Official Title:	**Pembrolizumab in Treating Participants With Metastatic, Recurrent or Locally Advanced Cancer and Genomic Instability**
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TITLE: A Basket Trial of Pembrolizumab in Patients with Advanced Solid Tumors and Genomic Instability

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Version Date: 04-January-2023

PROTOCOL AMENDMENTS

The current version of the protocol was released on 01-04-2023 and includes the following Amendments, 1-18 into the version approved by Rutgers IRB on 10/06/2017.

Amend-ment #	Section	Page(s)	Protocol Change
1.	4.2.1	13 15 15	Insertions, "Alterations in DNA response and repair genes have been shown to correlate with clinical outcome to immune checkpoint therapy" "There is mounting evidence that tumors with additional DNA repair defects will also be susceptible to immune checkpoint therapy." "As well, reports examining the relationship between a panel of DNA damage and repair alterations and response to PD-1/PD-L1 blockade in bladder cancer suggested that the presence of any DDR alteration was associated with a higher response rate, and that this was enhanced when tumors harbored known or likely deleterious DDR alterations compared with DDR alterations of unknown significance and in those whose tumors were wild-type for DDR genes, an observation that warrants additional study 22,23. Thus, tumors with BRCA1 or BRCA2 mutations or DDR alterations will be explored in a second cohort."
2.	5.1.2	17 18	#3 changed as "Have a diagnosis of a tumor with evidence of genomic instability or DNA repair deficiency on CLIA certified genomic testing, inclusive of mutations in POLE, POLD1 for Arm 1 and in BRCA1 and BRCA2 for Arm 2 as well as additional DNA repair abnormalities including RAD51, FANCA or FANCC, ATM, ATR, PALB2. In Arm 2, enrollment of breast and ovarian histologies will be limited to a total of 10 patients. Non-BRCA DNA repair abnormalities will be reviewed in consult with the Precision Medicine Initiative at Rutgers to confirm suitability for inclusion. #5 changed as "If adequate tissue is not present the patient may consent to a newly obtained biopsy in consultation with the PI and treating physician of risks and benefits of the biopsy".

3	5.1.3	19	#2 changed as "Has a diagnosis of immunodeficiency that consistently requires more than 10 mg daily of prednisone or equivalent or has resulted in life threatening episodes previously regardless of current treatment."
4	5.1.3	20	#9 changed as "Has active autoimmune disease which currently requires disease modifying agents that include corticosteroids > 10 mg of prednisone or equivalent or immunosuppressive drugs. Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment."
5	5.1.3	20	#10 changed as "Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis."
6	5.1.3	21	#19 changed as "Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection."
7	5.1.3	21	"MSI unknown will be excluded" appended to exclusion criteria #18
8	6.1	34	Table footnote modified for clarity
9	11.5.1.1	69	Blood collection into Red Top tubes added
10	11.5.1.2	69	Minor text edits on tumor tissue handling
11	11.5.2.1	70	Processing of peripheral blood into Red Top tubes appended (bullet b)©
12	11.5.2.2; bullet (a)	71	Genetic analyses specified (next generation sequencing)
13	11.5.2.2; bullet (e)	72	'RNA later' replaced with 'TRIzol'
14	11.5.2.2; Table 9	73	Text modifications
15	11.5.3; bullet (e)	73	Address change
16	11.5.4.4	76	Appended "nanostring, single cell RNA-sequencing, exosome sequencing, multispecial immunostain"
17	2.1 6.1 7.1.2.6.2	10 37 38 43	Required number of slides updated to 15 Modified to read: Patients will be evaluated for response by RECIST 1.1 and irRC criteria after every 9 weeks (3 cycles) of treatment until cycle 8, then subsequently after every 12 weeks (4 cycles) of treatment. Updated the schedule for collection of Circulating cell-free DNA Footnote #2 – added: All screening labs should be performed within 10 days of treatment initiation (refer to inclusion criteria #7, Table 1). Footnote #3 modified to read: "Prior to Cycles 4" Footnote #6 – added: every 6 weeks of treatment Deleted: Andrew Zloza, MD PhD Added: Joshua Vieth, PhD, Managing Deleted: a flow cytometry multiplex assay (LegendPlex) detecting IL-2, IL-4, IL-10, IL-6, IL-17A, TNF-α, sFas, sFasL, IFN-γ, granzyme A, granzyme

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	7.2.3.1 10.7 11.5	44 51 63	B, perforin and granulysin Added: Luminex Multiplexe Analysis against a panel of 48 human cytokines/chemokines. Moved (insertion) Added: "Rutgers Cancer Institute of New Jersey (Sponsor) Office of Human Research Services via OnCore within 24 hours of site notification. The completed SAE report (signed by the Investigator) must be sent to the Rutgers CINJ OHRS QA department." Deleted: Sites will contact the Rutgers Cancer Institute of New Jersey's OHRS Registration Desk (732) 235-7530 and fax (732) 235-7690 to register subjects. Added: Sites will register and enroll patients through OnCore® the Clinical Trials Management System for this study. Removed text from Sections 11.5, 11.5.1, 11.5.1.1, 11.5.1.2, 11.5.2, 11.5.2.1, 11.5.2.2, Table 9, 11.5.3, 11.5.4, 11.5.4.1, 11.5.4.2, 11.5.4.3, 11.5.4.4 Updated Section 11.5 with the biospecimen collection requirements and updated to include reference to the 051709 Correlative Research Laboratory Manual for collection, processing, and shipping details.
18	2.1 4.1 4.2.1		Changed irRC to iRECIST Deleted: Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475. Added: Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD 1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD 1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure (IB). Deleted: entire section Added: The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by: • Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg

	5.1.2		Q3W to 10 mg/kg every 2 weeks (Q2W), , representing an approximate 5- to 7.5-fold exposure range (refer to IB, Section 5.2.2) • Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W • Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and • Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W Deleted: without transfusion or EPO dependency (within 7 days of assessment) Added: without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks. Changed ≥60 mL/min to ≥30 mL/min
	5.1.3		Deleted Inclusion Criteria #10: Male subjects should agree to abstinence or use of an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy. Exclusion criteria #4 modified to read: Has severe hypersensitivity (≥Grade 3) to pembrolizumab or any of its excipients. Exclusion criteria #10 modified to read: Has a history of (non-infectious) pneumonitis/ interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease. Deleted: or father children from exclusion criteria # 14. Added to exclusion criteria #15: or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX 40, CD137). Deleted from exclusion criteria #17: 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 Exclusion criteria # 17 modified to read: Has received a live vaccine or live-attenuated vaccine within 30 days of planned start of study therapy. Administration of killed vaccines is allowed.
	5.2.1.1.		Deleted: Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.
	5.2.1.2.		Changed Section 5.2.1.2 heading to read: Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab and combination therapy Added: including coadministration with additional compounds,

			Added: and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in Table 3 below.
	5.2.2		Deleted: The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.
	5.3.2.		Added: Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
	5.4.1.		Deleted references to the ECI guidance document. Added: in Section 5.2.1.2, Table 3. Changed Management of Infusion Reactions section to read: Dose modification and toxicity management of infusion-reactions related to pembrolizumab Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.
	5.5.2.		Deleted: Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Deleted: and male Deleted: or impregnating a partner, respectively
	6.1		Added: PD-L1 analysis during screening Added: footnote 7Refer to the MISP Sample Handling Manual for collection and shipping instructions.
	7.1.2.1.		Deleted: For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document regarding the identification, evaluation and management of potential irAEs.

