Penn Medicine
University of Pennsylvania School of Medicine

CLINICAL RESEARCH PROTOCOL

A Phase Ib Tissue Collection Study of Pembrolizumab (MK-3475) in Subjects with Resectable Advanced Melanoma

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1.0 TRIAL SUMMARY

Abbreviated Title	Phase Ib Tissue Collection Study of Pembrolizumab (MK-3475) in Subjects with Resectable Advanced Melanoma
Trial Phase	Ib
Clinical Indication	Clinical stage III (nodal and/or intransit disease) or resectable stage IV melanoma (MEL), with or without prior treatment
Trial Type	Clinical study
Type of control	None
Route of administration	IV
Trial Blinding	None
Treatment Groups	1
Number of trial subjects	30 evaluable subjects
Estimated duration of trial	7 years
Duration of Participation	5 years

2.0 TRIAL DESIGN

2.1 Trial Design

Subjects will undergo a tumor tissue collection biopsy prior to treatment, followed by one dose of pembrolizumab 200 mg, then undergo a curative intent resection of all remaining disease 3 weeks after the initial dose of pembrolizumab. Post-operatively, subjects will receive up to 1 year of pembrolizumab 200 mg every 3 weeks.

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objectives & Hypothesis

1. To collect tumor tissue pre- and post- treatment with MK-3475 for studies of mechanism of action of PD-1 blockade with pembrolizumab.

Hypothesis: Collection of paired tumor tissue samples will be feasible. Pembrolizumab will result in a measurable change in tumor immune biomarkers. This is a hypothesis generating trial which will evaluate change in numerous tumor immune biomarkers.

2. To evaluate safety, tolerability and adverse experience profile of pembrolizumab in the peri-operative setting.

Hypothesis: Safety profile of pembrolizumab administered in the peri- and post-operative setting is similar to the overall safety profile with an infrequent occurrence of grade 3/4 adverse events related to the surgical procedure.

3.2 Secondary Objectives

1. To define the effect of PD-1 blockade with pembrolizumab on other immune regulatory pathways, including T cell activation, in paired tumor tissue samples from subjects with melanoma before and after treatment with a single dose of pembrolizumab.

2. To define the effect of PD-1 blockade with pembrolizumab on immune biomarkers in PBMC and serum from subjects before and at serial time points after treatment with a single dose of pembrolizumab.

3. To evaluate the disease-free-survival (DFS) in subjects with high risk MEL receiving pembrolizumab peri-operatively.

4. To evaluate overall survival (OS) in subjects with high risk MEL receiving pembrolizumab peri-operatively.

5. To describe the rate of recurrence and time to disease recurrence for subjects on the study.

4.0 BACKGROUND & RATIONALE

4.1 Background

Advanced melanoma remains a very high risk malignancy with high mortality despite recent advances and new drug approvals. Even after curative intent surgery, up to 70% of patients with high risk stage III melanoma develop recurrent metastatic disease and die.

Immune directed therapy has been effective in treating advanced unresectable melanoma with recent promising results with PD-1 blocking agents, which may affect an anti-tumor response by blocking the PD-1 mediated negative regulation on T lymphocytes, which are prime mediators of tumor immunosurveillance. Programmed Death-1 (PD-1) signaling involves binding to several discrete ligands, including PD-L1 and PD-L2. PD-L1 is often expressed within the tumor microenvironment including cancer cells and macrophages whereas PD-L2 is expressed primarily on professional antigen presenting cells. PD-1 negatively regulates the effector phase of the T cell response after ligation of PD-L1 to the receptor. Antibodies that block the PD-L1/PD-1 interaction prevent the down-regulation of the anti-tumor immune response, hence releasing the cytotoxic function of tumor-specific T cells.

Additional studies in human tumor tissue are needed to define the mechanism of action of PD-1 inhibition. Defining the mechanism is the first step in developing predictors of response to treatment with PD-1 inhibition, which would allow for more strategic development of combination immune therapy strategies, ultimately with the goal of increasing objective response rates to immune therapy in melanoma and other tumor types. This proposed study will generate mechanism of action data, as well as, neoadjuvant and adjuvant administration feasibility data.

Currently, standard options for patients with resected high risk melanoma include close observation, high dose interferon, or clinical trials, as high dose interferon has not demonstrated a definite overall survival benefit in this patient population. Effective therapies that reduce the risk of recurrence and improve survival are needed.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [1]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [2; 3; 4; 5; 6]. 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) [7; 8]. The structure of murine PD-1 has been resolved [9]. 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 [7; 10; 11; 12]. 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 [13; 14]. 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 [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. 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 [18; 19; 20; 13]. 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 [13]. 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) [21]. 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 (previously known as SCH 900475 and MK-3475) 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.

4.1.2 Preclinical and Clinical Trial Data

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

4.2 Rationale

Pembrolizumab is a highly selective, humanized monoclonal antibody (IgG4/kappa isotype) that directly blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2, with safety and efficacy demonstrated in melanoma and other advanced solid tumors. Unfortunately, little is known about the mechanism of action of pembrolizumab in tumor tissue and its interaction with other immune regulatory pathways.

Currently, standard options for patients with resected high risk melanoma include close observation, high dose interferon, or clinical trials, as high dose interferon has not demonstrated a definite overall survival benefit in this patient population. Effective therapies that reduce the risk of recurrence and improve survival are needed.

Anti-cancer activities by anti- PD-1mAb seen in MEL patients thus far provide a strong rationale for evaluation of pembrolizumab in MEL patients with high risk resectable disease. Pembrolizumab has been well tolerated with dose levels up to 200 mg and has shown a promising response rate of 41% in patients with advanced melanoma, which is much higher than the 10-15% response rate observed in ipilimumab registration trials. The existing data from the ongoing pembrolizumab study PN001 support the evaluation of the safety and efficacy of pembrolizumab in patients with resectable high risk MEL, for whom additional treatment options are needed.

Additional studies in human tumor tissue are needed to define the mechanism of action of PD-1 inhibition. Defining the mechanism is the first step in determining factors that predict response to treatment with PD-1 inhibition, which would allow for more strategic development of combination immune therapy strategies, ultimately with the goal of increasing objective response rates to immune therapy in melanoma and other tumor types. We do not expect any increased toxicity with pembrolizumab in the peri-operative period. This proposed study will generate mechanism of action data, as well as, neoadjuvant and adjuvant administration feasibility data.

