Protocol Title

Single-arm phase 2 study to examine pembrolizumab and concurrent radiation to induce an abscopal effect in patients with previously treated carcinoma of unknown primary (CUP16-268)

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PROTOCOL SIGNATURE PAGE

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I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable institutional review board(s).

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Signature of Site Investigator Date

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Site Investigator Name (printed)

_____

Site Investigator Title

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Name of Facility

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SYNOPSIS

TITLE	Single-arm phase 2 study to examine pembrolizumab and concurrent radiation to induce an abscopal effect in patients with previously treated carcinoma of unknown primary (CUP16-268)
SHORT TITLE	Phase 2 study of pembrolizumab and concurrent radiation in patients with previously treated carcinoma of unknown primary
PHASE	II
OBJECTIVES	Primary Objective: Evaluate the abscopal response rate in CUP patients treated with the combination of pembrolizumab plus radiotherapy. Secondary Objectives: • Determine the response rate by RECIST 1.1 and informed by irRECIST, of non-irradiated metastatic sites when pembrolizumab is combined with radiotherapy. • Evaluate treatment-related toxicity. • Evaluate PFS, OS, TTP, and DCR • Explore the association between RR and other endpoints (e.g., OS, PFS) Exploratory Objectives: • Evaluate the association of MSI status and mutation load with clinical efficacy • Evaluate the association of tissue PDL1 expression with clinical efficacy • Evaluate the association of soluble PD-L1 with clinical efficacy • Evaluate changes in the CD11a^{high} PD-1^{high} CD8+ T effector population in association with pembrolizumab-based therapy • Evaluate changes and predictive value of BIM expression in T cells in circulation and the predictive value of BIM/PD-1 expression in tumor infiltrating lymphocytes • Evaluate the prognostic and predictive value of tumor tissue of origin testing
STUDY DESIGN	This is a proof-of-principle single-arm phase 2 study in patients with previously treated CUP. All patients receive pembrolizumab combined with RT to a metastatic site, so as to induce an abscopal tumor response. The treatment combination will be repeated with RT delivery to a second metastatic site in a non-overlapping RT field. The results will be compared with historical control. The primary endpoint is the confirmed response rate (RR) in a non-irradiated site based on best responding abscopal lesion. This study will also evaluate the

	following secondary endpoints: RR in a non-irradiated site based on RECIST 1.1, adverse events, progression-free survival (PFS), overall survival (OS), time-to-progression (TTP), and disease control rate (DCR).
ELIGIBILITY CRITERIA	**Inclusion Criteria** 1. Written informed consent and HIPAA authorization for release of personal health information prior to registration. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately. 2. Age ≥ 18 years at the time of consent. 3. ECOG Performance Status of ≤ 2 within 28 days prior to registration. 4. Archival tissue must be available and identified during screening and shipped prior to Day -21. If archival tissue is not available and the subject is not undergoing a standard of care biopsy, the subject must undergo a research biopsy to obtain fresh tissue prior to start of treatment. 5. Carcinoma of unknown primary after the following diagnostic procedures have been performed if clinically indicated and are unrevealing of the primary site: • Complete history and clinically appropriate physical • CT scan of chest, abdomen, and pelvis • Directed evaluation of symptomatic areas • Mammogram in women • Colonoscopy in patients with liver metastasis or an elevated CEA • Direct pathologic comparison with prior tumor specimens, where possible, even if prior tumor is early-stage or clinically remote from current disease **NOTE:** Immunohistochemical stains will be performed according to institutional standards. If a primary carcinoma is identified, the patient should undergo treatment as appropriate for that primary tumor and not be enrolled in the study. The above diagnostic workup does not need to be performed if: (1) it was previously completed at the time of original diagnosis or (2) the investigator does not believe the workup has clinical utility at the current time, given that the patient has received interval therapy. 6. Histologic confirmation of metastatic adenocarcinoma, poorly differentiated non-small cell carcinoma, or poorly differentiated squamous carcinoma. **NOTE:** Pathology consultation at Mayo Clinic is recommended if clinically indicated. One scenario is where unknown primary is the most likely diagnosis but immunostains show

relatively site-specific marker staining (e.g., CD45, TTF1, chromogranin, GATA3, PAX8, PSA, melanocytic markers). Information provided for pathology consultation should include recent H&P and imaging reports.

7. If and when available, submission of genomic sequencing and expression profiling results is mandatory. Results of testing are not required prior to treatment.

8. At least one measurable lesion (per RECIST 1.1) outside the planned RT fields.

9. Stable or progressive disease after, or was unable to tolerate, at least one line of prior anticancer therapy for this disease. **NOTE:** For patients with stable disease, it is strongly encouraged to confirm the presence of active disease (eg, demonstrating FDG avidity via PET or repeat biopsy).

10. Radiation oncology consultation at enrolling site ≤ 56 days prior to registration to confirm at least two metastatic lesions which are targetable by RT at doses and schedule prescribed in this study and which reside in non-overlapping RT fields.

11. Demonstrate adequate organ function as defined in the table below. All screening labs to be obtained within 28 days prior to registration.

System	Laboratory Value
Hematological	
Absolute Neutrophil Count (ANC)	≥ 900 K/mm^3
Hemoglobin (Hgb)	≥ 8.5 g/dL without transfusion or/ EPO dependency (≤ 7 days prior to assessment)
Platelets	≥ 90,000 / mcL
Renal	
Creatinine **OR** Calculated creatinine clearance[l]	≤ 1.5 X upper limit of normal (ULN) **OR** ≥ 60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Bilirubin	Total bilirubin ≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN OR total bilirubin ≤2 X ULN if liver metastases are present
Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	> 2.5 g/dL

[1] Cockcroft-Gault formula will be used to calculate creatinine clearance (See SPM)

10. Females of childbearing potential must have a negative serum pregnancy test within 72 hours prior to registration. **NOTE:** If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. **NOTE:** breast milk cannot be stored for future use while the mother is being treated on study

11. Females of childbearing potential and males must be willing to abstain from heterosexual activity (abstinence) or use effective methods of contraception as described in Section 5.5 from the time of informed consent until 120 days after treatment discontinuation. **NOTE:** Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months

12. Willingness to return to the enrolling institution for follow up

13. Willingness to provide tissue and blood samples for correlative research purposes

Exclusion Criteria

14. Prior radiation to an area of the body which, if included in the current radiation field, poses an unacceptably high risk of toxicity in the opinion of the investigator. NOTE: A prior field that overlaps with the current field, by itself, does not exclude the patient.

15. Any of the following
    - Melanoma. **NOTE:** Positive tumor staining for S-100 or HMB45 alone does not exclude patients.
    - If immunostains are performed, and any of the below tests are positive:
        - Hematologic CD45+ (others such as CD2, CD20, CD30, CD43 also suggest hematologic origin)
        - Lung or thyroid origin (Thyroid Transcription Factor [TTF-1]). **NOTE:** Patients with biopsy proven TTF-1 positive tumor who do not have clinical evidence for either lung or thyroid cancer (e.g. a dominant lung mass) are still eligible.

16. Progressed on 4 or more lines of prior chemotherapy for this cancer. NOTE: Bisphosphonates and neoadjuvant/adjuvant anticancer therapies (including locally directed therapies) do not count as a line of therapy with regard to this exclusion criteria.

	17. Co-morbid systemic illnesses or other severe concurrent disease which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens. 18 Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment. 19 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. 20 Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. 21 Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies). 22 Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). 23 Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. **NOTE:** Inhaled steroids or steroid injections for joint disease are allowed. 24 Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). 25 Prior severe allergic reactions to a monoclonal antibody or hypersensitivity to pembrolizumab or any of its excipients. 26 Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier. 27 Other active malignancy which requires current treatment and which in the opinion of the site investigator is likely to interfere with evaluation of disease assessment. **NOTE:** Continuation of hormonal therapies is allowed.

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

29 Major surgery ≤ 4 weeks from registration. **NOTE:** Diagnostic laparoscopy (without other intervention) and/or biopsies (needle aspirate, core biopsy, open biopsy, etc…) are not considered major surgery

30 Uncontrolled intercurrent illness which in the opinion of the investigator poses unacceptably high risk when combined with study treatment, including but not limited to the following:

- Symptomatic congestive heart failure
- Unstable angina pectoris
- Severely impaired lung function
- Known history of active TB (Bacillus Tuberculosis)
- Uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN (**NOTE:** Optimal glycemic control should be achieved before starting trial therapy.)
- Significant underlying liver disease such as cirrhosis or severe hepatic impairment
- Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

31 Has an active infection requiring systemic therapy.

32 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.

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

	34 For patients in whom planned RT fields will include the heart, any of the following heart conditions, if in the opinion of the investigator they pose unacceptably high risk when combined with study treatment: • Prior symptomatic congestive heart failure • Documented myocardial infarction ≤6 months prior to registration (pretreatment ECG evidence of infarct only will not exclude patients) • Prior significant ventricular arrhythmia requiring medication • Prior 2nd or 3rd degree heart block or other types of clinically significant conduction delay ≤ 6 months prior to registration • Clinically significant pericardial disease (including pericardial effusion, pericarditis) or cardiac valvular disease ≤12 months prior to registration **NOTE:** As part of history and physical, all patients must be assessed for signs or symptoms of cardiac disease, or for prior history of cardiac disease. These conditions include but are not limited to diseases related to cardiac valves, pericardium, myocardium, atrioventricular delays or arrhythmias. It is strongly recommended that signs or symptoms of potentially clinically significant disease be evaluated with comprehensive cardiac echo. 35 For patients in whom planned RT fields during the study will include the chest, any of the following, if in the opinion of the site investigator they pose unacceptably high risk when combined with study treatment: • Prior fistula within thorax, including bronchoalveolar or esophageal. • Respiratory condition that required oxygen supplementation ≤ 3 months prior to registration • Clinically significant pulmonary hypertension ≤ 12 months prior to registration • Pneumonia requiring treatment ≤ 1 month prior to registration • Pulmonary embolism requiring treatment ≤ 6 months prior to registration • Pleural effusion requiring drainage ≤ 12 months prior to registration 36 Has known history of or any evidence of active, non-infectious pneumonitis 37 Currently uncontrolled hyper/hypothyroidism or hyper/hypocortism if in the opinion of the investigator they pose unacceptably high risk when combined with study treatment

	38 Received live vaccine ≤ 30 days prior to registration. **NOTE:** Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
TOTAL NUMBER OF SUBJECTS	N = 34
ESTIMATED ENROLLMENT PERIOD	Estimated 23 months
ESTIMATED STUDY DURATION	Estimated 36 months

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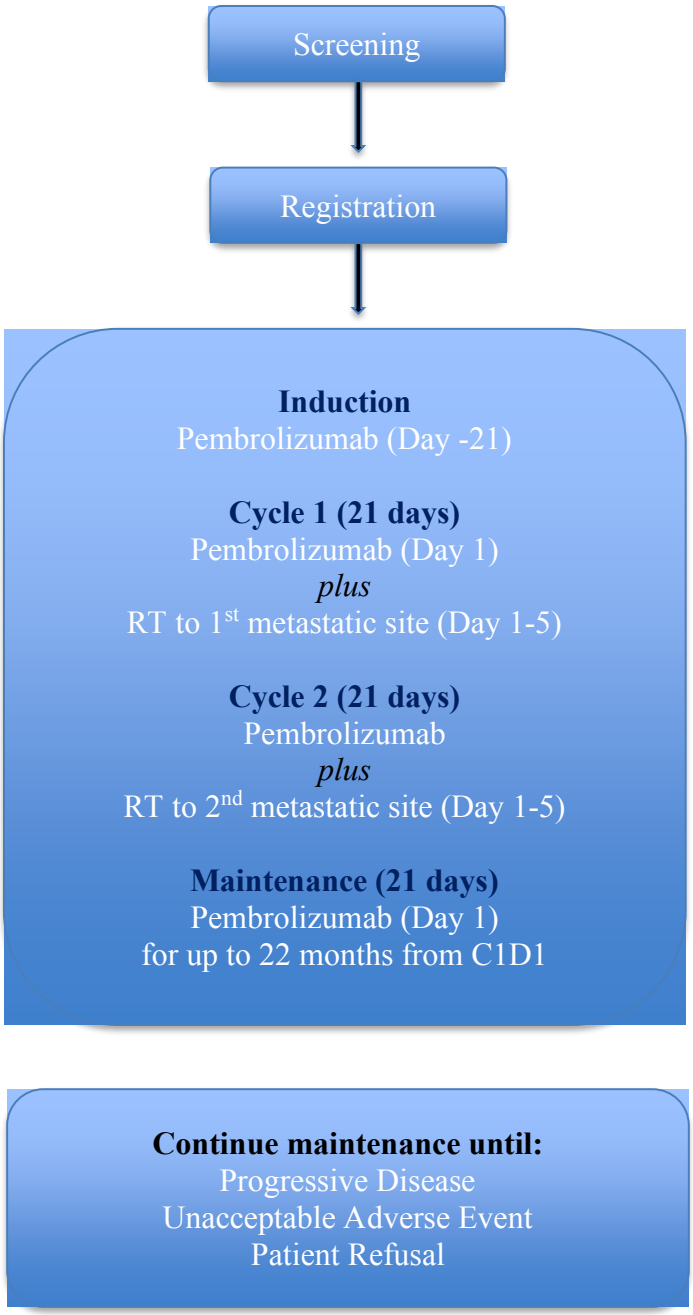
SCHEMA


Screening
Registration
Induction
Pembrolizumab (Day -21)
Cycle 1 (21 days)
Pembrolizumab (Day 1)
plus
RT to 1st metastatic site (Day 1-5)
Cycle 2 (21 days)
Pembrolizumab
plus
RT to 2nd metastatic site (Day 1-5)
Maintenance (21 days)
Pembrolizumab (Day 1)
for up to 22 months from C1D1
Continue maintenance until:
Progressive Disease
Unacceptable Adverse Event
Patient Refusal

1. BACKGROUND AND RATIONALE

1.1 Cancer of unknown primary (CUP) is an area of unmet need

In the U.S., approximately 31,430 cases of CUP are diagnosed per year (ACS, 2015). CUP is defined as biopsy-proven metastasis in the absence of an identifiable primary site. While no standard 1st-line regimen exists, platinum-, taxane-, or gemcitabine-based therapies, such as carboplatin/paclitaxel, are most commonly used. In clinical trials, including those led by Mayo (Holtan et al., 2012; Yoon et al., 2016), median overall survival (OS) from standard first-line therapies is 7-12 months. OS in the 2nd-line setting is 3-5 months.

