TITLE: Phase II study of pembrolizumab and ipilimumab following initial anti-PD1/L1 antibody

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This study is being conducted by institutional members of the Personalized Cancer Care Consortium (PCCC), as well as additional sites.

1.0 TRIAL SUMMARY

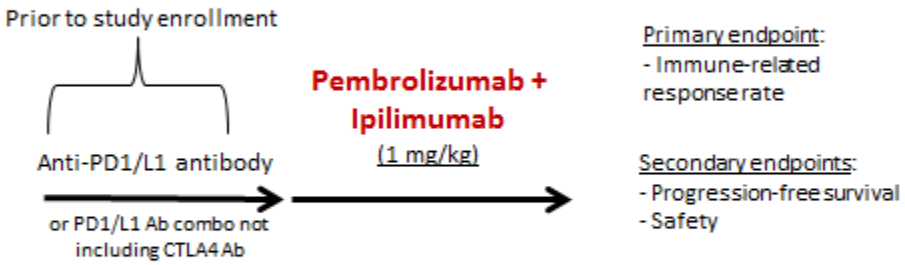
Abbreviated Title	Pembrolizumab and ipilimumab after anti-PD1/L1 antibody
Trial Phase	II
Clinical Indication	Melanoma
Trial Type	Therapeutic
Type of control	Historical
Route of administration	IV
Trial Blinding	None
Treatment Groups	Progression on anti-PD1/L1 antibody (or combination not containing anti-CTLA4),
Number of trial subjects	70
Estimated enrollment period	2 years
Estimated duration of trial	4 years
Duration of Participation	2 years

2.0 TRIAL DESIGN

2.1 Trial Design

Phase II study evaluating the benefit of the combination of anti-PD1 (pembrolizumab) and anti-CTLA4 (ipilimumab) antibodies in advanced melanoma. The study will determine the response rate of the combination and evaluate other clinical parameters such as progression-free survival and safety of the combination following anti-PD1/L1 antibody. The study will also provide the opportunity to investigate blood or tumor based factors that may predict response to anti-PD1 antibody in combination with anti-CTLA4.

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To determine the irRECIST* response rate of pembrolizumab with ipilimumab following initial progression to anti-PD1/L1 antibody (or combination not containing anti-CTLA4) in subjects with advanced melanoma.

*For review of irRECIST see Appendix – Section 13.3

Hypothesis: The combination of pembrolizumab and ipilimumab will induce tumor responses in patients who have been previously treated with anti-PD1/L1 antibody

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To summarize the progression-free survival (RECIST v1.1 and irRC) of the combination following prior treatment with anti-PD1/L1 antibody

Hypothesis: The combination will lengthen progression-free survival relative to historical data for ipilimumab alone.

(2) **Objective**: To assess the safety of the combination following prior treatment with anti-PD1/L1 antibody

Hypothesis: The combination will demonstrate a manageable safety profile in patients who have been previously treated with anti-PD1/L1 antibody

3.3 Exploratory Objective

(1) **Objective:** To evaluate changes in the tumor microenvironment and other biospecimens before and after adding ipilimumab to pembrolizumab.

Hypothesis: Changes in the tumor microenvironment will be observed that may correlate with a more favorable response to combination immunotherapy.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on PEMBROLIZUMAB.

4.1.1 Pharmaceutical(s) and Therapeutic Background

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

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

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

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

Ipilimumab (Yervoy®) is an immunoregulatory agent that blocks CTLA -4 to promote anti-tumor immunity. In a Phase III study of 676 subjects with unresectable or metastatic melanoma whose disease had progressed while receiving prior therapy for metastatic disease, ipilimumab administered at 3 mg/kg Q3W for up to 4 doses with or without a gp100 peptide vaccine was compared with gp100 alone[1]. The median overall survival was 10.1 months and 10.0 months for the ipilimumab alone and ipilimumab plus gp100 arms respectively, while the median overall survival was 6.4 months for the gp100 alone arm ($p<0.001$). An objective response rate of 10.9% was observed in ipilimumab alone arm, while the ipilimumab plus gp100 arm and gp100 alone arm had lower response rates, 5.7% and 1.5%, respectively. The median duration of response was 11.5 months in the ipilimumab plus gp100 arm, and not reached in the ipilimumab alone arm with a median follow -up period of 27.8 months. The median time to progression was 2.86 months for the ipilimumab alone arm and 2.76 months for the other two arms. In the ipilimumab alone arm, 97% of the subjects experienced an AE and grade 3/4 AEs occurred in 46% of the subjects. The most common AEs related to ipilimumab were immune-related events, which occurred in approximately 60% of the subjects treated with ipilimumab and 32% of the subjects treated with gp100. The frequency of grade 3/4 immune-related AEs was 15%. The immune-related AEs most often affected the skin and gastrointestinal tract, including pruritus, rash, vitiligo, diarrhea, and colitis.

Based on the above Phase III trial, ipilimumab was approved by the FDA for treatment of subjects with unresectable or metastatic melanoma and by the European Commission for treatment of subjects with previously-treated advanced melanoma. The recommended regimen is 3 mg/kg administered Q3W for a total of four doses.

Refer to the approved labeling for detailed background information on ipilimumab.

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

Melanoma (MEL) is the sixth most common malignancy in men and the seventh most common malignancy in women. The incidence of MEL is increasing worldwide, with a growing fraction of subjects with advanced disease for which prognosis remains poor. The median survival for subjects with metastatic MEL has been under 1 year. The 5-year survival rate of subjects with visceral involvement is under 10%. Treatment options for metastatic MEL have been limited to chemotherapeutic agents such as dacarbazine and high-dose interleukin-2 immunotherapy in a small percentage of subjects. In the past few years there has been steady progress in the development of targeted therapy and immunotherapy for metastatic MEL. Vemurafenib, a BRAF inhibitor, was approved in 2011 for the treatment of subjects with unresectable or metastatic MEL with BRAFV600E mutation (only about 50% of all melanoma subjects). IPI, an anti-CTLA4 monoclonal antibody, was also approved in 2011 for the treatment of subjects with unresectable or metastatic MEL. More recently, a second BRAF inhibitor (dabrafenib) and a first MEK inhibitor (trametinib) were approved for treatment of metastatic melanoma; the median survival for subjects treated with these drugs in pivotal trials was still less than 6 months. In spite of these newly approved therapies, overall outlook for subjects with metastatic MEL remains dismal and the development of new effective therapy is still needed.

