SUMMARY OF CHANGES

For Protocol Amendment 9, Version 2.0

NCI Protocol #: CITN-09
Local Protocol #: CITN-09

Protocol Date: May 22, 2018

I. Changes Requested by CTEP

#	Section	Pages	Revision

1.	7.1.1	71-76	Updated CAEPR content to version 2.4 per CTEP Request for Rapid Amendment dated 4/26/18. Updated risks following CAEPR table. The following changes are incorporated:
			• Added New Risk:
			  • Less Likely: Immune system disorders - Other (sarcoidosis)
			  • Rare but Serious: Immune system disorders - Other (acute graft-versus-host-disease); Metabolism and nutrition disorders - Other (diabetic ketoacidosis); Metabolism and nutrition disorders - Other (type 1 diabetes mellitus); Nervous system disorders - Other (non-infectious encephalitis); Nervous system disorders - Other (non-infectious meningitis); Palmar-plantar erythrodysesthesia syndrome
			• Increase in Risk Attribution:
			  • Changed to Less Likely from Also Reported on MK-3475 Trials But With Insufficient Evidence for Attribution: Abdominal pain; Back pain; Cough; Hyponatremia
			• Provided Further Clarification:
			  • Footnotes have been reordered.
			  • Footnote #2 has been updated and now reads: "Immune-mediated adverse reactions have been reported in patients receiving MK-3475 (pembrolizumab). Adverse events potentially related to MK-3475 (pembrolizumab) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of MK-3475 (pembrolizumab), administration of corticosteroids and supportive care."
			  • Footnote #3 has been added and reads: "Acute graft-versus-host disease has been observed in patients treated with MK-3475 (pembrolizumab) who received hematopoeitic stem cell transplants."
			  • Thrombotic thrombocytopenic purpura, previously listed under the IMMUNE SYSTEM DISORDERS SOC *(CTCAE 4.0 language)*, is now listed under the BLOOD AND LYMPHATIC SYSTEM DISORDERS SOC.

#	Section	Pages	Revision
			• Endocrine disorders - Other (hypophysitis, hypopituitarism) *(CTCAE 4.0 language)* is now reported as Hypophysitis and Hypopituitarism. • Infusion related reaction, previously listed under the GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS SOC *(CTCAE 4.0 language)*, is now listed under the INJURY, POISONING AND PROCEDURAL COMPLICATIONS SOC. • Nervous system disorders - Other (Guillain-Barre syndrome) *(CTCAE 4.0 language)* is now reported as Guillain-Barre syndrome. • General disorders and administration site conditions - Other (generalized edema) *(CTCAE 4.0 language)* is now reported as Generalized edema • Renal and urinary disorders - Other (nephrotic syndrome) *(CTCAE 4.0 language)* is now reported as Nephrotic syndrome

II. Comments Requiring a Response– Administrative & Editorial Issues:

#	Section	Comments
2.	7.1.1	In the CAEPR table, "Erythroderma" should not have the footnote 2 attached. **PI Response:** Agree. Corrected.
3.	7.1.1	GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS is missing the G. **PI Response:** Agree. Corrected.

III. Changes Initiated by P.I.

2.	Title page	Revised Amendment number, version number and date.
3.	Through out	Revised date in header.

NCI Protocol #: CITN-09: Merkel Cell Carcinoma – MK-3475

Local Protocol #: CITN-09: Merkel Cell Carcinoma – MK-3475

TITLE: A Phase II Study of MK-3475 in Patients with Advanced Merkel Cell Carcinoma (MCC)

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Study Coordinator: N/A

Responsible Research Nurse: N/A

Responsible Data Manager: N/A

NCI-Supplied Agent(s): MK-3475 (pembrolizumab, SCH 900475) (NSC # 776864).

Other Agent(s): N/A

Non-NCI Supplied Agent(s)/Supplier(s): N/A

IND Sponsor: CTEP

Protocol Type / Version # / Version Date: Amendment 9/Version 2.0/May 22, 2018

IND #: 123618

IND Sponsor: DCTD, NCI

<table>
<tr><th colspan="3">CONTACT INFORMATION</th></tr>
<tr><th>To submit site registration documents:</th><th>For patient enrollments:</th><th>To submit study data</th></tr>
<tr><td>Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal:

Regulatory Submission Portal (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.)

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support.

Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.</td><td>Please refer to the patient enrollment section for instructions on using the Oncology Patient Enrollment Network (OPEN) at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org.

Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.</td><td>Data collection for this study will be done via Medidata Rave (FHCRC/AXIO Research, Inc.). Please refer to the data submission section of the protocol for Medidata Rave instructions.</td></tr>
<tr><td colspan="3">The most current version of the study protocol and all supporting documents must be downloaded from the protocol-specific Web page of the CTSU Member website located at https://www.ctsu.org. Access to the CTSU members' Web site is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.</td></tr>
<tr><td colspan="3"><u>For clinical questions (i.e. patient eligibility or treatment-related):</u>
<u>Contact the CITN Central Operations and Statistical Center at citn@fhcrc.org or 206-667-7607</u></td></tr>
<tr><td colspan="3"><u>For nonclinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission)</u> contact the CTSU Help Desk by phone or email:
CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.</td></tr>
<tr><td colspan="3">The CTSU Web site is located at https://www.ctsu.org.</td></tr>
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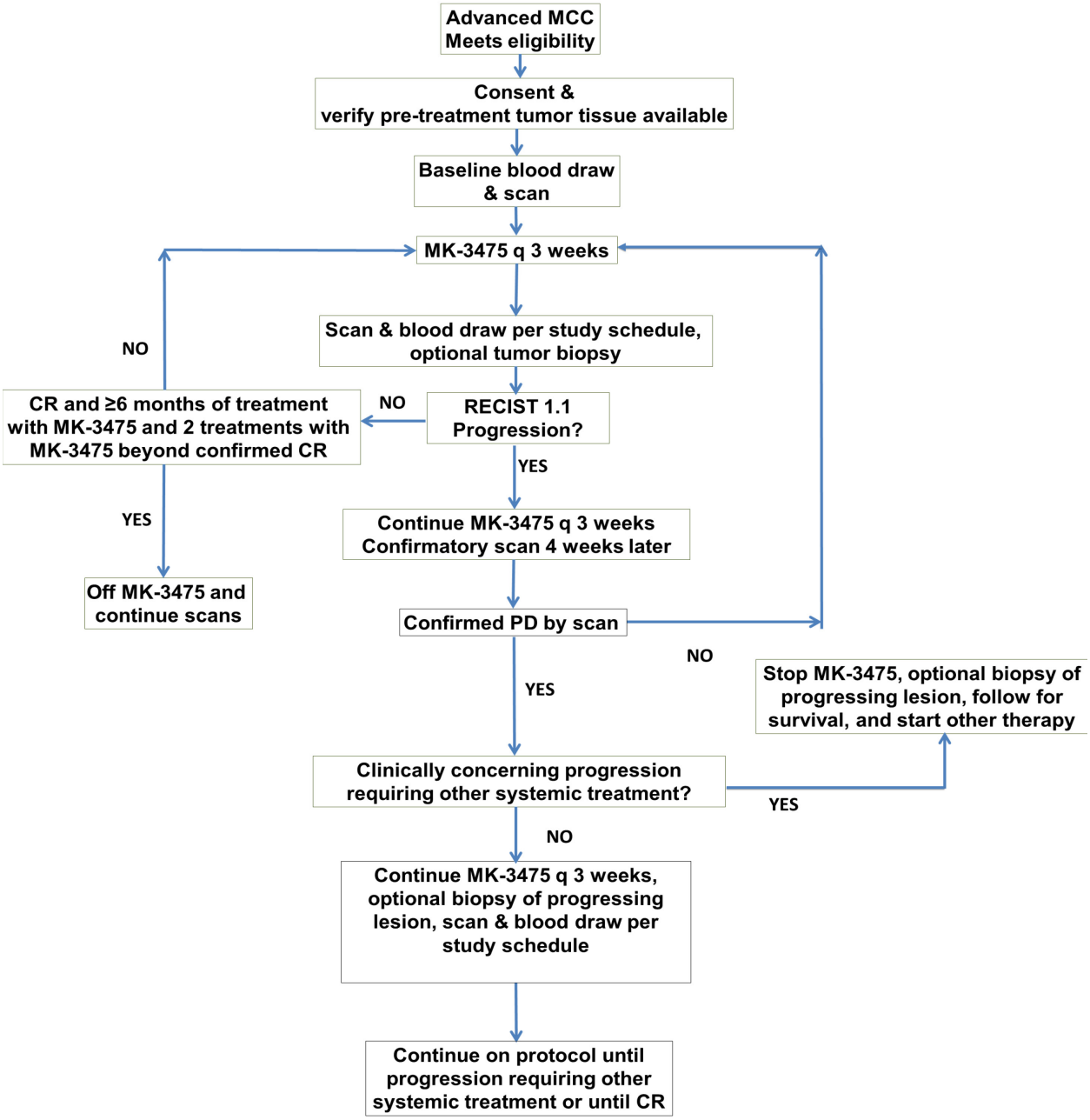
STUDY SUMMARY

Abbreviated Title	Merkel Cell Carcinoma (MCC) Therapy with MK-3475
Trial Phase	Phase II
Clinical Indication	Advanced stage MCC: Locally advanced or metastatic
Trial Type	Single Arm, Simon Two-Stage, Therapeutic
Type of control	Nonrandomized trial with primary end point of overall response rate (ORR). Secondary endpoint of progression-free survival (PFS) will be compared to recent historical controls receiving standard chemotherapy.
Route of administration	Intravenous (IV)
Trial Blinding	None
Treatment Group	MK-3475, 2 mg/kg every 3 weeks (q3wks)
Number of trial patients	Initial enrollment included 26 patients. Twenty-four (24) additional patients -were enrolled to provide an expanded safety and efficacy profile.
Estimated duration of trial	The first 26 patients were enrolled over 11 months. The additional 24 patients were enrolled over the following 18 months. Study to continue for up to 2 years beyond enrollment plus follow-up of last subject.
Duration of Participation	Each patient will participate in the trial from the time the Informed Consent Form (ICF) is signed through final protocol-specified contact. After a screening phase for eligibility, patients will receive MK-3475 every 3 weeks. Treatment for patients that achieve a stable disease (SD) or a partial response (PR) can continue for a maximum of 2 years. If progression is confirmed at week 17 or beyond, patients will be eligible to continue treatment with MK-3475 if otherwise clinically stable. Patients that achieve a complete response (CR) can discontinue treatment after 6 months of therapy provided the patient has had at least 2 cycles past validation of CR. At the discretion of the investigator, these patients will be eligible for retreatment if they experience disease progression, as long as they meet the criteria for retreatment and the trial is ongoing. Patients who have received 2 years of treatment on study and have a PR or SD and then progress, may be eligible for retreatment. Administration of MK-3475 will be stopped with: documented disease progression warranting alternative systemic therapy, ≥25% increase in tumor burden following initial confirmation of PD, unacceptable adverse event(s), intercurrent illness that prevents further administration of

	treatment, investigator's decision to withdraw the patient, patient withdraws consent, pregnancy of the patient, noncompliance with trial treatment or procedure requirements, or administrative reasons. After the end of treatment, each patient will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment with investigator attribution as to whether the SAE is associated with MK-3475 or subsequent therapies). After the Post-Treatment Safety Follow-Up Visit, patients will be followed for survival via phone contact every 12 weeks. Patients who discontinue study therapy without documented disease progression should continue to be monitored for disease status by radiologic imaging according to the guidelines described in the Study Schema for post-treatment follow-up.

Overall Trial Schema **(PD1 Trial Timeline_v10Jun16)**



PD1OverallTrialSchema10Jun16.pptx

Patient Visit Timeline (dosing, scans and biomarkers)

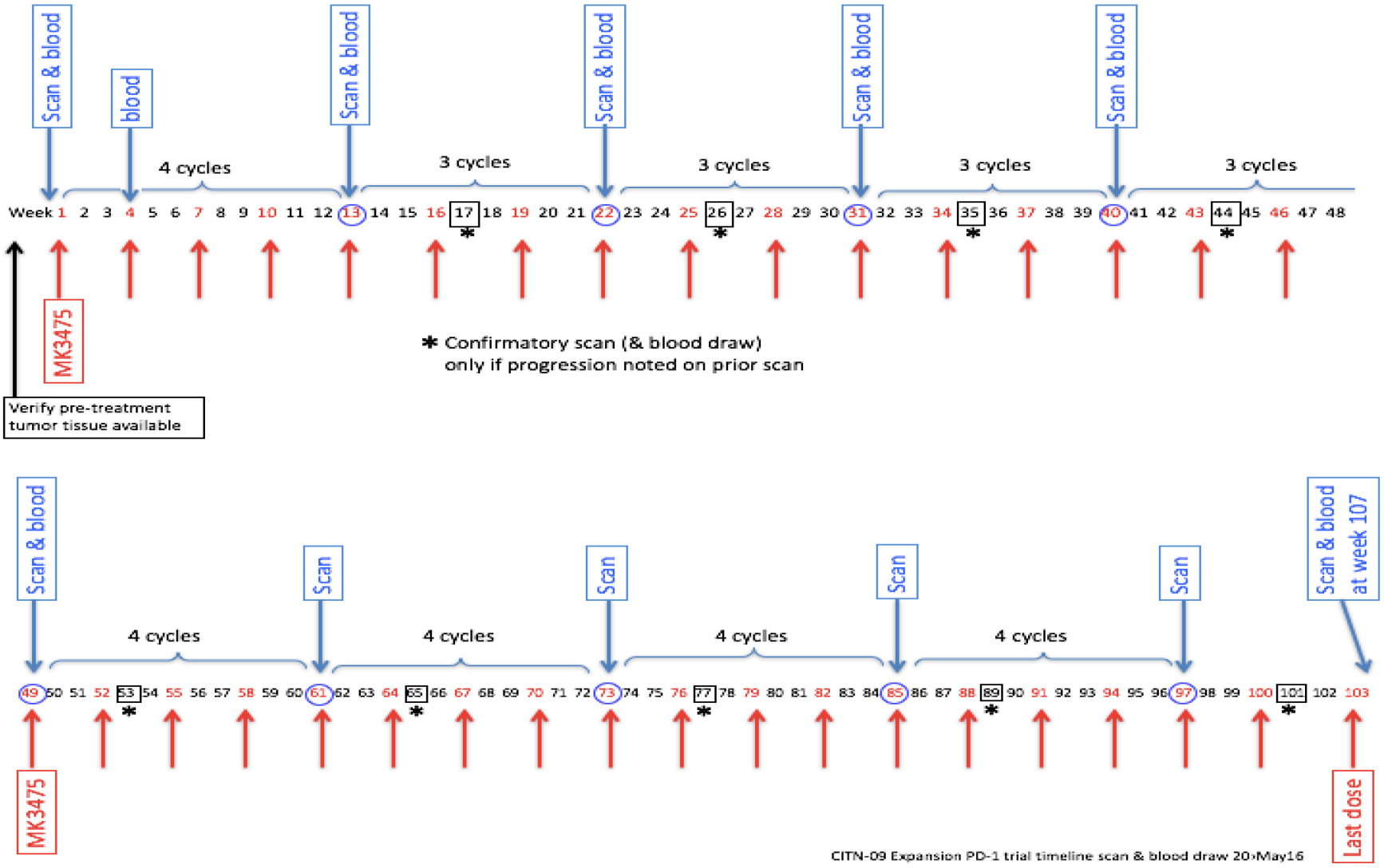


TABLE OF CONTENTS

1. OBJECTIVES

The goal of this trial is to determine the antitumor efficacy of MK-3475 in patients with advanced Merkel cell carcinoma (MCC). The trial is an open-labeled, non-randomized trial with a Simon two-stage design. Patients who have not received any previous systemic therapy for their disease will be treated with MK-3475 2 mg/kg administered every 3 weeks. Objective responses and disease progression will be monitored by computed tomography (CT) scans and assessed by Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1).

1.1 Primary Objectives

To determine the clinical efficacy of MK-3475 as the first systemic intervention for patients with advanced MCC.

The primary endpoint will be objective response rate (ORR) as measured by RECIST 1.1.

Hypothesis: Cellular immunity plays a major role in the outcome of MCC. Thus, therapy with MK-3475 will induce substantial objective responses and a portion of the responses will be durable.

1.2 Secondary Objectives

To determine the clinical activity of MK-3475 as the first systemic intervention for patients with advanced MCC.

The secondary endpoints will be progression-free survival (PFS), duration of response (DOR) and overall survival (OS), as measured by RECIST 1.1. The PFS will be determined at 16 months and compared to the historical PFS for patients treated with chemotherapy, which is 5% at 16 months.

- Progression-free survival (PFS)
- Duration of response (DOR)
- Overall survival (OS)

Hypothesis: Because of the strong links between cellular immunity and MCC survival, responses to MK-3475 are likely to be more durable, as assessed at 16 months, than responses to the selected comparator, recent historical controls treated with systemic chemotherapy, which tends to be immunosuppressive.

1.3 Exploratory Objectives

To determine the immune correlates of the clinical activity of MK-3475

The endpoints will include immunohistochemical (IHC) and gene expression analysis, whole exome sequencing, and neoantigen identification focusing on delineating the immune components and immunologic milieu within the tumor before therapy. The IHC analytes will include, but are not limited to Merkel cell polyomavirus (MCPyV) tumor antigen (T-Ag), programmed cell death 1 (PD-1), and its ligand 1 (PD-L1), CD8, CD3, CD68, CD45, and CD56. In addition, MCPyV viral DNA will be quantified in the tumor and T cell receptor sequencing in tumor infiltrating lymphocytes (TIL) will be performed where pre- and post-treatment biopsies

are available. Cellular and humoral MCPyV-specific immune responses will be assessed with Enzyme-Linked ImmunoSpot (ELISpot) serology assays in blood samples at baseline, after initiating MK-3475 therapy, and correlated with clinical responses over time. Among patients with corresponding major histocompatibility complex (MHC)-peptide tetramers, pre-and post-treatment samples of circulating MCPyV-specific CD8 T cells will be isolated by flow cytometry and subjected to deep immunophenotyping by messenger RNA (mRNA) expression analysis.

Hypothesis: MK-3475 will enhance functional activity of MCC-specific lymphocytes and facilitate tumor regression. Thus, the clinical endpoints associated with tumor regression and durability of response will be correlated with the pretreatment levels and type of immune cell infiltration in the tumor, PD-1 and PD-L1 expression and association in the tumor, or systemic measures of immune response to tumor-specific viral MCPyV antigens.

2. BACKGROUND

2.1 Study Disease

MCC is an aggressive skin cancer with a 5-year disease-associated mortality of 46% [Lemos 2010]. Nearly 50% of patients with MCC develop distant disease typically within 2 to 3 years of diagnosis. Systemic chemotherapy is commonly used, but is fairly toxic with limited, transient efficacy. The reported incidence of MCC has quadrupled in the past 20 years to an estimated ~1600 cases/year in the United States [Hodgson 2005; Albores-Saavedra 2010]. This increasing incidence is partly due to improved detection using a specific IHC marker, cytokeratin-20,[Moll 1992] but may also be due to the higher prevalence of known risk factors for MCC: chronic T-cell immune suppression[Penn 1999; Engels 2002; Heath 2008] and the number of Caucasians over 50 years of age with extensive prior sun exposure[Heath 2008]. Despite its growing health impact and aggressive behavior, very few clinical trials have focused on this disease. There is an unmet need for biology-driven therapy in MCC.

MCC is causally associated with the MCPyV [Feng 2008, Houben 2010, Rodig 2012]. The viral T-Ag oncoproteins, present in approximately 80% of MCC tumors, are required for ongoing tumor growth [Houben 2010]. The cellular immune response is strongly linked to outcome in MCC. Specifically, intratumoral CD8+ lymphocyte infiltration is associated with improved survival independent of stage at presentation [Paulson 2011]. MCPyV-specific T cells have been isolated from the blood and tumors of patients with MCC and track with disease burden.

PD-1 is likely playing a key role in MCC immune evasion in many patients [Afanasiev 2013]. The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T cells is to downmodulate unwanted or excessive immune responses, including autoimmune reactions. Over 90% of MCPyV-specific T cells express PD-1. The fact that PD-1 expression is high on these T cells, in combination with frequent expression of related T-cell immunoglobulin mucin-3 (TIM-3), suggests T-cell exhaustion/dysfunction is prevalent. Although these virus-specific T cells are present in many patients with MCC, they often fail to control progression of MCC, potentially due to immune evasion and exhaustion.

2.1.1 *Merkel cell polyomavirus (MCPyV) and MCC*

The discovery of the MCPyV in 2008, and its causal association with ~80–95% of

MCCs, [Feng 2008; Houben 2010; Rodig 2012] has provided new insight into MCC pathogenesis and underscores the importance of characterizing MCPyV-specific immune responses. MCC typically requires persistent expression of immunogenic polyomavirus T-Ag oncoproteins for ongoing growth and survival [Houben 2012]. The necessary and persistent [Houben 2010] expression of MCPyV T-Ag oncoproteins in MCC tumors provides a unique opportunity, not available for most cancers, to study antitumor immunity and immune evasion by assessing responses against virus-derived, tumor-specific antigens.

2.1.2 *Cellular Immunity and MCC*

There is substantial evidence that cellular immune function is important for survival from MCC. Intratumoral CD8+ lymphocyte infiltration is associated with improved survival as first reported by the Nghiem group [Paulson 2011]. This association has been validated in a second, large, independent cohort[Sihto 2012]. In a similar vein, patients with profound T-cell dysfunction,[Penn 1999; Engels 2002; Heath 2008] such as hematologic malignancies, HIV/AIDS, and immunosuppressive medications for autoimmune disease or transplant, have 10- to 30-fold increased MCC risk, and MCC sometimes spontaneously regresses after improvement in immune function in those patients. Moreover, patients with systemic immune suppression have decreased median survival in MCC independently of stage [Paulson 2013].

2.1.3 *Cellular Immune Response against MCPyV in MCC*

The persistent expression of MCPyV oncoproteins in MCC provides a unique opportunity to characterize immune evasion mechanisms in this cancer. Patients with MCPyV-positive MCC tumors frequently have T cells specific for these persistently expressed oncoproteins [Iyer 2011]. We hypothesized that these MCPyV-specific T cells, although present in the blood of patients with MCC, were unable to eliminate MCC tumors, either because they were dysfunctional and/or due to the other immune evasion mechanisms. We determined the presence and functional status of tumor-infiltrating lymphocytes (TIL, n=7) and virus-specific peripheral blood T cells from 18 patients with MCC and 10 control patients. MCPyV-specific CD8 T cells were detected directly ex vivo from the blood of 7 of 11 (64%) patients with MCPyV-positive tumors. In contrast, 0 of 10 control patients had detectable levels of these cells in their blood ($P<0.01$) [Afanasiev 2013]. MCPyV-specific T cells in serial blood specimens increased with MCC disease progression and decreased with effective disease therapy. Because T-cell number increases with disease burden, there is a need for careful interpretation of immunotherapy efficacy data aimed at increasing tumor-specific T-cell frequency. The presence and expansion of MCC-specific T cells with increasing tumor burden is highly suggestive that tumor immune escape mechanisms are active in MCC.

