Confidential

Title: Imaging the flare response with FDG PET/CT in patients with advanced metastatic melanoma on pembrolizumab.

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1.0 TRIAL SUMMARY

Abbreviated Title	Imaging the flare response from pembrolizumab with FDG PET/CT.
Trial Phase	*Correlative*
Clinical Indication	Patients with advanced metastatic melanoma who are starting pembrolizumab.
Trial Type	Prospective observational cohort study.
Type of control	No control.
Route of administration	n/a
Trial Blinding	None
Treatment Groups	n/a
Number of trial subjects	30 evaluable subjects
Estimated enrollment period	*1 year*
Estimated duration of trial	*2 years*
Duration of Participation	6 months
Estimated average length of treatment per patient	n/a

2.0 TRIAL DESIGN

2.1 Trial Design

This is a single institution, prospective, observational cohort study of FDG PET/CT as an early measure of response in patients with advanced metastatic melanoma scheduled to start pembrolizumab. Patients may participate in this study if they are greater than 18 years of age; most participants will be receiving care at the clinical practices of the University of Pennsylvania. Patients that may meet eligibility criteria will be approached about study participation regardless of race or ethnic background.

We anticipate enrolling up to 35 participants with metastatic melanoma who meet eligibility requirements for this study. The target accrual goal is to reach 30 evaluable patients, defined as those who complete two (Baseline and 1st Post-therapy) of the three planned FDG PET/CT scans. Thus, accrual is estimated to occur over approximately 1 year.

FDG PET/CT imaging will be used to evaluate glycolytic activity at sites of metastatic disease using the FDA approved clinical Positron Emission Tomography (PET) radiotracer, [^{18}F]Fluorodeoxyglucose (FDG). Study imaging will be performed in a manner identical to standard clinical FDG PET/CT imaging procedures used at the Hospital of the University of Pennsylvania using a dedicated whole-body PET/CT scanner. For each PET scan static images from the vertex of the skull to toes will be acquired approximately 60 minutes after injection of FDG. Imaging data will be processed as per standard protocol. The study will be performed under the regulatory approval of the Penn Institutional Review Board (IRB).

A total of three FDG PET/CT exams will be performed as follows:

- Baseline: A restaging FDG PET/CT will be obtained no more than 4 weeks prior to initiation of pembrolizumab to assess baseline, pre-therapy tumor glycolytic activity. This exam will be obtained for study purposes, although in some cases it may be obtained as clinical standard of care for restaging of disease prior to initiation of a new line of therapy. If this exam is obtained as clinical standard of care, it may occur prior to consent and enrollment in this study.

- 1st Post-therapy: at approximately 3 weeks (± 3 weeks) following the start of pembrolizumab, a post-therapy FDG PET/CT will be obtained. This exam will be obtained for study purposes, to assess glycolytic activity at sites of metastatic disease at an early time-point.

- 2nd Post-therapy: When clinically indicated, as per the judgment of the patient's oncologist, a restaging FDG PET/CT will be obtained at > 10 weeks following the start of pembrolizumab. This exam is clinical standard of care for restaging of disease after initiation of a new line of therapy, and would be performed even if the patient was not enrolled in the study. In some cases MRI or CT imaging may be performed as standard of care for restaging disease instead of FDG PET/CT.

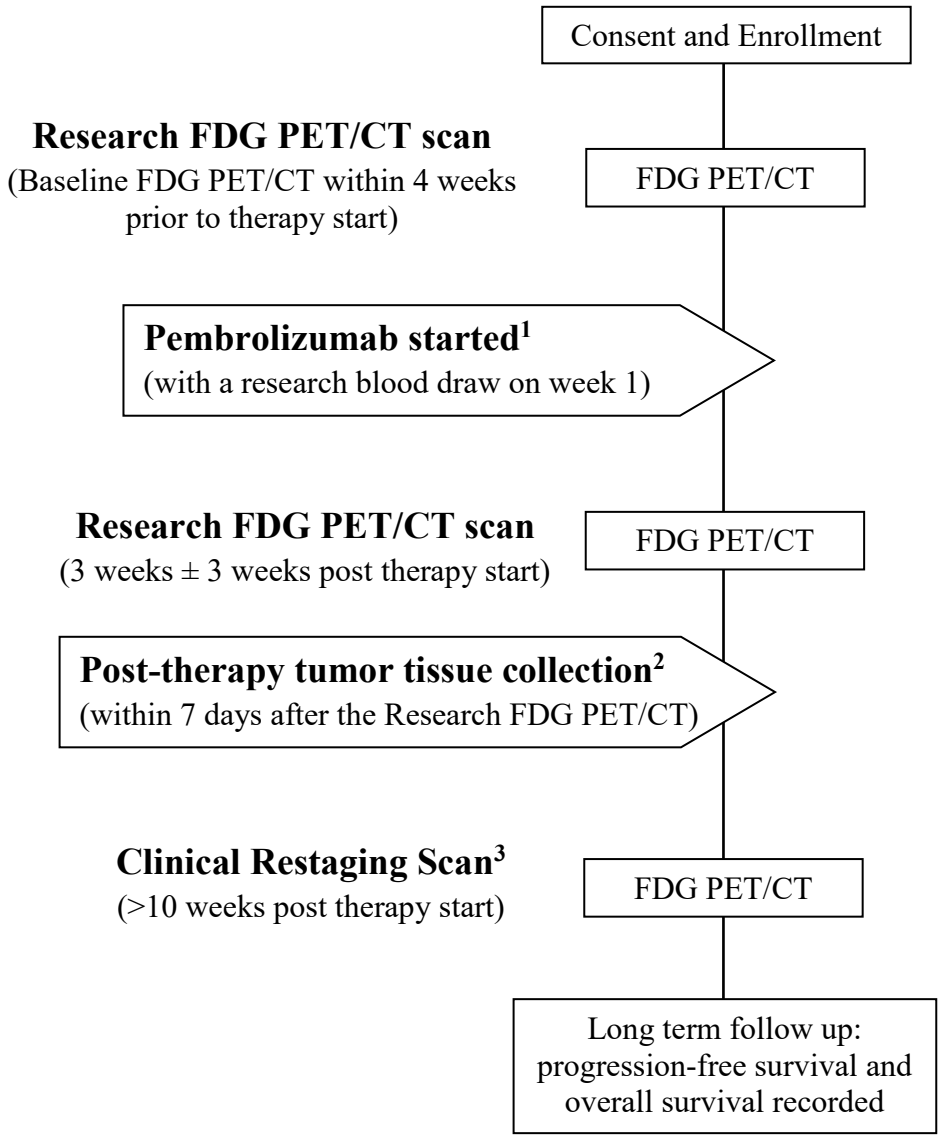
Almost all of the patients treated for melanoma in the University of Pennsylvania Health System (UPHS) have consented to participate in the Penn Melanoma Tissue Collection Program (UPCC #08607, IRB #703001). For patients participating in this program, blood samples will be collected, processed, and stored according to the program's standard operating procedure. Currently, this includes drawing less than 65 mL of blood according to the following schedule (after initiation of pembrolizumab): weeks 0, 3, 6, 9, 12, 15, and at 1 year. For the purposes of this study, patients will have an additional sample of blood drawn at approximately 1 week after starting pembrolizumab; this sample will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no blood samples will be drawn for the purposes of this study. Analysis of blood samples will include, but is not limited to, analysis of serum cytokines, chemokines, and T cell markers. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which some or all of these blood draws are not possible, in this case it will be documented in the CRF and the blood draws may be omitted and it will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

