A pilot study combining pembrolizumab with locally delivered radiation therapy for the treatment of metastatic esophageal cancers

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disclosure. Persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them

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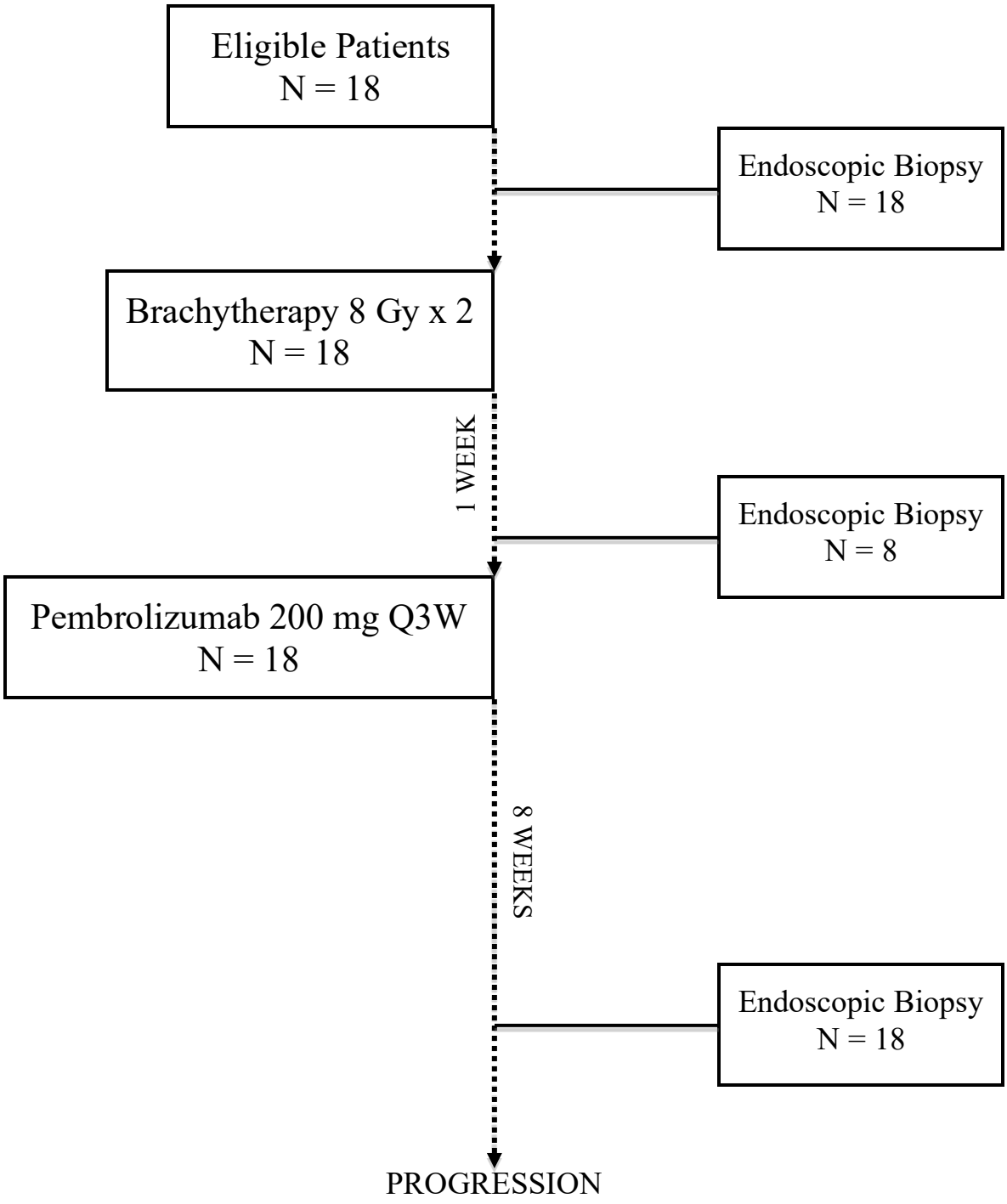
SCHEMA



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1.0 BACKGROUND AND RATIONALE

1.1 Esophageal Cancers

In 2014, it is estimated that there will be 18,170 new cases of esophageal cancer diagnosed and 15,450 deaths in the United States. Alarmingly, esophageal adenocarcinomas are increasing in incidence more rapidly than other malignancies and most notably in patients younger than 40 years of age. Unfortunately 80-90% of newly diagnosed patients present with regional or distant metastatic disease. Standard front-line therapy for patients with metastatic esophageal cancers is palliative systemic chemotherapy, usually with a fluoropyrimidine and a platinum agent, and for patients presenting with profound dysphagia at diagnosis, an abbreviated course of radiation therapy followed by the systemic chemotherapy or radiation therapy concurrently with the chemotherapy may also be given. Despite these treatments, the response rate for first-line therapies is generally about 40%, and the median survival is less than one year. Therefore, new therapeutic strategies are needed.