2.1.4 *Active Immune Evasion Mechanism in MCC*

In MCC tumors, PD-L1 expression within the tumor microenvironment has been observed and is positively correlated with the number of infiltrating CD8 lymphocytes using both histologic and mRNA-based analyses in independent cohorts [Afanasiev 2013; Lipson 2013]. Over 90% of MCPyV-specific T cells express PD-1 (as compared with approximately 30% of all CD8+ T cells, approximately 50% of cytomegalovirus [CMV]-specific T cells, and approximately 80% of Epstein-Barr virus [EBV]-specific T cells).

Furthermore, PD-1 surface expression on MCPyV-specific T cells is >2-fold higher compared to EBV- or CMV-specific T cells and it ligand, PD-L1, was present in 9 of 13 (69%) of MCC tumors [Afanasiev 2013]. PD-1 and TIM-3 are simultaneously co-expressed on the majority MCC-specific T cells, a combination that is often associated with dysfunction [Fourcade 2010; Sakuishi 2010]. T-cell dysfunction mediated by surface expression of these inhibitory molecules on T cells may, at least in part, explain why MCC tumors grow despite the presence of an immune response. These findings are consistent with prior reports indicating that chronic antigen exposure to tumor and viral antigens leads to the upregulation of inhibitory receptors such as PD-1 and TIM-3, which results in reversible T-cell dysfunction [Barber 2006; Jones 2008; Fourcade 2010; Greenough 2010; Wherry 2011]. In contrast to prior studies of virus- or tumor-specific CD8-T cells that show upregulation of PD-1 with acute infection[Greenough 2010] or with increasing tumor stage,[Kronig 2012] PD-1 expression on MCPyV-specific T cells was maintained at high levels throughout the MCC disease course even during declining T-cell absolute numbers during remission [Afanasiev 2013].

In melanoma, most circulating melanoma-specific CD8 T cells have been reported to be functionally active, whereas those derived from tumor lesions are functionally tolerant [Zippelius 2004]. In contrast, in MCC, T cells derived from the blood and tumor compartments have similar phenotypic profiles, both indicative of T-cell dysfunction [Afanasiev 2013]. Activation and inhibitory receptors tend to be induced simultaneously on MCC-specific T cells, consistent with chronic antigen exposure likely triggering immune tolerance, in agreement with prior studies of HIV-specific CD8 T cells[Trautmann 2006; Sauce 2007].

PD-1 upregulation on tumor-targeting CD8 T cells is particularly relevant if its ligand is expressed within the tumor microenvironment, which can lead to inhibitory pathway activation. PD-L1 is selectively expressed on many tumors[Hamanishi 2007; Zou 2008; Hino 2010] and on cells within the tumor microenvironment [Curiel 2003] and blockade of the PD-1/PD-L1 pathway has been recently shown to effectively induce durable tumor regression and stabilization of disease in a subset of several diverse types of cancer[Sakuishi 2010; Fisher 2012].

In summary, MCPyV-specific T cells: (1) dynamically correlate in frequency with clinical disease burden and with antibodies against the viral oncoprotein (T-Ag), (2) co-express markers of exhaustion, PD-1 and TIM-3, at far higher levels than T cells specific for other common human viruses such as EBV, (3) display minimal interferon gamma (IFN-γ) production in response to cognate peptide, which can be augmented after culture with inhibitory receptor blocking agents, and (4) are likely to encounter the relevant inhibitory receptor ligand, PD-L1, within the MCC tumor microenvironment.

2.1.5 *MCC as a Candidate for Testing MK-3475*

MK-3475 is a potent and highly selective humanized monoclonal antibody (mAb) of the immunoglobin G4 (IgG4)/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target T cells to facilitate tumor regression and ultimately immune rejection.

The expression of PD-1 and PD-L1 support the testing of the antibody to PD-1 (anti-PD-1) as a targeted approach to overcome tumor immune evasion mechanisms in MCC. The direct relationship of T-cell and antibody responses to the burden of MCC provide clinical biomarkers of disease status and may also serve as a potential indicator of response to anti-PD-1.

2.1.6 *Orphan Disease with Important Unmet Medical Needs*

Despite the strong rationale for investigation of immunotherapy in this virus-associated cancer, there are no systemic immune therapies approved or currently being investigated [Miller 2013]. The chances of successful immunotherapy for MCC are fairly high, in part due to its obligate viral protein expression [Houben 2010].

2.1.7 *Treatment with Cytotoxic Chemotherapy*

There are no FDA-approved therapies for MCC. However, systemic chemotherapy has been commonly used to manage metastatic MCC, despite toxicity and limited, transient efficacy. The National Comprehensive Cancer Network (NCCN) Merkel Cell Carcinoma Guidelines Version 1.2012 [Page MS-4] states "Data are insufficient to assess whether chemotherapy regimens improve either relapse-free or overall survival in MCC patients with distant metastatic disease." Data from patients treated by the Nghiem group at the Fred Hutchinson/University of Washington Cancer Consortium (FH/UW Consortium) shows that responders have a prolonged PFS compared to nonresponders, but without randomized data for the disease there is no firm evidence that chemotherapy improves OS.

If chemotherapy is used, the NCCN recommends cisplatin or carboplatin with or without etoposide [NCCN 2012]. Most patients with metastatic MCC will respond to chemotherapy, but will progress between 3 and 5 months and have a median time to death of 9 months after initial metastasis. Accordingly, the NCCN concludes "Clinicians should exercise independent medical judgment in choosing the chemotherapeutic regimen. Although the NCCN panel recognized that MCC is a rare disease that precludes robust randomized studies, enrollment in clinical trials is encouraged whenever available and appropriate."

The MCC team at the FH/UW Consortium has analyzed data from 58 patients in the Seattle cohort who developed distant metastatic disease, received first-line chemotherapy, and had evaluable metastatic lesion(s) that were not also treated with radiation or surgery (allowing the effects of chemo-mono-therapy to be evaluated). The median time to progression following chemotherapy in the Seattle cohort with "-platin"–based regimens was 98 days for the whole treated population and 145 days for the responding patients (details below).

TOTAL PATIENTS RECEIVING FIRST-LINE CHEMOTHERAPY (n = 58)

Complete response (CR): 8 of 58 (14%)
Partial response (PR): 24 of 58 (41%)
Stable disease (SD): 4 of 58 (7%)
TOTAL CR/PR/SD: 36 of 58 (64%)

Total Progressed at time of analysis (November 2013): 56 of 58 (97%)
Total not progressed 2 of 58 (1 CR and 1 PR) (3%)

Time to progression among all 58 evaluable chemo-treated cases:
50% progressed (Median time from start of chemotherapy) = 98.5 days
80% progressed = 222 days
90% progressed = 290 days
95% progressed = 428 days

Note*:* the progression from 0% to 95% looks like a straight line on a semi-log plot with no evidence of a plateau.

TOTAL PATIENTS THAT RESPONDED TO FIRST-LINE CHEMOTHERAPY (CR/PR/SD) (n = 36)

Progressed: 34 of 36 (94.4%)
Not progressed: 2 of 36 (5.5%) (1 CR and 1 PR)

Time to progression among "chemo responders":
50% (median) progressed 145 days
    50% (median) of CR patients = 303 days
    50% (median) of PR patients = 145 days
    50% (median) of SD patients = 132 days
80% progressed = 288 days
90% progressed = 316 days
94.4% progressed = 466 days

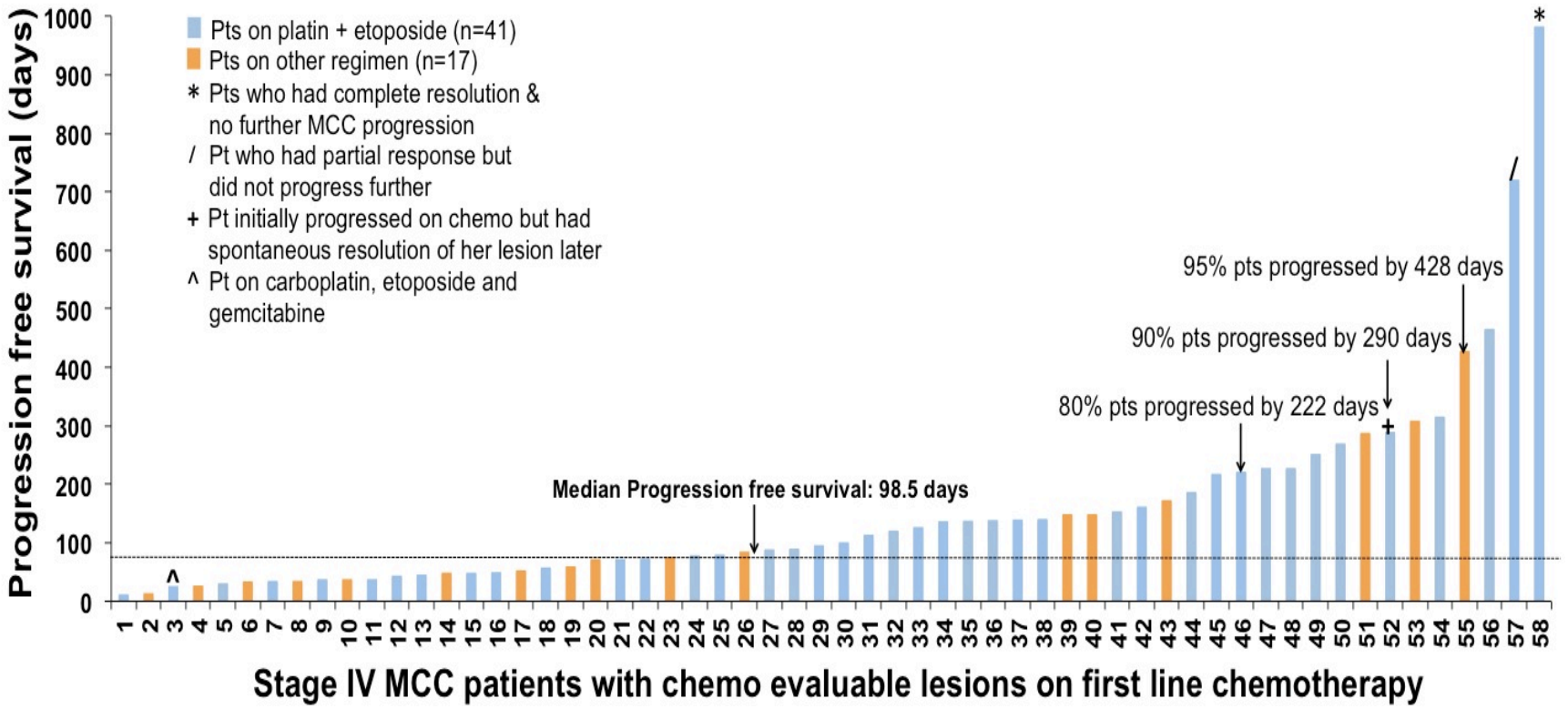


Figure 1: First-line chemotherapy and progression-free survival (PFS) in MCC patients with metastatic disease.
These data are derived from 58 patients in the Seattle cohort who developed distant metastatic disease, received first-line chemotherapy, and had evaluable metastatic lesion(s) that were not also treated with radiation or surgery (allowing the effects of chemo-mono-therapy to be evaluated). The vertical axis represents the number of DAYS between the start of first-line chemotherapy and progression (or death). Etoposide was combined with a "platin" in the 40 cases shown as blue bars. Among these 40 cases, the "platin" was carboplatin in 29 cases and cisplatin in 11. Patients 57 and 58 (asterisks) are the only ones who did not progress on chemotherapy or die, as of most recent data. Median PFS was 98.5 days from start of chemotherapy to progression or death. These 58 patients are a subset of the 179 patients we recently reported in which median OS was 289 days (9.5 months) from the date when distant disease was identified (Figure 3).

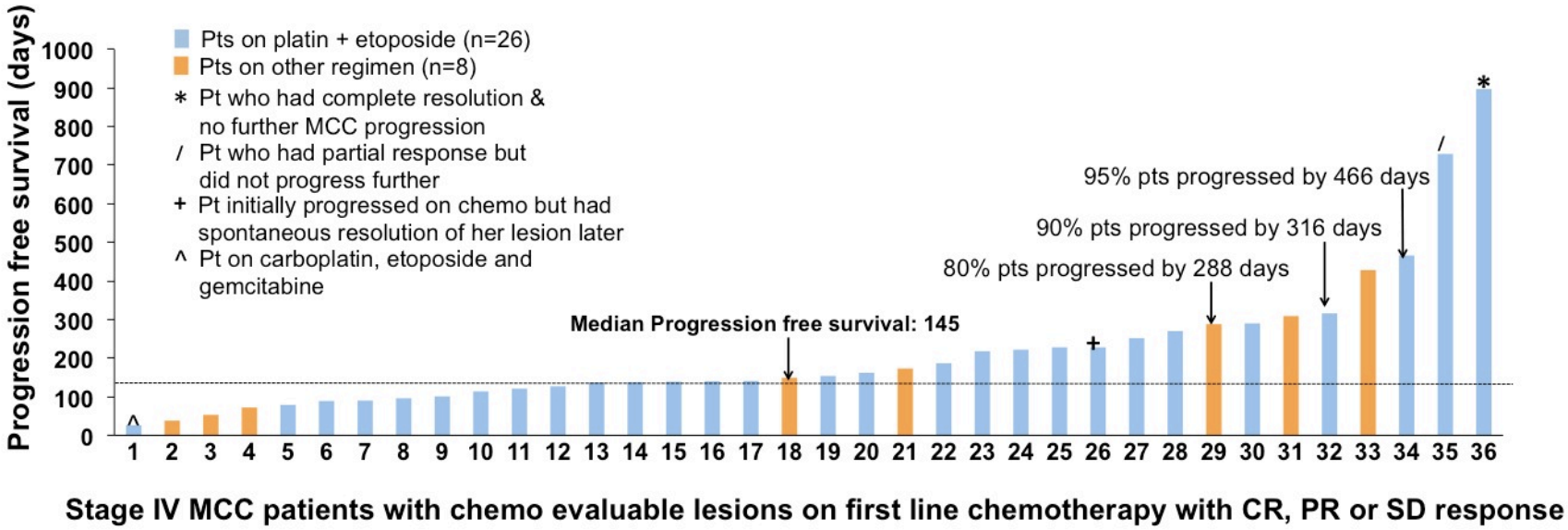


Figure 2: Progression-free survival (PFS) in MCC patients with metastatic disease, treated with first-line chemotherapy and responded with CR, PR, or SD.
These data represent the 36 "chemo responders" among the full cohort of 58 chemotherapy-treated patients in the Seattle cohort shown in Figure 1. Specifically, these 36 patients developed distant metastatic disease, received first-line chemotherapy, and achieved a complete or partial response. The vertical axis represents the number of DAYS between the start of first-line chemotherapy and progression (or death). Median PFS was 145 days from start of chemotherapy to progression or death. These 36 patients are a subset of the 179 patients with distant disease shown in Figure 3.

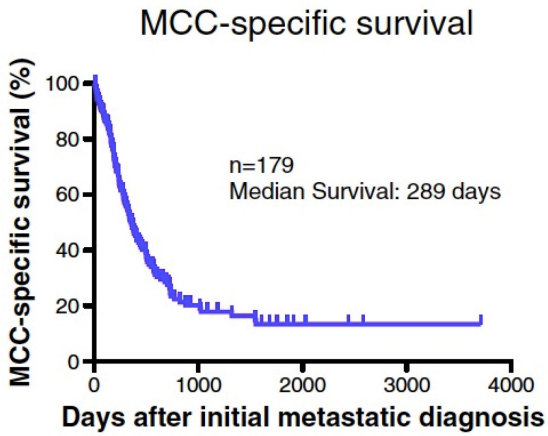


Figure 3: Disease-specific survival in patients who developed metastatic MCC.
Survival data are shown from 179 patients with metastatic MCC who were followed at the FH/UW Consortium [Miller 2013]. Median survival was 289 days from initial diagnosis of metastatic disease. When measured starting at the time of developing metastatic disease, there were no significant differences in survival based on the initial stage at presentation (data not shown). However, stage greatly influenced the likelihood of and median time to developing metastatic disease.

Given that cytotoxic chemotherapy-induced responses are typically very short in duration and there is no proven beneficial effect of cytotoxic chemotherapy on OS, the proposed trial will be conducted in chemotherapy-naïve patients and patients who received prior adjuvant chemotherapy more than 6 months before study participation. The likelihood of success with MK-3475 should be greater if given to patients who are immunocompetent (more likely in chemotherapy-naïve patients). In general, response rates and DOR are expected to be diminished with each subsequent line of therapy. This was indeed the case for MCC, with median PFS of 98 days in first line that decreased to 61 days in second-line chemotherapy, and overall response rate that fell from 54% to 23% in second line (Iyer, Nghiem, et al, unpublished). Finally, the antitumor activity of PD-1 in several malignancies, melanoma, renal cell carcinoma (RCC), and non–small cell lung cancer (NSCLC), is greater than the efficacy of chemotherapy in MCC, further supporting the study of anti-PD-1 as first-line therapy. Subjects who are treated with anti-PD-1 therapy are therefore unlikely to give up a substantially meaningful benefit from front-line chemotherapy. This will be explained to patients in detail in the consent form.

Radiation therapy is commonly used to treat MCC, but is not considered to be a systemic therapy in this protocol. Radiation therapy is considered useful as a treatment option for all stages of MCC [NCCN 2015]. For radiation as adjuvant to surgery, a meta-analysis comparing surgery alone with surgery plus adjuvant radiation revealed that the use of adjuvant radiation after complete excision lowered the risk of local and regional recurrences [Lewis 2006]. For radiation to the primary tumor or regional lymph nodes, a review of 83 cases diagnosed between 1992 and 2004 showed that administration prolonged time to recurrence and survival [Jabbour 2007]. For primary therapy, radiation is considered acceptable in select cases when complete excision is not feasible or refused by the patient. An Australian retrospective review of 43 patients reported an in-field tumor control rate of 75% using radiation alone [Veness 2010]. Radiation may also be

useful in the palliative setting.

2.2 CTEP IND Agent: MK-3475

MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

PD-1 is an immune-checkpoint receptor expressed on T cells that can suppress antitumor immunity when bound to either of its ligands, PD-L1 or PD-L2. Some tumor cells upregulate the PD-1 ligands to evade active T-cell immune surveillance. MK-3475 is a potent and highly selective humanized mAb designed to directly block the interaction between PD-1 and its ligands, thereby enhancing tumor regression and ultimately immune rejection.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to downmodulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4), which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [Usubutun 1998; Talmadge 2007].

The structure of murine PD-1 has been resolved [Al-Shibli 2008]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail, which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). After T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ, and ZAP70, which are involved in the CD3 T-cell signaling cascade [Diez 1998; Galon 2006; Talmadge 2007; Deschoolmeester 2010].

The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [Nobili 2008; Hiraoka 2010]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T cells, B cells, T regulatory cells (T regs), and natural killer (NK) cells [Kloor 2009; Hodi 2010]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T cells as well as subsets of macrophages and dendritic cells [Hillen 2008]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including nonhematopoietic tissues as well as in various tumor [Nishimura 2000; Lee 2008; Leffers 2009; Hiraoka 2010]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various nonhematopoietic 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 [Hiraoka 2010]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in patients with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

In mouse models, blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-γ, granzyme B, and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [Korman 2007]. In addition, the combination of gemcitabine and anti-PD-L1 mAb demonstrated synergy in the rejection of pancreatic mouse tumors [Nomi 2007]. Merck in-house experiments have confirmed the in vivo efficacy of PD-1 blockade as a monotherapy. Therapeutic studies in mouse models show that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T cells and leads ultimately to tumor rejection, either as a monotherapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 have demonstrated antitumor responses as a monotherapy in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, and colorectal carcinoma. Blockade of the PD-1 pathway effectively unleashes a T-cell response when used alone as well as in combination with chemotherapy in syngeneic mouse tumor models.

MK-3475 is a humanized IgG4 anti-PD-1 mAb with similar preclinical characteristics as BMS-936558. Both MK-3475 and BMS-936558 contain the S228P stabilizing mutation. MK-3475 is a pure PD-1 antagonist. MK-3475 potency in PD-1 binding, inhibition of ligand binding, and inhibition of PD-1 function has been similar or up to several-fold higher than that of an analogue of BMS-936558. Modeling of MK-3475 pharmacokinetics (PK) in monkeys vs BMS-936558 PK reported in humans suggested comparable concentration-time curves at various dose levels. A 1 month, repeat-dose, Good Laboratory Practice toxicity study with 4-month observation after dosing of MK-3475 revealed no major safety findings. The “No observed adverse effect level” (NOAEL) was ≥200 mg/kg.

Recent data of MK-3475 have validated PD-1 as an attractive target for clinical intervention and have provided proof of concept for anti-PD-1 mAbs in melanoma [Hamid 2013]. Patients with advanced melanoma were treated with MK-3575 with 10 mg/kg every 2 or 3 weeks. The response rate by RECIST was 38%. Responses were durable in the majority of patients (median follow-up, 11 months among patients who had a response); 81% of the patients who had a response (42 of 52) were still receiving treatment at the time of last published analysis in March 2013. The overall median PFS among the 135 patients was longer than 7 months. Common adverse events (AEs) attributed to treatment were fatigue, rash, pruritus, and diarrhea; most of the AEs were low grade (Trial detailed below in Section 2.2.1.1).

These data are similar to data in melanoma and renal carcinoma published for a similar agent, BMS-936558 [Sznol 2010]. BMS-936558 has shown an overall response rate of approximately 30% in patients with advanced melanoma and RCC who had failed prior therapy. Responses were of long duration, and the agent was generally well tolerated.

2.2.1 *MK-3475*

MK-3475 (SCH 900475) is a humanized immunoglobulin (Ig) G4 monoclonal antibody (mAb) which binds the programmed death 1 (PD-1) receptor, thus inhibiting the interaction with its ligands, PD-L1 or PD-L2 (Investigator's Brochure, 2014). PD-1 is an immune-checkpoint receptor expressed by T cells. When bound to either PD-L1 or PD-L2, the PD-1 pathway negatively regulates T-cell effector functions. The PD-1 pathway functions to limit unwanted or excessive immune responses, including autoimmune reactions. PD-L1 is typically expressed at low levels on various non-hematopoietic tissues, and PD-L2 is only detectably expressed on antigen-presenting cells in the lymphoid tissue or chronic inflammatory environments.