	7.1.2.5.4.		Added: iRECIST is based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by the investigator to assess tumor response and progression and make treatment decisions. When clinically stable, participants should not be discontinued until progression is confirmed per iRECIST. Added: If repeat imaging does not confirm PD per iRECIST (below), as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule Added: Confirmation of Progression per iRECIST Progression is considered confirmed, and the overall response will be confirmed progressive disease per iRECIST (iCPD), if ANY of the following occurs: • Any of the factors that were the basis for the initial unconfirmed progressive disease (iUPD) show worsening o For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point o For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1 o For new lesions, worsening is any of these: An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point Visible growth of new non-target lesions The appearance of additional new lesions • Any new factor appears that would have triggered PD by RECIST 1.1
	7.2.		Changed the Worldwide Product Safety; FAX to 215- 661-6229
	7.2.1.		Deleted: All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)
	7.2.3.1.		Added: o Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious. o Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. Deleted: Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified

			in the previous paragraph also must be reported immediately to the Sponsor and to Merck.
	7.2.3.2.		Deleted: recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor (CINJ) and CINJ will report within 2 working days Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) Deleted: The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent). Deleted: 1.Additional adverse events: A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document can be found in Appendix 4 and provides guidance regarding identification, evaluation and management of ECIs and irAEs. ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor (CINJ) and CINJ will report within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines. Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.
	10.6.1.		Deleted: annual review Added: quarterly review
	10.7.		Added: This protocol is available at the Rutgers Cancer Institute of New Jersey (CINJ)/Robert Wood Johnson University Hospital (RWJUH) in New Brunswick and NYU Langone Health.
	11.3.2.		Changed pulmonitis to pneumonitis
	11.4.		Deleted entire section
	11.5.		Changed the section numbering to 11.4 and subsections were updated to 11.4.1 – 11.4.3

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19	2,3	16	Trial Diagram updated to reflect closure of arm 1
	4.2	22	Added: Rationale for Protocol Modification In this modification (v.04JAN2023), we are closing Arm 1 due to low accrual and removing the limitation of enrollment of patients with breast and ovarian histologies in Arm 2. The power of study will not be affected since the study was not powered to pick up differences in response between breast/ovarian and non breast/ovarian patients. There is no mention of breast/ovarian-to other cohort comparisons. Mutations of HRD pathway are the scientific factor of interest, not ovarian/breast cohort, so there is not statistical issue with proposed change.
	5.1.2.	23	Removed: "POLE, POLD1 for Arm 1 and in" and "In Arm 2, enrollment of breast and ovarian histologies will be limited to a total of 10 patients." from inclusion criteria #3.
	5.1.3.	27	Exclusion criteria 19 updated to read: 19. Has a known **active** history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. **If patient has past hepatitis B virus or resolved HBV infection (defined as the presence of hepatitis B core antibody [HBcAb) and absence of HBsAg), patients may be enrolled provided that HBV DNA is negative.**

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1.0 TRIAL SUMMARY

Abbreviated Title	Basket Trial of Pembrolizumab
Trial Phase	II
Clinical Indication	*BRCA*, *POLE* and *POLD1* mutant tumors
Trial Type	Therapeutic
Type of control	None
Route of administration	IV
Trial Blinding	None
Treatment Groups	*BRCA*, *POLE* and *POLD1* mutant tumors
Number of trial subjects	40
Estimated enrollment period	18-24 months
Estimated duration of trial	30-36 months
Duration of Participation	30-36 months

2.0 TRIAL DESIGN

2.1 Trial Design

This will be a phase II trial that will enroll two cohorts of patients: a) Patients with advanced solid tumors with *POLE* and *POLD1* mutations whose tumors exhibit a microsatellite stable phenotype and b) Patients with advanced solid tumors with *BRCA1/2* mutations. The primary endpoint will be response rate, which we anticipate to be higher than that seen historically in unselected solid tumors. Progression free survival will also be measured.

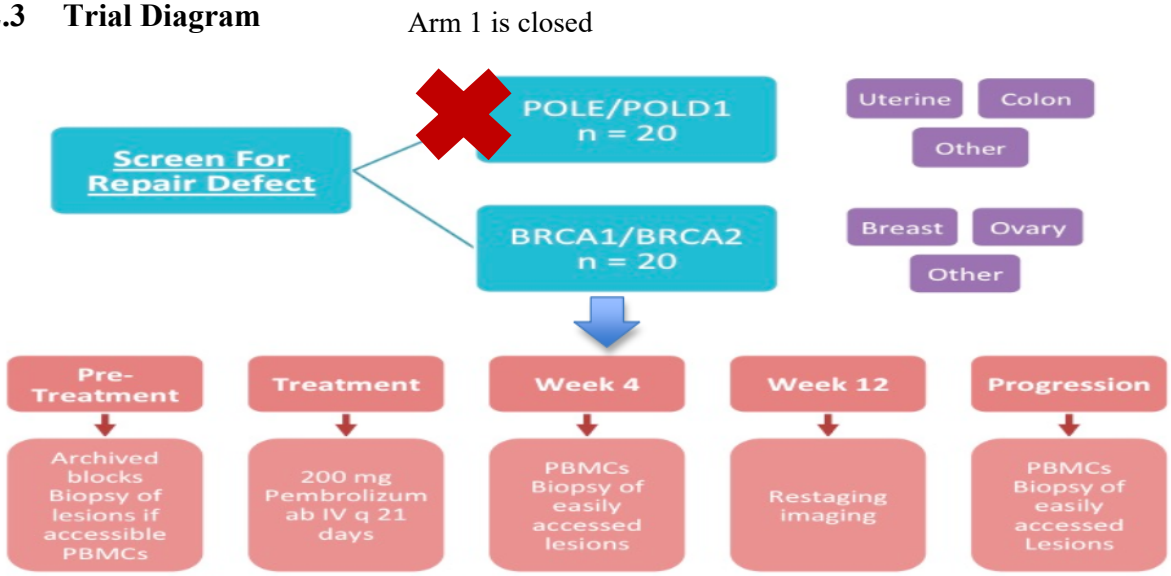
Patient Selection and Recruitment: Patients referred for this trial must submit pre-treatment tumor specimens for next generation sequencing analysis to characterize the presence of *POLE, POLD1,* or *BRCA1/2* mutations. Enrollment will be guided by presence of tumor mutations (as opposed to tumor histology). Patients with *BRCA1/2* mutations will be identified through standard of care testing for these mutations. If Foundation One next generation sequencing panel, or other similarly validated analysis, is ordered commercially prior to the patient's referral for study this analysis will be acceptable for enrollment to this protocol.
Patients with *POLE/POLD1* mutations will likely be identified through Foundation One next generation sequencing panel, or other similarly validated analysis, ordered commercially prior to the patient's referral for study. This analysis will be acceptable for enrollment to this protocol. Tumors harboring hotspot mutations in *POLE/POLD1* that result in a hypermutator phenotype are expected to be microsatellite stable (MSS). Testing for microsatellite instability will be done in all *POLE* mutant cases prior to enrollment. Tumors harboring non-hotspot *POLE* or *POLD1* mutations that show clear evidence of MSI will be excluded.

Archived tumor tissue will be retrieved for correlative analyses from each patient (20 unstained slides preferred, minimum of 15 required). If inadequate archived tissue is available, a fresh tumor biopsy must be performed.

Mandatory tumor assessments include collection of archived or fresh tumor at baseline and a biopsy of tumor upon progression. Fresh tumor biopsies or archived tumor tissue will be collected at baseline as well as peripheral blood mononuclear cell (PBMC) samples. Patients will be treated with pembrolizumab dosed at 200 mg intravenous infusion every three weeks (1 cycle = 21 days). Patients will be evaluated for response by RECIST 1.1 and iRECIST criteria after every 9 weeks (3 cycles) of treatment until cycle 8, then subsequently after every 12 weeks (4 cycles) of treatment. PBMCs (tumor optional) will be sampled to assess treatment effect on the immune response at the following time points: baseline and after week 4 of pembrolizumab. Mandatory submission of PBMC tumor biopsies at progression will occur provided that the biopsy of the progressing tumor does not impose significant risk in the opinion of the investigator. Plasma samples for analysis of cell-free DNA will also be collected prior to initiation of treatment, and every 6 weeks of treatment. The above design and assessments are depicted in the schema below.