1.2 Immune Therapy

1.2.1 Immunosurveillance

Immunosurveillance is a term used to describe the role of the host immune system in controlling tumor development. The role of immunosurveillance in the control and/or development of tumors has been described in three steps termed the three E's: Elimination, Equilibrium, and Escape. During the elimination phase, the immune system is able to recognize aberrantly expressed antigens or molecules that signal to the immune system that the cell has undergone a transformative event and should be eliminated. Unfortunately, transformed cells eventually adopt evasive properties that allow it to subvert detection or destruction by the immune system (Equilibrium) permitting its eventual outgrowth into a clinically evident tumor (Escape).[2] Thus, immunotherapy refers to strategies that manipulate the immune response in an attempt to re-establish an effective immunosurveillance mechanism to control tumor growth. Another benefit of cancer immunity is that the immune system represents a relatively tumor-specific effector mechanism that can simultaneously target multiple disease sites including the CNS and distant metastatic sites of disease, and the response can be long-lived often persisting for years.[3]

One mechanism by which tumors are able to evade immunologic detection is through the upregulation of inhibitory signals. For example, PD-L1 and PD-L2 are molecules frequently expressed on many tumor types. Their cognate receptor, PD-1, is expressed on the surface of many activated immune cell populations, including tumor-infiltrating T lymphocytes, and binding of PD-1 to either of these inhibitory ligands results in an "exhausted" or suppressed effector phenotype of infiltrating T cells permitting tumor escape.[4] Under normal conditions, these inhibitory

molecules serve to control chronic immune activation representing a "checkpoint" mechanism to prevent unregulated inflammatory responses that may be potentially detrimental to the host.[5] In the context of cancer immunity, however, the tumor microenvironment has coopted this checkpoint pathway to permit immune escape.[4,5] Therefore, one approach to bypass this immunosuppressive environment to reestablish immunosurveillance is through the use of blocking monoclonal antibodies specific to these inhibitory molecules to allow for the re-activation and expansion of endogenous anti-tumor T cells. This approach has been termed "checkpoint blockade" and initial phase I studies using either anti-PD-1 or anti-PD-L1 antibodies have demonstrated significant objective, long-term responses in patients with various tumor types.[6-10]

1.2.2 Immunosuppression in Esophageal Cancer

The immune system is composed of both pro-inflammatory effector cells and anti-inflammatory suppressive cells. The balance between these two opposing cell populations dictates the overall outcome of the immune response. In esophageal cancer, a predominance of effector cytotoxic $CD8^+$ Tcells is associated with improved patient survival [11], while a significantly higher number of the principle suppressive cell populations, myeloid derived suppressor cells (MDSCs) and regulatory T cells (Tregs), is associated with a poor prognosis [12,13]. Moreover, larger tumor burden and more advanced stage disease are correlated with a higher level of circulating MDSCs[13]. These data suggest that the relative balance between effector:regulatory immune cells determines the efficacy of immunosurveillance in controlling esophageal cancer.

The exact mechanism by which esophageal cancer subverts immune detection is presently unknown. Interestingly, MDSCs isolated from esophageal tumors were noted to have dramatically upregulated expression of PD-L1 [13]suggesting that the PD-1 pathway may be involved. In further support of this point, PD-L1 is expressed in 35-45% of esophageal cancers [14,15], while the other ligand for PD-1, PD-L2, is present in over 80% of tumors [15]. Furthermore, $PD\text{-}1^+$ tumor-infiltrating lymphocytes (TILs) were detected in approximately 60% of esophageal tumor samples [15]. The expression of PD-1 or its ligands, PD-L1 or PD-L2, are also associated with more advanced disease and a poorer outcome compared to patients that do not have evidence of upregulated expression of this pathway[14,15]. As such, inhibition of the PD-1/PD-L1 pathway may have therapeutic implications in the treatment of esophageal cancer.

1.3 Radiotherapy and the Immune Response

Radiation therapy is a standard treatment modality for many patients with esophageal cancer and is often used in the palliative setting to alleviate symptoms of dysphagia associated with locally progressive disease.

Intraluminal brachytherapy is a specific means of delivering radiotherapy to the luminal

surface of the esophagus with a specially designed catheter, typically with large hypofractionated doses delivered over only a few fractions[16,17]. This can be given at any point within an obstructed esophagus due to the small caliber of the introducer. The main advantage of brachytherapy over stent insertion is its potential to provide lasting control of tumor-related symptoms, and its main advantage of external beam radiotherapy is its ability to safely deliver large doses of radiation with great spatial precision while shortening treatment time and sparing surrounding organs-at-risk. Modern brachytherapy is typically delivered with a high-dose rate (HDR) afterloader. Histologic findings after such esophageal brachytherapy include necrosis, keratin formation, fibrosis, and giant cell reaction in all layers of the esophageal wall [17]. As such, the rapid shrinkage of luminal tumor masses results in prompt resolution of symptomatic dysphagia. While the ideal dosing of intraluminal brachytherapy in esophageal cancer remains unclear, a dose of 16 Gy in 2 fractions over 2 weeks gave identical results as 18 Gy in 3 fractions over 3 weeks [17,18].

Radiotherapy has also been shown to lead to significant alterations in the tumor microenvironment, including effects on immune cells. This effect is thought to primarily be due to the release of tumor-derived antigens and pro-inflammatory cytokines [19-21], a process known as immunologic cell death, which leads to the induction of an antitumor immune response [22]. Radiation-induced cell death results in release of reactive oxygen species and damage-associated molecular patterns (DAMPs) which activate local antigen-presenting cells to enhance the priming of tumor-specific T cells [23-26]. Furthermore, the local induction of a radiation-induced tumor-specific T cell response has been shown to result in reduction of systemic, non-radiated, tumor burden in a CD8+ T cell dependent manner [27]. The generation of a systemic anti-tumor immune response associated with local tumor therapy is referred to as the abscopal effect [28].

