TITLE: A phase II evaluation of pembrolizumab, a humanized antibody against PD-1, in the treatment of persistent or recurrent hypermutated/ultramutated endometrial cancer identified by next generation sequencing (NGS) and comprehensive genomic profiling (CGP), A Pilot Study

STUDY CONTACTS: Reference the Study Contact List

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TRIAL SUMMARY

Abbreviated Title	Pembrolizumab in ultramutated and hypermutated endometrial cancer
Trial Phase	II
Clinical Indication	Recurrent Hypermutated and ultramutated endometrial cancer
Trial Type	Open label
Type of control	none
Route of administration	IV
Trial Blinding	NA
Treatment Groups	Single arm Phase II trial in recurrent endometrial cancer patients
Number of trial subjects	Max 25
Estimated enrollment period	36 months
Estimated duration of trial	July 1, 2016 – June 30, 2020
Estimated average length of treatment per patient	24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication

TRIAL DESIGN

Patients must have persistent, recurrent or metastatic Ultra-mutated or Hyper-mutated endometrial cancer (as detected by NGS and CGP) with documented disease progression (disease not amendable to curative therapy).

Patients must have had one prior systemic chemotherapeutic regimen for management of persistent, recurrent or metastatic disease

Pembrolizumab 200 mg (fixed dose) IV every 3 weeks (+/- 3 days) until progression or adverse effects prohibit therapy

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1.0 OBJECTIVES

Primary Objectives & Hypothesis

Hypothesis: Because POLE/POLD1-ultramutated and/or hypermutated/MMR-defective endometrial tumors harbor a large number of novel mutations and are heavily infiltrated with TIL, anti-PD1 therapy with Pembrolizumab may represent an ideal treatment option for these patients to re-invigorate pre-existing immune responses and eliminate residual/recurrent chemotherapy and radiation resistant disease.

1.1 Primary Objectives:

1.1.1 To assess the antitumor activity (proportion of objective response by RECIST 1.1 criteria) of pembrolizumab with objective tumor response in patients with persistent, recurrent or metastatic endometrial cancer harboring an ultra-mutated or hyper-mutated (MMR gene-defective) phenotype identified by next generation sequencing (NGS) and comprehensive genomic profiling (CGP).

1.1.2 To determine the nature and degree of toxicity of pembrolizumab as assessed by CTCAE in patients with persistent, recurrent or metastatic endometrial carcinoma

1.2 Secondary Objective(s)

1.2.1 To estimate the duration of progression-free survival (PFS) and overall survival (OS).

1.3 Exploratory Objective

1.3.1 A) To prospectively evaluate and compare the sensitivity/specificity of NGS and comprehensive genomic profiling (CGP) to assess MMR gene deficiency/microsatellite instability (MSI) to standard PCR-based DNA microsatellite instability (MSI) and immunohistochemistry (IHC) testing for the identification of patients responsive to pembrolizumab. B) To evaluate the tumor mutation burden (TBN) and individual characteristics of somatic mutations identified using whole exome sequencing (WES) in ultra and hypermutated patients and their correlations to objective response, PFS and OS in pembrolizumab-treated patients.

1.3.2 To explore the composition of immune infiltrates in POLE/POLD1 and MMR defective tumor specimens/biopsies from primary and/or metastatic/recurrent sites with selected markers including (but not limited) to CD4+,CD8+, FoxP3, CD25, LAG-3, TIM-3, and ICOS and their correlations to objective response, PFS and OS in pembrolizumab -treated patients.

1.3.3 To systematically evaluate PD-1 and B7-H1 (i.e., PD-1 Ligand) expression in tumor infiltrating lymphocytes (TILs) and tumor cancer cells and explore their correlations with objective response, PFS, and OS in pembrolizumab -treated patients with PD-1 and B7-H1 scoring results.

1.3.4 To explore the association with treatment and subject response in peripheral blood populations in ultramutated and hypermutated patients before and during pembrolizumab treatment, including absolute lymphocyte counts, number of T cells, T-cell subsets, NK cells, and B cells as well as their cellular phenotypes by flow cytometry.

2.0 BACKGROUND & RATIONALE

2.1 Background

Endometrial cancer is the most common gynecologic malignancy with approximately 54,870 new cases and 10,170 estimated deaths related to the disease in the United States annually (1). Recently, using a comprehensive genetic investigation, The Cancer Genome Atlas (TCGA) Research Network provided compelling evidence that endometrial cancers result from heterogeneous somatic mutations and classified endometrial cancers into four categories: 1) polymerase epsilon (POLE)-ultramutated, 2) microsatellite instability hypermutated (MMR-defective), 3) copy-number low and 4) copy-number high, serous-like (2). In this landmark study, patients harboring POLE-ultramutated (7%) were found to have the best prognosis (Figure 1 Left panel below and reference #2). After the initial TCGA report, multiple studies from our group as well as others have confirmed that despite commonly being of high histological grade, ultra/hyper-mutation is correlated with a more favorable prognosis in both Type I and Type II endometrial cancer patients (3-6). Unfortunately, however, as recently demonstrated by the whole exome sequencing (WES) of 243 patients with endometrial disease enrolled in GOG86P (Figure 1 below, right panel, ref. # (7) and Douglas Levine MD personal communication) over 25% of patients developing recurrence, which was fatal in the majority of the cases, harbored an ultramutated or hypermutated tumor phenotype. Novel, effective treatment options for ultramutated and hypermutated recurrent endometrial cancer patients remain therefore desperately needed.

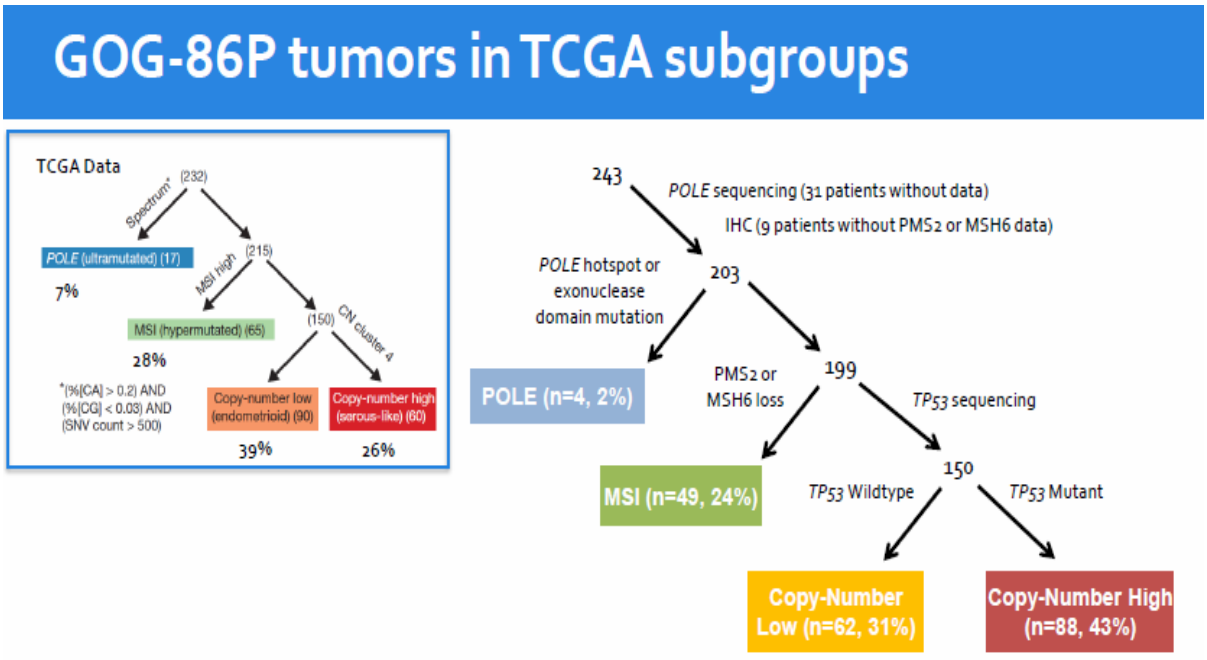

Figure 1. Molecular stratification of 232 WES endometrial cancer patients (TCGA report, left Panel, ref. #2) vs 243 endometrial cancer patients with recurrent disease sequenced using WES enrolled in GOG 86P (right panel, ref#7). In GOG86P patients with ultramutated and hypermutated tumors accounted for 2% and 24% of all recurrences, respectively.