Pembrolizumab has shown clinical activity in several cancers with response rates and disease control rates previously unknown to immunotherapy. While impressive as a single agent in melanoma, with a response rate of 38% demonstrated in the phase I experience[2], those patients that do not achieve response continue to have poor outcomes. In this context, there continues to be an area of unmet need in this non-responding melanoma patient population.

The T cell-inflamed tumor microenvironment may be a predictive biomarker for response to multiple immunotherapies including therapeutic vaccines, cytokines and anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibodies[3-8]. Tom Gajewski, MD, PhD at the University of Chicago has analyzed the tumor microenvironment in patients with melanoma and described that approximately 40% of cases show evidence of spontaneous priming of anti-tumor T cells leading to migration of CD8+ effector T cells into tumor sites. This phenotype has been designated the T cell-inflamed tumor microenvironment and is characterized by expression of T cell markers, chemokines for T cell recruitment, and transcripts indicative of type I interferon (IFN) signaling[4,5,7]. A gene expression signature that captures this phenotype has been developed including a group of 13 immune related genes and preliminary analysis suggests that this signature very closely predicts which tumors respond to anti-PD1 antibody treatment (data unpublished).

In addition to response prediction, this gene signature is also potentially useful in identifying those that will not respond. Patients harboring non-inflamed tumors therefore require

treatment strategies which may modulate the immune response to facilitate the influx of TIL and conversion from a non-inflamed to an inflamed tumor. Several tumor localized treatments have been suggested in preclinical models to facilitate such a shift including but not limited to radiation[9], immune agonists such as those directed against the STING pathway[10] and oncolytic tumor viruses[11]. While locally effective, these treatments are impractical in patients with wide spread metastatic disease.

As opposed to local treatment, a systemic treatment that can drive a T cell response into the tumors may be CTLA4 blocking antibodies. Phase III clinical trials evaluating the anti-CTLA4 antibodies tremelimumab and ipilimumab have documented single agent response rates on the order of 10%[1,12]. For both tremelimumab and ipilimumab, pre- and post-treatment biopsies have demonstrated a significant increase in CD8+ TIL in tumor lesions after anti-CTLA4 antibody treatment[13,14]. In some studies[15], there is also indication of modulation with the intra-tumoral *FoxP3*+ regulatory T cell population; however this has not been seen uniformly.

Preclinical investigations have supported the concept of combined blockade of PD-1 and CTLA-4 achieving more pronounced anti-tumor activity than blockade of either pathway alone[16]. Specifically, combined PD-1 and CTLA-4 blockade increases effector T-cell infiltration of tumors, overall intra-tumoral IFN-ɤ levels, as well as the frequency of CD8+ T cells that secrete both IFN-ɤ and TNF-α. These observations were the basis for the phase I study of the ipilimumab with the anti-PD1 antibody nivolumab. In this study, an increased response rate relative to historical controls for either agent was observed and patients who had previously progressed on ipilimumab alone were noted to have responses[17]. No patients were reported who had progressed on anti-PD1 antibody alone; however it was specifically noted that tumors from patients that tested negative by immunohistochemistry for PD-L1 (possibly a surrogate for the non-inflamed tumor phenotype) obtained responses at only a slightly lower response rate compared to those who tested positive for PD-L1[18]. This suggests that the addition of anti-CTLA4 to anti-PD1 therapy may be able to overcome the non-inflamed tumor phenotype.

4.2.2 Rationale for Dose Selection/Regimen/Modification

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

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB).
Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is

durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

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

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

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

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

In this study, pembrolizumab will be evaluated in combination with 1 mg/kg of ipilimumab. The dose schedule of ipilimumab will follow the approved drug label of Q3W. In a Phase 1 study, updated by Sznol et al at ESMO 2013, efficacy of nivolumab 3 mg/kg + ipilimumab 1 mg/kg ([ORR 40% (95% CI 16-68%) and Aggregate Clinical Benefit Rate (defined as CR + PR + uCR+ uPR+ irPR+ SD $\geq$24 week + irSD $\geq$24 week) 73%] appeared to have similar efficacy as nivolumab 1 mg/kg + ipilimumab 3 mg/kg [ORR 53% (95% CI 28-77%) and ACBR 65%]. In addition, the combination of nivolumab 3 mg/kg + ipilimumab 1 mg/kg showed lower

rate of drug related Grade 3-4 AEs compared with nivolumab 1 mg/kg + ipilimumab 3 mg/kg (44% versus 65%, respectively) [66].

The combination of pembrolizumab with ipilimumab was evaluated in a phase I study presented at ASCO 2015 (KEYNOTE-029)[19]. In this study pembrolizumab was administered at 2 mg/kg every 3 weeks in conjunction with 1 mg/kg of ipilimumab every 3 weeks x 4 doses. Of 19 patients evaluable for dose-limiting toxicity (DLT), 6 patients experienced ≥ 1 DLT. All of these were grade 3 in severity except for one episode of grade 4 lipase elevation. The only event that occurred in ≥1 patient was ALT elevation. Two patients experienced 2 DLTs each including elevated pancreatic enzymes and hyperthyroidism in one patient and increased ALT and AST in the other patient. All DLTs resolved with exception of elevated lipase (on-going at time of presentation). There were no treatment-related deaths and adverse events of any attribution led to discontinuation of both pembrolizumab and ipilimumab in 3 (13.6%) of patients and ipilimumab only in 5 (18.2%) of patients. At least 1 grade 3-4 treatment related adverse event was observed in 54.5% of patients. The most common AEs in decreased order of frequency were: fatigue (27.3%), diarrhea, liver enzyme elevations, colitis, lipase, nausea, abdominal pain, hypothyroidism, rash, hypophysitis, puritis, pyrexia, vitiligo, hyperthyroidism, hyponatremia, elevated pancreatic enzymes, pneumonitis, syncope and uveitis (4.5%). At the time of data cut off, 17/22 patients had at least one disease assessment evaluable for response. Partial response per RECSITv1.1 by investigator review was reported for 3 patients.

The overall dosing strategy is intended to emphasize dose intensity with pembrolizumab rather than ipilimumab, due to the known safety and efficacy profile of both agents.