Patients may undergo tumor biopsy as part of their clinical care prior to initiation of pembrolizumab. For patients participating in the Penn Melanoma Tissue Collection Program, these pre-therapy tissue samples will be collected, processed, and stored according to the program's standard operating procedure. For the purposes of this study, patients will have a post-therapy tumor biopsy which will be scheduled within 7 days after the 1st Post-therapy FDG PET/CT; this tissue will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no tumor biopsies will be performed for the purposes of this study. The biopsy techniques preferred for this study include core biopsies or surgical biopsies. The same biopsy techniques should be used as routinely used for a given tumor lesion/location. When possible, the

post-therapy tumor biopsy should target the same lesion that was biopsied pre-therapy; if the original lesion cannot be biopsied, then a lesion with a significant change in FDG activity should be targeted for biopsy. Analysis of tissue samples will include, but is not limited to, analysis of tumor-infiltrating lymphocytes (TILs) and macrophages. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which a pre- or post- therapy tumor biopsy is not performed; those cases will be documented in the CRF and will not be considered a protocol deviation. In some cases tissue may be collected outside of the 7 day window following the 1st Post-therapy FDG PET/CT; this will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

For patients participating in the Penn Melanoma Tissue Collection Program, we will follow the standard operating procedure regarding archival tissue access for patients undergoing a biopsy or surgery on melanoma or other skin cancer for diagnosing, treating or research purposes, but for which there is no applicable left over fresh tissue, or patients who had a biopsy or surgical procedure done on melanoma or other skin cancer for diagnosing, treating or research purposes at the University of Pennsylvania Health System (UPHS) or at an institution outside of UPHS. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no archival tissue will be requested.

2.2 Trial Diagram



[1] Pembrolizumab therapy will be given as part of the standard clinical care as determined by the treating oncology team. The blood draw at 1 week will be performed only if the patient is participating in this study and has also agreed to participate in the Penn Melanoma Tissue Collection Program, and may be omitted if it cannot be collected. The patient will remain evaluable for the primary endpoint even if the blood draw is omitted.

[2] Tumor tissue will be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then a tumor biopsy will not be performed for the purposes of this study. In some patients the tumor biopsy may not be performed, or may be performed outside of the 7 day window following the Research FDG PET/CT; omission/delay of the tumor biopsy will not be considered a protocol deviation.

[3] The 2nd Post-Therapy scan will be ordered as a clinical restaging scan. In some cases MRI or CT imaging may be performed as standard of care for restaging disease instead of FDG PET/CT.

3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objectives & Hypotheses

(1) **Objective:** To determine the distribution of changes from baseline in tumor FDG uptake on PET/CT at 3 weeks and >10 weeks after initiation of pembrolizumab.

Hypothesis: None, descriptive statistics.

(2) **Objective:** To evaluate the ability of FDG PET/CT to serve as an early measure of objective response to therapy in patients with melanoma on pembrolizumab.

Hypothesis: Melanoma metastases demonstrate changes in FDG uptake on a 3 week PET/CT scan that are associated with objective response to pembrolizumab.

3.2 Secondary Objectives & Hypotheses

(1) **Objective**: Based on changes in tumor FDG uptake at 3 weeks, determine which patients have FDG "flare" (an early increase in tumor FDG PET/CT uptake), and assess whether FDG "flare" is predictive of progression-free survival and overall survival in patients with melanoma on pembrolizumab.

Hypothesis: A flare response on FDG PET/CT performed 3 weeks after initiation of pembrolizumab is predictive of progression-free survival and overall survival in patients with melanoma.

(2) **Objective:** To collect tumor tissue pre- and post- treatment with pembrolizumab for correlation with changes in FDG uptake and objective response to therapy, and for studies of mechanism of action of PD-1 blockade with pembrolizumab.

Hypothesis: Treatment with pembrolizumab will result in a measurable change in tumor immune biomarkers, which will correlate with changes in tumor FDG uptake (including a "flare" response) and objective response to therapy, and will contribute to a better understanding of the mechanism of action of pembrolizumab.

(3) **Objective**: To measure immune biomarkers in blood from subjects before and at serial time points after treatment with pembrolizumab, for correlation with changes in FDG uptake and objective response to therapy, and for studies of mechanism of action of PD-1 blockade with pembrolizumab.

Hypothesis: Treatment with pembrolizumab will result in a measurable change in blood immune biomarkers, which will correlate with changes in tumor FDG uptake (including a "flare" response) and objective response to therapy, and will contribute to a better understanding of the mechanism of action of pembrolizumab.

(4) **Objective**: To correlate changes in tumor FDG uptake (including a "flare" response), serum immune biomarkers, and tissue immune biomarkers with objective response to therapy, progression-free survival, and overall survival. Changes in FDG uptake within immune related tissues (lymph nodes, spleen, bone marrow, and thymus) will also be correlated with serum and tissue immune biomarkers, and response.

Hypothesis: Serum and tissue immune biomarkers provide additional predictive value regarding objective response to therapy, progression-free survival, and overall survival when combined with FDG PET/CT imaging data.