2.2

2.3 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To evaluate the response rate of pembrolizumab in patients with evidence of genomic instability classified as follows: 1) All solid tumors with *POLE* and *POLD1* mutations 2) All solid tumors with *BRCA1/2* mutations

Hypothesis: Tumors with evidence of genomic instability such as those with mutations in *POLE, POLD1* and *BRCA1* and *BRCA2* will have higher response rates than tumors that do not exhibit these mutations.[1-7]

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To compare the complete and partial response rate, and response durability (immune related progression free survival), to historical cohort information of unselected patients treated with pembrolizumab

(2) **Hypothesis**: PFS rate will be improved in patients whose tumors have *POLE* and *POLD1* mutations, or *BRCA1/2* mutations, compared with unselected patients

3.3 Exploratory Objective

(1) Objective: To evaluate potential tumor and T cell biomarkers predictive of response through the following correlates:

- To evaluate the $CD4^+$ and $CD8^+$ T cell response in the tumor microenvironment and peripheral blood of patients treated on this study as well as the frequencies, activation/differentiation, functionality, and co-inhibitory molecule expression of immune cell populations in peripheral blood and tumor, before and after treatment with systemic pembrolizumab
- To measure PD-L1 expression in pretreatment tumor biopsies and in post treatment tumor tissue, as well as on biopsies taken at progression, to capture data on the relationship between PD-L1 expression and patient outcome
- To perform deep sequencing for detection of PD-1 and PD-L1 polymorphisms that may correlate with clinical outcomes as well as identification of mutations in immunoregulatory genes that are potential predictors of response to these therapies.
- To perform exome sequencing of pre-treatment tumor specimens to determine if the presence of immunogenic neoantigens is associated with response.
- To perform RNA sequencing to determine if expression of checkpoint genes, immune-regulatory modules, or non-coding RNAs including repetitive RNAs and retroelements are associated with response.

(2) Hypothesis: *POLE, POLD1* and *BRCA1/2* deficient tumors will have high incidence of expression of PD-L1 and evidence of CD8+ lymphocyte infiltration, levels and intensity of which will correlate with clinical benefit obtained. Responses will be associated with specific immune-related gene expression and effector T cell patterns and function.

4.0 BACKGROUND & RATIONALE

4.1 Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD 1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD 1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure (IB).

4.1.1 Pharmaceutical and Therapeutic Background

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

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

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

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor, and for non small cell lung cancer which express PD-L1 and are refractory to chemotherapy, and for head and neck squamous carcinomas that are refractory to platinum based chemotherapy.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Tumors with intrinsic defects in DNA repair mechanisms may have a high burden of potentially immunogenic mutations and thus be vulnerable to immune checkpoint blockade, a hypothesis that is the subject of multiple current investigations[1-3]. **We hypothesize that patients with mutations in DNA Polymerase Epsilon (*POLE*), part of the DNA polymerase complex responsible for DNA replication during S-phase, and *POLD1*, which is part of the main lagging strand polymerase, will result in a "hyper-mutator" phenotype. Similarly, cancers with mutations in *BRCA1/2* also have an underlying mutator phenotype which may lead to generation of immunogenic mutations. Thus, these tumors will be rendered especially vulnerable to therapy with pembrolizumab.**

Mutational Burden and Immunogenic Neo-epitopes. Immune checkpoint therapy with antibodies to programmed cell death receptor-1 (PD-1) can lead to prolonged remissions in multiple tumor types[8-16], but many do not benefit. While programmed death-ligand 1 (PD-L1) expression may predict response, it is not definitive, and its accuracy may be enhanced by the

discovery of additional predictors[17]. This is critical as combination therapy with checkpoint inhibitors enters the landscape of treatment for lung and melanoma cancers[18,19]. This approach raises the response and durable remission rate, but is complicated by a significantly higher incidence of grade 3 or 4 side effects; as well, increased financial burden is conferred. **Thus, elucidating predictive biomarkers for response is enormously beneficial, particularly if patients who require single agent PD-1 blockade for treatment of their disease could be identified.**

Acquired or germline defects in DNA repair pathways can result in very high mutational burdens. DNA repair defects in human cancer, include defects in mis-match repair (MMR) that result in a micro-satellite instability (MSI) phenotype, are associated with robust lymphocytic infiltration with CD8+ T-cells and evidence of immune checkpoint engagement[20,21]. Alterations in DNA response and repair genes have been shown to correlate with clinical outcome to immune checkpoint therapy [22,23], and clinical trials in malignancies with specific MMR repair deficiencies revealed that MMR repair status predicted clinical benefit[3].

A specific class of potentially predictive DNA repair defects includes defects in proofreading function of DNA polymerases, such as DNA polymerase Epsilon and DNA polymerase Delta (*POLE* and *POLD1*). Missense mutations in *POLE* and *POLD1* are seen as rare germline mutations associated with variant Lynch syndrome, or as acquired somatic mutations. Loss of *POLE* or *POLD1* proofreading function leads to an "ultra-mutator" phenotype with a mutation burden that exceeds even that seen in tumors with MSI phenotype[4]. *POLE* mutations occur in up to 10% of endometrial cancers, and are also found in colon, lung, breast and glial tumors. *POLE* mutant tumors show immune activation with a brisk lymphocytic infiltration and high expression of immune checkpoint genes such as PD-L1[21,24-27].

We identified a patient with *POLE* mutant endometrial cancer who experienced an exceptional response to pembrolizumab, supporting the hypothesis that *POLE* mutant cancers may be susceptible to immune checkpoint therapy[28]. Our patient's primary tumor showed a readily apparent peritumoral lymphocytic infiltrate (Fig 1 A, B[26]). She experienced immediate, sustained partial response to therapy still ongoing after 10 months (Fig. 1D[26]). The *POLE* mutation was identified through clinical grade targeted genomic profiling (Foundation OneTM) of a pre-treatment tumor sample. This tumor was associated with an ultramutator phenotype, with 32 likely pathogenic sequence variants in the primary tumor and 33 potentially pathogenic variants identified in the LN recurrence; 28 changes were present in both samples. Both samples harbored a mutation in the exonuclease domain of *POLE* that affects proofreading function (V411L) as well as a separate nonsense mutation in *POLE* (R114*), consistent with inactivation of the wild-type allele; these features are associated with an ultramutator phenotype[20,25]. In addition, there were a large number of single nucleotide variants classified as "variant of unknown significance" (VUS): 116 in the primary sample, and 159 in the recurrence, with only 83 shared between the tumors (Fig. 1E[26]). For comparison in 252 deidentified endometrioid endometrial cancers that underwent genomic profiling with the Foundation OneTM assay, 23 (9.1%) had sequence variants in *POLE*. Indeed, *POLE* mutant

endometrial cancers from TCGA have the highest mutation burden (Fig 2A[26]**.** Clustering analysis using a set of immune-related genes (Fig. 2B[26]) demonstrated that *POLE*-mutant endometrioid endometrial cancers have high expression of a large set of immunerelated genes compared with MSS cancers; MSI tumors appear to have an intermediate phenotype. To further determine if *POLE* mutant cancers were associated with an immune signature, analysis of RNA sequencing data from endometrioid endometrial cancers in TCGA was performed. *POLE* mutant cancers have higher expression of several genes encoding for immune checkpoint-related proteins, including PD-L1 and PD-L2, than either MSI or microsatellite stable (MSS) endometrioid cancers (Fig. 3A,B[26]). *POLE* mutant cancers also showed higher expression of T-cell markers such as CD8A, CD3G, PD-1 and CTLA-4, suggesting the presence of a preexisting T-cell infiltrate. Analysis of histologic image data from TCGA confirmed that *POLE* mutant cancers had presence of a robust lymphocytic infiltrate (Fig 3C[26]). It is of note that at least half of *POLE* mutant cancers, and the majority of those with hotspot mutations in the proofreading domain, do not show an MSI phenotype and thus would not be detected just by using an MSI assay. Importantly, the mutation burden conveyed by *POLE/POLD1* mutations is often **several orders of magnitude higher** than those noted in MSI phenotype cancers[27], implying these tumors may be even more sensitive to pembrolizumab than MSI phenotype tumors. **Thus *POLE* mutation identifies a subset of micro-satellite stable cancers with a high mutational burden. These tumors may be excellent candidates for immune checkpoint therapy**. These *POLE* and *POLD1* mutant tumors that are microsatellite stable mutant tumors will be the focus of one of the cohorts of our trial.