1.1.1 Barriers in drug development in CUP

- Trials in CUP examining various cytotoxic chemotherapy combinations, or biologic agents in molecularly unselected populations, have failed to improve outcomes.
- An alternative approach is to test novel agents within subgroups possessing a specific targetable alteration. However, trials which further shrink the study population in this way are not feasible, due to the low overall incidence of CUP, and the low frequency (<5% typically) of most potentially targetable genomic alterations in CUP (Ross et al., 2015).
- Alternatively, CUP patients may be "piggybacked" onto larger "basket" trials (e.g., M-PACT, MATCH) in which the same agent is given to patients from diverse tumor types sharing a specific alteration (e.g., BRAF mutation). However, it is unclear whether such data will be generalizable to CUP patients, who are likely to be poorly represented. The complex cross talk and signaling pathways may differ among tumor types, as suggested by the lack of single-agent efficacy of vemurafenib in BRAF-mutated melanoma vs BRAF-mutated colorectal cancer (Chapman et al., 2011; Mao et al., 2013).
- In addition, >15% of CUP patients do not have a genomic alteration that is potentially targetable with available therapies (Ross et al., 2015).

The current phase 2 trial attempts to overcome these barriers through the use of an innovative small trial based on recent data suggesting that immunotherapy in combination with radiation may induce an abscopal effect that can improve patient outcomes (Golden et al., 2015). The trial also attempts to take advantage of recent unpublished data that CUPs may have a relative high total mutation burden compared to other malignancies. Total mutation burden is a putative biomarker that predicts enhanced efficacy to anti-PD-1 therapy (Hodi et al., 2016; Rizvi et al., 2015).

1.2 Rationale for Therapeutic Approach

1.2.1 Trial Design

In this phase 2 study, patients with previously treated CUP will receive pembrolizumab in combination with concurrent RT, so as to induce an abscopal tumor response. The treatment combination will be repeated with RT delivery to a second metastatic site in a non-overlapping RT field.

This trial design is modeled after the proof-of-principle trial examining GM-CSF plus RT in advanced solid tumors (see Clinical data for abscopal effect, below), in which RT to metastatic sites was delivered 1 week after administration of GM-CSF (Golden et al., 2015).

1.2.2 Abscopal Effect

1.2.2.1 Pre-Clinical Data for Abscopal Effect

Recent data, including from lab-based Mayo Clinic collaborators in the current trial (Park et al., 2015), have generated the hypothesis that ionizing radiation (RT) can increase the production and presentation of tumor antigens in poorly immunogenic tumors. This, in turn, could augment the antitumor immune responses elicited by anti–PD-1/PD-L1 agents (Demaria et al., 2005; Reits et al., 2006; Zhang et al., 2007). The combination of anti-PD-L1 plus RT has been shown to induce an abscopal effect on non-irradiated distant tumors in a CD8-dependent, tumor antigen-specific manner (Demaria et al., 2005; Deng et al., 2014). RT can induce tissue PD-L1 expression, a predictive biomarker of PD-1 blockade (Ansell et al., 2015; Herbst et al., 2014; Powles et al., 2014; Taube et al., 2014; Topalian et al., 2012; Topalian et al., 2014). As shown by our collaborators, the combination of RT plus anti-PD-1 therapy elicited a 66% reduction in size of nonirradiated, secondary tumors outside the RT field (abscopal effect), compared to RT or PD-1 blockade alone (Park et al., 2015).

1.2.2.2 Clinical data for abscopal effect

A recent proof-of-principle clinical trial from New York University (NYU) was performed in patients with stable or progressing metastatic solid tumors (n=41, mostly lung and breast cancer) (Golden et al., 2015). Patients were treated with a combination of RT (35 Gy over 10 fractions to one metastatic site, repeated to a second metastatic site) plus GM-CSF. RT to metastatic sites was delivered 1 week after administration of GM-CSF. GM-CSF (granulocyte-macrophage colony-stimulating factor) is a potent immune adjuvant to dendritic cell maturation that has been shown to induce the differentiation of dendritic cells from bone marrow precursor cells (Blom et al., 2000; Inaba et al., 1992). GM-CSF by itself is not expected to have a significant contributory role to the responses observed in the trial. The primary endpoint was the abscopal response rate, defined as the proportion of patients with a response in the best responding abscopal lesion. An abscopal response was defined as a decrease in the longest diameter of at least 30% in any measurable (≥1 cm) non-irradiated lesion from baseline. In patients with more than three lesions, the non-irradiated lesions were measured individually for response to treatment. The best abscopal responding lesion was reported. The primary endpoint was achieved in 27% of patients. As the investigators from this trial concluded, optimization of the strategy probably needs integration of many distinct and sequential signals to correct the preexisting immune suppression or tolerance of metastatic tumors. Whereas RT with GM-CSF actively recruits an inflammatory response within the irradiated field, T-cell exhaustion remains an obstacle for long-term anti-tumor immunity because acquired resistance to RT was shown to be mitigated by PD-1/PD-L1 axis blockade (Dovedi et al., 2014; Twyman-Saint Victor et al., 2015).

These data represent a promising approach of utilizing RT to act as an "*in-situ* vaccine" to augment anti-tumor activity of immune blockade, including with anti-PD-1 antibodies. This approach has not been examined in CUP and forms the framework for our study.

1.2.2.3 Abscopal Response Rate

Importantly, primary endpoint in the NYU trial was the abscopal response rate (defined above), not RECIST 1.1. However, interestingly, a significantly better overall survival was noted in the patients with abscopal responses compared with those without an abscopal response: median overall survival was 21.0 (95% CI 11.1 - 31.0) months for abscopal responders vs 8.3 (95% CI 5.0 – 13.3) months for non-responders (HR 2.06, 95% CI 1.04 – 4.11). Median follow up was 5.6 years. A correlation between abscopal response rate and response per RECIST 1.1 was not reported.

The abscopal response rate, as defined in the NYU study, does not distinguish between nodal and non-nodal lesions. In our study, we will use RECIST criteria for defining a measurable or target lesion, which distinguishes between nodal and non-nodal lesions.

The limitations of standard RECIST in measuring benefit from immunotherapies are increasingly recognized. A significant number of patients have mixed responses (ie, some lesions shrink while others enlarge or remain stable). It is possible that the abscopal response rate may be a better predictor of long-term outcome than RECIST 1.1 when studying the combination of PD-1 inhibition and RT. The risk of relying on RECIST 1.1 alone is that potentially benefitting patients may be overlooked. Our purpose in this small phase 2 trial is to examine this issue in more detail. In addition, our trial will use irRECIST for determination of progression.

1.2.3 Radiation as Standard of Care

The dose and fractionation of radiation planned within the current study is an accepted standard for the treatment of metastatic lesions in CUP patients, as supported by the NCI (NCI, 2016). RT in combination with pembrolizumab is hypothesized to enhance an abscopal effect (see above, abscopal effect).

1.2.3.1 Fractionated (vs single dose) Radiotherapy has Greater Synergy with Immune Therapy

Dewan et al tested the hypothesis that the type of dose fractionation regimen of RT can determine the ability of RT to synergize with anti-CTLA-4 antibody(Dewan et al., 2009). In a mouse model where colon or breast cancer cells were injected into both flanks, single-dose or fractionated RT led to growth delay of the primary tumor, regardless of whether anti-CTLA-4 antibody was administered, but had no effect on secondary tumors outside the RT field. However, an abscopal effect on the secondary tumor outside the RT field only occurred in mice treated with the combination of antibody plus fractionated RT, not single-dose RT.

1.2.4 Induction Dose of Pembrolizumab on Day -21

A major gap in knowledge in the field is the optimal timing of checkpoint inhibition in relation to RT and/or chemotherapy other therapies in the induction of an abscopal effect. The available data suggests that the timing of the first dose of checkpoint inhibition in relation to other therapies may be critical (Dewan et al., 2009), including data from randomized controlled trials comparing the initiation of checkpoint inhibition concurrently vs non-concurrently with chemotherapy(Lynch et al., 2012; Reck et al., 2013). Dewan et al. found that delaying the initiation of anti-CTLA-4 antibody until a few days after the completion of RT reduced the therapeutic effect on the primary and secondary (non-irradiated) tumor, as compared to initiating

antibody treatment on the first or final day of RT (RT was given in 3 fractions of 6 Gy each) (Dewan et al., 2009). To date, no study has directly compared the initiation of checkpoint blockade on the same day as RT initiation *vs* prior to RT initiation.

Some data support the initiation of checkpoint blockade on the same day as RT. Deng et al demonstrated an abscopal effect from the combination of PD-L1 blockade and RT, and PD-L1 blockade (administered every 3 days for a total of 4 times) was initiated on the day of IR or 1 day before IR (Deng et al., 2014). However, the initiation of PD-1 axis blockade on the same day as (*vs* prior to) RT/chemotherapy can theoretical have a detrimental effect: cytotoxic T cells which become newly primed and recruited via anti-PD-1 therapy may be destroyed by RT and/or chemotherapy. Deng et al did not directly compare different administration schedules of PD-1/L1 with regard to RT.

Other data or concepts support the initiation of checkpoint blockade prior to RT:

- Collaborators in the current trial demonstrated an abscopal effect from the combination of PD-1 blockade and RT, and pharmacologic PD-1 blockade was initiated one day prior to RT (Park et al., 2015). While Park et al did not directly compare different administration schedules of PD-1 Ab with regard to RT, the investigators found a similar abscopal effect in PD-1 knockout models, suggesting that baseline inhibition of PD-1 can induce an abscopal response.
- Administration of carboplatin/paclitaxel in patients requires pre-medication with corticosteroids to reduce the likelihood and severity nausea/vomiting and hypersensitivity reactions. However, the well-known immunosuppressive effect of corticosteroids, despite their short half-life, may blunt the effect of immune checkpoint inhibition. The administration of pembrolizumab prior to the administration of cytotoxic agents may avoid this issue.

1.3 CUP Patient Population

In addition to the poor prognosis of CUP patients despite treatment, CUP was selected because these tumors exhibit molecular characteristics that may increase their susceptibility to PD-1 blockade. Recent examination of 70 CUP cases revealed PDL1-expressing cancer cells in 21% of tumors (Gatalica et al., 2014), a frequency comparable to that found in other tumor types where single-agent PD-1 therapy has shown activity. A high mutation load was detected in 13% of tumors of unknown primary; this frequency was the 16th highest out of >50 tumor types profiled using targeted next generation sequencing of >300 cancer genes (personal communication, unpublished data). A high mutation load has been shown to correlate with pembrolizumab activity in NSCLC (Rizvi et al., 2015).

At most cancer centers with a high volume of CUP patients, including at the Mayo Clinic, patients generally start standard chemotherapy with their local community oncologist prior to being seen at the larger center. This referral pattern is primarily for logistic reasons – i.e., inability to secure an immediate appointment after the completion of the diagnostic workup, which itself can be time-consuming.

Accordingly, the vast majority of CUP patients seen at large centers have already initiated first-line therapy. While there is no standard front-line regimen, platinum-, taxane-, and/or gemcitabine combination regimens are common, and one regimen has not been shown to be superior compared to another.

The rationale in the current study is to take advantage of this referral pattern by allowing the enrollment of patients who have started front-line therapy.

1.4 Potential Safety Concerns

1.4.1 Pembrolizumab

For pembrolizumab monotherapy, in clinical trials completed to date, most immune related adverse events associated with pembrolizumab were mild (grade 1–2), some were severe, and a few were lethal. Refer to the Investigator's Brochure (IB) for detailed safety information on MK-3475. In brief, the most recent IB (updated 2/17/2017) presents safety data available from the Sponsor's Reference Safety Dataset (n=2799), a locked and verified dataset with pooled data from clinical trials in melanoma and NSCLC, as part of the Sponsor's product development of pembrolizumab. The Reference Safety Dataset comprises safety data from locked KN001, KN002, KN006, and KN010 [Sec. 1]. The Reference Safety Dataset is the dataset from which frequencies in this IB are derived. The 5 most frequently reported AEs were: fatigue (37.3%), nausea (24.5%), decreased appetite (22.5%), diarrhea (22.3%), and cough (22%). The 5 most frequently reported serious AEs (SAE) were pneumonia (3.0%), pleural effusion (1.7%), pneumonitis (1.6%), dyspnea (1.6%) and pulmonary embolism (1.5%). The 5 most frequently reported AEs considered drug-related by the investigator were fatigue (24.2%), pruritus (16.7%), rash (13.8%), diarrhea (12.3%), and nausea (10.9%). The 5 most frequently reported SAEs considered drug-related by the investigator were pneumonitis (1.6%), colitis (0.9%), diarrhea (0.6%), pyrexia (0.4%), and autoimmune hepatitis (0.3%).