4.0 BACKGROUND & RATIONALE

4.1 Rationale

Recent clinical trials have shown great promise for a range of immunotherapy drugs, including anti-PD-1 therapy, in a variety of cancers. These therapies all share the common approach of activating the patient's immune system towards the tumor. This differs from conventional cytotoxic systemic therapies which non-specifically target dividing cells, including immune cells. For example, anti-PD-1 drugs such as pembrolizumab target PD-1, a receptor expressed on immune cells, which mediates an immunosuppressive effect when allowed to interact with its ligands PDL1 and/or PDL2. Since many tumors express PDL1/PDL2, therapies such as pembrolizumab block this interaction and have the potential to unmask the tumor to the immune system. When successful, this leads to an immune infiltration of the tumor and ultimately tumor cell death.

From the radiological perspective, following cancer response to immunotherapy presents unique challenges compared to conventional cytotoxic chemotherapies. Studies of anti-CTLA4 therapy with ipilimumab in melanoma revealed that a significant subset of responding tumors may exhibit non-traditional patterns of response that can confound interpretation on CT (computed tomography).[1] An example of a non-traditional response is the initial increase in size of the tumor over the course of several months with a later decrease in tumor size. In these cases, the initial increase in size of the tumor is thought to be on the basis of tumor infiltration by immune cells and can be mistaken for tumor growth on CT. Another example of a non-traditional tumor response to immunotherapy is a decrease in the size of the primary tumor with the development of new small lesions. This pattern has also been observed to correspond with favorable outcome in the setting of immunotherapy but would be considered disease progression by conventional measures.

An apparent increase in tumor burden that later resolves or decreases has been called a flare response, and is hypothesized to result from a transient immune-cell infiltrate in the tumor stroma. Several studies provide support for this hypothesis, including a case study of an ipilimumab-treated patient with apparent progressive disease at 12 weeks, in which the histological analysis of a lung nodule demonstrated a T-cell infiltrate, extensive necrosis, and no residual tumor cells.[2] Thus, a different set of response criteria have been developed, termed immune-related response criteria (irRC), that incorporate the possibility of a transient flare response, as well as a delayed immune response, into the assessment.[1,3] While irRC has

been shown to be an improvement over conventional RECIST (Response Evaluation Criteria In Solid Tumors) in the setting of immunotherapy, irRC still does not accurately identify all responders and non-responders. This is partly due to the fact that morphological changes to the tumor from immunotherapy can take several months to manifest. Thus, a more timely and accurate measure of response to immunotherapy is needed.

FDG PET/CT as a marker of cancer and inflammation:

2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG) is an ^{18}F analogue of glucose that serves as a PET radiotracer of cellular metabolism. FDG enters the cell through cell surface glucose transporters and becomes trapped within the cell following enzymatic phosphorylation by hexokinase. For decades, FDG has been employed to detect cancer exploiting a phenomenon known as the Warburg effect in which most malignancies preferentially produce ATP through a boost in extra-mitochondrial glycolysis, rather than mitochondrial oxidative phosphorylation, despite aerobic conditions. This metabolic switch, termed aerobic glycolysis, has a greater rate of glucose utilization albeit overall lower ATP yield, and provides the generation of key carbon precursors needed for the synthesis of nucleic acids, phospholipids, fatty acids, cholesterol and porphyrins.[4] As a result of the increased rate of glycolysis characteristic of the Warburg effect, most malignancies utilize more glucose (and FDG) than their non-transformed neighboring cells, which has led to the use of FDG PET/CT as a highly sensitive and moderately specific tool for the detection of occult metastatic disease.

It has recently been recognized that immune activation results in a similar cellular metabolic switch from oxidative phosphorylation to aerobic glycolysis. This is occurs with both the innate and adaptive immune activation. For example, when activated using TLR ligands or pro-inflammatory cytokines, neutrophils, dendritic cells, and macrophages have been demonstrated to switch their metabolism from oxidative phosphorylation to aerobic glycolysis.[5] Similarly, following TCR stimulation, lymphocytes increase glucose utilization by several orders of magnitude and switch glucose metabolism from oxidative phosphorylation to aerobic glycolysis.[6,7] Interestingly anti-inflammatory subpopulations of both the innate and adaptive immune system, such as M2 macrophages, regulatory T cells and quiescent memory T cells, demonstrate a predominant use of oxidative phosphorylation rather than aerobic glycolysis as opposed to their inflammatory counterparts such as activated macrophages and T-helper 17 cells.[8]

The upregulation of aerobic glycolysis in immune activation is readily visualized with FDG PET/CT imaging. Inflammatory conditions such as infection, rheumatoid arthritis and sarcoidosis yield intense avidity for FDG that compares with or exceeds that of most malignancies.[9,10] In fact, a significant limitation to the use of FDG PET/CT in cancer restaging has been interference of the tumor FDG signal by post-therapeutic inflammatory tissues including surgical changes, talc pleurodesis, and radiation therapy.[11]

In this study we propose utilizing FDG PET/CT to assess the immunotherapy response by detecting the superimposed increased metabolism within the tumor presented by a therapy-induced infiltrating population of glucose avid activated immune cells. Of note, a flare

phenomenon has been described in several cancer types following therapy; in patients with breast cancer on endocrine therapy, a clinical flare response was identified in 4 of 17 responders and 1 of 34 nonresponders, and an FDG PET flare response was seen in 15 of 17 responders and none of the 34 nonresponders.[12] These results suggest that FDG PET is more sensitive and specific for detecting a flare response compared to clinical criteria, and the results also suggest that a flare response is predictive of response to therapy.