PD-L1 is also expressed in the tumor microenvironment of various cancers (Zou and Chen, 2008). Activation of the PD-1 pathway may be a critical mechanism to evade T-cell mediated tumor rejection (Dong *et al.*, 2002; Pardoll, 2012). High levels of PD-L1 expression are correlated with poor prognosis and survival in renal cell carcinoma (RCC) (Thompson *et al.*, 2007), pancreatic carcinoma (Nomi *et al.*, 2007), hepatocellular carcinoma (HCC) (Gao *et al.*, 2009), and ovarian carcinoma (Hamanishi *et al.*, 2007).

Immune-checkpoint inhibition of another inhibitory T-cell receptor, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), with the mAb ipilimumab demonstrated significant prolongation of overall survival (OS) in patients with melanoma in two phase 3 trials (Hodi *et al.*, 2010; Robert *et al.*, 2011; Ribas, 2012). As an immunotherapy target, PD-1 is distinct from CTLA-4 because it can be activated directly by the cancer and it regulates the effector phase of T-cell response, whereas CTLA-4 regulates the initial stage of T-cell activation (Pardoll, 2012; Ribas, 2012). Antibodies targeting the PD-1 pathway have demonstrated durable objective responses in phase 1 and 2 trials. Nivolumab showed an overall response rate (ORR) of approximately 28% in subjects with advanced melanoma, 27% in subjects with RCC, and 18% in subjects with non-small cell lung cancer (NSCLC) who had failed prior therapy (Topalian *et al.*, 2012). MK-3475 has shown an ORR of approximately 38% in patients with melanoma (Hamid *et al.*, 2013) and ~20% in patients with NSCLC (Investigator's Brochure, 2014).

2.2.1.1 Clinical Development of MK-3475

Clinical data are derived from an ongoing, first-in human phase 1 study (PN001, NCT01295827) to evaluate the safety and clinical activity of MK-3475 as a monotherapy, sponsored by Merck Sharp & Dohme. There are five parts to this study (Parts A-D and F) (Investigator's Brochure, 2014).

Part A was a 3+3 dose-escalation study in subjects with solid tumors to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics, and to determine a maximum tolerated dose (MTD) or preliminary recommended phase 2 doses (RP2Ds). Doses were 1, 3, and 10 mg/kg every 2 weeks (Q2W); doses of either 2 mg/kg or 10 mg/kg were also administered every 3 weeks (Q3W). All 3 dose levels were well tolerated and no dose-limiting toxicities (DLTs) were observed; therefore, the MTD was

not determined. The RP2D was determined by the sponsor based on safety, PK, and pharmacodynamic measurements, along with the strength of antitumor activity signals observed.

The remaining four parts aim to characterize the safety profile and tolerability of MK-3475 and to evaluate the clinical activity of MK-3475 in the following patient populations:

Part B: Advanced melanoma patients who have either received prior ipilimumab (IPI-treated) or were naïve to prior ipilimumab (IPI-naïve). Patients in Part B receive MK-3475 at three dose levels: 2 mg/kg Q3W, 10 mg/kg Q3W, and 10 mg/kg Q2W.

Part C: NSCLC patients. Patients in Part C receive MK-3475 at 10 mg/kg Q3W.

Part D: Advanced melanoma patients that are IPI-naïve. Patients in Part D receive MK-3475 at 2 mg/kg Q3W and 10 mg/kg Q3W.

Part F: NSCLC patients with and without prior systemic therapy whose tumors express PD-L1 when exposed to MK-3475. Patients in Part F receive MK-3475 at 2 mg/kg or 10 mg/kg Q3W, or 10 mg/kg Q2W.

Pharmacokinetics
The half-life ($t_{1/2}$) of MK-3475 is approximately 4 weeks and there is no indication of dose dependency of half-life in the three dose groups (1, 3, and 10 mg/kg) (Investigator's Brochure, 2014). The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.

There was a dose-related increase in exposure from 1 to 10 mg/kg (Investigator's Brochure, 2014). Serum concentrations of MK-3475 were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W (Hamid *et al.*, 2013, Investigator's Brochure, 2014). Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.

Anti-Drug Antibodies (ADA) Data
The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of MK-3475 to elicit the formation of ADA (Investigator's Brochure, 2014). No impact of ADA on MK-3475 exposure has been observed.

Efficacy
When treated with MK-3475 monotherapy, the ORR for IPI-treated patients with melanoma (Part B) was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRC), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve patients with melanoma (Parts B and D) was 39%/43% by RECIST/investigator-assessed irRC, respectively. The majority of responses were seen in patients with melanoma by 16 weeks of therapy with MK-3475; however, some responses have been reported after 24

weeks or more of therapy with MK-3475. Responses can be delayed, and in some patients, a RECIST-defined progression followed by a response has been observed.

The preliminary objective response rate for 38 patients with NSCLC (Part C) was 21%/24% by RECIST/investigator-assessed irRC, respectively (Investigator's Brochure, 2014).

Pharmacodynamics/Biomarkers
PD-L1 is being investigated as a predictive biomarker for MK-3475 treatment. At the 15th World Conference on Lung Cancer, Garon *et al.* presented preliminary data on a subset of patients suggesting that higher levels of tumor PD-L1 expression are associated with increased clinical activity (Garon *et al.*, 2013). Objective responses by RECIST 1.1 occurred in 4 out of 7 patients with higher levels of PD-L1 expression (57%, 95% confidence interval [CI] 18-90%) vs. 2 out of 22 patients with lower levels of PD-L1 expression (9%, 95% CI 1-29%). These data are extremely preliminary, and PD-L1 is not being used for patient selection.

Biomarkers to evaluate immune modulation and markers in the tumor microenvironment, such as T-cell infiltration, the baseline expression of markers of T-cell suppression FoxP3 or the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) in tumor biopsies, were associated with a high response rate (Berman *et al.*, 2009; Hamid *et al.*, 2009).

2.2.1.2 Safety data

The most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritus, diarrhea, and rash (Investigator's Brochure, 2014). Most AEs were not considered serious. The most commonly reported immune-related AEs were rash, pruritus, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis.

Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritus and neuropathy.

2.3 Other Agent(s): N/A

2.4 Rationale for Trial of MK-3475 in Merkel Cell Carcinoma Patients

Patients with advanced and progressing MCC will receive MK-3475 as first systemic therapy. MK-3475 is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection.

Cellular immune response is strongly linked to outcome with intratumoral CD8+ lymphocyte infiltration associated with improved survival. PD-1 expression is likely playing a key role in MCC immune evasion: The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface

of activated T cells under healthy conditions, is to downmodulate unwanted or excessive immune responses, including autoimmune reactions.

The necessary and persistent [Houben 2010] expression of MCPyV T-Ag oncoproteins in MCC tumors provides a unique opportunity. The viral T-Ag oncoproteins are present in 80% of MCC tumors and are required for ongoing tumor growth. There is often a T cell response specifically against virus positive cells. Over 90% of MCPyV-specific T cells express PD-1, the level of PD-1 expression is relatively high on a given cell, and it is frequently co-expressed with TIM-3, a combination that is strongly associated with immune dysfunction [Afanasiev 2013].

MCC-specific T cells co-express markers of exhaustion, PD-1 and TIM-3 at far higher levels than T cells specific for other common human viruses, and are likely to encounter the relevant inhibitory receptor ligand, PD-L1, within the MCC tumor microenvironment. Specifically, in MCC tumors, using both histologic and mRNA-based analyses in independent cohorts, PD-L1 expression within the tumor microenvironment has been observed and is positively correlated with the number of infiltrating CD8 lymphocytes [Afanasiev 2013; Lipson 2013]. The data suggest that the PD-1/PD-L1 inhibitory axis is a likely immune evasion strategy in MCC tumors. Importantly, the blockade of the PD-1/PD-L1 pathway has been recently shown to effectively induce durable tumor regression and stabilization of disease in a subset of several diverse types of cancer [Brahmer 2012; Topalian 2012, Hamid 2013].

The expression of PD-1 and PD-L1 support the testing of anti-PD-1 as a targeted approach to overcome tumor immune evasion mechanisms in MCC. MK-3475 offers the possibility of "unleashing" T cell responses to viral antigens expressed by MCC cells as well as cancer antigens. The trial also offers the opportunity to study antitumor immunity and immune evasion by assessing responses against a virus-derived, tumor-specific antigen. These studies could lead to the elucidation of other potentially effective immunotherapy regimens.

ORR will be the primary endpoint; PFS will be determined and reported and compared at 16 months to the historical control of first-line chemotherapy (5% PFS at 16 months). The expectation is that MK-3475 will induce durable responses in a substantial proportion of patients and that MK-3475 will demonstrate a real and substantial patient benefit compared to recent historical controls.

Cellular immunity is known to play a major role in the outcome of MCC. Although the majority of patients respond to standard chemotherapy, virtually all relapse, most often within a few months as shown in Figures 1-3 above. There is thus a good possibility that responses to MK-3475 may be more substantial and durable than responses to chemotherapy. Because systemic cytotoxic chemotherapy is associated with immune suppression and is not well tolerated in this older population, MK-3475 may have many advantages over this traditional approach.

Finally, the antitumor activity of PD-1 in several malignancies, melanoma, RCC, and NSCLC, is greater than the efficacy of chemotherapy in MCC, further supporting the use of anti-PD-1 as first-line therapy. The subjects thusly treated are unlikely to give up any substantially meaningful benefit from front-line chemotherapy. This is explained to patients in detail in the consent form.

2.4.1 *Rationale for expansion of enrollment*

Rationale for expanding enrollment from 26 to 50 patients in order to provide an expanded safety and efficacy profile:

Data to date on this trial (CITN-09 trial of MK-3475 as first systemic intervention in advanced MCC) has been positive enough to consider applying to the FDA for indication approval. The data has been assessed from the Simon two-stage trial design used in its initial enrollment phase. Stage 1 was to enroll 9 patients and stage 2 was to enroll an additional 15 patients. The trial was to be suspended and any further enrollment stopped if no responses were seen in stage 1. Since responses were observed among the first 9 patients enrolled, the study was allowed to proceed to stage 2 and enroll 15 additional patients. Treatment was discontinued in 2 patients due to drug-related adverse events after receiving 1 dose of MK-3475, and enrollment of an additional 2 patients was allowed per protocol, bringing the total enrollment to 26 patients.

Preliminary results of this trial were presented at the European Cancer Congress 2015 on September 27, 2015 [Nghiem 2015], and at the American Association for Cancer Research Annual Meeting on April 19, 2016 [Nghiem 2016], and summarized in the New England Journal of Medicine [Nghiem 2016]. Of the 26 pts who had received at least 1 dose of pembrolizumab, 25 had undergone at least one radiologic and clinical response assessment. Of these 25pts, 4 had a complete response (CR), , 10 had a partial response (PR), 1 had an unconfirmed PR, and 9 progressed and are off trial. The objective response rate (calculated on confirmed responses) cut was 14/25 (56%).

One patient had grade 4 myocarditis after 1 dose of MK-3475, and one had grade 4 transaminase elevation after 2 doses, with improvement in both patients after discontinuation of MK-3475 followed by steroid administration. Strikingly, both of these patients had favorable tumor responses (skin metastases regressed in one; the other has an ongoing PR of extensive visceral metastases).In summary, among 25 radiographically evaluable MCC pts treated with MK-3475 alone as first systemic intervention, 15 showed evidence (14 confirmed and 1 unconfirmed by subsequent scans) of response to PD-1 pathway blockade. The authors speculated that the high response rate may in part be due to the immune response to antigens from the polyomavirus that often drives MCC.

Since the rate of response thus far in this trial is greater than anticipated and more favorable than historical data on response to cytotoxic chemotherapy regimens, this protocol is being amended to include up to 50 patients for whom MK-3475 will be the first systemic treatment for MCC. The additional data will provide for a more robust safety and efficacy profile.

2.4.2 *Rationale for continuing MK-3475 in patients with tumors that progress*

It is expected that many Merkel cell tumors will fail to respond to MK-3475. Patients with documented progressing MCC will have the option of continuing MK-3475, because a small subset of patients with melanoma respond to continued therapy. Patients being treated with MK-3475 for melanoma have experienced late responses with continued therapy. In the KEYNOTE-001 melanoma trial using MK-3475 there was an additional 3.6% response rate with continued therapy. This category of response in that trial was defined as an “unconventional response”

with "delayed pseudoprogression: ≥25% increase in tumor burden at any assessment after week 12 that was not confirmed as progressive disease per irRC at the next assessment". [Hodi 2014].

2.5 Correlative Studies Background

The links between the immune system and MCC are particularly strong due to the frequent presence of the MCPyV and the documented improved survival of MCC patients based on T-cell immune function. It is therefore plausible that MK-3475 responses will correlate with the nature of the immune response in the tumor microenvironment including PD-L1 expression. In addition, there are several unique aspects of the correlative studies proposed for this trial. These include the ability to study MCPyV-specific T cells (in a subset of patients with corresponding MHC-peptide tetramers) in terms of their number, functional status, and surface receptor phenotype and gene expression profile. These correlative studies will attempt to address the following questions:

1) Are MCPyV-positive tumors more likely to respond to PD-1?
2) Does the MCPyV DNA copy number or extent of viral protein expression by IHC predict responsiveness?
3) Is intratumoral CD8 T-cell infiltration a marker for patients that will respond to MK-3475?
4) Are patients with antibodies to the MCPyV oncoprotein more likely to respond to MK-3475?
5) Do antibody titers to the viral oncoprotein provide greater or different insights into responses than gold-standard imaging studies?
6) Do responsive patients (as compared to non-responders) demonstrate changes in cell surface exhaustion parameters (PD-1/TIM-3) for virus-specific T cells?
7) Are virus-specific or neoantigen-specific T cells more active following PD-1 therapy? (as assessed by interferon and other cytokine production after peptide stimulation and assay by ELISpot and/or intracellular cytokine analysis)
8) What immune evasion mechanisms are active in tumors that fail to respond to MK-3475? (Biopsies will be carried out when feasible, on accessible tumor sites.)
9) How does the mutanome landscape of MCPyV-positive patients compare to that of MCPyV-negative patients? How does mutational load rank within the NIH Cancer Genome Atlas (TCGA)?
10) How does predicted immunogenicity of neoantigens differ between MCPyVpos and MCPyVneg tumors? Does this difference correlate with response to pembrolizumab?
11) How does the T cell response to either viral antigens or neoantigens change over the course of treatment with pembrolizumab?
12) What is the phenotype of neoantigen-reactive or virus-reactive T cells and how does this phenotype change over the course of pembrolizumab therapy? Does a particular phenotype correlate with response?

2.5.1 ***Evaluation of Tumor Biopsies for Immune Cell Infiltrate—Laboratory Correlative Study #1***

All patients recruited in the study must have an available archival tumor biopsy for biomarker analyses. If sufficient archival tumor tissue (at least 0.5 × 0.5 × 0.5 cm) collected before MK-3475 therapy is not available, a baseline biopsy will be obtained before enrolling in the study, assuming this can be carried out safely. Because tumor-infiltrating immune cells are associated with clinical outcome in MCC tumors, infiltrates derived from pre-therapy tumor tissue and from optional biopsies of responding, stable or progressing lesions in patients who have not achieved CR will be assessed by IHC to determine extent and the nature of the T-cell infiltration and the immune milieu of the tumor microenvironment. PD-L1 is a critical ligand for PD-1 and may be associated with response to MK-3475, hence, PD-L1 expression will be quantitated in the baseline formalin-fixed paraffin-embedded tumor specimens from all cases at a Merck-designated contract research organization (CRO). The following cellular markers will also be analyzed by IHC analyses in this study: CD3, CD8, PD-1, MHC Class 1, and anti-MCPyV T-Ag.

Additionally, with a view to further characterize the immune cell infiltrate (pending discussions between investigators and Merck in light of study results and current literature), tumors may also be assayed by IHC for a variety of other markers including CD4 (T cells), CD56 and CD16 (NK and NK T cells—because many MCC tumors are CD56-positive themselves, CD16 is an important additional marker), CD45 (leukocytes), CD19 (B cells), CD68, glucocorticoid-induced tumor necrosis factor receptor family–related gene (GITR), TIM-3, forkhead box P3 (FOXP3), and lymphocyte-activation gene 3 (LAG3). Tumor tissue may also be tested for gene expression (NanoString Technologies).

In patients who have not achieved CR (either responding, stable or progressing lesions) a biopsy sample of a tumor may be obtained if feasible and safe (core, punch or excisional biopsy). This biopsy is highly preferred, but optional. The biopsy will be analyzed as outlined above. All studies indicated above maybe carried out for the biopsy.

2.5.2 ***Merkel Cell Polyomavirus DNA and Protein Expression in Tumor Biopsies—Laboratory Correlative Study #2***

To determine whether MCPyV-positive MCCs are more likely to respond to anti-PD-1 and if the DNA copy number or extent of viral protein expression by IHC predict responsiveness, tumor biopsies will be analyzed for presence of the MCPyV in 2 ways: (1) determine MCPyV oncoprotein expression in tumor cells using an optimized IHC protocol and (2) determine DNA status (and copy number) via polymerase chain reaction (PCR) of tumor DNA. Optimized protocols were described by Rodig and colleagues [Rodig 2012]. The IHC studies have been optimized and validated at University of Washington (UW) pathology laboratories. The DNA-PCR assay has been established by Dr. Meei-Li Huang in a clinical grade virology laboratory (Vaccine and Infectious Disease Division at Fred Hutchinson Cancer Research Center [FHCRC]) with oversight by Dr. Nghiem's laboratory.

These studies will be conducted on pretreatment biopsy samples and on biopsy samples

collected from patients who have not achieved CR (either responding, stable or progressing lesions), when available, as described for Laboratory Correlative Study #1.

2.5.3 *Merkel Cell Polyomavirus–Specific Cellular Immune Responses—Laboratory Correlative Studies #3–#5*

Dr. Nghiem's lab has developed several MHC-peptide tetramer reagents, which will enable the identification and isolation of circulating MCPyV-specific CD8 T cells [Iyer 2011; Afanasiev 2013]. To determine whether and which of these tetramer reagents would be applicable for a given patient, a low-resolution human leukocyte antigen (HLA) Class I typing assay will be performed after enrollment on study. Although MCPyV-positive MCC patients often have circulating MCPyV-specific CD8+ T cells, these cells are often functionally "exhausted" and display a PD-1hiTIM-3hi phenotype, which likely comprise a representative subset of the target cell population in MCC. The phenotype of MCPyV-positive T cells or neoantigen-reactive T cells will be followed over the course of treatment. To comprehensively evaluate cellular immune responses in these patients, the following correlative studies will be conducted:

2.5.3.1 *Laboratory Correlative Study #3: Immunophenotyping of Merkel Cell Polyomavirus–specific CD8+ T cells*

In this study, peripheral blood tumor-specific CD8 T cells will be evaluated by multiparametric flow cytometry at baseline and at multiple time points after initiation of MK-3475 therapy. These studies will be performed by the Nghiem Laboratory and CITN Immune Monitoring Laboratory at the UW.

2.5.3.2 *Laboratory Correlative Study #4: mRNA Expression Analysis of Merkel Cell Polyomavirus–specific CD8+ T cells*

In this study, (to be performed if mutually agreed to be feasible and indicated by Merck & Investigators) peripheral blood Merkel cell polyomavirus–specific CD8 T cells will be isolated using fluorescence-activated cell sorting (FACS) from frozen cells that were harvested at baseline and at multiple time points after initiation of MK-3475 therapy. FACS purification and mRNA expression analysis by Nanostring would be performed in collaboration with Merck Research Laboratories–Palo Alto.

We estimate that ~25% patients enrolled in the study will have a tetramer-matching HLA type and produce MCPyV-oncoprotein–specific T cells detectable by tetramer. In these cases, this will be a powerful direct way to assess number and function of virus- and tumor-specific T-cells (through Laboratory Correlative Studies #3 and #4).

2.5.3.3 *Laboratory Correlative Study #5: Analysis of Merkel Cell Polyomavirus–specific T cell specificities by interferon-gamma ELISpot or by intracellular cytokine staining (ICS)*

In this study, all patients, regardless of HLA type, will have cryopreserved peripheral blood mononuclear cells (PBMC) tested for responses to MCPyV peptides using standard ELISpot and/or ICS assays. These experiments will determine whether epitope spreading has occurred and whether it correlates

with viral status and clinical response.

Key questions that these correlative studies will address include: (1) Will the surface marker phenotype and/or transcriptional state of a patient's "exhausted" tumor-specific PD-1+ CD8 T cell predict response to MK-3475?; (2) Does the surface marker phenotype and/or transcriptional state of a patient's "exhausted" tumor-specific PD-1+ CD8 T cells correlate with features such as CD8 infiltration in the pretreatment tumor? And, are these features modulated with MK-3475 treatment?; and (3)Will PD-1+ T cells from nonresponding patients be characterized by a specific pattern of gene regulation, mutanome landscape or neoantigen array compared to those of responders? Specifically, are additional counter-regulatory immune modulators expressed to compensate for PD-1 blockade? If so, these secondary counter-regulatory immune modulators may represent relevant targets to overcome resistance to PD-1 therapy.

ELISpot analysis will be performed by the CITN Immune Monitoring Laboratory under the direction of Dr. Steven Fling. ICS analyses will be performed by the laboratory of Dr. Julie McElrath at FHCRC.

2.5.4 ***Merkel Cell Polyomavirus–Specific Antibody Responses—Laboratory Correlative Studies #6***

Because antibody titers for the T-Ag oncoprotein are a marker of disease burden and immune responsiveness to the virus, we will also test for these antibodies at baseline [Paulson 2010]. If positive, this assay will be carried out on serum collected at the time of scans. We anticipate that 50% of patients will make antibody to the MCPyV oncoprotein. Key questions include (1) Are seropositive patients more likely to respond to anti-PD-1? and (2) Do antibody titers provide greater or different insights into responses than gold-standard imaging studies? In several cases, rises in oncoprotein antibody have occurred before detection of disease by CT and may herald early immune recognition of recurrent disease. This serologic assay will be performed by Luminex and carried out by the CLIA-certified Clinical Immunology Laboratory, Department of Laboratory Medicine, at the UW in conjunction with Dr. Paul Nghiem's lab.

2.5.5 ***Quantification of PBMC and T-cell Subsets—Laboratory Correlative Study #7***

The effect of MK-3475 on lymphocyte number and phenotype will be assessed at baseline and at defined time points during the trial. The frequency and percentage of PBMC and T-cell subsets (CD4 and CD8 effector memory, central memory and Treg subsets, NK cells and myeloid-derived suppressor cells) will be determined using multiparameter flow cytometric analysis on whole blood and/or cryopreserved PBMC. Assays will be performed under the direction of Dr. Steven Fling in the CITN Central Laboratory in collaboration Dr. Nghiem's laboratory.

