CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: COMBINING PEMBROLIZUMAB AND PALLIATIVE RADIOTHERAPY IN GASTROESOPHAGEAL CANCER TO ENHANCE ANTI-TUMOR T CELL RESPONSE AND AUGMENT THE ABSCOPAL EFFECT

CITY OF HOPE PROTOCOL NUMBER: 16099 **Protocol Dated: 10/16/2019**

COH Initial Submission:	Protocol dated 04/08/2016	Version: 00
COH Amendment 01	Protocol dated 02/10/2017	Version: 01
COH Amendment 02	Protocol dated 07/26/2017	Version: 02
COH Amendment 03	Title Page dated 02/27/2018	Version: 03
COH Amendment 04	Protocol dated 10/16/2019	Version: 04
COH Amendment 05 at Cont.	Protocol dated 10/16/2019(tp)	Packet: 05

DISEASE SITE: Esophageal, gastric, and gastroesophageal cancers
STAGE (if applicable): IV
MODALITY: Intravenous
PHASE/TYPE: Phase II/Prospective biomarker analysis

PRINCIPAL INVESTIGATOR: Joseph Chao, MD

COLLABORATING INVESTIGATOR(S) Yi-Jen Chen, MD,

PARTICIPATING CLINICIANS: Vincent Chung, MD, Marwan Fakih, MD, Daneng Li, MD, Dean Lim, MD

DIAGNOSTIC RADIOLOGIST: .

STUDY STATISTICIAN: Paul Frankel, PhD

EXTERNAL COLLABORATING INVESTIGATOR(S): Greg Caporaso, Ph.D.,

Joanne Weidhaas, M.D., Ph.D.

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SPONSOR/IND NUMBER: 131134
DISEASE SITE: Esophageal, gastric, and gastroesophageal cancers
STAGE (if applicable): IV
MODALITY: Intravenous
PHASE/TYPE: Phase II/Prospective biomarker analysis

PRINCIPAL INVESTIGATOR:	Joseph Chao, M.D. City of Hope
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PARTICIPATING CLINICIANS:	Vincent Chung, M.D., Marwan Fakih, M.D., Daneng Li, M.D., Dean Lim, M.D., City of Hope
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STUDY SPONSOR AND MONITOR:	City of Hope

TABLE OF CONTENTS

1.0 TRIAL SUMMARY

Abbreviated Title	Pembrolizumab and palliative radiotherapy in metastatic gastroesophageal cancer
Trial Phase	II
Clinical Indication	SCC and/or adenocarcinoma of the esophagus, GEJ, and stomach
Trial Type	Prospective; Biomarker analysis
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	All patients will be assigned to the same therapy of palliative radiotherapy of 30 Gy over 10 fractions for symptoms from their primary tumor or a single target metastatic site in combination with pembrolizumab at a fixed dose of 200 mg every 3 weeks.
Number of trial subjects	14
Estimated enrollment period	24 months
Estimated duration of trial	36 months from the first patient signing informed consent until the last patient's last visit.
Duration of Participation	Patients will complete radiation therapy of 30 Gy in 10 fractions and will continue pembrolizumab until confirmed disease progression, or intolerable toxicity, or an initial 35 administrations (approximately 2 years). Patients who stop pembrolizumab with stable disease or better or 35 administrations of therapy without progression may be eligible at the discretion of the investigator to receive up to an additional year of pembrolizumab therapy. Patients with evidence of progressive disease (PD) on imaging and are clinically stable may continue to be treated at the discretion of the investigator.

2.0 TRIAL DESIGN

2.1 Trial Design

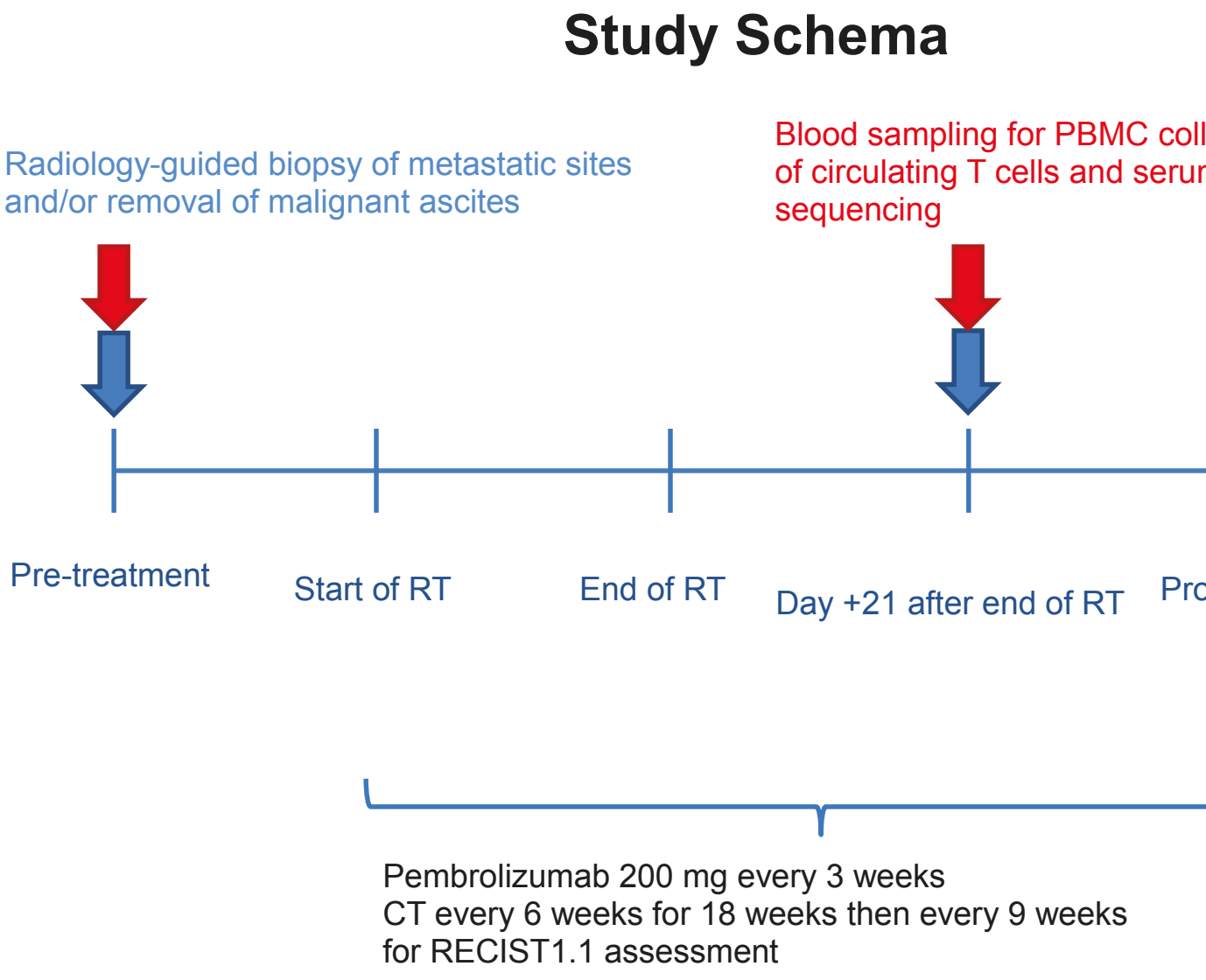
This is a single-arm, prospective, phase II, biomarker analysis trial of pembrolizumab in combination with palliative radiotherapy for patients with metastatic squamous cell or adenocarcinoma of the esophagus, stomach, or gastroesophageal junction (GEJ). We will enroll 14 patients within our institution who are in need of palliative radiation for symptoms related to their primary tumor including pain, dysphagia, and bleeding. Patients with a single metastatic site in need of palliative radiation (e.g., pain from a bony metastasis, enlarged lymph nodes, liver metastasis, etc.) will also be eligible provided they have other measurable metastatic sites outside of the radiation field. The exception will be patients who require palliative radiation to a symptomatic central nervous system (CNS) metastasis. The first dose of palliative radiation and first dose of pembrolizumab will commence on cycle 1, day 1.

