submitted to the IND Sponsor and Merck GS.

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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner who has provided written consent to provide information regarding pregnancy, that occurs during the trial or within 120 days of completing the trial. All 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 to Merck GS.

SAE reports and any other relevant safety information are to be forwarded to Merck GS facsimile number: [redacted]

7.5.2 Institutional Review Board (IRB)

Participating sites will be responsible for reporting to their IRB. All serious adverse events will be reported to the IRB per institutional standards. Upon receipt, follow-up information will be given to the IRB (as soon as relevant information is available) per institutional standards.

7.5.3 Food and Drug Administration (FDA)

All reporting to the FDA will be completed by the IND Sponsor (Dr. Le).

7.5.3.1 Expedited IND Safety Reports:

7 Calendar-Day Telephone or Fax Report:
The IND Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the investigational agent. Such reports are to be telephoned or faxed (301-827-9796) to the FDA within 7 calendar days of first learning of the event.

15 Calendar-Day Written Report:
The IND Sponsor is required to notify the FDA of any serious adverse event that is unexpected and possibly related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report

should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event.

7.5.3.2 IND Annual Reports

In accordance with the regulation 21 CFR § 312.33, the Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.33 for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the Sponsor-Investigator.

8. PHARMACEUTICALINFORMATION (MK-3475)

8.1 Agent Accountability

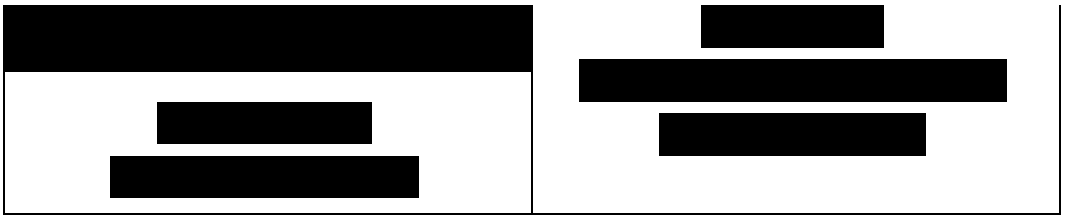
The sponsor/investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

8.2 Mode of Action

[redacted]

8.3 Description

[redacted]



8.4 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements. Open label kits will be provided for patient dosing.

8.5 Preparation

Refer to Procedures Manual for preparation instructions.

8.6 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.

Refer to Procedures Manual for Storage conditions.
Clinical supplies may not be used for any purpose other than that stated in the protocol.

8.7 Stability

Refer to Procedures Manual for Stability information.

8.8 Route of Administration

The reconstituted product is intended for IV administration.

8.9 Patient Care Implications

[redacted]

[redacted]

[redacted]

8.10 Agent Ordering

[redacted]

8.11 Returns and Reconciliation

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

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

9. CORRELATIVE/SPECIAL STUDIES

Sample collection, storage, and shipment instructions will be provided in the Procedures Manual.

9.1 Tumor Tissue Studies

Tumor biopsies will be collected (if a patient's tumor is thought to be reasonably safe and easy to biopsy) at baseline and prior to Cycle 3 (4-6 cores per timepoint). Additional optional biopsies may be obtained later in the course of therapy. Archival tumor samples will also be collected for every patient (slides and/or blocks). Fine needle aspiration will not be acceptable. Additional archival tissue from patients no longer receiving treatment due to disease progression may also be collected in order to evaluate resistant lesions. Detailed instructions for tissue collection, processing and shipment are provided in the procedures manual.

9.1.1 To explore the association of MSI positive, PD-L1 positivity, and tumor infiltrating lymphocyte characteristics with clinical responses, archived tumor tissue and tumor tissue obtained at baseline and during treatment (prior to cycle 3) will be compared. PD-L1 expression may predict response to anti-PD-1 [28, 51]. However, PD-L1 is also upregulated in response to IFN-γ released by infiltrating T cells and could potentially be a predictor of response to any active immunotherapy. Pre and post-treatment tumor biopsies will also be analyzed for PD-1 expression as well as infiltration of immune cells (effector T cells, Tregs, B cells, dendritic cells, etc). Characterization of immune checkpoint expression as well as immune infiltrates may be predictive of response to therapy and may also give insight into next generation combinatorial approaches. Preliminary data from a pancreatic cancer immunotherapy study suggests that induction of a $T_h1$and T_h17 phenotype at the tumor itself predicts response. Furthermore, upregulation of other inhibitory molecules such as IL-10 and TGF-β may identify other targets for combinatorial strategies.

Slides from a newly obtained biopsy sample of at least one tumor lesion at baseline or from archived tissue will be submitted for PD-L1 and biomarker staining. Biopsy or archival tissue samples should have proper size to enable IHC analysis of PD-L1.

9.1.2 The Ludwig Center for Cancer Genetics will perform whole-exome sequencing using next-generation sequencing on DNA from tumors and matched normal tissue. As with all targeted therapies, we expect a subset of patients will respond to

immunotherapy and there may be genetic determinants that define this response. These studies will define the genetic landscape of these tumors and may determine an 'immune responsive' phenotype. Furthermore, since each mutation is a potential neo-antigen that may drive an immune response, we will evaluate each mutation for antigenic potential in our model [58].

9.1.3 In cases, where the genes listed are known to be mutated, we will test for MSI status since there is a correlation between MSI status and these mutations.

Deleterious mutations in the following genes:

- MLH 1
- MSH 2
- MSH 6
- PMS2
- BRAF pV600E
- TGFBR2

9.2 Peripheral Blood Lymphocytes (PBLs)

Post-treatment expression of PD-1 and other lymphocyte activation markers will be measured and correlated with OS and MSI positivity. PBL will be collected as described in **Section 10**. PBL are isolated and stored frozen until use.

9.3 Serum and Plasma Marker Studies

Sera and plasma will be collected as described in **Section 10** to identify potential therapeutic targets, biomarkers, and predictors of response and autoimmune toxicity through proteomic approaches. In addition, we will look at alternative methods to identify MSI in circulating tumor DNA. Whole blood will be collected in a 10 milliliter Serum Separator Tube (SST tube) at the designated time points and processed using standard laboratory procedures. Using a pipette, aliquots of 1 mL of serum should be transferred to cryogenic vials and stored at -80ºC.

We will also collect whole blood in two 10 mL plasma preparation tubes with EDTA (PPT, BD Vacutainer, Franklin Lakes, NJ) and gently swirl tubes to mix blood with EDTA. Within two hours of collection, the sample will be processed using standard procedures for plasma separation. Plasma will be divided into 1 mL aliquots and stored at -80ºC. Pellets from this separation procedure will be washed in PBS and then divided into aliquots and also stored at -80ºC.

9.4 Normal tissue based studies

To assess the baseline characteristic of the subjects enrolled and to correlate these molecular and clinicopathologic criteria with treatment response and toxicity. DNA

will be extracted from whole blood (collection and processing described in 9.3) and used to evaluate for any germline mutations that may correlate with response or toxicity. A SNP array may be performed on each case using the Affymetrix Genomewide Human SNP Array 6.0 (Santa Clara, CA).