1.4.2 Combination of Pembrolizumab and RT

In the current trial, because RT may be given to lung or liver metastases, potential lung and liver toxicities of pembrolizumab are described further here:

- Pneumonitis in association with pembrolizumab (according to IB 22-May-2015):
    - Frequency/grade: Pneumonitis was quantitatively assessed using the following adverse events of special interest (AEOSI) preferred terms: acute interstitial pneumonitis, interstitial lung disease, pneumonitis, and idiopathic pneumonia syndrome. Out of a total of 1562 subjects with melanoma or lung cancer (P001 and P002) treated with pembrolizumab (2 mg/kg q3w, 10 mg/k2 q3 w, or 10 mg/k2 q2w), AEOSI of pneumonitis were identified in 45 subjects (2.9%), of which 42 (2.7%) were considered drug related. Serious AEOSI were identified in 1.2% of subjects, and all serious AEOSI were considered by the Investigators to be drug related. One subject died due to pneumonitis (interstitial lung disease, dose level 10 mg/k2 q3w)). By worst toxicity grade, 14 subjects (0.9%) experienced Grade 1 pneumonitis, 15 subjects (1.0%) experienced Grade 2 pneumonitis, 11 subjects (0.7%) experienced Grade 3 pneumonitis, and 2 subjects (0.1%) experienced Grade 4 pneumonitis. For "interstitial lung disease," 1 subject (0.1%) experienced Grade 1 interstitial lung disease, 1 subject (0.1%)

experienced Grade 3 interstitial lung disease, and 1 subject (0.1%) experienced Grade 5 interstitial lung disease.

  - Time to development of pneumonitis: Data are available from the 38 patients with previously-treated NSCLC treated with MK-3475 10mg/kg every 3 weeks in P001, of whom 2 patients have been reported with pneumonitis and one patient has been reported with steroid-responsive pulmonary edema. Two cases were grade 2; the case of pulmonary edema was grade 3. These cases began as early as four days after the first dose to several cycles (Week 18) after the first dose.

- Hepatic abnormalities in association with pembrolizumab (according to IB 22-May-2015): Hepatic AEOSI was quantitatively assessed using the following AEOSI preferred terms: hepatitis, autoimmune hepatitis, hepatitis acute, hepatitis fulminant and drug-induced liver injury. Out of a total of 1562 subjects, hepatic AEOSI were identified in 8 subjects (0.5%). All the hepatic AEOSI were considered drug-related. Serious hepatic AEOSI were identified in 2 subjects (0.1%); both were considered by the Investigators to be drug related. By worst toxicity grade, hepatitis was reported for 2 subjects (0.1%). The 2 instances were Grade 1 and Grade 3. Autoimmune hepatitis was reported for 6 subjects (0.4%). Two subjects (0.1%) experienced Grade 2 autoimmune hepatitis, 3 subjects (0.2%) experienced Grade 3 autoimmune hepatitis, and 1 subject (0.1%) experienced Grade 4 autoimmune hepatitis.

Delivery of RT to lung or liver lesion(s) in the current trial may theoretically cause an exacerbation of organ-specific toxicities and thus are considered in more detail as follows.

Radiation pneumonitis has been reported in patients who have undergone mediastinal radiation therapy for lung cancer, Hodgkin's lymphoma (HL), breast cancer, and other cancers that require radiation therapy to the thorax (Carver et al., 2007). A retrospective analysis of 1,911 patients who underwent combined-modality therapy for lung cancer demonstrated that the overall risk of radiation pneumonitis was 7.8% (Roach et al., 1995). Clinical factors that may increase the risk of radiation pneumonitis include concomitant chemotherapy, previous irradiation, and recent withdrawal of steroids (McDonald et al., 1995). Patient factors, such as lung function, age, and sex, do not adequately select patients at high risk for radiation pneumonitis or fibrosis (Dehing-Oberije et al., 2009). Radiation pneumonitis is typically a delayed acute reaction, usually occurring 1 to 3 months after completing mediastinal radiation therapy (McDonald et al., 1995). Patients who experience acute radiation pneumonitis will often have a self-limited course, with complete resolution of this process. However, a minority of patients may develop progressive pulmonary fibrosis, usually 6 to 24 months after treatment. Late complications of pulmonary fibrosis include cor pulmonale and respiratory failure (Carver et al., 2007). Both the V20 (ie, the percentage volume of both lungs minus the planning target volume [PTV] receiving 20 Gy) and the mean lung dose (MLD) correlate with the risk for radiation pneumonitis (De Ruysscher et al., 2010). In a landmark study (Graham et al., 1999), V20 was the single independent predictor of pneumonitis in a multivariate analysis. In this study, 42% of the patients received concurrent chemotherapy, and the incidence of grade >=2 pneumonitis was 7% when the V20 was 22 – 31%. With the radiation doses we are proposing in this study, we expect V20 to be < 20% and therefore, the risk of radiation-related pneumonitis will be minimal. However, as a precaution to

reduce risk for thoracic toxicities following RT plus pembrolizumab, the following measures will be implemented:

- Strict limits on normal tissue doses and other aspects of radiation delivery will be mandated per the most updated standard of care and as recommended(Tang et al., 2014).
- Patients with recent serious pulmonary illness will be excluded (see section 3).
- Monitoring will include late lung toxicities, given that pneumonitis often does not appear until several months after radiation treatment is completed.

Radiation-Induced Liver Disease (RILD) usually develops 1-2 months after RT and describes a clinical syndrome composed of fatigue, ascites, elevated LFTs and abdominal pain. Emami et al (Emami et al., 1991) estimated the acceptable liver tolerance dose (TD5/5; tolerance dose that may cause 5% complication rate within 5 years) of 3000, 3500, and 5000 cGy when whole, 2/3, and 1/3 of the liver was radiated, respectively. This task force estimation is limited by the 2D treatment technique employed at the time. The Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) (Marks et al., 2010) provides the more recent guidelines using the 3D dose/volume/outcome data. In this analysis, preexisting liver dysfunction (Child-Pugh B or C), mean liver dose and V30 (volume receiving 30 Gy or higher) were associated with RILD (Pan et al., 2010). Accordingly, patients with primary liver tumors have a lower radiation dose/volume threshold for developing RILD than patients with liver metastases. RILD data for partial liver radiation has been studied using the Lyman-Kutcher-Burman model by the investigators at the University of Michigan (Dawson et al., 2002). In this study, the rate of RILD grade 3 or higher within 4 months in 203 patients treated with 3D conformal radiation and concurrent hepatic arterial chemotherapy were analyzed. Although some patients received 90 Gy, no RILD was noted when the mean liver dose was <31 Gy. Therefore, in the current study, we will use the following liver constraints as described in QUANTEC to minimize liver toxicity: mean liver <= 30 Gy and >=700 ml of normal liver to receive <=15 Gy (which is a stricter constraint used for 5 fraction SBRT regimen).

The safety and tolerability of lung/liver RT in combination of pembrolizumab has not been extensively examined and, with the implementation of appropriate safeguards (see below: Eligibility, Study Design), is a focus of the current trial. The combination of pembrolizumab + concurrent radio(chemo)therapy is being examined in multiple trials in other tumor types:

- **Pembrolizumab, Combination Chemotherapy, and Radiation Therapy Before Surgery in Treating Adult Patients with Locally Advanced Gastroesophageal Junction or Gastric Cardia Cancer That Can Be Removed by Surgery NCT02730546 – This study is led by the PI of the current study (Yoon HH)**
  - Tumor: locally advanced gastroesophageal junction (GEJ) adenocarcinoma
  - Treatment: carboplatin/paclitaxel weekly for 5 weeks plus pembrolizumab every 3 weeks, plus concurrent RT
  - Location/schedule of RT: RT to GEJ at 41 Gy over 23 fractions
  - Estimated enrollment, N = 30
  - Dates study start, primary completion: Aug 2016, April 2018
  - Primary Outcome: Response

- Phase I Trial of MK-3475 and Concurrent Chemo/Radiation for the Elimination of Small Cell Lung Cancer (NCT02402920; for brevity, only "Part B" for extensive stage is shown):
    - Tumor: extensive SCLC (ECOG PS 0-2)
    - Treatment: Cis(carbo)platin/etoposide Days 1-3 (every 3 weeks) for up to 4 cycles, plus pembrolizumab on Day 1, plus concurrent RT
    - Location/schedule of RT: RT to chest at 45 Gy once a day for 15 days.
    - Estimated enrollment, N = 80
    - Dates study start, primary completion: July 2015, July 2023
    - Primary Outcome: MTD of MK 3475.
- A Randomized Multicenter Phase Ib/II Study to Assess the Safety and the Immunological Effect of Chemoradiation Therapy (CRT) in Combination with Pembrolizumab (MK-3475) Compared to CRT Alone in Patients with Resectable or Borderline Resectable Pancreatic Cancer (UVA-PC-PD101; NCT02305186):
    - Tumor: Resectable or borderline resectable pancreatic cancer (ECOG PS 0-1)
    - Treatment:
        - Experimental arm: Neoadjuvant capecitabine (825 mg/m2 orally twice daily, M-F on days of RT) and RT plus pembrolizumab 200 mg IV every 3 weeks on days 1, 22, and 43 during concurrent chemoradiation.
        - Control arm: Chemoradiation alone.
    - Location/schedule of RT: RT to pancreas at 50.4 Gy in 28 daily fractions
    - Estimated enrollment, N = 56
    - Dates study start, primary completion: March 2015, June 2017).
    - Primary Outcome: Number of Tumor Infiltrating Lymphocytes (TILs) per high powered field (hpf) in resected pancreatic tissue and incidence of DLTs.
- Phase I/II Trial of MK-3475 and Hypofractionated Stereotactic Radiation Therapy in Patients with Non-Small Cell Lung Cancer (NSCLC) (NCT02444741)
    - Tumor: metastatic NSCLC
    - Treatment – phase 1: Pembrolizumab (starting at 100 mg) on Day 1 of each 3-week cycle + SBRT to lung (50 Gy, 4 fractions)
    - Treatment – phase 2: Pembrolizumab (at MTD determined in phase 1) + SBRT, or pembrolizumab + wide-field radiation therapy (WFRT).
    - Location/schedule of RT: SBRT to lung at 50 Gy over 4 fractions, or WFRT to lung at 45 Gy in 15 daily fractions
    - Estimated enrollment, N = 104
    - Dates study start, primary completion: Sept 2015, Sept 2020
    - Primary Outcome: MTD (phase 1), PFS/response (phase 2)

Reported results from the above studies will be followed to inform potential modifications, if appropriate, in the current trial.

1.6 How Study Results Might Impact Future Trials or Practice

This proof-of-principle study is intended to demonstrate that the combination of pembrolizumab with RT has the potential to induce an abscopal response. If results are promising, the question of whether this therapeutic approach enhances anti-tumor activity through an abscopal response can be confirmed in future randomized trials. A future trial in the 2nd-line setting may randomize patients to placebo *vs* placebo plus pembrolizumab *vs* pembrolizumab plus RT.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

Evaluate the abscopal response rate in CUP patients treated with the combination of pembrolizumab plus radiotherapy.

2.1.2 Secondary Objectives

- Determine the response rate by RECIST 1.1 and informed by irRECIST, of non-irradiated metastatic sites when pembrolizumab is combined with radiotherapy.
- Evaluate treatment-related toxicity.
- Evaluate PFS, OS, TTP, and DCR
- Explore the association between RR and other endpoints (e.g., OS, PFS)

2.1.3 Correlative/Exploratory Objectives

- Evaluate the association of MSI status and mutation load with clinical efficacy
- Evaluate the association of tissue PDL1 expression with clinical efficacy
- Evaluate the association of soluble PD-L1 with clinical efficacy
- Evaluate changes in the CD11a^{high} PD-1^{high} CD8+ T effector population in association with pembrolizumab-based therapy
- Evaluate changes and predictive value of BIM expression in T cells in circulation and the predictive value of BIM/PD-1 expression in tumor infiltrating lymphocytes
- Evaluate the prognostic and predictive value of tumor tissue of origin testing

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

Subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Written informed consent and HIPAA authorization for release of personal health information prior to registration. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.

2. Age ≥ 18 years at the time of consent.

3. ECOG Performance Status of ≤ 2 within 28 days prior to registration.

4. Archival tissue must be available and identified during screening and shipped prior to Day -21. If archival tissue is not available and the subject is not undergoing a standard of care biopsy, the subject must undergo a research biopsy to obtain fresh tissue prior to start of treatment.

5. Carcinoma of unknown primary after the following diagnostic procedures have been performed if clinically indicated and are unrevealing of the primary site:
    - Complete history and clinically appropriate physical
    - CT scan of chest, abdomen, and pelvis
    - Directed evaluation of symptomatic areas
    - Mammogram in women
    - Colonoscopy in patients with liver metastasis or an elevated CEA
    - Direct pathologic comparison with prior tumor specimens, where possible, even if prior tumor is early-stage or clinically remote from current disease

    NOTE: Immunohistochemical stains will be performed according to institutional standards. If a primary carcinoma is identified, the patient should undergo treatment as appropriate for that primary tumor and not be enrolled in the study. The above diagnostic workup does not need to be performed if: (1) it was previously completed at the time of original diagnosis or (2) the investigator does not believe the workup has clinical utility at the current time, given that the patient has received interval therapy.

6. Histologic confirmation of metastatic adenocarcinoma, poorly differentiated non-small cell carcinoma, or poorly differentiated squamous carcinoma. **NOTE:** Pathology consultation at Mayo Clinic is recommended if clinically indicated. One scenario is where unknown primary is the most likely diagnosis but immunostains show relatively site-specific marker staining (e.g., CD45, TTF1, chromogranin, GATA3, PAX8, PSA, melanocytic markers). Information provided for pathology consultation should include recent H&P and imaging reports.

7. If and when available, submission of genomic sequencing and expression profiling results is mandatory. Results of testing are not required prior to treatment.

8. At least one measurable lesion (per RECIST 1.1) outside the planned RT fields.

9. Stable or progressive disease after, or was unable to tolerate, at least one line of prior anticancer therapy for this disease. **NOTE:** For patients with stable disease, it is strongly encouraged to confirm the presence of active disease (eg, demonstrating FDG avidity via PET or repeat biopsy).