Participation in this trial will be dependent on patients having a metastatic site amenable to ultrasound and/or CT-guided biopsy, inclusive of patients with malignant ascites amenable to ultrasound-guided aspiration, and agreeing to provide pre- and post-treatment biopsies for the

biomarker analysis primary endpoint of the study. Patients who are undergoing palliative radiation for a single target metastatic site, and only the primary tumor is amenable to biopsy are also eligible to participate if they consent to endoscopic pre- and post-treatment biopsies.

Treatment will continue until documented clinical PD, unacceptable AE(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the patient, patient withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, completion of 35 administrations (approximately 2 years) of treatment with study medication or achievement of a complete response.

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To establish that the combination of pembrolizumab and traditional external beam multifractionated RT to the primary tumor or a single target metastatic site of patients with metastatic gastric, esophageal, and/or GEJ cancers will lead to an increase in tumor infiltrating cytotoxic T-cells and circulating cytotoxic T cells and a reduction in immunosuppressive Tregs and MDSCs in metastatic sites.

Hypothesis: The combination of pembrolizumab and traditional external beam multifractionated RT to the primary tumor or a single target metastatic site will lead to increased immune-mediated tumor cytoxicity via a demonstrable increase in tumor infiltrating and circulating cytotoxic T cells and a reduction in immunosuppressive Tregs and MDSCs within metastatic sites post-therapy.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To establish that the combination of pembrolizumab and RT is feasible in this patient population and evaluate toxicities per NCI CTCAE ver.4.03.

Hypothesis: The combination of pembrolizumab and RT will be feasible with an acceptable safety profile to warrant future studies.

(2) **Objective**: To evaluate overall response rate (ORR) per RECIST1.1 and irRECIST in this treatment population and correlate with tumor T-cell response.

Hypothesis: The combination of pembrolizumab and RT will demonstrate clinical efficacy with a promising response rate compared to historical controls to warrant future studies.

(3) **Objective**: To evaluate progression-free (PFS) and overall survival (OS) in this treatment population.

Hypothesis: The combination of pembrolizumab and RT will demonstrate promising PFS and OS to warrant future studies.

3.3 Exploratory Objectives

(1) **Objective:** To measure changes in whole genome serum miRNA signatures before and after protocol therapy and correlate with tumor/immune/stromal cell miRNA expression profiling determined by deep sequencing.

(2) **Objective:** To measure changes in fecal and oral microbiomic diversity and correlate with ORR, PFS, and OS.

(3) **Objective:** To assess germline mutations in a panel of miRNA regulatory genes using the MiraDx assay as predictors of response and toxicity to pembrolizumab and RT.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab (aka MK-3475).

4.1.1 Pharmaceutical and Therapeutic Background

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

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

of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

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

4.1.2 Preclinical and Clinical Trial Data

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

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Metastatic squamous cell carcinomas (SCC) and adenocarcinomas of the esophagus, stomach, and gastroesophageal junction (GEJ) account for a high proportion of worldwide cancer-related mortality. While some progress has been made for HER2 and VEGFR2 targeted therapies in adenocarcinomas,[1,2] options for metastatic SCC remain dismal. Evidence for cytotoxic agents in SCC typically stem from a small proportion of patients enrolled in large phase III trials predominated by patients with adenocarcinoma histologies.[3] Novel targeted therapeutic approaches for both subtypes are desperately needed.

Pembrolizumab has demonstrated encouraging preliminary evidence of activity in small cohorts of gastric and esophageal cancer patients within the KEYNOTE-012 and KEYNOTE-028 trials, respectively.[4,5] Both trials selected for patients with evidence of PD-L1 expression within tumor stroma and/or tumor cells. KEYNOTE-012 reported a 22.2% overall response rate by central review, while KEYNOTE-028 noted a 30.4% overall response rate when assessed by investigator review. The activity of pembrolizumab in gastroesophageal cancer patients without tumor PD-L1 expression remains unknown, though lack of PD-L1 expression does not necessarily translate to an absolute lack of response to immune checkpoint inhibitors in pooled analyses of patients with melanoma, NSCLC, and genitourinary malignancies.[6] Given the promising early evidence of clinical efficacy in gastroesophageal cancers, strategies should be further developed to enhance the potency of immune checkpoint inhibitors, even in patients whose tumors are currently not considered to have detectable PD-L1 expression.

The abscopal effect is a rarely observed though well annotated clinical phenomenon in cancer radiotherapy (RT), in which non-irradiated metastatic lesions distant from the primary tumor site directly subjected to radiation are noted to regress. This phenomenon has been attributed to immune-mediated effects in preclinical models,[7] and provides rationale that immune checkpoint inhibition and RT could act synergistically. In a recent preclinical study, PD-1 was reported to suppress RT antitumor response, though delivery of a PD-1 blocking antibody

enhanced antitumor response at the primary radiated site as well as non-irradiated metastatic lesions.[8] Furthermore, radiation therapy has been shown to drive up the expression of PD-L1 tumor expression in preclinical models.[8,9,10] Mounting evidence also supports that mutational load in tumors correlate with clinical benefit from immune checkpoint inhibition.[11] Results from The Cancer Genome Atlas (TCGA) have demonstrated both esophageal SCC and gastric adenocarcinomas demonstrate a high rate of somatic mutations.[12,13] Furthermore, the development of esophageal SCC is commonly attributed to tobacco smoking, a risk factor shared with NSCLC in which anti-PD-1 strategies have been successful. It is not uncommon for patients with metastatic disease to undergo palliative, traditional external beam multifractionated RT to their primary tumor for the treatment of dysphagia, pain, or gastrointestinal bleeding or a single metastatic site such as a bone metastasis for the treatment of pain. The concordant delivery of pembrolizumab during palliative RT thus serves as an attractive window-of-opportunity trial to determine if the abscopal effect may be augmented with immune checkpoint blockade.

We propose a prospective trial to assess pre- and post-treatment biopsies of patients' metastatic sites with the hypothesis that tumor cytotoxic T cells will be increased with combining pembrolizumab and palliative RT with corresponding improvement in clinical activity. As we will only be radiating a single target lesion in this study population, assessment of response among non-irradiated metastatic sites will serve as a true measure of the abscopal effect. Toxicities are not anticipated to be overlapping for these two modalities, though safety should still be assessed in this early cohort of patients to ensure immune-mediated or radiation-induced toxicities are not exacerbated by the combination. At City of Hope where patients will be solely recruited for this trial, we have extensive experience in the conduct of pre- and post-treatment biopsy studies having completed accrual to a pilot trial of the novel nanopharmaceutical CRLX101 in this disease subset (Clinicaltrials.gov, NCT01612546). We also have a broad gastroesophageal cancer patient population evaluating more than 60 new cases per year due to our institution serving the diverse, multicultural population of the San Gabriel Valley in Los Angeles County. Dr. Peter Lee, MD, Chair of the Department of Immuno-Oncology, will serve as a collaborating investigator on this study given his laboratory's expertise in translational analyses of tumor/immune/stromal cell interactions.

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

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

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

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

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

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

The clinical efficacy of combining pembrolizumab and palliative radiotherapy will be measured by assessing overall response rate by RECIST 1.1 and irRECIST. To truly measure the abscopal effect, patients will be required to have metastatic disease sites outside the field of palliative radiation that are considered measurable by RECIST 1.1.

4.2.3.2 Biomarker Research

Analysis of pre- and post-treatment biopsies

Radiology-guided biopsies will utilize 20-gauge core biopsy needles. Biopsies obtained via an endoscopic approach will utilize standard endoscopic biopsy forceps in which specimens are typically 3-6 mm in diameter. As such tissue samples will be composed of both tumor cells and associated tumor stroma. Fresh tumor samples will be immediately delivered to the laboratory of Dr. Peter Lee for both ex vivo tumor/immune/stromal cell culture and expansion as well as histologic analyses. Patients with fresh malignant ascites collected will also be immediately delivered to the lab for ex vivo tumor/immune/stromal cell culture and expansion alone. Dr. Lee's lab will utilize quantitative spatial image analysis to examine changes in tumor cell-stromal interactions including CD4, CD8, Treg, and MDSC populations pre- and post-therapy.