2.5.6 ***Whole Exome Sequencing and RNA Sequencing--Laboratory Correlative Study #8***

In order to investigate the role of T cell mediated immunity directed towards tumor-specific mutant antigens, whole exome sequencing (WES) and RNA sequencing will be performed on MCC tumor samples collected at baseline and on PBMC DNA (to establish non-mutated sequence.)

The mutanome landscape in MCPyV-positive patients and MCPyV-negative patients will be compared. Correlation of these results to patients' responses to MK-3475 will be investigated. Results will also be compared to data on mutational load in other cancers currently documented in the NIH Cancer Genome Atlas (TCGA).

Studies using tumor tissue will be conducted on pretreatment biopsy samples, obtained as described for Laboratory Correlative Study #1.

2.5.7 ***Neoantigen Identification—Laboratory Correlative Study #9***

Using results of exome sequencing and RNA sequencing performed on baseline tumor samples and matched PBMC, neoantigen identification will be performed using Neon Therapeutics' neoantigen prediction MIKEY algorithm or similar technology and algorithms to predict and rank-order immunogenic epitopes from MCPyV-positive and MCPyV-negative tumors. Neon Therapeutics or another designated vendor will synthesize candidate peptides. The following will be identified, as indicated: number of predicted neo-antigens, characteristics of the predicted peptides (including affinity values, RNA expression values of the mutated genes, and variant allele frequency).

We will compare predicted immunogenicity of neoantigens between MCPyV-positive and MCPyV-negative tumors and assess correlation of immunogenicity with responses to pembrolizumab.

2.5.8 ***T cell Receptor Sequencing and Functional Phenotyping—Lab Correlative Study #10***

In parallel to neoantigen identification and to help address questions 10-12 raised in Section 2.5, we will perform T cell receptor (TCR) sequencing of tumor-infiltrating lymphocytes (TIL) where pre-treatment and post-treatment biopsies are available. We hypothesize that expansion of specific T cell clones in TIL (exhibited by higher TCR clonality) will be associated with improved response to PD1 therapy. Two methods of sequencing will be employed, depending on the availability of tissue type for a given subject. In the first, TCR beta chain (TCRB) high throughput sequencing will be done on genomic DNA extracted and PCR amplified from fixed tumor samples (pre- and post-treatment) using the ImmunoSeq hsTCRB kit (Adaptive Biotechnologies), and sequenced on the Illumina MiSeq platform in collaboration with Dr. Edus Warren (FHCRC) and analyzed using 'LymphoSeq' software, or using similar technologies.

In a second approach, where fresh biopsies are available, we will sequence TCRα and TCRβ genes in single T cells (sorted by flow cytometry) using the approach pioneered by Han et al [Han 2014] and now established in the Edus Warren Lab at FHCRC. Using this technology, we will be amplifying functional genes characteristic of different T cell subsets, which can then be associated with specific T cell clones. This information will be used to determine the T cell phenotype associated with clinically beneficial responses. PBMC samples will also be sequenced as a surrogate of TCR usage in the non-TIL T cell population, in order to help delineate tumor specific TCRs. In subjects where vital correlations are observed, we will use the same single cell sorting and phenotyping technology to investigate the development of these responses in serial PBMC samples collected pre-treatment and at several time points post treatment.

2.5.9 ***Kynurenine/Tryptophan (Kyn/Trp) Ratio***
Over expression of indoleamine 2,3-dioxygenase (IDO) results in increases in the Kyn/Trp ratio in blood and in the subsequent suppression of T-cell responses [Liu 2009]. IDO is expressed by some tumors, but probably more importantly, it is expressed by lymphocytes as a normal mechanism to dampen immune responses. Expression of IDO is one likely mechanism of anti-PD-1 failure. Kyn/Trp ratios will be analyzed at baseline, during treatment, and at end of treatment.

2.5.10 ***Samples for CITN Repository***
Any samples (serum, plasma, PBMC, tissue) collected for correlative assays will be stored in the CITN Repository at the CITN Central Laboratory before use, as will residual samples after use. Ancillary studies may be proposed, approved by the relevant CITN Committee, and conducted by proposing investigators according to the CITN Manual of Operations Guidelines for Ancillary Studies.

3. PATIENT SELECTION

3.1 Eligibility Criteria

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

3.1.1 Patients must have biopsy-proven metastatic MCC or locoregional MCC that has recurred following standard locoregional therapy with surgery and/or radiation therapy.

3.1.2 Patients must have measurable disease per RECIST 1.1 assessed by CT scan, or for skin lesions not measurable by CT scan, measurements may be performed with caliper or flexible ruler.

Note: Stage IV no evidence of disease (NED) is excluded by this criterion.

3.1.3 Be ≥18 years of age on day of signing informed consent

Note: Because no dosing or AE data are currently available on the use of MK-3475 in patients <18 years of age, children are excluded from this study.

3.1.4 Have a performance status of ≤1 on the Eastern Cooperative Oncology Group (ECOG) Performance Scale (see Appendix A)

3.1.5 Life expectancy of greater than 6 months

3.1.6 Patients must have normal organ and marrow function (all screening labs should be performed within 14 days of treatment initiation) as defined below:

System	Laboratory value
– leukocytes	≥2,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥9 g/dL OR ≥5.6 mmol/L

– serum total bilirubin	≤1.5 X upper limit of normal (ULN) OR direct bilirubin ≤ ULN for patients with total bilirubin levels >1.5 X ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN OR ≤5 X ULN for patients with liver metastases
– serum creatinine	≤2.5X ULN OR
– Measured or calculated[a] creatinine clearance (CrCl) (Glomerular filtration rate [GFR] can also be used in place of creatinine or CrCl)	≥30 mL/min for subject with creatinine levels >2.5 X institutional ULN
– Thyroid Stimulating Hormone (TSH)	Within Institutional Limits (ie: Normal). If TSH is greater or less than institutional limits patients may participate if their T4 is WNL. Patients may be on a stable dose of replacement thyroid medication. Dose adjustments are allowed if needed.

[a]Creatinine clearance should be calculated per institutional standard.

3.1.7 Patients must provide tissue from an archival tumor sample or newly obtained core, punch or excisional biopsy of a tumor lesion if deemed relatively safe and technically feasible.

Note: Newly obtained biopsy is preferable.

3.1.8 Female patients of childbearing potential must have a negative urine or serum pregnancy within 72 hours before receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Note: The effects of MK-3475 on the developing human fetus are unknown. For this reason and because anti-PD-1agents may be teratogenic, women of child-bearing potential must agree to use 2 methods of birth control, or be surgically sterile, or abstain from heterosexual activity beginning with the screening visit and for the duration of study participation, through 120 days beyond last dose of MK-3475 administration. Patients of childbearing potential are those who have not been surgically sterilized or have not been free from menses for >1 year.

Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

3.1.9 Men treated or enrolled on this protocol must agree to use 2 adequate methods of contraception starting with the screening visit, for the duration of study participation, and through 120 days after the last dose of MK-3475 administration.

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

3.2 Exclusion Criteria

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

3.2.1 Has had prior systemic therapy for MCC

Note: Prior systemic cytotoxic chemotherapy will be allowed if it was administered in the adjuvant setting (no clinically detectable MCC at the time) and treatment concluded more than 6 months prior to beginning study treatment.

3.2.2 Is currently participating in or has participated in a study of an investigational systemic agent to treat MCC; or is using an investigational device within 4 weeks of the first dose of treatment

Note: If patient received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention before starting therapy.

Toxicity from surgery or associated interventions that has not recovered to ≤Grade 1 is allowed if it meets the inclusion requirements for laboratory parameters.

3.2.3 Patients with locoregional disease that have not received appropriate standard locoregional therapy with surgery and/or radiation therapy

3.2.4 Has had radiation therapy within 2 weeks of beginning study treatment

3.2.5 Toxicity from prior radiation therapy has NOT resolved to grade 1 or less.

3.2.6 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.

3.2.7 Has had a prior monoclonal antibody for treatment of MCC.

3.2.8 Has had a prior monoclonal antibody for a non–cancer therapy indication within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to agents administered more than 4 weeks earlier.

3.2.9 Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

3.2.10 Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. These patients should be excluded because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other AEs.

Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 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 7 days prior to trial treatment.

3.2.11 Has a history of allergic reactions attributed to compounds of similar chemical or biologic composition to MK-3475.

3.2.12 Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Patients that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. The use of physiologic doses of corticosteroids may be approved after consultation with the Protocol PI and CITN. Patients with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.

3.2.13 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator

3.2.14 Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

3.2.15 Has uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, interstitial lung disease, non-infectious pneumonitis, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

3.2.16 Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of MK-3475 administration.

***Note**:* Pregnant women are excluded from this study because MK-3475 is an agent with the potential for teratogenic or abortifacient effects. Because of unknown but potential risks for AEs in nursing infants secondary to treatment of the mother with MK-3475, breastfeeding should be discontinued if the mother is treated with MK-3475.

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.

Men and nonpregnant, non–breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as (1) surgically sterilized, or (2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or (3) not heterosexually active for the duration of the study. The 2 birth control methods can be barrier method or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from the

screening visit throughout the study period up to 120 days after the last dose of study therapy.

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

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 and during the follow-up period described above. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Pregnancy: 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 without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). 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. If a male patient impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported and followed as described in Section 7.7.

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

3.2.17 Patient is Human Immunodeficiency Virus (HIV) positive.

Note: Patients who are Human Immunodeficiency Virus (HIV) positive may participate IF they meet the following eligibility requirements:

1. They must be stable on their anti-retroviral regimen, and they must be healthy from an HIV perspective.
2. They must have a CD4 count of greater than 250 cells/mcL.
3. They must not be receiving prophylactic therapy for an opportunistic infection.

3.2.18 Has known active Hepatitis B (*e.g.*, HBsAg reactive) or Hepatitis C (*e.g.*, HCV RNA [qualitative] is detected).

Note*:* A positive hepatitis B serology indicative of previous immunization (i.e., HBsAb-positive and HBcAb-negative) or a fully resolved acute hepatitis B infection is not an exclusion criterion.

3.2.19 Has active non-infectious pneumonitis ≥ grade 2; or history of grade 3 non-infectious pneumonitis within the past 12 months; or any history of grade 4 non-infectious pneumonitis.

3.2.20 History of other pulmonary disease such as emphysema or chronic obstructive pulmonary disease (COPD), (FEV_1<60% of predicted for height and age). Pulmonary function tests (PFTs) are required in patients with prolonged smoking history or symptoms of respiratory dysfunction.

3.2.21 Cardiovascular disease that meets one of the following: congestive heart failure (New York Heart Association Class III or IV), active angina pectoris, or recent myocardial infarction (within the last 6 months)

3.2.22 Prior organ allograft or allogeneic transplantation, if the transplanted tissue is still in place

3.2.23 Has had live vaccines within 30 days before the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, seasonal flu, H1N1 flu, rabies, BCG, and typhoid vaccine.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 Investigator (IVR), Non-Physician Investigator (NPIVR), and Associate Plus (AP) Registration with CTEP

4.1.1 CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE, or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training (GCP training certificate must be dated within 3 years)	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval

NCI Person Registration must be completed using the CTEP's Registration and Credential Repository (RCR) system. IVR, NPIVR, and AP will use their CTEP-IAM credentials to access RCR (https://ctepcore.nci.nih.gov/rcr) and to electronically sign and submit required registration documents.
Additional information can be found on the CTEP website at < https://ctep.cancer.gov/investigatorResources/default.htm >. For questions, please contact the RCR Help Desk by email at < RCRHelpDesk@nih.gov >.

4.1.2 CTEP IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application required to be used by all registration types (IVR, NPIVR, AP, Associate (A) and Associate Basic (AB)). IAM provides a solution for CTEP Enterprise and CTSU applications with the primary goal of streamlining user provisioning.

Additional information can be found on the CTEP website at https://ctepcore.nci.nih.gov/iam/index.jsp
For questions, please contact the ***CTEP Registration Help Desk*** by email at CTEPRegHelp@nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain Institutional Review Board (IRB) approval for this protocol and submit all required regulatory documents (including any protocol specific documents) to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to: an active Federal Wide Assurance (FWA) number, an active roster affiliation with the Lead Network or a participating organization, a valid IRB approval, and compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

4.2.1 Downloading Lead Protocol Organization Documents

Site registration forms may be downloaded from the CITN-09 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, the users must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the CITN link to expand, followed by protocol #CITN-09
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

4.2.2 Requirements for CITN-09 Site Registration:

IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance

Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

4.2.3 Submitting Regulatory Documents

Submit your IRB approval, model Informed Consent Form and other protocol-specific regulatory documentation to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission

When applicable original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

Sites can verify their registration status on the members' section of the CTSU Web site.

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password.
- Click on the Regulatory tab.
- Click on the Site Registration subtab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements outlined by the Lead Network (LPO). It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the LPO or participating organization roster. Registrars must hold a minimum of an AP registration type. Patient enrollment data entered by Registrars in OPEN will automatically transfer to the NCI's clinical data management system, Medidata Rave.

To assign an IVR or NPIVR as the treating, crediting, consenting, drug shipment (IVR only), or investigator receiving a transfer in OPEN, the IVR or NPIVR must list on their Form FDA 1572 in RCR the IRB number used on the site's IRB approval.

Enrollment will be facilitated using the Slot-Reservation System in conjunction with the Registration system in the Oncology Patient Enrollment Network (OPEN). Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot-reservation confirmation is obtained, site staff may then proceed to enroll patients to this study.

Enrollment will be facilitated using the Slot-Reservation System in conjunction with the Registration system in the Oncology Patient Enrollment Network (OPEN). Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot-reservation confirmation is obtained, site staff may then proceed to enroll patients to this study.

Prior to accessing OPEN CITN member site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- CITN member site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for the CTSU members' web site.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the CITN roster.

Note: The OPEN system will provide the site with a printable confirmation of registration information and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment as soon as possible.* Issues that would cause treatment delays should be discussed with the Principal Investigator. 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.

5. TREATMENT PLAN

This is a multi-institution, CITN Cooperative trial of MK-3475 in patients with advanced MCC, who have not received prior systemic therapy for their disease. The trial is open-labeled and nonrandomized. Patients who have not received any previous systemic therapy for their disease will be treated with MK-3475, given every 3 weeks.

ORR, PFS, and DOR will be monitored by CT scans at week 13 and then at 9-week intervals. At 1 year, the scan frequency will be decreased to every 12 weeks. Responses and progression will be assessed by RECIST 1.1 criteria. OS will be chronicled and reported.

Patients who appear to progress on the initial scan, occurring at week 13 of therapy, will be allowed to continue on therapy if otherwise stable, until a confirmatory scan is obtained at 17 weeks. If the 17-week scan confirms progression, the study treatment will be discontinued and the patient will be followed for survival. Alternatively, MK-3475 treatment may be continued in the setting of progressive disease if the patient is otherwise clinically stable as defined by the following criteria:

- Absence of clinical symptoms or signs indicating clinically significant disease progression
- ECOG performance status ≤1
- Absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention

Patients who appear to progress on any subsequent scan will be allowed to continue on therapy if otherwise stable, until a confirmatory scan is obtained 4 weeks later. If the scan repeated 4 weeks later confirms progression, the study treatment will be discontinued and the patient will be followed for survival, or alternatively, continue MK-3475 treatment, if patient is otherwise clinically stable as described above.

Therapy after progression is at the discretion of the patient and primary physician.

Patients with stable disease (SD) or partial response (PR) can be treated up to 2 years at patient and investigator discretion. Patients that achieve a complete response (CR) can discontinue treatment after 6 months of therapy provided that patient has had at least 2 cycles past validation of CR. Patients that recur off of therapy will be eligible for retreatment at patient and investigator discretion, provided they meet the criteria for retreatment and the trial is ongoing.

Administration of MK-3475 will be stopped with documented disease progression warranting alternative systemic therapy, unacceptable AE(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the patient, patient withdraws consent, pregnancy of the patient, noncompliance with trial treatment or procedure requirements, or administrative reasons. In patients with documented disease progression, treatment can continue until there is an increase in tumor burden of 25% or more following initial confirmation of progression.

The primary objective is to determine the clinical efficacy of MK-3475 as the first systemic intervention for patients with advanced MCC. The primary endpoint will be ORR as measured by RECIST 1.1.

The secondary objectives are to determine the clinical activity of MK-3475 as the first systemic intervention by determining PFS, DOR, and OS. The secondary endpoints will be PFS and DOR as measured by RECIST 1.1. PFS will be compared at 16 months to the historical PFS for patients treated with standard chemotherapy (5% PFS at 16 months). OS will be chronicled and reported.

The exploratory objectives are to determine the immune correlates of the clinical activity of MK-3475 (Section 1.3).

The protocol will follow the standard Simon two-stage design. Stage 1 will enroll 9 patients and stage 2 will enroll 15 additional patients. The trial will be suspended and any further enrollment stopped if no responses are seen in the first stage. The protocol initially enrolled 9 patients in Stage 1, and enrolled an additional 15 patients in Stage 2, achieving enrollment of 24. Treatment was discontinued in 2 patients due to drug-related adverse events after receiving 1 dose of MK-3475, and enrollment of an additional 2 patients was allowed per protocol, bringing the total enrollment to 26 patients.

Protocol Amendment 4 increased the total number of patients from 26 to 50 to provide an expanded safety and efficacy profile.

Patients who have confirmed progression of disease on MK-3475 therapy will stop study therapy but will continue to be followed for survival. Alternatively, MK-3475 treatment may continue in patients with confirmed progression, if they are otherwise clinically stable, as described above. Additional therapies will be chronicled and reported, but will not be considered part of this protocol.

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1.1 *CTEP IND Agent: MK-3475*

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Study Calendar (Section 10). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

MK-3475 treatment will be administered on an outpatient basis.

MK-3475 will be administered as a 30-minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 6.1). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. A window of −5

minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: −5 min/+10 min).

Study Treatment

Drug	Dose Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period
MK-3475	2 mg/kg	every 3 weeks	IV infusion	Day 1 of each cycle

Note: Calculate the required dose amount (2 mg/kg) based on subject's baseline weight. The dose amount should be recalculated if the subject's weight changes by more than 10% from the baseline weight measurement. If the patient's weight changes again by 10% or more (either increase or decrease) from the most recent weight used for dose calculation, recalculation of the dose is required.

Protocol Section 8.1 provides specific instructions for MK-3475 dose preparation, and drug administration.

Treatment with MK-3475 study drug may continue until one of the following events occurs:

- Documented disease progression warranting alternative systemic therapy
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse experiences (see Section 6.1).
- Need for >2 dose delays due to toxicity as per the dose modification guidelines described in Section 6
- Patient withdraws consent
- If in the opinion of the investigator and CITN Coordinating Center, a change or temporary or permanent discontinuation of therapy would be in the best interest of the patient.
- Pregnancy in patient
- Patient completes 24 months of treatment with MK-3475
- In patients with documented disease progression, treatment with MK-3475 can continue until one of the above occurs or until there is an increase in tumor burden of 25% or more following initial confirmation of progression.

Note*:* Patients will receive up to 24 months of trial treatment calculated from the date of first dose. Subjects who stop MK-3475 after 24 months may be eligible for up to 1 year of additional trial treatment if they progress after stopping trial treatment provided they meet the requirements detailed in Section 5.10.

The first radiologic assessment of tumor response status will be performed at Week 13 (as consistent with standard of care). This assessment shall occur before drug administration scheduled for Week 13. However, if a patient is found to be progressing on MK-3475 at this initial scan, the patient can continue on MK-3475 if otherwise stable (see criteria below). A confirmatory scan will be performed at week 17, as immunomodulatory therapies may result in an early increase in lesion size due to influx

of immune cells. This immune activation and infiltration reflects the therapeutic mechanism and, therefore, an early increase in tumor size in this context may herald a therapeutic response rather than progression.

Thus, it is recommended that a patient with an unconfirmed early (<17 weeks) progression should continue therapy with MK-3475 waiting for confirmation of disease progression, but ONLY if the following criteria are met:

- Absence of clinical signs and symptoms (including worsening of laboratory values) indicating clinically significant disease progression
- ECOG performance status ≤ 1
- Absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention

As depicted in the Overall Trial Schema, an MK-3475-treated patient whose progression is confirmed at the 17-week scan will discontinue therapy. Alternatively, if patient is otherwise clinically stable per criteria above, they may be continued on MK-3475 treatment. The next scan will be performed at Week 22 and then every 9 or 12 weeks per the Patient Visit Timeline. MK-3475 will be discontinued if the tumor burden increases by 25% or more following initial confirmation of progression.

In patients without progression and in patients with progression who continue treatment with MK-3475, disease radiologic monitoring should continue to be assessed at 9-week intervals through the first year of treatment and then at 12-week intervals throughout the duration of the study. Section 5.4 describes criteria for discontinuing treatment.

After a patient is discontinued from study therapy, an end of therapy visit should be performed as soon as possible following the decision to stop study drug treatment or in conjunction with last treatment visit if discontinuation of therapy is anticipated. A mandatory safety follow-up visit should be performed approximately 30 days after the last infusion of study medication. All AEs occurring within 30 days after the last dose of trial treatment will be recorded.

All patients who discontinue treatment due to progression will be followed for at least 30 days after their last dose of study drug or until initiation of a new anticancer treatment, whichever occurs first. After the safety follow-up visit, patients will be followed for survival via phone contact every 12 weeks.

Patients who discontinue study therapy without documented disease progression will have a mandatory safety follow-up visit approximately 30 days after the last infusion of study drug, and should continue to be monitored for disease status by radiologic imaging according to the guidelines described in the Visit Requirements and Study Calendar for post-treatment follow-up. Imaging may be performed at the investigative site or locally; scans will be collected and RECIST 1.1 assessments performed. Collection of disease assessments will continue until progression, start of a new anticancer therapy, death or end of study, whichever occurs first. Thereafter, patients who progressed or started new treatment will be followed for survival via phone contact every 12 weeks ± 1 week.

AE reporting with begin with administration of first dose of study medication. All AEs occurring within 30 days after the last dose of study treatment will be reported. Serious adverse events (SAE) related and unrelated to trial treatment will be collected for 90 days after the last dose of trial treatment or the start of new anticancer treatment, whichever comes first. Afterwards, only SAEs that are related to trial treatment will be reported. All SAE should be reported to the Sponsor via CTEP Adverse Event Reporting System (CTEP AERS).

Patients who are discontinued from the study due to an unacceptable drug-related AE will be followed for resolution of the AE to Grade 0–1 or stabilization, and monitored for disease status by radiologic imaging until progression or initiation of a new therapy for their cancer, whichever occurs first.