10. Radiation oncology consultation at enrolling site ≤ 56 days prior to registration to confirm at least two metastatic lesions which are targetable by RT at doses and schedule prescribed in this study and which reside in non-overlapping RT fields.

11. Demonstrate adequate organ function as defined in the table below. All screening labs to be obtained within 28 days prior to registration.

System	Laboratory Value
Hematological	
Absolute Neutrophil Count (ANC)	≥ 900 K/mm^3
Hemoglobin (Hgb)	≥ 8.5 g/dL without transfusion or EPO dependency (≤ 7 days prior to assessment)
Platelets	≥ 90,000 / mcL
Renal	
Creatinine **OR** Calculated creatinine clearance[1]	≤ 1.5 X upper limit of normal (ULN) **OR** ≥ 60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Bilirubin	Total bilirubin ≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN OR total bilirubin ≤2 X ULN if liver metastases are present
Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	> 2.5 g/dL

1 Cockcroft-Gault formula will be used to calculate creatinine clearance (See SPM)

12. Females of childbearing potential must have a negative serum pregnancy test within 72 hours prior to registration. **NOTE:** If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. **NOTE:** breast milk cannot be stored for future use while the mother is being treated on study

13. Females of childbearing potential and males must be willing to abstain from heterosexual activity (abstinence) or use effective methods of contraception as described in Section 5.5 from the time of informed consent until 120 days after treatment discontinuation. **NOTE:** Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months

14. Willingness to return to the enrolling institution for follow up

15. Willingness to provide tissue and blood samples for correlative research purposes

3.2 Exclusion Criteria

Subjects meeting any of the criteria below may not participate in the study:

16. Prior radiation to an area of the body which, if included in the current radiation field, poses an unacceptably high risk of toxicity in the opinion of the investigator. **NOTE:** A prior field that overlaps with the current field, by itself, does not exclude the patient.

17. Any of the following
    - Melanoma. **NOTE:** Positive tumor staining for S-100 or HMB45 alone does not exclude patients.
    - If immunostains are performed, and any of the below tests are positive:
        - Hematologic CD45+ (others such as CD2, CD20, CD30, CD43 also suggest hematologic origin)
        - Lung or thyroid origin (Thyroid Transcription Factor [TTF-1]). **NOTE:** Patients with biopsy proven TTF-1 positive tumor who do not have clinical evidence for either lung or thyroid cancer (e.g. a dominant lung mass) are still eligible.

18. Progressed on 4 or more lines of prior chemotherapy for this cancer. **NOTE:** Bisphosphonates and neoadjuvant/adjuvant anticancer therapies (including locally directed therapies) do not count as a line of therapy with regard to this exclusion criteria.

19. Co-morbid systemic illnesses or other severe concurrent disease which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens.

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

21. 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.

22. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

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

24. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. **NOTE:** Inhaled steroids or steroid injections for joint disease are allowed.

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

26. Prior severe allergic reactions to a monoclonal antibody or hypersensitivity to pembrolizumab or any of its excipients.
27. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

28. Other active malignancy which requires current treatment and which in the opinion of the site investigator is likely to interfere with evaluation of disease assessment.
    NOTE: Continuation of hormonal therapies is allowed.

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

30. Major surgery ≤ 4 weeks from registration. **NOTE:** Diagnostic laparoscopy (without other intervention) and/or biopsies (needle aspirate, core biopsy, open biopsy, etc…) are not considered major surgery. If subject received surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

31. Uncontrolled intercurrent illness which in the opinion of the investigator poses unacceptably high risk when combined with study treatment, including but not limited to the following:
    - Symptomatic congestive heart failure
    - Unstable angina pectoris
    - Severely impaired lung function
    - Known history of active TB (Bacillus Tuberculosis)
    - Uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN (**NOTE:** Optimal glycemic control should be achieved before starting trial therapy.)
    - Significant underlying liver disease such as cirrhosis or severe hepatic impairment
    - Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

32. Has an active infection requiring systemic therapy.

33. 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.

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

35. For patients in whom planned RT fields will include the heart, any of the following heart conditions, if in the opinion of the investigator they pose unacceptably high risk when combined with study treatment:
    - Prior symptomatic congestive heart failure
    - Documented myocardial infarction ≤6 months prior to registration (pretreatment ECG evidence of infarct only will not exclude patients)
    - Prior significant ventricular arrhythmia requiring medication
    - Prior 2nd or 3rd degree heart block or other types of clinically significant conduction delay ≤ 6 months prior to registration
    - Clinically significant pericardial disease (including pericardial effusion, pericarditis) or cardiac valvular disease ≤12 months prior to registration

    NOTE: As part of history and physical, all patients must be assessed for signs or symptoms of cardiac disease, or for prior history of cardiac disease. These conditions include but are not limited to diseases related to cardiac valves, pericardium, myocardium, atrioventricular delays or arrhythmias. It is strongly recommended that signs or symptoms of potentially clinically significant disease be evaluated with comprehensive cardiac echo.

36. For patients in whom planned RT fields during the study will include the chest, any of the following, if in the opinion of the site investigator they pose unacceptably high risk when combined with study treatment:
    - Prior fistula within thorax, including bronchoalveolar or esophageal.
    - Respiratory condition that required oxygen supplementation ≤ 3 months prior to registration
    - Clinically significant pulmonary hypertension ≤ 12 months prior to registration
    - Pneumonia requiring treatment ≤ 1 month prior to registration
    - Pulmonary embolism requiring treatment ≤ 6 months prior to registration
    - Pleural effusion requiring drainage ≤ 12 months prior to registration

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

38. Currently uncontrolled hyper/hypothyroidism or hyper/hypocortism if in the opinion of the investigator they pose unacceptably high risk when combined with study treatment

39. Received live vaccine ≤ 30 days prior to registration. **NOTE:** Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

40. History of organ or stem cell transplantation

4. SUBJECT REGISTRATION

All subjects must be registered through HCRN's electronic data capture (EDC) system. A subject is considered registered when an "On Study" date is entered into the EDC system. Subjects must be registered prior to starting protocol therapy.

5. TREATMENT PLAN

5.1 Pre-Medications

There are no pre-medication or hydration requirements for pembrolizumab administration. For radiation therapy please use institutional standards for pre-medications.

5.2 Study Treatment Administration

Treatment	Dose	Route	Schedule	Cycle Length
Pembrolizumab	200 mg	Intravenously (IV) over 30 minutes	Day -21 (induction) then Day 1 of each cycle. A maximum of 24 continuous months of pembrolizumab (35 cycles) will be administered.	21 days
RT	20-30 Gy over 5 fractions	Extracorporeal	Cycle 1- Day 1-5; 1st metastatic lesion Cycle 2- Day 1-5; 2nd metastatic lesion	

5.2.1 Pembrolizumab

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). Additional information can be found in the pharmacy manual.

A window of **± 3 days** may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs). After the completion of the period of concurrent RT, pembrolizumab monotherapy may be given up to **± 7 days** of the scheduled dose on Day 1 of each cycle. All trial treatments are administered on an outpatient basis.

NOTE: Dosing of pembrolizumab may be withheld in the case of medical/surgical events or logistical reasons (i.e. elective surgery, unrelated medical events, subject vacation, holidays) not related to study therapy. Patients should be placed back on study therapy within 3 weeks of the scheduled interruption to remain aligned with the Q3W dosing interval. The reason for withholding dosing of either pembrolizumab should be documented in the subject's study record.

5.2.2 Radiation Therapy

Palliative external beam radiation therapy is administered to 2 separate non-overlapping sites of metastatic disease. Radiation therapy is administered to the 1st site of metastatic disease on Days 1-5 of Cycle 1 and to the 2nd site of metastatic disease on Days 1-5 of Cycle 2. Here are guidelines for treatment:

- The treating radiation oncologist will select sites to be treated with radiotherapy with preference given to locations that are currently symptomatic or may become symptomatic if there is local tumor progression. Lesions that may provide palliative benefit may also be selected.
- The entire lesion does not need to be completely encompassed within the radiation field.
- If there are > 2 candidate metastatic sites, the treating radiation oncologist should consider treating sites with lower risk of treatment related adverse effects. No more than 2 metastatic sites may be treated.
- A third measurable lesion located outside the planned RT fields must NOT be radiated.

5.2.2.1 Dose specifications and technical factors

The trial administers a total dose of 20, 25, or 30 Gy over 5 fractions per metastatic lesion. The treating radiation oncologist may select the prescription dose to meet the tumor coverage requirements and the organ at risk constraints (OAR) as specified below. It is recommended that the highest dose possible be administered, so long as all mandatory organs at risk (OAR) criteria are met. General recommendations for dose based on lesion site are described below.

Total dose (Gy)	Fractions	Dose per fraction	Example sites
20-30	5	4-6	Liver, lung, bone (non-spine)
20-25	5	4-5	Abdomen/pelvis
20	5	4	Spine

Dose should be prescribed such that ≥ 95% of the PTV receives the prescription dose. All mandatory OAR criteria must be met at a given radiotherapy dose level, or else the dose must be lowered to the next lowest dose level.

The energy of the treatment beam will be at least 6 MV and no greater than 18 MV. The use of intensity-modulated radiation therapy (IMRT) is allowed, however, proton beam radiation therapy and intraoperative radiotherapy (IORT) are NOT allowed. Radiation dose prescriptions will be calculated using heterogeneity corrections to take into account tissue in homogeneities in treatment planning.

5.2.2.2 Immobilization and Simulation

After study enrollment, patients will undergo CT simulation. All patients will undergo high-resolution CT-based (≤ 2 mm slices) 3D treatment planning in custom made immobilization devices. IV contrast and other contrast agents can be used at physician's discretion and as per standard of care. Respiratory motion can be assessed and managed when clinically indicated (i.e. lung, liver, and adrenal gland metastases) with a 4D CT scan. Respiratory gating techniques (including breath hold techniques) may be employed for sites with tumor motion greater than 1 cm.

5.2.2.3 Treatment Planning and Target Volumes

When available, the RT planning process can incorporate diagnostic image fusion (MRI, PET/CT) with planning CT as needed to delineate the target volume and OAR. Gross tumor volume (GTV) will cover the entire metastasis to be treated. For metastases prone to respiratory motion, an internal target volume (ITV) may be generated based on the 4D CT images. Clinical target volume (CTV) expansion will be 5 mm on the GTV or ITV. For spinal lesions, the entire involved vertebra(e) should be included in the CTV. The CTV may be modified to exclude overlap with uninvolved adjacent OAR. The PTV will be a 5 mm uniform expansion of the CTV.

5.2.2.4 Treatment Delivery

Daily verification of the treatment setup will be performed with kilovoltage x-ray (2D) images or volumetric (3D) imaging (kilovoltage or megavoltage CT). CT volumetric imaging (3D to 3D match) is recommended for lung, liver, soft tissue and lymph nodes metastases. For bone metastases, a pair of kV orthogonal (2D) imaging can be used. RT treatments will be delivered on consecutive weekdays. RT treatments can be delivered on weekends and holidays but is not required.

5.2.2.5 Radiation Compliance Criteria

Target coverage

PTV: D95 ≥ 95% and max dose (0.03 cc) ≤120% (while hotspots in GTV is encouraged, hotspots in PTV should be avoided where it overlaps with organs at risk.)

Organs at risk

All mandatory organs at risk (OAR) constraints must be met for treatment on this protocol. If these constraints cannot be met at a given total dose, then the dose must be lowered (Lowest dose is 20 Gy in 5 fractions). For example, if the mandatory constraints cannot be met at a prescription dose of 30 Gy in 5 fractions, then the dose must be lowered to 25 Gy in 5 fractions. Questions regarding radiation planning can be directed to Dr. Sean Park (Park.Sean@mayo.edu) or Dr. Christopher Hallemeier (hallemeier.christopher@mayo.edu).

Mandatory OAR

Optic chiasm: D0.03cc[Gy] < 23 Gy
Optic nerves: D0.03cc[Gy] < 23 Gy
Cochlea: D0.03cc[Gy] < 23 Gy
Brainstem: D0.03[Gy] < 23 Gy
Spinal cord: D0.03[Gy] < 23 Gy
Cauda equina: D0.03[Gy] < 28 Gy
Brachial plexus: D0.03[Gy] < 28 Gy
Sacral plexus: D0.03[Gy] < 28 Gy
Esophagus: D0.03[Gy] < 28 Gy
Trachea: D0.03[Gy] < 33 Gy
Proximal bronchial tree: D0.03[Gy] < 33 Gy
Heart: D0.03[Gy] < 33 Gy
Stomach: D0.03[Gy] < 28 Gy
Duodenum: D0.03[Gy] < 28 Gy

Small bowel: D0.03[Gy] < 28 Gy
Large bowel: D0.03[Gy] < 28 Gy
Rectum: D0.03[Gy] < 33 Gy
Bladder: D0.03[Gy] < 33 Gy
Skin: D0.03[Gy] < 33 Gy
Lung: V20 < 10%
Liver: CV21Gy[cc] > 700 cc
Kidney total: V12.5Gy[%] < 67 %, Mean[Gy] < 12.5 Gy

Recommended (but not required) OAR
Esophagus: V19.5Gy[cc] < 5 cc
Trachea: V16.5Gy[cc] < 4 cc
Proximal bronchial tree: V16.5Gy[cc] < 4 cc
Stomach: V18Gy[cc] < 10 cc
Duodenum: V18Gy[cc] < 5 cc, V12.5Gy[cc] < 10 cc
Small bowel: V19.5Gy[cc] < 5 cc
Large bowel: V25Gy[cc] < 20 cc
Rectum: V25Gy[cc] < 20 cc
Bladder: V18.3Gy[cc] < 15 cc
Femoral heads: V30Gy[cc] < 10 cc

5.2.2.6 Treatment monitoring requirements

During radiation therapy, all patients will be seen by a healthcare provider for a radiation therapy management visit at least once weekly, at which time pertinent history and physical exam will be noted, in addition to the patient's weight and performance status.