9.5 Diagnostic Tissue Samples

Tissue, fluid, or blood may be collected from standard of care procedures used to treat or diagnoses immune related toxicities. Detailed instructions for tissue collection and shipment are provided in the procedures manual.

9.6 Microbiome Studies

Stool and saliva samples will be collected for microbiome studies at baseline and with each scan at select clinical trial sites. Detailed instructions for sample collection, processing, storage, and shipment are provided in the procedures manual.

10. STUDY CALENDAR

10.1 Cohorts A-C

Study Procedures	**Pre-Study[19]**		**Cycle (14 days) [20]**	**20 Weeks[21]**	**Off Treatment**	**Off Study[24]**
Visit Windows (days)[1]	N/A	-28 to -1	+7	**+/- 14**	+/- 7	+/- 14
MK-3475			X			
Pre-screening consent[2]		X				
Informed consent		X				
Inclusion/exclusion criteria		X				
Demographics		X				
MSI Testing	X					
Medical history		X				
Cancer History[3]		X				
Concurrent meds		X	X	**X**	X[22,23]	X
Physical exam[4]		X	X	**X**		X
Vital signs[5]		X	X	**X**		X

Height	X					
Weight		X	X	**X**		X
Performance status (PS)		X	X	**X**		X
Hematology profile[6, 12]		X	X	**X**	X[22,23]	X
Chemistry profile[7, 12]		X	X	**X**	X[22,23]	X
TSH, T3, FT4[8, 12]			X	**X**	X[22,23]	X
Urinalysis[9, 12]		X				
B-HCG[10, 12]		X				
Serum Tumor Markers[11, 12]		X	X	**X**	X[22,23]	X
Adverse event evaluation			X	**X**	X[22,23]	X
Radiologic evaluation[13]		X	X	**X**	X[23]	X
Tumor measurements[13]		X	X	**X**		X
Whole blood for PBL[14]			X	**X**	X[23]	
Serum (approximately 10cc) [14]			X	**X**	X[23]	
Whole Blood Sample for Plasma (approximately 20cc) [14]			X	**X**	X[23]	
Stool/Saliva Sample[15]		X	X			
Archival Tissue[16]		X	X		X	X
Tumor Biopsies[17]		X	X			
Buccal Swab/purple top tube[18]		X	X			

1: Longer delays to be approved by the IND Sponsor.
2: Pre-screening informed consent for MSI testing (Cohort C only).
3: Cancer history includes: primary site of cancer, gross location of primary tumor, secondary sites of cancer, histology, histologic grade, date of initial diagnosis, date of metastatic diagnosis, prior cancer therapy regimens.
4: Complete physical exam will be completed at baseline; focused physical examinations will be conducted thereafter. Exams, concomitant medication, AE assessments can be made up to 3 days prior to infusion.
5: Temperature, respiration rate, blood pressure, and pulse should be taken at baseline, prior to MK-3475 infusion, and at the end of the infusion. Pulse oximetry should be taken at baseline and prior to each MK-3475 infusion.
6: CBC with differential including absolute eosinophil count, absolute neutrophils, absolute lymphocytes, and platelets.

7: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

8: TSH, Total T3, and Free T4 will be collected at baseline (Day -28 to Day 1), 12 weeks (+/- 2 week), every 8 weeks (+/- 2 week) through week 52, and then every 12 weeks (+/- 2 week) thereafter including the off study evaluation. Weeks are in reference to calendar week and should not be adjusted due to dosing delays.

9: Bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, color, protein, RBC and WBC count, and specific gravity.

10: Serum or urine pregnancy test (women of childbearing potential).

11: Disease specific tumor markers where appropriate.

12: Labs may be collected within a window of up to 3 days prior to dosing.

13: Radiologic evaluations and tumor measurements will be performed at baseline (within 14 days prior to the first dose of MK-3475), 12 weeks (+/- 2 week), every 8 weeks (+/- 2 week) through week 52, and then every 12 weeks (+/- 2 week) thereafter including the off study evaluation. Weeks are in reference to calendar week and should not be adjusted due to dosing delays.

14: See Correlative Studies table below for further details.

15: Samples to be obtained at baseline and with each scan [12 weeks (+/- 2 week), every 8 weeks (+/- 2 week) through week 52, and then every 12 weeks (+/- 2 week) thereafter] at select clinical trial sites. Detailed instructions regarding collection and processing are located in the Procedures Manual.

16: Attempts to obtain archival tumor samples will be made for every patient until the sample is obtained or documentation that the sample cannot be obtained. The tissue sample should have proper size to enable IHC analysis of PD-L1. Fine needle aspirates will not be acceptable. Detailed instructions for tissue collection, processing and shipment are provided in the Procedures Manual.

17: Tumor biopsies to be taken (if a patient's tumor is thought to be reasonably safe and easy to biopsy) at baseline and prior to Cycle 3 (4-6 cores per timepoint). The biopsy performed prior to Cycle 3 has a -7 day window prior to the Cycle 3 visit. Additional optional biopsies may be obtained later in the course of therapy. The tissue sample should have proper size to enable IHC analysis of PD-L1. Fine needle aspiration will not be acceptable. Detailed instructions for tissue collection, processing and shipment are provided in the Procedures Manual.

18: Buccal swabs and/or purple top tube will be obtained only for those patients who do not have enough normal tissue for MSI testing. This collection can occur any time after consent.

19: Patients who stop MK-3475 with SD or better may be eligible for up to one year of additional MK-3475 therapy if they progress after stopping MK-3745, they meet the criteria listed in **Section 5.7.2,** and undergo the following evaluations: interval medical and cancer history, physical exam, vitals, weight, PS, hematology and chemistry profile, autoimmune and endocrine panel, urinalysis, serum or urine pregnancy test, serum tumor markers, radiographic evaluations, and tumor measurements. The following samples will be collected at baseline and while on study: peripheral blood, serum, whole blood for plasma, and biopsy. Subjects who restart treatment after relapse should resume at the same dose and cycle interval which they were receiving prior to discontinuation.

20: For cycle 1, study procedures do not need to be repeated if they were conducted within 3 days of the pre-study evaluations.

21: Week 20 evaluations (calculated from the date of the first dose), which include additional blood, CT/MRI scans and tumor measurements, may coincide with a cycle visit. All week 20 evaluations must be completed at the Week 20 time point.

22: Patients will undergo the Off Treatment Evaluation 30 days (+/- 7 days) after the last infusion of MK-3475 if they completed 24 months of MK-3475 treatment or the patient has discontinued MK-3475 after an investigator determined confirmed CR (**Section 5.7.1**). The assessment of AEs and medications can be made either as part of a clinical visit or over the phone. Patients will also continue to be monitored for the resolution of drug-related AEs to ≤ Grade 1. SAEs that occur within 90 days of the last infusion of MK-3475 or before initiation of a new antineoplastic treatment should also be followed and recorded.

23: While off treatment (**Section 5.7.1**), patients should continue to be monitored for disease status by radiologic imaging and tumor marker(s) every 12 weeks (+/- 2 weeks) for 1 year starting from the last scan. After 1 year, disease status should be evaluated every 24 weeks (+/- 2 weeks). These evaluations can be done locally if approved by the IND Sponsor. In addition, we will also continue to collect clinical labs, as well as peripheral blood, serum, and plasma samples every 12 weeks (+/- 2 weeks) for 1 year starting from the last research blood draw as described below. After 1 year, clinical labs and research bloods should be collected every 24 weeks (+/- 2 weeks).