An area of active interest is focused on combining local therapy with immunotherapy to optimize the abscopal effect in metastatic cancer [29-33]. Preclinical models have supported a synergistic effect between local radiotherapy and checkpoint blockade; however, these studies have largely been performed using CTLA-4 checkpoint blockade, another T cell inhibitory molecule[34-38]. Interestingly, several recent studies have suggested that PD-1/PD-L1 expression actually mediates resistance to fractionated radiotherapy with amelioration of the abscopal response, and that inhibition of the PD-1/PD-L1 axis can overcome this resistance leading to improved systemic immune responses against non-radiated metastatic lesions following local fractionated radiotherapy [39,40]. Thus, these data support the use of local fractionated radiation combined with checkpoint blockade as a rationale therapeutic strategy for the treatment of metastatic cancer.

1.4 Pembrolizumab (MK-3475)

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

and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

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

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

1.4.1 Rationale for Dosing

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

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

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

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

with tumor type.

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

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

1.5 Study Rationale

The abscopal effect is a phenomenon in which local radiotherapy is associated with the regression of metastatic lesions distant to the irradiated site, and is thought to be mediated by a systemic anti-tumor immune response generated by local immune activation[41]. The intensity of the radiotherapy-elicited immune response has been shown to be positively associated with the local response [42]. Therefore, hypofractionated radiotherapy would be expected to more effectively elicit an anti-tumor immune response than standard radiation, and this has been supported in preclinical studies [43]. Furthermore, increased expression of PD-1 and PD-L1 have been shown to abate the generation of an abscopal response following fractionated radiotherapy in preclinical mouse models but regression of non-radiated metastases is restored when hypofractionated radiotherapy is combined with PD-1/PD-L1 blockade [39,40] supporting the rationale for combining hypofractionated local radiation with checkpoint blockade in the treatment of metastatic cancer.

Unfortunately, there is a paucity of data evaluating the effect of this combinatorial approach in esophageal cancer.

High dose-rate intraluminal brachytherapy is a well-established tool for palliation of dysphagia in patients with metastatic esophageal cancer [44]. Compared with external beam radiotherapy, brachytherapy has the appeal of delivering highly localized, intense doses of hypofractionated radiation to the tumor tissue with minimal exposure of surrounding normal tissues to radiation. Limiting exposure to surrounding normal tissue has additional appeal in the use of immune stimulatory approaches, as recent reports have demonstrated a correlation between radiation dose to normal tissues (such as lung) and lymphopenia with a resultant decrease in overall survival [45]. Furthermore, esophageal cancers have been shown to have high expression of PD-L1 and an immunosuppressive microenvironment. Therefore, it is our hypothesis that a short course of intense radiation therapy will generate a systemic tumor-specific T cell response that will be enhanced by PD-1 inhibition resulting in systemic antitumor efficacy.

We therefore propose to treat patients with metastatic esophageal cancers and dysphagia with two fractions of brachytherapy followed by pembrolizumab. The brachytherapy is hypofractionated and will provide a radiation dose of sufficient intensity to induce the release of tumor-derived antigens and trigger an antitumor immune response. The simplicity of the design should maximize the chance to examine the hypothesis that radiotherapy can induce an immune response, which can then be augmented by pembrolizumab treatment. Success in this study would provide the impetus to conduct further trials aimed at developing this unique strategy as a more broadly applicable therapeutic option in the treatment of patients suffering from these deadly cancers, and will provide important mechanistic insights into the relationship between radiation treatment and immune therapy augmentation.

Taken together, these data indicate that targeting the PD-1/PD-L1 axis in esophageal cancers in combination with radiation therapy may be a rational treatment strategy for these cancers.

1.6 Correlative Studies Background

The clinical correlates of this study will have two aims:

- Aim 1: Identify tumor-associated and systemic biomarkers that predict response to the combination of pembrolizumab and brachytherapy in patients with esophageal cancer.
- Aim 2: Characterize the immune response at baseline (pre-brachytherapy), post-brachytherapy, and post-pembrolizumab to identify immune parameters associated with protection and the development of an abscopal effect.

Peripheral blood samples will be drawn at baseline (pre-brachytherapy), Day 1 (pre-pembrolizumab), and post-pembrolizumab on Day 22, 3 months, 6 months, 12 months, and/or at time of progression (no further blood samples will be drawn after progression if occurs before 12 months). Peripheral blood samples will be used to characterize and quantify the immune response following brachytherapy and pembrolizumab.

Endoscopic evaluation with biopsies will be performed prior to brachytherapy (all patients), after brachytherapy/pre-pembrolizumab (8 of 18 patients), and at 2-6 months post-pembrolizumab (all patients) to assess the local immune environment.

