Protocol Cover Page

“Multicenter, Randomized Phase II study evaluating Pembrolizumab in combination with chemotherapy and chemoradiation in locally advanced esophageal”

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Joint Clinical Trials Office (JCTO)

Multicenter, Randomized Phase II study evaluating Pembrolizumab in combination with chemotherapy and chemoradiation in locally advanced esophageal adenocarcinoma

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INVESTIGATOR AGREEMENT

Protocol Version 10.0 Dated: October 10, 2022

I have read the protocol entitled "Multicenter, Randomized Phase II study of Pembrolizumab in combination with chemotherapy or chemoradiation in locally advanced esophageal adenocarcinoma"

I agree to conduct the study as detailed herein and in compliance with ICH Guidelines for Good Clinical Practice and applicable regulatory requirements and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

_____

Principal investigator printed name

_____ _____

Principal investigator signature **Date**

_____

_____

_____

Investigational site or name of institution and location (printed)

Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCMC.

List of Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
ECI	Events of Clinical Interest
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBAF	Human Research Billing Analysis Form
ICF	Informed Consent Form
IND	Investigational New Drug
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious AdverseReaction
UAP	Unanticipated Problem
WCMC	Weill Cornell Medical College

Protocol Summary

Full Title:	Multicenter, Randomized Phase II study of Pembrolizumab in combination with chemotherapy and chemoradiation in locally advanced esophageal adenocarcinoma
Short Title:	Phase II study of Pembrolizumab in locally advanced esophageal adenocarcinoma
Clinical Phase:	II
Principal Investigator:	Manish A. Shah
Sample Size:	N= 42 patients
Accrual Ceiling:	This study will enroll 42 patients and screen up to 80 patients
Study Population:	locally advanced esophageal adenocarcinoma
Accrual Period:	3.5 years
Study Design:	multicenter, random assignment study
Study Duration:	4.5 years (projected end date July 2021).
Study Agent/ Intervention Description:	Pembrolizumab

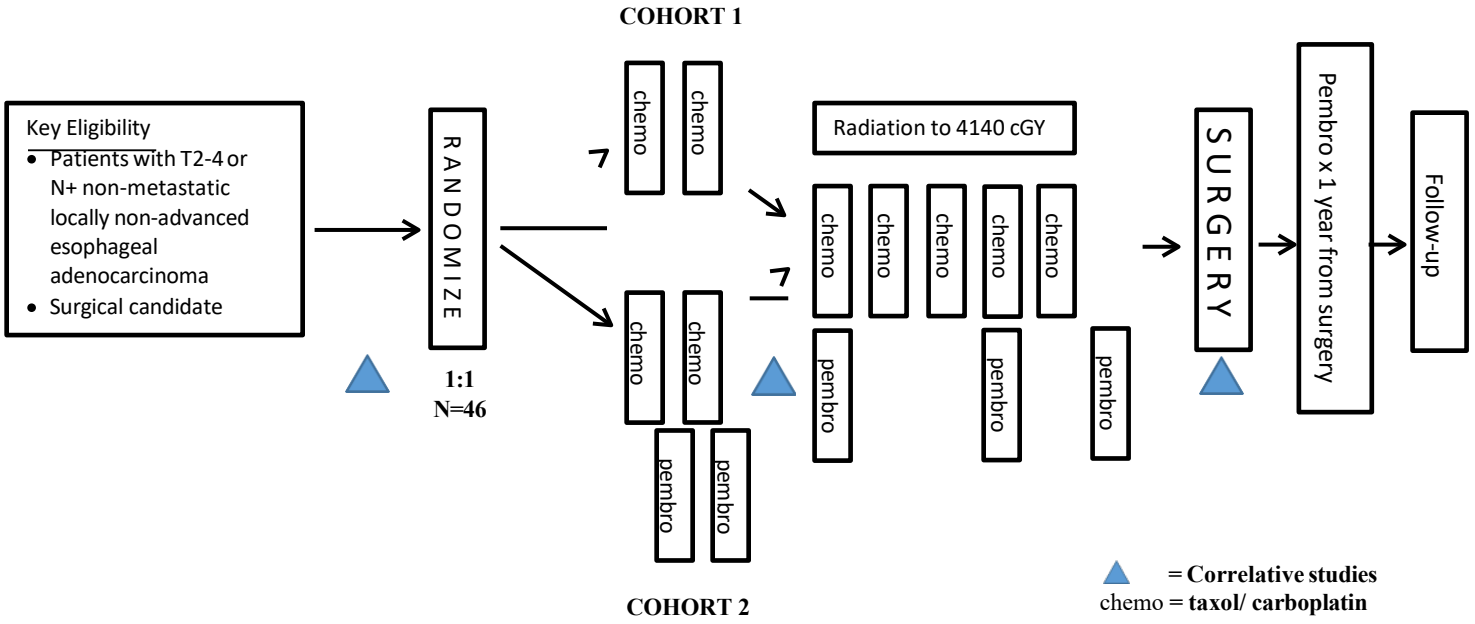
Primary Objective: To examine the safety and efficacy of the combination of pembrolizumab with chemotherapy and chemoradiation in locally advanced esophageal adenocarcinoma as assessed by the major pathologic response rate (defined as complete response or near complete response).