5.2.2.7 Treatment interruptions

Treatment interruptions should be minimized through the use of supportive care measures. Interruptions for life-threatening adverse events (CTCAE Grade 4) are allowed.

5.3 Concomitant Medications

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient's primary physician.

5.3.1 Acceptable Concomitant Medications

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

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

5.3.2 Prohibited Concomitant Medications

The following medications are **not** permitted during the screening and treatment phase (including retreatment for post-complete response relapse) of this trial:

- Anti-neoplastic systemic chemotherapy or biological therapy. Continuation of hormonal therapies is allowed.
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, intranasal influenza, 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. However, exceptions may be possible if approved after consultation with the sponsor-investigator.

Patients who, in the assessment by the site investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Patients may receive other medications that the investigator deems to be medically necessary.

5.4 Supportive Care

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

5.4.1 Blood products and growth factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the Journal of Clinical Oncology, Vol 24, No 18 (June 20), 2006: pp. 2932-2947.

5.4.1.1 Neutropenia

Prophylactic use of colony-stimulating factors including Granulocyte Colony-Stimulating Factor (G-CSF), pegylated G-CSF or Granulocyte Macrophage Colony-Stimulating Factor GM-CSF is not allowed in this study. Therapeutic use of G-CSF is allowed in patients with Grade 3-4 febrile neutropenia.

5.4.1.2 Anemia
Transfusions and/or erythropoietin may be utilized as clinically indicated for the treatment of anemia, but should be clearly noted as concurrent medications.

5.4.1.3 Thrombocytopenia
Transfusion of platelets may be used if clinically indicated. ITP should be ruled out before initiation of platelet transfusion.

5.4.2 Anti-Infectives
Patients with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating Investigator for a given infectious condition, according to standard institutional practice.

5.4.3 Corticosteroids
Patients requiring chronic steroid administration (excluding inhaled steroids) are excluded from the trial. Patients may continue on inhalation therapy. Corticosteroids are known immunosuppressive agents that can mitigate the effects of pembrolizumab. Steroids should be generally reserved to treat side effects of pembrolizumab.

5.4.4 Antiemetics
Antiemetics may be used at the discretion of the attending physician. Nausea and vomiting should be treated aggressively, and consideration should be given to the administration of prophylactic antiemetic therapy according to standard institutional practice. Patients should be strongly encouraged to maintain liberal oral fluid intake. Volume depletion should be corrected before initiation of study drug.

5.4.5 Anti-Diarrheals
Patients should be monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered.

All patients who experience diarrhea 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.

NOTE: Loperamide/diphenoxylate/atropines should NOT be used for diarrhea symptoms unless: (1) it is believed that pembrolizumab-related enterocolitis is unlikely to be present after detailed evaluation by gastroenterology, including endoscopy; PLUS (2) approval is documented by a gastroenterology specialist.

5.5 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

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

Female patients will be considered of non-reproductive potential if they are either:
(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.); **OR**

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening; **OR**

(3) has a congenital or acquired condition that prevents childbearing.

Female and male patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence from heterosexual activity; Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, **OR**
post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

5.5.1 Acceptable Methods of Contraception

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)

- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for patients participating at sites in this country/region.

Patients 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 patients of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a patient of childbearing potential will not reliably comply with the requirements for contraception, that patient should not be entered into the study.

5.5.2 Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with pembrolizumab, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. Please see Section 11 for reporting guidelines.

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to HCRN and to Merck as described below.

5.5.3 Use in Nursing Women

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

6. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v4 will be used to grade adverse events.

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Study Calendar & Evaluations.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation as specified in Study Calendar & Evaluations.

6.1 Dose Delays for Pembrolizumab

There are no dose reductions for pembrolizumab; the 200 mg flat dose is the only dose that will be administered.

Strictly follow the modifications in this table for the first two cycles, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose.

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

Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event. If pembrolizumab-related toxicity does not resolve to Grade 0-1 within 12 weeks after last administration of study drug, study therapy discontinuation is recommended.

With sponsor-investigator agreement, patients with a laboratory adverse event still at Grade 2 may continue in the study only if asymptomatic and controlled. In patients who continue on study therapy after experiencing an Adverse Event warranting potential dose delay, if considered drug-related by the site investigator.

For patients who experience a recurrence of the same severe AEs listed above with rechallenge of pembrolizumab, a consultation with the sponsor-investigator will occur to determine whether the patient should continue in the study. A patient who experiences the same SAE of the same NCI CTCAE grade or higher with rechallenge of pembrolizumab must discontinue pembrolizumab immediately.

Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembro

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 corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks c 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 immunosuppre 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 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for s symptoms of pneumoniti • Evaluate participants wit pneumonitis with radiogr initiate corticosteroid trea • Add prophylactic antibio opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for s of enterocolitis (ie, diarrh pain, blood or mucus in s without fever) and of bow (ie, peritoneal signs and i • Participants with ≥ Grade suspecting colitis should consultation and perform rule out colitis. • Participants with diarrhea advised to drink liberal q fluids. If sufficient oral f feasible, fluid and electro substituted via IV infusio
	Grade 4	Permanently discontinue		

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
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver functi weekly or more frequentl enzyme value returned to stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/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 other signs and symptom
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and sy hypophysitis (including adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and sy disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and sy disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal
	Grade 3 or 4	Permanently discontinue		

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
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluati etiology and/or exclude c
	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 evaluati etiology and/or exclude c
	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 when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1D

6.2 Immunotherapy-related toxicities

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

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

Patients should be monitored for signs and symptoms of immunotherapy-related toxicities, which include but are not limited to the following:

Pneumonitis

- For Grade 2 events, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For Grade 3-4 events, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

Diarrhea/colitis

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

- All patients who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For Grade 2 diarrhea/colitis, administer oral corticosteroids.
- For Grade 3 or 4 diarrhea/colitis, treat with intravenous steroids followed by high dose oral steroids. Recommended dose of 1 to 2 mg/kg/day of prednisone or equivalent.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

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

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

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

Hypophysitis

- For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

Hyperthyroidism or hypothyroidism

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

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

Hepatic

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

Renal failure or nephritis

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

6.3 Infusion Reaction

Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of study treatment administration. Below are guidelines for management of Infusion Reactions

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 treatment 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. **Patients 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 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

6.4 Protocol Therapy Discontinuation

In addition to discontinuation from therapy related to toxicities as outlined above, a subject will also be discontinued from protocol therapy and followed per protocol under the following circumstances outlined below. The reason for discontinuation of protocol therapy will be documented in the EDC system.

- Documented disease progression
- Site investigator determines a change of therapy would be in the best interest of the subject
- Subject requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - In a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if he or she may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.
- Female subject becomes pregnant
- Protocol therapy is interrupted for ≥ 12 weeks.
- A maximum of 24 continuous months of pembrolizumab (35 cycles) was administered.

6.5 Protocol Discontinuation

If a subject decides to discontinue from the protocol (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. A complete final evaluation at the time of the subject's protocol withdrawal should be made with an explanation of why the subject is withdrawing from the protocol. If the reason for removal of a subject from the study is an adverse event, it will be recorded on the eCRF.

7. STUDY CALENDAR & EVALUATIONS

Cycle = 21 days	< 28 days prior to Registration	Treatment				End of treatment[13] ~ 30 days after last dose of pembro
		Pre-RT (induction)	During RT		Cycle 3 and subsequent Day 1	
			Cycle 1	Cycle 2		
		D -21	D1	D1		
REQUIRED ASSESSMENTS						
Informed Consent	X					
Medical History[1]	X					
Physical Exam	X		X	X	X	X
Vital signs and ECOG PS [2]	X		X	X	X	X
ECG	X					
AE and concomitant medications review	X	X	X	X	X	X
LABORATORY ASSESSMENTS						
CBC with differential and platelet count	X	X	X	X	X	X
Chemistry panel[3]	X	X	X	X	X	X
T3, Free T4, TSH[4]	X			X	X[4]	X
Pregnancy test[5]	X					
Urinalysis for protein, glucose, blood	X					
DISEASE ASSESSMENT						
CT (chest, abd/pelvis)[6]	X[6]				X[6]	X[6]
Mammogram (women only)[7]	X[7]					
Colonoscopy (if liver metastasis or suspicion of colon primary)[8]	X[8]					
SPECIMEN COLLECTION						
MANDATORY Blood for correlative research[9]		X	X	X	X[9]	X
MANDATORY Blood for somatic baseline[10]		X				
MANDATORY: Archival Tissue, Research Biopsy or New Standard of Care Biopsy[11]		X				
MANDATORY: Tumor Tissue from Standard of Care Biopsy[12]						X[12]
FOLLOW UP						
Survival, Subsequent Therapy[14]						

Key Footnotes:

1: Medical History to include a smoking history, how the subject learned about the study and prior anti-cancer treatment. If available, s
sequencing or expression profiling results is mandatory. If and when available, submission of genomic sequencing and expression prof
Results of testing are not required prior to treatment.

2: Vital signs to include blood pressure, weight, and height (screening only) and ECOG performance status.

3: AST, ALT, alkaline phosphatase, total bilirubin (direct bilirubin if total is elevated), BUN, Cr, creatinine clearance, calcium, phosph
bicarbonate, uric acid, chloride. If there is a clinical need for more frequent testing, it may be performed as needed.

4: Thyroid tests are collected every other treatment cycle, starting with the second cycle after the completion of RT

5: For women of childbearing potential only. Must be done within 72 hours prior to registration

6: Tumor measurements will be performed at the following time points: (1) ≤ 28 days prior to registration, (2) after the completion of R
initiation of post-RT therapy), and (3) every 9 weeks (± 7 days) afterward until the subject comes off study treatment. Tumor measurer
once every 12 weeks (± 2 weeks) after 6 months of study treatment and once every 4 months (± 3 weeks) after 12 months of study treat
increased time intervals may be possible after discussion with and agreement by the sponsor-investigator. The type of tumor measurem
discretion of the site investigator although preferred is CT scan with contrast; if this cannot be done then a PET/CT; if this cannot be do
performed. The type of modality used should be consistent throughout the study treatment. All radiology imaging tests will be submitt
review once funding is available. Please see SPM regarding shipping of radiology imaging.

7: Performed ≤ 60 days prior to original diagnosis or at any time thereafter. A mammography should be performed if initial histology o
possible diagnosis of breast cancer. Also, mammography should be performed if the clinical presentation suggests a possible diagnosis
particularly important if there is no readily accessible site for biopsy. The cost of the mammogram should be covered if the treating phy
"diagnostic" as opposed to "screening." A mammogram is not necessary for squamous carcinoma or neuroendocrine tumors where a br
clinically being considered.

8 Performed within one year prior to original diagnosis or at any time thereafter.

9: Whole blood samples for correlative studies will be sent to the Haidong Dong Lab at Mayo Clinic after collection at the following 5
treatment Day -21 (within 21 days prior to initiating treatment), (2) prior to treatment Cycle 1 Day 1 (- 5 days), (3) prior to treatment C
(4) prior to treatment Cycle 3 Day 1 (first pembrolizumab after completion of RT (- 5 days) and (5) at the time of treatment discontinua
can be found in the Correlative Laboratory Manual (CLM)

10: Whole blood samples for somatic baseline will be collected at any time prior to treatment Day -21 and after registration. See CLM

11: Archival tissue must be identified during screening and shipped prior to Day -21. If archival tissue or standard of care biopsy is not
biopsy will be performed. Please see SPM for additional details.

12: If a subject has a biopsy at progression as per standard of care, some of this tissue will be stored for research particularly directed at
resistance.

13: The end of treatment visit should only occur when subjects permanently stop study treatment for whatever reasons (toxicity, progre
site investigator) and should be performed 30 days (±7 days) after the last dose of treatment. Subjects who have an ongoing Grade ≥ 2
this visit will continue to be followed until the AE resolves to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new
starts, whichever is earlier.

14:

8. BIOSPECIMEN STUDIES AND PROCEDURES

Summary Tables of Blood specimens

Type of biospecimen to submit	Mandatory or optional	When to submit	Collection tube description and/or additive (color of tube top)	Volume to collect per tube (number of tubes to be collected)	Blood product being processed and submitted by participating site	Additional processing required at site after blood draw?	Storage/ shipping conditions	Reason for submission (background/methodology section)
Blood for correlative research (Haidong Dong Lab)[a]	Mandatory	Pre-treatment Day -21, C1D1, C2D1, C3D1, EOT; See calendar above for additional details	EDTA (purple top)	10 ml (2)	Whole blood	No	Ambient temperature (DO NOT FREEZE)	Defined translational studies (Section 1.5)
Blood for somatic baseline	Mandatory	Pre-treatment Day -21	EDTA (purple top)	10 ml (1)	Whole blood	No	Frozen to HCRN	Stored for futu somatic baselin testing

*CLM = Correlative Laboratory Manual

Summary Table of Tissue Specimens

Type of tissue biospecimen to submit	Mandatory	When to submit	Reason for submission (background/methodology section)	Where to find specifi details for biospecim submission
Formalin-fixed paraffin-embedded (FFPE) tissue blocks with corresponding H&E (OR unstained slides with corresponding H&E); archival or fresh biopsy	Mandatory	Prior to Day -21	CLM	CLM
Formalin-fixed paraffin-embedded (FFPE) tissue block with corresponding H&E (OR unstained slides with corresponding H&E)	Mandatory	At time of tumor recurrence during standard of care biopsy.	CLM	CLM

8.1 Whole Blood Collection

Please see Tables above and CLM for additional information regarding blood collection. Collection of these samples should occur Monday- Thursday.