FDG PET/CT studies are typically performed at approximately 12 weeks following the initiation of pembrolizumab, and the probability of seeing a flare response on these clinical studies is very low; this is supported by data presented at ASCO in 2014, in which 3.6% of patients with melanoma demonstrated a flare response on imaging (≥25% increase in tumor burden) at 12 weeks. We propose looking for a flare response at about 3 weeks following the start of immunotherapy. The choice of this time point is considered appropriate for the activation and expansion of immune cells and is supported by data on pembrolizumab in non-small cell lung cancer, which revealed that circulating cytotoxic T cells specific to tumor neoantigens peak at around 3 weeks from the initiation of therapy (Figure 1).[13] Additional data on pembrolizumab in multiple other tumor types also found that activated cytotoxic T cells peak at around 3 weeks.[14]

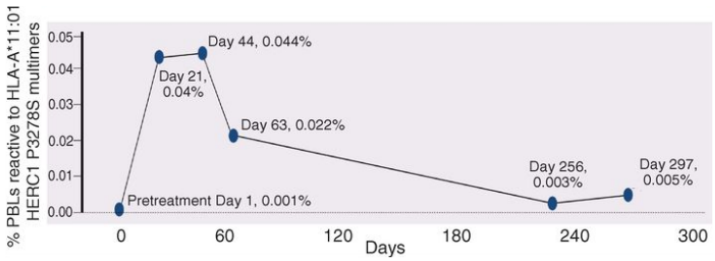


Figure 1: Neoantigen-specific T cell response to pembrolizumab in non-small cell lung cancer. In a study by Rizvi *et al*, the magnitude of the neoantigen (HERC1 P3278S) reactive CD8+ T cell response was measured in peripheral blood of patients with non-small cell lung cancer receiving pembrolizumab by determining proportion of CD8+ T cell population in serially collected autologous peripheral blood lymphocytes recognizing the HERC1 P3278S neoantigen (ASNASSAAK) before and during pembrolizumab treatment. In this study the neoantigen specific T cells begin to peak at day 21 of anti-PD1 therapy. (Naiyer A. Rizvi et al. Science 2015;348:124-128.)

No significant change is expected in the tumor's inherent basal glycolytic activity over the short interval between the baseline FDG PET/CT and the 3 week PET/CT. Therefore we hypothesize that the flare response will be able to be visualized with FDG PET/CT by detection of a superimposed burst of tumoral glycolytic activity resulting from immunotherapy induced immune infiltration. If successful, this study will help to determine the ideal time point for visualization of the flare response, test the potential for the flare response to serve as a biomarker for detecting an early response to therapy, and contribute to our understanding of pembrolizumab's mechanism of action.

In addition, studies in human tumor tissue are needed to define the mechanism of action of PD-1 inhibition, and provide an explanation for observed changes in tumor FDG uptake. In this study we propose collecting pre- and post- treatment tumor tissue in which the post-treatment tumor tissue is acquired within 7 days following the 3 week PET/CT. We also propose acquiring blood samples pre-therapy, and at serial time-points during therapy (including a 1 week sample, which is earlier than other studies have explored to date). Analysis of these samples will include, but is not limited to, serum cytokines, chemokines, and T cell markers, and tumor-infiltrating lymphocytes (TILs) and macrophages; some of

these studies will be performed upon receipt of additional funding. Of note, some of these studies will be informed by, and/or modeled after the current study at Penn titled, "A Phase Ib Tissue Collection Study of Pembrolizumab (MK-3475) in Subjects with Resectable Advanced Melanoma." This data will be correlated to patient response as well as changes in tumor FDG uptake. Thus, we expect the proposed study to generate mechanism of action data, and help identify factors that predict response to treatment with PD-1 inhibition, which may allow for more strategic development of combination immune therapy strategies, and ultimately may help to increase objective response rates to immune therapy in melanoma and other tumor types.

4.1.1 Dose Rationale and Risk/Benefits Assessment

2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG) is FDA approved for imaging in clinical oncology, and has been extensively used in oncologic PET imaging with no evidence of pharmacological effect. No adverse events (AEs) have been reported in the large number of published studies of human experience for FDG at the dose to be used in this study. In this study we will administer approximately 15 mCi of FDG according to the standard procedures used for clinical FDG PET/CT at the Hospital of the University of Pennsylvania.

Risk/Benefit

Participants will undergo up to two additional FDG PET/CT examinations for the purposes of this study. This will be in addition to other clinically indicated CT and/or PET/CT examinations. FDG is a positron emitting radiopharmaceutical. As such, it poses an intrinsic radiation exposure risk. However, when administered in low tracer doses as a PET imaging agent, as described in this protocol, this risk is felt to be extremely small. In addition to the radioactive FDG tracer, a low-dose CT of the chest, abdomen and pelvis is typically performed with the FDG PET/CT for the purpose of anatomical registration and attenuation correction of PET images. There are no separate diagnostic CT scans performed as part of this scan. FDG PET/CT scans are a standard clinical procedure for patients with metastatic melanoma. This research involves exposure to radiation that could affect a fetus or pregnant woman, due to this risk a urine pregnancy test will be performed for women of childbearing potential at the time of screening. Women who are pregnant at the time of screening will not be included in this study.

The dose will be delivered intravenously by skilled clinical professionals who typically administer FDG for clinical purposes; venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. It is possible that bruising, dizziness or fainting could occur in some subjects. There is a risk of phlebitis or infection, which is very remote. Also, the PET/CT scan takes place in a small, enclosed space and therefore can be uncomfortable for some people with claustrophobia or musculoskeletal disorders (such as arthritis). Subjects will be made as comfortable as possible and staff will be available throughout the imaging to address any discomfort.

No psychological, social or legal risk is expected. While loss of confidentiality is possible, it is felt to be very unlikely due to the small number of professionals involved in the study with

knowledge of this information. All clinicians and research staff involved are well trained in HIPAA practices. There is no anticipated additional financial risk to the patient due to participation in this study since the two additional study FDG PET/CT's, 1 week blood draw (if performed), and post-therapy tumor biopsy (if performed) will be paid for through study funds. The clinical restaging FDG PET/CT examinations and pre-therapy tumor biopsy (if performed) will be paid for through the patient's insurance as per routine clinical care for their cancer. Additional blood draws described in this protocol outside of the 1 week sample will be paid for by the Penn Melanoma Tissue Collection Program.

The risk to the patient is felt to be minimal with only the added radiation considered the likely risk posed to the patient. It is not unusual for oncologic patients to receive multiple radiological tests, including multiple PET/CT scans, in the course of clinical management and the addition of two FDG PET/CT scans is not considered to add a large risk to the subject. This study proposes the addition of two PET/CT scans to the patient's care. This study also proposes the addition of one tumor biopsy to the patient's care; this procedure carries a low risk of bleeding or infection at the biopsy site, thus the additional risk to the patient from this procedure is felt to be minimal.

There is no anticipated benefit to study subjects as a result of their participation in this study. There is potential benefit to general society if FDG PET/CT proves to be a useful imaging agent for detecting an early response to immunotherapy for cancer, or helps in the understanding of pembrolizumab's mechanism of action.