There is mounting evidence that tumors with additional DNA repair defects will also be susceptible to immune checkpoint therapy. Defects in the *BRCA1/2* related homologous recombination (HR)-mediated DNA repair pathway represent a separate category of tumors which may have genomic instability and thus, respond well to checkpoint inhibitor therapy. Loss of *BRCA1/2* function is a hallmark of tumor that arises in germline carriers of *BRCA1/2* mutation, but functional loss of *BRCA1/2* is also seen in a subset of sporadic cancers as well. Tumors that lack *BRCA1/2* have a profound defect in homology-mediated DNA repair (HMDR) and show evidence of dramatic genomic instability. Interestingly *BRCA1/2* mutant tumors have a huge burden, not of point mutations, but of gross chromosomal rearrangements, and copy number variants. The junctions of rearrangement events can lead to fusion proteins and deletions/truncations that can have novel, potentially immunogenic junction sequences. Similar to MMR-deficient tumors, *BRCA1* mutant tumors show brisk lymphocytic infiltrate and expression of immune checkpoint proteins[6,7,29]. These data suggest that *BRCA1/2* mutant cancers may also be good candidates for immune checkpoint blockade**. As well, reports examining the relationship between a panel of DNA damage and repair alterations and response to PD-1/PD-L1 blockade in bladder cancer suggested that the presence of any DDR alteration was associated with a higher response rate, and that this was enhanced when tumors harbored known or likely deleterious DDR alterations compared with DDR alterations of unknown significance and in those whose tumors were wild-type for DDR genes, an observation that warrants additional study**

[22,23]. Thus, tumors with BRCA1 or BRCA2 mutations or DDR alterations will be explored in a second cohort.

Rationale for Dose Selection/Regimen/Modification

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W), , representing an approximate 5- to 7.5-fold exposure range (refer to IB, Section 5.2.2)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Rationale for Protocol Modification

In this modification (v.04JAN2023), we are closing Arm 1 due to low accrual and removing the limitation on enrollment of patients with breast and ovarian histologies in Arm 2. The power of the study will not be affected since the study was not powered to pick up differences in response between breast/ovarian and non breast/ovarian patients. There is no mention of breast/ovarian-to other cohort comparisons. Mutations of HRD pathway are the scientific factor of interest, not ovarian/breast cohort, so there is not statistical issue with proposed change.

4.2.2 Rationale for Endpoints

4.2.2.1 Efficacy Endpoints

It has been published in a phase 2 trial of pembrolizumab that tumors with defects in mismatch repair deficiency have superior response rates and immune related progression free survival compared with tumors that are mismatch repair proficient[3]. We similarly expect that tumors with *POLE, POLD1* and *BRCA1/2* mutations will have superior response rates and progression free survival compared with cohorts of patients whose tumors are not selected for genomic instability. We have thus selected the primary clinical endpoint of response rate and secondary endpoints of immune related progression free survival to be evaluated in this basket trial.

4.2.2.2 Biomarker Research

We expect that tumors which are *POLE, POLD1* and *BRCA1/2* deficient will have high incidence of expression of PD-L1 and evidence of CD8+ lymphocyte infiltration, levels and intensity of which will correlate with clinical benefit obtained and that responses will be associated with specific immune-related gene expression and effector T cell patterns and function. We also expect patients whose tumors harbor high mutational loads to benefit most from immunotherapy. Mutational load may be both point mutations (seen in *POLE* and *POLD1* mutants) as well as genomic rearrangements, duplications and deletions (seen in *BRCA1/2* mutants). We will examine immunologic parameters that may indicate response to treatment using advanced multi-parameter flow cytometric approaches and immunochemistry. We will employ genomic sequencing analyses of pre-treatment specimens and specimens collected at progression. This is important information to collect as analysis may lead to the selection of biomarkers that will further assist in the selection of patients for immunotherapeutic approaches and as well, will allow us to potentially develop markers to assist in monitoring of response durability in patients on therapy.

We will also evaluate plasma samples collected before and during treatment with pembrolizumab for presence of circulating cell-free tumor DNA. We will evaluate the measurement of mutant-tumor DNA allele-fractions as a surrogate marker of response or progression to immune therapy. Several studies have shown that quantitative assessment of tumor-DNA in plasma can herald progression; this may allow one to separate radiographic pseudo-progression from true progression.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

5.1.2 Subject Inclusion Criteria

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

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have a diagnosis of a tumor with evidence of genomic instability or DNA repair deficiency on CLIA certified genomic testing, inclusive of mutations in *BRCA1* and *BRCA2* for Arm 2 as well as additional DNA repair abnormalities including RAD51, FANCA or FANCC, ATM, ATR, PALB2. Non-BRCA DNA repair abnormalities will be reviewed in consult with the Precision Medicine Initiative at Rutgers to confirm suitability for inclusion.

4. Have advanced cancer (metastatic, recurrent or locally advanced) and measurable disease based on RECIST 1.1.

5. Be willing to provide archived tumor tissue. Tissue from the most obtained core or excisional biopsy of a tumor lesion is preferred. 20 unstained slides are preferred but a minimum of 15 slides will be acceptable. If adequate tissue is not present the patient may consent to a newly obtained biopsy in consultation with the PI and treating physician of risks and benefits of the biopsy.

6. Have a performance status of 0 or 1 on the ECOG Performance Scale.

7. Demonstrate adequate organ function as defined in **Table 1**, all screening labs should be performed within 10 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 erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.
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** ≥30 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)	≤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
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
[a]Creatinine clearance should be calculated per institutional standard.	

8. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

9. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.5.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

5.1.3 Subject Exclusion Criteria

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

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

2. Has a diagnosis of immunodeficiency that consistently requires more than 10 mg daily of prednisone or equivalent or has resulted in life threatening episodes previously regardless of current treatment.

3. Has a known history of active TB (Bacillus Tuberculosis)

4. Has severe hypersensitivity (≥Grade 3) to pembrolizumab or any of its excipients.

5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer, or malignancies that have been inactive for three years or exceptionally indolent. Any current diagnosis of second malignancy requires approval from Principal Investigator and Sponsor.

8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline) and have no evidence of new or enlarging brain metastases. Patients who had oligometastatic disease treated with stereotactic radiation or gamma knife therapy may receive treatment 14 days after therapy as long as they are not requiring steroids. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.

9. Has active autoimmune disease which currently requires disease modifying agents that include corticosteroids > 10 mg of prednisone or equivalent or immunosuppressive drugs. Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease.

11. Has an active infection requiring systemic therapy.

12. 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.

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

14. Is pregnant or breastfeeding, or expecting to conceive 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.

15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX 40, CD137).

16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

17. Has received a live vaccine or live-attenuated vaccine within 30 days of planned start of study therapy. Administration of killed vaccines is allowed.

18. Tumors harboring non-hotspot *POLE* or *POLD1* mutations that show clear evidence of MSI will be excluded. In addition, MSI unknown will also be excluded.

19. Has a known active history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. If patient has past hepatitis B virus or resolved HBV infection (defined as the presence of hepatitis B core antibody [HBcAb) and absence of HBsAg), patients may be enrolled provided that HBV DNA is negative.

5.2 Trial Treatments

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

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background & Rationale.
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5.2.1.2 Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab and combination therapy

Adverse events (both non-serious and serious) associated with pembrolizumab exposure, including coadministration with additional compounds, may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided inTable 3 below.

Table 3. Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
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)[1]	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.
Hypophysitis	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
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	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	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
Renal Failure or Nephritis	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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
All Other Drug-Related Toxicity[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

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

[1] 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 patients should hold therapy and have imaging to assess for progression. If no progression is seen and abnormalities persist for at least 1 week then patients should be discontinued.

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

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.

5.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle. Patients may be seen separately for physical exam and treatment evaluations if needed no more than 48 hours apart, and a repeat toxicity assessment by study staff must occur on day of treatment.
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).

5.2.3 Trial Blinding/Masking

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

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications

or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.3.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.3.2 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. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the 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.

5.4 Rescue Medications & Supportive Care

5.4.1 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 and in Section 5.2.1.2, Table 3. 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 to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to the details below and Section 5.2.1 for dose modification and supportive care.

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

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** that persists greater than 3 days, administer oral corticosteroids.
For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, 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.

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

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires 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	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).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	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.**	Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
<u>Grades 3 or 4</u> 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.		

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

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)
- vasectomy of a female subject's male partner

- 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)
- male condom or 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.