Secondary Objectives: 1) To examine other measures of efficacy of pre-operative therapy in combination with pembrolizumab, including the pathologic complete (or near complete) response rate, R0 resection rate, and median and 1-year disease free and overall survival rates.
2) To explore the effect of the combination of pembrolizumab with chemotherapy as assessed by the local immune infiltration and clinical response in each treatment group.

Exploratory Objectives: 1) To examine whether anti PD1 therapy is associated with a transcriptomic signature of intra-tumoral immune activation predictive of response and 1-year survival.
2) To examine whether chemotherapy is associated with induction of PD-L1 expression in tumors.
3) To examine whether anti PD1 therapy is associated with increased intra-tumoral immune cell infiltration

Endpoints: Major pathologic response rate**,** 1yr disease free and overall survival rate

SCHEMA



Cohort 1

1. Induction chemotherapy with taxol/carboplatin (q 3 week)
2. Chemoradiation – taxol/carboplatin weekly, with Pembrolizumab
3. Resection
4. Pembrolizumab every 6 weeks for 1 year following surgery (beginning at3-14 weeks following resection)

Cohort 2

1. Induction chemotherapy with taxol/ carboplatin (q 3 weeks), with Pembrolizumab administered the week after chemotherapy. (i.e. weeks 2 and 5).
2. Chemoradiation – taxol/carboplatin weekly, with Pembrolizumab
3. Resection
4. Pembrolizumab every 6 weeks for 1 year following surgery (beginning at 3-14 weeks following resection)

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1 STUDY OBJECTIVES

1.1 Primary Objectives

To examine the safety and efficacy of the combination of Pembrolizumab with chemotherapy and chemoradiation in locally advanced esophageal adenocarcinoma as assessed by the major pathologic response rate (defined as complete response or near complete response).

1.2 Secondary Objectives

To examine other measures of efficacy of pre-operative therapy in combination with pembrolizumab, including the pathologic complete (or near complete) response rate, R0 resection rate, and median and 1-year disease free and overall survival rates.

To explore the effect of the combination of pembrolizumab with chemotherapy as assessed by the local immune infiltration and clinical response in each treatment group.

1.3 Exploratory Objectives

To examine whether anti PD1 therapy is associated with a transcriptomic signature of intra-tumoral immune activation predictive of response and improved 1 year survival.

To examine whether chemotherapy is associated with induction of PD-L1 expression or induction of an inflammatory signature in tumors.

To examine whether anti PD1 therapy is associated with increased intra-tumoral immune cell infiltration.

2 BACKGROUND

2.1 Disease

Esophageal cancer is the 8th most common cancer worldwide, and the 5th most common gastrointestinal malignancy in the U.S., with an estimated 18,170 new cases and 15,450 deaths in 2013[1,2]. Esophageal cancer histology varies by location, with squamous cell carcinoma (SCC) more prevalent in the upper and middle thirds of the esophagus and more prevalent worldwide, and adenocarcinoma (ADC) more prevalent in the lower third of the esophagus and at the gastroesophageal junction (GEJ) and more prevalent in the West. There are two accepted treatment paradigms for the adjuvant therapy of esophageal/ GEJ carcinoma: (1) peri-operative chemotherapy for esophageal/GEJ ADC, and (2) pre-operative chemoradiation for both SCC and ADC. Our primary aim is to examine the safety and efficacy of pembrolizumab in both pre-operative treatment paradigms for esophageal/GEJ carcinoma. The specific rationale for our study design is rooted in three unanswered questions: (1) does the addition of

an immune check-point inhibitor (pembrolizumab) enhance the efficacy of cytotoxic therapy (chemotherapy with chemoradiation) as determined by response rates, nodal own-staging and 1 year disease free survival in comparison to historical controls, (2) what are the pathological effects of combining pembrolizumab with chemotherapy alone, and (3) what are the molecular (PD-L1 expression), immunological (TILs extent) and gene- expression signatures associated with the efficacy of pembrolizumab in the neoadjuvant setting.