While it is currently not understood why patients with ultramutated and hypermutated phenotype may have improved outcomes when compared to the others groups it is possible that the large number of somatic mutations present in these tumors, similarly to what has recently been demonstrated in melanoma and lung cancer patients (8), may render these cancers highly immunogenic for the host due to the large number of mutated epitopes. Consistent with this view, POLE mutated and MMR gene defective endometrial cancers are characterized by a high rate of infiltration of T lymphocytes (9-11). Moreover, results from our group have recently demonstrated that ultramutated endometrial tumors identified using WES, unlike copy-number low and copy-number high serous-like tumors, may trigger activation of both the T helper arm and the cytotoxic arms of the immune system (12). In this regard, the inability to mount a potent and long lasting antitumor immune response against human tumors has often been attributed to the lack of generation of sufficient tumor-specific T cell help (13,14).

Endometrial cancer with a MSI phenotype may occur secondary to different genetic events such as germline mutations in one of the MMR genes (as observed in patients with Lynch syndrome) or because of epigenetic silencing of the promoters of both alleles of the MLH1 gene. Moreover, another group of tumors with MSI phenotype (also referred as “Lynch like” tumors) exist that exhibit loss of expression of 1 or more of the MMR genes secondary to somatic mutations. Accordingly, PCR-based MSI and/or IHC testing are currently used in the clinic to identify tumors with mismatch repair gene defect. However, both MSI and IHC may have a 10-15% false negative rate. In addition the sensitivity of MSI testing for the identification of MSH6 mutated patients (ie, a mutation more prevalent in hyper-mutated endometrial cancer when compared to colon carcinoma) can be as low as 55% (15-17). These data imply that a significant number of endometrial cancer patients harboring hyper-mutated tumors potentially eligible to pembrolizumab may be missed using standard MSI testing because associated with a positive IHC staining and/or displayed low or no MSI by standard PCR testing (15-17). **With the goal to overcome the current limitations in the identification of endometrial cancer patients potentially responsive to immunotherapy,** in collaboration with FM (Foundation Medicine, Inc. Cambridge, MA) we recently developed and validated a novel computational method to assess microsatellite instability (MSI) and tumor mutation burden (TMB) using next generation sequencing (NGS) data and applied it to a series endometrial carcinoma (EA) patients assayed with **comprehensive genomic profiling** (CGP)(18,19). Briefly, DNA was extracted from 257 FFPE EA clinical specimens and CGP was performed on hybridization-captured libraries of 315 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. **114 intronic homopolymer repeat loci** (10-20bp long in the human reference genome) were analyzed for length variability and compiled into an overall MSI score via principal components analysis. Predictions of MSI-H (High) or MSS (MSI-Stable) and tumor mutation burden (TMB) were validated against colorectal and EA in which MSI status was previously determined by PCR or IHC (ie, current standards), showing an overall concordance of 97% (65/67). **Importantly, NGS and assessment of tumor MSI status by CGP was able to detect 3 times as many patients who may potentially benefit from immunotherapy (i.e., hyper-mutated) compared to genomic alteration in MMR pathway genes alone, while simultaneously detecting POLE ultra-mutated and hyper-mutated MMR gene-defective MSI stable endometrial cancer patients (18,19).**

Recent data from immune check point inhibitors immunotherapy trials **in melanoma patients** have hypothesized that the mutational burden of the tumor may play a critical role for the identification of patients most likely to respond to immunotherapy. Consistent with this view, in these studies the mutational burden was higher in melanoma patients experiencing a sustained clinical benefit than in those without a sustained benefit (8). In this regard, because only a tiny proportion of predicted neo-epitopes are actually presented on the cell surface with MHC and are targets of endogenous T cell responses, it is likely, that the number of predicted

mutation-associated neoantigens is proportionate to the number of actual mutation-associated neoantigens, and tumors with a high number of actual mutation-associated neoantigens are more likely to stimulate the immune system to react against the tumor (8). These recent data strongly suggest that the presence of a specific threshold of mutations in hyper-mutated endometrial cancer patients may be potentially critical for the identification of patients experiencing maximal/sustained clinical benefit to immune check point inhibitors. Consistent with this view, as shown in Figure 2 below for the 65 MMR gene defective endometrial carcinoma cases studied in the TCGA report, (reference # 2, data downloadable from the TCGA online resource: https://tcga-data.nci.nih.gov/tcga/), hypermutated tumors varied greatly in the number of total mutations.

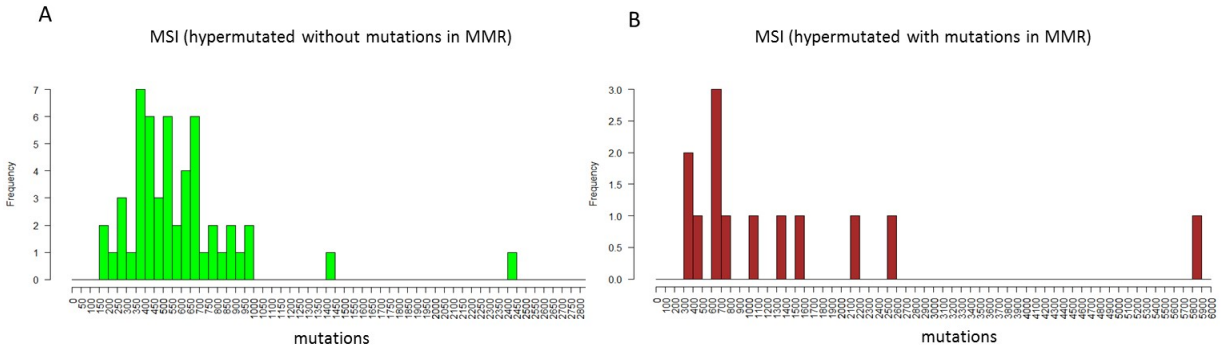

Figure 2. Density plots of the MSI hypermutated endometrial carcinoma patients from TCGA (ref. 2). We divided the MSI hypermutated original category, composed of n=65 patients in two sub-categories: 1) MSI MMR-defective secondary to MLH1 promoter methylation without mutations in MMR genes (ie, 52 patients: Panel A) and 2) MSI MMR-defective with at least one mutation in one of the 4 MMR genes (ie, 13 patients: Panel B). We found **MSI high patients with MMR gene mutations to harbor a 2.39 folds higher number of mutations (mean mutation number = 1404 vs 587, respectively) when compared to MSI high MLH1 promoter methylated patients.**

Accordingly, **to evaluate whether a specific threshold of mutations is necessary to induce a response and/or whether in addition to the number, a specific signature of DNA mutations** (for example a high prevalence of C:G>G:C transversions or enrichment with two separate signatures [TCT→A] and [TCG→, i.e., typical of POLE exonuclease mutations) is necessary to trigger strong pembrolizumab immune responses, we are planning to perform whole exome sequencing (WES) in all patients selected by NGS and CGA and treated with pembrolizumab within the clinical trial.

In this regard, because pre-existing immunity is present in a significant subset of endometrial cancer patients and because tumor infiltrating lymphocytes (TIL) are often suppressed by the PD1/PD-1L axis the use of Pembrolizumab may be highly effective in re-invigorating pre-existing immunity in this selected cancer patient population. Consistent with this view, compelling evidence indicates that B7 molecules (i.e., B7-1/CD80, B7-2/CD86, B7-H1/PDL1, B7-H2/L-ICOS, B7-DC, B7-H3 and B7-H4) and their ligands (i.e., CTLA-4, CD28, PD-1, ICOS) not only provide crucial positive signals to stimulate and support T-cell activation, but can also offer negative signals that control and suppress potentially protective T-cell responses against spontaneously arising and virally-induced human tumors (20). Expression of these molecules on the surface of endometrial tumor cells, tumor associated macrophages (TAM) and/or DC, may attenuate or abrogate the ability of the immune system to successfully eliminate strongly antigenic (i.e., hypermutated and ultramutated) tumors (20). Because these negative signals in multiple human solid tumors have been shown to be largely provided by PD-1 or programmed death-1, blockade of PD-1/PD-L1 co-inhibitory pathways by novel monoclonal antibodies may represent an