5.5.3 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. If a male subject impregnates his female partner the study personnel at the site must be informed

immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.5.4 Use in Nursing Women

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

5.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be discontinued from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4.

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

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

  Note: For unconfirmed radiographic disease progression, please see Section 7.1.2.5.4 A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.5.5

- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6.0 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.6.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks or PR for 48 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial response was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.5.5.

5.7 Subject Replacement Strategy

5.8 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.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Treatment Cycles**									**End of Treatment**		
						To be repeated beyond 8 cycles						
Treatment Cycle/Title:	Screening	1	2	3	4	5	6	7	8	Discontinue[5]	Safety Follow-up[5]	Long term Follow up
Scheduling Window (Days):	-28	± 3 d	± 3d	± 3d	± 3d	± 3d	± 3d	± 3d	± 3d	At time of Discontinue	30 days post discontinue	Q12 weeks from Safety F/U visit
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Demographics and Medical History	X											
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	
Trial Treatment Administration		X	X	X	X	X	X	X	X			
Post-study anticancer therapy status											X	X
Survival Status												X
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	
Full Physical Examination	X	X	X	X	X	X	X	X	X[1]	X	X	
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X.	X.	
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	
Pregnancy Test – Urine or Serum	X											
PT/INR and aPTT[2]	X											

Trial Period:	**Treatment Cycles**									**End of Treatment**		
						To be repeated beyond 8 cycles						
Treatment Cycle/Title:	Screening	1	2	3	4	5	6	7	8	Discontinue[5]	Safety Follow-up[5]	Long term Follow up
Scheduling Window (Days):	-28	± 3 d	± 3d	± 3d	± 3d	± 3d	± 3d	± 3d	± 3d	At time of Discontinue	30 days post discontinue	Q12 weeks from Safety F/U visit
CBC with Differential[2]	X	X	X	X	X	X	X	X	X	X	X	
CMP, LDH, Uric Acid, Phos, Mg, D. Bili (if t.bili > ULN)[2]	X	X	X	X	X	X	X	X	X	X	X	
Urinalysis	X											
T3, FT4 and TSH[2]	X	X	X	X	X		X		X		X	
Tumor Imaging[3]	X				X			X		X		
Tumor Markers	X				X			X		X		
PD-L1 analysis	X[7]											
Archival or Newly Obtained Tissue Collection[4]	X		X[4]							X[4]		
Correlative Studies Blood Collection (PBMC and serum)	X		X[4]							X[4]		
Circulating Cell-free tumor DNA (Plasma)	X			X		X		X[6]		X		

[1]After 24 weeks, PE to be performed once every six weeks.
[2] All screening labs should be performed within 10 days of treatment initiation (refer to inclusion criteria #7, Table 1). CBCD, CMP to be performed prior to each treatment. See Table 5 for complete list of chemistries. After cycle 4, thyroid function tests to be performed every six weeks.

[3]Tumor imaging (CT chest/abdomen/pelvis preferred; MRI may be used in select instances if felt to be clinically appropriate by investigator) to be performed after every three cycles until commencement of eight cycles, when imaging will be performed after every four cycles (Prior to Cycles 4 and 7, then prior to Cycles 11, 15, 19, etc). Tumor imaging to be completed within 7 days of next scheduled cycle. Patients who enroll with known prior disease will require MRI brain repeat imaging every 12 weeks.
[4] Archived tumor tissue is required for enrollment of all patients (20 unstained slides preferred, minimum of 15 required). Newly obtained biopsy may occur if inadequate tissue is available. Optional biopsies will be obtained after 4 weeks and at mandatory biopsies at progression. PBMCs will be drawn at baseline, Cycle 2, and at progression. Correlative tumor and blood may be collected within a +/- 7 day window.
[5]Tumor imaging and RECIST measurements will continue past end of treatment until time of progression.
[6]Plasma samples for circulating cell-free tumor DNA will be collected every 6 weeks of treatment, whenever scheduled times coincide, at the time of PBMC or standard of care blood draws. Samples may be collected within a +/- 7 day window.
[7]Refer to the MISP Sample Handling Manual for collection and shipping instructions.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator. Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial.

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

Specifics about a trial and the trial population will be added to the consent form template at the protocol level. The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 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.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 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. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.6 Assignment of Screening Number

The coordinating site will assign each patient a screening number.

7.1.1.7 Trial Compliance (Medication/Diet/Activity/Other)

The investigator or qualified designee will review trial compliance regarding medication, contraception, scheduled assessments, other.

7.1.2 Clinical Procedures/Assessments

All clinical procedures/assessments will be performed according to the Trial Flow Chart (Section 6.0).

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart (Section 6.0) 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 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

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

7.1.2.3 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.

7.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart (Section 6.0).

7.1.2.5 Tumor Imaging and Assessment of Disease

All tumor assessments will be performed according to the Trial Flow Chart (Section 6.0). If a patient discontinues treatment for any reason other than progression, tumor assessments will be done every 12 weeks (± 14 days) for duration of study disease monitoring of 24 months unless after cycle 8 in which case they will be performed every 16 weeks (±.14 days).

7.1.2.5.1 Methods of Tumor Assessment

The preferred imaging modality is CT scan. Other modalities (MRI, PET-CT, ultrasound, endoscopy, laparoscopy, cytology, histology) may be used as adjuncts. If there is a compelling reason to do PET/CT scans or MRI (e.g. patient has allergy to iodinated contrast), the PI should

be contacted for permission.

All measurements should be taken and recorded in metric notation using a ruler or calipers. The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Combinations of clinically measurable and radiographically measurable are permissible. For example, the patient may have dermal lesions that are most accurately measured by clinical evaluation with a ruler or calipers, and lymph nodes that are most accurately measured on CT scan.

Cytology and histology may be helpful to differentiate a complete response from a partial response.

7.1.2.5.2 RECIST version 1.1

The primary set of response criteria for this trial is RECIST v1.1[30]. See Appendix 11.3 for criteria for complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

RECIST v1.1 will be modified for this trial. To account for tumor pseudo-progression that can sometimes occur with immunotherapy, it is required that RECIST PD be confirmed on a subsequent assessment performed 4-6 weeks later (see Section 7.1.2.5.4 for details).

7.1.2.5.3 Evaluation of Best Overall Response (BOR)

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

7.1.2.5.4 Confirmation of PD

iRECIST is based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by the investigator to assess tumor response and progression and make treatment decisions. When clinically stable, participants should not be discontinued until progression is confirmed per iRECIST. Allowing patients to continue treatment despite the initial radiologic progression takes into account the observation that some patients can have a transient tumor flare in the first few months after start of immunotherapy with subsequent disease response. The minimal criteria must be met to continue treatment in patients with radiological PD. Such criteria may include the following:

1. No symptoms or signs (including worsening of laboratory values) indicating disease progression

2. No decline in ECOG performance status or symptomatic clinical deterioration

3. No evidence of rapid progression of disease or of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

If a patient does not meet all of the requirements above, the patient should be removed from trial immediately for unconfirmed PD with clinical demise. Every effort should be made to document the objective progression even after discontinuation of treatment. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time (not evaluated by confirmatory scan) should be reported as "symptomatic clinical deterioration."

For patients who are clinically well and meet the criteria above, the patient may remain on treatment at the discretion of the investigator, and imaging should be repeated no less than 4 weeks but no more than 6 weeks later (4-6 weeks). If repeat imaging does not confirm PD per iRECIST (below), as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule.

If the repeat assessment shows demonstrates PD (relative to baseline or the nadir tumor burden, whichever is smaller) as per iRECIST criteria below, treatment will be discontinued. The development of confirmed PD and/or the development of symptomatic clinical deterioration constitutes confirmed progressive disease. In the case of confirmed progressive disease, study treatment should be discontinued.