24: 30 days after their last dose of study drug or within 7 days prior to initiation of a new anti-cancer treatment, whichever comes first. Patients who come off study should continue to be followed as described in **Section 5.9.**

Correlative Studies While On study							
	Baseline	**2 Weeks**	**4 Weeks**	**12 Weeks**	**20 Weeks**	**Q 8 Weeks (Weeks 28-52)**	**Q 12 Weeks (week 64 and beyond)**
Whole blood for PBL [1]	X[2]	X[2]	X[2]	X[3]	X[3]	X[3]	X[3]
Serum (approximately 10cc) [1]	X	X	X	X	X	X	X
Whole Blood Sample for Plasma (approximately 20cc) [1]	X	X	X	X	X	X	X

1: Blood and serum samples will be collected at baseline (Day -28 to Day 1), 2 weeks (+/- 1 week), 4 weeks (+/- 1 week), 12 weeks (+/- 2 week), every 8 weeks (+/- 2 week) through week 52, and then every 12 weeks (+/- 2 week) thereafter. Weeks are in reference to calendar week and should not be adjusted due to dosing delays. 2: Up to 64 cc
3: Up to 100 cc

Correlative Studies While Off Treatment (per Section 5.7.1)		
	Q 12 Weeks (for first 52 weeks off treatment)	**Q 24 Weeks (after first 52 weeks off treatment)**
Whole blood for PBL (up to 100cc)	X	X
Serum (approximately 10cc)	X	X
Whole Blood Sample for Plasma (approximately 20cc)	X	X

10.2 Cohort D

Study Procedures	**Pre-Study[19]**	**Cycle (21 days) [20]**	**21 Weeks[21]**	**Off Treatment**	**Off Study[24]**

Visit Windows (days)[1]	N/A	-28 to -1	-3/+7	**+/- 14**	+/- 7	+/- 14
MK-3475			X			
Pre-screening consent[2]		X				
Informed consent		X				
Inclusion/exclusion criteria		X				
Demographics		X				
MSI **and** mutator phenotype testing	X					
Medical history		X				
Cancer History[3]		X				
Concurrent meds		X	X	**X**	X[22,23]	X
Physical exam[4]		X	X	**X**		X
Vital signs[5]		X	X	**X**		X
Height	X					
Weight		X	X	**X**		X
Performance status (PS)		X	X	**X**		X
Hematology profile[6, 12]		X	X	**X**	X[22,23]	X
Chemistry profile[7, 12]		X	X	**X**	X[22,23]	X
TSH, T3, FT4[8, 12]			X	**X**	X[22,23]	X
Urinalysis[9, 12]		X				
B-HCG[10, 12]		X				
Serum Tumor Markers[11, 12]		X	X	**X**	X[22,23]	X
Adverse event evaluation			X	**X**	X[22,23]	X
Radiologic evaluation[13]		X	X	**X**	X[23]	X
Tumor measurements[13]		X	X	**X**		X

Whole blood for PBL[14]			X	**X**	X[23]	
Serum (approximately 10cc) [14]			X	**X**	X[23]	
Whole Blood Sample for Plasma (approximately 20cc) [14]			X	**X**	X[23]	
Stool/Saliva Sample[15]		X	X			
Archival Tissue[16]		X	X		X	X
Tumor Biopsies[17]		X	X			
Buccal Swab/purple top tube[18]		X	X			

1: Longer delays to be approved by the Sponsor or Protocol Chair.
2: Pre-screening informed consent for MSI testing (Cohort C only).
3: Cancer history includes: primary site of cancer, gross location of primary tumor, secondary sites of cancer, histology, histologic grade, date of initial diagnosis, date of metastatic diagnosis, prior cancer therapy regimens.
4: Complete physical exam will be completed at baseline; focused physical examinations will be conducted thereafter. Exams, concomitant medication, AE assessments can be made up to 3 days prior to infusion.
5: Temperature, respiration rate, blood pressure, and pulse should be taken at baseline, prior to MK-3475 infusion, and at the end of the infusion. Pulse oximetry should be taken at baseline and prior to each MK-3475 infusion.
6: CBC with differential including absolute eosinophil count, absolute neutrophils, absolute lymphocytes, and platelets.
7: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
8: TSH, Total T3, and Free T4 will be collected at baseline (Day -28 to Day 1), 12 weeks (+/- 2 week), every 9 weeks (+/- 2 week) through week 57, and then every 12 weeks (+/- 2 week) thereafter including the off study evaluation. Weeks are in reference to calendar week and should not be adjusted due to dosing delays.
9: Bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, color, protein, RBC and WBC count, and specific gravity.
10: Serum or urine pregnancy test (women of childbearing potential).
11: Disease specific tumor markers where appropriate.
12: Labs may be collected within a window of up to 3 days prior to dosing.
13: Radiologic evaluations and tumor measurements will be performed at baseline (within 14 days prior to the first dose of MK-3475), 12 weeks (+/- 2 week), every 9 weeks (+/- 2 week) through week 57, and then every 12 weeks (+/- 2 week) thereafter including the off study evaluation. Weeks are in reference to calendar week and should not be adjusted due to dosing delays.
14: See Correlative Studies table below for further details.
15: Samples to be obtained at baseline and with each scan [12 weeks (+/- 2 week), every 9 weeks (+/- 2 week) through week 57, and then every 12 weeks (+/- 2 week) thereafter] at select clinical trial sites. Detailed instructions regarding collection and processing are located in the Procedures Manual.
16: Attempts to obtain archival tumor samples will be made for every patient until the sample is obtained or documentation that the sample cannot be obtained. The tissue sample should have proper size to enable IHC analysis of PD-L1. Fine needle aspirates will not be acceptable. Detailed instructions for tissue collection, processing and shipment are provided in the Procedures Manual.
17: Tumor biopsies to be taken (if a patient's tumor is thought to be reasonably safe and easy to biopsy) at baseline and prior to Cycle 3 (4-6 cores per timepoint). The biopsy performed prior to Cycle 3 has a -7 day window prior to the Cycle 3 visit. Additional optional biopsies may be obtained later in the course of therapy. The tissue sample should have proper size to enable IHC analysis of PD-L1. Fine needle aspiration will not be acceptable. Detailed instructions for tissue collection, processing and shipment are provided in the Procedures Manual.
18: Buccal swabs and/or purple top tube will be obtained only for those patients who do not have enough normal tissue for MSI testing. This collection can occur any time after consent.

19: Patients who stop MK-3475 with SD or better may be eligible for up to one year of additional MK-3475 therapy if they progress after stopping MK-3745, they meet the criteria listed in **Section 5.7.2,** and undergo the following evaluations: interval medical and cancer history, physical exam, vitals, weight, PS, hematology and chemistry profile, autoimmune and endocrine panel, urinalysis, serum or urine pregnancy test, serum tumor markers, radiographic evaluations, and tumor measurements. The following samples will be collected at baseline and while on study: peripheral blood, serum, whole blood for plasma, and biopsy. Subjects who restart treatment after relapse should resume at the same dose and cycle interval which they were receiving prior to discontinuation.