4.2.3 Rationale for Endpoints

Here we propose a clinical trial testing the hypothesis that the addition of anti-CTLA4 antibody (ipilimumab) to pembrolizumab will generate clinical responses in patients who have had initial progressive disease, or only disease stabilization, to anti-PD1/L1 antibody alone. Preclinical and prior clinical data support this hypothesis and from a practical standpoint, deferral of the toxicities associated with anti-CTLA4 in those patients who do not require exposure to anti-CTLA4 is particularly attractive.

We propose to continue pembrolizumab beyond progression on an anti-PD1/L1 antibody as we believe that single agent ipilimumab will overcome the non-inflamed tumor microenvironment in only a small percentage of patients while the combination of ipilimumab and pembrolizumab may induce responses in as many as 40% of patients based on prior studies. The results of this study may suggest that continuous PD-1 blockade is required to optimize therapy for advanced melanoma and, through our correlative analysis, we hope to propose a model by which we can suggest which patients would benefit from upfront pembrolizumab alone or pembrolizumab in combination with anti-CTLA4 antibody.

4.2.3.1 Efficacy Endpoints

The primary efficacy objective of this trial is to evaluate the anti-tumor activity of the combination of pembrolizumab and ipilimumab in subjects with advanced melanoma who

have previously been treated with an anti-PD1/L1 antibody. Response rates per irRECIST will be evaluated.

irRECIST as assessed by the investigator will be used as the primary response rate efficacy endpoint. irRECIST will also be used by the local site to determine eligibility and make treatment decisions. The primary efficacy endpoint is response rate based on irRECIST. Secondary endpoints are listed in section 3.2.

Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses which may be functionally anergic. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST criteria may not provide a complete response assessment of immunotherapeutic agents such as pembrolizumab therefore the irRECIST have been developed to encompass this.

Broadly, irRECIST includes the concept of treatment beyond progression, if radiologic imaging shows PD, tumor assessment should be repeated $\geq$ 4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms progressive disease, subjects will be discontinued from trial therapy. In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions as well as non-target lesions.

In subjects who have initial evidence of radiological PD, it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained a minimum of 4 weeks later. This decision should be based on the clinical judgment of the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

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

When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease.

4.2.3.2 Biomarker Research

Our group has previously described the T cell-inflamed tumor microenvironment as a potential predictive biomarker for response to multiple immunotherapies including therapeutic vaccines, anti-CTLA-4, and anti-PD-1/PD-L1 antibodies[3-8,20]. Our analysis of the tumor microenvironment in patients with melanoma suggests that approximately 35-50% of cases show evidence of spontaneous priming of anti-tumor T cells leading to migration of CD8+ effector T cells into tumor sites. This phenotype has been designated the T cell-inflamed tumor microenvironment and is characterized by expression of T cell markers, chemokines for T cell recruitment, and transcripts indicative of type I IFN signaling[4,5,7].

In addition to response prediction, this gene signature is also potentially useful in identifying those that will not respond. Patients harboring non-inflamed tumors therefore require treatment strategies which may modulate the immune response to facilitate the influx of tumor infiltrating lymphocytes (TIL) and conversion from a non-inflamed to an inflamed tumor.

Beyond the tumor microenvironment, aspects of the host (patient) and commensal environment may also have an essential impact on immunity in response to both radiation and anti-PD1 antibody. Host factors of interest regarding the immune response include circulating immune subsets as well as germline DNA sequence and polymorphisms. Further, a growing literature supports a role for commensal microbiota in the immune response to infection and cancer including development of acute versus chronic immune responses and regulatory mechanisms to prevent collateral tissue damage[21]. We and others have proposed that manipulation of the microbiome has the potential ability to modulate the anti-tumor immune response and promote the development of an inflamed tumor microenviroment[22,23].

Considering this background, we propose to collect peripheral blood for serum, peripheral blood mononuclear cells, germline DNA, as well as pre-treatment fecal sample and baseline tumor tissue on all patients. Additionally, it is requested that any patients who undergo tumor biopsy during the study have any available tissue forwarded for analysis in comparison of pretreatment and on-/post-treatment tumor microenvironmental factors.

Pending available tissue, biomarker analysis may include the following assays listed below:

Biomarker name **(Facility performing assay)**	**Assay**	**Tissue/Body Fluid Tested** **and Timing of Assay**
Interferon inducible genes (IDO, PD-L1, FoxP3 etc.) University of Chicago Human Immunological Monitoring Core	Gene Array	Pre- and post-treatment tumor tissue
PD-L1, CD3, CD8, FoxP3, Ki67, exome sequencing University of Chicago Human Immunological Monitoring Core	IHC, exome sequencing	Pre-treatment tumor tissue (or archived FFPE tissue)
Germline DNA	Genotyping for a genome-wide association study (results pooled with subjects from other studies)	Pre-treatment blood

Biomarker name (Facility performing assay)	Assay	Tissue/Body Fluid Tested and Timing of Assay
University of Chicago Human Immunological Monitoring Core		
Gut microbiome sequencing University of Chicago Human Immunological Monitoring Core	Gut microbiome sequencing (results pooled with subjects from other studies)	Pre-treatment stool
Peripheral blood T cell functional activity University of Chicago Human Immunological Monitoring Core	ELISA for IFN-γ after co-culture with Staphylococcal enterotoxin A	Blood before treatment and after 4 doses of pembrolizumab

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

1. Unresectable or metastatic melanoma with known BRAF mutation status

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 for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Have experienced disease progression during treatment with an anti-PD1/L1 antibody as the treatment regimen immediately prior to accrual to this study or disease progression within 6 months of adjuvant anti-PD1 antibody.
4. Have measurable disease based on irRECIST 1.1.
5. Have a performance status of 0 or 1 on the ECOG Performance Scale.
6. Demonstrate adequate organ function as defined in Table 1.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥8 g/dL or ≥4.96 mmol/L
Renal	
Serum creatinine **OR**	≤1.5 X upper limit of normal (ULN) **OR**

Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
[a]Creatinine clearance should be calculated per institutional standard.	

7. 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.

8. 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 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

5.1.3 Subject Exclusion Criteria

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

1. Has received study therapy (including investigational device) as part of a clinical trial within 4 weeks of the first dose of treatment, with the exclusion of an anti-PD1/L1 antibody given as either a single agent or non-CTLA-4 antibody containing combination.