4.2.1 Rationale for the Trial and Selected Subject Population

A total of 30 evaluable subjects will be enrolled in this study. Patients with a diagnosis of clinical stage III or resectable stage IV melanoma may be considered for enrollment. This patient population is selected as their risk of recurrence is very high, but there are no effective systemic therapy options that prolong survival in this population. Subjects will be recruited from the Clinical Practices of the University of Pennsylvania. We plan to consent sufficient subjects such that 30 evaluable subjects are enrolled. Subjects will be considered evaluable if they undergo both tumor tissue collection procedures and receive at least 1 dose of pembrolizumab.

4.2.2 Rationale for Dose Selection/Regimen/Modification

The dose of pembrolizumab, 200mg intravenous every 3 weeks is selected as it has been observed to be safe and effective in prior clinical studies of pembrolizumab. Subjects will receive one dose of neoadjuvant therapy followed by one year of adjuvant pembrolizumab 200 mg intravenous every 3 weeks. One year of adjuvant therapy is selected based on the rationale that subjects with advanced disease who are treated with pembrolizumab for at least 6 months and have a complete response are able to stop therapy with an ongoing durable clinical response. Durable complete responses have not been observed with stopping pembrolizumab at an earlier time point. Furthermore, 1 year is the standard

duration of treatment for adjuvant high dose interferon, which is the only approved adjuvant therapy for melanoma.

Biosamples (blood and tumor) obtained from subjects before and at serial time points during treatment will be analyzed. High priority assays will be performed as indicated below. Second priority assays will be performed as the budget allows.

High priority assays:

1. Peripheral blood mononuclear cells (PBMC) will be obtained at various time points at baseline and during this protocol, including before and after treatment and before and after surgery. PBMCs will be analyzed by flow cytometry to measure important T cell subsets. This assay will include intracellular staining for multiple T cell cytokines and transcription factors in order to determine multifunctionality. Expression of inhibitory receptors including PD-1 and others will be examined in PBMC populations to test whether changes in such pathways on T occurs following treatment.

2. Tumor-infiltrating lymphocytes (TIL) will be harvested from tumor biopsies before and after treatment to analyze treatment-induced effects on TILs using a similar multiparameter flow cytometry assay described above for PBMC.

Second priority assays:

1. Inflammatory cytokines/chemokines. Serum obtained at various times at baseline and during the protocol will be analyzed using Luminex bead array and Invitrogen 30-plex kits, which can simultaneously quantify/determine concentrations of 30 important factors, including IL-1, TNF-alpha, IL-6 and others.

2. T-cells within TIL can also be further expanded in vitro when possible using artificial APC systems routinely used in the laboratory to facilitate further study T-cell specificity and function.

3. Deep sequencing of the TCR (along with tumor and germline DNA) will also be performed when feasible from blood and tumor as a novel way to measure tumor-specific T-cell responses induced by treatment. This method does not require a priori understanding of a particular tumor antigen or MHC. Of particular interest will be evidence of TCR clones increasing in the circulation after therapy that are also found in the subject's tumor, per novel analytic methodologies noted in the preliminary data section. Such observations would be consistent with a vaccine effect of therapy.

4. Antigen-specific T-cell responses will be measured when possible using established assays in which PBMC pre and post therapy will be tested in vitro in T-cell responder assays. For patients who are HLA-A2+, functional responses and peptide-MHC quantification will be measured for a panel of melanoma associated antigens, as well as, viral antigens as a control. Functional measurements can include cytokine production, CD107a mobilization, and lysis against peptide-pulsed targets or tumor cells lines expressing the melanoma antigen. In addition, for functional assessments of T cells without regard to the patients MHC, we will use autologous CD40-activated B cells electroporated with viral or tumor antigen mRNA as in vitro stimulators.

5. New antibodies with reactivity to defined antigens will be measured in serum collected before and during treatment. When possible, we will use the ProtoArray protein array, which uses chip-based technology to profile 9,000 proteins. Analysis will be by Prospector software. The induction of high titer antibodies to defined tumor antigens not only reflects a humoral immune response to treatment but also a cellular response.

6. Circulating tumor cells (CTCs) and circulating tumor DNA will be measured in the blood pre- and post-treatment using promising cell surface markers in collaboration with Dr. Erica Carpenter. Levels of circulating tumor material will be correlated to tumor burden and can potentially afford important information with regards to response to therapy and prognosis.

Assays noted above may be performed by the specific collaborators currently indicated or other academic/industry collaborators as relationships are developed in the future.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial:

1. The subject must have clinical stage III or resectable stage IV MEL. Subject's may not have a diagnosis of uveal or mucosal melanoma.

2. The subject must be expected to have an adequate amount of tumor burden to yield 2-4 pre-operative research core biopsy (14-gauge needle) specimens or the equivalent amount of tissue (4-6 mm punch biopsy), in addition, to the tissue required for diagnostic purposes.

3. The subject must be expected to have an adequate amount of residual tumor after their pre-operative research tumor tissue collection, such that their operative research tumor collection will also yield at least 4-6 research core biopsy specimens or the equivalent amount of tissue.

4. The subject must be willing to undergo the two paired tumor tissue biopsy procedures to obtain samples for biomarker analysis. Tissue obtained must not be previously irradiated.

5. Either the subject or the subject's legal representative must be willing and able to provide written informed consent for the trial.

6. The subject must be $\geq$ 18 years of age on day of signing informed consent.

7. The subject must have a performance status of 0 or 1 on the ECOG Performance Scale.

8. The subject must demonstrate adequate organ function as defined in Table 1, all screening labs must be performed within 10 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/mcL

Platelets	≥100,000/ mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥50 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
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

9. Female subjects of childbearing potential must have a negative serum pregnancy test within 72 hours prior to receiving the first dose of study medication.

10. The subject must be willing to use protocol defined method(s) of contraception:

Female subjects of childbearing potential must be willing to use 2 methods of contraception, or abstain from heterosexual sexual intercourse for two weeks before the time of the first dose of study medication, while on study, through 120 days after the last dose of study medication.

Female subjects of childbearing potential are defined as those women who have not been surgically sterilized or have not been free from menses for > 1 year.

Male subjects must agree to use an adequate method of contraception starting with the first dose of study medication, while on study, through 120 days after the last dose of study medication.