8.1.1 Return of Genetic Testing Research Results

Since the results generated by the somatic baseline genetic testing included in this section are not currently anticipated to have clinical relevance to the patient or their family members, the genetic results will not be disclosed to the patients or their physicians. Sharing of research data with individual patients should only occur when data have been validated by multiple studies and testing has been done in CLIA-approved laboratories.

8.2 Tissue Collection Information

8.2.1 Pretreatment Tissue

Pretreatment tumor tissue must be submitted for this trial. Archived tumor tissue is sufficient and tissue collected as close as possible to study treatment is strongly encouraged. A documented attempt to locate and retrieve archival tissue should be made during screening. Please see the CLM for documentation form. If archival tissue is identified, it should be shipped prior to Day -21. If archival tissue is not available and a standard of care biopsy is not being performed, a research biopsy is required for study eligibility.

Submitted tissue samples will be analyzed for the following biomarkers:

- TOO assay
- Immune status using such assays as mismatch repair, microsatellite instability, mutational load, genomic profile, PD-L1, BIM-PD-1 expression, tumor infiltrating lymphocytes, and others
- Molecular pathways that are deemed to be relevant for prognosis or ability to predict outcomes from immune and/or radiotherapy

The rationale for these tissue studies is explained below.

8.2.2 Tissue from Standard of Care Biopsy at Progression

If a subject has a biopsy at progression as per standard of care, some of this tissue will be stored for research particularly directed at mechanisms of resistance such as but not limited to BIM/PD-1 expression, and CD3, CD8, granzyme B by IHC and other molecular markers in the PD-1 pathway.

8.3 Sample Analyses

8.3.1 MSI and Mutation Load

Recent data implicate the potential value of MSI (mismatch repair) status (Le et al., 2015) and mutation load (Le et al., 2015; Rizvi et al., 2015) in predicting anti-PD-1/PD-L1 blockade. As described above, CUPs appear to have a relatively high proportion of tumors with high mutation load. MSI data are recently available on certain genomic profiles, which also provides information on alterations in hundreds of cancer-related genes and mutational load. Genomic testing is commercially available and has been obtained as part of clinical care in Mayo Clinic

patients to facilitate treatment options.
Given that genomic testing is often routinely ordered in clinical settings, some subjects may already have had the necessary analyses performed. Subjects who have had previous genetic results including MSI and mutation load must consent to permit use of those previous findings by the investigators and therefore not need additional genomic testing. If previous results are not available, subjects must consent to providing tissue for this analysis.

8.3.2 Tissue Expression of PD-L1 and Immune Markers

PDL1 expression is an important marker that has potential predictive value for pembrolizumab therapy. The density of markers of adaptive immunity such as CD3, CD8, FoxP3, and CD45RO infiltrating intratumoral/stromal tissue compartments have been associated with survival in patients with colorectal cancer, as we (Yoon et al., 2012) and others (Galon et al., 2006; Pages et al., 2005) have shown. It is possible that adaptive immune markers in TILs, apart from PD-L1 expression, can have predictive value for immunomodulating agents (Kluger et al., 2015).

Pre-treatment tissue and tissue from standard of care biopsy at progression will be analyzed for multiple markers including PD-L1, CD3, CD8, FoxP3, CD45RO via IHC.

8.3.3 Soluble PD-L1

Release of inhibitory coregulatory proteins into the circulation may represent one mechanism by which tumors thwart immune responses. A collaborator in the current trial (Haidong Dong Laboratory) identified a soluble form of PD-L1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma (Frigola et al., 2011). This lab developed an ELISA for quantification of PD-L1 in biological fluids. Soluble PD-L1 was detected in the cell supernatants of some PD-L1-positive tumor cell lines. Protein sequencing established that the measured soluble PD-L1 retained its receptor-binding domain and could deliver proapoptotic signals to T cells. Higher preoperative soluble PD-L1 levels were associated with larger tumors ($P < .001$), tumors of advanced stage ($P = .017$) and grade ($P = .044$), and tumors with necrosis ($P = .003$). A doubling of soluble PD-L1 levels was associated with a 41% increased risk of death ($P = .010$). These observations suggest that soluble PD-L1 may be detected in the sera of cancer patients and that it may systemically impair host immunity, thereby fostering cancer progression and subsequent poor clinical outcome.

Peripheral blood is collected for soluble PD-L1 analyses at the following 5 time points: (1) prior to treatment Day -21 (2) prior to treatment Cycle 1 Day 1 (- 5 days), (3) prior to treatment Cycle 2 Day 1 (- 5 days), (4) prior to treatment Cycle 3 Day 1 (first pembrolizumab after completion of RT' - 5 days) and (5) at the time of treatment discontinuation.

8.3.4 Tumor-Reactive CD11ahighPD-1high CD8+ T Effector Population Following RT

T cell receptor (TCR) tetramers can detect tumor-specific immunity but have limitations. Both the down regulation of the TCR induced by persistent tumor antigen encounter and the weak affinity of $CD8^+$ T cells for tumor antigens contribute to the underestimation of anti-tumor responses measured by tetramers. In addition, TCR tetramers are not capable of detecting $CD8^+$ T cell responses to subdominant epitopes. Therefore, a novel method of detecting tumor-related T cell response is necessary in situations where the identity of tumor antigens are unknown or suboptimal to be detected by tetramers. CD11a expression is required in the rejection of tumors

(Schmits et al., 1996). A collaborator laboratory recently established that the CD11a^{high} PD-1^{+} CD8^{+} T cell population represent tumor-reactive CD8^{+} T cells(Liu et al., 2013). In addition, work from this lab determined that CD11a^{high}CD8+ T cells inside tumors are tumor-specific functional effector cells and that their function is enhanced by stereotactic ablative radiotherapy in PD-1 knockout mice(Park et al., 2015). In the current study, we propose to investigate whether the CD11a^{high} PD-1^{+} CD8^{+} T cell population in GEJ cancer patients following the initiation of chemoRT plus pembrolizumab can identify tumor-related T cell response.

PBMCs and plasma will be collected at the following 5 time points: (1) prior to treatment Day -21 (2) prior to treatment Cycle 1 Day 1 (- 5 days), (3) prior to treatment Cycle 2 Day 1 (- 5 days), (4) prior to treatment Cycle 3 Day 1 (first pembrolizumab after completion of RT' - 5 days) and (5) at the time of treatment discontinuation.

- *T cell activation marker assay* PD-1^{+} CD11a^{high} CD8^{+} T cells will be stained with antibodies for CD69, CD25, CD45RO and CD62L which are upregulated upon T cell activation.
- *T cell proliferation assay* The proliferation of PD-1^{+} CD11a^{high} CD8^{+} T cells will be measured by their larger forward light scatter (FSC) and intranuclear expression of Ki67.
- *T cell differentiation assay* The expression of transcription factors that controls T cell differentiation into effector or memory cells will be analyzed. We will measure the levels of T-bet (for effector T cells) and Eomes (for memory T cells) in PD-1^{+} CD11a^{high} CD8^{+} T cells.
- *T cell function assay.* Cytotoxic (CTL) T cell effector functions of CD11a^{high} PD-1^{+} CD8^{+} T cells will be assessed by measuring degranulation (CD107a expression) and intracellular production of IFN-gamma following a 4-hour *ex vivo* stimulation with PMA/ionomycin or with any known prostate antigen peptides. The expression of effector molecule of CTL (granzyme B) and its inducer transcription factors (Bcl6 and Runx3), will be examined in PD-1^{+} CD11a^{high} CD8^{+} T cells.

8.3.5 Balance of Circulating CD8^{+} Effector Cell Subpopulation

Given that the antigen spectrum for a given tumor is usually incomplete, it is possible that *neo* antigens could be generated and released from tumor cells following RT, which would not be identified by commercially available tetramers. Since the establishment of antigen specificity for the entire T-cell population is not possible, this study will ascertain whether the antigen burst and/or inflammatory factors released following RT would selectively expand or reduce T cell subpopulation. While RT-induced changes are initiated locally at tumor sites, RT can impact the systemic immune system as observed in the abscopal effect. We hypothesize that tumor antigens and/or inflammatory factors released from RT-induced tumor cell necrosis will trigger an efflux of effector or memory T cells in the peripheral blood since these subpopulation of T cells are antigen-primed and may have a rapid response to altered antigen stimulation in context with inflammatory cytokines. It has been reported that RT increases the serum levels of IL-1beta and IL-6 and reduces the serum levels of TGF-beta in prostate cancer patients after 1-2 weeks of RT. Among the cytokines, IL-6 is responsible for expansion of effector memory CD8^{+} T cells and IL-1beta and TGF-beta regulate the transmigration or tissue retention of effector and memory CD8^{+} T cells. In this portion of the project, fluctuations in frequencies among naïve (CCR7^{+}CD45RA^{+}), central memory (CCR7^{+}CD45RA^{-}), effector/effector memory (CCR7^{-} CD45RA^{-}), and terminally differentiated (CCR7^{-}CD45RA^{+}) CD8^{+} T cells will be determined and

evaluated together with the development of anti-gastric cancer immunity in relation to the timing of RT. PBMCs and plasma will be isolated at (baseline), after RT (day 8, 15, pre-surgery), after adjuvant therapy, and at tumor recurrence. Cells will be co-stained with antibodies for CD3, CD8, CD45RA, CCR7 to identify $CD8^+$ T cells and their phenotype. The percentage of each subset will be determined based on the total $CD8^+$ T cells when analyzed by flow cytometry. We will compare the frequency of each subset of $CD8^+$T cells and their PD-1 expression in relation to the different time points following RT. If we identify any significant changes of T cell subset, we will measure the cytokine profiles of these patients at that time point using multiplex methods.

8.3.6 BIM Expression in Circulating T Cells and Tumor-Infiltrating Lymphocytes

The Haidong Dong lab recently reported that Bim is a downstream signaling molecule of the PD-1 pathway, and its detection in T cells is significantly associated with expression of PD-1 and effector T cell markers (Dronca et al., 2016). High levels of Bim in circulating tumor-reactive ($PD\text{-}1^+CD11a^{hi}CD8^+$) T cells were prognostic of poor survival in patients with metastatic melanoma who did not receive anti–PD-1 therapy and were also predictive of clinical benefit in patients with metastatic melanoma who were treated with anti–PD-1 therapy. Moreover, this circulating tumor-reactive T cell population significantly decreased after successful anti–PD-1 therapy. This study supports a crucial role of Bim in both T cell activation and apoptosis as regulated by PD-1 and PD-L1 interactions in effector $CD8^+$ T cells. Measurement of Bim levels in circulating T cells of patients with cancer may provide a less invasive strategy to predict and monitor responses to anti–PD-1 therapy. This lab also has experience evaluating BIM/PD-1 expression in tumor infiltrating T cells using double-stained IHC.

8.3.7 Expression Profiling to Identify Tissue of Origin in CUP

Accurate identification of the tissue of origin (TOO) via molecular profiling and subsequent site-specific therapy has been theorized to improve outcomes. Gene expression profiling has accurately identified the tissue of origin in ~ 85% of patients in patients with known primary, as a collaborator in the current study (Kerr et al., 2012) and others (Erlander et al., 2011; Monzon et al., 2009; Pillai et al., 2011) have shown. In retrospective studies, molecular profiling rendered a prediction in the majority of CUP patients that was consistent with clinical and pathologic features (Horlings et al., 2008; Varadhachary et al., 2008). In CUP patients whose primary was detected later, molecular testing predicted the primary in 75% of cases (Greco et al., 2013).

Our group recently found that a commercially available tumor gene expression profiling assay that predicts tissue of origin identified CUP patients who responded to carboplatin/paclitaxel-based chemotherapy (Yoon et al., 2016). In this multicenter NCI-sponsored cooperative group trial (N0871), patients with a tissue of origin where platinum/taxane is a standard had a higher response rate (53% vs 26%) and significantly longer progression free survival (6.4 vs 3.5 months; HR 0.47; 95% CI 0.24–0.93; P =.026) and overall survival (17.8 vs 8.3 months, HR 0.37; 95% CI 0.18–0.76; P =.005), compared with patients with a tissue of origin where platinum/taxane is not standard. These data suggest the possible prognostic and predictive importance of evaluating tissue of origin in CUP patients. Multiple commercially available TOO assays (CancerType ID, BioTheranostics; Cancer Genetics) can be ordered for clinical purposes that can aid in diagnosis and treatment decisions. Pre-treatment tissue (archival or biopsy) will be submitted for TOO assay analyses.

8.3 Storage of Biospecimens

At the completion of the study, any unused/remaining material will be stored for future research in the area of cancer. Patients will be asked permission to store leftover samples of tissue or blood.

8.4 Confidentiality of Biospecimens

Samples that are collected will be identified by a subject's sequence ID assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's sequence ID.

9. CRITERIA FOR DISEASE EVALUATION

RECIST 1.1 will be adapted to account for the unique tumor response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can induce a clinical response after an apparent initial increase in tumor volume or even the appearance of new lesions. Standard RECIST may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab. Immune-related RECIST (irRECIST) is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immuno-therapeutics. In addition, an improved long-term survival has been observed when using the best abscopal response as the predictor variable.

The version of RECIST used in this study will be the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) by Eisenhauer et al. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline.

9.1 Tumor Imaging and Assessment of Disease

Each participating site will determine subject eligibility and response. All CT, PET, and MRI scans, scheduled and unscheduled, should be submitted to central imaging as soon as possible. Treatment decisions will be based on the site investigator's radiographic assessment of disease, and not based on Research Base's assessment. There will be no real-time confirmation from central review. Retrospectively, the Research Base will review radiologic images for a retrospective analysis of subject eligibility and response.