Patients in whom MK-3475 is stopped due to AE, can have the agent restarted (rechallenged) after resolution of the AE to Grade 1. If the same AE occurs at the same grade, MK-3475 will be discontinued.

VISIT REQUIREMENTS (also refer to Study Calendars):

Screening phase (pre-study; Day -28 to day 0):

- Obtain informed consent
- Record demographics
- Obtain medical history
- Tumor imaging
- Tumor biopsies/archival tissue collection*

*If sufficient archival tumor tissue (at least 0.5 × 0.5 × 0.5 cm) collected before MK-3475 therapy is not available, a baseline biopsy (core biopsy, punch biopsy or excisional biopsy) will be obtained before enrolling in the study assuming this can be carried out safely.

Screening phase (pre-study, Day -14 to day 0)

- Record concomitant medications
- Physical exam
- Vital signs
- Performance status
- 12-lead electrocardiogram (EKG)
- Pulmonary function testing (for patients with a history of pulmonary disease, prolonged smoking history, or symptoms of respiratory dysfunction).

- Laboratory tests:
    - Urine or serum B-HCG in women*
    - Complete blood count (CBC) with differential
    - Comprehensive serum chemistry panel
    - Urinalysis
    - T3, FT4, and TSH

*For women of reproductive potential, a urine pregnancy test should be performed within 72 hours before first dose of trial treatment. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test performed by the local study site laboratory will be required.

Treatment Cycles (to be repeated every 3 weeks)

Cycle 1
Day 1 ± 3, prior to MK-3475 administration

- AE evaluation
- Physical examination
- Vital signs, height and weight (height required only for first treatment visit)
- Performance status
- Laboratory tests:
    - Kyn/Trp ratio
    - IFN-γ ELISpot
    - Immunophenotyping
    - T cell gene expression and phenotyping
    - MCPyV antibody levels
    - Serum, plasma, and PBMC storage

On Day 1 ± 3, MK-3475 is administered after above procedures completed.

After MK-3475 infusion, a 12-lead EKG is performed within 30 minutes of end of infusion.

Subsequent Cycles without Scheduled Tumor Imaging (every 3 weeks)*
*See study Patient Visit Timeline and Study Calendar
Day 1 ± 3, prior to MK-3475 administration:

- Concomitant medications
- Review of AEs
- Physical examination (limited)
- Vital signs and weight
- Performance status
- Laboratory tests:
    - CBC with differential
    - Comprehensive serum chemistry panel
    - Urinalysis*
    - T3, FT4, and TSH*
- Optional tumor biopsy/tissue collection if safe and feasible (core biopsy, punch biopsy or excisional biopsy) in patients who have not achieved CR (either responding, stable or progressing lesions).

*Urinalysis and thyroid function testing to be performed at Baseline, Cycle 6, and then every 6 cycles after baseline. After baseline, this will correlate with visits when scans and correlative blood draws are not scheduled.

On Day 1 ± 3, MK-3475 is administered after above procedures completed.

Subsequent Cycles with Tumor Imaging Scheduled*
*See Patient Visit Timeline and Study Calendar
Day 1, within 7 days, before MK-3475 administration.

- Tumor imaging [Frequency of imaging is every 9 weeks ± 3 days (every 3rd cycle) for first year of treatment, and then every 12 weeks ± 7 days (every 4th cycle) during second year of treatment].

Day 1 ± 3, prior to MK-3475 administration*:
*Blood will be drawn for correlative studies during Year 1 of treatment; during Year 2, correlative blood draws are required only around time of confirmation of progressive disease or at time of discontinuation of study drug. Safety labs (CBC and comprehensive serum chemistry) will occur with every cycle.

- Concomitant medications
- Review of AEs
- Physical examination
- Vital signs and weight
- Performance status
- 12-lead EKG*
- Laboratory tests:
    - CBC with differential
    - Comprehensive serum chemistry panel
    - Kyn/Trp ratio
    - IFN-γ ELISpot
    - Immunophenotyping
    - T cell gene expression and phenotyping
    - MCPyV antibody levels
    - Serum, plasma, and PBMC storage
- Optional tumor biopsy/tissue collection if safe and feasible (core biopsy, punch biopsy or excisional biopsy) in patients who have not achieved CR (either responding, stable or progressing lesions).

On Day 1 ± 3, MK-3475 is administered after above procedures completed.

*In Cycle 8 (and Cycle 1), 12-lead EKG is performed within 30 minutes after end of MK-3475 infusion.

Confirmatory Tumor Imaging
When tumor imaging is performed to confirm progressive disease (PD; refer to Patient Visit Timeline), the following laboratory tests should be performed. If not possible to schedule a blood draw around the time of confirmatory scan, blood for these laboratory tests will be collected at the end-of-therapy visit.

- CBC with differential
- Comprehensive serum chemistry panel
- Kyn/Trp ratio
- IFN-γ ELISpot
- Immunophenotyping

  - T cell gene expression and phenotyping
  - MCPyV antibody levels
  - Serum, plasma, and PBMC storage
  - Optional tumor biopsy/tissue collection (core biopsy, punch biopsy or excisional biopsy) if PD is confirmed.

End of Therapy

- (The End of Therapy Visit is performed as soon as possible following the decision to stop study drug treatment or in conjunction with last treatment visit if discontinuation of therapy is anticipated).
- Concomitant medications
- Review of AEs
- Physical exam
- Vital signs and weight
- 12-lead EKG
- Performance status
- Laboratory tests:
  - Urine or serum HCG in women
  - CBC with differential
  - Comprehensive serum chemistry panel
- Laboratory tests (if not collected at time of determination of CR, PR, or disease progression):
  - Kyn/Trp ratio
  - INF-γ ELISpot
  - Immunophenotyping
  - T cell gene expression and phenotyping
  - MCPyV antibody levels
  - Serum, plasma, and PBMC storage
- Tumor imaging (not required if scan was performed within prior 4 weeks).
- Optional tumor biopsy/tissue collection if safe and feasible (core biopsy, punch biopsy or excisional biopsy), in patients who have not achieved CR (either responding, stable or progressing lesions) (Section 2.5.1).

Post-Treatment Follow-Up

Post-Treatment Safety Follow-Up (30 Days Post Treatment ± 3 days). (This visit may be combined with End of Treatment Visit if decision to discontinue treatment is delayed).

- Concomitant medications
- Review of AEs
  - All AEs occurring within 30 days after the last dose of study treatment will be reported. Serious adverse events (SAE) related and unrelated to trial treatment will be collected for 90 days after the last dose of trial treatment or the start of new anticancer treatment, whichever comes first. Afterwards, only SAEs that are related to trial treatment will be reported.
- Physical exam
- Vital signs and weight
- Performance status
- Laboratory tests:
  - Complete blood count (CBC) with differential
  - Comprehensive serum chemistry panel
  - Urinalysis
  - T3, FT4, and TSH

Post-Treatment Disease Assessments

- Upon discontinuation of treatment in patients without documented progression, radiologic imaging for disease assessment will continue either at the investigative site or locally. Imaging will be collected and evaluated per RECIST 1.1.

- Year 1 Post-Treatment: Imaging will be performed Q 3 months (+/-14 days) or as clinically indicated for the first year post-completion of treatment.

- Years 2 and 3 Post-Treatment: Imaging will be performed Q 6 months (± 1 month) or as clinically indicated during years 2 and 3 post-treatment.

- If the study remains open, imaging will be performed annually or as clinically indicated once patient has completed 3 years of follow up for disease assessment.

- Collection of standard of care disease assessments will continue until progression, start of a new anticancer therapy, death or end of study, whichever occurs first.

Survival Follow-Up Phone Contacts (every 12 weeks ± 1 week)
Begin survival follow-up phone contacts after discontinuing MK-3475 due to disease progression or start of a new anticancer therapy (initiate 12 weeks ± 1 week following the Post-Treatment Safety Follow-Up Visit). Survival follow-up will continue until death or the end of the study, whichever occurs first.

5.1.2 ***Other Agent(s): N/A***

5.2 Definition of Dose-Limiting Toxicity: N/A

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 MK-3475 Concomitant Medication

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

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

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

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

- Anti-cancer systemic chemotherapy or biological therapy.
- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Investigational agents other than MK-3475.
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with Protocol P.I and CITN. Any lesion treated with radiation can no longer be considered as a target lesion for evaluating MK-3475 efficacy.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (*e.g.* Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be

approved after consultation with the Protocol P.I.

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

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

5.4 Duration of Therapy

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

- Disease progression warranting alternative systemic therapy,
- Intercurrent illness that prevents further administration of treatment,
- Patient decides to withdraw from the study.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- In patients with documented disease progression, treatment with MK-3475 can continue until one of the above occurs or until there is an increase in tumor burden of 25% or more following initial confirmation of progression.
- Unacceptable adverse event(s), including:
    - Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
    - Grade 3 drug-related autoimmune or inflammatory event including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
    - Any Grade 3 or 4 drug-related laboratory imbalance or electrolyte abnormality, not associated with underlying organ pathology and that do not require treatment except for electrolyte replacements do not require treatment discontinuation, with the following exceptions with approval of the Principal Investigator:
        - Hypophysitis or pan-hypopituitarism any grade should discontinue treatment.
        - Grade 4 amylase or lipase abnormalities that are not associated with DM, associated liver or gall bladder inflammation clinical manifestations of pancreatitis and which decrease to <Grade 4 within 1 week of onset may stay

on study.

- Any drug-related liver function test (LFT) abnormality that meets the following criteria requires discontinuation: Grade 3 AST or ALT (>5 x ULN) and total bilirubin >3 x ULN.

- Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.

- Any patient requiring systemic steroid or other immunosuppressive treatment.

- For patients with skin-only toxicity, when symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Discontinue MK-3475 if unable to reduce corticosteroid dose for irAEs to ≤10 mg. MK-3475 treatment may be restarted and the dose modified as specified in the protocol.

- Patients with peripheral thyroiditis and no other autoimmune/inflammatory event may be restarted after a short course of steroids on a stable replacement regimen.

- Any dosing interruption lasting >12 weeks with the following exceptions:
  - Dosing interruptions >12 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >12 weeks, the Principal Investigator must be consulted.
  - Tumor assessments should continue as per protocol even if dosing is interrupted.

5.5 Duration of Follow Up

Upon discontinuation of therapy in patients without documented disease progression, every effort should be made to continue monitoring patients' disease status by radiologic imaging either at the investigative site or locally. Imaging will be collected and evaluated per RECIST 1.1.

- Year 1 Post-Treatment: Imaging will be performed Q 3 months (+/-14 days) or as clinically indicated for the first year post-completion of treatment.

- Years 2 and 3 Post-Treatment: Imaging will be performed Q 6 months (± 1 month) or as clinically indicated during years 2 and 3 post-treatment.

- If the study remains open, imaging will be performed annually once patient has completed 3 years of follow up for disease assessment.

- Collection of standard of care disease assessments will continue until progression, start of a new anticancer therapy, death or end of study, whichever occurs first.

Patients removed from protocol therapy for unacceptable AE(s) will be followed for resolution or stabilization of the AE or 1 year, whichever is longer, in addition to following for disease assessments.

Disease assessments will be collected until (1) the start of new anticancer treatment, (2) documented disease progression, (3) death, or (4) the end of the study, whichever occurs first.

After progression or start of a new anticancer treatment, patients will be followed for survival (Section 5.1.1). Time to death will be chronicled for all patients.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the applicable criteria, including progressive disease, withdrawal, or inability to follow study protocol as listed in Section 5.4. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.6.1 Patient Replacement Strategy

Patients that have received only 1 dose of MK-3475 will not be replaced unless therapy was stopped due to toxicity.

5.7 Criteria to Resume Treatment

For non-autoimmune or inflammatory events, patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of Grade 2 fatigue.
- Patients with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Patients with combined Grade 2 AST/ALT AND total bilirubin values meeting study parameters outlined in Section 5.4 should have treatment permanently discontinued.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies (not including drug-related adrenal insufficiency or hypophysitis) adequately controlled with only physiologic hormone replacement may resume treatment after replacement correction and clinically stable regimen.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the 12 week delay period.

If treatment is delayed >12 weeks, the patient must be permanently discontinued from study therapy, except as specified in Section 5.4 (Duration of Therapy).

5.8 Treatment Beyond Progression

Immunotherapeutic agents such as MK-3475 may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

If radiologic imaging shows progressive disease (PD), tumor assessment will be repeated by the site approximately 4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms PD, patients will be discontinued from study therapy, if alternate therapy is needed. Alternatively, patients who are otherwise clinically stable may be continued on MK-3475, per investigator discretion. Even if the patient remains otherwise stable, MK-3475 treatment will be discontinued if tumor burden increases by 25% or more following initial confirmation of progression.

In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions. The decision to continue study treatment after the first evidence of disease progression, and upon confirmation of PD, determined by radiologic imaging, is at the Investigator's discretion based on the clinical status of the patient as described in the table below.

Patients may receive study treatment while waiting for confirmation of PD and if continuing MK-3475 after confirmation of PD, if they are otherwise clinically stable as defined by the following criteria:

- Absence of clinical symptoms or signs indicating disease clinically significant disease progression
- ECOG performance status ≤ 1
  Absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat imaging at approximately 4 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at approximately 4 weeks to confirm PD if possible	Discontinue treatment
Repeat scan	No additional	Discontinue	No additional	N/A

confirms PD	imaging required if treatment discontinued. If treatment continues, regularly scheduled imaging assessments continue every 9 or 12 weeks	treatment or continue treatment at the Investigator's discretion	imaging required	
Repeat scan shows SD, PR, or CR	Continue regularly scheduled imaging assessments every 9 or 12 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 9 or 12weeks	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion
Tumor imaging will be assessed first at week 13, then every 9 weeks (63±3 days) during the first year of treatment. Subsequently, tumor imaging will be performed every 12 weeks (84 ±7 days).				

5.9 Discontinuation of Treatment Following Complete Response

Discontinuation of treatment may be considered for patients who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks with MK-3475 and had at least two treatments with MK-3475 beyond the date when the initial CR was declared.

5.10 Retreatment with MK-3475 of patients with recurrence

Some patients may be eligible to receive MK-3475 in the Retreatment Period of this study if the study remains open and the patient meets the following conditions:

- Patient stopped initial treatment with MK-3475 after attaining an investigator-determined confirmed CR on MK-3475 therapy
  - Was treated for at least 24 weeks with MK-3475 before discontinuing therapy;
  - Received at least 2 treatments with MK-3475 beyond the date when the initial CR was declared; or
- Patient with confirmed SD or PR completed 2 years of MK-3475 study treatment.
- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with MK-3475
- Did not receive any anticancer treatment since the last dose of MK-3475

- Meets safety parameters in Eligibility/Exclusion Criteria outlined in Section 3.1 and 3.2

  Note: Patients that relapse on MK-3475 and are eligible for retreatment are encouraged to be retreated, however, the final decision is up to the treating physician and the patient.

Patients who restart treatment will be retreated at the same dose and frequency as when they last received MK-3475.

5.11 Beginning and End of the Trial

The study begins when the first patient signs the informed consent. The end of the study may be designated as the time point when all patients have discontinued the study or are a minimum of 6 months post-completion of study medication administration. At this point, a database lock of the trial may occur to allow the analysis of the study data. (Prior to the final database lock at the end of the study, interim database locks may occur to allow interim analysis of study data). Any remaining patients may continue to receive study medication and be seen by the investigator per usual standard of care for this patient population. In addition, the investigator will be expected to monitor for and report any SAEs, or pregnancies, as detailed in Section 7. The patient is considered on study until such time that he/she meets any of the discontinuation criteria and written notification is given to the Protocol P.I./CITN.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 MK-3475 Dose Modifications

6.1.1 General MK-3475 Dose Modifications

MK-3475 will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥Grade 3 including laboratory abnormalities, and severe or life-threatening AEs.

The table below includes general guidelines for toxicities that are not listed in the AE-specific table (see section 6.1.2).

General Dose Modification Guidelines for Drug-Related Adverse Events.

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Hematological Toxicity	1, 2, 3	No	N/A	N/A	N/A
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Non-hematological toxicity Note: Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference Section 6.1.2.	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to Grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	3	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	4	Yes	N/A	N/A	Subject must be discontinued

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued. With Principal Investigator 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. Patients who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of MK-3475 should be discontinued from trial treatment.

Additionally, immune-related adverse events (irAEs), defined as adverse events of unknown etiology, associated with drug exposure and consistent with an immune phenomenon, may be predicted based on the nature of the MK-3475 compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. All AEs of unknown etiology associated with drug exposure should be evaluated to determine if they are possibly immune-related. If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAE.

The table below includes guidelines for managing irAEs that are not listed in the AE-specific table (see section 6.1.2).

General Dose Modification Guidelines for Drug-Related Immune-Related Adverse Events

irAE	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold MK-3475	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold MK-3475 Discontinue if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 12 weeks of toxicity	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks.

6.1.2 AE-specific MK-3475 Dose Modifications and Supportive Care Guidelines

The table below includes recommendations on the management of specific AEs and when to hold and/or discontinue MK-3475. These guidelines are intended to be applied when the investigator determines the events to be treatment-related. Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance. Therefore, these recommendations should be seen as guidelines and the treating physician should exercise individual clinical judgment based on the patient.

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Colitis events • **Bowel obstruction** • **Colitis** • **Colitis microscopic** • **Enterocolitis** • **Enterocolitis hemorrhagic** • **Gastrointestinal (GI) perforation** • **Necrotizing colitis** **Diarrhea** *All patients who experience diarrhea should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.*	≤Grade 1	No change in dose	• For diarrhea, treat symptomatically (loperamide, oral hydration, electrolyte substitution and ADA colitis diet). Endoscopy is recommended if symptoms persist. • Grade 1 diarrhea that persist for >1 week should be treated with the addition of oral diphenoxylate hydrochloride and atropine sulfate four times daily and budesonide 9 mg daily.	Symptoms may include (but not limited to): • Abdominal pain, cramping and/or bloating • Blood and/or mucus in stool with or without fever • Constipation • Diarrhea • Ileus • Nausea and/or vomiting • Peritoneal signs consistent with bowel perforation • Rectal bleeding • With or without fever Patients with diarrhea should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as	All attempts should be made to rule out other causes such as metastatic disease, bacterial or parasitic infection, viral gastroenteritis, or the first manifestation of an inflammatory bowel disease by examination for stool leukocytes, stool cultures, and a *Clostridium difficile* titer.
	Grade 2	Hold until ≤Grade 1. Resume at same dose level. May increase dosing interval by 1 week if it takes more than 4 weeks for toxicities to resolve.	• GI consultation and endoscopy is recommended to confirm or rule out colitis for grade 2 diarrhea that persists >1 week or grade 1-2 diarrhea with rectal bleeding (additional guidelines for the treatment of persistent colitis are provided below). • Grade 2 diarrhea should be treated with the addition of oral diphenoxylate hydrochloride and atropine sulfate four times daily and budesonide 9 mg daily. • Grade 2 diarrhea with diffuse ulceration and bleeding seen on endoscopy may require oral steroids with prolonged taper and represent an increased risk for the development of bowel perforation. • When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. • In patients with Grade 2 enterocolitis, MK-3475 should be withheld and anti-diarrheal treatment should be started. If symptoms are persistent for more than one week, systemic corticosteroids should be initiated (*e.g.*, 0.5 mg/kg/day of prednisone or equivalent). When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month.		

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
	Grade 3-4	Withhold MK-3475 Discontinue if unable to reduce corticosteroid dose to <10 mg per day prednisone equivalent within 12 weeks of toxicity	• In patients with Grade 3 enterocolitis, MK-3475 will be permanently discontinued and treatment with systemic corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month. For Grade 3-4 diarrhea (or Grade 2 diarrhea that persists after initial steroid treatment), • Rule out bowel perforation. Imaging with plain films or computed tomography (CT) can be useful. • Consider consultation with gastroenterologist and confirmation biopsy with endoscopy. • Treat with intravenous (IV) steroids (methylprednisolone 125 mg) followed by high-dose oral steroids (prednisone 1-2 mg/kg once per day or dexamethasone 4 mg every 4 hours). When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Taper over 6-8 weeks in patients with diffuse and severe ulceration and/or bleeding. • If IV steroids followed by high-dose oral steroids does not reduce initial symptoms within 48-72 hours, consider treatment with infliximab at 5 mg/kg once every 2 weeks. Discontinue infliximab upon symptom relief and initiate a prolonged steroid taper over 45-60 days. If symptoms worsen during steroid reduction, initiate a retapering of steroids starting at a higher dose of 80 or 100 mg followed by a more prolonged taper and administer infliximab. CAUTION: infliximab is contraindicated in patients with bowel perforation or sepsis[b]. • If symptoms persist despite the above treatment a surgical consult should be obtained.	peritoneal signs and ileus). In symptomatic patients, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered.	