4.1.2 Rationale for Endpoints

4.1.2.1 Primary Endpoint

Objective response to therapy at 6 months, based on imaging and the clinical evaluation of the patient, is an acceptable primary endpoint in this prospective, observational cohort study of FDG PET/CT as an early measure of response in patients with advanced metastatic melanoma. Response will be determined by RECIST version 1.1. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study. Patients will be categorized as responders (i.e., complete response or partial response) or nonresponders (stable disease or progressive disease).

4.1.2.2 Secondary Endpoints

Progression free survival (PFS), is defined as the time from initiation of Pembrolizumab to documented disease progression, death due to any cause or last patient follow-up that documented lack of disease progression.

Overall survival, is defined as start date of Pembrolizumab until death from any cause or last patient contact alive. Survival data will be obtained from the electronic medical records.

4.1.2.3 Biomarker Research

Almost all of the patients treated for melanoma in the University of Pennsylvania Health System (UPHS) have consented to participate in the Penn Melanoma Tissue Collection Program (UPCC #08607, IRB #703001). For patients participating in this program, blood samples will be collected, processed, and stored according to the program's standard operating procedure. Currently, this includes drawing less than 65 mL of blood according to the following schedule (after initiation of pembrolizumab): weeks 0, 3, 6, 9, 12, 15, and at 1 year. For the purposes of this study, patients will have an additional sample of blood drawn at approximately 1 week after starting pembrolizumab; this sample will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no blood samples will be drawn for the purposes of this study. Analysis of blood samples will include, but is not limited to, analysis of serum cytokines, chemokines, and T cell markers. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which some or all of these blood draws are not possible, in this case it will be documented in the CRF and the blood draws may be omitted and it will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

Patients may undergo tumor biopsy as part of their clinical care prior to initiation of pembrolizumab. For patients participating in the Penn Melanoma Tissue Collection Program, these pre-therapy tissue samples will be collected, processed, and stored according to the program's standard operating procedure. For the purposes of this study, patients will have a post-therapy tumor biopsy which will be scheduled within 7 days after the 1st Post-therapy FDG PET/CT; this tissue will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no tumor biopsies will be performed for the purposes of this study. The biopsy techniques preferred for this study include core biopsies or surgical biopsies. The same biopsy techniques should be used as routinely used for a given tumor lesion/location. When possible, the post-therapy tumor biopsy should target the same lesion that was biopsied pre-therapy; if the original lesion cannot be biopsied, then a lesion with a significant change in FDG activity should be targeted for biopsy. Alternatively, the largest lesion or most accessible lesion may be biopsied. Analysis of tissue samples will include, but is not limited to, analysis of tumor-infiltrating lymphocytes (TILs) and macrophages. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which a pre- or post- therapy tumor biopsy is not performed; those cases will be documented in the CRF and will not be considered a protocol deviation. In some cases tissue may be collected outside of the 7 day window following the 1st Post-therapy FDG PET/CT; this will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

For patients participating in the Penn Melanoma Tissue Collection Program, we will follow the standard operating procedure regarding archival tissue access for patients undergoing a biopsy or surgery on melanoma or other skin cancer for diagnosing, treating or research purposes, but for which there is no applicable left over fresh tissue, or patients who had a biopsy or surgical procedure done on melanoma or other skin cancer for diagnosing, treating or research purposes at the University of Pennsylvania Health System (UPHS) or at an institution outside of UPHS. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no archival tissue will be requested.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Melanoma.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial:

1. The subject must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures.

2. The subject must be ≥ 18 years of age on day of signing informed consent.

3. The subject must have biopsy proven or clinically documented advanced stage metastatic melanoma.

4. The subject must have measurable disease (per RECIST 1.1) that is seen on CT, MRI, or FDG PET/CT.

5. The subject must be recommended to start pembrolizumab.

5.1.3 Subject Exclusion Criteria

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

1. Females who are pregnant or breast-feeding at the time of screening will not be eligible for this study. Female participants of child-bearing potential will have a urine pregnancy test at the time of the screening visit.

2. Subject is not able to tolerate imaging procedures in the opinion of the investigator or treating physician.

3. 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.

4. Subject has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent as assessed by medical record review and/or self-reported.

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

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

7. 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.

8. Subject has known active central nervous system 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 receiving pembrolizumab 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.

5.2 Subject Recruitment and Screening

Patients will generally be offered enrollment at the time of their office visit to oncology at the Hospital of the University of Pennsylvania. Study personnel or an investigator will present the patient with information regarding the study and enrollment. Enrollment into the study will be presented as optional and will not impact clinical management or choice of therapeutic intervention. It will be emphasized that enrollment in the study is not mandatory and that failure to enroll will not result in prejudice by the treating clinicians and will not affect patient care.

All patients being considered for the study and eligible for screening must sign an informed consent for the study prior to any study specific procedures. Patients will generally have their baseline FDG PET/CT performed as a research scan. Occasionally, the baseline FDG PET/CT may be ordered by a treating physician as part of standard medical care, in which case the scan may occur before the patient is consented and enrolled in this study.

Information from this baseline FDG PET/CT scan will be compared to the post-therapy FDG PET/CT scans. FDG PET/CT scans performed at an institution outside of University of Pennsylvania health system will not be able to be used in this study.

5.3 Subject Withdrawal/Discontinuation Criteria

The criteria for enrollment must be followed explicitly. If a subject who does not meet enrollment criteria is inadvertently enrolled, that subject should be discontinued from the study. A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. If a subject is prematurely discontinued from participation in the study for any reason, at any time, at either the investigator's discretion or the subject's request, an effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study.

The primary reason for discontinuing participation in the study may include, but is not limited to, one of the following:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Development of an intercurrent illness, injury, or medical condition likely to interfere with subject safety or the overall assessment.
- Noncompliance with protocol, e.g., the patient fails to appear at one or more imaging procedures.
- Development of any condition for which the investigator feels study withdrawal is justified.
- The subject has a confirmed positive serum pregnancy test
- The subject is lost to follow-up.
- Termination of the study.

Follow-up information will be obtained for subjects who discontinue the study. Additional details are listed in Section 7.1.4 (Visit Requirements).