Acceptable forms of birth control include condoms, diaphragms, cervical cap, an intra-uterine device (IUD), surgical sterility (tubal ligation or a partner that has undergone a vasectomy), or oral contraceptives, OR the subject must agree to completely abstain from heterosexual intercourse. Abstinence at certain times of the cycle only, such as during the days of ovulation, after ovulation and withdrawal are not acceptable methods of birth control.

5.1.3 Subject Exclusion Criteria

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

1. Subject has unresectable disease; i.e. in the opinion of the surgical oncologist, all of the subject's melanoma cannot be completely removed with a clear margin.

2. Subject is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of study treatment.

3. Subject has a known hypersensitivity to pembrolizumab or any of its ingredients.

4. Subject has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to enrollment.

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

6. Subject has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) agents (including ipilimumab), interferon, high dose IL-1 or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways.

7. Subject has had prior chemotherapy, targeted small molecule therapy, 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

8. For subjects who have received major surgery, the subject must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study therapy.

9. Subject has had radiation therapy to the tumor selected for research collection, or has had radiation therapy to any site within 4 weeks prior to study Day 1.

10. Subject has received transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, GM-CSF or recombinant erythropoietin) within 4 weeks prior to study Day 1.

11. Subject has a known additional malignancy that is progressing or requires active treatment.

12. Subject has known active central nervous system (CNS) metastases and/or carcinomatous meningitis.

> Note: Subjects with previously treated brain metastases will be eligible to 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.

13. Subject has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents.

> Note: Subjects with vitiligo or resolved childhood asthma/atopy are an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.

14. Subject has evidence of interstitial lung disease, active, non-infectious pneumonitis or has a history of non-infectious pneumonitis which required steroids.

15. Subject has an active infection requiring systemic therapy, including active tuberculosis

16. Subject 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.

17. Subject has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

18. Subject is pregnant or breastfeeding, or expecting to conceive or father a child within the projected duration of the trial participation (from 2 weeks prior to the first dose of study treatment, while participating on the study and through 120 days after the last dose of trial treatment).

19. Subject has severe cardiovascular disease, i.e. arrhythmias, requiring chronic treatment, congestive heart failure (NYHA Class III or IV) or symptomatic ischemic heart disease.

20. Subject has hepatic decompensation (Child-Pugh score >6 [class B and C]).

21. Subject has uncontrolled thyroid dysfunction

22. Subject has uncontrolled diabetes mellitus

23. Subject has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) as determined by medical record review.

24. Subject has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) as determined by medical record review.

25. Subject has received a live vaccine within 30 days prior to the first dose of trial treatment.

5.2 Trial Treatments

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

Table 2 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Pembrolizumab	200 mg	Q 3 wks	IV infusion	1 year	Experimental
The pembrolizumab dosing interval may be increased due to toxicity as described in Section 5.2.1.2.					

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.

5.2.1.2 Dose Modification

Dose Limiting Toxicity (DLT): Surgical outcomes will be evaluated as the effect of Pembrolizumab after surgery, and the effect of pembrolizumab on surgical complications is unknown. Dose Limiting toxicity will be defined as Grade 3 non-hematologic, and Grade 4 hematologic toxicity that is not expected for surgery alone or pembrolizumab alone. DLTs will be monitored for up to 6 weeks post-surgery.

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs as per Table 3 below.

Table 3: Dose modification guidelines for drug-related adverse events.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
	3-4 or Recurrent Grade 2	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 (Grade 2 for pneumonitis) drug-related AE that recurs or any life-threatening event.

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

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to Table 4– Infusion Treatment Guidelines for further management details.

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

With investigator and medical monitor agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic. For information on the management of adverse events, see Section 5.4.1.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be permanently discontinued from trial treatment.

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 5 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab will be administered as a 30 minute IV infusion every 3 weeks. Every effort should be made to target infusion timing to be as close to 30 minutes as possible.

The treatment cycle interval may be increased by 1 week when restarting pembrolizumab that was held due to:

- Grade 2 toxicities that did not resolve to Grade 0-1 within 4 weeks
- Grade 3 or 4 toxicities

Trial Blinding/Masking

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

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

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

5.3.1 Acceptable Concomitant Medications

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

All concomitant medications will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date will also be included on the CRF.

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

5.3.2 Prohibited Concomitant Medications

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

1. Anti-cancer systemic chemotherapy, immune or biological therapy
2. Investigational agents other than pembrolizumab
3. Radiation therapy

   Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with Sponsor-Investigator and medical monitor.

4. Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed

virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

5. Oral glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor-Investigator and medical monitor. Inhaled and topical steroids are permitted.

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 or qualified designee deems to be medically necessary.

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

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

5.4 Rescue Medications & Supportive Care

5.4.1 Supportive Care Guidelines

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

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

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

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

- For Grade 3-4 events, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.

- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

Diarrhea/Colitis:

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

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

- For Grade 2 diarrhea/colitis, administer oral corticosteroids.

- For Grade 3 or 4 diarrhea/colitis, treat with intravenous steroids followed by high dose oral steroids.

- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

  - For **T1DM** or **Grade 3-4** Hyperglycemia

    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

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

  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid

taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

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

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

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

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

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

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

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, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5 h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion and Monitor Symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen 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

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Appropriate resuscitation equipment will be available in the room and a physician readily available during the period of drug administration.		

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has 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. Male subjects of reproductive potential must agree to avoid impregnating a partner while receiving study drug and for 120 days after the last dose of study drug either by practicing total abstinence from heterosexual activity or with a female partner using 2 methods of birth control. 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, 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 7.6.2-Reporting of Pregnancy and Lactation to the Sponsor-Investigator and to Merck. 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.

5.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor-Investigator and to Merck without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor-Investigator and to Merck and followed as described above and in Section 7.6.2.

5.5.4 Use in Nursing Women

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

5.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.2 – Other Procedures.

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

1. The subject or legal representative withdraws consent.
2. Confirmed radiographic disease progression
3. Unacceptable adverse experiences as described in Section 5.2.1.2
4. Intercurrent illness that prevents further administration of treatment
5. Investigator's decision to withdraw the subject
6. The subject has a confirmed positive serum pregnancy test
7. Noncompliance with trial treatment or procedure requirements

8. The subject is lost to follow-up

9. Administrative reasons

After the end of treatment (C4 of pembrolizumab per trial flow chart, section 6.0), each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2). Subjects will have post-treatment follow-up for disease status until disease recurrence, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.7 Clinical Criteria for Early Trial Termination

The study will be terminated early if there is evidence that the rate of DLTs is greater than 33%. A DLT is defined as any grade 3 or higher hematologic or non-hematologic toxicity that is at least possibly related to Pembrolizumab and is not an expected adverse event for Pembrolizumab or surgery and which occurs anytime from the initiation of study therapy to 6 weeks post-surgery.