innovative, potentially highly effective therapeutic approach to reverse immune suppression while inducing tumor-specific immunity in POLE/POLD1-ultramutated and/or hypermutated/MMR-defective endometrial cancer patients. Consistent with this hypothesis exciting results have recently been reported in the clinical setting against multiple human cancers including hypermutated endometrial cancer patients by the use of fully-human antibodies that target the inhibitory receptor PD1 expressed on activated T-cells (21-23). Taken together, these studies strongly validate the importance of the PD-1-PD-L1 pathway for the treatment of patients harboring highly immunogenic tumors such as ultramutated/hypermutated endometrial cancers. Accordingly, in this study we are planning to systematically evaluate PD-1 and B7-H1 (i.e., PD-1 Ligand) expression in tumor infiltrating lymphocytes (TILs) and POLE/POLD1 and MMR defective cancer cells and explore their correlations with objective response, PFS, and OS in pembrolizumab-treated patients with PD-1 and B7-H1 scoring results. We will also explore the composition of immune infiltrates in tumor specimens/biopsies from primary and/or metastatic/recurrent sites with selected markers including (but not limited) to CD4+,CD8+, FoxP3, CD25, LAG-3, TIM-3, and ICOS and their correlations to objective response, PFS and OS in pembrolizumab -treated patients. Finally, we will explore the association with treatment and subject response in peripheral blood populations in ultramutated and hypermutated patients before and during pembrolizumab treatment, including absolute lymphocyte counts, number of T cells, T-cell subsets, NK cells, and B cells as well as their cellular phenotypes by flow cytometry.

2.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane

receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

2.2 Rationale

2.2.1 Rationale for the Trial and Selected Subject Population

Because POLE/POLD1-ultra-mutated and/or hyper-mutated/MMR-defective endometrial tumors harbor a large number of novel mutations and are heavily infiltrated with TIL, anti-PD1 therapy with Pembrolizumab may represent an ideal treatment option for these patients to re-invigorate pre-existing immune responses and eliminate residual/recurrent chemotherapy and radiation resistant disease.

The use of a well validated next generation sequencing (NGS) method (Foundation Medicine, Inc. Cambridge, MA)(18,19)(ie, the test is already standardized and performed in CLIA-certified laboratories without need for assay development) that allows assessment of tumor mutation burden (TMB) followed by a comprehensive genomic profiling analysis (CGP)(18,19) may represent the ideal approach to identify the population of ultra-mutated (i.e., POLE-mutated MSI stable) and hyper-mutated (i.e., MMR-defective MSI High, MSI low and MSI stable) patients most likely to respond to pembrolizumab therapy. Moreover, the use of comprehensive whole exome sequencing (WES) in the patient populations selected for pembrolizumab treatment by FM testing will allow a direct correlation of the total number and the characteristics of the individual mutations identified in these patients with clinical responses to the immune check point inhibitor.

2.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a

lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

3.0 PATIENT ELIGIBILITY

3.1 Entry Criteria

Recurrent ultramutated/hypermutated endometrial cancer patients with measurable disease.

3.2 Subject Inclusion Criteria

3.2.1 Patients must have histologically confirmed endometrial cancer that is recurrent or progressive following at least one prior chemotherapy regimen.

3.2.2 Patients with the following histologic epithelial cell types are eligible: Endometrioid adenocarcinoma, serous adenocarcinoma, clear cell carcinoma, undifferentiated carcinoma, mixed epithelial carcinoma, carcinosarcoma, and adenocarcinoma not otherwise specified (N.O.S.).

3.2.3 Tumors must demonstrate ultramutation (POLE/POLD1 -mutation) and/or hyper-mutation (due to MMR gene defect) in a representative primary or metastatic tumor site by next generation sequencing (NGS) and Comprehensive Genomic Profiling (CGP) testing performed at Foundation Medicine and/ or standard PCR-based DNA microsatellite instability (MSI) and immunohistochemistry (IHC).

3.2.4 All patients must have measurable disease by RECIST 1.1.

3.2.5 Patients must have a ECOG performance status of 0 or 1.

3.2.6 Women of childbearing potential must have a negative urine and serum pregnancy test within 72 hours prior to receiving first dose and must be willing to use contraceptive through 120 days of last dose of Pembrolizumab.

3.2.7 Patients must have recovered from effects of recent surgery, radiotherapy, or chemotherapy. Patients with ≥ Grade 2 neuropathy are eligible.

3.2.8 Patients may have received prior radiation therapy for treatment of endometrial cancer. Prior radiation therapy may have included pelvic radiation therapy, extended field pelvic/para-aortic radiation therapy, intravaginal brachytherapy and/or palliative radiation therapy. All radiation therapy must be completed at least 4 weeks prior to the first date of study therapy.

3.2.9 Patients may have received prior hormonal therapy for treatment of endometrial carcinoma. All hormonal therapy must be discontinued at least one week prior to the first date of study therapy.

3.2.10 Patients may have received prior therapy (including chemotherapy, biologic/targeted therapy and immunotherapy) for treatment of endometrial cancer. All therapy must be discontinued at least 3 weeks prior to the first date of study therapy. Any investigational agent must be discontinued at least 30 days prior to the first date of study therapy.

3.2.11 Chemotherapy administered in conjunction with primary radiation as a radio-sensitizer WILL be counted as a systemic chemotherapy regimen.

3.2.12 Patients are allowed to receive, but not required to receive, up to 4 additional lines of therapy.

3.2.13 At least 4 weeks must have elapsed since the patient underwent any major surgery (e.g., major: laparotomy, laparoscopy) There is no delay in treatment for minor procedures (e.g., tumor FNA or core biopsy, venous access device placement).

3.2.14 Have demonstrated adequate organ function as defined in Table 1. All screen labs should be performed within 14 days of treatment initiation

Table 1 . Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

3.2.15 Patients must have signed an approved informed consent and authorization permitting release of personal health information.

3.2.16 Patients must be 18 years or older

3.3 Ineligible Patients

3.3.1 Patients who have had prior therapy with nivolumab, pembrolizumab or with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune check point pathways.

3.3.2 History of severe hypersensitivity reaction to any monoclonal antibody.

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

3.3.4 Patients who are pregnant or nursing. The effects of pembrolizumab on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) must agree to use adequate

contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. WOCBP should use an adequate method to avoid pregnancy for 23 weeks after the last dose of investigational drug. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IV/L or equivalent units of HCG) within 24 hours prior to the start of pembrolizumab. Women must not be breastfeeding.

3.3.5 Women who are not of childbearing potential (i.e., who are postmenopausal or surgically sterile or have undergone definitive radiation) do not require contraception.

3.3.6 Patients with known brain metastases or leptomeningeal metastases are excluded unless the following conditions are met: Metastases have been treated and there is no evidence of progression by CT scan or magnetic resonance imaging (MRI) prior to the first dose of pembrolizumab administration). There must also be no requirement for immunosuppressive doses of systemic corticosteroids (>10 mg/day prednisone equivalents) for at least 1 week prior to study drug administration.

3.3.7 Patients should be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS). Patients should be excluded if they have a positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection (e.g., HCV RNA [qualitative] is detected).

3.3.8 Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids, should be excluded. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IRB), Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease. Patient with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjogren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

NOTE: Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger (precipitating event).

3.3.9 Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses <10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if <10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (e.g., contrast dye

allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

3.3.10 Patients who have had evidence of active or acute diverticulitis, intra-abdominal abscess, abdominal/pelvic fistula, gastrointestinal perforation, GI obstruction and/or who require parenteral hydration and/or nutrition..

3.3.11 Has a known history of active TB (Bacillus Tuberculosis)

3.3.12 Hypersensitivity to pembrolizumab or any of its excipients.

3.3.13 Prior invasive malignancy (except non-melanomatous skin cancer such as basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer) unless disease free for a minimum of 3 years.

3.3.14 Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

3.3.15 Has an active infection requiring intravenous systemic therapy.

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

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

3.3.18 Has not recovered from adverse events to <Grade 1 or prior treatment level due to a previously administered agent. Subjects with Grade <2 neuropathy or alopecia of any grade are an exception to this criterion and may qualify for the study.

3.3.19 Has a known additional malignancy that progressed or required active treatment within the last five years. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

3.3.20 Is pregnant or breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

3.3.21 Patients should not have used investigational agents or device within 4 weeks prior to first dose.

3.3.22 Has received a live vaccine within 30 days of planned start of study therapy.

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

3.4 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial.