20: For cycle 1, study procedures do not need to be repeated if they were conducted within 3 days of the pre-study evaluations.

21: Week 21 evaluations (calculated from the date of the first dose), which include additional blood, CT/MRI scans and tumor measurements, may coincide with a cycle visit. All week 21 evaluations must be completed at the Week 21 time point.

22: Patients will undergo the Off Treatment Evaluation 30 days (+/- 7 days) after the last infusion of MK-3475 if they completed 24 months of MK-3475 treatment or the patient has discontinued MK-3475 after an investigator determined confirmed CR (**Section 5.7.1**). The assessment of AEs and medications can be made either as part of a clinical visit or over the phone. Patients will also continue to be monitored for the resolution of drug-related AEs to ≤ Grade 1. SAEs that occur within 90 days of the last infusion of MK-3475 or before initiation of a new antineoplastic treatment should also be followed and recorded.

23: While off treatment (**Section 5.7.1**), patients should continue to be monitored for disease status by radiologic imaging and tumor marker(s) every 12 weeks (+/- 2 weeks) for 1 year starting from the last scan. After 1 year, disease status should be evaluated every 24 weeks (+/- 2 weeks). These evaluations can be done locally if approved by the IND Sponsor. In addition, we will also continue to collect clinical labs, as well as peripheral blood, serum, and plasma samples every 12 weeks (+/- 2 weeks) for 1 year starting from the last research blood draw as described below. After 1 year, clinical labs and research bloods should be collected every 24 weeks (+/- 2 weeks).

24: 30 days after their last dose of study drug or within 7 days prior to initiation of a new anti-cancer treatment, whichever comes first. Patients who come off study should continue to be followed as described in **Section 5.9.**

Correlative Studies While On Study							
	Baseline	**3 Weeks**	**6 Weeks**	**12 Weeks**	**21 Weeks**	**Q 9 Weeks (Weeks 30-57)**	**Q 24 Weeks (week 81 and beyond)**
Whole blood for PBL [1]	X[2]	X[2]	X[2]	X[3]	X[3]	X[3]	X[3]
Serum (approximately 10cc) [1]	X	X	X	X	X	X	X
Whole Blood Sample for Plasma (approximately 20cc) [1]	X	X	X	X	X	X	X

1: Blood and serum samples will be collected at baseline (Day -28 to Day 1), 3 weeks (+/- 1 week), 6 weeks (+/- 1 week), 12 weeks (+/- 2 week), every 9 weeks (+/- 2 week) through week 57, and then every 12 weeks (+/- 2 week) thereafter. Weeks are in reference to calendar week and should not be adjusted due to dosing delays. 2: Up to 64 cc

3: Up to 100 cc

Correlative Studies While Off Treatment (per Section 5.7.1)

	Q 12 Weeks (for first 52 weeks off treatment)	Q 24 Weeks (after first 52 weeks off treatment)
Whole blood for PBL (up to 100cc)	X	X
Serum (approximately 10cc)	X	X
Whole Blood Sample for Plasma (approximately 20cc)	X	X

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be evaluated for response at the 12 week timepoint, every 8 weeks through week 52, and then every 12 weeks thereafter including the off study evaluation. Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [**Appendix E**] and the immune related Response criteria (irRC) [**Appendix F**]. Tumor assessments in the second expansion cohort will be evaluated by RECIST 1.1 criteria only. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. RECIST and irRC response assessments will be performed locally for the trial and may be requested for central review.

11.1.1 Definitions

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

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

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

11.1.2 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

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

PET-CT: A PET-CT can be used to obtain the images as long as the CT is of diagnostic quality.

11.1.3 Duration of Response as per RECIST 1.1 and irRC

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements. In order to be considered durable, Stable Disease must be maintained for at least 6 weeks (this will be attained by the 9 week time point).

11.1.4 Progression-Free Survival (PFS) as per RECIST 1.1 and irRC

PFS is defined as the duration of time from start of treatment to time of first documented progression or death, whichever occurs first.

11.1.5 Overall Survival (OS)

OS is defined as the duration of time from start of treatment to time of death.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Management

All information will be collected on study-specific case report forms (CRFs) by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator at each site.

Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE) Reviewing data from all sites.

Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.

- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

12.2 Safety Meetings

Scheduled meetings will take place weekly and will include the protocol principal investigator, study coordinator(s), data manager(s), sub-investigators (as appropriate), collaborators (as appropriate), and biostatisticians (as appropriate) involved with the conduct of the protocol. During these meetings matters related to the following will be discussed: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for objectives.

Monthly teleconferences will be scheduled to include the Investigator and Merck representatives. During these meetings, the Investigator shall provide Merck with study progress updates. The Investigator will provide a summary of key points from the weekly meetings with a focus on safety of the protocol participants, enrollment status, and progress of data for objectives. In addition, Merck will provide safety and applicable program updates to the Sponsor.

12.3 Monitoring

This is a DSMP Level II study under the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data Safety Monitoring Plan (DSMP, 12/6/2012, **Appendix G**). Eligibility for all sites will be monitored by the JHU SKCCC CRO. Data monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. Additional data and safety monitoring oversight will also be performed by the SKCCC Safety Monitoring Committee (SMC - as defined in the DSMP). The protocol will be monitored internally by the Principal

Investigator at each site. External monitoring will occur according to the following guidelines:

Johns Hopkins SKCCC: The protocol will be monitored externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

Participating site(s): The protocol will be monitored by the internal CRO at each site. A report of the reviews will be submitted to the Johns Hopkins principal investigator and SKCCC CRO.

Authorized representatives of the Coordinating Center may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

Dr. Le is holding the IND for this study. She will comply with all regulated reporting requirements to the FDA.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a multicenter, open-label, two-stage, phase 2 study to evaluate the clinical activity of MK-3475 in MSI positive and MSI negative solid tumors. We will enroll three cohorts of patients: patients with MSI positive colorectal adenocarcinomas (Cohort A); patients with MSI negative colorectal adenocarcinomas (Cohort B); and patients with MSI positive solid tumor malignancies but not colorectal adenocarcinoma (Cohort C); all cohorts will receive MK-3475. Each cohort will be assessed separately. This design aims to evaluate MSI as a biomarker and evaluate treatment benefit simultaneously.

The primary endpoints for Cohorts A and B are immune-related progression-free survival (irPFS) rate at 20 weeks and objective response rate (irORR) assessed using immune related response criteria (irRC); irPFS is defined as the proportion of subjects alive and free of disease progression at 20 weeks per irRC; and irORR is the proportion of subjects whose best overall response is a CR or PR.

The primary endpoint for Cohort C is immune-related progression-free survival (irPFS) rate assessed using immune related response criteria (irRC) at 20 weeks, defined as the proportion of subjects alive and free of disease progression at 20 weeks per irRC. Each cohort will be assessed separately.

The primary endpoint for Cohort D will be objective response rate (ORR), which is defined as the proportion of subjects who have response of CR or PR.