PD-L1 expression within the tumor, on cancer cells and stromal cells, will be analyzed by immunohistochemistry (IHC). PD-1 expression on tumor infiltrating T cells and circulating peripheral blood mononuclear cell (PBMC) T cells will be analyzed by a combination of IHC and flow cytometry. Immune cell populations will be further analyzed via high dimensional flow cytometry to measure levels of Tregs and MDSCs, which will allow us to calculate the ratio of effector T cells to Tregs and MDSCs, respectively. Ex vivo isolated T cells will also be analyzed for proliferative capacity (CFSE dilution) and cytokine production (IFN-γ and IL-17) after stimulation with anti-CD3 and/or PMA and Ionomycin. Furthermore, by use of multi-color IHC we will determine influences of pembrolizumab+RT treatment on the magnitude and proximity of CD8 T cells to cancer cells within the tumor. Lastly, we will analyze changes in the diversity of T cell repertoire in both PD-1 positive and PD-1 negative T cell populations following pembrolizumab+RT treatment via deep sequencing of the TCR Vβ CDR3 region. Such changes in T cell repertoire can be suggestive of potential epitope spreading for anti-tumor antigens and reversal of tumor antigen-specific T cell anergy. Genomic analyses utilizing City of Hope's Integrative Genomics Core will also be performed on the corresponding cancer/stromal/immune cell populations via total RNA-seq for coding and non-coding RNAs (including miRNAs).

Performance of these studies will require several highly developed techniques that have been routinely conducted in Dr. Peter Lee's lab. A prototype BD microfluidic FACS instrument allows for 12-color analysis of low cell numbers, thereby allowing maximal data acquisition out of small sample sizes. A powerful quantitative, spatial image analysis system will enable

us to analyze immune and cancer cells in tissues via 8-color histology. Lastly, Dr. Lee's lab has considerable expertise in TCR repertoire analysis via deep sequencing, which can yield information on T cell specificity to unknown antigens.

Serum circulating miRNA sequencing

10 mL of whole blood will be collected from each patient at prespecified time points and serum separated per standard procedures. Total RNA will be extracted using Trizol and sent to the Integrative Genomics Core at City of Hope for miRNA-seq. The Integrative Genomics Core is a shared resource at City of Hope and is equipped with all major state-of-the-art microarray and sequencing platforms for genomic studies, including Illumina's Hiseq 2500 and Life Technology's Ion Proton sequencers, Illumina's Hiscan Beadchip system, Affymetrix's GeneChip system, and Agilent's microarray system. With a highly experienced team of bioinformaticians integrated within the core, efficient genomic data analysis services will be provided to convert high throughput data into biologically interpretable results. They have extensive expertise in utilizing both commercial and open-source software tools for sequencing data analysis including but not limited to R/Bioconductor, IGV, Bowtie, TopHat, Cufflinks, Novoalign, MACS, MEME, Transfac, BWA, GATK, Samtools, Gene Set Enrichment Analysis (GSEA), Ingenuity Pathway Analysis and DAVID.

Sequence reads that match human miRNAs in Sanger mirBase will be calculated and analyzed. Circulating whole genome miRNA signatures will be compared to miRNAs sequenced from total RNA-seq from the corresponding tumor biopsy timepoints to aid in biomarker discovery of serum miRNAs that may predict for immunotherapy response or resistance. miR-23a will be of particular interest given its description as a gastric cancer oncomir and function as a repressor of T cell cytotoxicity in preclinical miRNA screens.[14,15] miR-34a and miR-200 will be other miRNAs of interest to measure given reporting of their ability to suppress PD-L1 expression in other experimental systems.[16,17]

5.0 METHODOLOGY

5.1 Entry Criteria

Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values and/or to determine pre-eligibility, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented, and the prospective participant must receive a copy of the signed informed consent document. All screening procedures and their respective windows are detailed in the Study Activity Calendar.

Participant Enrollment

COH DCC Availability and Contact Information

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope.

DCC staff are available between the hours of 8.00 am and 5.00 pm PST, Monday through Friday (except holidays). DCC contact information is as follows:

- Phone: (626) 256-4673 ext. 83968
- E-mail: DCC@coh.org

Eligible subjects must be registered **prior** to start of protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, the subject's registration on the study may be canceled after discussion with the PI. The Data Coordinating Center should be notified of cancellations as soon as possible.

Registration Process

To register a participant the subsequent procedure is to be followed:

1. The study team should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The protocol nurse or CRC will email a copy of the following documents to the DCC:
    - Completed eligibility checklist (printed from Section 5.1 of the protocol)
    - Signed Informed Consent
    - Signed subject's bill of Rights
    - Signed HIPAA authorization form and
    - Provide copies of source documentation only if not readily available as a finalized record in the COH Electronic Medical Record (EMR).
3. After having received all transferred documentation, the DCC will complete the review the documents to verify eligibility, working with the study team as needed to resolve any missing required source elements. A participant failing to meet all protocol eligibility requirements will not be registered.
4. Once eligibility has been confirmed, DCC staff will register the participant by: assigning a subject accession number, register the subject on study centrally into a COH clinical trials management system (e.g. MIDAS), and enter the subject into the eCRF system, Medidata RAVE.

5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form within 24 hours, including the participant study number to:
    - The site study team: Principal Investigator, treating physician, protocol statistician, protocol nurse, CRC and COH IDS Pharmacy.
    - the COH sponsor team designees

Screen Failures and Registered Participants Who Do Not begin Study Treatment

The DCC is to be notified of all participants who sign consent but do not meet eligibility criteria or do not initiate protocol therapy.

5.1.1 Inclusion Criteria

Participants must meet all of the following criteria on screening examination to be eligible to participate in the study:

Patient MRN:	Patient Initials: (F,M,L):

Informed Consent and Willingness to Participate

___ 1. Documented informed consent of the participant

___ 2. Willing to provide tumor tissue amenable to ultrasound or CT-guided biopsy for biomarker analyses.

- Patients with malignant ascites are permitted to participate and provide ascites samples for biomarker analyses.
- Patients receiving radiation to a single metastatic site in which only the primary tumor is accessible for biopsy by endoscopy will also be eligible.

Age Criteria, Performance Status and Life Expectancy

___ 3. Age: ≥ 18 years

___ 4. ECOG performance status of ≤ 1

___ 5. Life expectancy of ≥ 3 months

Nature of Illness and Treatment History

___ 6. Diagnosis of metastatic squamous cell carcinoma and/or adenocarcinoma of the esophagus, gastroesophageal junction, or stomach in need of palliative radiotherapy to the primary tumor or a single metastatic site for symptoms such as pain, dysphagia, and/or gastrointestinal bleeding.

- Patients with adenocarcinoma histology and known HER2-overexpressing disease are permitted to participate if they progressed or are intolerant of prior trastuzumab-containing therapy.

___ 7. Measurable metastatic sites of disease outside of the target lesion undergoing palliative radiation based on RECIST 1.1 as assessed by the investigator.

___ 8. Have no limits on prior lines of therapy or may be treatment-naïve if in need of palliative RT provided the patient has not received prior anti-PD-1, anti-PD-L1, or anti-PD-L2 therapy.