1.6.1 Aim 1: Biomarker assessment

PD-L1 expression on tumor cells has been used as a clinical biomarker to correlate with response to PD-1 checkpoint blockade. However, there remains debate regarding an appropriate cut-off to define positivity. Moreover, there are a substantial percentage of patients who benefit from therapy who are considered negative for PD-L1 expression, which may be associated with uninhibited interactions between PD-1/PD-L2 or PD-L1/CD80, which also induce immune regulation. Furthermore, it is not clear what should be considered an appropriate definition of positivity. Lastly, there have been no studies to date that have

demonstrated concordance/discordance in checkpoint inhibitor expression between primary and metastatic lesions. Therefore, Aim 1 will address two primary goals:

- Quantify expression of PD-1, PD-L1, PD-L2, and CTLA-4 (an alternative checkpoint pathway) by IHC on tumor cells and tumor infiltrating lymphocytes (TILs) to determine if there is an association between response or resistance to brachytherapy + pembrolizumab. If an association is detected, the level of expression on either tumor and/or TIL that adequately defines positivity of associated molecule will be defined to predict patients that would most likely benefit from brachytherapy and pembrolizumab therapy.
- Compare checkpoint inhibitor expression levels of PD-1, PD-L1, PD-L2, and CTLA-4 in paired primary and metastatic biopsies. This data will allow determination of frequency of concordant or discrepant expression of various mechanisms of checkpoint inhibition between primary and metastatic disease sites. This information can help clarify potential etiology of any mixed clinical responses (primary response and metastatic lesion progresses, or vise versa). It would also help determine associations that would predict the potential development of an abscopal effect.

1.6.2 Aim 2: Immune characterization

The tumor infiltrating lymphocytes (TIL) have been studied in esophageal cancer and are generally felt to be associated with good prognosis.[46-49] The presence of TILs has been used as justification for the presence of immunosurveillance and thus the rationale for the use of immunotherapy. While the presence of CD8+ cytotoxic T cells within the TIL have predominantly been associated with protection, there is often the presence of immunosuppressive subsets such as regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) that are associated with poor prognosis.[50,51] The interplay between these effector and suppressive subsets in determining the response to checkpoint inhibitor therapy has yet to be elucidated. Therefore, Aim 2 will characterize the tumor immune infiltrate and circulating immune cells using the following approaches:

- TCR profiling by spectrotyping and flow cytometry to assess changes in relative frequency of TCR gene utilization as a surrogate of clonal expansion and selection of the CD4 and CD8 T cell repertoire (i.e. tumor-specific T cell response) following brachytherapy and pembrolizumab.
- Flow cytometry and CyTOF to identify and quantify the immunophenotype of various effector immune subsets to determine changes in immune profile between baseline, post-brachytherapy, and post-pembrolizumab. Correlations between treatment response and resistance will be determined to predict potential patients that will benefit from combined brachytherapy and pembrolizumab. The following immune subsets will be assessed:
    - T cell: CD3, CD8, CD4, Foxp3 (Tregs), t-bet (Th1), ROR-γ (Th17), effector memory T cells (Tem), central memory T cells (Tcm); intracellular staining for IFN-γ, TNF-α, IL-2, IL-17, IL-4, IL-10 will be determined to assess functional capacity of T cells before and after brachytherapy and pembrolizumab.

    - NK cell: CD56, CD16
    - MDSC: CD14, HLA-DR
    - Activation markers: ICOS, OX40, CD137, CD25, GITR
    - Inhibitory markers: PD-1, PD-L1, PD-L2, CTLA-4, TIM-3, LAG-3
- Multiplex analysis and QPCR to assess changes in cytokine profile: IFN-α, IFN-β, IFN-γ, IL-2, TNF-α, IL-2, IL-4, IL-5, IL-13, IL-10, IL-6, TGF-β.
- Tumor cells will be microdissected from tissue biopsies and sent for whole exome sequencing and RNAseq to quantify the mutational landscape as a surrogate of antigenic burden. As antigenic burden has been correlated with response to checkpoint inhibitor therapy, we will determine if response to brachytherapy and pembrolizumab is associated with mutational burden.[52-54]

2.0 OBJECTIVES

2.1 Primary Objective

To determine the tolerability of localized esophageal hypofractionated brachytherapy administered in two fractions when combined with pembrolizumab in patients with metastatic esophageal carcinoma.

2.2 Secondary Objectives

1. To assess the local antitumor effect of hypofractionated brachytherapy followed by pembrolizumab as determined by endoscopic measurements of change in tumor length and esophageal lumen diameter and by grade of dysphagia per CTCAE criteria.
2. To assess the systemic efficacy by irRC-based criteria of hypofractionated brachytherapy to the esophagus combined with systemic pembrolizumab on non-radiated metastatic lesions.
3. To determine the preliminary antitumor efficacy of pembrolizumab combined with local hypofractionated brachytherapy in patients with metastatic esophageal carcinoma.

2.3 Exploratory Objective

1. To determine the expression profile of activating and inhibitory molecules in tumor samples after hypofractionated brachytherapy and pembrolizumab therapy to identify biomarkers correlated with response or resistance.
2. To annotate the local and distant immune response after hypofractionated brachytherapy and pembrolizumab to determine mediators of protection and development of an abscopal effect.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Any patient with metastatic esophageal cancer that is deemed a candidate for brachytherapy for local control or treatment of dysphagia as determined by treating physician.