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

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

4. Hypersensitivity 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 (excluding commercial or investigational anti-PD1 or anti-PD-L1 antibodies as single agents) or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

Patients who have autoimmune adverse events controlled by replacement therapy (i.e. hypothyroidism) due to previous treatment are eligible provided replacement therapy has been initiated and toxicity has returned to Grade 1.

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.

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), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. Patients with previous grade III/IV toxicity from immunotherapy that led to treatment discontinuation are excluded.

10. Patients with uveal/ocular melanoma are excluded.

11. Has known history of, or any evidence of active, non-infectious pneumonitis.

12. Has an active infection requiring systemic therapy.

13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the 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.

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

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

16. Has received prior therapy with an anti-CTLA4 agent in the unresectable or metastatic disease setting. Prior treatment with ipilimumab in the adjuvant setting is allowed

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

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

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

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

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
Pembrolizumab	200 mg per dose	Q3W	IV infusion	Day 1 of each 3 week cycle
Ipilimumab	1 mg/kg	Q3W	IV infusion	Day 1 of each 3 week cycle x 4 treatments

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 and Rationale.

The dose amount required to prepare ipilimumab will be based on the subject's weight in kilograms (kg).

5.2.1.2 Dose Modification

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

threatening AEs as per Table 3 below. See Section 5.6.1 for supportive care guidelines, including use of corticosteroids.

All Grade 3-5 adverse events considered possibly, probably or definitely related to study agent(s) must be discussed with the Principal Investigator. All treating clinicians involved in the protocol are welcomed to discuss complicated cases at any time with the Principal Investigator:

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Table 3

Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject (Includes both drugs unless stated)
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. For Grade 3 diarrhea/colitis, permanently discontinue ipilimumab
	4	Permanently discontinue both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
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 both pembrolizumab and ipilimumab
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. Permanently discontinue ipilimumab.
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 both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
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 both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab and ipilimumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
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 both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
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 both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab
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 both pembrolizumab and ipilimumab	Permanently discontinue both pembrolizumab and ipilimumab

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 and lasts 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). Ipilimumab should be permanently discontinued for subject's who fail to complete the full treatment course of ipilimumab within 16 weeks from administration of first dose.

If a subject's study treatment has been interrupted for more than 2 doses of pembrolizumab and/or ipilimumab, the investigator must contact the Principal Investigator to review the subject's condition in order to resume the treatment. Subjects being treated on combination therapy with pembrolizumab plus ipilimumab who require discontinuation of ipilimumab due to toxicity may continue treatment with pembrolizumab after approval from the Principal Investigator. The reason for discontinuation must be recorded.

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 due to administrative reasons.

All trial treatments will be administered on an outpatient basis. Patients will be monitored per individual site institutional standard of care policy during administration of ipilimumab and pembrolizumab.

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). Pembrolizumab should always be administered first when in combination with ipilimumab.

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

Ipilimumab will be administered once every 3 weeks for a total of 4 doses. Ipilimumab may be administered as either a 30 or 90 minute IV infusion. For additional instructions regarding dosing please refer to the ipilimumab label.

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 Randomization or Treatment Allocation

There is no randomization within the study however study participants will be allocated to a treatment arm based on prior progressive disease on anti-PD1/L1 antibody alone or PD1/L1 combination not including CTLA4 Ab.

5.4 Stratification

None

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

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

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded 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 recorded.

5.5.2 Prohibited Concomitant Medications

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

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

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.

Local surgery resulting from disease progression is prohibited. However, if indicated for palliative measure and after Principal Investigator approval, local surgery may be permitted after the Week 12 tumor assessment. 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.

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

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

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** consider early administration of oral corticosteroids, especially if symptoms persists greater than 3 days,.
- For **Grade 3 or 4 diarrhea/colitis** consider early administration of corticosteroids and if symptoms persist > 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.

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

  Infusion reactions to ipilimumab will be managed by the investigators according to the product labels.

  Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab. Infusion reactions to ipilimumab will be managed by the investigators according to the product labels.

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);	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (PEMBROLIZUMAB) with:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
prophylactic medications indicated for < =24 hrs	Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Diphenhydramine 50 mg po (or equivalent dose of antihistamine). 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.		

- **Management of Stevens-Johnson syndrome and Toxic Epidermal Necrolysis:**

To date, approximately 11,000 patients in clinical trials and 27,000 patients in the post-marketing setting have been treated with KEYTRUDA®. One fatal case of SJS in a clinical trial and one fatal case of TEN in the post-marketing setting have been reported in patients treated with KEYTRUDA®. Including these cases, there have been 8 cases of SJS (6 in clinical trials, and 2 post-marketing) and 2 cases of TEN (both post-marketing) all of which were serious.

- For signs or symptoms of SJS or TEN, withhold KEYTRUDA® and refer the patient for specialized care for assessment and treatment.
- If SJS or TEN is confirmed, permanently discontinue KEYTRUDA®.

- **Management of Immune-mediated myocarditis:**

A total of 6 cases of myocarditis have been reported in patients treated with KEYTRUDA® in clinical trials or in an expanded access program. There were was 1 fatal case reported in a clinical trial.

- For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies, and administer corticosteroids as appropriate.

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

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

5.7.2 Contraception

Pembrolizumab and/or ipilimumab may have adverse effects on a fetus *in utero*. Furthermore, it is not known if pembrolizumab or ipilimumab have transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

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

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 they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to the Principal Investigator. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab and/or ipilimumab, 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 Principal Investigator and to Merck without delay and within 24 hours to the Principal Investigator 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 Principal Investigator. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Principal Investigator and to Merck and followed as described above and in Section 7.2.2.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab and/or ipilimumab 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.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Principal Investigator 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 – Other Procedures.

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: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved. Such cases should be discussed by the treating physician with the Principal Investigator.
- 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 (which includes both doses of pembrolizumab and ipilimumab together for 4 doses followed by pembrolizumab monotherapy), 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 protocol requirements.

- Administrative reasons

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. 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 until death, initiating subsequent treatment, withdrawal of consent, or the end of the study, whichever occurs first.

5.8.1 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 with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR 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, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.4.2.