Tumor imaging may be performed by computed tomography (CT) (preferred) or magnetic resonance imaging (MRI), but the same imaging technique, acquisition, and processing parameters should be used in a subject throughout the trial. Scans performed as part of routine clinical management are acceptable for use as the baseline scan if they are of diagnostic quality. Scans performed as part of routine clinical management are acceptable for use as the baseline scan if they are of diagnostic quality.

Imaging should follow calendar days and not be delayed for any dose interruptions that may occur.

Imaging should continue to be performed until whichever one of the following occurs first:

- disease progression
- start of new systemic anti-cancer treatment
- withdrawal of consent
- death
- end of study

9.1.1 Timing of Repeat Imaging to Confirm Response

Best abscopal response should be confirmed by a repeat radiographic assessment not less than 4 weeks from the date the response was first documented.

9.2 Assessment of Disease

9.2.1 Determination of Response (tumor shrinkage)

Best abscopal response will be applied as the primary measure for assessment of tumor shrinkage, following the NYU study by Golden et al (see Background 1.2.2).
Best abscopal response is defined as either of the following:

- Non-nodal lesions: Decrease in the longest diameter by at least 30% in any measurable (≥1 cm) non-irradiated lesion from baseline
- Nodal lesions: Decrease in the short-axis diameter of any measurable (≥1.5 cm) non-irradiated nodal lesion from baseline

RECIST 1.1 will be applied as a secondary measure for assessment of tumor response.

9.2.2 Determination of Progressive Disease (irRECIST)

irRECIST is applied only in situations when first radiographic progression per RECIST 1.1 has occurred. After initial radiographic progression per RECIST 1.1, irRECIST will be used by site investigators to assess tumor progression and to make treatment decisions.

If radiologic imaging verifies initial disease progression per RECIST 1.1, treatment may continue at the discretion of the site investigator until repeat imaging ≥ 4 weeks later. The site investigator's decision to continue treatment while awaiting repeat imaging should be based on the subject's overall clinical condition guided by the following criteria:

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

Assessment of repeat imaging after initial RECIST 1.1-defined disease progression:
Subject will be considered as not having disease progression as defined by irRECIST if repeat imaging shows:

- < 20% increase in tumor burden compared to nadir, and
- Stable or improved previous new lesion (if identified as cause for initial disease progression), and
- Stable/improved non-target disease (if identified as cause for initial disease progression).

- *Treatment plan:* Treatment may be continued/resumed.

Subject will be considered as having disease progression as defined by irRECIST if repeat imaging confirms disease progression due to any of the scenarios listed below:

- Tumor burden remains ≥20% and at least 5 mm absolute increase compared to nadir, or
- Non-target disease resulting in initial disease progression is worse (qualitative), or
- New lesion resulting in initial disease progression is worse (qualitative), or
- Additional new lesion(s) since last evaluation
- *Treatment plan:* Subjects will be discontinued from study therapy

In determining whether or not the tumor burden has increased or decreased, site investigators should consider all target lesions as well as non-target lesions.

In cases where irRECIST-defined progression is occurring at the same time as a best abscopal response, study treatment should generally be discontinued. However, if the subject is achieving a clinically meaningful benefit, an exception to continue treatment may be considered.

The guidance described above regarding treatment continuation/discontinuation and repeat imaging per irRECIST is described in the table below.

When feasible, study treatment should continue until progression is confirmed. Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease. Subsequent tumor imaging follows protocol schedule.

Plan for Imaging and Treatment after 1st Radiologic Evidence of Progressive

Plan for Imaging and Treatment after 1st Radiologic Evidence of Progressive Disease (application of irRECIST)				
Scenario	**Clinically stable**		**Clinically unstable**	
	Imaging plan	**Treatment plan**	**Imaging plan**	**Treatment plan**
1st radiologic evidence of PD as defined by RECIST 1.1	Perform repeat scan at ≥ 4 weeks to confirm PD.	May continue study treatment at Investigator's discretion while awaiting repeat scan	Perform repeat scan at ≥ 4 weeks to confirm PD per Investigator discretion only	Discontinue study treatment
Repeat scan confirms PD as defined by irRECIST	No additional scans required.	Discontinue treatment (exception is possible upon consultation with Research Base)[1]	No additional scans required	N/A
Repeat scan shows SD, PR, or CR as defined by irRECIST	Continue regularly scheduled scans.	Continue study treatment at Investigator's discretion	Continue regularly scheduled scans.	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion
CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease [1] If a subject has confirmed radiographic progression per irRECIST (i.e., 2 scans at least 4 weeks apart demonstrating progressive disease), but the subject is achieving a clinically meaningful benefit (e.g., experiencing a response in a non-irradiated lesion), an exception to continue treatment may be considered following consultation with the Research Base.				

9.3 Definitions of Measurable and Non-Measurable Disease according to RECIST 1.1

9.3.1 Measurable Disease

A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥ 2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

A superficial non-nodal lesion is measurable if its longest diameter is ≥ 1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).
NOTE: Tumor lesions in a previously irradiated area are not considered measurable disease.

9.3.2 Non-Measurable Disease

All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

NOTE: 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. In addition, lymph nodes that have a short axis < 1.0 cm are considered non- pathological (i.e., normal) and should not be recorded or followed.

9.3.3 Guidelines for Evaluation of Measurable Disease according to RECIST 1.1

Measurement Methods:
All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.

The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.

Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

9.3.4 Acceptable Modalities for Measurable Disease

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: If the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

Chest X-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scans are preferable.
Physical Examination: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

FDG-PET: FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A ‘positive’ FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered ‘negative.’ New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

- No FDG-PET at baseline and a positive FDG-PET at follow-up:
    - If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
    - If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal PDG-PET scan.
    - If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, it is not classified as PD.

9.3.5 Measurement at Follow-up Evaluation

A subsequent scan must be obtained not less than 4 weeks following initial documentation of an objective status of either complete response (CR) or partial response (PR).

In the case of stable disease (SD), follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of not less than 6 weeks.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain.)

9.4 Measurement of Effect per RECIST 1.1

9.4.1 Target Lesions & Target Lymph Nodes

Measurable lesions up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. Lesions to be monitored for assessment of response must lie outside the RT field. These lesions can be non-nodal or nodal, where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected. **NOTE:** If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.

Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.

The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

9.4.2 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. Lesions to be monitored for assessment of response must lie outside the RT field.

9.5 Response criteria per best abscopal response

A novel aspect of this study is the examination of best abscopal response. Response criteria per best abscopal response are identical to RECIST-defined response criteria, with the exception that tumor shrinkage by > 20% in only a single representative measurable lesion (not a sum of lesions) is sufficient to count as a best abscopal response. In addition, up to 10 measurable lesions per organ may be recorded. Best abscopal response is a binary variable: present or not present. A subject can experience a best abscopal response at the same time as CR, PR, SD, or PD as defined by irRECIST/RECIST 1.1.

See 9.2.2 for guidance on management of cases when a best abscopal response co-occurs with PD as defined by irRECIST/RECIST 1.1.

9.6 Response Criteria per RECIST 1.1

All target lesions and target lymph nodes followed by

- CT/MRI/PET-CT/Chest X-ray/physical examination must be measured on re-evaluation. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

NOTE: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

Evaluation of Target Lesions

- Complete Response (CR): All of the following must be true:
  - Disappearance of all target lesions.
  - Each target lymph node must have reduction in short axis to <1.0 cm.
- Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD.
- Progression (PD): At least one of the following must be true:
  - At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
  - At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.
  - See Section 11.1 on incorporation of irRECIST in determining whether PD on RECIST counts as true progression.

- See Section 11.32 for details regarding the requirements for PD via FDG-PET imaging.

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

Evaluation of Non-Target Lesions & Non-target Lymph Nodes

- Complete Response (CR): All of the following must be true:
  - Disappearance of all non-target lesions.
  - Each non-target lymph node must have a reduction in short axis to <1.0 cm.
- Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.
- Progression (PD): At least one of the following must be true:
  - At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
  - Unequivocal progression of existing non-target lesions and non-target lymph nodes. (**NOTE:** Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)
  - See Section 11.1 on incorporation of irRECIST in determining whether PD on RECIST counts as true progression
  - See Section 11.32 for details regarding the requirements for PD via FDG-PET imaging.

9.6.1 Overall Objective Status per RECIST 1.1

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

Symptomatic Deterioration: Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. A patient is classified as having PD due to "symptomatic deterioration" if any of the following occur that are not either related to study treatment or other medical conditions:

- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

9.7 Descriptive Factors

- RT to bone on this study: ever vs. never
- Number of metastases: 3 vs. 4-6 vs. 7-10 vs. 11 or more
- Number of anatomic regions involved (1-3: thoracic, abdominal/pelvic, or bone)
- Type of anatomic region involved (thoracic [yes/no], abdominal/pelvic [yes/no], bone [yes/no])

9.8 Treatment/Follow–Up Decision at Evaluation of Patient

9.8.1 CR, PR or SD

Patients who are CR, PR, or SD will continue treatment per protocol. Patients who achieve a CR should receive at least 2 further cycles of study treatment beyond the confirmation of CR. While subsequent continuation of study treatment is at the Investigator's discretion, it is strongly encouraged for pembrolizumab monotherapy to be continued.

9.8.2 PD

Patients who develop PD based on RECIST and, if appropriate, irRECIST while receiving therapy will continue to be monitored. These patients should be treated, at the discretion of their treating physician, with alternative chemotherapy if their clinical status is good enough to allow further therapy.

- Patients who develop PD in the CNS only should receive whole brain radiotherapy (WBRT) and continue treatment on study after completion of WBRT.
- Patients who develop non-CNS PD by RECIST and irRECIST at any time should go to event monitoring.

A maximum of 24 continuous months of pembrolizumab (35 cycles) can be administered. If study treatment is discontinued while a patient is experiencing CR, PR, or SD, the patient should

undergo active monitoring. If the cancer recurs, up to 12 months of further pembrolizumab monotherapy can be administered if deemed appropriate by the site investigator.

A patient is deemed ineligible if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment off-protocol at the discretion of the treating physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly to the event-monitoring phase of the study (or off study treatment, if applicable). If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. If the patient never received treatment, on-study material must be submitted.

A patient is deemed a major violation, if protocol requirements regarding treatment in Cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will continue to be monitored per protocol. The patient may continue treatment off-protocol at the discretion of the treating physician as long as there are no safety concerns, and the patient was properly registered.

A patient is deemed a cancel if he/she is removed from the study for any reason before any study treatment is given. No further data submission is necessary.

10. DRUG INFORMATION

10.1 Pembrolizumab (Keytruda®)

Please refer to the current version of the Investigator's Brochure (IB) for additional information regarding this drug.

Pembrolizumab is a potent and highly selective humanized 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 subjects with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. It is also approved for the treatment of subjects with PD-L1 positive metastatic NSCLC who have disease progression on or after platinum chemotherapy. Please refer to the current version of the Keytruda® prescribing information and the pembrolizumab Investigator's Brochure (IB) for additional information regarding this drug.

10.1.1 Supplier/How Supplied

Merck will supply pembrolizumab at no charge to subjects participating in this clinical trial, as summarized in the table below.

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

10.1.2 Preparation
Please refer to the Pharmacy Manual for a comprehensive description of pembrolizumab preparation.

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

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

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

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

10.1.6 Use in Pregnancy and Nursing Women
Based on its mechanism of action, pembrolizumab can cause fetal harm when administered to a pregnant woman. Subjects who are pregnant or planning to become pregnant are not eligible for enrollment. It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

10.1.7 Adverse Events
Please refer to the current prescribing information for pembrolizumab, and to the current version of the Investigator's Brochure for a complete list of adverse events.

The most common adverse reactions (reported in ≥20% of subjects in clinical trials of pembrolizumab in melanoma subjects) included fatigue, pruritus, rash, constipation, diarrhea, nausea and decreased appetite. The most common adverse reactions (reported in ≥20% of subjects in clinical trials of pembrolizumab in NSCLC subjects) included fatigue, decreased appetite, dyspnea and cough.

Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events (IMAEs) are of primary concern. Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis, colitis, thyroid disorders (hypothryoidism/hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitus, uveitis, and nephritis. After a recent review of data, events newly characterized as identified risks also include pancreatitis, myositis, and severe skin reaction. The majority of IMAEs was mild to

moderate in severity, manageable with appropriate care, and rarely required discontinuation of therapy.

Further details around frequency, reporting, and management of IMAEs can be found in the current version of the Investigator's Brochure. In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated; these are also further described in the current IB.

11. ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE v4 will be utilized for AE assessment. A copy of the CTCAE v4 can be downloaded from the CTEP website at http://ctep.cancer.gov. All forms for AE/SAE recording and reporting can be found in the Study Procedure Manual or in the EDC system (Documents and Information Tab).

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence whether or not considered related to the study drug that appears to change in intensity during the course of the study. The following are examples of AEs:

- Unintended or unfavorable sign or symptom
- A disease temporally associated with participation in the protocol
- An intercurrent illness or injury that impairs the well-being of the subject

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

11.1.2 Serious Adverse Event (SAE)

A SAE is an adverse event that meets any of the following:

- Results in death. **NOTE:** Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization for ≥ 24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events. Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered an SAE

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is a new cancer (that is not a condition of the study)
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.1.3 Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.1.4 Relatedness

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

Unrelated	Adverse Event is ***not related*** to the study drug(s)
Unlikely	Adverse Event is ***doubtfully related*** to the study drug(s)
Possible	Adverse Event ***may be related*** to the study drug(s)
Probable	Adverse Event is ***likely related*** to the study drug(s)
Definite	Adverse Event is ***clearly related*** to the study drug(s)

11.2 Reporting

11.2.1 Adverse Events

- AEs will be recorded from time of signed informed consent until 30 days after discontinuation of study drug(s).
- AEs will be recorded regardless of whether or not they are considered related to the study drug(s).
- All AEs that are possibly, probably, or definitely related will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- AEs considered related to study drug(s) will be followed until resolution to Grade ≤ 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever occurs first.
- Asymptomatic laboratory abnormalities that do not require treatment will not be collected as adverse events.