3.5 Identification of eligible patients

Archived tumor samples or newly obtained biopsies will be used for determining patient's eligibility. Identification of ultra-mutated (POLE/POLD1-mutation) and/or hyper-mutated (due to MMR gene defect) patients by standard PCR-based DNA microsatellite instability (MSI) and immunohistochemistry (IHC), and/or next generation sequencing (NGS) and Comprehensive Genomic Profiling (CGP) testing that is already standardized and performed in CLIA-certified laboratories at Foundation Medicine (18,19) without need for assay development.

4.0 STUDY MODALITIES AND TREATMENT MODIFICATIONS

4.1 The treatment to be used in this trial is outlined below in Table 2

Table 2 Trial Treatment

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

Please refer to the Investigator's Brochure for Preclinical and Clinical data on Pembrolizumab.

LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

Clinical Supplies will be provided by Merck as summarized in Table 3.

Table 3 Product Descriptions

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

Packaging and Labeling Information

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

Clinical Supplies Disclosure

Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

Storage and Handling Requirements

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

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

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

Returns and Reconciliation

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

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

4.2 Dose Selection/Modification

4.2.1 Dose Selection

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

4.2.2 Dose Modification (Escalation/Titration/Other)

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

Table 4 Dose Modification Guidelines for Drug-Related Adverse Events

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

irAE Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication[d]
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Nephritis (grading according to increased creatinine or acute kidney injury)	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Neurological Toxicities	2	Toxicity resolves to Grade 0-1	Based on severity of AE administer corticosteroids
	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	1	Toxicity resolves to Grade 0	Based on severity of AE administer corticosteroids
	2-4	Permanently discontinue	Permanently discontinue
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS		Based on severity of AE administer corticosteroids
	Confirmed SJS, TEN, or DRESS	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	Persistent Grade 2, 3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis;

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

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

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to Infusion Treatment Guidelines for further management details.

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

[d] Patients with Grade 2 toxicity may continue therapy with pembrolizumab at the discretion of the Principal Investigator.

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

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

4.3 Timing of Dose Administration

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

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

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

4.4 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

4.5 Concomitant Medications/Vaccinations (allowed & prohibited)

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

4.5.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for serious adverse events (SAEs) and events of clinical interest (ECIs).

4.5.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

4.6 Rescue Medications & Supportive Care

4.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment

guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

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

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

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

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**:

- For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

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

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

Table 5 Infusion Reaction Treatment Guidelines

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

4.7 Diet/Activity/Other Considerations

4.7.1 Diet

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

4.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero.

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female subjects of reproductive potential must agree to avoid becoming pregnant, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- female condom (cannot be used together)

- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

4.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor.

4.7.4 Use in Nursing Women

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

4.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

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

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

- Confirmed radiographic disease progression
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment.

- Administrative reasons
- In case of radiographic complete response confirmed with 2 consecutive CAT scans 12 weeks apart, patient may be discontinued from study.

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

4.9 Subject Replacement Strategy

Early drop-outs and ineligible patients: If an enrolled subject drops out of the study before receiving treatment or is found to be ineligible, she will be replaced.

4.10 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

If subject discontinues they will not be replaced.

5.0 CORRELATIVE STUDIES

TUMOR TISSUE COLLECTION AND BLOOD SAMPLING

Specimen Requirements

Required Specimen	Collection Time Point	Ship Specimens to
FFPE Primary Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	**Foundation Medicine** for next generation sequencing (NGS) and comprehensive genomic profiling (CGP) and **Yale University** for pathology review, MSI testing, WES and correlative studies
FFPE Metastatic Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	
FFPE **Recurrent Primary** Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	
FFPE **Recurrent Metastatic** Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	
FFPE **Persistent Primary** Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	
FFPE **Persistent Metastatic** Tumor 1st Choice: block 2nd Choice: 20 unstained slides (charged, 5µm)	Prior to all treatment	
Pre-treatment plasma prepared from **10mL** of blood drawn **EDTA top tube**	Prior to study treatment	**Yale University School of Medicine** the day the specimen is collected
Pre-treatment Immune Whole Blood **10mL drawn into green top (sodium heparin) tube(s)**		**Yale University School of Medicine** the day the specimen is collected
Treatment Serum prepared from 10mL of blood drawn **EDTA top tube**	To be drawn every other cycle after start of study treatment	**Yale University School of Medicine** the day the specimen is collected
Treatment Immune Whole Blood **10mL drawn into green top (sodium heparin) tube(s)**		**Yale University School of Medicine** the day the specimen is collected

Dr. Alessandro Santin, c/o Naomi O'Leary, Yale University School of Medicine, 330 Cedar St, LSOG 305, New Haven, CT 06520, Phone: 203-737-4450, Email: naomi.oleary@yale.edu

Correlative studies and Specimen Procedures

MSI Testing

Standard micro-satellite instability testing of tumor specimens (ie, immunohistochemistry (IHC) and PCR based tests) will be performed in the CLIA certified molecular lab located in the Pathology Department at Yale University.

PD-L1/B7-H1 and PD-1 Immunohistochemistry
Upon trial completion, unstained sections of formalin-fixed, paraffin-embedded (FFPE) tumor will be tested by immunohistochemical analysis for PD-L1/B7-H1 and PD-1 IHC at Yale University and/or Qualtek.

Tumor Infiltrating Lymphocytes
Upon trial completion, unstained sections of FFPE tumor will be analyzed for immunohistochemical analysis of tumor infiltrating lymphocytes (TILs) at Yale University.

Whole exome sequencing (WES)
Whole exome sequencing of tumor specimens from Pembrolizumab treated patients will be performed at the west campus facility at Yale University as previously described by our research group (4).

Peripheral Blood Flow cytometry studies
PBL collected at different time points (ie, before treatment and at 6 and 12 weeks during pembrolizumab treatment) will be evaluated for absolute lymphocyte counts, number of T cells, T-cell subsets, NK cells, and B cells as well as different actication markers (ie, CD25, HLA-DR) by flow cytometry.

6.0 STUDY PARAMETERS

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

6.1 Observations and Tests

The following observations and tests are to be performed and recorded before, during, and after treatment (**Table 6: Study Calendar**):

Study week	**Pre-Treatment** *****	**Treatment Period** **Cycle 1**	**Treatment Period** **Cycle 2 and higher**	**End of treatment visit**
Cycle Day		21	21	
Study Procedures Window	**(-28 to 1 Days)**	**± 3**	**± 3**	
History, PE, Performance Status, Toxicity Evaluation, weight	X	X	*** X	X
CBC /Diff/Platelets	X	X	***X	X
Chemistries:Serum Cr,BUN,K,Mg,Bili	X	X	***X	X
PT/INR and aPTT	X			
Urinalysis	X		****as clinically indicated	****as clinically indicated
SGOT,SGPT,Alk Phos	X	X	X	X
Vital signs	X	X	X	X
EKG	X		****as clinically indicated	****as clinically indicated
Toxicity	X	X	X	X

Study week	**Pre-Treatment** *****	**Treatment Period** **Cycle 1**	**Treatment Period** **Cycle 2 and higher**	**End of treatment visit**
Cycle Day		21	21	
Study Procedures Window	**(-28 to 1 Days)**	**± 3**	**± 3**	
History, PE, Performance Status, Toxicity Evaluation, weight	X	X	*** X	X
CBC /Diff/Platelets	X	X	***X	X
Chemistries:Serum Cr,BUN,K,Mg,Bili	X	X	***X	X
PT/INR and aPTT	X			
******Radiographic tumor evaluation and measurement (Chest/Abd/Pelvis)	X		X	
*CA 125	X		X	
*TSH	X		X	
** collection of blood for Translational Studies	X		Every other cycle after study initiation	X

* CA 125, and TSH should be performed after 6 weeks (end of cycle 2), 12 weeks (end of cycle 4), and every 12 weeks thereafter until end of treatment on protocol. CA 125 shall be performed per standard of care following end of treatment per protocol. TSH shall be performed per endocrinologist/PCP for thyroid toxicities.

** See Appendix C for collection and shipping information.

*** All activities performed every 3 weeks

**** During the treatment period, EKG and urinalysis will be performed on screening and as clinically Indicated thereafter.

***** All screen labs should be performed within 14 days of treatment initiation. Laboratory assessments at Cycle 1 Day 1 are not required if the previous screen labs are within 14 days prior to treatment initiation.

****** Repeat CT scan or MRI should use similar equipment and techniques to ensure consistent measurements. Tumor evaluation should be performed after 6 weeks (end of cycle 2), 12 weeks (end of cycle 4), and every 12 weeks (+/- 14 days) thereafter.