Preoperative Chemoradiation for Esophageal Carcinoma:

Based on the Dutch CROSS trial, tri-modality therapy is also a standard option for the management of potentially resectable esophageal carcinoma, particularly SCC[3]. This study enrolled 366 patients, randomized to preoperative chemoradiation with paclitaxel and carboplatin versus surgery alone. Preoperative chemoradiotherapy improved OS (median OS 49.4 vs. 24.0 months, 3-year OS 58% vs. 44%, p=0.003). Patients with SCC of the esophagus derived the most benefit with trimodality therapy, with HR 0.42 (95% CI 0.226-0.788), whereas patients with esophageal ADC had HR 0.74 (95% CI 0.536-1.024)[3].

Induction Chemotherapy and the Effects on Tumor Infiltrating Lymphocytes

Induction chemotherapy (eg. chemotherapy given prior to the initiation of chemoradiation) is commonly employed to initiate therapy while the treatment planning for radiation is underway. It is associated with dysphagia relief, and is a standard practice, accepted by the US Intergroup (eg. induction carboplatin/taxol is one of the arms of the intergroup study CALGB 80101). In our own experience, when given without radiation, carboplatin and paclitaxel was associated with high response rates, including a 11% pathology complete response rate seen in esophageal adenocarcinoma.[4] Chemotherapy is known to affect the infiltrating tumor lymphocytes. In a study evaluating T cell subpopulations in peripheral circulation of patients with ovarian carcinoma receiving taxol/carboplatin, it was noted that there was a profound increase in the cytotoxic T cell response beginning about 12-14 days following chemotherapy in these patients[5]. Specifically, the proportions of Th1, Tc1, CD45RO memory T, NKT cells and the ratio of Tc1 to Tc2 cells increased significantly 12-14 days following chemotherapy, associated with a decrease in regulatory T cells[5]. Given the significant potential of carboplatin/ paclitaxel to increase the antigenic load (based on the induction of apoptosis), and the known effects of augmenting a CD8 infiltrate, there is significant potential for pembrolizumab to have an added effect when given following chemotherapy. This justifies the randomization during induction therapy.

Pathologic Node+ Esophageal Cancer Patients Have a High Risk of Relapse:

Unfortunately, despite pre-operative therapy, the majority of patients with esophageal adenocarcinoma eventually will recur and die of their disease. Pathologically involved lymph nodes have a particularly poor prognosis. Patients with persistent node-positive disease at surgery (ypN+) have significantly worse disease-free survival compared to ypN0 patients (10–11 months vs. 30-32 months)[6]. This provides the rationale to continue with post-operative pembrolizumab following resection.

PD-1 and Pembrolizumab:

Programmed death ligand 1 (PD-L1) is a transmembrane protein that was first identified for its role in the maintenance of self-tolerance and prevention of autoimmunity[7]. Engagement of PD-L1 on dendritic cells with the programmed death 1 (PD-1) receptor on T cells delivers an inhibitory signal that promotes T cell anergy or apoptosis[8,9]. Tumor cells often over-express

PD-L1 (or PD-L2), resulting in T-cell anergy and escape from immunosurveillance in the tumor microenvironment. Blockade of the interaction between PD-L1 or PD-L2 on tumor cells and PD-1 on T cells reverses T cell suppression within tumors, thereby promoting effective anti-tumor immune responses. Pembrolizumab is a highly selective, humanized monoclonal IgG4-kappa isotype antibody against PD-1 that successfully blocks the negative regulatory signaling of the PD-1 receptor expressed on T cells[10], and has received FDA approval in September 2014 for its activity in melanoma[11].

Immunosurveillance in Esophageal/GEJ carcinoma:

Several investigators have demonstrated the significance of immunosurveillance in esophageal cancer[12-14]. Specifically, tumor infiltrating lymphocytes (TILs) correlated with improved survival[13,14], and expression of PD-L1 and PD-L2 was associated with poor survival in esophageal carcinoma[12,13]. Notably, 43% of esophageal SCC and 70% of esophageal adenocarcinoma express PD-L1[13], and its expression is independently associated with worse survival [12].