The secondary efficacy endpoints include overall survival, progression-free survival according to RECIST 1.1 (PFS), best overall response (BOR), duration of objective response (DOOR), time to objective response (TDOR), and disease control rate (DCR) per RECIST 1.1, and irPFS, irBOR, irDOOR, irTDOR, irDCR per irRC.

We will also evaluate safety and toxicity of MK-3475. We will evaluate exploratory endpoints of pharmacogenomics and predictive biomarkers for responses.

13.2 Sample Size/Accrual Rate

For Cohort A and B, a total of 25 evaluable patients are planned.

MK-3475 will be considered to be inactive and of no interest for further evaluation if the irPFS rate at 20 weeks is 5% (p_{10}) or less or irORR is 5% (p_{20}) or less, and considered active if the irPFS rate at 20 weeks is 25% or greater (p_{11}) and irORR is 21% or greater (p_{21}). We will apply a step-down gatekeeping procedure to preserve a study-wise type I error rate at 0.05 when evaluating co-primary endpoints of irPFS and irORR for Cohort A and B. Specifically, the endpoints will be tested in the hierarchical order first with irPFS at 20 weeks. If the null hypothesis of 20-week irPFS being 5% is rejected (i.e. the result reaches statistical significance), we will proceed to test for irORR. If irPFS is not significant at the 0.05 level (one-sided test), the statistical testing for irORR will not occur. Twenty-five patients in Cohort A and B would provide 90% power to detect an improved irPFS rate at 20 weeks from 5% to 25%, a 20% increase, at significance level of 0.05 based on a one-sided test. For an improved irORR from 5% to 21%, a total of 25 patients would provide 80% power to detect an of 16% increase using a one-sided exact binomial test at a 0.05 significance level.

For Cohort A and B, there will be 1 interim and 1 final analysis for irPFS, and 1 final analysis of irORR. An interim analysis will be conducted in order to assess futility after irPFS at 20 weeks are available for the first 15 evaluable patients. According to Green-Dahlberg design, if 0 irPFS are observed, enrollment in that cohort will be terminated. If at least one patient is free of disease progression at 20 weeks per irRC, an additional 10 evaluable subjects will be enrolled into that cohort. If 3 or fewer patients are progression free at 20 weeks in the total of 25 evaluable patients enrolled in that cohort, then the regimen will be considered inactive. If 4 or more patients are progression free at 20 weeks, we proceed to test for the endpoint of irORR for that cohort. If 4 or more responders (CR or PR) are observed, we conclude the regimen is promising with that cohort and warrants further study.

There is a 1.3% probability of stopping for futility under the alternative hypothesis p_{11} (i.e. incorrectly stopping at the interim when the drug is efficacious) and a 46%

probability of stopping for futility under the null hypothesis p_{10} (i.e. correctly stopping at the interim when the drug is not efficacious).

For Cohort D, the regimen would be considered of insufficient activity for further study if the response rate is 5% or less, and the minimum required level of efficacy that would warrant further study with the proposed regimen is a 21% response rate. A two-stage Green-Dahlberg design is planned. A total of 20 subjects will be entered in the first stage. If zero response is observed, that cohort will be terminated and we will conclude the regimen is ineffective. If $\geq$1 subjects respond, then additional 15 subjects will be studied for that cohort. If $\leq$ 4 subjects respond in stage one and two combined, we consider this regimen ineffective. If $\geq$5 responses are observed, we conclude the regimen is promising and warrant further study. Each cohort could also be terminated as soon as 5 responses are observed before the total cohort is enrolled or evaluated in that cohort. The maximum sample size will be 35 for each cohort. This design provides 90% power to detect an absolute difference of 16% of response with a type I error of 0.05. There is a 36% chance to stop the trial early for futility under 5% response rate. Tumor assessments for Cohort D will be evaluated by RECIST 1.1 criteria only.

For Cohort C, a total of 21 evaluable patients are planned, respectively. MK-3475 will be considered to be inactive and of no interest for further evaluation if the irPFS rate at 20 weeks is 5% (p_0) or less and considered active if the irPFS rate at 20 weeks is 25% or greater (p_1).

A two-stage Green-Dahlberg design is used for Cohort C. An interim analysis will be conducted in order to assess futility after irPFS at 20 weeks are available for the first 14 evaluable patients. If 0 irPFS are observed, enrollment in Cohort C will be terminated. If at least one patient is free of disease progression at 20 weeks per irRC, an additional 7 evaluable subjects will be enrolled into Cohort C. If 3 or fewer patients are progression free at 20 weeks in the total of 21 evaluable patients enrolled in Cohort C, then the regimen will be considered inactive in that cohort. If 4 or more patients are progression free at 20 weeks, we conclude the regimen is promising and warrants further study this patient population.

This design provides 81% power to detect an improved irPFS rate at 20 weeks from 5% to 25% in Cohort C respectively, a 20% increase, with a type I error of 0.05. There is a 1.8% probability of stopping for futility under the alternative hypothesis p_1 (i.e. incorrectly stopping at the interim when the drug is efficacious) and a 49% probability of stopping for futility under the null hypothesis p_0 (i.e. correctly stopping at the interim when the drug is not efficacious).

As of 28Jan2015, four irPFS at 20 weeks and four responses have been observed in each Cohort A and Cohort C. Since the emerging clinical data suggest promising clinical activity, up to an additional 50 patients will be enrolled in each of these cohorts under a second expansion in Amendment 7. Tumor assessments in the second expansion cohort will be evaluated by RECIST 1.1 criteria only.

13.3 Stratification Factors

We will enroll four cohorts of patients based on disease and MSI or mutator phenotype status: patients with MSI positive colorectal adenocarcinomas (Cohort A); patients with MSI negative colorectal adenocarcinomas (Cohort B); and patients with MSI positive solid tumor malignancies but not colorectal adenocarcinoma (Cohort C); and patients with MSI negative solid tumor malignancies that have a documented mutation burden level measured at $\geq$ 20 mutations per megabase pairs (MB) (Cohort D).

13.4 Analysis of Primary Endpoints

For Cohort A and B, immune-related progression-free survival (irPFS) rate at 20 weeks and objective response rate (irORR) assessed using immune related response criteria (irRC) are co-primary endpoints. irPFS rate at 20 weeks will be estimated as the proportion of patients alive and free of disease progression at 20 weeks per irRC, along with its 95% confidence interval (CI); irORR will be estimated as the proportion of subjects whose best overall response is either a CR or PR with corresponding 95% CI. The patients who drop out of the study due to toxicity and do not have a follow-up scan will be considered non-responders. The formal statistical testing for irORR will take place only if 20-week irPFS is statistically significant. For Cohort C, irPFS will be the primary endpoint.

As of 28Jan2015, four irPFS at 20 weeks and four responses have been observed in each Cohort A and Cohort C. Since the emerging clinical data suggest promising clinical activity, up to an additional 50 patients will be enrolled in each of these cohorts under a second expansion in Amendment 7. Tumor assessments in the second expansion cohort will be evaluated by RECIST 1.1 criteria only, and ORR will be the primary endpoint for the expanded Cohort A and Cohort C.