The screening procedures include:

- A review of the patient medical history including a list of current medications and dosing schedules, previous therapies for disease, and residual treatment toxicities.
- Physical examination, height, weight and vital signs (pulse, temperature, respiration, and blood pressure). A pelvic examination may be required if this method is used to measure patients disease.
- An electrocardiogram (ECG) will be performed for baseline assessment.
- A CT scan of chest, abdomen and pelvis will be performed or an MRI if necessary. The scans may not have to be repeated if performed within 28 days.
- Blood test for CA 125
- Blood test for thyroid-stimulating hormone (TSH)
- Lab work: CBC, Chemistry (electrolyte, magnesium, SGOT, SGPT, Alk. Phos), Coagulation (PT/INR and aPTT)

- Approximately 3 teaspoons (20 mL) of blood will be collected to evaluate the number and activation of B cells, T cells and NK cells
- woman of child-bearing potential, a pregnancy test will be performed from either urine or blood sample.

Treatment Procedures:
The following will take place at different times during the treatment period:

- At each study visit (every 3 weeks) a physical examination including weight and vital signs (pulse, temperature, respirations and blood pressure). A pelvic examination may be required if this method is used to measure your disease.
- Toxicity assessment (weekly during first cycle of treatment)
- Routine lab work such as complete blood count, serum chemistries, and electrolytes will be obtained
- A CT or MRI of the chest abdomen and pelvis will be performed after 6 weeks, 12 weeks, and every 12 weeks (+/- 14 days) thereafter or as medically indicated thereafter.
- Blood test for CA 125 Protein levels, and TSH will be drawn after 6 weeks, 12 weeks, and every 12 weeks thereafter until end of treatment on protocol.
- Blood samples to test the number and activation of different subsets of lymphocytes will be collected at baseline and every other cycle.

Note: In order to more precisely determine time of progression, the investigator is encouraged to obtain radiologic assessments earlier than 12 weeks if there is a strong clinical suspicion of progression of disease to either confirm or refute the clinical impression.

End-of- Treatment Visit:

- Physical examination will be performed. A pelvic examination is required if this method is used to measure your disease.
  - A CT scan of the chest, abdomen and pelvis will be performed, if not done within prior 12 weeks.
  - Blood test for CBC, Chemistry,

Follow-up:
Subjects will need to see their doctor every 3 months to check the status of their disease for 2 years following study treatment. Afterwards, they will see the doctor every 6 months for 3 years and then yearly.

Patients who have completed study treatment or who have discontinued study treatment for reasons other than Progressive Disease per RECIST 1.1 will continue to be evaluated with tumor assessments every 12 weeks (+/- 14 days) until documentation of Progressive Disease, withdrawal of consent or until the patient starts subsequent anti-cancer therapy, whichever comes first.

Patients will be followed every 3 months for survival only after disease progression.
This may be performed by telephone follow up.

6.1.1 Administrative Procedures

6.1.1.1 Informed Consent

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

General Informed Consent

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

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

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

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

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

6.1.1.2 Inclusion/Exclusion Criteria

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

6.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

6.1.1.4 Prior and Concomitant Medications Review

6.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial.

Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

6.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial.

6.1.1.5 Disease Details and Treatments

6.1.1.5.1 Disease Details

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

6.1.1.5.2 Prior Treatment Details

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

6.1.1.5.3 Subsequent Anti-Cancer Therapy Status

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

6.1.1.6 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

6.1.1.7 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history.

12-Lead Electrocardiogram (ECG)
A standard 12-lead ECG will be performed using local standard procedures once at screening. Clinically significant abnormal findings should be recorded as medical history. Additional time points may be performed as clinically necessary.

6.1.1.8 Directed Physical Exam

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

6.1.1.9 Vital Signs

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

6.1.1.10 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

6.1.1.11 Tumor Imaging and Assessment of Disease

For this study, imaging of chest abdomen, and pelvis are required at Screening and at each scheduled disease assessment visit after enrollment. Computed tomography (CT) is the modality strongly preferred for chest. For abdomen and pelvis CT or magnetic resonance imaging (MRI) are both acceptable. However, the same imaging modality and technique, such as use of contrast should be used consistently for an anatomic region in a subject throughout the trial. At Screening, imaging scans must be performed within 28 days prior to date of allocation to confirm the subject has measurable disease per RECIST 1.1. After that tumor evaluation should be performed after 6 weeks (end of cycle 2), 12 weeks (end of cycle 4), and every 12 weeks thereafter. Imaging assessments should continue until disease progression per RECIST 1.1. The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit.

6.1.1.12 End of Treatment and Follow-up Tumor Imaging

In subjects who discontinue trial treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (after 6 weeks (end of cycle 2), 12 weeks (end of cycle 4), and every 12 weeks (+/- 14 days) thereafter) to monitor disease status until the start of new anti-cancer treatment, disease progression, death, or the end of the study, whichever occurs first.

ASSESSMENTS DURING TREATMENT

7.0 EVALUATION CRITERIA

7.1 Objective Response

The major parameters of response to be assessed include complete and partial responses, Progression-Free Survival, Overall Survival, documentation of Sites of Recurrence, and Treatment Related Toxicity. Treatment response will be based on RECIST v1.1 Guidelines for Measurable Lesions.

Measurable Lesions- Only recurrent patients with measurable lesions will be enrolled in the study. All target lesions must be assessed using the same technique as baseline. Included in the evaluations are the following standard response criteria for target lesions:

- Complete Response (CR): Disappearance of all target lesions. No new lesions. Lymph nodes must be <10mm short axis.

- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum of the longest diameters. No new lesions.

- Progressive Disease (PD): The appearance of a new lesion, or at least a 20% increase in the sum of the longest diameters of the target lesions, taking as the reference the smallest sum of the longest diameters recorded since treatment started, and at least 5mm increase.

- Stable Disease (SD): Target lesions do not qualify for CR, PR, or progression. No new lesions.

All non-target lesions must be assessed using the same technique as baseline. Included in the evaluations are the following standard response criteria for non-target lesions:

- Complete Response: The disappearance of all non-target lesions.
- Incomplete Response/Stable Disease: The persistence of one or more non-target lesion(s).
- Progression Disease: The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Objective Response (OR) will be defined as Complete or Partial Response using CR or PR as described by the above RECIST v1.1 Response Criteria.

Progression-Free Survival (PFS) will be defined as date from entry to a particular protocol to date of reappearance or increasing parameters of disease on imaging (CT scan or MRI) or by clinical exam or death from any cause, or is censored at date of last disease assessment.

Overall Survival (OS) will be defined as observed length of life from entry to a particular protocol to death or, for living patients, date of last contact.

Treatment Toxicity: The grade level of the various toxicities will be classified using the Common Toxicity Criteria, version 4 (CTC, v.4) guidelines. Acute toxicities will be scored if occurring </= 30 days from treatment completion, and chronic if > 30 days. Frequency and duration of treatment interruptions due to the treatment toxicity will be assessed.

8.0 STUDY MONITORING AND REPORTING PROCEDURES

8.1 Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal

investigator with the help of the Remote Monitoring Coordinator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator, the IRB or the Yale Cancer Center Data and Safety Monitoring Committee (DSMC) have the authority to stop or suspend the study or require modifications.

8.2 The risks associated with the current study are deemed moderate for the following reasons:

1. We do not view the risks associated with pembrolizumab as minimal.
2. Given the now established safety and validity of studies performed using the FDA-approved drug pembrolizumab, we do not view the proposed study as high risk.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

8.3 Attribution of Adverse Events:
Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator Dr. Alessandro Santin, according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

8.4 Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

8.5 Plan for Determining Seriousness of Adverse Events:

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

8.6 Plan for reporting Reportable Adverse Events and other unanticipated problems involving risks to subjects or others to the IRB

The principal investigator will report the following types of events to the IRB: a) adverse events that are serious or life-threatening AND unanticipated (or anticipated but occurring with a greater frequency than expected) AND possibly, probably or definitely related to the drug/device/intervention; and b) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website.

Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g.,Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

X All Co-Investigators listed on the protocol.

X Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

X Merck Pharmaceuticals

The principal investigator Alessandro Santin will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

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

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of Sponsor's product or vaccine, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met. If a dose of Sponsor's product or vaccine meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect." All reports of overdose with and without an adverse event must be reported by the investigator within 24 hours to the Sponsor either by electronic media or paper.