Early termination rules will be used to monitor DLTs during the enrollment and treatment of patients. Toxicity data will likely be reviewed after groups of 5 patients have been treated and followed for 6 weeks post-surgery. Enrollment will be terminated if the data indicate that the rate of DLTs, as defined above, is >33%. There are published data on the safety of Pembrolizumab in other populations of melanoma patients. Thus, we will assume a modestly informative beta (.5,1.5) prior which is equivalent to information on 2 treated patients. Given the prior and the observed data, the Bayesian posterior probability that the toxicity rate exceeds 33%, is calculated. If the number of patients with a DLT **equals or exceeds** the number in the table below, then it is likely that the toxicity rate is unacceptable, as noted by Bayesian posterior probabilities.

Bayesian Early Termination Rules for Toxicity						
Patients treated	5	10	15	20	25	30
Patients with toxicity	3	5	7	9	11	13
Posterior Prob[tox rate >33%]	0.81	0.81	0.82	0.83	0.84	0.85
Action	Terminate accrual, re-evaluate trial					

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

Study Therapy																
	Screening [1] (Within 28 days of cycle 1)	Week/Cycle (21 Days ± 5 Days)														Post-Treatment Visit (notes 13-16)
Week		0	3	6	9	12	15	18	21	24	27	30	33	36	Until Wk52	N
Cycle		1	2[2]	3	4	5	6	7	8	9	10	11	12	13	N	
Study Procedures																
Informed Consent [3]	X															
Inclusion/Exclusion Criteria	X															
Demographics/Medical History/ Prior Medications [4]	X															
Vital Signs/Weight [5]	X	X	X	X	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	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Study Therapy																
	Screening [1] (Within 28 days of cycle 1)	Week/Cycle (21 Days ± 5 Days)														Post-Treatment Visit (notes 13-16)
Week		0	3	6	9	12	15	18	21	24	27	30	33	36	Until Wk52	N
Cycle		1	2[2]	3	4	5	6	7	8	9	10	11	12	13	N	
Review Adverse Events [6]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tissue collection [7]	X[7]		X[7]													X (recurrence)[7]
Research Blood Collection (up to 120mL per draw)	X[17]	X	X	X	X	X			X			X			X (every 3rd cycle)	X (recurrence)[17]
CBC with Differential [8]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Serum Chemistry Panel [9]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test -Serum β-HCG [10]	X															

Study Therapy																
	Screening [1] (Within 28 days of cycle 1)	Week/Cycle (21 Days ± 5 Days)														Post-Treatment Visit (notes 13-16)
Week		0	3	6	9	12	15	18	21	24	27	30	33	36	Until Wk52	N
Cycle		1	2[2]	3	4	5	6	7	8	9	10	11	12	13	N	
HLA type[11]		X														
T3, FT4 and TSH[12]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Survival status[13]																X[15]
Tumor Imaging[14]	X					X				X				X		
Pembrolizumab		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood collection (up to 10 mL) for DNA[7]		X														

1 Routine laboratory tests (serum chemistry; hematology) for screening should be performed within 10 days of Cycle 1 Day 1.

2 Cycle 2 pembrolizumab may occur up to 9 weeks after cycle 1 to allow for full surgical recovery. All subsequent cycles will be every 21 days (+/-5 days) up to week 52.

3 Written consent must be obtained prior to performing any protocol specific procedure. Results of a test performed as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g. within 28 days prior to Cycle 1, Day 1).

4 Includes history of treatment for the primary diagnosis, including prior systemic, radiation treatment and surgical treatment, and best response to prior systemic treatments. Date of last prior cancer treatment must be documented. Radiographic studies performed prior to study entry may be collected for review by the investigator. Report complete medication history for 30 days prior to the screening visit (Visit 1).

5 Vital signs to include temperature, pulse, respiratory rate and blood pressure.

6 Adverse experiences and laboratory safety measurements will be graded per NCI CTCAE version 4.0. All adverse experiences, whether gradable by CTCAE or not, will also be evaluated for seriousness.

7 Tumor tissue collection sample #1 to be collected during screening. C1 (Week 0) treatment to occur within 7 days of sample #1 collection. Collection of sample #2 (complete surgical resection of all remaining tumor) is to be performed approximately 3 weeks after administration of pembrolizumab C1 (Week 0). There will be a 7 day window of flexibility to receive the first dose of pembrolizumab (up to 14 days after the initial screening biopsy). A tissue sample of at least one tumor lesion is mandatory at baseline (prior to Cycle 1) and at the time of complete resection. Additional biopsy samples at disease progression are highly desirable when it is feasible. The tissue samples should have proper size to enable all planned biomarker analyses. Fine needle aspiration will not be acceptable. The biopsy techniques allowed in the study include core biopsies or surgical biopsies. The same biopsy techniques should be used as routinely used for a given tumor lesion/location and specimens will be processed and stored according to the standard process. For patients who give consent to the optional Future Biological Research, the remaining tissue samples after the study biomarker analyses will be retained for future biological analyses. DNA sample for analysis should be obtained pre-dose, on Day 1 (or with any subsequent scheduled blood draw).

8 PT/INR and aPTT should be collected at Screening. Coagulation parameters should be determined throughout the study when clinically indicated. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing.

9 Comprehensive serum chemistry panel must include sodium, potassium, BUN, Creatinine, AST, ALT, alkaline phosphatase, total bilirubin and lactate dehydrogenase. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing.

10 For women of reproductive potential, a serum pregnancy test will be performed within 72 hours of the first dose. Women with amenorrhea for <1 years and who have not had a hysterectomy and oophorectomy will only be considered not to be of reproductive potential if they have a documented FSH value in the postmenopausal range.

11 HLA can be drawn once, at the time of any scheduled blood draw, or may be omitted, per PI decision.

12 Analysis of T3, FT4 and TSH will be performed by the local study site laboratory. TSH is recommended with each cycle; T3 and T4 as indicated.