2. Presence of an evaluable metastatic lesion (locoregional lymph nodes are acceptable).

3. At least 18 years of age.

4. ECOG performance status 0-2 (see Appendix A)

5. Adequate bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500/mcL
   b. Platelets ≥ 100,000/mcL
   c. Hemoglobin ≥ 9 g/dL
   d. Total bilirubin ≤ 1.5 x IULN
      OR
      Direct bilirubin ≤ IULN for patients with total bilirubin > 1.5 x IULN
   e. AST(SGOT)/ALT(SGPT) ≤ 2.5 x IULN (or ≤ 5 x IULN for patients with liver metastases)
   f. Serum creatinine ≤ 1.5 x IULN
      OR
      Creatinine clearance by Cockcroft-Gault ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels > 1.5 x IULN
   g. INR or PT ≤ 1.5 x IULN unless patient is receiving anticoagulant therapy as long as INR or PTT is within therapeutic range of intended use of anticoagulants
   h. aPTT ≤ 1.5 x IULN unless patient is receiving anticoagulant therapy as long as INR or PTT is within therapeutic range of intended use of a anticoagulants

6. Sexually active women of childbearing potential and men must agree to use contraceptive methods as described in Section 5.5 prior to study entry, for the duration of study participation, and for 120 days after the last dose of pembrolizumab. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

7. Either enrolled in HRPO# 201107221 ("Tissue and Blood Acquisition for Genomic Analysis and Collection of Health Information for Patients with Gastrointestinal Cancers"), which facilitates the collection of specimens for correlative studies, or consenting to collection of blood and tissue as part of this protocol for research testing.

8. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Prior treatment with an anti-PD-1, anti-PD-L1 or anti-PD-L2 agent.

2. Received a live vaccine within 30 days prior to the first dose of pembrolizumab. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette-Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. FluMist) are live attenuated vaccines and are not allowed.

3. Presence of a concurrent active, incurable malignancy that may alter the outcome of the treatment for esophageal cancer as determined by the treating physician.

4. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of pembrolizumab.

5. Currently receiving any other investigational agents, has participated in a study of an investigational agent, or use of an investigational device within 4 weeks of the first dose of pembrolizumab.

6. Has received systemic therapy within 4 weeks of the first dose of pembrolizumab.

7. Known active central nervous system metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 weeks prior to the first dose of MK-3475 and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

8. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab or other agents used in the study.

9. Uncontrolled intercurrent illness that would limit compliance with study requirements. This would include, but is not limited to: ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, immunosuppression, autoimmune conditions, underlying pulmonary disease, or psychiatric illness/social situations.

10. Has an active autoimmune disease requiring systemic treatment within the past 2 years (i.e. with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

11. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.

12. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 72 hours of study entry.

13. Known history of hepatitis B (defined as hepatitis B surface antigen [HBsAg] reactive) or known active hepatitis C virus (defined as HCV RNA [qualitative] is detected).

14. Known history of active TB.

15. Known history of HIV (HIV 1/2 antibodies).

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

This is a single institution, open-label, single-dose-level, pilot study combining hypofractionated brachytherapy with standard dose pembrolizumab.

5.1 Premedication Administration

No premedications are required, but antiemetics may be given as per institutional practice if needed.

5.2 Agent Administration

Brachytherapy can be given at any point within the esophagus. It will be delivered using a high-dose-rate Ir-192 afterloader via a dedicated esophageal applicator. The prescription dose will be 16 Gy delivered in 2 fractions of 8 Gy per fraction, separated by a 7 to 10 day interval between fractions. The dose will be prescribed at 1 cm from the source axis, with a 1 to 2 cm proximal and distal margin at the discretion of the treating radiation oncologists. The maximum total active length is not to be > 10 cm. CT simulation is preferred, though fluoroscopic simulation is acceptable.

Pembrolizumab will be started within 1 week (± 3 days) after completion of brachytherapy at a standard dose of 200 mg administered as an intravenous infusion over 30 minutes (-5 minutes/+10 minutes). It will be given every 3 weeks.

5.3 Evaluability

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death.

All patients are evaluable for disease response unless they discontinue treatment due to treatment related adverse events(s) prior to completion of Cycle 2 and have not had any disease assessment.

5.4 General Concomitant Medication and Supportive Care Guidelines

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

Patients are prohibited from receiving the following therapies while in screening for and enrolled in this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol

- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy other than brachytherapy as dictated in this protocol
- Live vaccines within 30 days prior to the first dose of treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved.

5.4.1 Management of Infusion Reactions

Pembrolizumab may cause severe or life-threatening reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

The table below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Infusion Reaction Treatment Guidelines

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

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum or urine pregnancy test within 72 hours prior to study entry and again within 72 hours prior to the first dose of pembrolizumab.

Pembrolizumab may have adverse effects on a fetus in utero.

For this trial, male subjects will be considered to be of non-reproductive potential if they

have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below). Women in the following categories are not considered of childbearing potential:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

    Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

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

<u>Male Participants</u>:

Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined timeframe:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in the table below when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

- Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in the table below during the protocol-defined timeframe.

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of < 1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen-containing) hormonal contraception [b, c] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b, c] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant [b, c] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e. when used consistently and correctly). b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least [X days, corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential] after the last dose of study treatment. c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test.

Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected, after the last dose of study treatment, and as required locally.

Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.7 Duration of Follow-up

Patients will be followed every 3 weeks while receiving pembrolizumab. After progression, patients will be followed every 3 months for 12 months or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in the table below.

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent grade 2	Permanently discontinue		
Diarrhea / colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (i.e. diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e. peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with 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.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased Bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (e.g. propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or Permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (e.g. levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation		

		include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		
1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. **NOTE:** For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).				

For information on the management of infusion reactions, see Section 5.4.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 7.4. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

Merck & Co, Inc. requires that all serious adverse events, suspected unexpected serious adverse reactions, pregnancies, incidences of lactation, and events of clinical interest (refer to Section 7.5.2) be reported as outlined in Section 7.5.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

Study team will only collect those lab results that are clinically significant as determined by the investigator.