8.8 ADVERSE EVENT REPORTING

This study will utilize the CTC version 4.0 for toxicity and Adverse Event Reporting. A copy of the CTC version 4.0 can be downloaded from the CTEP home page (Table 7).

Toxicity Grade	Type[a]	Local IRB	Study Coordinators Via email/fax/phone

4,5	Unknown	Yes	Yes
5	Known	No	Yes*
2,3	Unknown	No	No
4 (non-myelo)	Known	No	No
4 (myelo[b])	Known	No	No

* If clearly related to the commercial agent(s)
[a] Type (Known or unknown) is based on toxicities included in the package insert or literature of known toxicities associated with the study drug(s).
[b] Myelosuppression, which includes neutropenia, anemia, thrombocytopenia

8.9 ASSESSMENT OF SAFETY

8.9.1 SPECIFICATION OF SAFETY VARIABLES

Safety assessments will consist of monitoring all adverse events and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to **pembrolizumab**, all events of death, and any study specific issue of concern.

8.9.1.1 Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with advanced recurrent endometrial carcinoma that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

8.9.2 Serious Adverse Events

Refer to Section 9.0 of the study protocol.

8.9.3 Methods and Timing for Assessing and Recording Safety Variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in Section 9, are collected and reported to the appropriate IRB(s), and Merck in accordance with FDA regulations at 21CFR 312.32 (a)(IND Safety Reports).

8.9.4 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

8.9.5 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately.

Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to pembrolizumab (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

> There is a plausible temporal relationship between the onset of the AE and administration of pembrolizumab and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to pembrolizumab and/or the AE abates or resolves upon discontinuation of pembrolizumab or dose reduction and, if applicable, reappears upon re-challenge.

No

> Evidence exists that the AE has an etiology other than pembrolizumab (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to pembrolizumab administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are **listed** or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those **not listed** in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

8.10 PROCEDURES FOR ELICITING AND RECORDING ADVERSE EVENTS

8.10.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

"How have you felt since your last clinical visit?

"Have you had any new or changed health problems since you were last here?"

8.10.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations. All information will be recording using Oncore Clinical Trials Management System.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the conduct of the research must be reported to the Yale Principal Investigator and the Remote Monitoring Coordinator immediately (if possible) to ensure that all over site committees are properly informed. All information will be entered into Oncore Clinical Trials Management System and updated appropriately. Further determination regarding the need to submit to the subjects local IRB, Yale University HHRP following IRB Policy 710 for reporting Unanticipated Problems Involving Risk to Subjects and Merck Pharmaceuticals, Inc. Drug Safety Committee will be

reviewed.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history.

A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE.

If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions

Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or

Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 30 days of awareness after the last dose of study drug, a report should be completed and expeditiously submitted to Merck Pharmaceuticals, Inc. Follow-up to obtain the outcome of the pregnancy should also occur.

Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and

expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to pembrolizumab should be reported as an SAE. A negative pregnancy outcomes should be reported to Merck as SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior pembrolizumab exposure.

If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

Sponsor is responsible to monitor successful transmission of SAE reports to Merck.

8.10.3 STUDY CLOSE-OUT REPORTS

Any Clinical Study Report (final study report), abstracts or literary articles that are a result of the study should be sent to Merck Pharmaceuticals for review.

9.0 PROCEDURES FOR REPORTING UNANTICIPATED PROBLEMS INVOLVING RISKS TO SUBJECTS OR OTHERS, INCLUDING ADVERSE EVENTS (UPIRSOs)

9.1 Expedited Reporting of UPISROs Occurring at Yale

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

Serious Adverse Event (SAE)
Any adverse event that results in any of the following outcomes:

- death,
- a life-threatening experience,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- a congenital anomaly/birth defect, or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

The PI will promptly investigate all safety information related to an adverse experience. If the results of the PI's investigation show an adverse drug experience not initially determined to be reportable (based on whether the event is serious, unexpected, and associated with drug administration) is so reportable, the PI will report

such experience. Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

SAEs will be reported in accordance with all applicable institutional (HIC), Sponsor, and FDA requirements. The Multi-site PI (Sponsor) will adhere to 21CFR56.108.

Reporting to the Yale Human Investigation Committee

Timeframe for Reporting

1. Events that may require a temporary or permanent interruption of study activities by the Principal Investigator or sponsor to avoid potential harm to subjects should be reported to the IRB **immediately** (if possible), followed by a written report to the IRB using the UPIRSO Reporting Form (710 FR 4) **no more than 5 calendar days** after the Yale Principal Investigator becomes aware of the event**.**

2. Internal Events (defined above) should be reported to the IRB using the UPIRSO Reporting Form (710 FR 4) **within 5 calendar days** of the Principal Investigator becoming aware of the event.

3. External Events (defined above) should be reported to the IRB using the UPIRSO Reporting Form (710 FR 4) **within 15 calendar days** of the Yale University Principal Investigator (PI) becoming aware of the event ONLY IF either of the following are true:

   (a) The Yale PI has concluded that an immediate change to the protocol is necessary to address the risks raised by the event, and BI agreed to the changes OR

   (b) A monitoring entity (e.g., an external IRB at the site where the problem or event occurred, the sponsor, or the Data Safety Monitoring Board) has required modifications/amendments to the research protocol or consent documents as a result of the event.

For all reports of external events, the UPIRSO Reporting Form (710 FR 4) must include the following information:

(a) a clear explanation of why the event or series of events has been determined to meet criteria for reporting;

(b) a description of the proposed protocol changes and any corrective actions to be taken by the PI in response to the external event; and

(c) any aggregated data and an analysis or summary from the sponsor or DSMB, when applicable and available, sufficient to explain the significance of the event or series of events in order to ensure the information is interpretable and relevant to the IRB's task of protecting the rights and welfare of human participants.

Reporting to Investigators at Collaborating Sites

The Yale PI will notify all participating investigators in a written safety report of any adverse experience **associated with the use of the drug** that is both **serious** and **unexpected** as soon as possible and in no event later than 15 calendar days after the sponsor's (PI's) initial receipt of the information. [21CFR312.32(c)]

9.2 SUB SITE INVESTIGATOR SAE REPORTING REQUIREMENTS

The collaborating investigator (Sub Site PI) in a multi-center trial will report **serious**, **unexpected** adverse events occurring at their site to the Yale Principal Investigator **regardless of attribution** immediately (if possible) of knowledge of the event. Full written reports should be submitted to the Yale PI within 5 calendar

days of initial knowledge of the report. This information will also need to be recorded in Oncore Clinical Trials Management System in the SAE section.

Send SAEs to the Yale Principal Investigator:

Name: Alessandro Santin, MD
Phone: 203-737-4450
Email: alessandro.santin@yale.edu
Fax: 203-737-4339

9.3 SAE reporting to Merck

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

- within two (2) calendar days upon receipt of initial and follow-up SAEs containing at least one fatal or immediately life-threatening event;
- within ten (10) calendar days upon receipt of any other initial and follow-up SAEs.

At all times, the Remote Monitoring Coordinator is available to facilitate submissions and answer any questions regarding the process for all sub site staff.

10.0 MULTI-SITE MANAGEMENT AND COORDINATION

10.1 Overview

This is a multi-site trial where **Dr. Alessandro D. Santin** is the lead Principal Investigator for Yale University and for all non-Yale sites. The Gynecological Oncology research team at Yale University consisting of a Multi-site Research Coordinator, Remote Monitoring Coordinator, Multi-site Data Manager, Clinical Research Nursing Coordinator, Specimen Procurement Coordinator and a Multi-site Administrative Assistant will provide multicenter research support for all sites involved in this project. The YCCI clinical trials management support staff will provide clinical database systems support and training through the use of Oncore Clinical Trials Management System.

10.2 Initiation of Study

Once sub-site IRB approval has been obtained and all required start up documents have been submitted, Dr. Alessandro D. Santin will perform a Site Initiation Visit (SIV) prior to enrollment of study subjects. This will be either on-site or via teleconference with each participating site PI and staff available. Members of the Gynecologic Oncology research team will be available to answer all protocol questions. Once this has taken place with the participating site they may enroll subjects into the protocol. All pre and post SIV documents will be collected and stored in accordance with the Gynecologic Oncology Research SOP for collection of regulatory documents.