<u>Confirmation of Progression per iRECIST</u>

Progression is considered confirmed, and the overall response will be confirmed progressive disease per iRECIST (iCPD), if ANY of the following occurs:

- Any of the factors that were the basis for the initial unconfirmed progressive disease (iUPD) show worsening
    - For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point
    - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
    - For new lesions, worsening is any of these:
        - An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point
        - Visible growth of new non-target lesions
        - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

7.1.2.5.5 Confirmation of PR or CR

The subject's first instance of a PR or CR should be confirmed on a repeat assessment no less than 4 weeks later (4-6 weeks); however, it is acceptable to delay the repeat assessment until the next scheduled interval imaging study (12 weeks ± 7 days) at the discretion of the treating investigator, or if the patient prefers not to have an extra CT scan for this purpose. A biopsy of any residual lesion may be used to confirm a CR at the discretion of the treating investigators.

7.1.2.5.6 Determining the date of progression or response

For all patients who experience disease progression on study, the date noted for disease progression is the time of the scan when it was originally determined, and not the later date of the confirmatory scan.

For all patients who experience complete or partial response on study, the date noted for disease response is the time of the scan when it was originally determined, and not the later date of the confirmatory scan.

7.1.2.6 Tumor Tissue Collection and Correlative Studies Blood Sampling

7.1.2.6.1 Genomics/Sequencing:

In collaboration with Shridar Ganesan, MD, PhD, Director of the Functional Genomics Shared Resource, we propose to perform genomic sequencing analysis of pre-treatment biopsy specimens, and specimens, if available at progression. Next generation DNA sequencing of exomes will commence, and RNA sequencing will evaluate selected gene expression. Tumor samples, and where available, matched normal tissue or peripheral blood lymphocytes, will be assayed by whole exome sequencing to determine the set and amount of genomic variants present. In addition to standard variant analysis, we will also implement algorithm such as NASeek to look for presence of immunogenic neo- antigens. For cancer specimens from *BRCA1/2* mutation carriers, where there is likely a high burden of genomic rearrangements, we will also look for presence of gene fusion using the NUGEN SPET approach. *BRCA1/2* mutant specimens will also be analyzed by CGH to assay for specific patterns of gross genomic alterations (allelic imbalance, large scale transitions, etc). By this approach we hope to determine if the nature and/or pattern of genomic changes present, as well as the presence of potential immunogenic neoantigens, will correlate with response to pembrolizumab.

7.1.2.6.2 Tissue/Blood:

In collaboration with Joshua Vieth, PhD, Managing Director of the Immune Monitoring Core, we propose to assess the frequencies, activation/differentiation, functionality, and co-inhibitory molecule expression of immune cell populations in peripheral blood and tumor, before and after treatment with systemic pembrolizumab. We will measure the following immunologic parameters that may be indicative of responsiveness to treatment using advanced multi-parameter flow cytometric approaches and immunohistochemistry:

1) The frequencies of T and NK cell subsets will be analyzed by flow cytometry using antibodies for CD3, CD4, CD8, CD11b, CD11c, CD14, CD16, CD19, CD25, CD56,

and FoxP3. Special attention will be paid to CD8 T cell:CD4 Treg and NK:CD4 Treg cell ratios. We hypothesize that increased effector (CD8 and NK cell subsets versus Treg subsets) following pembrolizumab administration will be a biomarker positively associated with responsiveness to treatment and other major prognostic indicators for survival.

2) Activation and differentiation of T cell subsets will be analyzed by flow cytometry using additional antibodies for HLA- DR, CD38, CD57, CCR7, CD45RA and CD45RO. We hypothesize that activation and differentiation toward effector and memory responses versus exhaustion following pembrolizumab administration will be a biomarker positively associated with responsiveness to treatment and other major prognostic indicators for survival.

3) Immune cell functionality before and after pembrolizumab will be analyzed by assessing intracellular cytokines and effector molecules in serum via Luminex Multiplexe Analysis against a panel of 48 human cytokines/chemokines. We hypothesize that increased expression of immune-stimulatory cytokines and effector molecules (versus inhibitory cytokines) following pembrolizumab administration will be a biomarker positively associated with responsiveness to treatment and other major prognostic indicators for survival.

4) Expression of the T cell inhibitory molecules PD-1, TIM-3, 2B4, Lag-3, and CTLA-4 on CD8+ T cells and their ligands (PD-L2, Gal9, CD48, CD80, CD86) will be evaluated on antigen presenting cells (MHC-II+) and tumor cells (CD45-) using respective monoclonal antibodies to these molecules and analyzed by flow cytometry. PD-L1 will be assessed from formalin-fixed paraffin-embedded by immunohistochemistry via Qualtek/Dako assay (Carpinteria, CA). We hypothesize that reduced expression of other T cell co-inhibitory molecules will be seen following pembrolizumab administration.

If samples are available, some or all of the following analyses may also be performed: nanostring, single cell RNA-sequencing, exosome sequencing, multispecial immunostain, proteomics and grafting of fresh tissue into experimental animals using blood and tumor tissue. The experimental protocol will follow laboratory SOPs and published procedures.

Leftover research specimens will be stored for future research for subjects who have provided permission.

7.1.2.6.3 Plasma cell-free DNA:

Samples of plasma will be collected prior to treatment, then every 6 weeks. Plasma will be analyzed for quantitative presence of circulating cell-free tumor DNA (ctDNA). This will be done either by custom quantitative PCR assays for presence of known tumor-associated mutations identified on tumor sequencing, or using panel sequencing platforms such as Guardant360. Correlation of level of ctDNA with clinical and radiographic response will be determined. The aim is to determine if ctDNA can be a sensitive early predictor of response and resistance to pembrolizumab, and whether it can differentiate true progression from

radiographic pseudoprogression.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

- Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

- Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5. Laboratory Tests

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

† Perform on women of childbearing potential only.
‡ If considered standard of care in your region.

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

7.1.4 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 12 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening Period

During the screening period (28 days prior to treatment initiation), the following data will be collected and assessed for each patient: informed consent, whether the patient meets the inclusion and exclusion criteria of the trial, demographics and medical history, a review of prior or concomitant medications, a post-study anticancer therapy status, the patient's survival status, a review of adverse events, a full physical examination of the patient, vital signs and weight, ECOG performance status, a pregnancy test assessing urine or serum β-HCG, prothrombin time (PT)/international normalized ratio (INR) and activated partial thromboplastin time (aPTT), a complete blood count (CBC) with differential, a comprehensive metabolic panel (CMP), a urinalysis, a test of triiodothyronine (T3), free throxine (FT4), and thyroid stimulating hormone (TSH), tumor imaging, an MRI of the brain, and archival of newly obtained tissue (preferably 20 unstained slides but minimum of 10; exceptions may be allowed at discretion of PI in conjunction with study sponsor; newly obtained biopsy may commence if inadequate tissue). Peripheral blood mononuclear cells (PBMCs) will be drawn at baseline, after 4 weeks of therapy, and at progression(s). Plasma will be drawn at baseline, after 4 weeks of therapy, then aligning with blood draws every six weeks. Correlative tumor and blood may be collected with a +/- 7 day window.

7.1.5.2 Treatment Period

The treatment period consists of 8 treatment cycles, which repeat from cycle 5 if treatment continues beyond cycle 8. At each cycle throughout the treatment period, the following are to be performed and/or assessed for each patient: a review of prior and concomitant medications,

administration of trial treatment, survival status, a review of adverse events, a full physical examination (after 24 weeks, a physical exam will be performed once every 6 weeks), the patient's vital signs and weight, the patient's ECOG performance status, CBC with differential, and CMP (both to be performed prior to each treatment). T3, FT4, and TSH will be measured at cycle 1 through cycle 4 (after cycle 4, these thyroid function tests are to be performed every 6 weeks). Tumor imaging **(**CT chest/abdomen/pelvis preferred; MRI may be used in select instances if felt to be clinically appropriate by investigator) is to be performed after every 3 cycles until commencement of 8 cycles, after which time imaging will be performed after every 4 cycles. An MRI of the brain will be required every 12 weeks for patients who enroll with known prior disease. Optional biopsies will be obtained after 4 weeks and at mandatory biopsies at progression**.** PBMCs will be drawn after 4 weeks of therapy, and at progression. Correlative tumor and blood may be collected with a +/- 7 day window.

7.1.5.3 Post-Treatment Visits

Visits following trial treatment will assess the following conditions for each patient at the time of treatment discontinuation: a review of prior and concomitant medications, survival status, a review of adverse events, a full physical examination, vital signs and weight, ECOG performance status, CBC with differential, and CMP.