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death

- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- A new cancer
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Suspected Unexpected Serious Adverse Reaction

Definition: any serious adverse event, the nature, severity or frequency of which is not consistent with information in the most current investigator's brochure, or with respect to a marketed product the most current Summary of Product Characteristics (SPC) or Package Insert.

7.1.7 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.8 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.9 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies

as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

7.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 7.1.4) associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 7.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information.

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

7.5 Reporting to Merck & Co., Inc.

The PI shall forward to Merck's Global Safety group (FAX 215-661-6229) any SAE (Section 7.1.2) and SUSAR (Section 7.1.6) information, including, but not limited to, all

initial and follow-up information involving any study subject in the study within 2 business days or 3 calendar days (whichever comes first) of learning of the SAE or SUSAR.

7.5.1 Reporting Pregnancy and Lactation

Although pregnancy and infant exposure during breastfeeding 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) that occurs during the trial.

Pregnancies and infant exposures during breastfeeding that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and infant exposures during breastfeeding that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

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

7.5.2 Reporting Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 661-6229).

Events of clinical interest for this trial include:

- an overdose of Merck product, that is not associated with clinical symptoms or abnormal laboratory results.
- an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal,

as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

7.5.3 Reporting Overdose

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

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

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

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 661-6229)

7.6 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment.

Study team will only collect those lab results that are clinically significant as determined by the investigator.

8.0 PHARMACEUTICAL INFORMATION

8.1 Pembrolizumab

8.1.1 Pembrolizumab Description

Pembrolizumab is a potent humanized IgG4 mAb with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is being advanced for clinical development as an IV immunotherapy for advanced malignancies.

8.1.2 Clinical Pharmacology

Refer to Section 5.2 of the IB.

8.1.3 Pharmacokinetics and Drug Metabolism

Refer to Section 5.2 of the IB.

8.1.4 Supplier

Pembrolizumab will be provided free of charge by Merck & Co., Inc.

8.1.5 Dosage Form and Preparation

Merck will provide pembrolizumab as a liquid drug product.

8.1.6 Storage and Stability

Pembrolizumab should be stored under refrigerated conditions (2°C - 8°C).

If not used immediately, vials and/or IV bags may be stored at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours.

8.1.7 Administration

Pembrolizumab will be given intravenously over the course of 30 minutes (-5 min/+10 min) on an outpatient basis.

9.0 CORRELATIVE STUDIES

9.1 Collection of Specimen(s)

For patients who have consented to HRPO# 201107221, all tumor and peripheral blood samples will be collected under that consent, as it is a tissue bank which authorizes permission for cellular, molecular, and genomic characterization and return of information.

For patients who have not consented to HRPO# 201107221, all specimens will be collected as part of this protocol. All samples will be transported and stored at the Tissue Procurement Core unless otherwise indicated.

9.1.1 Endoscopic biopsy

All enrolled patients will undergo endoscopic biopsy per standard of care protocol at time of initial diagnosis/time of enrollment and 2-6 months after initiation of pembrolizumab. At this time, further passes will be made so that 4 to 8 additional cores will be taken for research purposes. At 1-2 weeks after initiation of brachytherapy, eight consenting patients will undergo a research-related optional endoscopic biopsy (not coinciding with a SOC endoscopy) consisting of 4 to 8 cores. These biopsies will be used to assess changes in the local tumor microenvironment following brachytherapy.

All specimens will be taken to the Tissue Procurement Core fresh and then processed according to the instructions in HRPO# 201107221 (frozen in OCT).

9.1.2 Peripheral blood

Peripheral blood samples will be collected at the following time points:

- Pre-brachytherapy
- Post-brachytherapy but pre-pembrolizumab (on day 1)
- Day 22 after the start of pembrolizumab
- 3, 6, and 12 months (+/- 2 weeks) after the start of pembrolizumab
- Time of progression

Six collection tubes (BD Vacutainer® sodium heparin (green top), REF 367874, 10 mL each for a total of approximately 60 mL) are filled by venipuncture at each time point. Blood samples will be transported to the Tissue Procurement Core within one hour of collection. PBMC will be obtained by Ficoll-Hypaque gradient centrifugation and cryopreserved in 10% DMSO according to standard procedures.

Nucleic acid isolation: DNA will be isolated from PBMC by the LTP for exome sequencing at The Genome Institute to serve as reference for germline mutations. To identify somatic mutations, DNA and RNA will be extracted from OCT-embedded banked tissue. The OCT block will be delivered to the LTP where the block will be sectioned and stained in order to confirm the presence of tumor, determine tumor/normal ratio, and guide isolation of tumor cells by, for instance, laser capture microdissection (LCM). All tissue selected for sequencing will be processed into a single-cell suspension by mechanical and enzymatic digestion, and used to extract nucleic acids. Tumor DNA + RNA will then undergo tumor exome and tumor cDNA-capture sequencing, respectively, at The Genome Institute.

Plasma and lymphocyte separation: PBMC will undergo centrifugation with Ficoll to separate the plasma and cellular (lymphocyte) layers from RBC. Both the plasma and cellular components will be frozen and stored at -80°C for further studies.

9.2 Immune Monitoring

9.2.1 Immune profiling

Patient-derived PBMC will be used to quantify and characterize the immunologic landscape at baseline and at various time points post-brachytherapy and pembrolizumab. Single cell suspensions will be isolated as described above. All correlative studies will performed in the Immune Monitoring Laboratory at the Center of Human Immunology and Immunotherapy Programs at Washington University.