13 Once a subject completes 12 months of pembrolizumab therapy, experiences disease recurrence or starts a new antineoplastic therapy, the subject will complete the below noted safety follow up and move into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status.

14 Tumor imaging will be performed at timepoints in accordance with standard of care anticipated to be within 30 days prior to enrollment and every 3-6 months on and post-treatment.

15 The mandatory Safety Follow-Up visit will be conducted within 4 weeks after the last dose of study drug or before the initiation of a new antineoplastic treatment, whichever comes first. Patients with an AE of Grade >1 will be further followed until the resolution of the AE to Grade 0-1 or until beginning of a new antineoplastic therapy, whichever occurs first.

16 AEs occurring within 30 days after the last dose of study drug should be recorded. After this time, record only AEs that are considered related to study drug. 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.

17 Whole blood samples will be drawn on biopsy and resection days, every cycle for the first 5 cycles, then every 3 cycles while on pembrolizumab, with final sample as recurrence sample for PBMC analysis if available.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor-Investigator and/or Merck for reasons related to subject safety. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

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 IRBs 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 process will adhere to IRB requirements, applicable laws and regulations and Sponsor-Investigator requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial. Waivers to the eligibility criteria will not be granted.

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

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

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy prior to 4 weeks after the last dose, 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.

7.1.1.6 Adverse Event (AE) Monitoring

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

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 (irAE).

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

7.1.1.7 Full Physical Exam

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

7.1.1.8 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, and blood pressure. Height will be measured at screening only.

7.1.1.9 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

7.1.1.10 Tumor Imaging and Assessment of Disease

Investigators may use CT, PET or MRI imaging for baseline screening imaging and to assess for disease recurrence in accordance with standard of care.

7.1.1.11 Tumor Tissue Collection and Correlative Studies Blood Sampling

Biosamples (blood and tumor tissue) obtained from subjects before and at serial time points during treatment will be analyzed. Tissue will be collected according to the Trial Flow Chart - Section 6.0.

7.1.1.12 Laboratory Procedures/Assessment

Laboratory tests for screening or entry must 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.

The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject may vary depending upon clinical course of the subject and length of time on trial.

In melanoma patients, presence of tumor infiltrating lymphocytes correlates with improved prognosis with CD8 T cells being the primary mediators of tumor lysis, while CD4+FoxP3+ Tregs result in immune suppression. As a result, a high CD8:Treg ratio correlates with a balance in favor of anti-tumor immunity. Indeed, CD8:Treg ratio has been shown to correlate with survival in ovarian cancer (Sato PNAS 2005), and therefore is an ideal primary endpoint that is reasonable biologically and may be meaningful clinically.

We will analyze a population of terminally exhausted T cells defined as $Eomes^{hi}$ $PD1^{hi}$ by flow cytometry. Previous work from the Wherry lab has shown differential exhaustion profiles including a terminally exhausted $Eomes^{hi}$ $PD1^{hi}$ population of cells which do not respond to anti-PDL1 therapy in vitro in contrast to a $Tbet^{hi}iPD1^{int}$ (less exhausted phenotype) population of T cells that do respond well to PD-L1 blockade in vitro. (Paley, Wherry Science 2012) Quantification of the terminally exhausted T cell population ($Eomes^{hi}$ $PD1^{hi}$ population) may be of prognostic significance as we hypothesize that a higher percentage of $Eomes^{hi}$ $PD1^{hi}$ T cells pre and post PD-1 therapy would be expected to correlate with treatment resistance. We will also do functional assessment of these T cell subsets pre and post anti PD-1 therapy via flow cytometry with Ki67 and Granzyme B to see if pembrolizumab is able to restore T cell function in the terminally exhausted ($Eomes^{hi}$ $PD1^{hi}$ population) T cell populations, as indicated by increased Ki67 and Granzyme B in these T cells after PD-1 blockade.

Analysis of multiple other inhibitory T cell markers including CTLA4, LAG3, TIM3, 2B4, and CD160 will be done with quantification of MFI. Tumor specific T cells from melanoma TILs show significant exhaustion with upregulation of multiple inhibitory markers. (Baitsch J Clin Invest 2011) PD-1 blockade may have differential effects on these markers (may increase or decrease their expression). For example, disruption of the PD1-PDL1 axis by anti -PDL-1

therapy results in decreased expression of LAG-3 and upregulation of PD-1 in a T cell exhaustion model (chronic viral infection model). In this T cell exhaustion model, combination therapy with anti- LAG3 and anti –PDL1 increased antigen specific T cells, T cell function, as well as viral control. (Blackburn, Wherry Nat Immunol 2009). As a result, assessing the effect of PD-1 blockade on other inhibitory molecules will be valuable in determining immune target candidates for combination immunotherapies with PD-1 blockade.

7.1.2 Other Procedures

7.1.2.1 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.

7.1.3 Visit Requirements

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

7.1.3.1 Screening

Screening requirements are outlined in Section 6.0 - Trial Flow Chart. The first tumor biopsy is collected during the screening period after informed consent. If the subject is able to undergo collection of the screening period tumor specimen and meets all other eligibility requirements, the subject can be enrolled in the study.

Subjects may be rescreened after initially failing to meet the inclusion/exclusion criteria. Results from assessments performed during the initial screening period are acceptable in lieu of a repeating a screening test if performed within the specified time frame and the results meet the inclusion/exclusion criteria.

7.1.3.2 Treatment Period

Subjects who have signed informed consent, are eligible for the study and who have undergone the screening tumor biopsy can be enrolled on the study and must receive the week 0 (cycle 1) treatment with pembrolizumab within 1 week of enrollment.

There will be a 7 day window of flexibility to receive the first dose of pembrolizumab (up to 14 days after the initial screening biopsy). If therapy cannot be started within this time frame, the patient will be removed from the study and replaced.

Subjects must then undergo a complete resection of their remaining tumor within 3 weeks (up to 1 week later, or up to 3 days earlier) after C1 of pembrolizumab treatment date.

In the event that there is a complete response after the first dose of study drug, and there is no surgical tissue available, the patient will have the option to continue with the study drug for up to one year, but will be considered non-evaluable and replaced for the study.