On 23May2017, FDA approved MK-3475 (pembrolizumab) for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors that are MSI-H or mismatch repair deficient. The recommended dose is 200mg for adults every 3 weeks. After the approval patients may receive this new lower dose. We will continue follow-up of the patients for disease progression and death after FDA approval. The analysis for PFS will be performed in two ways; the primary analysis will censor all patients at the date of the last tumor assessment prior to starting the treatment at the FDA-approved lower dose, if they do not have disease progression at or prior to that date. A secondary analysis will define PFS as the time from the first day of study treatment to the date of the first documented tumor progression or death due to any cause, whichever occurs first, during the entire follow-up period. Subjects who did not progress or die will be censored on the date of their last evaluable tumor assessment.

The evaluable population includes all subjects who receive at least one dose of MK3475 and have MSI results confirmed using the MSI Analysis System from Promega (**section**

3.4). This test will determine MSI status through the insertion or deletion of repeating units in the five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24). At least 2 MSI loci are required to be evaluable in Cohorts A and C. Patients may be assigned to a new cohort and/or replaced based on the Promega test results. With amendment 9, the decision was made to not require retesting with Promega if MSI status is confirmed by IHC or other PCR.

The primary endpoint for Cohort D will be response rate. The evaluable population for Cohort D includes all subjects who receive at least one dose of MK-3475 and have solid tumors that have a documented mutation burden level measured at $\geq$ 20 mutations per megabase pairs (MB) using the Foundation Medicine (FoundationOne.com) targeted sequencing panel.

13.5 Analysis of Secondary Endpoints

Overall survival (OS) is the time from the first day of study treatment with MK-3475 to the date of death due to any cause. A subject who has not died will be censored at last known date alive. Kaplan-Meier curves will be used to summarize OS.

The analyses described below will be conducted on the basis of responses as assessed by both RECIST v1.1 and irRC. Summary statistics will be provided for each cohort as well as for each tumor type for Cohort C. The analysis will also be conducted in PD-L1+ versus PD-L1- protein expression subgroups.

PFS is defined as the time from the first day of study treatment to the date of the first documented tumor progression or death due to any cause, whichever occurs first. Subjects who did not progress or die will be censored on the date of their last evaluable tumor assessment. Kaplan-Meier curves will be used to summarize PFS.

ORR is defined as the proportion of subjects whose best overall response (BOR) from baseline is either a CR or PR. BOR is determined by the best response designation recorded between the date of the first study treatment and the date of objectively documented progression or the date of subsequent anti-cancer therapy, whichever occurs first. For subjects without documented progression or subsequent anticancer therapy, all available response designations will contribute to the BOR determination. For subjects who continue MK-3475 beyond progression, the BOR should be determined based on response designations recorded at the time of the initial progression. DCR is the proportion of patients with CR, PR, or SD.

Among patients with an objective response, DOOR is defined as the time between the date of initial complete or partial response to the date of the first documented tumor progression or death due to any cause. Subjects who neither progress nor die will be censored on the date of their last assessment. TTOR is defined as the time from the first day of study treatment to the date of the first documented CR or PR. DOOR and TTOR will be evaluated for responders (CR or PR) only.

13.6 Safety Analysis

The safety analysis will be performed in all treated subjects. Toxicity will be tabulated by type and grade.

13.7 Biomarker Analysis

Potential relationships between biomarker data and efficacy or safety endpoints will be investigated as part of an analysis plan aimed at identifying baseline biomarkers that may be used to prospectively identify subjects likely (or not likely) to respond toMK-3475 and to identify subjects who may be predisposed to having adverse reactions to treatment. These exploratory predictive biomarker analyses will be completed with biomarkers measured in blood and in tumor samples and will focus primarily- as outlined in the exploratory objectives- on germline mutations and SNPs, and on MSI positive, PD-L1 positivity, and tumor infiltrating lymphocyte in tumor specimens. Similar analyses will be completed with peripheral blood samples. We will also explore standard protein biomarkers such as CEA, CA19-9, and other exploratory circulating biomarkers.

Associations between biomarkers and efficacy measures will be analyzed on all subjects with available biomarker data. Efficacy measures will include OS and standard and immune criteria of response and PFS. Demographic and case-history factors will be examined to determine whether stratification or adjustments should be made within the subsequent statistical analyses, and if necessary, the appropriate stratification or adjustment will be made. Biomarkers will be summarized graphically as they relate to efficacy and safety endpoints, as applicable. Summary statistics will be tabulated. The relationships between binary measures (e.g. response) and candidate biomarkers will be investigated using logistic regression. Associations will be summarized in terms of point and interval estimates of hazard ratios, odds ratios, or other statistics, as appropriate for the analyses completed. Models to predict clinical activity based on combinations of biomarkers may also be investigated.

The dynamics of tumor growth trajectories are often ignored in evaluation of treatment efficacy, which often leads to underpowered studies and biased comparisons. To address this issue, we constructed a Bayesian mixture model of tumor growth based on longitudinal measures of serum biomarkers. This model assumes natural growth and drug induced decay are two independent latent processes underlying tumor growth among responders of treatment, whereas the growth process is the sole driver of tumor growth among non-responders. The data we obtained from collaboration will be used to test the effectiveness of the proposed method in comparing treatment effects based on longitudinal serum biomarkers. This dataset will also help us to develop novel statistical methods to better identify subgroups that are more responsive to a given treatment, where patient subgroup is often defined based on genetic profiles. This work

will be done in collaboration with the Medical University of South Carolina (Dr. Elizabeth Garrett-Mayer and Wei Wei).

Additional post hoc statistical analyses not specified in the protocol, such as alternative modeling approaches may be completed. All analyses described in this section are based on the availability of the data.

REFERENCES

1. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Rüschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res. 1997: 57:4749-56.

2. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998:58:5248-57.

3. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer. 2001:91:2417-22.

4. Grogg KL, Lohse CM, Pankratz VS, Halling KC, Smyrk TC. Lymphocyte-Rich Gastric Cancer: Associations with Epstein-Barr Virus, Microsatellite Instability, Histology, and Survival. Mod Pathol. 2003:16:641-51.

5. Leach FS. Microsatellite instability and prostate cancer: clinical and pathological implications. Curr Opin Urol. 2002:12:407-11.

6. Kouso H, Yoshino I, Miura N, Takenaka T, Ohba T, Yohena T, et al. Expression of mismatch repair proteins, hMLH1/hMSH2, in non-small cell lung cancer tissues and its clinical significance. J Surg Oncol. 2008:98:377-83.

7. Skarda J, Fridman E, Plevova P, Hajduch M, Radova L, Ofek E, et al. Prognostic value of hMLH1 and hMSH2 immunohistochemical expression in non-small cell lung cancer. A tissue microarray study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2006:150:255-9.

8. Agaram NP, Shia J, Tang LH, Klimstra DS. DNA mismatch repair deficiency in ampullary carcinoma: a morphologic and immunohistochemical study of 54 cases. Am J Clin Pathol. 2010:133:772-80.

9. Garg K, Leitao MM Jr, Kauff ND, Hansen J, Kosarin K, Shia J, Soslow RA. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient

age and tumor morphology enhances detection of mismatch repair abnormalities. Am J Surg Pathol. 2009:33:925-33.