The T cell repertoire pre- and post- brachytherapy and pembrolizumab will be further characterized by analyzing the TCR gene utilization of circulating PBMCs. This will be done by multi-parametric flow cytometry, spectratyping, and sequencing. MHC class I and II tetramers will be generated either in the Immune Monitoring Laboratory at the Center of Human Immunology and Immunotherapy Programs at Washington University, or the NIH Tetramer Core Facility.

Phenotypic and polyfunctional characterization of tumor-specific T cells will be performed by ELISPOT, multi-parametric flow cytometry, and mass cytometry (CyTOF) in the Immune Monitoring Laboratory at the Center of Human Immunology and Immunotherapy Programs at Washington University.

Serum will be analyzed for changes in cytokine expression following brachytherapy and pembrolizumab. Cytokine levels will be quantified using ELISA, QPCR, and multiplex analysis and performed in the Immune Monitoring Laboratory at the Center of Human Immunology and Immunotherapy Programs at Washington University.

9.2.2 Microenvironment characterization

Tumor samples obtained from primary and metastatic biopsies will be analyzed for biomarkers (PD-1, PD-L1, PD-L2, and CTLA-4) that are associated with response to brachytherapy and pembrolizumab to identify individuals who may benefit most from this therapeutic approach. These will be sent-out to contracted pathology laboratories for analysis.

PD-L1 staining will be performed by QualTek Molecular Laboratories. Five unstained slides from FFPE tissue samples should be shipped cold (2-8°C) using the materials provided by QualTek to:

MISP Receiving

QualTek Molecular Laboratories
300 Pheasant Run
Newtown, PA 18940

IHC staining of OCT embedded tissue will characterize the location of various infiltrating lymphocytes relative to tumor. Furthermore, single cell suspensions can be made from fresh frozen tissue to characterize the phenotype of various populations of infiltrating leukocytes (T cells, B cells, NK cells, monocytes/macrophages, dendritic cells) by multi-parametric flow cytometry and mass cytometry. Expression of various activation and inhibitory markers on the TIL will also be assessed.

Mutation burden will be determined by whole exome sequencing. PBMC will be used to obtain germline sequences for comparison to isolated tumor from tissue biopsies (somatic mutations). Isolated tumor DNA + RNA will then undergo tumor exome and tumor cDNA-capture sequencing, respectively, at The Genome Institute or through a commercially-available, CLIA/CLEP-certified assay.

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted no more than 4 weeks prior to start of protocol therapy. Cycles of pembrolizumab are 21 days. There is a +/- 3 day window for each visit.

	Screening / baseline	**Pre-brachy**	**Brachy**	**Post-brachy**	**C1D1**	**C2D1**	**Day 1 of each subsequent cycle**	**End of every 3rd cycle**	**3 mos[1]**	**6 mos[1]**	**12 mos[1]**	**EOT**	**F/U[12]**
Informed consent	X												
H&P, ECOG PS	X				X	X	X						
CBC	X				X	X	X						
CMP	X				X	X	X						
aPTT, PT/INR	X												
β-hCG[2]	X[3]				X[5]								
T3, FT4, TSH	X				X		X[13]						
Urinalysis	X				X		X[13]						
PET/CT or CT or MRI	X							X				X	
Brachytherapy			X[4]										
Pembrolizumab					X[6]	X	X						
Endoscopic biopsy		X		X[7, 14]					X[8,9]				
Blood for PBMCs		X		X[7]		X[10]			X	X	X	X[11]	
Survivor Follow-up													X[15]
AE assessment	Patients will be followed for AEs for 30 days following the last dose of pembrolizumab.												

1. After the start of pembrolizumab.
2. Women of childbearing potential only.
3. No more than 72 hours before start of brachytherapy.
4. 2 fractions with 7-10 days between fractions.
5. No more than 72 hours before start of pembrolizumab.
6. Treatment with pembrolizumab will start approximately 1 week following the end of brachytherapy.
7. Prior to first dose of pembrolizumab.
8. 8 patients only.
9. 2 to 6 months after the start of pembrolizumab.
10. Prior to that day's dose of pembrolizumab.
11. Time of progression.
12. Every 3 months for 12 months after patient comes off study.
13. Every odd-numbered cycle.
14. Optional
15. Follow-up can be done via med review in the electronic medical record, phone calls, or medical request from an outside facility.

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Brachytherapy Form	With each fraction of brachytherapy
Treatment Form	End of each cycle
Toxicity Form	Continuous
Correlatives Form	Prior to brachytherapy Post-brachytherapy End of Cycle 2 Beginning of Cycle 4 3 Months 6 Months 12 Months
Treatment Summary Form	Completion of treatment
Follow Up Form	Every 3 months for 12 months
MedWatch Form	See Section 7.0 for reporting requirements

*We are not capturing abnormal labs unless they are clinically significant as deemed by the investigator

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, response and progression will be evaluated in this study using the Immune-Related Response Criteria (irRC) guidelines.