If the resection surgery is delayed by more than one week after the 3 week window (more than 28 days after the first dose of pembrolizumab), the patient may continue with surgery and pembrolizumab afterwards. Patients are expected to initiate adjuvant pembrolizumab within 9 weeks of cycle 1 to allow for post-surgical recovery. Patients will be evaluated by both the surgeon and the oncologist or qualified designee to determine adequate surgical recovery before initiation of study drug. In the event of delays, there is no timeframe restriction in terms of when adjuvant pembrolizumab is initiated.

Up to 12 months of pembrolizumab are given every 3 weeks after C1 per the Trial Flow Chart, Section 6.0.

7.1.3.3 Post-Treatment Visits

Subjects are evaluated within 4 weeks of their last dose of pembrolizumab and then according to the Trial Flow Chart, Section 6.0.

7.1.3.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted within 4 weeks 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.

7.1.3.4 Follow-up Visits

Subjects will move into the Follow-Up Phase after completing pembrolizumab and should be assessed every 3-6 months by radiologic imaging to monitor disease status (Section 6.0 - Trial Flow Chart). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study.

7.1.3.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by

telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.3.4.2 Definition of Evaluable Patients

A patient who has both adequate tissue collected prior to surgery and adequate tissue collected at surgery, will be deemed evaluable. Patients who do not have adequate tissue collected at either time point or who do not undergo surgery, are deemed unevaluable and will be replaced. If such patients received Pembrolizumab, then they will be evaluated in all safety analyses.

7.1.3.4.3 Definition of Time-to-event Outcomes

Disease-free survival (DFS) is defined as the time from date of surgery to date of first documented disease progression, death due to any cause or last date that patient was documented to be disease-free (i.e., scan date). DFS is the preferred outcome because patients are rendered NED from surgery. Overall survival (OS) is defined as the time from date of surgery to date of death due to any cause or last patient contact alive. Both DFS and OS outcomes will be measured from date of surgery, and not date of pre-surgery single dose Pembrolizumab, in order to compare DFS and OS estimates to published outcomes from other trials in this population. Both DFS and OS will be estimated by the Kaplan-Meier method.

7.2 Assessing and Recording Adverse Events

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

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.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case

report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2. 1.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.1 Applicable reporting to Abramson Cancer Center Data and Safety Monitoring Committee (DSMC) will occur as follows:

On-Site subjects (this includes any subjects enrolled at other sites on an in-house study)

Every effort should be made to report an event as a diagnosis, not as a list of symptoms. Symptoms that led to the diagnosis should be included in the event description, but should not be the actual event.

1. Unless covered by exclusions below Grade 3 or higher events must be reported within 10 days of knowledge.
2. All unexpected deaths within one business day of knowledge.
3. All others deaths within 30 days of knowledge. Deaths of subjects off-study for greater than 30 days from the last study treatment/intervention are not reportable unless a longer time frame is specified in the protocol.

EXCEPTIONS to AE/SAE Reporting:

a. Grade 3 or 4 events that are judged by a study investigator to be clearly unrelated to protocol therapy. The reason for determining that the event is unrelated must be clearly documented in the EMR.
b. Grade 3 or 4 events that are probably or definitely related to progression of disease as judged by a study investigator. The fact that this event is related to disease progression must be clearly documented in the EMR.
c. Grade 3 or 4 events that are probably or definitely related to an FDA approved agent. The fact that this event is related to the FDA approved agent must be clearly documented in the EMR.

7.3 7.3 Exceptions

A **one time**, **intentional** action (planned prospectively) or process that departs from the IRB and CTSRMC approved study protocol, intended for **one** occurrence. Advance documented IRB and DSMC approval is required.
For in-house studies with a Medical Monitor or Safety Monitoring Committee (not DSMB), approval must be obtained from the Medical Monitor or Safety Monitoring Committee prior to submitting the exception request to the DSMC. The following information must be contained in your exception request:

- When it is needed and why it is needed in that timeframe
- Has the Medical Monitor or Sponsor approved and provide the documentation of approval
- Is this an exception from eligibility, treatment, disease progression, study calendar windows, etc.
- Why the exception is needed (cite the section(s) of the protocol) along with the full clinical details of the subject. This must be determined by the sub-Investigator or PI.
- The reason why the protocol currently doesn't allow the situation for which an exception is being requested. This must be determined by the sub-Investigator or PI.
- If there are plans to amend the protocol and if not, why not.
- If additional follow-up or interventions will be required in order to protect the subject as a result of this exception.

Study Exceptions the DSMC may Reject:

Exceptions to eligibility, treatment/dosing, contraindicated treatment/therapies/ interventions or safety tests for the following types of studies may be rejected by the DSMC:1. Any investigator-initiated treatment study.
2. Any treatment study involving on-campus manufacturing of any component, regardless of sponsor.

To seek approval, you must provide the DSMC with strong and compelling scientific and clinical information to support your request. You should also include a statement explaining whether or not the protocol will be amended. If the protocol will not be amended your reasoning must be provided. If this situation is likely to happen again, the DSMC will require a protocol amendment.

7.4 7.4 Deviation

Any **unintentional** action or process that departs from the IRB and DSMC approval and is **identified retrospectively**. The deviation is reportable to the DSMC and the IRB within 10

days from the time the event becomes known to the study team only when: one or more participants were placed at increased risk of harm, or, the event has the potential to occur again, or the event has the potential to qualify as serious or continuing noncompliance.

If the PI determines that a deviation has any potential to impact participant safety (harm and/or risk), or the integrity of data produced from the participant, or some other overall impact on the study, the PI must report the deviation to the IRB and DSMC as described above. The IRB will make the final assessment of the impact. The DSMC will assess for additional safety and scientific integrity concerns.

The following information must be contained in your deviation report:

- When it happened? When the study team (any member) became aware
- The full description of the deviation including important dates, test results, actions taken towards the subject, etc. Also, why it happened and how it was identified.
- Was the Medical Monitor or Sponsor notified. If so, their response?
- The PIs assessment of the impact on risk, safety and/or outcome. If no impact, why. If impact, what and what will happen next.
- The corrective actions that have been implemented to date and the impact of those corrective action plans.
- Future corrective action plans (if applicable) and the impact of those plans.
- If there are plans to amend the protocol (if applicable to prevent future deviations) and if not, why not.

If the PI determines that the event had no potential to impact participant safety (harm and/or risk) or the integrity of data produced from the participant, the PI must fully document his/her rationale for each category (risk, harm, and participant data).