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The investigator may retain and continue to use any data collected before such withdrawal of consent.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

	Screening Phase	Baseline FDG PET/CT[4]	1st Post-therapy FDG PET/CT	Tumor Tissue Collection[5]	2nd Post-therapy FDG PET/CT[6]
Scheduling window relative to pembrolizumab initiation:		< 4 weeks prior	3 weeks after (± 3 weeks)		> 10 weeks after
Administrative Procedures					
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Demographics and Medical History	X				
Clinical Procedures/Assessments					
Review Adverse Events[1]			X	X	
Laboratory Procedures/Assessments: analysis performed on site					
Pregnancy Test – Urine or Serum β-HCG	X				
Tumor Imaging and Assessment of Disease					
FDG PET/CT[2]		X	X		X
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood					
Archival or Newly Obtained Tissue Collection	X			X	
Correlative Studies Blood Collection[3]					

[1]Adverse events related to the research PET/CT will only be assessed for the period of time from the injection of FDG to the completion of the imaging exam. AEs related to the clinical treatment will not be collected or reported as part of this study.
[2]All FDG PET/CT scans will be performed as per routine clinical procedure.
[3]Less than 65 mL of blood will be collected after initiation of pembrolizumab according to the following schedule: weeks 0, 1, 3, 6, 9, 12, 15, and at 1 year; specimens will be processed and stored according to the Penn Melanoma Program tissue collection Standard Operating Procedure. The week 1 blood sample will be collected only for the purposes of this study. If blood samples cannot be collected they will be omitted and not considered a protocol deviation.
[4]This scan will usually be performed as a research exam. Occasionally, it may be performed as a clinically indicated restaging exam, which may occur prior to consent and enrollment in this study.
[5]Tumor tissue is to be collected within 7 days after the 1st Post-therapy FDG PET/CT. The biopsy techniques preferred for this 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 Penn Melanoma Program

tissue collection Standard Operating Procedure. When possible, the post-therapy tumor biopsy should target the same lesion that was biopsied pre-therapy; if the original lesion cannot be biopsied, then a lesion with a significant change in FDG activity should be targeted for biopsy. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then a tumor biopsy will not be performed for the purposes of this study. In some patients the tumor biopsy may not be performed, or may be performed outside of the 7 day window following the 1st Post-therapy FDG PET/CT; omission/delay of the tumor biopsy will not be considered a protocol deviation.

[6]This scan is a clinically indicated restaging exam. In some cases MRI or CT imaging may be performed as standard of care for restaging disease instead of FDG PET/CT.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

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

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

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

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

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

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

A baseline medical history and physical exam will be collected from the medical record from standard clinical visits with the treating medical oncologist.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart. Adverse events that are grade 3 or higher will be recorded. Adverse events related to the research PET/CT will only be assessed for the period of time from the injection of FDG to the completion of the imaging exam. AEs related to the clinical treatment will not be collected or reported as part of this study.

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

7.1.2.2 Tumor Imaging and Assessment of Disease

The following procedures will be done at each imaging session, as per routine clinical practice for FDG PET/CT imaging.

- Injection of FDG performed as per routine clinical procedure.

All women of child-bearing potential will be asked on the day of the PET/CT scan if they might be pregnant; this is a standard question for all patients who will be undergoing PET/CT scans due to the radiation exposure associated with the scan. If the patient is unsure about whether she might be pregnant then a urine pregnancy test will be performed prior to the injection of FDG.

The patient will be made comfortable in a preparatory room. Approximately 15 mCi of FDG will be administered according to the standard procedures used for clinical FDG PET/CT at the Hospital of the University of Pennsylvania. All patients will undergo a vertex of skull to toes PET/CT scan starting at approximately 60 minutes after FDG injection. A brief low-dose CT scan will be acquired according to standard PET/CT imaging procedures; this is used for attenuation correction and anatomical localization of findings in the PET scan. This can be performed either before or after the PET transmission scan. There are no separate diagnostic CT scans performed as part of this research.

A total of three FDG PET/CT exams will be performed as follows:

- Baseline: A restaging FDG PET/CT will be obtained no more than 4 weeks prior to initiation of pembrolizumab to assess baseline, pre-therapy tumor glycolytic activity. This exam will be obtained for study purposes, although in some cases it may be obtained as clinical standard of care for restaging of disease prior to initiation of a new line of therapy. If this exam is obtained as clinical standard of care, it may occur prior to consent and enrollment in this study.

- 1st Post-therapy: at approximately 3 weeks (± 3 weeks) following the start of pembrolizumab, a post-therapy FDG PET/CT will be obtained. This exam will be obtained for study purposes, to assess glycolytic activity at sites of metastatic disease at an early time-point.

- 2nd Post-therapy: When clinically indicated, as per the judgment of the patient's oncologist, a restaging FDG PET/CT will be obtained at > 10 weeks following the start of pembrolizumab. This exam is clinical standard of care for restaging of disease after initiation of a new line of therapy, and would be performed even if the patient was not enrolled in the study. In some cases MRI or CT imaging may be performed as standard of care for restaging disease instead of FDG PET/CT.

7.1.2.3 Tumor Tissue Collection and Correlative Studies Blood Sampling

For patients participating in the Penn Melanoma Tissue Collection Program (UPCC #08607, IRB #703001), blood samples will be collected, processed, and stored according to the program's standard operating procedure. Currently, this includes drawing less than 65 mL of blood according to the following schedule (after initiation of pembrolizumab): weeks 0, 3, 6, 9, 12, 15, and at 1 year. For the purposes of this study, patients will have an additional sample of blood drawn at approximately 1 week after starting pembrolizumab; this sample will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no blood samples will be drawn for the purposes of this study. Analysis of blood samples will include, but is not limited to, analysis of serum cytokines, chemokines, and T cell markers; proposed cytokines and chemokines are listed in Table 1, and proposed T cell markers are listed in Table 2. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which some or all of these blood draws are not possible, in this case it will be documented in the CRF and the blood draws may be omitted and it will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

Patients may undergo tumor biopsy as part of their clinical care prior to initiation of pembrolizumab. For patients participating in the Penn Melanoma Tissue Collection Program, these pre-therapy tissue samples will be collected, processed, and stored according to the program's standard operating procedure. For the purposes of this study, patients will have a post-therapy tumor biopsy which will be scheduled within 7 days after the 1st Post-therapy