10. Maple JT, Smyrk TC, Boardman LA, Johnson RA, Thibodeau SN, Chari ST. Defective DNA mismatch repair in long-term (> or =3 years) survivors with pancreatic cancer. Pancreatology. 2005:5:220-8.

11. Disis ML. Immune regulation of cancer. J Clin Oncol 2010;28(29):4531-8.
12. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8(8):793-800.

13. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nature 2002;2:116-26.

14. Brown JA, Dorfman DM, Ma F-R, Sullivan EL, Munoz O, Wood CR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol 2003;170:1257-66.

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

16. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, et al. PD-1 expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Caner Res 2007;13(6):1757-61.

17. Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, 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(7):2151-7

18. Gao Q, Wang X-Y, Qiu S-J, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res 2009;15(3):971-9.

19. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. PNAS 2007;104(9):3360-5.

20. Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010;116:1757-66.

21. Fourcade J, Kudela P, Sun Z, Shen H, Land SR, Lenzner D, et al. PD-1 is a regulator of NYESO-1-specific CD8+ T cell expansion in melanoma patients. J Immunol 2009;182:5240-9.

22. Cai G, Karni A, Oliveira EML, Weiner HL, Hafler DA, Freeman GJ. PD-1 ligands, negative regulators for activation of naïve, memory, and recently activated human CD4+ T cells. Cell Immunol 2004;230(2):89-98.

23. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: and 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. PNAS 2002;99(19):12293-7.

25. Tsushima F, Tanaka K, Otsuki N, Youngnak P, Iwai H, Omura K, et al. Predominant expression of B7-H1 and its immunoregulatory roles in oral squamous cell carcinoma. Oral Oncol 2006;42:268-74.

26. Homsi J, Kashani-Sabet M, Messina JL, Daud A. Cutaneous melanoma: prognostic factors. Cancer Control 2005;12(4):223-9..

27. Korman A, Chen B, Wang C, Wu L, Cardarelli P, Selby M. Activity of anti-PD-1 in murine tumor models: role of "host" PD-L1 and synergistic effect of anti-PD-1 and anti-CTLA-4 [Abstract]. J Immunol 2007;178:48.37

28. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of Anti□PD-1 antibody in cancer. N Engl J Med 2012:doi: 10.1056/NEJMoa1200690.

29. Patnaik A, Kang SP, Tolcher AW, Rasco DW, Papadopoulos KP, Beeram M, et al. 2012 ASCO Annual Meeting: Phase I study of MK-3475 (anti-PD-1 monoclonal antibody) in patients with advanced solid tumors, 2012.

30. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363(8):711-23.

31. Chapman PB, Hauschild A, Robert C, Hannen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. New Engl J Med 2011;346(26):2507-16.

32. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364(26):2517-26.

33. Bellati F, Visconti V, Napoletano C, Antonilli M, Frati L, Benedetti Panici P, et al. Immunology of gynecologic neoplasms: analysis of the prognostic significance of the immune status. Curr Cancer Drug Targets 2009;9(4):541-65.

34. Ruffell B, DeNardo DG, Affara NI, Coussens LM. Lymphocytes in cancer development: polarization towards pro-tumor immunity. Cytokine Growth Factor Rev 2010;21:3-10.

35. Shirabe K, Motomura T, Muto J, Toshima T, Matono R, Mano Y, et al. Tumor-infiltrating lymphocytes and hepatocellular carcinoma: pathology and clinical management. Int J Clin Oncol 2010;15:552-8.

36. Al-Shibli K, Al-Saad S, Andersen S, Donnem T, Bremnes RM, Busund L-T. The prognistic value of intraepithelial and stromal CD3-, CD117- and CD138-positive cells in non-small cell lung carcinoma. APMIS 2010;118:371-82.

37. Clark CE, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. Cancer Lett 2009;279:1-7.
38. Diederichsen ACP, Hjelmborg JvB, Christensen PB, Zeuthen J, Fenger C. Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLADR expression on tumour cells. Cancer Immunol Immunother 2003;52:423-8.

39. Gao Q, Qiu S-J, Fan J, Zhou J, Wang X-Y, Xiao Y-S, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. J Clin Oncol 2007;25(18):2586-93.

40. Hillen F, Baeten CIM, van de Winkel A, Creytens D, van der Schaft DWJ, Winnepenninckx V, et al. Leukocyte infiltration and tumor cell plasticity are parameters of aggressiveness in primary cutaneous melanoma. Cancer Immunol Immunother 2008;57:97-106.

41. Laghi L, Bianchi P, Miranda E, Balladore E, Pacetti V, Grizzi F, et al. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastatsis: a longitudinal study. Lancet Oncol 2009;10:877-84.

42. Li JF, Chu YW, Wang GM, Zhu TY, Rong RM, Hou J, et al. The prognostic value of peritumoral regulatory T cells and its correlation with intratumoral cyclooxygenase-2 expression in clear cell renal cell carcinoma. BJU Intern 2008;103:399-405.

43. Nemolato S, Fanni D, Naccarato AG, Ravarino A, Bevilacqua G, Faa G. Lymphoepithelioma-like hepatocellular carcinoma: a case report and a review of the literature. World J Gastroenterol 2008;14(29):4694-6.

44. Nobili C, Degrate L, Caprotti R, Franciosi C, Leone BE, Trezzi R, et al. Prolonged survival of a patient affected by pancreatic adenocarcinoma with massive lymphocyte and dendritic cell infiltration after interleukin-2 immunotherapy. Report of a case. Tumori 2008;94:426-30.

45. Oshikiri T, Miyamoto M, Shichinohe T, Suzuoki M, Hiraoka K, Nakakubo Y, et al. Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. J Surg Oncol 2003;84:224-8.

46. Piersma SJ, Welters MJP, van der Burg SH. Tumor-specific regulatory T cells in cancer patients. Human Immunol 2008;69:241-9.

47. Rao UNM, Lee SJ, Luo W, Mihm Jr. MC, 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 2010;133:646-53.

48. Sasaki A, Tanaka F, Mimori K, Inoue H, Kai S, Shibata K, et al. Prognostic value of tumorinfiltrating FOXP3+ regulatory T cells in patients with hepatocellular carcinoma. EJSO 2008;34:173-9.

49. Shen Z, Zhou S, Wang Y, Li R, Zhong C, Liang C, et al. Higher intratumoral infiltrated Foxp3+ Treg numbers and Foxp3+/CD8+ ratio are associated with adverse prognosis in resectable gastric cancer. J Cancer Res Clin Oncol 2010;136:1585-95.
50. Hiraoka N. Tumor-infiltrating lymphocytes and hepatocellular carcinoma: molecular biology. Int J Clin Oncol 2010;15:544-51.

51. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, *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. 2010: 28:3167-75.

52. Parsons DW, Li M, Zhang X, et al. The genetic landscape of the childhood cancer medulloblastoma. Science 2011;331:435-9.

53. Berger MF, Hodis E, Heffernan TP, et al. Melanoma genome sequencing reveals frequent PREX2 mutations. Nature 2012:485:502-6.