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7.5 7.5 Events Requiring Prompt Reporting to the IRB including Unanticipated Problem Involving Risks to Subjects or Others Reporting Requirements

Federal Regulation 21CFR §56.108(b)(1) and 45 CFR 46.103(b)(5) require the IRB to "follow written procedures for ensuring prompt reporting to the IRB…any unanticipated problems involving risk to human subjects or others."

In alignment with 21 CFR 312, investigators are required to promptly report to the IRB:

(1) Unanticipated problems including suspected adverse reactions and adverse reactions.

- An event is considered a "suspected adverse reaction" when there is *reasonable possibility* that the drug/investigational product caused the adverse event. For these reporting purposes, *reasonable possibility* means there is evidence to suggest a causal relationship between the drug/investigational product and the event.

- **For University of Pennsylvania IRB reporting, this means an event should be considered *probably or definitely related to the research procedures.***

- An event is "unexpected" if it is not listed in the investigator's brochure/package insert, or, is not listed at the specificity or severity that has previously been observed with the specific drug/investigational product; if an investigator's brochure/package insert is not available, is not consistent with the risk information described in the general investigational plan.)

- "Unexpected" also refers to events that are mentioned in the investigator's brochure/package insert as occurring with a class of drugs or as anticipated, but, are not mentioned as to have been occurring (have been seen) with the particular drug/investigational product under study.

(2) Unanticipated adverse device reaction. Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

- For drug/investigational product and device events, "serious" is defined as any death, life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Other events that may be considered "serious" but not meet the prior criteria include: those events that may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes noted above.

(3) In addition to unanticipated problems, the IRB also requires prompt reporting of the following events:

- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- Violation or deviation (meaning an accidental or unintentional change to the IRB approved protocol) only when: one or more participants were placed at increased risk of harm, or, the event has the potential to occur again, the event represents serious or continuing noncompliance.

(4) Breach of confidentiality.

(5) Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.

7.6 7.6 Reporting to the Sponsor and to Merck

7.6.1 7.6.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose is any dose higher than 20% over the prescribed dose for pembrolizumab. No specific information is available on the treatment of overdose of pembrolizumab. Pembrolizumab should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of 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 Sponsor-Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.6.2 7.6.2 Reporting of Pregnancy and Lactation to the Sponsor-Investigator 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 or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; (FAX 215 993-1220)

7.6.3 7.6.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.6.3.1 7.6.3.1 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 another important medical event

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220 within two (2) business days of learning of the information.

Refer to Table 10 for additional details regarding each of the above criteria.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

Additionally, any serious adverse event, considered by an investigator who is a physician or qualified designee to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor-Investigator and to Merck.

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

7.6.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor 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.

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported within 24 hours to the Sponsor-Investigator and to Merck Global Safety within 2 working days.

7.6.4 Evaluating Adverse Events

An investigator who is a physician or qualified designee 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 10 Evaluating Adverse Events

An investigator who is a physician or qualified designee, 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	

Table 10 Evaluating Adverse Events

	†**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?

Table 10 Evaluating Adverse Events

<table>
<tr><td>Relationship to test drug</td><td colspan="2">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 physician or qualified designee. 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):</td></tr>
<tr><td></td><td>Exposure</td><td>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?</td></tr>
<tr><td></td><td>Time Course</td><td>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)?</td></tr>
<tr><td></td><td>Likely Cause</td><td>Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors</td></tr>
</table>

Table 10 Evaluating Adverse Events

	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?

Table 10 Evaluating Adverse Events

The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a physician or qualified designee according to his/her best clinical judgment, including consideration of the above elements.	
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.6.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 8.0 STATISTICAL CONSIDERATIONS

8.1 Design

This a single arm Phase 1b trial in patients with resectable advanced melanoma that will: 1) establish the safety and feasibility of Pembrolizumab as adjuvant therapy and 2) investigate the immunologic effects of Pembrolizumab in tumor and peripheral blood. Patients will have tumor tissue collected at study entry, and then they receive one dose of Pembrolizumab prior surgical tumor resection. After surgery, patients receive adjuvant Pembrolizumab for up to one year, during which time serial blood samples will be taken.

8.2 Objectives

The primary objectives are to:

1. Collect tumor tissue before and after a single dose of Pembrolizumab for exploratory analyses of the immunologic effects of Pembrolizumab and to generate testable and reasonable hypotheses. These analyses will examine immunologic components of tumor infiltrating lymphocytes (TILs).

2. Evaluate safety, tolerability and adverse events of Pembrolizumab when given peri-operatively and as adjuvant therapy.

The secondary objectives are to:

1. To examine the effects of Pembrolizumab on other immune regulatory pathways, from paired tumor tissue samples after treatment with a single dose of Pembrolizumab.

2. To examine the effects of Pembrolizumab on immune biomarkers in PBMC and serum at serial time points during and after treatment with Pembrolizumab.

3. To evaluate the disease-free-survival (DFS) and overall survival (OS) in patients receiving Pembrolizumab as adjuvant therapy.

4. To describe the rate of recurrence and time to disease recurrence in patients receiving Pembrolizumab as adjuvant therapy.

8.3 Endpoints

Evaluability. A patient will be deemed evaluable for immune biomarker analysis if the patient received one pre-operative dose of Pembrolizumab and had adequate tissue collected at both baseline and tumor resection. Non-evaluable patients will be replaced.

Toxicity. CTCAE version 4.0 grades will be employed. Any patient who receives any amount of Pembrolizumab will be included in the toxicity analysis.

Immune biomarkers. Priority assays will include: characterization of Tregs, CD8 T cells, exhausted T cells defined as $Eomes^{hi}PD1^{hi}$ and Ki67 and Granzyme B in TILs. A full description of all immunologic outcomes and hypotheses is included in Section 7.1.1.12.

Disease-free and overall survival. Disease-free survival (DFS) is defined as the time from date of surgery to date of first documented disease progression, death due to any cause or last date that patient was documented to be disease-free (i.e., a scan date). Overall survival (OS) is defined as the time from date of surgery to date of death due to any cause or last patient contact alive. Both DFS and OS outcomes will be measured from date of surgery to allow us to compare DFS and OS estimates with those of other adjuvant trials.