5.9 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:	**Screening Phase**		**Treatment Cycles[a]**								**End of Treatment**	**Post-Treatment**	
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4*	To be repeated beyond 8 cycles 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b] Every 12 weeks post discon
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	
Administrative Procedures													
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	X
Trial Treatment Administration		X	X	X	X	X	X	X	X	X			
Clinical Procedures/Assessments													
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination		X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs and Weight		X	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	X
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Pregnancy Test – Urine or Serum β-HCG		X											
CBC with Differential		X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Serum Chemistry Panel		X	X	X	X	X	X	X	X	X	X	X	
Urinalysis		X											
TSH (with reflexive T3, FT4 as indicated)		X	X	X	X	X	X	X	X	X	X	X	
Efficacy Measurements													

Trial Period:	**Screening Phase**		**Treatment Cycles[a]**								**End of Treatment**	**Post-Treatment**	
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4*	To be repeated beyond 8 cycles				Discon	Safety Follow-up	Follow Up Visits[b] Every 12 weeks post discon
							5	6	7	8			
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	
Tumor Imaging (window includes all of cycle following drug administration)		X				X				X			
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood													
Archival or Newly Obtained Tissue Collection[C]		X											
Correlative Studies Blood Collection[C]		X	X			X					X		
Fecal Sample		X				X							

a – Treatment cycles; *Treatment cycle 4 visit-to allow for the scan any time after infusion of the 4th dose.

b – Follow up visits for up to 2 years following drug discontinuation

c – Samples to be collected for correlative blood analysis are detailed in section 8.1.1.11

7.0 REGISTRATION PROCEDURES

7.1 General Guidelines

Prior to registration and any study-specific evaluations being performed, all patients must have given written informed consent for the study and must have completed the pre-treatment evaluations. Patients must meet all of the eligibility requirements listed in Section 3. Eligible patients will be entered on study centrally by the University of Chicago study coordinator. All sites should call the study coordinator at (773) 834-1746 or PhaseIICRA@medicine.bsd.uchicago.edu to verify availability of a slot.

7.2 Registration Process

When a potential patient has been identified, notify the CRA via phone or email to ensure a reservation on the study ((773) 834-1746 or PhaseIICRA@medicine.bsd.uchicago.edu). Reservations for potential subjects will only be held for subjects who have signed consent for that particular study.

When registering a subject, the following must occur:

- Confirm that the institution has a current IRB approval letter for the correct version of protocol/consent and has an annual update on file, if appropriate.

- Submit all required materials (Eligibility Checklist, Source documentation, & signed consent form) to confirm eligibility and required pre-study procedures to the CRA a minimum of 48 hours prior to the subject's scheduled therapy start date.

- Source documentation includes copies of all original documents that support each inclusion/exclusion criteria. The eligibility checklist does not serve as source documentation but rather as a checklist that original source documentation exists for each criterion.

- Communicate with the CRA to ensure all necessary supporting source documents are received and the potential subject is eligible to start treatment on schedule. If there are questions about eligibility, the CRA will discuss it with the PI. PI may clarify, but not overturn, eligibility criteria.

- Affiliate sites must confirm registration of subjects by obtaining a subject study ID number from the CRA via phone, fax or email.

- If a subject does not start on the scheduled day 1 treatment date, promptly inform the CRA as the delay in start may deem the subject ineligible and/or require further or repeat testing to ensure eligibility.

- The date the patient receives treatment for the first time will be considered the patient's "OnStudy Date." The patient's subject ID will be assigned and a confirmation of registration will be issued by the CRA on this date. Subjects that sign consent and do not go "OnStudy"

will be recorded in the database with the date they signed consent and the reason for not going "OnStudy" (e.g., Ineligible, Screen Failure or Withdrawn Consent).

8.0 TRIAL PROCEDURES

8.1 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 Principal Investigator 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 may require that additional informed consent be obtained from the subject.

8.1.1 Administrative Procedures

8.1.1.1 Informed Consent

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

8.1.1.1.1 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 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 requirements as well as applicable laws and regulations.

8.1.1.2 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.

8.1.1.3 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.

8.1.1.4 Prior and Concomitant Medications Review

8.1.1.4.1 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.

8.1.1.4.2 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 should be recorded as defined in Section 7.2.

8.1.1.5 Disease Details and Treatments

8.1.1.5.1 Disease Details

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

8.1.1.5.2 Prior Treatment Details

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

8.1.1.5.3 Subsequent Anti-Cancer Therapy Status

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

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

8.1.1.6 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

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).

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

8.1.1.7 Physical Exam

The investigator or qualified designee will perform a physical exam at protocol specified time points as per the Trial Flow Chart (Section 6.0). Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

8.1.1.8 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.

8.1.1.9 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 13.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

8.1.1.10 Tumor Imaging and Assessment of Disease

Baseline and restaging radiologic imaging will be performed to encompass all sites of metastatic disease. This will generally include at least CT CAP though the specific imaging study can be changed by the treating investigator depending on patient circumstances. Radiologic imaging modality should be consistent throughout the study for response

assessment. For patients who have known bone metastases at baseline, bone scan should additionally be pursued as standard of care for disease assessment.

8.1.1.11 Tumor Tissue Collection and Correlative Studies Blood Sampling

The following tables outline the requirements for tissue and blood banking. These will be collected and stored for batched future analysis.

Biospecimen type	**Collection Time Point**	**Number of samples per patient**
Fecal sample	Pre-treatment: obtained prior treatment on protocol On-treatment: obtained at dose 4 of treatment with pembrolizumab	2 samples
A paraffin-embedded tissue block of the primary tumor taken before initiation of treatment (if available).	Pre-treatment: obtained prior treatment on protocol	1 historical sample
Peripheral Blood Mononuclear Cells: ~60 ml of anti-coagulated whole blood in green top tube	Pre-treatment: 8-10 tubes taken within 21 days prior to initiation of treatment. Post treatment: 8-10 tubes taken day of dose 1 and dose 4 pembrolizumab and at time of progressive disease.	Up to 30 samples total over 3 time points
Whole blood for germline DNA: 8-10 mL of whole blood in PAXgene tube	Pre-treatment: taken 21 days prior to initiation of treatment.	1 sample
Whole blood for serum analysis: 8-10 mL of whole blood in red top tube	Pre-treatment: taken within 21 days prior to initiation of treatment.	1 sample

Biospecimens will be shipped and stored in the University of Chicago Human Immune Monitoring Core Facility.