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase. During the safety follow-up, the following conditions will be assessed for each patient: a review of prior and concomitant medications, survival status, a review of adverse events, a full physical examination, vital signs and weight, ECOG performance status, CBC with differential, CMP, and analysis of T3, FT4, and TSH.

7.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (72 ± 7 days) by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.5.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression. During the follow-up visits, the following conditions will be assessed for each patient: a review of prior and concomitant medications, survival status, a review of adverse events, a full physical examination, vital signs and weight, ECOG performance status, CBC with differential, and CMP.

7.1.5.4.1 Survival Follow-up

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

7.1.5.5 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

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

OR

    - Had SD, PR or CR and stopped pembrolizumab treatment after 12 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.2

- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.

- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.5.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.

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

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

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Trial Flow Chart.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

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

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

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

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-**661-6229**)

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

7.2.3.1 Serious Adverse Events

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

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose of pembrolizumab;
- Is another important medical event

    - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious.

    - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Refer to Table 6 for additional details regarding each of the above criteria.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported to the Rutgers Cancer Institute of New Jersey (Sponsor) Office of Human Research Services via OnCore within 24 hours of site notification. The completed SAE report (signed by the Investigator) must be sent to the Rutgers CINJ OHRS QA department. Rutgers CINJ will report within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs. **SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229**

The Sponsor will forward a copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

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

7.2.3.2 Events of Clinical Interest

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

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 6. Evaluating Adverse Events

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

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	

Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE

		RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL ANALYSIS PLAN

Sample size justification. To address the primary objective, we have calculated the 95% confidence intervals (CI's) to assess precision of expected response rate of pembrolizumab in treating patients with specified mutant tumors (e.g., *POLE*, *POLD1*, and *BRCA1* and *2*). Responses to single agent PD-1 inhibition are not seen in the majority of patients; available published data in pretreated triple negative breast, lung, ovarian tumors, histologies likely to be enrolled to this trial, have ranged from 11-19% in small studies[16,31]. Based on the data in MSI high tumors by Le et al[3], responses can be expected to rise significantly in tumors with genomic instability, with the lowest rate of 40% in their published series. We thus assume that the response rate of pembrolizumab in our trial, treating patients with *POLE* (or *POLD1*) and/or *BRCA1/2* mutant tumors, will similarly be at about 40%. For n=23 patients for each type of tumors ([*POLE* or *POLD1]* and [*BRCA1* and *2*]), we expect to yield a 95% CI of (0.20, 0.60), within the 40%+/- 20% range (Table 7). This confidence interval is above the response rate of using standard therapy in treating unselected cancer patients suggesting significant improvement of pembrolizumab over standard therapies.

To address the secondary objective, we used the same 95% CI calculations (Table 7), assuming no loss of follow-up, to assess the precision of expected PFS rate of pembrolizumab at 6 months in treating patients with specified mutant. Based on the data in Le et al[3] (Panel C Fig 2), the PFS rate at 6 months was at least 35% while the immune-related PFS at 20 weeks was at least 67%. Assuming the 6-month PFS rates in our study will be similarly at least 35%. With n=23 patients for each type of tumors ([POLE or POLD1] and [BRCA1 and 2]), we expect to yield a 95% CI of (0.15,0.55) for PSF of 35% and (0.46,0.84) for PSF of 65%, both within the +/- 20% range.

Table 7. 95% Confidence Intervals for Response and/or PFS* Rate Based on Estimated Rate (P) and Sample Size (n) for a Specific Mutant Tumor.

P							
n	**0.25**	**0.30**	**0.35**	**0.40**	**0.50**	**0.65**	**0.75**
20	(0.06,0.44)	(0.10,0.50)	(0.14,0.56)	(0.18,0.62)	(0.28,0.72)	(0.44,0.86)	(0.56,0.94)
23	(0.07,0.43)	(0.11,0.49)	(0.15,0.55)	(0.20,0.60)	(0.29,0.71)	(0.46,0.84)	(0.57,0.93)
25	(0.08,0.42)	(0.12,0.48)	(0.16,0.54)	(0.20,0.60)	(0.30,0.70)	(0.46,0.82)	(0.58,0.92)
26	(0.08,0.42)	(0.12,0.48)	(0.16,0.54)	(0.21,0.59)	(0.30,0.70)	(0.48,0.82)	(0.59,0.91)

28	(0.09,0.4)	(0.13,0.47)	(0.17,0.53)	(0.21,0.59)	(0.31,0.69)	(0.47,0.83)	(0.59,0.91)
30	(0.10,0.40)	(0.13,0.47)	(0.18,0.52)	(0.22,0.58)	(0.32,0.68)	(0.48,0.82)	(0.60,0.90)
***CI calculations for PFS assumed no loss of follow-up.**							

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.
Clinical Supplies will be provided by Merck as summarized in Table 8.

Table 8. Product Descriptions

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

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

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

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

Subjects' identities, names or any identifying characteristics will not be disseminated in publications, nor made public. The data forms will be de-identified, meaning that the patients identifying information will be matched to the research number, in a separate file.

10.2 Compliance with Financial Disclosure Requirements

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

The Rutgers University's Office of Research and Economic Development Integrity Office (RIO) reviews and manages research-related conflicts of interest. The Principal Investigator and Sub-Investigators must report conflicts of interest annually and within 30 days of a change in status, and when applicable, must have a documented management plan that is developed in conjunction with the Rutgers RIO and approved by the IRB/IEC.

Dr. Salma Jabbour, a sub-investigator on this study, has a financial relationship with Merck & Co., the company that provides the study drug, Pembrolizumab (KEYTRUDA). Please feel free to ask any further questions you might have about this matter.

10.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical

Practice (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.
All revisions to the protocol must be discussed with, and be prepared by, Rutgers Cancer Institute of New Jersey. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.
If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB for review and approval/favorable opinion
- Merck Sharp Dohme Corp
- Regulatory Authority(ies), if required by local regulations

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to Rutgers Cancer Institute of New Jersey Office of Human Research Services.
If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form must be revised and submitted to the IRB for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

If the revision is done via an administrative letter, investigators must inform their IRB.
The investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator by the Sponsor.

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.
Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation

and worksheets/case report forms. The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or subject files.

ICH Good Clinical Practice guidelines recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed. The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial.

Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

10.4 Issues with Minors

For minors, according to local legislation, one or both parents or a legally acceptable representative must be informed of the study procedures and must sign the informed consent form approved for the study prior to clinical study participation. The explicit wish of a minor, who is capable of forming an opinion and assessing this information to refuse participation in,

or to be withdrawn from, the clinical study at any time should be considered by the investigator. Minors who are judged to be of an age of reason must also give their written assent.

10.5 Compliance with Trial Registration and Results Posting Requirements

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

10.6 Quality Management System

10.6.1 Data Safety Monitoring Board

The Rutgers Cancer Institute Human Research Oversight Committee is responsible for quarterly data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor- investigator. The HROC in concert with the Quality Assurance Monitoring Team oversees the conduct of Rutgers CINJ cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

10.6.2 Monitoring

The Rutgers Cancer Institute of New Jersey's (Rutgers CINJ) Quality Assurance Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the Rutgers CINJ Data and Safety Monitoring Plan, the Quality Assurance Monitoring Team will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1 – 3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

The Rutgers CINJ Human Research Oversight Committee (HROC) will perform annual reviews on findings from the quality Assurance Monitoring Team visit and additional safety and toxicity data submitted by the Principal Investigator.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon

request of Rutgers CINJ leadership, the Scientific Review Committee, the Human Research Oversight Committee (HROC), the sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

10.7 Registration

This protocol is available at the Rutgers Cancer Institute of New Jersey (CINJ)/Robert Wood Johnson University Hospital (RWJUH) in New Brunswick and NYU Langone Health.

A copy of the institution's IRB-approved informed consent document and written justification for any changes made to the informed consent for this protocol will be on file at Rutgers Cancer Institute of New Jersey's Office of Human Research Services (OHRS) before any participating institutions may enter patients.