8.4 Monitoring Toxicity

The study will be terminated early if there is evidence that the rate of serious adverse events is greater than 33%. A serious adverse event is defined as any grade 3 or higher hematologic or non-hematologic toxicity that is at least possibly related to Pembrolizumab and is not an expected adverse event for either Pembrolizumab or surgery and which occurs anytime from the initiation of study therapy to 6 weeks post-surgery. Early termination rules are defined in Section 5.7.

8.5 Plans for Data Analysis

Patient Evaluability. Only evaluable patients will contribute to the primary analysis that characterizes the immunologic effects of Pembrolizumab in tumor tissue and secondary analyses on other immune regulatory pathways. All patients, regardless of time on study and procurement of tissue, will contribute to the primary analysis of safety and to the secondary analyses of immunologic effects of Pembrolizumab in peripheral blood and of disease-free and overall survival.

Toxicity: All observed adverse events which occur anytime from the initiation of study therapy to 30 days after the final dose of Pembrolizumab will be graded and tabled. All grade 3 or higher hematologic or non-hematologic toxicities that are at least possibly related to Pembrolizumab and not an expected adverse event for either Pembrolizumab or surgery and which occur anytime from the initiation of study therapy to 6 weeks post-surgery will be described separately.

Immune biomarkers: Immunologic outcomes will be described using scatter and box plots and descriptive statistics (e.g., mean, median, SD, range). Pre-treatment to post-treatment paired comparisons will employ paired Student's t-test or Wilcoxon signed ranks test, as appropriate. With several post-treatment blood samples intended, trends over time will be described using scatter and box plots, descriptive statistics (e.g., fold changes from baseline). Comparisons of post-treatment values to the baseline value will be performed by repeated measures ANOVA. Longitudinal modeling using linear mixed effects models will also be attempted, in order to extend this investigation to characterized trends over time and could include modeling jointly the inter-dependency among several longitudinally measured immunologic outcomes.

Disease-free and overall survival: will be estimated by the method of Kaplan and Meier. Median and 1-year DFS and OS rates and 95% CIs will be estimated.

8.6 Sample Size

This study will enroll 30 evaluable patients. Assuming that at most 5% of enrolled patients will not be evaluable, we expect that no more than 32 patients will be enrolled.

Primary objective: Treatment of 30-32 patients will firmly establish the safety of the treatment. If the true underlying serious adverse rate is as low as 10%, then the failure to observe at least one adverse event in 32 treated patients, is <5% (binomial probability is 0.042). If the observed serious adverse event rate is 16.7% (5 of 30 patients), then the true event rate is likely to be no higher than 30%, since the upper bound of the 2-sided 95% confidence interval is 30%.

Primary objective: For exploratory analyses of immunological effects of Pembrolizumab in tumor tissue, we anticipate conducting many paired comparisons. Due to multiple parameters tested, we will control the false positive rate by calculating adjusted p values using the Bonferroni-Holm method.

We have estimated the detectable effect sizes for two of our immunologic endpoints, CD8/Treg ratio and Granzyme B level using data on peripheral blood. In 17 melanoma patients treated with Pembrolizumab, the mean pre- and maximum post-treatment Granzyme B levels in peripheral blood were 27.49 and 41.72, respectively. The standard deviation of paired differences (s_d) was 15.90, yielding an effect size of $0.89s_d$. The mean pre- and maximum post-treatment CD8/Treg ratio in peripheral blood were 5.61 and 11.82, respectively. The standard deviation of paired differences (s_d) was 8.61, yielding an effect size of $0.72s_d$. With 30 evaluable patients, there is 99% power to detect an effect size of $0.89s_d$ and 96% power to detect an effect size of $0.72s_d$ using a paired Student's t-test at 2-sided 5% error.

These two endpoints were selected because they had the most dramatic post-treatment increases in peripheral blood. More heterogeneity and smaller effect sizes are possible in tissue. But we still have sufficient power to detect smaller effect sizes. For example,

an effect size of $0.55s_d$ can be detected with 82% power by a paired t-test at 2-sided 5% significance level.

8.7 Study Duration

With an estimated accrual of 5 patients per month, it is anticipated that accrual will continue for 6 months. We plan to follow patients for an additional 2 years after enrollment has been completed, in order to better estimate DFS and OS.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Clinical Supplies will be provided by Merck as summarized in Table 11.

Table 11 Product Descriptions

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

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. They will be stored in the PCAM Investigational Drug Pharmacy as part of the Abramson Cancer Center.

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

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why

- Who will use or disclose that information

- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 Compliance with Trial Registration and Results Posting Requirements

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

10.3 Quality Management System

The Data Safety and Monitoring Committee (DSMC) of the University of Pennsylvania's Abramson Cancer Center will monitor the data quality and adherence to safety rules. Additionally, the DSMC will review all safety/toxicity data for the trial and recommend trial suspension or termination as needed.

Specific details of monitoring and audit frequency will be included in the Monitoring Plan, but will be at least every 6 months.

Medical monitor

This study will be monitored in accordance with the DSMC monitoring plan. Dr. Arati Desai, an independent clinician in the Department of Medicine, Division of Hematology Oncology, will serve as medical monitor and will consult on decisions made as a part of this trial as noted above. The medical monitor will perform a regular review and assessment of the number and type of serious adverse events (at minimum yearly, but may occur more frequently as needed).

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events, as noted above, as well as the construction and implementation of a site data and safety monitoring plan.

Monitoring Plan:

Please see the DSMC Monitoring Plan.

10.4 Data Management

Data collection and management will be carried out by the staff of the Clinical Research Unit at Penn's Abramson Cancer Center. The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All entries will be entered into an electronic data capture system (EDC) via VELOS.

10.5 Compliance with Auditing and Inspecting

The investigator will permit the Cancer Center's Administrative Director of Compliance and Auditing or her designee, and the Abramson Cancer Center Data Safety and Monitoring Committee (DCOM), to review records, data and facilities at mutually agreeable times in accordance with the DSMC monitoring plan.

The investigator also agrees to allow IRB review and regulatory authority (FDA) inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor-Investigator and Merck as required by this protocol.

10.6 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely

responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.7 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures. This protocol and any amendments will be submitted to the University of Pennsylvania IRB and CTSRMC for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB and CTSRMC for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the patient or legally acceptable surrogate, and the physician-investigator obtaining the consent.

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12.0 APPENDICES

12.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.

Grade	Description
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.	

12.2 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. (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm)

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

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

* As published in the European Journal of Cancer:

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

In addition, volumetric analysis will be explored by central review for response assessment.