PD-1/PD-LI Inhibitors in Gastroesophageal Cancer:

There has been early demonstration of activity of PD-1/ anti-PD-L1 antibody immune checkpoint inhibitors in upper GI malignancies. Activity has been demonstrated with the PD-L1 antibody MEDI14736 in advanced gastric cancer. Segal et al evaluated MEDI4736 in 16 patients with gastroesophageal adenocarcinoma[15]. Four patients had a partial response. Several responses were also seen in pancreatic cancer – another disease type not typically associated with activity from immune therapy. Pembrolizumab also appears to have substantial activity in patients with advanced gastric cancer expressing PD-L1 as reported at ESMO 2014. Of the 39 patients enrolled, 19 were from Asia-Pacific and 20 were from other areas of the world. At a median follow-up of approximately 6 months, the ORR was 31% (31.6% in Asians and 30% in non-Asians). There were no complete responses and six partial responses each in Asians and non-Asians. In Asian patients, the median time to response was 8 weeks (range, 7-8 weeks) and six of the six responses are ongoing. The response duration ranged from 8+ to 16+ weeks and the median duration of response has not been reached. In the non-Asian group, the median time to response was 12 weeks (range, 7-17 weeks) and five of the six responses are ongoing. The response duration ranged from 9+ to 20+ weeks, and the median duration of response has also not been reached. There are planned studies of the addition of immune checkpoint inhibitors with chemotherapy. However, there is very little data any sequence dependency of combined therapy. One study in non-small-cell lung cancer suggests that chemotherapy prior to immune checkpoint inhibition (in this case, anti-CTLA-4 therapy with ipilimumab) was superior (as assessed by PFS) to a strategy of immediately administering chemotherapy/ipilimumab simultaneously[16]. Conversely, activating the anergic T cells prior to cytotoxic therapy also appears to be a rational approach, as it allows for T cell activation prior to the potentially negative impact of steroids and cytotoxic therapy on these cells[17]. Biomarkers predictive of immune checkpoint inhibition efficacy are also being investigated.

Pathologic Response and Pre-operative Chemoradiotherapy in Esophageal Cancer:

Histopathologic response has been established as a predictor of patient outcome following preoperative chemoradiotherapy. An assessment of vital residual tumor cells can be performed to determine the degree of pathologic response[23]. Across over 250 patients, the major pathologic response rate, defined as complete response or near complete response, i.e. < 10% residual tumor cells), for esophageal adenocarcinoma is approximately 30%[24,25]. Prognostically, this group of patients with a major histologic response have a much better

survival than non-responders[26], where the pooled hazard ratio was 0.46 (95% CI 0.32-0.66).

2.2 Investigational Agent or Device

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 IgVariable-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.

2.3 Study Rationale

Esophageal cancer is a prevalent and deadly malignancy. Because of early symptoms of dysphagia and weight loss, patients commonly present prior to the development of metastases, and thus, our opportunity to improve survival with improved immunosurveillance in the adjuvant and neo-adjuvant setting is substantial. PD-L1 and PD-L2 is overexpressed in upper gastrointestinal cancers, and there is preclinical and preliminary clinical evidence of a synergistic or additive effect of immune checkpoint inhibitors when combined with cytotoxic therapy in the advanced disease setting. Our considered neoadjuvant approach will address important questions of the sequence dependence of pembrolizumab when combined with chemotherapy, and will provide important safety and efficacy data of the combination of chemoradiation and pembrolizumab. Additionally, the neoadjuvant approach where tumor tissue and blood samples can be obtained prior to and after therapy, affords a unique opportunity for the evaluation of biomarkers predictive of response and resistance to pembrolizumab.

2.3.1 Rationale for Dose Selection of Pembrolizumab

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

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

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

the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type. The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

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

2.3.2 Rationale for Randomization

The primary endpoint of the study is to evaluate the addition of pembrolizumab to chemoradiation in patients with locally advanced esophageal cancer compared with historical control. The rationale for randomizing patients to chemotherapy + pembrolizumab versus chemotherapy alone during induction therapy is to examine the impact of pembrolizumab in augmenting tumor lymphocyte infiltration following chemotherapy. During the induction chemotherapy phase, patients will be randomly assigned to chemotherapy alone or chemotherapy followed 1 week later by pembrolizumab. The rationale is to examine the hypothesis that cytotoxic chemotherapy, by virtue of cell killing, will increase the breadth and density of neoantigens present for immune system recognition. Patients who receive pembrolizumab following chemotherapy may therefore have a more robust immune response, as assessed by the lymphocytic infiltrate in the tumor and possibly by a greater response to treatment as assessed radiographically. This is an exploratory hypothesis.