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Endocrine events • **Hyperthyroidism** • **Hypophysitis** • **Hypopituitarism** • **Hypothyroidism** • **Thyroid disorder** • **Thyroiditis**	Grade 1-2	No change in dose	• Monitor thyroid function or other hormonal level tests and serum chemistries more frequently until returned to baseline values.	Symptoms may include (but not limited to): • Abdominal pain • Abnormal thyroid function tests and/or serum chemistries (Thyroid-stimulating hormone increased [decreased], Free thyroxine increased, Tri-iodothyronine increased.) • Arrhythmias[c] • Cold or heat intolerance • Fatigue • Fever • Headache • Hypotension[c] • Loss of appetite • Mental status and/or behavior changes • Nausea and/or vomiting • Unusual bowel habits • Vision disturbances • Weakness	All attempts should be made to rule out other causes such as brain metastases, sepsis, and/or infection. An endocrinology consultation is recommended.
	Grade 3-4	Hold/discontinue MK-3475.	• Consider endocrine consultation. • Rule out infection and sepsis with appropriate cultures and imaging. • Treat with an initial dose of methylprednisolone 1-2 mg/kg IV followed by oral prednisone 1-2 mg/kg per day. When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.		
Endocrine events • **Adrenal insufficiency** • **Hypophysitis** • **Pan-hypopituitarism**	Grade 1-4	Discontinue MK-3475.	• Thyroid hormone and/or steroid replacement therapy to manage adrenal insufficiency. • If Grade 1-2 hypophysitis is considered, pituitary gland imaging should be considered (magnetic resonance imaging [MRIs] with gadolinium and selective cuts of the pituitary can show enlargement or heterogeneity and confirm the diagnosis). • Grade 3-4 hypophysitis with clinically significant adrenal insufficiency and hypotension, dehydration, and electrolyte abnormalities (such as hyponatremia and hyperkalemia) constitutes adrenal crisis. Hospitalization and IV methylprednisolone should be initiated.		
Eye event • **Uveitis**	Grade 1	Discontinue MK-3475 **if** symptoms persist despite treatment with topical immunosuppressive therapy	• Evaluation by an ophthalmologist is strongly recommended. • Treat with topical steroids such as 1% prednisolone acetate suspension and iridocyclitics.	Symptoms may include (but not limited to): • Blurred vision • Diffuse erythema and a prominent blush on the sclerae • Dryness of the eyes	All attempts should be made to rule out other causes such as metastatic disease, infection, or other ocular

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
	Grade 2	Discontinue MK-3475 **if** symptoms persist despite treatment with topical immunosuppressive therapy and does not improve to Grade 1 within the re-treatment period OR requires systemic treatment.	• Evaluation by an ophthalmologist is strongly recommended. • Treat with topical steroids such as 1% prednisolone acetate suspension and iridocyclitics.	• Pain • Photophobia	disease (*e.g.*, glaucoma or cataracts).
	Grade 3-4	Discontinue MK-3475.	• Treat with systemic corticosteroids such as prednisone at a dose of 1-2 mg/kg per day. When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.		
Hepatic events • **Hepatitis** • **Hepatitis, Autoimmune**	Grade 1-2	No change in dose	• Monitor liver function tests more frequently until returned to baseline values.	Symptoms may include (but not limited to): • Elevations in: o AST >2.5 times ULN o ALT >2.5 times ULN o Total bilirubin >1.5 X ULN • Fever • Malaise • Upper quadrant abdominal pain	All attempts should be made to rule out other causes such as metastatic disease, progressive liver disease, viral hepatitis, alternative drug toxicity, infectious causes and/or myositis.
	Grade 3-4	Discontinue MK-3475 when AST or ALT >5.0 times ULN and/or total bilirubin >3.0 times ULN.	• Consider appropriate consultation and liver biopsy to establish etiology of hepatic injury, if necessary. • Treat with high-dose IV glucocorticosteroids for 24-48 hours. When symptoms improve to grade 1 or less, a steroid taper with dexamethasone 4 mg every 4 hours or prednisone at 1-2 mg/kg should be started and continued over no less than 4 weeks. • If serum transaminase levels do not decrease 48 hours after initiation of systemic steroids, oral mycophenolate mofetil 500 mg every 12 hours may be given. Infliximab is not recommended due to its potential for hepatotoxicity[b]. • Several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased .		

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Nausea	≤Grade 1	No change in dose	• Nausea should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Patients should be strongly encouraged to maintain liberal oral fluid intake.		
	Grade 2	Hold until ≤Grade 1. Resume at same dose level. May increase dosing interval by 1 week if it takes more than 4 weeks for toxicities to resolve.			
	Grade 3	Hold until <Grade 2. May increase dosing interval by 1 week for each occurrence. Discontinue if toxicities do not resolve within 12 weeks.			
	Grade 4	Off protocol therapy			
Neutropenia	≤Grade 1	No change in dose			
	Grade 2	No change in dose			
	Grade 3	No change in dose			
	Grade 4	Hold until resolves to ≤Grade 1. May increase the dosing interval by 1 week. Discontinue if toxicities do not resolve within 12 weeks.			

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Pneumonitis events • **Pneumonitis** • **Interstitial lung disease** • **Acute interstitial pneumonitis**	Grade 1	MK-3475 may be continued with close monitoring.	• Radiologic findings should be followed on serial imaging studies. • Consider pulmonary consultation and/or bronchoscopy if clinically indicated.	Symptoms may include (but not limited to): • Abnormal breath sounds • Chest pain and/or tightness[c] • Dyspnea[c] • Dry cough • Fatigue • Fever • Hemoptysis	All attempts should be made to rule out other causes such as metastatic disease, bacterial or viral infection.
	Grade 2	Hold MK-3475	**To rule out other causes such as infection:** • Consider pulmonary consultation with bronchoscopy and biopsy/bronchoalveolar lavage (BAL). • Consider pulmonary function tests. **If the patient is determined to have study drug associated pneumonitis:** • Treat with systemic corticosteroids at a dose of 1-2 mg/kg/day prednisone or equivalent. When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. • Treatment with MK-3475 may be resumed if the event improves to ≤Grade 1 within 12 weeks and corticosteroids have been reduced to the equivalent of methylprednisolone 10 mg by mouth daily or less. Repeat chest imaging monthly as clinically indicated. **For Grade 2 pneumonitis that improves to ≤Grade 1 within 12 weeks, the following rules should apply:** • First episode of pneumonitis: May increase dosing interval by one week in subsequent cycles. • Second episode of pneumonitis: Discontinue MK-3475 if upon rechallenge the patient develops a second episode of ≥Grade 2 pneumonitis.		

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
	Grade 3-4	Discontinue MK-3475.	• Consider pulmonary function tests with pulmonary consult. • Bronchoscopy with biopsy and/or BAL is recommended. • Treat with IV steroids (methylprednisolone 125 mg). When symptoms improve to grade 1 or less, a high-dose oral steroid (prednisone 1-2 mg/kg once per day or dexamethasone 4 mg every 4 hours) taper should be started and continued over no less than 4 weeks. • If IV steroids followed by high-dose oral steroids does not reduce initial symptoms within 48-72 hours, treat with infliximab at 5 mg/kg once every 2 weeks. Discontinue infliximab upon symptom relief and initiate a prolonged steroid taper over 45-60 days. If symptoms worsen during steroid reduction, initiate a retapering of steroids starting at a higher dose of 80 or 100 mg followed by a more prolonged taper and administer infliximab.		
Renal events • **Nephritis** • **Nephritis autoimmune** • **Renal failure** • **Renal failure, Acute**	Grade 1	Consider withholding MK-3475 **if** Grade 1 does not improve with symptomatic treatment	• Provide symptomatic treatment.	Symptoms may include (but not limited to): • Fatigue • High blood pressure • Increased serum creatinine • Swelling	All attempts should be made to rule out other causes such as obstructive uropathy, progression of disease, or injury to other chemotherapy agents. A renal consultation is recommended.
	Grade 2	Consider withholding MK-3475.	• Systemic corticosteroids may be indicated.		
	Grade 3-4	Discontinue MK-3475.	• Renal consultation with consideration of ultrasound and/or biopsy as appropriate. • Treat with systemic corticosteroids at a dose of 1-2 mg/kg prednisone or equivalent once per day. • When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. • Discontinue MK-3475 if unable to reduce corticosteroid dose for irAEs to ≤10 mg. • MK-3475 treatment may be restarted and the dose modified as specified in the protocol.		

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Skin events • **Dermatitis exfoliative** • **Erythema multiforme** • **Stevens-Johnson syndrome** • **Toxic epidermal necrolysis** If they are considered to be immune related, ≥Grade 3 or result in dose modification or discontinuation: • **Pruritus** • **Rash** • **Rash generalized** • **Rash maculo-papular** • **Vitiligo**	Grade 1-2	No change in dose	• Symptomatic treatment should be given such as topical glucocorticosteroids (*e.g.*, betamethasone 0.1% cream or hydrocortisone 1%) or urea-containing creams in combination with oral antipruritics (e.g., diphenhydramine HCl or hydroxyzine HCl). • Treatment with oral steroids is at investigator discretion for grade 2 events.		All attempts should be made to rule out other causes such as metastatic disease, infection, or allergic dermatitis.
	Grade 3	Hold MK-3475.	• Consider dermatology consultation and biopsy for confirmation of diagnosis. • Treatment with oral steroids is recommended, starting with 1 mg/kg prednisone or equivalent once per day or dexamethasone 4 mg four times orally daily. When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.		
	Grade 4	Permanently discontinue MK-3475.	• Dermatology consultation and consideration of biopsy and clinical dermatology photograph. • Initiate steroids at 1-2 mg/kg prednisone or equivalent. When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.		
Thrombocytopenia	≤Grade 1	No change in dose			
	Grade 2	No change in dose			
	Grade 3	No change in dose	• Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.		
	Grade 4	Hold MK-3475 until resolves to ≤Grade 1. May increase the dosing interval by 1 week.	• Grade 4 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.		

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Vomiting	≤Grade 1	No change in dose	• Vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Patients should be strongly encouraged to maintain liberal oral fluid intake.		
	Grade 2	Hold until ≤Grade 1. Resume at same dose level. May increase dosing interval by 1 week if it takes more than 4 weeks for toxicities to resolve.			
	Grade 3	Hold until <Grade 2. May increase dosing interval by 1 week for each occurrence. Discontinue if toxicities do not resolve within 12 weeks.			
	Grade 4	Off protocol therapy			

Event(s)	CTCAE v5.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines	Symptoms[a]	Differential Diagnosis
Other events • **Autoimmune neuropathy** • **Demyelinating polyneuropathy** • **Guillain-Barre** • **Myasthenia gravis-like syndrome** • **Non-infectious myocarditis** • **Non-infectious pericarditis** • **Pancreatitis** • **Rapid onset of grade 3 fatigue in the absence of disease progression**	Grade 1	Consider withholding MK-3475 for Grade 1 that does not improve with symptomatic treatment.	• Provide symptomatic treatment.		All attempts should be made to rule out other causes. Therapeutic specialists should be consulted as appropriate.
	Grade 2	Consider withholding MK-3475.	• Systemic corticosteroids may be indicated. • Consider biopsy for confirmation of diagnosis.		
	Grade 3-4	Discontinue MK-3475.	• Treat with systemic corticosteroids at a dose of 1-2 mg/kg prednisone or equivalent once per day. • When symptoms improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. • Discontinue MK-3475 if unable to reduce corticosteroid dose for irAEs to ≤10 mg. • MK-3475 treatment may be restarted and the dose modified as specified in the protocol.		

[a] The signs and symptoms may be associated with any of the diagnoses in the associated "Event(s)" column.
[b] Janssen Biotech, Inc.: REMICADE (Infliximab) prescribing information revised September 2011.
http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Label_ApprovalHistory#labelinfo
[c] If symptoms indicate possible new or worsening cardiac abnormalities, additional testing and/or a cardiology consultation should be considered

6.1.3 Treatment Guidelines for Infusion Reactions

Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

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

Treatment Guidelines for Infusion 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 hours	**Stop Infusion and Monitor Symptoms.** Additional supportive care, as per institutional guidelines. 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). **Please note:** prior to restarting the infusion, confirm that the 4 hour room temperature stability from the time of the IV bag preparation will not be exceeded. 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 upon rechallenge despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of antipyretic).

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 and Monitor Symptoms. Subject is permanently discontinued from further trial treatment administration.** Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours PO or IV) for up to three days. The dexamethasone dose will then be reduced step-wise over up to four days. Additional supportive care, as per institutional guidelines. 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.	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For Further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

6.2 Delayed Visits for Reasons Other Than Toxicity

A schedule for return visits should be established at the first visit. If a participant misses a treatment, the missed treatment will be administered as soon as possible, so that the subsequent treatments are given in the appropriate intervals. Treatment may be continued for an additional time period, if needed. Participants who are treated outside of the established schedule should return to the original schedule as soon as possible.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

AE monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential AE associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with

bold and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the *CTEP, NCI Guidelines: Adverse Event Reporting Requirements* http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm for further clarification.

Note: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously via CTEP-AERS. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPRs for CTEP IND Agent(s): **MK-3475**

Comprehensive Adverse Events and Potential Risks list (CAEPR)

for

MK-3475 (pembrolizumab, NSC 776864)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3793 patients.* Below is the CAEPR for MK-3475 (pembrolizumab, NSC 776864).

Note: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.4, March 16, 2018[1]

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia[2]		
	Lymph node pain[2]		
	Thrombotic thrombocytopenic purpura[2]		
CARDIAC DISORDERS			
		Myocarditis[2]	

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
	Endocrine disorders - Other (thyroiditis)[2]		
	Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
EYE DISORDERS			
		Uveitis[2]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		
	Diarrhea[2]		***Diarrhea[2] (Gr 2)***
	Mucositis oral[2]		
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[2]		
	Small intestinal mucositis[2]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[2]		
Fatigue			***Fatigue (Gr 2)***
	Fever[2]		
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (autoimmune hepatitis)[2]		
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis[2]	
		Cytokine release syndrome[2]	
		Immune system disorders – Other (acute graft-versus-host disease)[2,3]	
		Immune system disorders - Other (hemophagocytic lymphohistiocytosis)[2]	
	Immune system disorders - Other (pseudoprogression/tumor inflammation)[2]		
	Immune system disorders – Other (sarcoidosis)[2]		
		Serum sickness[2]	
INFECTIONS AND INFESTATIONS			

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Infection[4]		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Infusion-related reaction	
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased		
	CPK increased		
		GGT increased	
		Serum amylase increased	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyponatremia		
		Metabolic and nutrition disorders – Other (diabetic ketoacidosis)[2]	
		Metabolism and nutrition disorders – Other (type 1 diabetes mellitus) [2]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		***Arthralgia[2] (Gr 2)***
	Arthritis[2]		
	Avascular necrosis[2]		
	Back pain		
	Joint effusion[2]		
	Joint range of motion decreased		
	Musculoskeletal and connective tissue disorder - Other (tenosynovitis)[2]		
	Myalgia[2]		
	Myositis[2]		
NERVOUS SYSTEM DISORDERS			
		Guillain-Barre syndrome[2]	
		Nervous system disorders - Other (myasthenic syndrome)[2]	
		Nervous system disorders - Other (neuromyopathy)[2]	

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Nervous system disorders – Other (non-infectious encephalitis)[2]	
		Nervous system disorders – Other (non-infectious meningitis)[2]	
		Nervous system disorders - Other (polyneuropathy)[2]	
		Paresthesia	
		Peripheral motor neuropathy[2]	
RENAL AND URINARY DISORDERS			
		Renal and urinary disorders - Other (autoimmune nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Pleuritic pain[2]		
	Pneumonitis[2]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Bullous dermatitis[2]		
		Erythema multiforme[2]	
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus[2]		***Pruritus*[2] *(Gr 2)***
	Rash acneiform[2]		
	Rash maculo-papular[2]		***Rash maculo-papular*[2] *(Gr 2)***
	Skin and subcutaneous tissue disorders - Other (dermatitis)[2]		
	Skin hypopigmentation[2]		
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis	
	Urticaria[2]		
VASCULAR DISORDERS			
		Vasculitis[2]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@ctep.nci.nih.gov. Your name, the name of the investigator, the protocol, and the agent should be included in the email.

[2] Immune-mediate adverse reactions have been reported in patients receiving MK-3475 (pembrolizumab). Adverse events potentially related to MK-3475 (pembrolizumab) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of MK-3475 (pembrolizumab), administration of corticosteroids and supportive care.

[3] Acute graft-versus-host disease has been observed in patients treated with MK-3475 (pembrolizumab) who received hematopoietic stem cell transplants.

[4] Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on MK-3475 (pembrolizumab) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MK-3475 (pembrolizumab) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation; Hemolysis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Chest pain - cardiac; Heart failure; Myocardial infarction; Pericardial effusion; Pericardial tamponade; Ventricular arrhythmia
EYE DISORDERS - Eye pain
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Constipation; Duodenal hemorrhage; Dysphagia; Gastritis; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intussusception); Oral pain; Rectal hemorrhage; Small intestinal perforation; Upper gastrointestinal hemorrhage; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Facial pain; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Malaise; Non-cardiac chest pain; Pain
INVESTIGATIONS - Cholesterol high; Creatinine increased; Fibrinogen decreased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (groin pain); Pain in extremity
NERVOUS SYSTEM DISORDERS - Aphonia; Depressed level of consciousness; Dysarthria; Edema cerebral; Encephalopathy; Headache; Hydrocephalus; Lethargy; Meningismus; Nervous system disorders - Other (brainstem herniation); Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Confusion

RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria; Renal and urinary disorders - Other (hydronephrosis); Nephrotic syndrome; Urinary incontinence; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Dyspnea; Hypoxia; Laryngeal inflammation; Pleural effusion; Pneumothorax; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Skin and subcutaneous tissue disorders - Other (drug eruption)
VASCULAR DISORDERS - Hypertension; Peripheral ischemia; Thromboembolic event

***Note**:* MK-3475 (pembrolizumab) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (i.e., those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

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

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The Coordinating Center of the Corresponding Organization is responsible for submitting to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease Progression"** under the system organ class (SOC) "General disorders and administration site conditions". Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Pregnancy loss

Pregnancy loss is defined in CTCAE as "Death in Utero".

Any pregnancy loss should be reported expeditiously, as Grade 4 "pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC.

A pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP AERS recognizes this event as a patient death.

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERSwithin the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol-specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions: N/A

mechanism (Section 7.4):

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the ***[form name]*** is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

7.7 Reporting of Pregnancy and Lactation to the Sponsor

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) that occurs during the trial or within 120 days of completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. 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.

8. PHARMACEUTICAL INFORMATION

A list of the AEs and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent

8.1.1 MK-3475 (SCH 900475) (NSC 776864)

Other Names: SCH 900475

Classification: Anti-PD-1 MAb

Molecular Weight: 148.9-149.5 KDa

CAS Number: 1374853-91-4

Mode of Action: The programmed cell death 1 (PD-1) receptor is an inhibitory receptor expressed by T cells. When bound to either of its ligands, PD-L1 or PD-L2, activated PD-1 negatively regulates T-cell activation and effector function. The pathway may be engaged by tumor cells to suppress immune control. MK-3475 blocks the negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

Description: MK-3475 is a humanized MAb of the IgG4/kappa isotype.

How Supplied: MK-3475 is supplied by Merck & Co., Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as single-use 50 mg vials containing a sterile, non-pyrogenic white to off-white lyophilized powder formulated in 10mM histidine buffer, pH 5.2-5.8, containing 7% sucrose and 0.02% polysorbate 80.

Preparation: Allow the required number of MK-3475 vials to equilibrate to room temperature. Reconstitute the lyophilized powder by adding 2.3 mL of Sterile Water for Injection (SWFI) to the vial to yield 2.4 mL of solution containing 25 mg/mL of MK-3475. The vial contains an excess fill of 10 mg (0.4 mL) to ensure recovery of 50 mg (2 mL) per vial. Add SWFI along the walls of the vial to avoid foaming. Swirl the vial, do not shake. Discard vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the final solution for IV administration add the dose volume of MK-3475 to an infusion bag containing 0.9% Sodium Chloride Injection, USP and gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL**.

> Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin

Storage: Store intact vials between 2°C - 8°C (36°F - 46°F). Do not freeze. If a storage temperature

excursion is identified, promptly return MK-3475 to between 2-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability testing of the intact vials is on-going**.**

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 20 hours. MK-3475 solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of reconstituted solution in vials, room temperature storage of admixture solutions in the IV bags, and the duration of infusion.

Route of Administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of Administration: Infuse over approximately 30 minutes (range: 25 - 40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required; however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer other drugs through the same infusion line.

Compatible infusion set materials: PVC plasticized with DEHP or DEHT, PVC and tri-(2-ethylhexyl) trimellitate, polyethylene lined PVC, polyurethane, or polybutadiene

Patient Care Implications: Refer to the protocol for information on evaluation and management of potential immune-related adverse events.

Dose Calculation: Calculate the required dose amount based on dose level and subject weight. The dose amount should be recalculated if the subject's weight changes by more than 10% from the baseline weight measurement. If the patient's weight changes again by 10% or more (either increase or decrease) from the most recent weight used for dose calculation, recalculation of the dose is required.

Availability: MK-3475 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

MK-3475 is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.2 Agent Ordering and Agent Accountability

8.2.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there

are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

8.2.2 Agent Inventory Records –The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.2.3 Investigator Brochure Availability - The current version of the IB will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB coordinator via email.

8.2.4 Useful Links and Contacts:

CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/

NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov

PMB policies and guidelines:
http://ctep.cancer.gov/branches/pmb/agent_management.htm

PMB Online Agent Order Processing (OAOP) application:
https://ctepcore.nci.nih.gov/OAOP/

CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/

CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov

PMB email: PMBAfterHours@mail.nih.gov

IB Coordinator: IBCoordinator@mail.nih.gov

PMB phone and hours of service: (240) 276-6575
Monday through Friday between 8:30 am and 4:30 pm (ET)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

The study calendars and lab manual describe blood draws for safety labs, research labs, and the CITN Central Laboratory Biorepository. In the interest of patient safety, we are including a provision to draw less blood if patients are anemic. A CBC will be performed as part of the safety labs each time research labs and biorepository blood needs to be drawn (safety labs drawn

for screening will used for this purpose prior to the first blood draws for research and the biorepository). The results of the CBC will be reviewed and the following blood volumes will be drawn based upon the patient's hemoglobin level:

- For a hemoglobin over 10.0 g/dL, draw the full volume of blood for safety labs, research labs, and biorepository. The biorepository volume of 10 mL will be used for cells.
- For a hemoglobin between 9.0 and 10.0 g/dL, draw the full volume of blood for safety labs, decrease blood volume drawn for yellow top tubes (from 130 mL to 110 mL(this decrease eliminates blood draws for the biorepository). Draw remaining research labs.
- For a hemoglobin less than 9.0 g/dL, draw the full volume of blood for safety labs, MCPyV antibody levels (10 ml or 5 ml red top), immunophenotyping (10 ml green top), and T cell functioning/phenotyping (10 ml yellow top).Eliminate other research labs including blood draw for biorepository.

9.1 Biomarker Studies

See Laboratory Correlative Studies, Section 9.2

9.2 Laboratory Correlative Studies

Hypotheses, rationale and literature citations are provided in Section 2.5 for the ancillary/exploratory immune correlative studies in this protocol.

9.2.1 *Evaluation of Tumor Biopsies for Immune Cell Infiltrate, Merkel Cell Polyomavirus, and mRNA expression—Laboratory Correlative Studies#1 and #2*

9.2.1.1 *Collection of Specimen(s)*

Formalin-fixed, paraffin-embedded archival tissue block(s) from tumor obtained before MK-3475 will be identified by the clinical site at the relevant pathology laboratories where they were processed and stored. Alternatively, patients will undergo a baseline biopsy (core, punch or excisional) as part of this protocol. In patients who have not achieved CR (either responding, stable or progressing lesions) a biopsy sample of a tumor may be obtained if feasible and safe (core, punch or excisional biopsy). This biopsy is highly preferred, but optional. The biopsies will be analyzed as outlined above. All studies indicated in Section 2.5.1 may be carried out on the archival tissue or baseline biopsies as well as biopsies collected from responding, stable or progressing lesions.

9.2.1.2 *Handling of Specimens(s)*

Formalin-fixed, paraffin-embedded archival tumor blocks will be requested by fax and phone by the clinical site from the relevant pathology laboratory by providing the patient's name, consent form, and date of collection. If patients undergo biopsy as part of this protocol, tissue will be placed in formalin for overnight shipping to the CITN Central Laboratory.