Laboratory Criteria (within 10 days of treatment initiation unless indicated otherwise)

___ 9. ANC ≥ 1,500/mm^3	ANC:	Date:
___ 10. Platelets ≥ 100,000/mm^3	Plts:	Date:
___ 11. Hemoglobin ≥ 9 g/dL ▪ May receive transfusion to meet this goal as it is anticipated a subset of patient will require palliative radiation to a bleeding primary tumor	Hgb:	Date:
___ 12. Total serum bilirubin ≤ 1.5 x ULN **OR** Direct bilirubin ≤ ULN if total bilirubin levels > 1.5 x ULN	ULN: Bil: Direct Bil:	Date:
___ 13. Albumin ≥ 2.5 mg/dL	Albumin:	Date:
___ 14. AST and ALT ≤ 2.5 x ULN **OR** ≤ 5.0 x ULN if liver metastases present	ULN: ALT:	Date:
	ULN: AST:	Date:
___ 15. Serum creatinine ≤ 1.5 x ULN **OR** Creatinine clearance[a] ≥ 60 mL/min if creatinine levels > 1.5 x ULN ▪ GFR can also be used in place of creatinine or CrCl [a] If measured or calculated per institutional standard	ULN: Serum Cr: Cr Cl:	Date:
___ 16. **If not receiving anticoagulants**: International Normalized Ratio (INR) **OR** Prothrombin (PT) ≤ 1.5 x ULN **If on anticoagulant therapy:** PT must be within therapeutic range of intended use of anticoagulants	ULN: INR: PT:	Date:
___ 17. **If not receiving anticoagulants**: Activated Partial Thromboplastin Time (aPTT) ≤ 1.5 x ULN **If on anticoagulant therapy:** aPTT must be within therapeutic range of intended use of anticoagulants	ULN: aPTT:	Date:

___ 18. Negative urine or serum pregnancy test (female of childbearing potential only) ▪ If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required	Urine test: Serum test:	Date:

Contraception

___ 19. Female of childbearing potential: willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication.

- Childbearing potential defined as not being surgically sterilized or have not been free from menses for > 1 year.

___ 20. Male: Use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.2 Exclusion Criteria

Prior Therapy

___ 1. Anti-PD-1, anti-PD-L1, or anti-PD-L2 agents

___ 2. Prior radiation therapy within the field of the target lesion that in the opinion of the treating radiation oncologist would preclude further palliative radiation to a dose of 30 Gy.

___ 3. 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.

___ 4. Chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

- Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
- Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

___ 5. Live vaccine within 30 days of planned start of study therapy.

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

___ 6. Immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

___ 7. Investigational device within 4 weeks of the first dose of treatment

Concomitant Therapy

___ 8. Currently receiving an investigational agent

___ 9. About to undergo palliative radiation for a symptomatic CNS metastasis

___ 10. Systemic steroid therapy

Other Illnesses and Conditions

___ 11. Hypersensitivity to pembrolizumab or any of its excipients

___ 12. Diagnosis of immunodeficiency

___ 13. 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 (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

___ 14. Known history of, or any evidence of active, non-infectious pneumonitis.

___ 15. Current active infection requiring systemic therapy

___ 16. Known history of active TB, HIV 1/2, Hepatitis B or Hepatitis C

___ 17. Known additional malignancy that is progressing or requires active treatment.

- Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

___ 18. 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.

___ 19. 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.

___ 20. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

___ 21. Pregnant or breastfeeding (female only).

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 1.

Table 1 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Radiotherapy	3 Gy	Daily	External Beam	Daily except weekends and holidays for 10 fractions	Standard of care and combination agent

Trial treatment should begin as close as possible to the date on which treatment is allocated/assigned. The first dose of pembrolizumab on cycle 1, day 1 should occur on the same day after the first fraction of radiation therapy.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of the dose to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (aka MK-3475) are provided in the Pharmacy Manual.

5.2.1.2 Dose Modification

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 2 below. See Section 5.4.1 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

Table 2: Dose Modification Guidelines for Drug-Related Adverse Event

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

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

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

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

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record. Dose reductions of pembrolizumab are not permitted.

5.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Study Calendar (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All pembrolizumab treatment will be administered on an outpatient basis. Palliative radiotherapy is permitted to be delivered as an inpatient if in line with patient standard-of-care and after discussion with the study PI.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

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

5.2.3 External Beam Radiotherapy

5.2.3.1 General Radiotherapy Information

The intent of treatment is to deliver a palliative course of radiotherapy to a total dose of 30 Gy in 10 fractions to the symptomatic primary disease sites in the esophageal, GE junction, or gastric areas or one of the metastases. Both 3D conformal radiotherapy and IMRT are allowed.

Megavoltage equipment with effective photon energies ≥6MV is required.

Technique: 3D conformal using opposed AP-PA portals or multifield techniques, such as AP, PA and opposed lateral fields or IMRT are recommended. ICRU-50 and ICRU-62 prescription methods and nomenclature shall be used in this trial. Tightly contoured targets should be used to spare as much bone marrow, small bowel, liver, heart, lung and kidney as possible.

The prescription volume is the planning target volume (PTV). The total dose is 30 Gy in 10 fractions. A minimum of 90% of the PTV will receive 30 Gy. No more than 10% of the PTV shall receive greater than 35 Gy.

5.2.3.2 Localization, Simulation, and Immobilization

Prior to the simulation, all pertinent radiographs, operative notes, and other diagnostic procedures such as endoscopic findings should be reviewed. This will allow an informed determination of target volume and treatment field borders.

Simulation is required for all patients and should be performed with the patient positioned in an individualized immobilization device in the treatment position on a flat hard table and it is better in the supine position. Simulation must be performed on a diagnostic quality radiation

therapy simulator, which reproduces the geometry of the treatment machine. Oral contrast can be used to help outlining the target volume in the esophageal/GE junction/gastric areas. CT-based treatment planning is required for this study. The planning CT should encompass the entire thoracic cavity for esophageal/GE junction cancer and the abdominal to a level below the bottom of the kidneys for gastric cancer. A maximum slice thickness of 3-5 mm is required through regions of the gross tumor volume (GTV).

5.2.3.3 Treatment Planning and Target Volumes

Gross Target Volume (GTV): The GTV is defined as the primary tumor in the esophageal/GE junction/gastric areas, the grossly enlarged lymph nodes, or the gross metastatic sites requiring a palliative course of radiotherapy.

Clinical Target Volume (CTV): Since the intent of radiotherapy is for palliation, there is no need to define a CTV for treatment.

Planning Target Volume (PTV): Margins shall be added to the GTV for set up error and movement as PTV. This expansion could be 0.5 to 2 cm and does not need to be uniform in all dimensions. 4DCT scan is allowed to customize PTV expansion.

3D conformal radiotherapy or IMRT should be planned to optimize the PTV coverage and a minimum of 90% of the PTV shall receive 30 Gy. No more than 10% of the PTV shall receive greater than 35 Gy. IMRT can be delivered by tomotherapy or TrueBeam using RapidArc technique.

5.2.3.4 Critical Structures and Compliance Criteria

Organs at risk including lungs, liver, heart, kidneys and spinal cord shall be contoured. Dose constraints must follow the standard guidelines. The treating physician must carefully consider the tolerance dose/volume to each critical normal structure. It is recommended to reduce doses to lung and heart as much as possible.

- Lung: mean dose $\leq$ 20 Gy, V30 $\leq$ 20%, V20 $\leq$ 25%, V10 $\leq$ 40% and V5 $\leq$ 50%
- Heart: mean dose $\leq$ 25 Gy, V30 $\leq$ 30%
- Kidney: V20 $\leq$ 30%
- Liver: mean dose $\leq$ 20 Gy, V30 $\leq$ 30%

5.2.3.5 Radiotherapy Adverse Events

Adverse effects related to radiation therapy will be recorded. These include nausea/vomiting, diarrhea, poor appetite, weight loss, fatigue, skin erythema, esophagitis, carditis, pneumonitis, myelosuppression, myelitis, late pulmonary fibrosis, and esophageal stricture.

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject'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 on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 8.0.

5.3.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy outside of the clinical target volume of the index target lesion
    - Note: Radiation therapy to a symptomatic lesion other than the index target lesion or to the brain may be allowed at the investigator's discretion if the radiation course occurs after radiation has completed to the index target lesion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

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

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

5.4 Rescue Medications & Supportive Care

5.4.1 Supportive Care Guidelines

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

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

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

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

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

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

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

    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**:

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

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

    - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):

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

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

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

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

Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 3 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids);	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
prophylactic medications indicated for < =24 hrs	Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Diphenhydramine 50 mg po or IV (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier

methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 8.6-Reporting of Pregnancy and Lactation to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 8.6.

5.5.4 Use in Nursing Women

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

5.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

  Note: For unconfirmed radiographic disease progression, please see Section 5.2.2

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 7.1.2.6.2
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.5.5
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6.0 (Study Calendar) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 8.0). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.6.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.5.5.