<u>Human Immune Monitoring Core:</u>

Contact: Yuanyuan Zha
University of Chicago - Human Immune Monitoring Core
910 E. 58th St. MKL057
Chicago, IL 60637
yzha1@bsd.uchicago.edu
Phone: 773-702-4812

8.1.2 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	
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	
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)
	Calcium		
	Chloride		Blood for correlative studies
	Creatinine		
	Glucose		
	Potassium		
	Sodium		
	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 urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.			

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.

8.1.3 Other Procedures

8.1.3.1 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 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.4.2. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit and then proceed to the Follow-Up Period of the study.

8.1.4 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.

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 7.1.4.2) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

8.1.4.1 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 (± 7 days) by radiologic imaging to monitor disease status up to 24 months after the final patients is enrolled or until receiving treatment outside of this protocol. 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 7.1.4.2. 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 7.1.4.2 will move from the follow-up phase to the Second Course Phase when they experience disease progression.

8.1.4.2 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 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with 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 5.7.2). 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.

8.2 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 first day of study drug administration 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.

8.2.1 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."

All reports of overdose with and without an adverse event must be reported within 24 hours to the UC CCC Cancer Clinical Trials Office and Principal Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

8.2.2 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 UC CCC Cancer Clinical Trials Office and Principal Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

8.2.3.1 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;
- Is an other important medical event

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 first day of study drug administration through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the UC CCC Cancer Clinical Trials Office and Principal Investigator and 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.

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 UC CCC Cancer Clinical Trials Office and Principal Investigator and to Merck.

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

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

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

8.2.3.2 Unexpected Events

Unexpected events are those not listed at the observed specificity or severity in the protocol, informed consent, investigator brochure, or FDA-approved package insert. An event is considered unexpected if it is listed as occurring within the class of drugs or otherwise expected from the drug's pharmacological properties but which has not been previously observed with this specific investigational agent.

8.2.3.3 Adverse Reactions

An adverse event is considered to be an adverse reaction if there evidence to suggest a causal relationship to the study agent. This may include a single occurrence of an event strongly associated with drug exposure (e.g. Stevens-Johnson Syndrome), one or more occurrence of an event otherwise uncommon is the study population, or an aggregate analysis of specific events occurring at greater than expected frequency.

8.2.3.4 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the UC CCC Cancer Clinical Trials Office and Principal Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)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, 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.

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.

8.2.4 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 STUDY PI 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.)

8.2.5 Serious Adverse Event Reporting to the Coordinating Center

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

All serious adverse events and all Events of Clinical Interest that have been specified to require expedited reporting occurring on this study require expedited reporting to the University of Chicago Comprehensive Cancer Center (UC CCC). The responsible Research Nurse or other designated individual at the treating site should report the SAE to the Principal Investigator and the Cancer Clinical Trials Office (CCTO) by the end of the business day when s/he becomes aware of the event. Events occurring after business hours should be reported to the CCTO by 12pm (noon) the next business day. Reports should be made using the 'Serious Event Report' Form. Please scan and send via email (preferred) or fax to the following:

University of Chicago Phase II CRA General:
PhaseIICRA@medicine.bsd.uchicago.edu
Phone: 773-834-1746
Fax: 773-702-4889

UC CCC Cancer Clinical Trials Office Quality Assurance:
qaccto@bsd.uchicago.edu

All unexpected adverse reactions must be reported to the IND holder so that the University of Chicago CCTO can inform the FDA. The responsible Research Nurse or other designated individual at the treating site should provide a complete written report using the FDA MedWatch 3500A form. The completed form should be sent to the CCTO at qaccto@bsd.uchicago.edu and to the Phase II CRA at PhaseIICRA@medicine.bsd.uchicago.edu within the specified timelines below regardless of whether all information regarding the event is available. If applicable, a follow-up report should be provided to the CCTO if additional information on the event becomes available.

Participating sites should not forward any adverse event reports directly to the FDA. The CCTO will report all events to the FDA as per the current FDA guidelines.

> *Fatal or Life-threatening Events*: within 4 calendar days from treating investigator knowledge of the event
>
> *All Other Reportable Events:* within 10 calendar days of treating investigator knowledge of the event

All serious adverse events should also be reported to the local IRB of record according to their policies and procedures.

8.2.6 Serious Adverse Event Reporting by the Coordinating Center

The designated UC CCC Regulatory Manager will notify all participating sites of all unexpected and serious adverse reactions that occur on this clinical trial and which are reported to the FDA and/or UC Institutional Review Board (IRB). When reported to the FDA, a copy of the completed Form 3500A (MedWatch) will be provided to the responsible Regulatory Manager by the CCTO IND Coordinator for distribution to all participating sites.

8.2.7 Serious Adverse Event Reporting to Merck

Any serious adverse event reported to the UC CCC, whether or not related to Merck product, must also be reported within 2 working days to Merck Global Safety by the responsible Research Nurse or other designated individual at the treating site.

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: +1-215-993-1220.

9.0 DATA REPORTING

Data reporting will be performed utilizing the eVelos electronic data capture system. The University of Chicago CRA will provide you with the applicable user registration information.

All required data must be recorded in the eVelos database at the completion of each cycle. AEs are to be entered in real time. SAEs are to be entered on the Serious Event Form within 24 hours of the site's knowledge of the event and sent via email (preferred) or fax to the University of Chicago (PhaseIICRA@medicine.bsd.uchicago.edu or qaccto@bsd.uchicago.edu; Fax: 773-702-4889). All case report forms must be completed by designated study personnel. Each screened (consented) patient is to be entered into eVelos within 48 hours of patient registration. In addition to direct data entry, you may be required to provide supporting source documentation. Source records are original documents, data, and records (e.g., medical records, raw data collection forms, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. Each site will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each subject enrolled in this clinical trial. Source records must be adequate to reconstruct all data transcribed onto the case report form.

10.0STATISTICAL ANALYSIS PLAN

10.1 Statistical Analysis Plan

The primary endpoint of the study will be response rate by irRECIST criteria. A Simon (Cont Clin Trials, 1980), optimal two-stage design will be employed to test the null hypothesis of a 10% response rate (based on historical ipilimumab alone) vs. a 30% alternative. Twelve

patients will be entered into the first stage and if 1 or fewer responses are observed then the trial will be terminated for lack of activity. Otherwise an additional 23 patients will be enrolled for a total of 35. If 5 or fewer responses are observed the treatment will be considered insufficiently active whereas if 6 or more (>17%) are seen the null hypothesis will be rejected and the treatment considered worthy of further testing. This design has an alpha level of 0.10 (1-sided), 90% power, and a 0.65 probability of stopping early if the true response rate is only 10%. With the closing of cohort 2, we will enroll an additional 35 patients who had initial progression if 6 or more patients respond among the initial 35. This will bring the total sample size to n=70, and provide additional precision for the estimated response rate and other secondary endpoints. For response rate, assuming a true rate of 30%, n=70 subjects will provide a 90% confidence interval width of ± 9.0%.