FDG PET/CT; this tissue will also be collected, processed, and stored according to the Penn Melanoma Tissue Collection Program standard operating procedure. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no tumor biopsies will be performed for the purposes of this study. The biopsy techniques preferred for this study include core biopsies or surgical biopsies. The same biopsy techniques should be used as routinely used for a given tumor lesion/location. When possible, the post-therapy tumor biopsy should target the same lesion that was biopsied pre-therapy; if the original lesion cannot be biopsied, then a lesion with a significant change in FDG activity should be targeted for biopsy. Analysis of tissue samples will include, but is not limited to, analysis of tumor-infiltrating lymphocytes (TILs), macrophages, and other myeloid subsets; changes in tumor cells will also be evaluated, and the fraction of necrotic cells will be calculated. TILs will be analyzed using multiparameter flow cytometry; proposed phenotypic markers are listed in Table 2. This data will be correlated to patient response as well as changes in tumor FDG uptake. Some of these studies will be performed upon receipt of additional funding. There may be patients for which a pre- or post- therapy tumor biopsy is not performed; those cases will be documented in the CRF and will not be considered a protocol deviation. In some cases tissue may be collected outside of the 7 day window following the 1st Post-therapy FDG PET/CT; this will not be considered a protocol deviation. Experimental pathology results will not be reported to the participant and will have no impact on the treatment decisions made by the treating physician.

For patients participating in the Penn Melanoma Tissue Collection Program, we will follow the standard operating procedure regarding archival tissue access for patients undergoing a biopsy or surgery on melanoma or other skin cancer for diagnosing, treating or research purposes, but for which there is no applicable left over fresh tissue, or patients who had a biopsy or surgical procedure done on melanoma or other skin cancer for diagnosing, treating or research purposes at the University of Pennsylvania Health System (UPHS) or at an institution outside of UPHS. If patients have not consented to participate in the Penn Melanoma Tissue Collection Program, then no archival tissue will be requested.

Chemokines	Inflammatory Cytokines	Inhibitory Cytokines
Mip1a	IFNg	IL4
Mip1b	TNF	IL10
Rantes	IL1	TGFbeta
IP10	IL2	
ITAC	IL6	
Fractalkine	IL12	
	IL17	
	IL23	

Table 1. Proposed cytokine / chemokine analysis.

Lineage Markers	Differentiation	Transcription Factors	Inhibitory Receptors	Function
CD3 CD4 CD8 FoxP3 CD14 CD16 CD19	CD27 CD45RA	Tbet Eomes	PD-1 Tim3 LAG-3 CD160 CTLA4 KLRG1 SAP CD244 TIGIT	Granzyme B Ki67 Perforin

Table 2. Proposed flow cytometric analysis.

7.1.3 Laboratory Procedures/Assessments

A urine pregnancy test will be performed on women of childbearing potential only at screening.

7.1.4 Visit Requirements

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

7.1.4.1 Screening

The investigator will provide for the protection of the subjects by following all applicable regulations. These regulations are available upon request from the sponsor. The sponsor and IRB must review the informed consent form used during the informed consent process, and it must be available for inspection.

Before any procedures specified in this protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent.
- Be given time to ask questions and time to consider the decision to participate.
- Voluntarily agree to participate in the study.
- Sign and date an IRB-approved informed consent form.

Patients will usually have their baseline FDG PET/CT performed as a research scan. Occasionally, the baseline FDG PET/CT may be ordered by a treating physician as part of standard medical care, in which case the scan may occur before the patient is consented and enrolled in this study. Information from this baseline FDG PET/CT scan will be compared to the post-therapy FDG PET/CT scans. FDG PET/CT scans performed at an institution outside of University of Pennsylvania health system will not be able to be used in this study.

The following additional patient data will be obtained: demographics (including gender, date of birth, height, weight, and race), histologic diagnosis, and year of diagnosis. Correlative pertinent imaging including CT, MRI, x-ray, ultrasound, FDG PET/CT, or bone scan may be reviewed if necessary to confirm eligibility. For patients referred from outside the University of Pennsylvania health system, patient records, biopsy material, and imaging will be reviewed to determine eligibility for the study. For women of childbearing potential a urine pregnancy test will be performed at the screening visit.

7.1.4.2 Clinical Follow-up

Clinical follow-up may include standard of care imaging, tumor markers and standard visits with a treating physician at intervals standard for metastatic melanoma follow-up after the start of new treatment, and usually at the time of symptomatic progression. The timing of clinical visits will be determined by the treating physician and is not mandated by this study.

Information will be collected through medical record review to assess for status of disease and the date of progression will be documented at the time of clinical progression. Clinical follow up for overall survival, by medical record review, will continue until loss to follow up or death. For those whose are cared for by a non-UPHS clinician during this period, this clinician will be contacted by phone for follow-up. The patients may also be contacted by phone for follow-up.

7.1.5 Image Interpretation

Static images will be reconstructed using standard procedure and analyzed by visual inspection and standardized uptake value (SUV) analysis. The tumor avidity, as estimated by the SUVmax, of the primary tumor and up to 5 metastatic lesions will be measured on each FDG PET/CT scan. Patients will be grouped into responders (partial/complete response) and nonresponders (stable disease or progressive disease) based on RECIST. Patients will also be grouped based on a positive flare response (greater than 20% increase in SUVmax) or negative flare response (less than 20% increase in SUVmax. An exploratory tumor-by-tumor FDG PET/CT response analysis will be performed for the primary tumor and up to 5 metastatic lesions in each patient. Changes in FDG uptake within immune related tissues (lymph nodes, spleen, bone marrow, and thymus) will also be measured as an exploratory objective using SUVmax and SUVmean.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject that develops or worsens in severity during the course of the study. 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 protocol-specified procedure, whether or not considered related to the 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 study period, is also an adverse event.

Progression of the cancer under study is not considered an adverse event.

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 study and AEs will be captured according to guidelines for standard AE reporting.

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

7.2.1.1 Serious Adverse Events

A serious adverse event is any adverse event occurring during participation in this study 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 an other important medical event

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

For the time period beginning when the consent form is signed until the completion of the post-therapy tumor tissue biopsy any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to the research FDG PET/CT scan procedure or post-therapy tumor tissue biopsy that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

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

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

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

7.2.2 Evaluating Adverse Events

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

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

Table 6 Evaluating Adverse Events

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

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	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 Merck product to be discontinued?	

Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	
	Time Course	
	Likely Cause	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was 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 Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to 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) Sponsor's 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 MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR 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 Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.
No, there is not a reasonable possibility of Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated

	AE.)

7.2.3 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL CONSIDERATIONS

8.1 Design:

This is a pilot correlative study for patients with metastatic melanoma receiving pembrolizumab on a standard regimen. The intent of this study is to evaluate FDG PET/CT as an early measure of response to immunotherapy. Tumor FDG uptake will be measured at baseline and at 3 and >10 weeks after initiation of pembrolizumab. 30 evaluable patients will be enrolled; evaluable implies that the patient had baseline and week 3 FDG PET/CT scans. This study will also include serum and tissue sampling for immune biomarkers.

8.2 Objectives:

The primary objectives are to:

1. Determine the distribution of changes from baseline in tumor FDG uptake on PET/CT at 3 weeks and >10 weeks after initiation of pembrolizumab.
2. Test for the association between change in tumor FDG uptake on PET/CT at 3 weeks and objective response (RECIST).

The secondary objectives are to:

1. Based on change in FDG uptake at 3 weeks, determine which patients have FDG "flare."
2. Test for the association between FDG flare (yes/no) and objective response.
3. Test for the association between FDG flare and progression-free and overall survival.
4. Test for correlation between change in FDG uptake and levels of immune biomarkers in serum and tissue.
5. Test for association between changes in immune biomarkers in serum and tissue with objective response, progression-free survival, and overall survival.

8.3 Endpoints:

1. Objective response is defined by RECIST. The clinical evaluation takes place at 3 months per standard pembrolizumab regimens. Patients will be categorized as responders (complete response or partial response) or nonresponders (stable disease or progressive disease).

2. The maximum standardized uptake value (SUVmax) will be analyzed for all tumor sites. Immune-related tissues will be analyzed with SUVmax and SUVmean.
3. Studies have shown that the baseline test/re-test variability is 15-20%. Thus, FDG "flare" will likely be defined by >20% increase in tumor FDG uptake at 3 weeks. This definition may be modified for the observed distribution of changes in tumor FDG uptake on our study.
4. Progression-free and overall survival are defined in Section 4.1.2.
5. Immune biomarkers (see Section 7.1.2.3).

8.4 Plans for Data Analysis:

This is a pilot correlative study of FDG PET/CT imaging to evaluate early response to immunotherapy. We will first begin with a descriptive analysis. For continuous variables, we will generate graphs and descriptive statistics (e.g., means, standard deviations, medians and inter-quartile range). Categorical variables will be described by frequencies, proportions and 95% exact confidence intervals. Change in tumor FDG uptake will be closely examined. It is likely that change in tumor SUVmax >20% will be classified as a FDG "flare" and the number of patients with a flare will be computed.

Next, associations between change in tumor FDG uptake with clinical outcomes will be conducted. Change in tumor FDG uptake at 3 weeks will be compared between responders and nonresponders by a two sample t-test. Also, objective response rates will be compared between patients with or without FDG "flare" by Fisher's exact test.

PFS and OS will be estimated by the method of Kaplan and Meier. Median values and 95% confidence intervals will be calculated. PFS and OS will be compared between patients with or without FDG "flare" by the log rank test. Cox regression may be used to assess the effect of continuously scaled measurements of FDG change. For all survival analyses, a landmark analysis will be performed. Here, PFS or OS are defined from a landmark, which is 3 weeks after initiation of pembrolizumab. The landmark method is necessary because the change in tumor FDG uptake is known at 3 weeks. Serum and tissue biomarkers will be correlated with change tumor FDG uptake, using Pearson correlation or Spearman's correlation, as appropriate. Within-patient pre-/post-treatment changes in biomarkers will be evaluated by paired Student's t-test or Wilcoxon signed ranks test, as appropriate. We will test for an association between post-/pre-treatment fold change in immune biomarkers in serum and tissue and objective response, progression-free survival, and overall survival using logistic regression (for response) and Cox regression (for time-to-event outcomes) using landmark survival analysis.

8.5 Sample Size Justification:

The target is 30 evaluable patients, which may require up to 35 enrolled patients. Based on our clinical experience, we assume a 50% objective response rate, thus there will be 15

responders and 15 nonresponders. A comparison of change in FDG SUV at 3 weeks between responders and nonresponders, will have 84% power to detect a 1.0SD difference in mean values, using 1-sided two sample t test at a 5% significance level. The 1-sided test is justified, since responders are expected to have greater changes in FDG SUV.

8.6 Study Duration:

This study will enroll 30 evaluable patients over 1 year.

9.0 ADMINISTRATIVE AND REGULATORY DETAILS

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (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).

All research personnel associated with this study have completed the University of Pennsylvania's Patient Oriented Research Training Program as well as HIPAA Compliance Training. Trained staff will assess eligibility, introduce the study rationale, procedures, study risks, and collect the combined informed consent/HIPAA authorization form. The study team will work to uphold the privacy of the participants in several ways. Communications made among study staff regarding participants will use ID numbers whenever possible and minimize the use of patient name or other identifying information except when necessary for conduct of the study. Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and

passwords. Whenever feasible, identifiers will be removed from study-related information. In data analysis sets, we will use ID numbers and/or patient initials only.

Precautions will be applied to protecting subject privacy and the protected health information detailed below:

1. Name
2. Address
3. Date of Birth
4. Telephone Number
5. Email address
6. Emergency contact number, name, and relationship
7. Medical Record Number
8. Health Plan ID numbers

Data will be accessible to the study investigators, all study staff, Department of Radiology IND office representatives, Radiation Research Safety Committee members, UPenn IRB and Office of Clinical Research, and the FDA (if desired).

9.2 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.

9.3 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9.4 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the study sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

9.5 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, 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 to the sponsor 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 for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

10.0 STUDY FINANCES

10.1 Subject Stipends or Payments

We will pay for the cost of parking, if not already covered through the hospital, on the day of the baseline PET/CT scan and on the day of the 1st post-therapy PET/CT scan. In addition, the patient will receive $100 after completion of the 1st post-therapy PET/CT scan, to cover the inconvenience of time and effort spent in participation of this study.

11.0 APPENDICES

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

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

11.2 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.

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