5.7 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 STUDY CALENDAR

6.1 Initial Treatment Phase

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-Treatment		
Treatment Cycle:	Main Study Screening	C1/D1	C1/D8	C1/D15	C2D1	C3D1	C4D1	C5D1	C6+D1[a]	Last Dose	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[c]
Scheduling Window (Days):	-21 to -1		± 1	± 1	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks post discon	Every 12 weeks
Administrative Procedures													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X		
Post-study anticancer therapy status												X	X
Survival Status													X
Clinical Procedures/Assessments													
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X	
Full Physical Examination	X									X			
Directed Physical Examination		X	X	X	X	X	X	X	X		X		
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X		
ECOG Performance Status[d]	X	X	X	X	X	X	X	X	X	X	X		
Trial Treatment Administration													
Pembrolizumab		X			X	X	X	X	X				
Palliative Radiotherapy[e]		X	X	X									
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													

Trial Period:	**Screening Phase**	**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle:	Main Study Screening	C1/D1	C1/D8	C1/D15	C2D1	C3D1	C4D1	C5D1	C6+D1[a]	Last Dose	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[c]
Scheduling Window (Days):	-21 to -1		± 1	± 1	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks post discon	Every 12 weeks
Pregnancy Test – Urine or Serum β-HCG[f]	X										X		
PT/INR and aPTT[d]	X												
CBC with Differential[d]	X	X	X	X	X	X	X	X	X	X	X		
Comprehensive Serum Chemistry Panel[d]	X	X	X	X	X	X	X	X	X	X	X		
Urinalysis[d]	X												
T3, FT4 and TSH[d,g]	X				X		X		X[g]		X		
Serum carcinoembryonic antigen (CEA)[h]	X					X		X	X[h]				
12-Lead EKG	X												
Efficacy Measurements													
Tumor Imaging[i]	X					X		X	X[i]			X	
Tumor Biopsies/Correlative Studies Blood													
Tumor Biopsies[j]	X					X							
Correlative Studies Blood/Stool/Oral Swab Collection[k]	X					X				X			

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-Treatment		
Treatment Cycle:	Main Study Screening	C1/D1	C1/D8	C1/D15	C2D1	C3D1	C4D1	C5D1	C6+D1[a]	Last Dose	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[c]
Scheduling Window (Days):	-21 to -1		± 1	± 1	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks post discon	Every 12 weeks

a. Patients will continue pembrolizumab for a maximum of 35 cycles for first course of therapy if no evidence of disease progression or intolerable toxicity.

b. In patients who discontinue study therapy without documented disease progression, every effort should be made to continue monitoring disease status by radiologic imaging every 6 weeks (± 7 days) until 1) the start of new anti-cancer treatment, 2) disease progression on imaging, 3) death, or 4) the end of the study, whichever occurs first.

c. After the start of new anti-cancer treatment or documented disease progression by imaging, the patient should be contacted by telephone every 12 weeks to assess for survival status.

d. Laboratory tests and ECOG PS are to be performed within 10 days prior to the first dose of trial treatment.

e. Palliative radiotherapy to a total dose of 30 Gy to the primary tumor will be delivered in 3 Gy fractions daily with the exception of weekends and holidays. The first fraction of radiation should be given on C1D1 prior to administration of pembrolizumab. Radiation may continue into cycle 2 if delayed for management of toxicity or administrative reasons.

f. For women of reproductive potential, a urine pregnancy test will be performed within 72 hours prior to the first dose of trial treatment. A serum test may be considered if urine test is not appropriate or inconclusive.

g. Thyroid function tests should be collected at screening and every 6 weeks in concordance with day 1 of even-numbered cycles.

h. Serum CEA should be collected at screening and every 6 weeks in concordance with day 1 of odd-numbered cycles.

i. Tumor imaging should be performed every 6 weeks through the first 6 cycles, then subsequently performed every 9 weeks thereafter. For patients who complete 35 cycles of therapy or 2 years of follow up without evidence of progression, scans can be spaced out to every 12-24 weeks at the discretion of the treating physician. The exception will be patients who demonstrate PD, PR, or CR by RECIST 1.1, in which event the next imaging will be performed at 6 weeks for confirmation. Refer to Section 7.1.2.6.2 for details.

j. Tumor biopsies will be performed prior to cycle 1/day 1, and at 21 days (± 3 days) after completion of palliative radiotherapy. Biopsies will be performed of metastatic sites amenable to access by CT or ultrasound-guidance. Malignant ascites accessible by ultrasound guidance will also be eligible as tumor biopsy specimens. For patients receiving RT to a metastatic site and only the primary tumor is accessible to biopsy, these will be obtained by endoscopy.

k. Blood/stool/oral swab collection for correlative studies will be collected at screening assessment, at 21 days (± 3 days) after completion of palliative radiotherapy, at timepoint of first progression by RECIST (if applicable and continued on protocol therapy), and at end of treatment.

6.2 Second Course Phase

Trial Period:	Treatment Cycles						End of Treatment	Post-Treatment		
Treatment Cycle:	C1/D1	C2/D1	C3/D1	C4D1	C5D1	C6+D1[a]	Last Dose	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[c]
Scheduling Window (Days):		± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks post discon	Every 12 weeks
Administrative Procedures										
Inclusion/Exclusion Criteria	X									
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X		
Post-study anticancer therapy status									X	X
Survival Status										X
Clinical Procedures/Assessments										
Review Adverse Events	X	X	X	X	X	X	X	X	X	
Full Physical Examination	X						X			
Directed Physical Examination		X	X	X	X	X		X		
Vital Signs and Weight	X	X	X	X	X	X	X	X		
ECOG Performance Status	X	X	X	X	X	X	X	X		
Pembrolizumab	X	X	X	X	X	X				
Laboratory Procedures/Assessments										
Pregnancy Test – Urine or Serum β-HCG	X							X		
PT/INR and aPTT	X									
CBC with Differential	X	X	X	X	X	X	X	X		

Trial Period:	**Treatment Cycles**						**End of Treatment**	**Post-Treatment**		
Treatment Cycle:	C1/D1	C2/D1	C3/D1	C4D1	C5D1	C6+D1[a]	Last Dose	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[c]
Scheduling Window (Days):		± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 6 weeks post discon	Every 12 weeks
Comprehensive Serum Chemistry Panel	X	X	X	X	X	X	X	X		
Urinalysis	X									
T3, FT4 and TSH[d]	X		X		X	X[d]		X		
Serum carcinoembryonic antigen (CEA)[e]	X		X		X	X[e]				
Efficacy Measurements										
Tumor Imaging[f]			X		X	X[f]				

a. Patients will continue pembrolizumab for a maximum of 17 cycles for second course of therapy if no evidence of disease progression or intolerable toxicity.

b. In patients who discontinue study therapy without documented disease progression, every effort should be made to continue monitoring disease status by radiologic imaging every 6 weeks (± 7 days) until 1) the start of new anti-cancer treatment, 2) disease progression on imaging, 3) death, or 4) the end of the study, whichever occurs first.

c. After the start of new anti-cancer treatment or documented disease progression by imaging, the patient should be contacted by telephone every 12 weeks to assess for survival status.

d. Thyroid function tests should be collected at C1/D1 and every 6 weeks in concordance with day 1 of odd-numbered cycles.

e. Serum CEA should be collected at C1D1 and every 6 weeks in concordance with day 1 of odd-numbered cycles.

f. Tumor imaging should be performed every 6 weeks through the first 6 cycles, then subsequently performed every 9 weeks thereafter. For patients who complete 35 cycles of second course therapy or 2 years of follow up without evidence of progression, scans can be spaced out to every 12-24 weeks at the discretion of the treating physician. The exception will be patients who demonstrate PD, PR, or CR by RECIST 1.1, in which event the next imaging will be performed at 6 weeks for confirmation. Refer to Section 7.1.2.6.2 for details.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

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

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in the clinical trial. Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

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

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

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

The informed consent will adhere to IRB requirements, applicable laws and regulations and COH requirements.

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

Prior Medications

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

Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 8.0.