2.4 Correlative Studies

(1) We will determine the frequency and character of immune cell infiltration before and after therapy with pembrolizumab by flow cytometry.

(2) We will perform whole tumor RNASeq and deconvolution to determine the frequency and activation state of tumor infiltration of stromal and immune cells.

2.5 Hypotheses

2.5.1 Primary Hypothesis

The addition of pembrolizumab to chemotherapy and chemoradiation will be associated with improved anti-tumor efficacy resulting in improved patient survival in patients with esophagus/GEJ carcinoma treated with a curative paradigm.

2.5.2 Secondary Hypotheses

Cytotoxic therapy can augment the activation of the immune system by pembrolizumab when these two treatment strategies are combined. We believe that we will be able to generate preliminary evidence of this relationship by assessing the character and extent of immune infiltration following combined therapy as well as assessing the clinical response to the tumor.

Pembrolizumab can be given safely with chemoradiation in the preoperative setting for localized esophageal squamous cell carcinoma.

2.5.3 Exploratory Hypothesis

Treatment with pembrolizumab is associated with a specific immune related gene-expression signature predictive of pembrolizumab efficacy as assessed by improved clinical outcome.

Chemotherapy and/or radiotherapy can induce PD-L1 expression and thus potentially enhance efficacy of pembrolizumab.

Clinical efficacy of pembrolizumab is associated with increased tumor infiltration by CD8+ lymphocytes.

3 SUBJECT SELECTION

3.1 Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

3.1.1 Patients must have histologically or cytologically confirmed esophageal or GEJ adenocarcinoma

3.1.2 Clinical tumor stage should be T2 Npositive M0 or T3-T4 Nany M0

3.1.3 Be willing and able to provide written informed consent/assent for thetrial

3.1.4 Be ζ 18 years of age on day of signing informed consent.

3.1.5 Be a candidate for surgical resection.

3.1.6 Be willing to provide tissue during endoscopic assessment of theirtumor.

3.1.7 Have a performance status of 0 or 1 on the ECOG Performance Scale.

3.1.8 Demonstrate adequate organ function asdefined in Table 1, all screening labs should be performed within 14 days of treatmentinitiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion within 7 days of assessment
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

3.1.9 Female subject of childbearing potential should have a negative urine or serum pregnancy within 7 days prior to receiving the first dose of studymedication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

3.1.10 Female subjects 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 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

3.1.11 Male subjects 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.

3.2 Exclusion Criteria

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

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

3.2.2. Evidence of metastatic disease.

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

3.2.4. Has active TB or a history of active TB within 10 years of registration (Bacillus Tuberculosis)

3.2.5. Hypersensitivity to pembrolizumab or any of its excipients.

3.2.6. Has had a prior anti-cancer treatment, including chemotherapy, radiation, or monoclonal antibody (mAb) for their current diagnosis of esophageal adenocarcinoma.

3.2.7. Has a known additional malignancy that is active. 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.

3.2.8. Has a previous invasive malignancy treated with curative intent less than 3 years from time of registration. Exceptions include prostate cancer, basal cell squamous skin cancer, and cervical cancer.

3.2.9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.10. Has known history of, or any evidence of active, non-infectiouspneumonitis.

3.2.11. Has an active infection requiring systemic therapy.

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

3.2.13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

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

3.2.15. Has received prior therapywith an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

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

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

3.2.18. Has received a 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.

4 REGISTRATION PROCEDURES

4.1 Patient Registration – Part 1 (WCMC Only)

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- First and last page of signed informed consent form

Registration must be completed within 24 hours of the signing of informed consent.

4.2 Patient Registration – Part 2 (All Sites)

Study participants will be centrally registered with the Weill Cornell Medicine Joint Clinical Trials Office (JCTO). To register a new study subject, email the following documents to JCTOIIT@med.cornell.edu :

- Completed WCM subject registration form
- First and last page of the fully executed informed consent form, plus additional pages if checkboxes for correlative studies are required.
- Fully executed HIPAA research authorization form (if separate from the consent document)
- Eligibility checklist signed and dated byinvestigator and research nurse
- Source documents verifying eligibility including
  - Pathology report confirming diagnosis
  - Radiology reports (CT or MRI scan)
  - Endocopy report (EGD or EUS) to confirm disease location
  - Laboratory reports confirming all required eligibility criteria have been met
  - On-study visit note documenting PS and consenting process
- Documentation of any eligibility waivers granted

Note that attachments larger than 4.5 MB are not accepted, so larger attachments should be split into more than one email.