9.2.1.3 *Shipping of Specimen(s)*

The clinical site will arrange for the formalin-fixed, paraffin-embedded archival tumor blocks to be shipped at ambient temperature to the CITN Central Laboratory. For biopsies done as part of this protocol, tissue in

formalin will be shipped overnight at ambient temperature to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory. All samples will then be delivered to FHCRC Core/Habecker Laboratory.

Following sectioning at the CITN Central Lab, unstained slides will be shipped by CITN Central Laboratory to Merck Research Laboratories in Palo Alto, CA, to Merck-designated CRO, or to PhenoPath, Seattle, WA. DNA extracted from the archival material will be provided to Dr. Meei-Li Huang clinical virology laboratory at FHCRC for DNA PCR analysis.

9.2.1.4 *Site(s) Performing Correlative Study*

The majority of the IHC studies will be performed at the FHCRC Core/Habecker Laboratory with oversight by Dr. Paul Nghiem's laboratory or at PhenoPath in Seattle, WA. Pre- and post-treatment levels of CD8+ T-cell tumor infiltrates will be assessed using criteria defined in Paulson, et al [Paulson 2011]. MCPyV protein expression in tumor tissue will also be assessed by IHC.

The DNA-PCR assay will be performed by Dr. Meei-Li Huang in a clinical grade virology laboratory (Vaccine and Infectious Disease Division at FHCRC).

PD-1 and PD-L1 expression will be tested by multiparametric IHC at Merck Research Laboratories in Palo Alto, CA, and PD-1 expression will also be tested with chromogenic IHC at a Merck-designated CRO. The multiparametric studies performed by Merck Research Laboratories will allow simultaneous determination of subcellular localization and identity of tumor/inflammatory cell types expressing PD-L1. When feasible, samples will also be assessed by Dr. Janis Taube at Johns Hopkins using multi-spectral immunofluorescence as detailed in the NEJM manuscript [Nghiem 2016]. The option will be left open to subsequently assess further analytes by IHC at PhenoPath or other collaborators based on mutual agreement with the investigators/CITN and Merck.

mRNA expression (Nanostring) will be performed at Merck Research Laboratories in Palo Alto using unstained tissue slide material or may be performed at NanoString Technologies in Seattle, WA, pending mutual agreement on feasibility and logistics between NanoString Technologies & Investigators/CITN.

9.2.2 *Merkel Cell Polyomavirus–Specific Cellular Immune Responses—Laboratory Correlative Studies #3–#5*

9.2.2.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects just before, during, and at end of treatment. These blood draws will typically be carried out on the same visit as imaging scans.

9.2.2.2 ***Handling of Specimens(s)***

Yellow-top tubes (for subsequent PBMC isolation) will be shipped on day of blood draw for overnight delivery to the CITN Central Laboratory.

9.2.2.3 ***Shipping of Specimen(s)***

Yellow-top tubes will be shipped overnight at ambient temperature to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory. The CITN Central Laboratory will coordinate the shipment of study samples for FACS-based sorting and T cell activation to the Nghiem and McElrath Labs, and samples for mRNA expression to Merck Research Laboratories in Palo Alto, pending mutual agreement on feasibility and logistics between Merck & Investigators/CITN.

9.2.2.4 ***Site(s) Performing Correlative Study***

Merkel cell polyomavirus–specific cellular immune responses will be assessed at the UW at the laboratory of Dr. Paul Nghiem and the CITN Central Laboratory. ICS analyses will be performed by the McElrath Lab at FHCRC.

mRNA expression (Nanostring) will be performed at Merck Research Laboratories in Palo Alto, pending mutual agreement on feasibility and logistics between Merck & Investigators/CITN.

9.2.3 ***Merkel Cell Polyomavirus–Specific Antibody Responses—Laboratory Correlative Study #6***

9.2.3.1 ***Collection of Specimen(s)***

Blood draws will be performed by venipuncture on study subjects just before, during, and at end of treatment. These blood draws will typically be carried out on the same visit as imaging scans.

9.2.3.2 ***Handling of Specimens(s)***

Serum specimens will be processed and frozen at the clinical collection site for batch shipment to the CITN Central Laboratory.

9.2.3.3 ***Shipping of Specimen(s)***

Serum samples will be batch shipped overnight on dry ice to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory. The CITN Central Laboratory will coordinate the shipment of serum samples to the UW Department of Laboratory Medicine.

9.2.3.4 ***Site(s) Performing Correlative Study***

Merkel cell polyomavirus–specific antibody responses will be assessed by Luminex by the UW Laboratory Medicine under the supervision of Dr. Mark Wener.

9.2.4 *Quantification of PBMC and T-cell Subsets—Laboratory Correlative Study #7*

9.2.4.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects just before, during, and at end of treatment. These blood draws will typically be carried out on the same visit as imaging scans.

9.2.4.2 *Handling of Specimens(s)*

Blood Samples will be collected at room temperature and shipped ambient to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the central laboratory within 24 hours of blood draw.

9.2.4.3 *Shipping of Specimen(s)*

Green-top tubes will be shipped at ambient temperature to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory.

9.2.4.4 *Site(s) Performing Correlative Study*

Quantification of PBMC and T-cell subsets will be performed under the direction of Dr. Morishima at the CITN Central Laboratory.

9.2.5 *Whole-Exome Sequencing and RNA Sequencing—Laboratory Correlative Study #8*

9.2.5.1 *Collection of Specimen(s)*

Testing will be performed on matched tumor collected at baseline and peripheral blood samples.

9.2.5.2 *Handling of Specimen(s)*

Blood samples will be collected at room temperature and shipped ambient to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the CITN Central Laboratory within 24 hours of blood draw.

Formalin-fixed, paraffin-embedded archival tissue block(s) from tumor obtained before MK-3475 treatment will be obtained as described in 9.2.1. If sufficient archival material for performance of WES and RNA sequencing is not available, additional archival material may be requested from the site.

9.2.5.3 *Shipping of Specimen(s)*

Blood tubes will be shipped at ambient temperature to the CITN Central Laboratory.

The clinical site will arrange for the formalin-fixed, paraffin-embedded archival tumor blocks to be shipped at ambient temperature to the CITN Central Laboratory. For biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory.

Following sectioning at the CITN Central Laboratory, unstained slides will be shipped by CITN Central Lab to Neon Therapeutics or equivalent designated collaborator.

9.2.5.4 ***Site(s) Performing Correlative Study***

Whole exome sequencing and RNA sequencing will be performed by Neon Therapeutics, Cambridge, MA, or equivalent designated vendor.

9.2.6 ***Neoantigen Identification—Laboratory Correlative Study #9***

9.2.6.1 ***Collection of Specimen(s): NA***

No additional specimens are required for this correlative study once WES and RNA sequencing are performed. Neoantigen prediction will be based on WES and RNA sequencing (Correlative Study #8, Section 9.2.5).

9.2.6.2 ***Handling of Specimen(s): NA***

9.2.6.3 ***Shipping of Specimen(s): NA***

9.2.6.4 ***Site(s) Performing Correlative Study***

Neoantigen identification will be performed by Neon Therapeutics, Cambridge, MA, or equivalent designated vendor.

9.2.7 ***T cell Receptor (TCR) Sequencing and Functional Phenotyping—Lab Correlative Study #10***

9.2.7.1 ***Collection of Specimen(s)***

Testing will be performed on tumor biopsies and peripheral blood samples collected at baseline and post-treatment.

9.2.7.2 ***Handling of Specimen(s)***

Blood samples will be collected at room temperature and shipped ambient to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the CITN Central Laboratory within 24 hours of blood draw.

Formalin-fixed, paraffin-embedded archival tissue block(s) from tumor obtained before MK-3475 treatment will be obtained as described in 9.2.1. If sufficient archival material for performance of TCR sequencing is not available, additional archival material may be requested from the site.

Fresh tumor biopsies at a minimum of 100 milligram will be collected, transported in appropriate media, and dissociated using mechanical and enzymatic digestion with collagenase, DNAase, and hyaluronidase. The resulting single cell suspension will be collected by centrifugation and cryopreserved viably in 10% dimethyl sulfoxide (DMSO) and either 12.5% HSA (or 12.5% FCS (Fetal calf serum) or CryoStor10. Samples will be stored at -80°C and then transferred appropriately to liquid nitrogen for long term

storage until shipment.

9.2.7.3 ***Shipping of Specimen(s)***

Blood sample tubes will be shipped at ambient temperature to the CITN Central Laboratory.

The clinical site will arrange for the formalin-fixed, paraffin-embedded archival tumor blocks to be shipped at ambient temperature to the CITN Central Laboratory. For formalin-fixed biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory.

The clinical site will arrange for cryo-preserved tumor biopsy specimens to be shipped on dry ice to the CITN Central Laboratory. Clinical sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory.

Biopsies and PBMC will be shipped by CITN Central Lab to the Warren Lab or equivalent designated collaborator.

9.2.7.4 ***Site(s) Performing Correlative Study***

T cell receptor sequencing and functional phenotyping will be performed by the Warren Lab, or equivalent designated vendor.

9.2.8 ***Kynurenine/Tryptophan (Kyn/Trp) Ratio--Laboratory Correlative Study***

9.2.8.1 ***Collection of Specimen(s)***

Blood draws will be performed by venipuncture on study subjects at time points before, during, and after treatment.

9.2.8.2 ***Handling of Specimens(s)***

Plasma will be isolated by the local laboratory and frozen at -80°C

9.2.8.3 ***Shipping of Specimen(s)***

Plasma samples will be batch shipped overnight on dry ice to the CITN Central Laboratory. Sites will utilize a web-based specimen system (BSI-Web) to communicate with the CITN Central Laboratory. The CITN Central Laboratory will coordinate the shipment of samples to Incyte Corporation.

9.2.8.4 ***Site(s) Performing Correlative Study***

Kynurenine and tryptophan levels will be assessed at Incyte Corporation.

9.2.9 ***CITN Central Laboratory Biorepository***

On days of blood draws for correlative studies, there is also a planned blood draw of 20 mL for the biorepository (PBMC storage). Blood that is drawn for the biorepository will be shipped to the CITN Central Laboratory for storage and future testing.

Any tissue remaining after testing has been completed will be kept in the CITN Central Laboratory Biorepository for storage and future testing.

9.3 Special Studies-N/A

9.3.1 *HLA Class I and Class II typing*

DNA will be extracted from residual blood collected at baseline, and assayed either at Puget Sound Blood Center or the laboratory of Dr. Dan Geraghty (FHCRC). CITN Central Laboratory will coordinate sample shipping to Puget Sound Blood Center or Geraghty Lab.

10. STUDY CALENDAR

All baseline evaluations with the exception of scans are to be conducted within 14 days before start of protocol therapy. Scans must be done ≤4 weeks before the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours before initiating the next cycle of therapy. **Treatment Year 1**

Trial Period	Screening Phase	Treatment Cycles Year 1 [a]				End of Tx	Post-treatment follow-up		
		To be repeated up to 2 years							
Tx Cycle/Title	Pre-Therapy	Cycle 1	Cycle 2	Cycles without scans (q 3 weeks)	Cycles with scans (Cycle 5 then q 9 weeks(through Cycle 17) [n]	D/C therapy	Post-Tx Safety FU	Post-Tx Disease Assessments [o,t]	Survival Follow-up [r]
Scheduling Window (Days)	Day -28 to Day 0	Day 1 ±3	Day 1 ±3	Day 1±3	Day 1 ±3	At time of D/C	30 days ± 3 days post D/C	Variable frequency[o]post-D/C	Every 12 weeks ±1week phone contact
MK-3475 administration [b]		X	X	X	X				
Administrative procedures									
Informed Consent	X								
Concomitant Medications [m]	X		X	X	X	X	X		X
Medical History, demographics, etc.	X								
Clinical Procedures/Assessments [a]									
Review of AEs [d, e]		X	X	X	X	X	X	X[e]	
Physical Exam [u]	X	X	X[u]	X[u]	X	X	X		
Vital Signs, Height and Weight [f]	X[f]	X	X	X	X	X	X		
12-Lead EKG [g]	X	X				X			
Pulmonary function [v]	X								
ECOG PS	X	X	X	X	X	X	X		
Laboratory Assessments (Safety Labs)[a,h,j,s]									
Pregnancy Test (Urine or Serum HCG) [h, i]	X					X			
CBC with Differential.	X		X	X	X	X	X [l]		
Comprehensive Serum Chemistry Panel [j]	X		X	X	X	X	X [l]		
Urinalysis [j, k]	X			X [k]			X [l]		
T3, FT4, and TSH [j, k]	X			X [k]			X [l]		

Trial Period	Screening Phase	Treatment Cycles Year 1 [a]				End of Tx	Post-treatment follow-up		
		To be repeated up to 2 years							
Tx Cycle/Title	Pre-Therapy	Cycle 1	Cycle 2	Cycles without scans (q 3 weeks)	Cycles with scans (Cycle 5 then q 9 weeks(through Cycle 17) [n]	D/C therapy	Post-Tx Safety FU	Post-Tx Disease Assessments [o,t]	Survival Follow-up [r]
Scheduling Window (Days)	Day -28 to Day 0	Day 1 ±3	Day 1 ±3	Day 1±3	Day 1 ±3	At time of D/C	30 days ± 3 days post D/C	Variable frequency[o]post-D/C	Every 12 weeks ±1week phone contact
Correlative Studies Blood Draws [c]									
Kyn/Trp ratio		X	X		X	X			
IFN-γ ELISpot/ICS		X	X		X	X			
Immunophenotyping		X	X		X	X			
T cell Gene Expression and Phenotyping		X	X		X	X			
MCPyV Antibody Levels		X	X		X	X			
Serum, plasma and PBMC storage		X	X		X	X			
Efficacy Measurements									
Tumor Imaging [n, o, p]	X				X	X		X	
Tumor Biopsies/Archival Tissue Collection									
Tumor Biopsies [q]	X					X			

See calendar footnotes following Treatment Year 2 Calendar.

Treatment Year 2

Trial Period	Treatment Cycles Year 2[a]		End of Tx	Post-treatment follow-up		
	To be repeated up to 2 years					
Tx Cycle/Title	Cycles without scans (q 3 weeks)	Cycles with scans, (q 12 weeks year 2)[n]	D/C therapy	Post-Tx Safety FU	Post-Tx Disease Assessments [o,t]	Survival Follow-up [r]
Scheduling Window (Days)	Day 1±3	Day 1 ±3	At time of D/C	30 days ±3 days post- D/C	Variable frequency[o]post-D/C	Every 12 weeks ±1 week phone contact
MK-3475 administration [b]	X	X				
Administrative Procedures						
Informed Consent						
Concomitant Medications [m]	X	X	X	X		X
Medical History, demographics, etc.						
Clinical Procedures						
Review of AEs [d, e]	X	X	X	X	X[e]	
Physical Exam [u]	X[u]	X	X	X		
Vital Signs, Height and Weight [f]	X	X	X	X		
12-Lead EKG [g]			X			
Pulmonary function [v]						
ECOG PS	X	X	X	X		
Laboratory Assessments						
Pregnancy Test (Urine or Serum HCG) [h, i]			X			
CBC with Differential.	X	X	X	X [l]		
Comprehensive Serum Chemistry Panel [j]	X	X	X	X [l]		
Urinalysis [j, k]	X [k]			X [l]		
T3, FT4, and TSH [j, k]	X [k]			X [l]		
Correlative Blood Draws						
Kyn/Trp ratio			X			
IFN-γ ELISpot/ICS			X			
Immunophenotyping			X			
T cell Gene Expression and Phenotyping			X			
MCPyV Antibody Levels			X			
Serum, plasma and PBMC storage			X			

Trial Period	Treatment Cycles Year 2[a]		End of Tx	Post-treatment follow-up		
	To be repeated up to 2 years					
Tx Cycle/Title	Cycles without scans (q 3 weeks)	Cycles with scans, (q 12 weeks year 2)[n]	D/C therapy	Post-Tx Safety FU	Post-Tx Disease Assessments [o,t]	Survival Follow-up [r]
Scheduling Window (Days)	Day 1±3	Day 1 ±3	At time of D/C	30 days ±3 days post- D/C	Variable frequency[o]post-D/C	Every 12 weeks ±1 week phone contact
Efficacy Measurements						
Tumor Imaging [n, o, p]		X	X		X	
Tumor Biopsies/Archival Tissue Collection						
Tumor Biopsies [q]			X			

Abbreviations: AE, adverse event(s); CBC, complete blood count; D/C, discontinuation/discontinue; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; EOC, end of cycle; FU, follow-up; HCG, human chorionic gonadotropin; Kyn/Trp, kynurenine/tryptophan; PS, performance status; TSH, thyroid-stimulating hormone; tx, treatment

a. In general, safety labs and assessments/procedures are to be performed on Day 1 and before the first dose of treatment for each cycle unless otherwise specified. In general, the window for each visit is ± 3 days unless otherwise noted. Treatment cycles are 3 weeks (21 days); however the treatment cycle interval may be increased due to toxicity according to the dose-modification guidelines provided in Section 6. If the interval is increased, all procedures except imaging should be performed based on the new dosing schedule.
b. MK-3475 will be administered as an IV infusion over 30 minutes. The dose is 2mg/kg every 3 weeks (21 days ± 3 days). The infusion is given in an out-patient setting. Patients who restart treatment after relapse from CR should resume at the same dose and cycle interval which they were receiving before discontinuation.
c. Blood for correlative studies will be drawn on Day 1 of Cycle 1, prior to drug administration, then prior to study drug infusion on day 1 of Cycle 2 and Cycle 5, then prior to drug administration every 3rd cycle (i.e., day 1, Cycle 8, 11, 14, etc.) during first year of treatment. After 1 year of therapy,blood for correlative studies is also drawn upon confirmed CR or PR and at the time of disease progression. If the correlative blood draw is not obtained at time of determination of CR, PR or disease progression, then this collection will be done at the end-of-therapy visit.
d. AEs and laboratory safety measurements will be graded per NCI CTCAE version 5.0. All AEs, whether gradable by CTCAE or not, will also be evaluated for seriousness.
e. Record all AEs occurring within 30 days after the last dose of trial treatment. Report all SAEs (related and unrelated to trial treatment) occurring up until 90 days after the last dose of trial treatment or the start of new anticancer treatment, whichever comes first. Afterwards, report only SAEs that are related to trial treatment.
f. Vital signs to include temperature, pulse, respiratory rate, weight, and blood pressure. Height will be measured at baseline only.
g. EKG should be performed at baseline, and then within 30 minutes of the end of infusion after dosing for Cycle 1 and Cycle 8 and at discontinuation.
h. Laboratory tests for screening are to be performed within 14 days before the first dose of trial treatment. See Section 5.1.1 for details regarding laboratory tests.
i. For women of reproductive potential, a urine pregnancy test should be performed within 72 hours before first dose of trial treatment. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test performed by the local study site laboratory will be required. Pregnancy tests (serum and/or urine tests) should be repeated if required by local guidelines.
j. After Cycle 1, lab samples can be collected up to 72 hours before the scheduled time point. See Section 5.1.1 for details regarding laboratory tests.
k. Urinalysis and thyroid function testing to be repeated every 6 cycles after baseline (cycles 6, 12, 18, etc.) and at the Post-Treatment Follow Up visit.
l. Unresolved abnormal labs that are drug related AEs should be followed until resolution. Labs do not need to be repeated after the end of treatment if labs are within normal range.
m. Concomitant medications – Enter new medications started during the trial through the safety follow-up visit. Record all medications taken for SAE reporting as defined in Section 5.3.1.
n. The initial tumor imaging will be performed within 28 days before the first dose of trial treatment. Scans performed as part of routine clinical management are acceptable for use as the screening scan if they are of diagnostic quality and performed within 28 days before the first dose of trial treatment. On-study imaging will be performed and reviewed at week 13 before drug administration. Patients who show progression at week 13 will continue on therapy and have a conformational scan at week 17. Patients with

no evidence of disease progression will have scans every 3 cycles (every 9 weeks ± 3 days) during the first year of treatment. Scan frequency will be decreased to every 12 weeks ± 7 days during second year of treatment. For cycles with scans scheduled, scans should occur within 7 days before scheduled drug administration so that the investigator may evaluate the patient for possible progression before administering the next dose of study medication. The same imaging technique should be used in a patient throughout the trial with the exception that patients who have discontinued treatment without evidence of progression may have scans performed either at investigative site or locally. Local reading (investigator assessment with site radiology reading) will be used to determine eligibility and for patient management; Sponsor may collect radiological assessments for retrospective analysis by a central vendor. (See Overall Trial Schema and Patient Visit Timeline).

o. In patients who discontinue study therapy without confirmed disease progression, a radiological evaluation should be performed at the time of treatment discontinuation (i.e., date of discontinuation ± 4 week window). If a previous scan was obtained within 4 weeks before the date of discontinuation, then a scan at treatment discontinuation is not mandatory. Every effort should be made to continue monitoring their disease status by radiologic imaging at the investigative site or locally as follows: Year 1 Post-Treatment: imaging will be performed Q 3 months (+/-14 days) or as clinically indicated. Years 2 and 3 Post-Treatment: imaging will be performed Q 6 months (± 1 month) or as clinically indicated. If the study remains open, imaging will be performed annually once patient has completed 3 years of follow-up for disease assessment. Collection of imaging for disease assessments will continue until (1) the start of new anticancer treatment, (2) documented disease progression, (3) death, or (4) the end of the study, whichever occurs first.
p. A scan must be performed within 28 days before restarting treatment with MK-3475 after relapse from CR. Imaging should continue to be performed at frequencies described in footnote "n" above from the first dose of trial treatment or more frequently if clinically indicated. The Sponsor may collect radiological assessments for retrospective analysis by a central vendor.
q. Tumor biopsy tissue (archival or fresh) is required per screening requirements. In patients who have not achieved CR (either responding, stable or progressing lesions), a biopsy sample of a tumor may be obtained if feasible and safe (core, punch or excisional biopsy). This post-treatment biopsy is highly preferred, but optional.
r. After the start of new anticancer treatment or documented disease progression, the patient should be contacted by telephone every 12 weeks ± 1 week to assess for survival status. Survival follow-up will continue until death or end of the study, whichever occurs first.
s. Laboratory tests for determining eligibility for retreatment in patients who relapse from CR are to be performed within 7 days before the first retreatment dose of MK-3475. See Section 3.1.6 for details regarding laboratory tests.
t. Patients who attain a CR and discontinue treatment or those who attain a PR or SD after completion of 2 years of treatment may restart trial treatment if they meet the criteria specified in Section 5.4.3.
u. After cycle 1, limited physical exam performed on visits not correlated with scans.
v. For patients with a history of pulmonary disease, prolonged smoking history, or symptoms of respiratory dysfunction.