10.2.1 Investigational Site Training

Dr. Alessandro D. Santin or an appointed designee will provide investigational staff training prior to study initiation. Training topics will include but are not limited to: Good Clinical Practice (GCP), AE reporting, study details and procedure, study documentation, specimen procurement and shipping, informed consent, and

enrollment. Sub sites are supplied with telephone and e-mail contact for all personnel and encouraged to contact with any questions regarding the conduct of the protocol.

10.2.2 Data Collection

Sites will use Oncore Clinical Trials Management System for all data associated with the protocol. Data managers at participating centers will receive Oncore access once they are designated by the site PI, complete an Oncore access request and submit HIPAA training certification. Individuals will receive Oncore training by a member of the YCCI clinical trials management support staff. Subjects entered into the system will be identified by a subject number assigned after randomization. The confidentiality of records that could identify subjects will be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s). The database will be monitored by the Multi-site Data manager and the Remote-site Monitoring Coordinator to ensure that data is entered in a timely manner.

10.3 Monitoring

Dr. Alessandro D. Santin or an appointed designee must be allowed to visit all study site locations periodically to assess the data quality and study integrity. On-site they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain compliant.

In addition, remote monitoring of data may be performed periodically requiring the site to submit de-identified patient data for comparison to the Remote-site Monitoring Coordinator. The study may be audited by the Yale University DSMB internal auditors. Yale University DSMB audit reports will be kept confidential.

10.4 Study Enrollment Procedures

Any modification of the original consent document provided to participating sites by the Coordinating Center must be approved in advance by the Coordinating Center. Rationale will be provided with any request for a change. A copy of each site's IRB and Coordinating Center approved informed consent document must be on file at the Gynecological Oncology Regulatory office before subjects may be enrolled.

To register eligible patients on this study, each site will contact the Multi-site Coordinator; Lisa Baker, RN (203-785-6398) or Martha Luther, RN (203-737-2781) and provide the signed and dated eligibility checklist, completed signature page of the consent form and additional source documents if requested by the Principal Investigator or Multi-site Coordinator. Once the Principal Investigator or Multi-site Coordinator verifies eligibility, a unique subject study number will be issued. The subject will not be identified by name.

10.5 Responsibilities of the Lead Investigator Dr. Alessandro D. Santin:

The lead investigator is responsible for the overall conduct of the study at all participating sites and for monitoring the safety and quality of the data as well as compliance to the protocol and with applicable federal regulations and Good Clinical Practice (GCP).

The lead investigator will monitor accrual rates at all sites for adequate progress.

The Lead Investigator will ensure appropriate coordination, submission and approval of the protocol as well as the consent documents and any subsequent amendments at all sites. There will be only one version of these documents that will be used by all participating institutions and the lead investigator is responsible for assuring that the correct versions are used by all participating institutions.

No additional sites will be added to the study without a proposed amendment and review and approval by the Yale IRB (HIC).

The lead investigator has the authority to suspend accrual at any site not complying with this protocol, including not submitting data in a timely manner. Any suspension of accrual will be reported to the Yale University Data Safety Monitoring Committee (DSMC) as well as to the Yale University HHRP. Site principal investigators must report the suspension to their IRB.

10.6 Responsibilities of the Coordinating Center

The coordinating center, under the direction of the lead investigator Dr. Alessandro D. Santin, MD, is responsible to ensure that each participating site has the appropriate assurance on file with the Office for Human Research Protection (OHRP) or their local/central Institutional Review Board (IRB). The coordinating center is responsible for obtaining copies of OHRP/IRB assurance for each site prior to enrollment of subjects at the site.

10.7 IRB Approvals

The Coordinating Center is responsible to ensure that no patients are entered on study without full IRB approval and that IRB re-approval is appropriately maintained. A copy of the IRB approval document from each participating institution will be obtained by the Coordinating Center prior to activation of the study at any site. Documentation of reapproval must be provided to the Coordinating Center in a timely manner or registration will be halted at any site in which a current continuing approval is not on file at the Coordinating Center.

10.8 Amendments and Consents

The Coordinating Center will maintain a copy of all amendments, consent forms, and approvals from each site. Consent forms will be reviewed and approved by the Coordinating Center to ensure consistency with the Yale IRB approved consent. Should changes to the protocol or consent become necessary, protocol amendments will be issued by the Coordinating Center to all sites for local site approval prior to implementation, unless there is an apparent immediate hazard to a subject or the subject's best interest is endangered. Any such deviation from the approved protocol will be promptly reported to the lead investigator.

10.9 Responsibilities of Participating Sites:

The principal investigator at each site is responsible for the overall conduct of the study at their site and for monitoring the safety and quality of the data as well as compliance to the protocol and with applicable federal regulations and (GCP).

The principal investigator at each site is responsible for assuring that all the required data is collected and entered onto the e-CRFs using Oncore Clinical Trials Management Systems in accordance with study-specific requirements and that the data submission and reporting timelines are met. The coordinating center will perform onsite monitoring periodically to ensure adherence to the protocol as well as regulatory compliance.

11 STATISTICAL ANALYSIS PLAN

a. Statistical Analysis Plan

This is a single-arm, open-label phase II study using the optimal flexible two-stage design of Chen *et al*. (24) to evaluate the efficacy of the study regimen through objective tumor response. There are no treatment comparisons involved and no known historical controls available.

The targeted accrual for the first stage of the study will be 12 eligible and evaluable patients but permitted to range from 8 to 15 for administrative reasons. The cumulative targeted accrual for the second stage will be 22 eligible and evaluable patients but permitted to range from 18 to 25 for administrative reasons.

If the true probability of tumor response is 5%, then the study has a 65% average probability of early termination and an expected probability of 10% of incorrectly declaring the study regimen interesting. If the true probability of tumor response is 25%, then the study has an expected 90% chance of correctly classifying the study regimen as being interesting.

11. 0 Justification of Design:

This study will employ the optimal flexible two-stage design since it not only minimizes the average expected number of patient exposure to inactive regimen, but also allows the actual study accrual to slightly deviate from the targeted study accrual in a multi-center trial while maintaining the levels of the average type I and II errors.

Study Endpoints

11.1 Primary Endpoints

1) Frequency of objective tumor response as assessed by RECIST. *Subjects who continue treatment beyond initial investigator-assessed, RECIST 1.1-defined progression will be considered to have investigator-assessed progressive disease at the time of the initial progression event but may be designated as delayed responses for purposes of determining proportion of objective response and counted as responses for two stage designs.*

2) Frequency and severity of adverse events as assessed by CTCAE v4.

Secondary Endpoints

1) Duration of progression-free survival (PFS) and overall survival (OS).

Translational Science Objectives

1) To prospectively evaluate and compare the sensitivity/specificity of NGS and comprehensive genomic profiling (CGP) to assess MMR gene deficiency/microsatellite instability (MSI) to standard PCR-based DNA microsatellite instability (MSI) and immunohistochemistry (IHC) testing for the identification of patients responsive to pembrolizumab. B) To evaluate the tumor mutation burden (TBN) and individual characteristics of somatic mutations identified using whole exome sequencing (WES) in ultra and hypermutated patients and their correlations to OR, PFS and OS in pembrolizumab-treated patients.

2) To explore the composition of immune infiltrates in POLE/POLD1 and MMR defective tumor specimens/biopsies from primary and/or metastatic/recurrent sites with selected markers including (but not limited to) CD4+,CD8+, FoxP3, CD25, LAG-3, TIM-3, and ICOS and their correlations to OR, PFS and OS in pembrolizumab -treated patients.

3) To systematically evaluate PD-1 and B7-H1 (i.e., PD-1 Ligand) expression in tumor infiltrating lymphocytes (TILs) and tumor cancer cells, and explore their correlations with OR, PFS, and OS in pembrolizumab -treated patients with PD-1 and B7-H1 scoring results.

4) To explore the association with treatment and subject response in peripheral blood populations in ultramutated and hypermutated patients before and during pembrolizumab treatment, including absolute lymphocyte counts, number of T cells, T-cell subsets, NK cells, and B cells as well as their cellular phenotypes by flow cytometry.

Primary Objectives Study Design

Primary Hypothesis and Endpoints

Primary Hypothesis:

The intent of this study is to evaluate the efficacy of the study regimen measured by objective tumor response. Tumor response is dichotomized as response (i.e., complete or partial response) vs. non-response (i.e., stable disease, progressive disease, or indeterminate disease) and is assumed to have a Bernoulli distribution with a probability equal to π. Given a sample size, the number of tumor responses is binomially distributed with a given sample size and probability equal to π. Statistically, the evaluation of the study regimen efficacy measured by tumor response will be formulated through hypothesis testing via tumor response.