7.1.1.5 Disease Details and Treatments

Disease Details

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

Prior Treatment Details

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

Subsequent Anti-Cancer Therapy Status

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

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

7.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique City of Hope screening number that will be used to identify the subject for all procedures that occur prior to treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects.

Any subject who is screened multiple times will retain the original screening number assigned at the initial screening visit.

7.1.1.7 Trial Compliance (Medication/Diet/Activity/Other)

Interruptions from the protocol specified treatment for greater than 12 weeks between treatments require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on subject management.

Administration of trial medication will be witnessed by the investigator and/or trial staff. The total volume of pembrolizumab infused will be compared to the total volume prepared to determine compliance with each dose of pembrolizumab administered. The instructions for preparing and administering pembrolizumab are provided in the Pharmacy Manual.

Treatment with standard of care palliative radiotherapy will be prepared and administered as per the approved Department of Radiation Oncology and institutional guidelines.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

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

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document in the Appendix regarding the identification, evaluation and management of potential irAEs.

Please refer to Section 8.0 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

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

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Study Calendar, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study Calendar (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Appendix 1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Study Calendar.

7.1.2.6 Tumor Imaging and Assessment of Disease

Tumor imaging may be performed by computed tomography (CT; preferred) or magnetic resonance imaging (MRI), but the same imaging technique should be used in a subject throughout the trial. CT scan is the more commonly used modality and is preferred for the majority of patients. An MRI can be utilized if clinically appropriate.

7.1.2.6.1 Initial Tumor Imaging

To meet screening criteria, initial tumor imaging must be performed within 21 days prior to start of cycle 1, day 1. This scan will be considered the baseline assessment for the study. The site study team must review pre-trial images to confirm the subject has at least one target lesion (i.e. meets measurability requirements) per RECIST 1.1.

Scans performed as part of routine clinical management are acceptable for use as the baseline scan if they are of diagnostic quality, include all required anatomy, and performed within 21 days prior to start of treatment.

7.1.2.6.2 Tumor Imaging on Study per Immune-related RECIST (irRECIST)

The first on-study imaging assessment should be performed at 6 weeks (42 days ± 7 days) after the first dose of trial treatment. Subsequent imaging should be performed every 6 weeks (42 days ± 7 days) through the first 6 cycles. After 6 cycles, imaging will be pursued every 9 weeks (63 days ± 7 days) until demonstration of PD, PR, or CR, in which event imaging will be performed in 6 weeks for confirmation.

RECIST 1.1 may not provide an accurate 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 immunotherapeutics.

Patients with 1st radiologic assessment of PD by RECIST 1.1 may continue treatment if clinically stable as per Table 4. If the repeat scan confirms PD, patients will be discontinued from treatment. Exception can be made if the Investigator feels the patient is deriving benefit from treatment and after discussion with the study PI and Sponsor. Subsequent imaging should be pursued every 6 weeks until demonstration of confirmed PR or CR in which event imaging will then be conducted every 9 weeks if a patients has completed at least 6 cycles of pembrolizumab. Imaging may be more frequent if clinically indicated.

Clinically stable is defined by the following criteria:

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

Table 4 Imaging and Treatment after 1st radiologic evidence of progressive disease (PD)

	Clinically Stable		Clinically Unstable	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD per RECIST 1.1	Repeat imaging at 6 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at ≥ 4 weeks to confirm PD if possible	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible after discussion with	No additional imaging required	N/A

		the PI and Sponsor)		
Repeat scan shows SD, PR, or CR	Continue regularly scheduled imaging assessments every 6-9 weeks dependent on cycle of therapy	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 6-9 weeks dependent on cycle of therapy	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood/Stool/Oral Swab Sampling

7.1.2.7.1 Analysis of pre- and post-treatment tumor biopsies

Tumor biopsies will be performed during the Screening Phase and 21 days after completion of palliative radiotherapy. CT or ultrasound-guided biopsies will utilize 20-gauge core biopsy needles. As such tissue samples will be composed of both tumor cells and associated tumor stroma. Fresh tumor samples will be immediately delivered to the laboratory of Dr. Peter Lee for both ex vivo tumor/immune/stromal cell culture and expansion as well as histologic analyses. Patients with fresh malignant ascites collected by ultrasound guidance will also be immediately delivered to the lab for ex vivo tumor/immune/stromal cell culture and expansion alone.

Dr. Lee's lab will utilize quantitative spatial image analysis to examine changes in tumor cell-stromal interactions including CD4, CD8, Treg, and MDSC populations pre- and post-therapy.

PD-L1 expression within the tumor, on cancer cells and stromal cells, will be analyzed by immunohistochemistry (IHC) locally in Dr. Lee's lab, as well as centrally through QualTek Laboratories. PD-1 expression on tumor infiltrating T cells will be analyzed by a combination of IHC and flow cytometry. Immune cell populations will be further analyzed via high dimensional flow cytometry to measure levels of Tregs and MDSCs. Dr. Lee's lab will also pursue multi-color IHC to determine influences of pembrolizumab+RT treatment on the magnitude and proximity of CD8 T cells to cancer cells within the tumor. Changes in the diversity of the T cell repertoire in both PD-1 positive and PD-1 negative T cell populations following pembrolizumab+RT treatment will be analyzed via deep sequencing of the TCR Vβ CDR3 region. Such changes in T cell repertoire can be suggestive of potential epitope spreading for anti-tumor antigens and reversal of tumor antigen-specific T cell anergy. Genomic analyses utilizing City of Hope's Integrative Genomics Core will also be performed on the corresponding cancer/stromal/immune cell populations via total RNA-seq for coding and non-coding RNAs (including miRNAs).

Performance of these studies will require several highly developed techniques that have been routinely conducted in Dr. Peter Lee's lab. A prototype BD microfluidic FACS instrument allows for 12-color analysis of low cell numbers, thereby allowing maximal data acquisition out of small sample sizes. A powerful quantitative, spatial image analysis system will enable analysis of immune and cancer cells in tissues via 8-color histology. Lastly, Dr. Lee's lab has considerable expertise in TCR repertoire analysis via deep sequencing, which can yield information on T cell specificity to unknown antigens.

7.1.2.7.2 Correlative Studies Blood Sampling

<u>Circulating T cell, Tregs, and MDSC Isolation and Characterization</u>

Blood Sample Collection

Blood samples will be collected from an indwelling venous catheter or by venipuncture into three 7 mL green-top tubes (sodium or lithium heparin) at three timepoints: (1) Screening visit, (2) 21 days after completion of palliative radiotherapy, and (3) at the time of first progression by traditional RECIST1.1 (to capture potential pseudoprogression and only applicable for patients continued on treatment beyond progression), and at end of treatment. To prevent blood clotting, tubes will be inverted several times and then immediately placed on ice for transportation to the CICSL (Clinical Immunobiology Correlative Studies) processing laboratory. The whole blood should be processed within 4 hours of collection. While awaiting processing, the blood should be kept on a rocker set at low speed to mimic circulation and avoid clot formation.

Sample Processing in CICSL

Plasma

- For plasma preparation, anti-coagulated whole blood (two 7 mL green-top tubes) will be processed by centrifugation for 10 minutes at 1000 x g at 4^{O}C. The resulting upper plasma layer from each tube will be drawn up sequentially into a sterile 5 mL syringe and pushed through a sterile 0.2/0.8 micron disposable filter (PALL Acrodisc PF, Cat. 4658). The filtered plasma will then be transferred in 500 µL aliquots into multiple appropriately-labeled Starstedt microfuge tubes (Starstedt Cat 72.692.005). To one aliquot, add 0.5 mL glycerol/0.02% sodium azide solution to dilute the plasma 50/50 v/v. **Keep the diluted plasma sample at -20˚C and do not freeze**. All the remaining plasma aliquots will be stored frozen at -80˚C until ready for testing.