11. MEASUREMENT OF EFFECT

Therapeutic response will be assessed by serial scans. Overall clinical response rate and tumor progression will be assessed by evaluating nonbiopsied lesions if present, using standard methods (RECIST), including physical exam and CT scans performed before therapy, at 13 weeks (before drug administration), and every subsequent 9 weeks through 1 year and every 12 weeks in year 2 according to standard practice. For cycles with scans scheduled, the scans shall occur before MK-3475 administration. Patients will be managed in accordance with RECIST guidelines as published by Eisenhauer [Eisenhauer 2009]. However, MK-3475-treated patients who appear to be progressing at the 13-week scan can continue therapy until progression is confirmed at the 17-week scan, provided the patient is otherwise stable.

11.1 Antitumor Effect—Solid Tumors

For the purposes of this study, patients with advanced MCC should be evaluated for response by spiral CT scan in accordance with standard practice at 9-week intervals, with the initial scan at week 13 of the trial, before drug administration. Throughout the trial, for cycles with scans scheduled, scans shall occur before scheduled drug administration so that the investigator may evaluate the patient for possible progression before administering the next dose of study medication. Response and progression will be evaluated in this study using the new international criteria proposed by the revised RECIST guideline (Version 1.1)[Eisenhauer 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in RECIST.

Immunotherapeutic agents such as MK-3475 may produce antitumor effects by potentiating endogenous cancer-specific immune responses that may be functionally anergic. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST guidelines may not provide a CR assessment of immunotherapeutic agents such as MK-3475, especially at early therapeutic time points. Because of the possibility that the initial scan at 13 weeks may misclassify MK-3475 responders as progressing on therapy, those MK-3475–treated patients who appear to be progressing at week 13 can continue therapy until progression is confirmed at the 17-week scan, providing they meet the guidelines stated above (Section 5.1.1). If the patient is found to be progressing on the 17-week scan, the investigator will determine whether to discontinue protocol therapy and begin other systemic therapy, such as cytotoxic chemotherapy, or alternatively to continue MK-3475 treatment. If MK-3475 therapy is discontinued, the patient will attend the end-of-treatment and 30-day follow-up visits. The time of progression will be reported as the first time that progression was noted. If patients regress or have SD as determined by the confirmatory 17-week imaging study, the time of eventual progression will be separately reported.

If progression is noted on subsequent routine disease monitoring scans (≥22 weeks), which will continue throughout the trial at 9- or 12-week intervals, a confirmatory scan will be obtained 4 weeks later. If this confirmatory scan identifies an MK-3475-treated patient as progressing on therapy, the investigator will determine whether to discontinue protocol therapy and begin other systemic therapy, such as cytotoxic chemotherapy, and follow patient as indicated above, or

alternatively to continue MK-3475 treatment. (See Overall Trial Schema and Patient Visit Timeline).

11.1.1 ***Definitions***

Evaluable for Objective Response. All patients that have received at least 1 injection of MK-3475 will be considered evaluable for response.

A patient on MK-3475 who is identified as progressing on therapy at the initial 13-week scan may continue receiving MK-3475 until progression is confirmed by a subsequent scan at 17 weeks. Patients may only receive treatment while waiting for confirmation of PD if the following criteria are met:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

If these criteria are met, it is advisable to keep patients on MK-3475 until confirmation of progression but is subject to the discretion of the treating physician. Patients that are deemed clinically unstable are not required to have repeat imaging for confirmation.

If progression is confirmed, then the patient will be discontinued from trial treatment. If progression is not confirmed, then the patient should resume/continue trial treatment and have their next scan according to the standard follow-up schedule on week 22 then every 9 weeks thereafter during the first year of treatment and every 12 weeks during the second year of treatment.

Alternatively, if progression is confirmed, patients who are clinically stable will be eligible to receive continued MK-3475. Patients who have not achieved CR (either responding, stable or progressing lesions) may have an optional biopsy of tumor prior to continuing therapy to assess potential reasons for the lack of response to MK-3475. Patients will then continue MK-3475 treatment and scans per the study calendar.

For patients who discontinue trial treatment for reasons other than disease progression, imaging during the post-treatment follow-up period is to continue as described in Section 5.1.1 until confirmed disease progression,start of a new antineoplastic therapy, death or end of study, whichever occurs first.

Local reading (investigator assessment with site radiology reading by RECIST 1.1 criteria) will be used to determine patient eligibility and for patient management. Radiologic images will be collected and stored for a possible retrospective analysis of patient eligibility and treatment response to be performed by a central vendor, including RECIST 1.1.

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 1 cycle of therapy, and have had their disease re-evaluated will be considered

evaluable for non–target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Patients who do not receive a post-baseline scan due to discontinuing treatment as a result of toxicity should be considered evaluable for response.

11.1.2 ***Disease Parameters***

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, magnetic resonance imaging (MRI), or 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 or in an area subjected to other locoregional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in the 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.

Nonmeasurable 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 nonmeasurable 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 nonmeasurable.

Note*:* Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor nonmeasurable) 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 noncystic 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 that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to

further characterize any objective tumor regression in the measurable dimension of the disease.

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

11.1.3 ***Methods for Evaluation of Measurable Disease***

In this study, all tumors will be measured using spiral CT scan.

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.

11.1.4 ***Response Criteria***

11.1.4.1 ***Evaluation of Target Lesions by RECIST***

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 progression).

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

11.1.4.2 ***Evaluation of Non-Target Lesions by RECIST***

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 PI).

11.1.4.3 ***Evaluation of Best Overall Response (RECIST)***

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD 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/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD /not evaluated	No	PR	
SD	Non-CR/Non-PD /not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR, or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

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

For Patients with Nonmeasurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* "Non-CR/non-PD" is preferred over 'stable disease' for non–target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 ***Progression-Free Survival***

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

11.1.6 ***Duration of 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 PD the smallest measurements recorded since the treatment started).

11.1.7 ***Overall Survival***

Status of patient survival will be reported to the CITN by the primary treating physicians on an every 6 months schedule.

11.1.8 ***Response Review***

Imaging studies will be collected for a possible expert review of responses. Investigator determined responses will be chronicled and reported unless or until a central expert review takes place.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate Rave roles in the CTSU Regulatory Support System (RSS). To access iMedidata/Rave the site user must have an active CTEP IAM account (check at < https://ctepcore.nci.nih.gov/iam >). In addition, site users that are members of the CITN must have the appropriate Rave roles (Rave CRA, site PI, or Read-Only) in RSS at the enrolling site. To hold the Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the CITN roster will be sent a study invitation email from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the will be listed in the upper right pane of the iMedidata screen.

Users who have not previously activated their iMedidata/Rave accounts at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave

tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site: (http://ctep.cancer.gov/reporting/cdus.html).

All AEs (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above.

12.1.2 Responsibility for Data Submission

For CITN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the CITN Sites understand the procedures for data submission for each CITN protocol and that protocol specified data are submitted accurately and in a timely manner to the CITN data management organization-via the electronic data capture system, Medidata Rave.

Data from Medidata Rave and CTEP-AERS is reviewed by the CITN data management organization on an ongoing basis as data is received. Queries will be issued by CITN data management organization directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the CITN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

The CITN data management organization will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with the CITN data management organization to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. The CITN data management organization will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions will be carried out by the CITN data management organization contractor, Axio. CDUS submissions are performed by Axio on a quarterly basis. The trial's lead institution is responsible for timely submission to the CITN data management organization via Rave, as above.

See Section 12.1.1 for details on CDUS reporting. As the data management center for this trial, Axio is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the PI and the Coordinating Center and the procedures for auditing are presented in Appendix B.

- The PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required. Submit documentation of reportable adverse events to CTSUprotocol@westat.com and state in the subject line "Safety Report for *NCI protocol #*" or "Action Letter for *NCI protocol #*", as appropriate. A brief summary cover page on Coordinating Center letterhead is encouraged. These documents will be posted to the CTSU protocol web page and included in the next CTSU bi-monthly broadcast.
- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Clinical Research Site to the CTSU Regulatory Office for entry in the Regulatory Support System (RSS) and transmission to CTEP.

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with

(an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication,

manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, but before the conduct of any analysis, changes are made to primary and/or key secondary hypotheses or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR. No separate Statistical Analysis Plan (SAP) will be issued for this study.

13.1 Responsibility for Analyses

The statistical analysis of the data obtained from this study will be the responsibility of the FHCRC as part of the CITN.

This trial is being conducted as an open-label study, i.e., patients, investigators, and trial personnel will be aware of patient treatment.

A single interim analysis will be conducted at the end of Stage 1 (Section 13.10). If at least 1 response is observed among the 9 enrolled patients, the trial will proceed to enroll an additional 15 patients in Stage 2.

13.2 Study Design/Endpoints

13.2.1 *Primary Objective*

- To determine the clinical efficacy of MK-3475 as the first systemic intervention for patients with advanced MCC

The primary endpoint will be ORR as measured by RECIST 1.1.

13.2.2 *Secondary Objectives*

- To determine the clinical activity of MK-3475 as the first systemic intervention for patients with advanced MCC

The secondary endpoints will be PFS, DOR, and OS, as measured by RECIST 1.1. The PFS will be determined at 16 months and compared to the historical PFS for patients treated with chemotherapy, which is 5% at 16 months.

13.2.3 *Exploratory Objectives*

- To determine the immune correlates of the clinical activity of MK-3475

The endpoints will include IHC and gene expression analysis focusing on delineating the immune components and immunologic milieu within the tumor before therapy. The IHC analyses performed will include, but is not limited to, MCPyV T-Ag, PD-1, CD8, and CD3. In addition, MCPyV viral DNA will be quantified in the tumor (Section 2.5.2).

Cellular and humoral MCPyV–specific immune responses will be assessed with ELISpot and serology assays in blood samples at baseline, after initiating MK-3475 therapy, and correlated with clinical responses over time. Among patients with corresponding MHC-peptide tetramers, pre- and post-treatment samples of circulating MCPyV-specific CD8 T cells will be isolated and subjected to deep immunophenotyping by mRNA expression analysis (Section 2.5.3).

13.3 Analysis Endpoints

13.3.1 *Efficacy Endpoints*

The primary and secondary efficacy endpoints are described below.

Objective response rate (ORR) is defined as the proportion of patients who have achieved CR or PR according to RECIST 1.1.

Progression-Free Survival (PFS) is defined as the time from randomization to PD or death, whichever occurs earlier, based upon investigator assessment using RECIST 1.1. Patients without documented PD/death will be censored at the last disease assessment date.

Duration of response (DOR) is defined as the time interval between the date of first response (CR/PR) and the date of progression.

Overall survival (OS) is defined as the time from randomization to death due to any cause. Patients without documented death at the time of analysis will be censored at the date last known to be alive.

13.3.2 *Safety Endpoints*

The primary safety endpoints are AEs graded using CTCAE (Version 5.0) criteria. Safety will be assessed by quantifying the toxicities and grades experienced by subjects who have received MK-3475 including AEs and SAEs. Safety will be monitored by cumulative data reviews throughout the trial.

Other safety endpoints include laboratory safety assessments, ECOG performance status, vital signs, and physical examinations.

***Note**:* Patients discontinuing MK-3475 due to toxicity will still be considered evaluable for response.

13.4 Analysis Populations

13.4.1 *Efficacy Analysis Population*

The population of eligible subjects who receive at least 1 dose of treatment will serve as the primary population for the analysis of efficacy data in this study.

13.4.2 *Safety Analysis Population*

The All Subjects as Treated (ASaT) population will be used for the analysis of safety data in this study. The ASaT population consists of all patients who received at least 1 dose of study treatment. . Patients who do not receive the study treatment will be excluded from analysis.

At least one laboratory or vital sign measurement obtained subsequent to at least 1 dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

13.5 Statistical Methods

The protocol has a standard Simon two stage design. The null hypothesis that the true response rate is 5% will be tested against a one-sided alternative. In the first stage, 9 patients will be accrued. If there are no responses in these 9 patients, the study will be stopped. Otherwise, 15 additional patients will be accrued for a total of 24. The null hypothesis will be rejected if 3 or more responses are observed in 24 patients. This design yields a type I error rate of 0.10 and power of 0.90 when the true response rate is 25%. PFS at 16 months will be estimated by Kaplan-Meier method.

The current protocol will increase the total number of patients from 26 to 50. The initial protocol enrolled nine subjects in Stage 1, and planned to enroll an additional 15 subjects in Stage 2 for a total enrollment of 24. Treatment was discontinued in 2 patients due to drug-related adverse events after receiving 1 dose of MK-3475, and enrollment of an additional 2 patients was allowed per protocol, bringing the total enrollment to 26 patients. The current protocol amendment will increase the total number to 50 to provide an expanded safety and efficacy profile.

Unless otherwise stated, all statistical tests will be conducted at the α=0.05(1-sided) level.

13.5.1 *Statistical Methods for Efficacy Analyses*

13.5.1.1 *ORR*

ORR will be estimated as the number of responders as a percent of the number of eligible participants who received at least 1 dose of treatment. If a substantial amount of primary endpoint data are missing (at least 1 value missing from more than 20% of participants), using nonparametric estimation to estimate the ORR requires the missing completely at random assumption may give misleading results. In this situation, analyses of the primary endpoint at the primary time point will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. A generalized linear model for the ORR will use a binomial error distribution. The model will include as covariates all available baseline predictors of the missing outcomes.

Note: Patients with confirmed progression will be declared as having progressive disease for statistical purposes even if they remain on study and respond continued MK-3575. Responses to continued MK-3575 will be chronicled and reported.

13.5.1.2 *PFS, DOR, and OS*

Survival curves for PFS, DOR, and OS will be estimated using the Kaplan-Meier

method.

For PFS, subjects without documented PD/death will be censored at the last disease assessment date. Any subject who is lost to follow-up will be included in the analysis and their PFS time will be censored on the last date that the subject was known to be progression-free, defined as the date of the last tumor assessment not indicating progression. As a sensitivity analysis, the primary analysis of PFS will be performed reconsidering subjects without documented PD or death who discontinued treatment or received new anticancer therapy to have been progressed at the date of treatment discontinuation or initiation of new anticancer therapy, whichever occurs later.

For OS, subjects without documented death at the time of analysis will be censored at the date last known to be alive.

For DOR, subjects who have not yet progressed by the last disease assessment will be censored at the last disease assessment—this is intended to describe censoring rules for the analysis where only responders are used.

Table 13.1 Analysis Strategy for Key Efficacy Variables

Endpoint/Variable (Description, Time point)	**Statistical Method**	**Analysis Population**	**Missing Data Approach**
Primary Hypothesis			
ORR	Simon Two Stage design analysis	Eligible subjects who receive at least 1 dose of treatment	See 13.5.1.
Secondary Objectives			
PFS	Estimation: Kaplan-Meier method for PFS curve estimation	Eligible subjects who receive at least 1 dose of treatment	Model based (censored at last assessment)
DOR	Summary statistics using Kaplan-Meier method	All responders	Nonresponders are excluded in analysis
OS	Summary statistics using Kaplan-Meier method	Eligible subjects who receive at least 1 dose of treatment	Model based (censored at last date)
Exploratory Objectives			
PFS and OS by the following biomarker categories: • pre-therapy CD8+ • pre-therapy CD3+ T-cell infiltration in tumor • pre-therapy PD-1 and PD-L1 expression in tumor, • * pre-therapy immune response to MCPyV	Descriptive statistics	Eligible subjects who receive at least 1 dose of treatment	censored at last assessment

ORR by the following biomarker categories: • pre-therapy CD8+ • pre-therapy CD3+ T-cell infiltration in tumor • pre-therapy PD-1 and PD-L1 expression in tumor, * pre-therapy immune response to MCPyV	Descriptive statistics	Eligible subjects who receive at least 1 dose of treatment	Missing value considered nonresponder

***Note**:* PD after 1 or more missed visits will be counted as PD as of the date of documented disease progression. An additional sensitivity analysis will be performed where subjects with 2 or more missed visits before progression will be censored at the last disease assessment before the ≥2 missed disease assessments.

13.5.2 ***Statistical Methods for Safety Analyses***

Safety and tolerability will be assessed by clinical review of all relevant parameters including adverse experiences, laboratory tests, vital signs, and ECG measurements.

Adverse experiences will be summarized as counts and frequencies by toxicity grade. Summary statistics (median and range) for time to onset of first drug-related toxicity will be provided.

Laboratory assessments, vital signs, and other safety endpoints will be summarized using descriptive statistics as appropriate.

13.5.3 ***Summaries of Baseline Characteristics, Demographics, and Other Analyses***

The number and percentage of subjects enrolled, and the primary reason for discontinuation will be displayed. Demographic variables (such as age) and baseline characteristics will be summarized either by descriptive statistics or categorical tables. No statistical hypothesis tests will be performed on these characteristics.

13.6 Multiplicity

The treatment comparison will be tested at one sided 5% alpha level and no multiplicity adjustment will be applied.

13.7 Sample Size/Accrual Rate and Power Calculations

To date, the trial has enrolled 26 patients. The initial plan was to "enroll between 9 and 24 subjects to receive MK-3475 in a two-stage Simon design with 90% power to reject the null hypothesis of a 5% true response rate against a 1-sided alternative when the true response rate is 25%. The study will enroll the first 9 patients over a 6-month period, followed by enrollment of 15 more subjects over 10 months".

"In the first stage, 9 patients will be accrued. If there are no responses in these 9 patients, the study will be stopped. Otherwise, 15 additional patients will be accrued for a total of 24. The null hypothesis will be rejected if 3 or more responses are observed in 24 patients. This design yields a type I error rate of 0.10 and power of 0.90 when the true response rate is 25%".

The current protocol amendment will increase the total number of patients to 50 to provide an expanded safety and efficacy profile. The analysis will utilize the same statistical test.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino		+		=	
Not Hispanic or Latino	21	+	29	=	50
Ethnic Category: Total of all subjects	21	+	29	=	(50)
Racial Category					
American Indian or Alaskan Native		+		=	
Asian	1	+	1	=	2
Black or African American		+	1	=	1
Native Hawaiian or other Pacific Islander		+		=	
White	20	+	27	=	47
Racial Category: Total of all subjects	21	+	29	=	(50)
	(A1 = A2)		(B1 = B2)		(C1 = C2)

13.8 Stratification Factors- N/A

13.9 Interim Analyses

There will be one interim analysis at the end of Stage 1, on the first 9 patients (Section 13.8). If there are no responses in these 9 patients, the study will be stopped. Otherwise, 15 additional patients will be accrued for a total of 24.

13.10 Compliance (Medication Adherence)

Drug accountability data for trial treatment will be collected during the study. Compliance with trial treatment administration will be measured by subjects: (1) receiving unscheduled study agent infusions/injections; (2) missing an infusion/injection. Numbers and percentages of subjects and infusion/injection visits with any deviation in these measures will be reported for the eligible subjects

13.11 Extent of Exposure

The extent of exposure will be summarized as duration of treatment in cycles. Dose intensity will also be summarized as appropriate.

13.12 Analysis of Secondary Endpoints

Analysis of secondary endpoints is addressed in Section 13.6.1.

13.13 Reporting and Exclusions

13.13.1 ***Evaluation of Toxicity***

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

3475.

13.13.2***Evaluation of Response***

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: (1) CR, (2) PR, (3) SD, (4) PD, (5) early death from malignant disease, (6) early death from toxicity, (7) early death because of other cause, or (9) unknown (not assessable, insufficient data).

Note*:* By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

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

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

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (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 CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP-sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Before the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site before the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are 2 options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of 2 ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and email address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C BIOASSAY TEMPLATES

Correlative objective	Assay	Tissue/Body Fluid Tested and Timing of Assay	M/O
mRNA expression profiling (Nanostring focused on immune genes) Jennifer Yearly, MD (Merck Research Labs)	Nanostring mRNA expression on approximately 400 immune genes	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Gene expression profiling (Nanostring) (NanoString Technologies)	Nanostring gene expression of up to 800 genes	Tumor: Prior to treatment , and at time of confirmed progression (if feasible)	M
Tumor PD-L1 Expression Mary Savage, PhD (Merck Research Labs)	Chromogenic IHC	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Tumor PD-1 & PD-L1 Expression Jennifer Yearly, DVM PhD (Merck Research Labs)	Multicolor IHC	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Tumor PD-1 & PD-L1 Expression Janis Taube MD (Johns Hopkins)	Multispectral immunofluorescence	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Immune cell infiltrate markers: CD3, CD8, and MHC Class I. Optional markers: CD4, CD56, CD16, CD45, CD19, CD68, GITR, TIM-3, FOXP3 and LAG3 Julie Habecker MD (FHCRC Core Lab or PhenoPath)	IHC	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Merkel cell polyomavirus (MCPyV) DNA Meei-Li Huang PhD (FHCRC)	PCR	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
Merkel cell polyomavirus (MCPyV) Protein Expression Julie Habecker MD (FHCRC Core Lab)	IHC	Tumor: Prior to treatment biopsy tissue and at time of confirmed progression (if feasible)	M
MCPyV specific T cell Immunophenotyping Paul Nghiem MD PhD (University of Washington)	MCPyV peptide specific tetramer isolation	PBMC: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy.	M
MCPyV specific T cell gene expression analysis Jennifer Yearly, MD (Merck Research Labs)	mRNA expression (Nanostring)	PBMC: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy	M
MCPyV specific T cell IFN-γ responses Steve Fling PhD (University of Washington)	IFN-γ ELISpot	PBMC: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy	M
MCPyV specific antibody specific for T-Ag oncoprotein & VP1 Mark Wener MD (University of Washington, Lab Medicine)	Luminex	Serum: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy.	M
PBMC and T cell subset quantification (frequency and percent) of CD4 T cells, CD8 effector memory and central memory, Treg, NK and MDSC. Steve Fling PhD (University of Washington)	Multiparameter flow cytometric analysis	PBMC: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy	M
T cell functional status/phenotype Julie McElrath MD PhD (FHCRC)	Intracellular cytokine staining	PBMC: Prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy	M

Correlative objective	Assay	Tissue/Body Fluid Tested and Timing of Assay	M/O
Exome sequencing and RNA sequencing Neon Therapeutics or equivalent vendor	Gene sequencing	Tumor: Prior to treatment	M
Neoantigen identification Neon Therapeutics or equivalent vendor	Bioinformatics	Tumor: Prior to treatment	M
T cell receptor sequencing Edus Warren Lab (FHCRC)	PCR	Tumor: Prior to treatment	M
PBMC functional phenotyping Edus Warren Lab (FHCRC)	Flow cytometry	PBMC: Prior to treatment	M
Kyn/Trp ratio Incyte Corporation	mass spectrometry	Plasma: prior to treatment & every 9 to 12 weeks (prior to cycles correlated with tumor imaging) and end of therapy	M

This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail. PMCID: PMCID

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