54. Lee W, Jiang Z, Liu J, et al. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 2010:465:473-7.

55. Timmermann B, Kerick M, Roehr C, et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. PLoS One 2010:5:e15661.

56. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma Multiforme. Science 2008:321:1807-12.

57. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, et al. Durable Cancer Regression Off-Treatment and Effective Reinduction Therapy with an Anti-PD-1 Antibody. Clin Cancer Res. 2013:19:462-8.

58. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, Vogelstein B, *et al*. Epitope landscape in breast and colorectal cancer. Cancer Res. 2008: 68:889-92.

APPENDIX A: Performance Status Criteria

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

APPENDIX B: Foundation One Tumor Mutational Burden (TMB) Testing

APPENDIX C: Adverse Event of Clinical Interest (ECI) Reporting Form

Adverse Event of Clinical Interest (ECI) Reporting Form

Please notify: [redacted]

Protocol Title:	**Phase 2 Study of MK-3475 in Patients with Microsatellite Unstable (MSI) Tumors**		
Protocol Number (MK-3475-016):	**Signature of PI:**	**Principal Investigator:**	**Date:**

Report Type:

☐ **Initial** ☐ **Follow-up** ☐ **Final Follow-up** ☐ **Addendum to:**

Section A: Subject Information

Subject ID:	**Subject Initial:**	**Subject Gender:** ☐ **Male** ☐ **Female**

Section B: Event Information

Event diagnosis or symptoms:	**Date of First Dose (MK-3475):**	**Action taken with the study drug (MK-3475):** ☐ **None** ☐ **Interrupted** ☐ **Discontinued** ☐ **Delayed**
	Date of Last Dose (MK-3475) prior to Event:	
	Number of Total Doses (MK-3475):	

Event Onset Date:	**Event End Date:**	**Date Event Discovered:**

Relationship to:	**MK-3475**	**Underlying Disease**
Unrelated	☐	☐
Probably Unrelated	☐	☐

Possible Related	☐	☐
Probably Related	☐	☐
Definitely Related	☐	☐

Section C: Brief Description of the Event: (please include relevant procedures and laboratory values)

Section D: Relevant Medical History

Section E: Concomitant Drug (Not related to ECI)

Name of the Drug	Start Date	Stop Date	Route	Dose	Frequency

Section F: Comments

Additional Documents: ☐ Please specify

APPENDIX D: SAE Reporting Form

Serious Adverse Event Reporting Form

Please notify: [redacted]

Protocol Title:	**Phase 2 Study of MK-3475 in Patients with Microsatellite Unstable (MSI) Tumors**		
Protocol Number (MK-3475-016):	**Signature of PI:**	**Principal Investigator:**	**Date:**
☐ **Report Type:** ☐ **Initial** ☐ **Follow-up** ☐ **Final Follow-up** ☐ **Death** ☐ **Addendum to:**	**Serious Criteria (check all that apply):** ☐ Death ☐ Life-threatening ☐ Hospitalization or Elongation of Existing Hospitalization ☐ Persistent or Significant Disability ☐ Congenital Anomoly ☐ Other Important Medical Event ☐ Cancer Overdose	**Hospital Admission Date:** **Hospital Discharge Date:**	**Date Event Discovered:**

Section A: Subject Information		
Subject ID:	**Subject Initial:**	**Subject Gender:** **Male** ☐ **Female** ☐

Section B: Event Information		
Event diagnosis or symptoms:	**Date of First Dose (MK-3475):**	**Action taken with the study drug (MK-3475):** ☐ **None** ☐ **Interrupted** ☐ **Discontinued** ☐ **Delayed**
	Date of Last Dose (MK-3475) prior to Event:	
	Number of Total Doses (MK3475):	

Event Onset Date:	Event End Date:

Relationship to:	MK- 475	Underlying Disease
Unrelated		
Probably Unrelated		
Possible Related		
Probably Related		
Definitely Related		

Section C: Brief Description of the Event:

Section D: Relevant Medical History

Section E: Concomitant Drug (Not related to AE)

Name of the Drug	Start Date	Stop Date	Route	Dose	Frequency

Section F: Comments
Additional Documents: ☐ Please specify

APPENDIX E: Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1 will be used in this study for assessment of tumor response. While either CT or MRI may be used utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable unless there is evidence of progression in the irradiated site. Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to

be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

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

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

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

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

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

Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute

increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

Evaluation of Non-Target Lesions

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

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

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

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

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

Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/NonPD	No	PR	≥4 wks. Confirmation**

CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

Reference

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

APPENDIX F: Immune Related Response Criteria

For all patients who experience disease progression on study, the date noted for of disease progression is the time of the scan where it is originally detected, and not the following date of the confirmatory scan.

Definitions of measurable and non-measurable disease

Measurable disease: Neoplastic masses that can be precisely measured in 2 in-plane perpendicular diameters. Both its longest diameter and its longest perpendicular must be greater than or equal to

10 mm. Lymph nodes must have a short-axis line-length of ≥ 15 mm. Malignant lymph nodes must be measurable in 2 perpendicular diameters. Both its longest diameter and its longest perpendicular must be greater than or equal to 15 mm. The quantitative endpoint will be defined as the product of the longest diameter with its longest perpendicular.

Non-measurable disease: Non-measurable lesions are those that are not suitable for quantitative assessment over time. These include:

1) Neoplastic masses that are too small to measure, because their longest uninterrupted diameter or longest perpendicular are less than 10 mm.

2) Neoplastic masses whose boundaries cannot be distinguished. This includes masses which cannot be demarcated from surrounding tissue because of inadequate contrast, masses with overly complex morphology, or those with highly heterogeneous tissue composition.

3) Other types of lesions that are confidently felt to represent neoplastic tissue, but difficult to quantify in a reproducible manner. These include bone metastases, leptomeningeal metastases, malignant ascites, pleural/pericardial effusions, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, ill-defined abdominal masses, etc.

For irRC, only target lesions selected at baseline and measurable new lesions are taken into account.

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all **index lesions** (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated.

At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (≥ 5 X 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total time-point **tumor burden.**

Overall response using irRC:

- **Complete Response (irCR):** Complete disappearance of all tumor lesions (whether measureable or not, and no new lesions). CR must be confirmed by repeated, consecutive assessments made no less than 4 weeks from the date first documented.

- **Partial Response (irPR):** Decrease in SPD of 50% or greater by a consecutive assessment at least 4 weeks after first documentation.

- **Stable Disease (irSD):** Failure to meet criteria for irCR or irPR, in absence of irPD.

- **Progressive Disease (irPD):** At least 25% increase in SPD relative to nadir (minimum recorded tumor burden) Confirmation by a repeat, consecutive assessment no less than 4 weeks from the data first documented.

Please note other key differences between irRC and the original WHO criteria:

New measurable lesions will be incorporated into the SPD

New non measurable lesions do not define progression but preclude irCR

Non-index lesions contribute to defining irCR (complete disappearance required).

REFERENCE

IrRC for the current protocol is adopted from the following reference:

Wolchok, JD, Hoos, A, O'Day S, et al., Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clinical Cancer Research, 2009 Dec 1;15(23):7412-20. Epub 2009 Nov 24.

APPENDIX G: Data Safety Monitoring Plan (DSMP)