12.2 Immune-Related Response Criteria [Ref Wolchok Clin Cancer Res 2009]

For the purposes of this study, patients should be re-evaluated for response/progression every 9 weeks. A confirmatory scan should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using immune-related response criteria. For the immune-related response criteria, only index and measurable new lesions are taken into account. At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions (5 lesions per organ, up to 10 visceral lesions and 5 cutaneous index lesions) is calculated. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of the index lesions and of new, measurable lesions (≥ 5 x 5 mm; up to 5 new lesions per organ: 5

cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden:

$$\text{Tumor Burden} = SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$$

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out immune-related progressive disease). Decreases in tumor burden must be assessed relative to baseline measurements. The immune-related response criteria were derived from WHO criteria and, therefore, the thresholds of response remain the same.

The overall response according to the immune-related response criteria is derived from time-point response assessments (based on tumor burden) as follows:

- irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions) – confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented
- irPR, decrease in tumor burden ≥ 50% relative to baseline – confirmed by a consecutive assessment at least 4 weeks after first documentation
- irSD, not meeting criteria for irCR or irPR, in absence of irPD
- irPD, increase in tumor burden ≥ 25% relative to nadir (minimum recorded tumor burden) – confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented

Patients are considered to have irPR or irSD even if new lesions are present as long as they meet the respective thresholds of response as described above. Furthermore, patients are not considered to have irPD if new lesions are present and the tumor burden of all lesions does not increase by at least 25%.

12.2.1 Treatment Beyond Progression

Accumulating evidence indicates that a minority of patients treated with immunotherapy may derive clinical benefit despite initial evidence of radiographic PD.

Patients will be permitted to continue on study treatment beyond initial radiographic PD, as long as they meet the following criteria:

• Patient had investigator-assessed clinical benefit.
• Patient has stable performance status.
• Patient is tolerating study treatment.
• Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases).

The decision to continue study treatment beyond initial investigator-assessed progression should be clearly documented in the study records. A follow-up scan

should be performed at the next scheduled imaging evaluation (but no sooner than 6 weeks) to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the patient is clinically deteriorating and unlikely to receive any benefit from continued study treatment. If the investigator feels that the patient continues to achieve clinical benefit by continuing study treatment, the patient should remain on the study.

12.3 12.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least monthly (or before each dose-escalation if occurring sooner than monthly), and provide a semi-annual report to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

14.0 STATISTICAL CONSIDERATIONS

14.1 Study Design

This single arm, phase I study will primarily investigate the tolerability of combining pembrolizumab with locally delivered radiation therapy for the treatment of patients with metastatic esophageal carcinoma.

14.2 Study Endpoints

The primary endpoint is the safety as measured by treatment related adverse events. Secondary endpoints are progression-free survival (PFS) and overall survival (OS) for all the metastatic esophageal carcinom**a** patients, and the evaluation of antitumor efficacy including the total tumor size of all the target lesions based on irRC criteria, the length of esophageal tumor, the lumen size, and the dysphagia grade measured prior to brachytherapy, after brachytherapy/pre-pembrolizumab, and at 8 weeks post-pembrolizumab. Exploratory endpoints are the quantified tumor-associated and systemic biomarkers and immune responses obtained through tumor tissue biopsies and peripheral blood at baseline (pre-brachytherapy), post-brachytherapy, and post-pembrolizumab.

14.3 Data Analysis

Demographic, clinical characteristics and treatment related adverse events will be summarized using descriptive statistics. PFS and OS will be analyzed by Kaplan-Meier (KM) method. Paired t-test and/or paired-sample Wilcoxon Signed Rank test will used to compare the total tumor size of all the target lesions, tumor length, lumen size, dysphagia grade, and antitumor immune responses before and after hypofractionated brachytherapy and pembrolizumab therapy.

14.4 Power Analysis and Sample Size

The primary objective of this study will be to evaluate the safety/tolerability of this novel radiation plus drug combination. A design has been chosen which will provide a reasonable ability to detect serious adverse events (SAE) and overall tolerability associated with the treatment. Sample size calculations for safety are expressed in terms of the ability to detect SAEs. The ability of the study to identify SAE/MTD is best expressed by the maximum true rate of events that would be unlikely to be observed and the minimum true rate of events that would very likely be observed. Specifically, for the sample size in the study (n=18 patients), there is at least 85% chance of observing at least 1 SAE/MTD if the true rate of such an event is at least 0.10. Conversely, there is at least 90% chance that we would not observe at least 1 SAE if the true rate is less than 0.006. The secondary objective of this trial is to evaluate the systemic response rate of the novel therapeutic approach. We have chosen to power the study to provide a reasonably reliable estimate of the ability of this approach to induce a positive outcome as measured by irRC criteria (please note that stable disease denotes efficacy by irRC criteria). As an example if we observe 5 responses out of 18 trial subjects, our 95% exact binomial confidence interval for the true rate will range from 0.10 - 0.53. If we observe 9 responses (50%), our 95% exact binomial confidence interval for the true rate will range from 0.26 to 0.74.

14.5 Accrual

It is estimated 18 patients will be enrolled within 2 and a half years' time.

14.6 Toxicity and Plans for Data and Safety Monitoring (Stopping Rule)

Toxicity will be reviewed on a continuous basis. Early stopping of this trial will be based on the excessive treatment related adverse events. By assuming that a toxicity rate of 1/6 or less is acceptable and that a toxicity rate of 1/3 or more would definitely be unacceptable, based on the sequential probability ratio test (SPRT) with 80% power and 0.05 significance level, the study will be halted if 5 of the first 8, or 6 of the first 12 patients experience unacceptable toxicities, or if the 7^{th} unacceptable toxicity is observed before the 15^{th} patient has completed the trial.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.