PBMCs

- Any blood remaining in the two 7 mL green-top tubes used to prepare plasma above will be diluted 1:1 with Hank's Balanced Salt Solution ("HBSS", Irvine Scientific, Cat. 9228 or equivalent) and combined with the whole blood from the unused green-top tube in a sterile 50 ml conical centrifuge tube. Peripheral blood mononuclear cells

(PBMC) will then be isolated from the combined whole blood sample by Ficoll-gradient separation as described below;

- Allow Accuspin-Histopaque tubes ("Accuspin", Sigma Cat. A6929 or A0561, for 12 or 100 tubes, respectively) and HBSS to warm to room temperature. Place a Mr. Frosty container in the refrigerator and prepare the freezing media by adding 10% DMSO to fetal calf serum and chill at 4˚C or on ice.
- Prepare Accuspin tubes by centrifuging at 1000 x g for 1 minute at room temperature (RT) with brakes on. Each tube can process up to 20 mLs of whole blood; prepare the appropriate amount of tubes necessary. After centrifugation, the Histopaque reagent should be below the barrier of the tube. Add 5 mLs of HBSS to the Accuspin tube. Add up to 20 mls of whole blood to each Accuspin tube until all the blood has been distributed.
- Centrifuge the blood sample at 800 x g for 15 minutes at RT with brakes on LOW. After centrifugation, three layers should be visible above the barrier of the tube: the plasma layer at the top, a cloudy layer in the middle where the PBMC are, and a clear Histopaque reagent layer right below. Using a pipette, remove the upper plasma layer to within 2 cm of the cloudy interphase. Carefully pipette the cloudy PBMC interphase and transfer to a sterile 50 mL centrifuge tube.
- Add HBSS up to the 45 mL mark in the centrifuge tube with the PBMC and spin at 400 x g for 10 minutes at RT with brakes on. Decant the supernatant and loosen the cell pellet before adding HBSS to the 45-mL mark again for a second wash. Centrifuge at 300 x g for 10 minutes at RT with brakes on. Decant the supernatant, loosen the cell pellet and then add a known volume of HBSS to resuspend the cells for counting. Mix the cell suspension up and down with a pipette several times before removing a small aliquot for cell count.

Centrifuge the cell suspension one final time at 300 x g for 10 minutes at RT with brakes on. PBMC should be frozen down at 0.5 – 1 x 10^7 cells/vial. Determine the volume of freezing media (fetal calf serum with 10% DMSO) needed to give a 1 x 10^7 cell/mL suspension. After the last centrifugation is complete, discard supernatant and loosen the cell pellet before adding freezing media slowly, a small volume at a time with mixing in between (vortex at low speed). Aliquot 0.5 - 1 mL of the final cell suspension into individually labeled cryovials. Transfer the cryovials into Mr. Frosty and store at -80˚C. Twenty four hours later, cryovials will be transferred to liquid nitrogen tanks for long-term storage.

Circulating T cell Characterization

PD-1 expression on circulating PBMC T cells will be analyzed by flow cytometry. Immune cell populations will be further analyzed via high dimensional flow cytometry to measure levels of Tregs and MDSCs, which will allow for calculating the ratio of effector T cells to Tregs and MDSCs, respectively. Ex vivo isolated T cells will also be analyzed for proliferative

capacity (CFSE dilution) and cytokine production (IFN-γ and IL-17) after stimulation with anti-CD3 and/or PMA and Ionomycin.

Serum Circulating miRNA Profiling

20 mL of whole blood will be collected from each patient into 2 Vacutainer red top tubes (no additive) at three timepoints: (1) Screening visit, (2) 21 days after completion of palliative radiotherapy, and (3) at the time of first progression by traditional RECIST1.1 (to capture potential pseudoprogression and only applicable for patients continued on treatment beyond progression), and at end of treatment. Blood will be allowed to clot on ice for 15-20 min, and then centrifuged at 1900g (3000 rpm) for 10 minutes at 4ºC using a swinging bucket rotor. The upper (yellow) serum phase will then be transferred to four separate labeled cryovials without disturbing the intermediate buffy coat layer. The cryovials will be placed on ice, and then delivered immediately for storage in the Fox North Building, Room N2002, freezer EFRU385. Serum samples will be stored frozen at -80 ºC until time of analysis.

Total RNA will be extracted using Trizol within the Integrative Genomics Core at City of Hope for miRNA-seq. Sequence reads that match human miRNAs in Sanger mirBase will be calculated and analyzed. Circulating whole genome miRNA signatures will be compared to miRNAs sequenced from total RNA-seq from the corresponding tumor biopsy timepoints to aid in biomarker discovery of serum miRNAs that may predict for immunotherapy response or resistance. miR-23a will be of particular interest given its description as a gastric cancer oncomir and function as a repressor of T cell cytotoxicity in preclinical miRNA screens.[14,15] miR-34a and miR-200 will be other miRNAs of interest to measure given reporting of their ability to suppress PD-L1 expression in other experimental systems.[16,17]

Germline miRNA biomarker analysis

Weidhass et al recently discovered that a new class of miRNA regulatory network disrupting germline mutations can act as biomarkers of response and toxicity to anti-PD-1 and anti-PD-L1 therapy.[18] Specifically a panel of germline mutations in 3' untranslated regions (3'-UTR) and miRNA promoter regions provided 89% specificity for response and 76% specificity for toxicity in a population of 85 patients with melanoma and non-small cell lung cancer treated with anti-PD-1 or anti-PD-L1 agents.

Leftover banked PBMC samples from any timepoint will be shipped batched via overnight courier on dry ice to:

MiraDx
Contact: Joanne Weidhaas, M.D., Ph.D
11600 Wilshire Blvd.
Los Angeles, CA 90025

DNA extraction from PBMCs will be performed at MiraDx and targeted sequencing will be performed for the panel of miRNA germline mutation biomarkers. Results will be correlated with RECIST1.1 and irRECIST responses as well as toxicities recorded per NCI CTCAE.

7.1.2.7.3 Correlative Studies Stool and Oral Swab Sampling

Stool Sampling

Patients will be provided a biospecimen bag and sterile swab (BD BBL™ CultureSwab™ Sterile, Media-free Swabs) and instructed on proper collection from a used toilet tissue paper the required stool sample. Patients will be contacted and reminded by the clinical research coordinator 48-24 hours in advance of the specified protocol visit to perform the stool collection and will be advised to leave the specimen at room temperature until the day of their visit. Patients will also have the option of swabbing and submitting the stool specimen during their protocol visit provided they are reporting daily regular bowel movements. Samples submitted to the clinical research coordinator will be stored promptly and banked at -20 ^{O}C until analysis. Samples will be labeled as "stool" and with the patient's deidentified protocol ID number and date of collection.

Oral Sampling

At the prespecified timepoints, patients will be asked during their protocol visit in clinic to perform an oral swab of both inner cheeks and tongue using a provided sterile swab (BD BBL™ CultureSwab™ Sterile, Media-free Swabs). These samples will also be collected by the clinical research coordinator and will be stored promptly and banked at -20 ^{O}C until analysis. Samples will be labeled as "oral swab" and with the patient's deidentified protocol ID number and date of collection.

Microbiomic Profiling

Banked stool and oral swab specimens at the time of planned analysis will be shipped via courier overnight on dry ice to the lab of Dr. Greg Caporaso of Northern Arizona University/TGen:

J. Gregory Caporaso, Ph.D.
Northern Arizona University
1350 S Knoles Drive
Flagstaff, AZ 86011, USA

Microbial DNA will be extracted from swab samples using the PowerSoil® DNA Isolation Kit (Mobio Carlsbard, CA). A 16S rRNA library for the MiSeq Illumina platform will be prepared according to the protocol from the Earth Microbiome Project (http://www.earthmicrobiome.org/emp-standard-protocols/). Microbiome sequencing data to analyze species diversity will be subsequently analyzed using Quantitative Insights Into

Microbial Ecology (QIIME) 1.9.1, biom-format version 2.1.5, VSEARCH version 1.7.0 (https://github.com/torognes/vsearch), SSU-ALIGN 0.1, and FastTree, as well as custom analytic software (source code at https://github.com/caporaso-lab/autism-fmt1) being prepared for release in QIIME 2.

7.1.3 Laboratory Procedures/Assessments

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		PK
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 8.0 – Data and Safety Monitoring Plan. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 – Study Calendar. Specific procedure-related details are provided in Section 7.1 – Trial Procedures.