Evaluable patients will be defined as any eligible patient who receives a dose of pembrolizumab in combination with ipilimumab.

Secondary endpoints will include estimation of progression-free survival using the Kaplan-Meier method and assessment of changes in biomarkers from pre-post treatment using paired t-tests or Wilcoxon signed-rank tests.

10.2 Sample Size and Expected Accrual

The total sample size will be 70 patients Patient accrual is projected to be approximately 3 patients per month. Thus accrual is likely to proceed for 8 to 24 months.

11.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

11.1 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 7.

Table 7 Product Descriptions

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

11.2 Packaging and Labeling Information

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

11.3 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.

11.4 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.

11.5 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.

12.0 ADMINISTRATIVE AND REGULATORY DETAILS

12.1 Institutional Review Board (IRB) Approval and Consent

Unless otherwise specified, each participating institution must obtain its own IRB approval. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

12.2 Food and Drug Administration (FDA) Approval

This study will be conducted under an IND held by Dr. Thomas Gajewski at the University of Chicago. The University of Chicago CCTO will be responsible for facilitating all communications with the FDA on behalf of the IND holder. Participating sites should not communicate directly with the FDA.

12.3 Required Documentation

Prior to the selection of a study site that is not a full member of the Personalized Cancer Care Consortium, the audit and trial oversight processes for the site must be reviewed and approved by the UC CCC Clinical Research Advisory Committee.

Before the study can be initiated at any site, the following documentation must be provided to the Cancer Clinical Trials Office (CCTO) at the University of Chicago Comprehensive Cancer Center.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Investigational drug accountability standard operating procedures

Additionally, before the study can be initiated at any site, the required executed research contract/subcontract must be on file with the University of Chicago.

12.4 Data and Safety Monitoring

This study will be remotely monitored by the designated University of Chicago Clinical Research Associate (CRA) in accordance with the University of Chicago, Section of Hematology/Oncology standard operating procedure titled Monitoring of Multi-Institutional Investigator Initiated Clinical Trials.

Prior to subject recruitment, and unless otherwise specified, a participating site will undergo a Site Initiation Teleconference to be conducted by the designated University of Chicago research team. The site's principal investigator and his or her study staff must attend the site initiation meeting.

Monitoring will be conducted to verify the following:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Compliance with regulations
- Submission of required source documents

Participating sites will also undergo a site close-out teleconference upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and to ensure that the site Investigator is aware of his/her ongoing responsibilities.

Unless otherwise specified, this protocol will undergo weekly review at the multi-institutional data and safety monitoring teleconference as per procedures specified by the UC CCC NCI-approved Data and Safety Monitoring Plan. The conference will review:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

Protocol deviations are to be documented using the Protocol Deviation Form and sent via email to PhaseIICRA@medicine.bsd.uchicago.edu. Deviations that are considered major because they impact subject safety or alter the risk/benefit ratio, compromise the integrity of the study data, and/or affect subjects' willingness to participate in the study must be reported within 7 days. Please contact the University of Chicago CRA (PhaseIICRA@medicine.bsd.uchicago.edu) if you have questions about how to report deviations. All major protocol deviations should also be reported to the local IRB of record according to their policies and procedures.

12.5 Auditing

In addition to the clinical monitoring procedures, the University of Chicago Comprehensive Cancer Center will perform routine Quality Assurance Audits of investigator-initiated clinical trials as described in the NCI-approved UC CCC DSM Plan. Audits provide assurance that trials are conducted and study data are collected, documented and reported in compliance with the protocol. Further, quality assurance audits ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements. The audit will review subjects enrolled at the University of Chicago in accordance with audit procedures specified in the UC CCC Data and Safety Monitoring plan.

For institutions who are formal members of the Personalized Cancer Care Consortium (PCCC), the UC CCC will conduct on site quality assurance audits on average every two years during the enrollment and treatment phase of the study.

Auditing procedures for participating sites that are not full members of the PCCC must be specified and approved by the UC CCC Clinical Research Advisory Committee. In general, for sites that are not full members of the PCCC, auditing responsibility will be delegated to the participating center, with the annual audit report forwarded to the University of Chicago for review.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the University of Chicago Cancer Clinical Trials Office and Regulatory Manager that such a request has been made.

12.6 Amendments to the Protocol

All modifications to the protocol, consent form, and/or questionnaires will be submitted to the University of Chicago IRB for review and approval. A list of the proposed modifications or amendments to the protocol and/or an explanation of the need of these modifications will be submitted, along with a revised protocol incorporating the modifications. Only the Study Lead PI can authorize any modifications, amendments, or termination of the protocol. Once a protocol amendment has been approved by the University of Chicago IRB, the Regulatory Manager will send the amended protocol and consent form (if applicable) to the affiliate institutions electronically. Upon receipt of the packet the affiliate institution is expected to do the following:

- The affiliate must reply to the email from the Regulatory Manager indicating that the amendment was received by the institution and that it will be submitted to the local IRB.

- The amendment should be submitted to the affiliate institution's IRB as soon as possible after receipt. The amendment **must** be IRB approved by the institution **within 3 months** from the date that it was received.

- **The University of Chicago version date and/or amendment number must appear on the affiliate consent form and on the affiliate IRB approval letter.** The version dates can be found on the footer of every page of the protocol and consent form. The amendment number can be found on the University of Chicago IRB amendment approval letter that is sent with the protocol/amendment mailing.

The IRB approval for the amendment and the amended consent form (if amended consent is necessary) for the affiliate institution must be sent to the designated UC Regulatory Manager as soon as it is received.

12.7 Annual IRB Renewals, Continuing Review and Final Reports

A continuing review of the protocol will be completed by the University of Chicago IRB and the participating institutions' IRBs at least once a year for the duration of the study. The annual IRB renewal approvals for participating institutions should be forwarded promptly to the Regulatory Manager. If the institution's IRB requires a new version of the consent form with the annual renewal, the consent form should be included with the renewal letter.