We will target an accrual of 12 eligible and evaluable patients in the first stage of the study, but permit the accrual to range from 8 to 15 for administration reasons. If there are more than 0 out of 8-13 or 1 out of 14-15 patients responding (complete or partial response) and medical judgment indicates, accrual to the second stage of the trial will be initiated. Otherwise, the study will be stopped and the treatment will be classified as clinically uninteresting. If the study advances to the second stage, then a cumulative accrual of 22 eligible and evaluable patients will be targeted, but permitted to range from 18 to 25 for administration reasons. If more than 1 out 18, or 2 out 19-25 patients respond and medical judgment indicates, then the study regimen will be considered worthy for further investigation.

Under the assumed accrual ranges of 8 to 15 (stage 1) and 18 to 25 (cumulatively after stage 2), and under the rejection rules associated with these accrual ranges, if the true probability of tumor response is 5%, then the study has a 65% average probability of early termination and an average 10% probability of incorrectly declaring the study regimen interesting; if the true probability of tumor response is 25%, then the study has a 90% average chance of correctly classifying the study regimen as being interesting.

The following table (**Table 8**) summarizes the accrual ranges and associated rejection rules.

Table 8: Accrual Ranges and Associated Rejection Rules.			
Stage of Accrual	**Targeted Cumulative Accrual**	**Limits of Actual Accrual**	**Max Number of Responses to Reject Study Regimen**
1	12	8-15	0/(8-13), 1/14-15
2	22	18-25	1/(18), 2/(19-25)

11.2 Definitions of Primary Endpoints and How These Will Be Analyzed

1) Proportion of objective tumor response as assessed by RECIST.

2) Frequency and severity of adverse events as assessed by CTCAE v4 and corresponding frequency table will be provided as descriptive statistics.

All patients who receive any therapy will be evaluated for both treatment efficacy and toxicity. Only those patients who are deemed "ineligible" or who receive no therapy will be eliminated from the analysis. While on occasion, circumstances may prevent the determination of treatment efficacy, such patients will be included in the analysis and labeled as "indeterminate." This category will be listed and be reflected in the calculation of the proportion responding.

11.3 Sample Size and Power Calculations:

The sample size and power calculations will be based on the optimal flexible two-stage method (24). This method assumes that the number of patients responding to the study regimen has binomial distribution and the accrual combination for stage 1 and 2 is uniformly distributed.

The minimum and maximum accruals for this study are 8 and 25 eligible and evaluable patients, respectively.

Under the assumed accrual ranges of 8 to 15 (stage 1) and 18 to 25 (cumulatively after stage 2), and under the rejection rules shown in **Table 8** for these accruals, the study has an average power of 90.0% at a target alpha=10% significance level to detect the 20% increase in the rate of tumor response from the null-hypothesis rate of 5% to a clinically interesting rate of 25% under the alternative hypothesis. The study's true alpha has an average of 9.5% with a 64.9% average probability of early termination and average expected sample size of 15.14 subjects under the null hypothesis.

11.4 Study Monitoring of Primary Objectives

There will be one formal futility interim analysis for treatment efficacy in the study.

If the study has an accrual of 12 eligible and evaluable patients in the first stage since the study is activated, which is permitted to range from 8 to 15, the accrual of the study will be temporarily suspended until the data of primary endpoint is matured to make a decision on whether to go forward to second stage accrual according to the guidelines. That is, if there are 0 out of 8-13 or at most 1 out of 14-15 patients responding (complete or partial response) and medical judgment indicates, the study will be stopped and the treatment will be classified as clinically uninteresting. Otherwise, accrual to the second stage of the trial will be initiated. As this is a two-stage multi-institutional phase II protocol, the initial overall review of toxicity is usually performed after completion of the first stage of accrual, at which point accrual is generally suspended pending formal analysis of response.

Accrual Goal

The projected minimum and maximum accruals for the study are 8 and 25 eligible and evaluable patients, respectively. The targeted accrual is 12 eligible and evaluable patients in the first stage of the study, and cumulatively 22 eligible and evaluable patients will be targeted in the second stage if the study advances.

Study Duration

1) Patients will receive therapy until disease progression or intolerable toxicity intervenes.

The patient can refuse the study treatment at any time.

2) All patients will be treated (with completion of all required case report forms) until disease progression, initiation of a subsequent cancer treatment or study withdrawal.

Patients will then be followed every three months for the first two years and then every six months for the next three years.

3) A patient is considered off study therapy when the patient has progressed or died, a non-protocol drug or therapy (directed at the disease) is initiated, or all study therapy is discontinued. Report all treatment received on Cycle Drug Information Forms and adverse events on Adverse Events Forms until the patient qualifies as being off study therapy.

Estimated Duration for Completion of Primary Endpoint: provision of timeframe, e.g. 24 months from activation

11.5 Dose Level Guidelines

Not Applicable.

11.6 Secondary or Exploratory Elements (including correlative science aims)

Secondary Hypotheses and Endpoints:

There are no specific secondary hypotheses regarding secondary objective. The purpose of secondary objective is to describe the survival functions for progression-free survival (PFS) and overall survival (OS).

Definitions of Secondary Endpoints and How These Will Be Analyzed

Progression-free survival is defined as the duration of time from study entry to time of progression, death, or the date of last contact, whichever occurs first. PFS is censored in patients who are alive and have not progressed.

Overall survival is defined as the duration of time from study entry to time of death or the date of last contact. OS is censored in patients who are alive.

The PFS and OS survival functions will be visualized using Kaplan-Meier curves, and summarized using medians of PFS and OS as well as proportions of PFS and OS at the one-year and 3-year follow-up times.

11.7 Exploratory Hypothesis and Endpoints

Dot plots and box plots in conjunction with Spearman's correlation analysis will be used to explore the associations of tumor expressions of PD-L1, PD-1, lymphocyte infiltration and other interesting biomarkers (such as CD4+,CD8+, FoxP3, CD25, LAG-3, TIM-3, and ICOS) with the ordered RECIST categories of tumor response. Logistic regression will be used to explore associations of the same tumor expressions and biomarkers with OR. Cox proportional-hazards (PH) regression will be utilized to evaluate the associations of these tumor expressions and biomarkers with PFS and OS.

The above analysis approaches will also be used to evaluate the effects of TBN and individual somatic mutations at baseline on RECIST response, OR, PFS, and OS. The effect on PFS and OS of TBN and individual somatic mutations that accumulate during treatment will be analyzed using either landmark analysis (25) or Cox regression with time-dependent covariates.

The sensitivity and specificity of NGS and CGP to assess MMR gene deficiency/MSI will be assessed using standard contingency-table methods with the gold standard being PCR-based DNA MSI-testing. Agreement between methods will also be assessed using Cohen's Kappa in conjunction with McNemar's test to test for systematic bias in the disagreements that occur.

The association of peripheral blood populations (absolute lymphocyte counts, number of T cells, T-cell subsets, NK cells, and B cells, as well as cellular phenotypes) with treatment and subject response will be explored using profile plots versus treatment cycle, in conjunction with longitudinal-analysis techniques such as mixed-models analysis or regression with generalized estimating equations to accommodate the non-independence among multiple measurements from the same subject over time.

11.8 Gender/Ethnicity/Race Distribution

	Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	2	0	2
Not Hispanic or Latino	23	0	23
Ethnic Category: Total of all	25	0	**25**
			Gender
Racial Category	**Females**	**Males**	**Total**
American Indian or Alaskan Native	0	0	0
Asian	0	0	0
Black or African American	5	0	5
Native Hawaiian or other Pacific	0	0	0
White	20	0	20
Racial Category: Total of all	25	0	**25**

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Appendix A:

FIGO Surgical Stages for Endometrial Cancer

IA **Tumor confined to the uterus, no or < 50% myometrial invasion**

IB **Tumor confined to the uterus, > 50% myometrial invasion**

II **Cervical stromal invasion, but not beyond uterus**

IIIA **Tumor invades serosa or adnexa**

IIIB **Vaginal and/or parametrial involvement**

IIIC1 **Pelvic node involvement**

IIIC2 **Para-aortic involvement**

IVA **Tumor invasion bladder and/or bowel mucosa**

IVB **Distant metastases including abdominal metastases and/or inguinal lymph nodes**

Appendix B:
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 C
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