Sites will register and enroll patients through OnCore® the Clinical Trials Management System for this study. Registration/Enrollment process:

- Registration: Any subject that has signed the consent will be entered into OnCore®.

- Enrollment: Once eligibility has been confirmed the subject will be enrolled (indicated as "On Study") through OnCore®. The completed, signed and dated eligibility checklist and supporting source documents (de-identified for participating centers) will be uploaded into OnCore®.

- **Patients will not start protocol treatment prior to enrollment.**

Trial treatment should begin within 10 days of registration or as close as possible to the date on which treatment is allocated/assigned.

10.8 Data Management

10.8.1 Study Documentation

Study documentation includes but is not limited to source documents, case report forms, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder", which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects" diaries

or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

10.8.2 Case Report Forms (CRFs)

The electronic CRF stored in Oncore® will be the primary data collection document for the study. The CRFs will be updated in a timely manner following acquisition of new source data. Only the key personnel delegated on the delegation of authority log are permitted to make entries, changes, or corrections in the CRF.

An audit trail will be maintained automatically by the electronic CRF management system. All users of this system will complete user training, as required or appropriate per regulations.

10.8.3 Data Management Procedures and Data Verification

Users of the electronic CRF will have access based on their specific roles in the protocol. Completeness of entered data will be checked automatically by the eCRF system, and users will be alerted to the presence of data inconsistencies. Additionally, the data manager and project manager will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

10.8.4 Study Closure

Following completion of the studies, the PI will be responsible for ensuring the following activities:
- Data clarification and/or resolution- Accounting, reconciliation, and destruction/return of used and unused study drugs - Review of site study records for completeness- Shipment of all remaining laboratory samples to the designated laboratories

10.9 Data Safety Monitoring Plan

The Rutgers Cancer Institute of New Jersey (RCINJ) has established a Data and Safety Monitoring Plan (DSMP) for the conduct of clinical trials in patients with cancer. The DSMP is enacted in part by the Human Research Oversight Committee (HROC). HROC is an established group within the Office of Human Research Services, and it is responsible for oversight of all therapeutic and non-therapeutic clinical trials conducted by RCINJ and it meets

regularly to review deviations. HROC will serve as the Data Safety Monitoring Board (DSMB) for this clinical trial. All unexpected and serious adverse events will be reported to the DSMB (as well as each institutional IRB and study sponsor) according to the reporting requirements detailed in Section 7.2 of this protocol and per the Rutgers University DSMP.

11.0 APPENDICES

11.1 ECOG Performance Status

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

11.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting.

11.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1 will be used in this study for assessment of tumor response, with one modification. To account for tumor pseudo-progression that can sometimes occur with immunotherapy, it is required that RECIST PD be confirmed on a repeat scan before a patient is removed from study (see Section 7.1.2.5 for details).

For full details, refer to the manuscript as published in the *European Journal of Cancer*[30]:

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

11.3.1 Measurable Disease

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

NOTE: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

NOTE: Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

11.3.2 Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, and lymphangitis cutis/pneumonitis, are considered as non-measurable. Non-measurable also includes lesions that are < 20 mm by chest x-ray.
NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

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

11.3.3 Target Lesions

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

diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

11.3.4 Non-target Lesions

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

11.3.5 Evaluation of Target Lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. Note: the appearance of one or more unequivocal new lesions is also considered progression.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.3.6 Evaluation of Non-Target Lesions

Complete Response (CR)	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis)
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.
Non-CR/Non-PD	Persistence of one or more non-target lesion(s).

11.3.7 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it a) increases in size to ≥ 15 mm in the short axis, or b) there is new pathological confirmation that it is disease (regardless of size).

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

11.3.8 Evaluation of Best Overall Response

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

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non- PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

11.4 Collection, Processing and Shipping of Blood and Tumor Tissue and Correlative Science*

Samples will be processed at CINJ according to the **051709 Correlative Research Laboratory Manual**.

Participating centers (non-CINJ sites) will not be processing specimens locally. All samples will be shipped and processed at CINJ according to the **051709 Correlative Research Laboratory Manual**.

11.4.1 Tissue Collection

11.4.1.1 Analysis of PD ligand expression (Qualtek analysis)

Formalin-fixed tissue (archival tissue collected at baseline, if adequate tissue is not present the patient may consent to a newly obtained biopsy) will be utilized for analysis of PD-L1. PD-L1 will be detected using the Dako IHC assay for PD-L1 developed by Qualtek (Santa Barbara, CA). Kits must be requested from Qualtek.

11.4.1.2 Tissue for Correlative Research

Tissue will be collected according to the 051709 Correlative Research Laboratory Manual. Newly obtained biopsy may occur if inadequate tissue is available. An optional biopsy will be obtained after 4 weeks and a mandatory biopsy at progression.

11.4.1.3 Tissue Collection Priority

The priority for tissue collection shall be as follows when tissue is available:

1) Diagnostic (non-research; sent to pathology)
2) Collection in HTS-FRS Media for processing and Genomic Analysis
3) Placed in formalin for IHC
4) Placed in HTS-FRS for TIL isolation and flow cytometry

11.4.2 Blood Collection

Blood samples will be collected for correlative research studies (PBMC and serum isolation) at baseline, C2 (after 4 weeks of treatment) and at discontinuation of treatment.

Blood samples will be collected for circulating cell-free tumor DNA at baseline, every 6 weeks during treatment and at discontinuation of treatment.

All blood samples will be collected according to the 051709 Correlative Research Laboratory Manual.

11.4.3 Safety Precautions

Universal precautions (*i.e.*, a method of infection control in which all human blood and body fluids are treated as if they are infectious for Hepatitis viruses, Human

Immunodeficiency virus (HIV), and other known and unknown infectious agents) will be utilized when handling all unfixed cells and tissues.

a. Hepatitis B and Hepatitis C viruses may be transmitted through blood and other body fluids, and are associated with acute hepatitis, chronic liver disease, and hepatocellular carcinoma in humans. The probability of seroconversion after needlestick exposure is estimated at 7%. Untreated virus can persist for up to one week at room temperature. All staff who work with human tissue must provide evidence of Hepatitis B vaccination.
b. HIV is a retrovirus that causes severe immunodeficiency. Infection increases the risk of developing malignancies, infection by opportunistic organisms, and death. The probability of seroconversion after needlestick exposure is estimated at 0.5%. Infectivity of untreated virus persists for up to one week at room temperature.
c. Other potentially infectious agents, both known and unknown, pose hazards to those working with human tissue. Included are tuberculosis, HTLV1, Coccidiomycosis, Creutzfeldt-Jacob disease, among others.
d. Individual institutional and OSHA guidelines must be followed when handling human cells and tissues, and referred to for additional information on blood-borne pathogens, laboratory safety, chemical safety, and biohazardous waste disposal. Briefly:
    i. Personal protective equipment (PPE) must be used at all times while working with human tissue. These include disposable latex or nitrile gloves, face shield, protective splash-resistant laboratory coat (disposable preferred), and covered protective shoes.
    ii. Gloves should be immediately removed and replaced in the event that they become torn or perforated. Gloves must be removed prior to leaving the work area, and disposed of in an appropriate waste disposal container. Hands must be washed in a "clean" sink after removal of gloves.
    iii. Face shields, goggles and masks should be worn whenever a potential for exposure to splashes, spray, splatter, droplets, aerosols of blood or tissue fluid, or other potentially infectious materials may be generated, and if there is a potential for eye, nose or mouth contamination. They should be worn at all times while handling tissue in the for processing.
    iv. Protective lab coats, preferably disposable types, must be donned while working with tissue. Contaminated clothing must be removed prior to leaving the work area, and appropriately laundered or discarded, as per individual institutional guidelines.
    v. All waste must be disposed of prior to leaving the work area. Biohazardous sharps must be properly disposed of in an approved "sharps" container. All other non-sharp waste must be disposed of in an approved orange or red biohazardous waste disposal bag.
    vi. After completion of work with human tissue, all work surfaces must be disinfected with a product that has been demonstrated to be effective against bacteria, viruses, pseudomonas, tuberculosis and fungi. Product literature should be referred to for appropriate use.

Any injuries or exposure to human tissue or potentially infectious biologic agents must be.

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