7.1.5.1 Screening

Within 21 days prior to start of treatment, potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in Section 5.1. Screening procedures may be repeated after consultation with the Sponsor.

Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame. Screening procedures are to be completed within 21 days prior to the first dose trial treatment except for the following:

Laboratory tests and ECOG PS are to be performed within 10 days prior to the first dose of trial treatment.

For women of reproductive potential, a urine pregnancy test will be performed within 72 hours prior to the first dose of trial treatment. A serum test may be considered if urine test is not appropriate or inconclusive.

Subjects may be rescreened after initially failing to meet the inclusion/exclusion criteria. Results from assessments performed during the initial screening period are acceptable in lieu of a repeat screening test if performed within the specified time frame and the inclusion/exclusion criteria is met. Subjects who are rescreened will retain their original screening number.

7.1.5.2 Treatment Period

Visit requirements are outlined in Section 6.0 – Study Calendar. Specific procedure-related details are provided in Section 7.1 – Trial Procedures.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 6 weeks (42 ± 7 days) by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 9 weeks (± 7 days). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.5.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression. Details are provided in Section 6.2 – Second Course Phase.

7.1.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.5.5 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. A repeat course of palliative radiotherapy to the primary tumor will not be given during this time. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
    - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
        - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
        - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

    - Had SD, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.1
- Female subject of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.5.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.

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

- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Patients will not receive another course of palliative radiotherapy to their primary tumor. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.2 – Second Course Phase.

8.0 DATA AND SAFETY MONITORING PLAN

8.1 Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because the study is an investigator-initiated phase II trial and involves a COH IND.

8.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

8.3 Adverse Events and Serious Adverse Events

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.0.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease,

disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA

in accordance with the approved City of Hope's Institutional policy [policy effective date: 05/14/14].

8.4 Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

8.5 Definition of an Overdose for This Protocol and Reporting of Overdose to Merck

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

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

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

All reports of overdose with and without an adverse event must be reported within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

8.6 Reporting of Pregnancy and Lactation to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

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

8.7 Reporting of Adverse Events to Merck

8.7.1.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

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

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

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

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

9.0 STATISTICAL ANALYSIS PLAN

9.1 Primary Objective

The primary objective of this study is to establish that the combination of pembrolizumab and a palliative course of traditional external beam multifractionated RT in patients with metastatic gastric, esophageal, and/or GEJ cancers will lead to an increase in tumor infiltrating cytotoxic T-cells and circulating cytotoxic T cells and a reduction in immunosuppressive Tregs and MDSCs in non-irradiated sites.

Each patient will serve as their own control, and tumor cytotoxic T cell infiltration and immunosuppressive Tregs will be compared pre- and post-treatment. As variability of the difference in the T cell populations pre- and post-treatment is unknown and still being established, the power calculation is based on the concept of "effect size" in this biomarker analysis study. Specifically, a sample-size of 14 patients will have 80% power to detect an effect size of 0.71 using a paired t-test with a 0.05 one-sided significance level. The effect size is the mean difference divided by the standard deviation of the difference.

A positive biological signal combined with acceptable secondary endpoints (response and toxicity) are sought for a clear indication of promise of this combination.

9.2 Secondary Objectives

Secondary objectives such as ORR per RECIST 1.1 and irRECIST, PFS, and OS are exploratory, though will also be documented, along with toxicity. All patients who receive at least one dose of pembrolizumab will be included in the safety analysis. Stool/oral microbiomic profiling and germline miRNA biomarker panel analyses will also be correlated with secondary outcome measures of ORR and toxicities.

While overlapping toxicities are not anticipated with the combination of pembrolizumab and RT, the initial treatment of patients within this trial warrants a careful review of toxicity:

- Toxicity-based Interim Analysis: A study hold will be placed for re-evaluation at the proposed starting dose as accrual proceeds on this phase II study according to the following rule: The study will hold pending an amendment if 3 patients discontinue therapy after cycle 1 due to treatment-related toxicity. This rule has a 97% chance of holding the study if the true discontinuation rate due to toxicity on cycle 1 is 50%. It has a 15% chance of holding if the true discontinuation rate on cycle 1 is 15%.
- Treatment-related mortality stopping rule: Any treatment-related death will hold the study accrual pending review by the PI, Sponsor, and DSMB/IRB.

With 14 patients, there is 96% power to detect at least one responder if the true response rate is 21% or higher, which is in the range of 22% in the phase I trial of pembrolizumab among gastric cancer patients with PD-L1 overexpression. As a result, failure to observe any response in the 14 patients would result in lack of enthusiasm for the combination of pembrolizumab and RT in this patient population. PFS and OS will be determined using the Kaplan-Meier method.

10.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

10.1 Investigational Product

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

Clinical Supplies will be provided by Merck as summarized in Table 7.

Table 7 Product Descriptions

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

10.2 Packaging and Labeling Information

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

10.3 Clinical Supplies Disclosure

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

10.4 Storage and Handling Requirements

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

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

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

10.5 Returns and Reconciliation

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

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

11.0 DATA REPORTING

11.1 Data Reporting

11.1.1 Confidentiality and Storage of Records

The original data collection forms will be sent to the CTO and stored in a locked area. This protocol uses Electronic Data Collection per institutional policy for investigator-initiated trials, and the data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

11.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

11.1.3 Data Collection Forms and Submission Schedule

All data will be collected 2 weeks prior using MediData Rave. Data will be sent to the location identified in Section 11.1.1 and stored in a secure location.

11.1.3.1 Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject.

11.1.3.2 Prior Therapy Forms and On-Study Forms

Within 2 weeks of registration, the clinical research associate will submit data in regards to prior therapies the patient has received, if applicable.

12.0 HUMAN SUBJECT ISSUES

12.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the investigator before the study is initiated.

The IRB will be informed of revisions to other documents originally submitted for review; serious unexpected or unanticipated adverse experiences occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Any amendment to the protocol document and accompanying informed consent document/template, as developed and provided by the PI, will require review and approval by the COH IRB before the changes are implemented in the study.

12.2 Recruitment of Subjects

The Gastrointestinal Medical Oncology Division will identify patients undergoing treatment for metastatic gastroesophageal cancer at City of Hope.

12.3 Study location and Performance Sites

This study will be performed at COH.

12.4 Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed participant authorization informing the participant of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, ant the

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

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of participants will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. Source documents provided to coordinating center for the purpose of auditing or monitoring will be de-identified and labeled with the study number, participant ID, and participant initials.
The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring/ auditing, IRB reviews, and FDA/regulatory authority inspections. The participant's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

12.5 Financial Obligations and Compensation

The investigational drug, pembrolizumab, will be provided free of charge by City of Hope and Merck. Should this drug or device become commercially available during the course of treatment, the research participant and/or the insurance carrier may be asked to pay for the costs of the drug.

The standard of care drug(s) and procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

12.6 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Prospective participants will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Prospective participants will be afforded sufficient time to consider whether or not to participate in the research.

After the study has been fully explained, written informed consent will be obtained from either the prospective participant or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the prospective participant or his/her legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the participant or his/her legally authorized representative. The original signed consent must be maintained by the investigator and available for inspection sponsor designated representatives, or regulatory authority at any time. Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation.

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APPENDIX 1: ECOG PERFOMANCE STATUS

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

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

APPENDIX 2: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST) 1.1 AND IMMUNE RECIST (IR-RECIST)

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

* As published in the European Journal of Cancer:

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

irRECIST** will be utilized to account for the unique tumor responses associated with immunotherapeutics.

**As published in Clinical Cancer Research:

Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. Clin Cancer Res. 2013 Jul 15;19(14):3936-43. Epub 2013 Jun 6.

APPENDIX 3: EVENTS OF CLINICAL INTEREST GUIDANCE DOCUMENT

The most recent version (5.0, 12/18/2014) of the Events of Clinical Interest (ECI) Guidance Document provides study sites with further guidance on the identification and management of ECI for the pembrolizumab program and reporting guidelines to Merck.

APPENDIX 1: ECOG PERFOMANCE STATUS

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

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

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