11.2.2 Serious Adverse Events (SAEs)

11.2.2.1 Site Requirements for Reporting SAEs to HCRN

- The time period for expedited reporting begins at registration and continues through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier.
- SAEs will be reported on the SAE/ECI Submission Form.
- For timelines of reporting, please see the table below.
- SAEs include events related and unrelated to the study drug(s).
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.

REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to HCRN **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). HCRN will then report to Merck. The definition of a serious adverse event can be found above in Section 11.1.2.

ALL SERIOUS adverse events that meet the above criteria **in Section 11.1.2** be immediately reported within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframe	Grade 2 Timeframes	Grade 3 Timeframe	Grade 4 & 5 Timeframe
Resulting in Hospitalization ≥ 24 hrs	7 Calendar Days			24-Hour; 3 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		7 Calendar Days	

Expedited AE reporting timelines are defined as;

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

The site will submit the completed SAE/ECI Submission Form to HCRN **within the timeframes described in the table above.** The form may be submitted to HCRN electronically to safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies as per local requirements.

The original copy of the SAE/ECI Submission Form and the email correspondence must be kept within the study file at the study site.

Until the SAE has resolved (see resolution guidelines listed in 11.2.2.1), sites must submit follow-up SAE/ECI Submission Forms when the AE has decreased by at least 1 Grade level to HCRN electronically to safety@hoosiercancer.org.

11.2.3 Overdose

For purposes of this trial, an overdose 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. In the event of overdose, pembrolizumab should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event is associated with the overdose of pembrolizumab, the adverse event is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of pembrolizumab 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 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 1 business day** to HCRN and **within 1 business day** to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

11.2.4 Reporting of Pregnancy and Lactation

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation 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 lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of pembrolizumab, 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.

The outcome of the pregnancy will be reported to Hoosier Cancer Research Network (HCRN) **within 1 business day** and HCRN will report to Merck **within 1 business day** if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

11.2.5 Events of Clinical Interest (ECI)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the SAE/ECI Submission Form. ECIs (both non-serious and serious adverse events) identified from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, will be reported from the site to HCRN **within one business day**. HCRN will report to Merck **within one business day**.
Events of clinical interest for this trial include:

1. an overdose of Pembrolizumab, as defined in Section 10.3 - Definition of an Overdose and Reporting Guidelines, that is not associated with clinical symptoms or abnormal laboratory results.

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

   ***NOTE:** These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial Master File (or equivalent).

11.3 HCRN Requirements for Reporting SAEs to Merck

HCRN will report all SAEs to Merck **within 1 business day** of receipt of the SAE/ECI Submission Form from a site. Follow-up information will be provided to Merck as it is received from site. Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220).

11.4 Sponsor-Investigator Responsibilities

HCRN will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

11.5 HCRN Responsibilities to FDA

For protocols exempt from the requirements of an IND, the above stated requirements are not applicable. HCRN will continue to facilitate compliance of applicable requirements for the sponsor-investigator in relation to this study. This includes but is not limited to 21 CFR 50.20 informed consent, 21 CFR Part 56 IRB, and pertinent sections of the Public Health Service Act and FDAAA.

12. STATISTICAL METHODS

12.1 Study Design

This is a proof-of-principle single-arm phase 2 study in patients with previously treated CUP. All patients receive pembrolizumab combined with RT to a metastatic site, so as to induce an abscopal tumor response. The treatment combination will be repeated with RT delivery to a second metastatic site in a non-overlapping RT field.

The results will be compared with historical control. The primary endpoint is the confirmed response rate (RR) in a non-irradiated site based on best responding abscopal lesion. This study will also evaluate the following secondary endpoints: RR in a non-irradiated site based on RECIST 1.1, adverse events, progression-free survival (PFS), overall survival (OS), time-to-progression (TTP), and disease control rate (DCR).

12.2 Endpoints

12.2.1 Definition of Primary Endpoint

The primary endpoint for this study will assess the confirmed response rate in the best responding abscopal lesion in a non-irradiated site. Best abscopal response is defined as the frequency of patients whose best responding abscopal lesion demonstrates at least a 30% decrease in its longest diameter from baseline (Golden et al., 2015). Responses need to be confirmed (2 consecutive responses at least 4 weeks apart) to count as a response (see Section 11). All eligible patients who are registered and start treatment will be evaluable for response.

The study is powered based on the confirmed response rate. In the largest second-line CUP trials where patients received cytotoxic therapy, patients received gemcitabine alone (N = 39) (Hainsworth et al., 2001) or gemcitabine plus irinotecan (N = 40) (Hainsworth et al., 2005). RRs were 8% and 10%, PFS was not reported, and OS was 3 months and 4.5 months— all respectively.

A Simon's optimal two-stage design will be utilized (see below). This design has 80% power to test the hypothesis of 15% improvement in RR (i.e. 24%) from a historical control of 9%, at one-sided significance level of 0.10. The 2-stage design details are below, where accrual will continue during the interim analysis phase unless we observe a much higher than expected accrual rate or toxicity rate.

Interim Analysis: Enroll 14 eligible patients. If at least 2 confirmed responses are observed in the first 14 eligible patients, we will continue the trial to the second stage and continue the study to a full accrual of 29 eligible patients. Otherwise, the study will be permanently closed due to lack of efficacy.

Final Analysis: If the trial is a success during the interim analysis, we will enroll another 15 eligible patients to the second and final stage (29 eligible total). If at least 5 confirmed responses are observed in the first 29 eligible patients (17%), the treatment will be considered worthy of further investigation. Otherwise, the study will be permanently closed due to lack of efficacy.

12.2.2 Definition and Analysis of Secondary Endpoints

- Response rate (RR) by RECIST 1.1.

- Adverse events: The maximum grade for each type of adverse event will be summarized using CTCAE version 4.0. The frequency and percentage of grade 2+ adverse events will be assessed. We will also explore the treatment related adverse events as well (toxicities).

- Progression-free survival (PFS) is defined as the time from registration to the first of either disease progression or death from any cause, where disease progression will be determined based on RECIST 1.1 criteria. PFS will be estimated using the Kaplan-Meier method, where patients that are alive and progression-free will be censored on their last tumor evaluation date.

- Overall Survival (OS) is defined as the time from registration to death from any cause. OS will be estimated using the Kaplan-Meier method.

- Time-to-Progression (TTP) is defined as the time from registration to disease progression, where patients that are progression-free will be censored on their last tumor evaluation date. TTP will be estimated using the cumulative incidence method, where deaths will be treated as competing risks.

- Disease Control Rate (DCR) is defined as the frequency (%) of patients who have a best response of PR, CR, or Stable disease.

- The association between RR (using RECIST 1.1 (informed by irRECIST)) with other clinical endpoints (e.g., OS, PFS, etc.) will be performed. For time-to-event endpoints, we will use Cox proportional hazards models and Kaplan-Meier methodology. This analysis of RR as a predictor of OS, PFS, etc. will be based on a landmark analysis at various time points of interest post-baseline that correspond to expected tumor assessment time points. This will eliminate the possible response bias of patients living longer or doing better because they lived long enough to respond to treatment. For any associations of RR with categorical data, we will use Chi-square or Fisher's exact tests along with logistic regression models. Descriptive statistics and graphical techniques will be used to summarize this data. Given the small sample size, all these analyses will be considered hypothesis generating and exploratory.

12.3 Sample Size and Accrual

A maximum of 29 evaluable patients will be accrued onto this phase II study unless the study is closed early for excessive toxicity or lack of efficacy. To account for potential ineligibles, major treatment violations during Cycle 1, or cancellations, we will enroll an additional 5 patients, for a total of 34 patients. The expected accrual rate is about 1.5 patients per month across the Hoosier sites. With this accrual rate, we expect to finish accrual within about 23 months, assuming we accrue 34 total patients. We anticipate that the study will take approximately 36 months to complete. This allows a 9-month follow-up for the final patient enrolled, along with data entry, data clean-up, and analysis. Given the expected accrual rate is around 1.5 patients per month, it is expected that the study will take around 23 months to fully accrue. We plan to monitor the accrual continually and if we only end up accruing 6 patients or less in the first year (after study activation), we will consider stopping the trial for slow accrual.

12.4 Assessment of Safety

Any subject who receives at least one dose of treatment on this protocol will be evaluable for toxicity. The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4 will be used. The Study Calendar shows the schedule of toxicity assessments.

12.5 Assessment of Efficacy

All eligible patients who are registered and receive at least one dose of trial drug will be evaluable for all efficacy endpoints.

12.6 Translational Studies

We plan to correlate the tumor markers (MSI, tissue PDL1 expression, mutational load, genomic mutation analysis, BIM/PD-1 expression in tumor infiltrating T cells, and other immune markers, and tumor tissue of origin testing) and plasma-based markers (soluble PD-L1 and T cell subpopulations) with clinical endpoints like confirmed response, best responding abscopal lesion, time-to-event outcomes, and adverse events. Statistical and graphical techniques will be used to evaluate the relationships between tumor markers and clinical data. For time-to-event endpoints, we will use Cox proportional hazards models, and for confirmed response we will use Logistic regression models. In addition, we will use the Chi-square or Fisher's exact tests to test the association between categorical marker data and confirmed response/adverse events. Continuous marker data will be assessed using 2-sample t-tests (or the nonparametric equivalent for non-normal data). Descriptive statistics and graphical techniques will be used to summarize this data. Given the small sample size, all these analyses will be considered hypothesis generating and exploratory.

12.7 Interim Analysis/Criteria for Stopping Study

Interim Analysis (based on primary endpoint): Enroll 14 eligible patients. If at least 2 confirmed responses are observed in the first 14 eligible patients, we will continue the trial to the second stage and continue the study to a full accrual of 29 eligible patients. Otherwise, the study will be permanently closed due to lack of efficacy. Accrual will continue during the interim analysis phase unless we observe a much higher than expected accrual rate or toxicity rate.

Final Analysis (based on primary endpoint): If the trial is a success during the interim analysis, we will enroll another 15 eligible patients to the second and final stage (29 eligible total). If at least 5 confirmed responses are observed in the first 29 eligible patients (17%), the treatment will be considered worthy of further investigation. Otherwise, the study will be permanently closed due to lack of efficacy.

Adverse Event Stopping Rule: The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual after any temporary suspension or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may also choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e., an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy any of the following criteria:

- If at any time, 4 of the initial 10 treated patients or 40% or more of all patients (i.e. when accrual is greater than 10 patients) have experienced a Grade 4 non-hematologic adverse event.
- If at any time, 2 of the initial 10 treated patients or 20% or more of all patients (i.e., when accrual is greater than 10 patients) have experienced a Grade 5 adverse event (non -progressive disease).

13. TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted with guidance from the Mayo Clinic Cancer Center's DSMP.

HCRN oversight activities include:

- Review all adverse events requiring expedited reporting as defined in the protocol
- Provide trial accrual progress, safety information and data summary reports to the sponsor-investigator
- Submit data summary reports to the DSMB for review according to DSMB Charter

13.2 Data Safety Monitoring Board

This study will have a Data and Safety Monitoring Board (DSMB). This information will also be provided to HCRN who will distribute to the site investigator/participating sites for submission to their respective IRB according to the local IRB's policies and procedures.
The DSMB review will include but is not limited to:

- Adverse event summary report
- Audit results if applicable
- Data related to stopping/decision rules described in study design
- Study accrual patterns
- Protocol deviations

During the active treatment phase of this trial, the DSMB will review the above information at least every 6 months. The DSMB will provide a recommendation to the sponsor-investigator after all information is reviewed.

13.3 Data Quality Oversight Activities

Remote validation of the EDC system data will be completed on a continual basis throughout the life cycle of the study. Automated edit check listings will be used to generate queries in the EDC system and transmitted to the site to address in a timely fashion. Corrections will be made by the study site personnel.

The trial site may also be subject to quality assurance audit by Merck or its designee as well as inspection by appropriate regulatory agencies.

13.3.1 Onsite Monitoring

There will be at least one routine visit per site per year for sites that have accrued. Additional for cause visits may occur as necessary. Selected source documents will be reviewed for verification of agreement with data entered into the EDC system. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by HCRN or its designee.

The trial site may also be subject to quality assurance audit by Merck or its designee as well as inspection by appropriate regulatory agencies.

13.4 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-investigator 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. All results of primary and secondary objectives must be posted to CT.gov within a year of completion. The sponsor-investigator has delegated responsibility to HCRN for registering the trial and posting the results on 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 study site contact information.

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management

HCRN will serve as the Clinical Research Organization for this trial. Data will be collected through a web based clinical research platform, a system compliant with Good Clinical Practices and Federal Rules and Regulations. HCRN personnel will coordinate and manage data for quality control assurance and integrity. All data will be collected and entered into the EDC system by study site personnel from participating institutions.

14.2 Case Report Forms and Submission

Generally, clinical data will be electronically captured in the EDC system and correlative results will be captured in the EDC system or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives.

The completed dataset is the sole property of the sponsor-investigator's institution and should not be exported to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without permission from the sponsor-investigator and HCRN.

14.3 Record Retention

To enable evaluations and/or audits from Health Authorities/HCRN, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with the site contract with HCRN. No records will be destroyed until HCRN confirms destruction is permitted.

14.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel.
Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, HCRN, Merck, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

15 ETHICS

15.1 Institutional Review Board (IRB) Approval

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator must submit written approval by the IRB to HCRN before he or she can enroll subjects into the study.

The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB, as local regulations require.

Progress reports and notifications of serious and unexpected adverse events will be provided to the IRB according to local regulations and guidelines.

15.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

15.3 Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The site investigator must store the original, signed informed consent form. A copy of the signed informed consent form must be given to the subject.

16 REFERENCES

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