12.8 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

12.9 Obligations of Study Site Investigators

The Study Site Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Study Site Principal Investigator is responsible for personally overseeing the treatment of all study patients. He/she must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Study Site Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the CRFs. Periodically, monitoring visits or audits will be conducted and he/she must provide access to original records to permit verification of proper entry of data.

13.0 APPENDICES

13.1 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.

13.2 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. (http://ctep.cancer.gov/reporting/ctc.html)

13.3 Evaluation of Tumor Response by immune-related Response Evaluation Criteria in Solid Tumors (irRECIST)

Tumor response will be assessed by the irRECIST (Nishino et al, 2013)[24]. This standard is a reconciliation of RECIST guidelines v1.1 with the original immune-related Response Criteria (irRC) (Wolchok et al, 2009)[25]. The latter standard incorporated principles important for assessment of immune-checkpoint blocking immunotherapy for cancer, but fundamentally differed from RECIST in using 2-dimensional tumor assessment. In contrast, irRECIST is based on 1-dimensional tumor measurement, but it differs from RECIST guidelines v1.1 in the following key ways:

- Under RECIST guidelines v1.1, the appearance of new lesions indicates PD. Under irRECIST, new measurable lesions are incorporated in the tumor burden, which is used to determine immune-related progressive disease (irPD), immune-related partial

response (irPR), and immune-related complete response (irCR). New non-measurable lesions preclude irCR.

- Under RECIST guidelines v1.1, there is no confirmation for PD. In addition, responses and irPDs must be confirmed by consecutive scans at least 4 weeks apart, assuming no clinical deterioration.

The following sub-sections describe irRECIST in detail.

Tumor Burden

At baseline, the tumor burden is the sum of single diameters (short axis for nodal lesions, longest diameter for other lesions) for the target lesions. In subsequent scans, the diameters of new measurable lesions are added to the tumor burden. If a subject is retreated, then up to 5 target lesions (perhaps different from the original lesions) will be selected and a new baseline tumor burden will be established.

Overall Response at a Single Time Point

The table below outlines determination of disease response at a single assessment based on irRECIST.

irRECIST: Overall Response

Tumor Burden (Baseline and New)	Non-Target Lesions (Baseline and New)	Response
Disappearance of non-nodal lesions. All pathologic lymph nodes < 10 mm (short axis)	Disappearance of non-nodal lesions. All pathologic lymph nodes < 10 mm (short axis)	irCR[a]
≥ 30% decrease from baseline	Any	irPR[a]
≥ 20% increase from nadir and at least 5 mm	Any	irPD[a]
Neither sufficient decrease to qualify for PR, nor sufficient increase to qualify for PD	Any	irSD
Disappearance of all non-nodal lesions. All pathologic lymph nodes < 10 mm	Any other than disappearance of all non-nodal lesions and reduction of pathologic lymph nodes < 10 mm	irPR[a]
Not all evaluated[b]	Any	irNE

a Selection as best response requires confirmation by 2 consecutive measurments at least 4 weeks apart.
b If some lesions are measured, response may be inferred from available measurements. For example, growth in evaluated target lesions may be sufficient for irPD regardless of status of non-evaluated lesions.

REFERENCES

1. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med 2010:NEJMoa1003466.
2. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013;369:134-44.
3. Spranger S, Spaapen RM, Zha Y, et al. Up-Regulation of PD-L1, IDO, and Tregs in the Melanoma Tumor Microenvironment Is Driven by CD8+ T Cells. Sci Transl Med 2013;5:200ra116.
4. Gajewski TF, Louahed J, Brichard VG. Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy. Cancer J 2010;16:399-403.
5. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013;14:1014-22.
6. Gajewski TF, Woo SR, Zha Y, et al. Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. Curr Opin Immunol 2013;25:268-76.
7. Harlin H, Meng Y, Peterson AC, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res 2009;69:3077-85.
8. Ji RR, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2011.
9. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 2014;124:687-95.
10. Woo SR, Fuertes MB, Corrales L, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 2014;41:830-42.
11. Zamarin D, Holmgaard RB, Subudhi SK, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Science translational medicine 2014;6:226ra32.
12. Ribas A, Kefford R, Marshall MA, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 2013;31:616-22.
13. Ribas A, Comin-Anduix B, Economou JS, et al. Intratumoral immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase in patients with melanoma undergoing CTLA4 blockade. Clin Cancer Res 2009;15:390-9.
14. Tarhini AA, Edington H, Butterfield LH, et al. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One 2014;9:e87705.
15. Hamid O, Schmidt H, Nissan A, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 2011;9:204.
16. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.
17. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122-33.

18. Sznol M, Kluger H, Callahan M, et al. Survival, response duration, and activity by BRAF mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). J Clin Oncol 32:5s, 2014 (suppl; abstr LBA9003^).
19. Michael B. Atkins TKC, F. Stephen Hodi, John A. Thompson, Wen-Jen Hwu, David F. McDermott, Melissa Brookes, Alessandra Tosolini, Scot Ebbinghaus, Zijiang Yang, Christine K. Gause, Rodolfo F. Perini, Antoni Ribas. Pembrolizumab (MK-3475) plus low-dose ipilimumab (IPI) in patients (pts) with advanced melanoma (MEL) or renal cell carcinoma (RCC): Data from the KEYNOTE-029 phase 1 study. J Clin Oncol 33, 2015 (suppl; abstr 3009).
20. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer. N Engl J Med 2012.
21. Goldszmid RS, Dzutsev A, Trinchieri G. Host immune response to infection and cancer: unexpected commonalities. Cell host & microbe 2014;15:295-305.
22. Zitvogel L, Galluzzi L, Viaud S, et al. Cancer and the gut microbiota: an unexpected link. Science translational medicine 2015;7:271ps1.
23. Goldszmid RS, Dzutsev A, Viaud S, Zitvogel L, Restifo NP, Trinchieri G. Microbiota Modulation of Myeloid Cells in Cancer Therapy. Cancer immunology research 2015;3:103-9.
24. Nishino M, Jagannathan JP, Ramaiya NH, Van den Abbeele AD. Revised RECIST guideline version 1.1: What oncologists want to know and what radiologists need to know. AJR Am J Roentgenol 2010;195:281-9.
25. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clinical Cancer Research 2009;15:7412-20.