Central registration information is reviewed and entered into the centralized research database. Documentation of subject registration will be faxed to the Investigational Pharmacy to allow for release of study agent.

5 Study Procedures

The Schedule of Evaluations - Section 5.1 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below

5.1 Schedule of Assessment: Cohort 1

	Screening Phase		Treatment Phase											Surgery	Post-Surgery					End of Treatment	Post-Treatment
Treatment Title	Part 1 (-28 to -1)	Part 2 (-14 to -1)	Wk 1	Wk 2	Wk 4	Wk 5	Wk 6-7	Wk 8[6]	Wk 9	Wk 10	Wk 11	Wk 12	Wk 14	Wk 15-19	Every 6 weeks x 12 mo	3 mo	6 mo	9 mo	12 mo	At time of discon	Every 6 months post discon
Informed Consent	X																				
Inclusion/Exclusion Criteria	X																				
Demographics and Medical History	X																				
Concomitant Medication Review	X		X					X									X		X		
Randomization			X																		
Physical Examination		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Vital Signs and Weight		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
ECOG Performance Status		X	X		X			X			X		X			X	X	X	X		
Pregnancy Test – Urine or Serum β-HCG		X[7]											X								
Mouth Wash		X																			
PT/INR and aPTT		X			X								X								
CBC with Differential, Comprehensive Serum Chemistry Panel, Liver Function Tests+ T bili		X		X	X	X	X	X	X	X	X	X	X		X					X	X
CEA & CA19-9		X	Collect as clinically indicated																		
Urinalysis		X					X						X			X	X	X	X		
T3, FT4 and TSH		X					X						X			X	X	X	X		
Tumor Imaging[1, 2, 3, 4]	X[1]						X[2]							X[3]		Every 6 months and as clinically indicated[4]				X	
RECIST 1.1 Assessment[8]	X						X							X						X	
Tissue Biopsy[5]		X					X							X							
Correlative Blood Collection		X					X							X		X	X	X	X		
Chemotherapy Administration[10]			X		X			X	X	X	X	X									
Radiation Administration								X	X	X	X	X									
Pembrolizumab Administration[9,10]								X			X		X		X						
Review Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X					X	

5.2 Schedule of Assessments: Cohort 2

	Screening Phase		Treatment Phase											Surgery	Post-Surgery					End of Treatment	Post-Treatment
Treatment Title	Part 1 (-28 to -1)	Part 2 (-14 to -1)	Wk 1	Wk 2	Wk 4	Wk 5	Wk 6-7	Wk 8[6]	Wk 9	Wk 10	Wk 11	Wk 12	Wk 14	Wk 15-19	Every 6 weeks x 12 mo	3 mo	6 mo	9 mo	12 mo	At time of discon	Every 6 months post discon
Informed Consent	X																				
Inclusion/Exclusion Criteria	X																				
Demographics and Medical History	X																				
Concomitant Medication Review	X		X					X									X		X		
Randomization			X																		
Physical Examination		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Vital Signs and Weight		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
ECOG Performance Status		X	X		X			X			X		X			X	X	X	X		
Pregnancy Test – Urine or Serum β-HCG		X[7]											X								
Mouth Wash		X																			
PT/INR and Aptt		X			X								X								
CBC with Differential, Comprehensive Serum Chemistry Panel, Liver Function Tests+ T bili		X		X	X	X	X	X	X	X	X	X	X		X					X	X
CEA & CA19-9		X	Collect as clinically indicated																		
Urinalysis		X					X						X			X	X	X	X		
T3, FT4 and TSH		X					X						X			X	X	X	X		
Tumor Imaging[1, 2, 3, 4]	X[1]						X[2]							X[3]		Every 6 months and as clinically indicated[4]				X	
RECIST 1.1 Assessment[8]	X						X							X						X	
Tissue Biopsy[5]		X					X							X							
Correlative Blood Collection		X					X							X		X	X	X	X		
Chemotherapy Administration[10]			X		X			X	X	X	X	X									
Radiation Administration								X	X	X	X	X									
Pembrolizumab Administration[9, 10]				X		X		X			X		X		X						
Review Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X					X	

1. Tumor imaging during screening can be performed as one procedure, PET/CT with diagnostic quality, or can be performed as 2 individual procedures.
2. Tumor Imaging performed during week 6-7 should be PET-CT without diagnostic quality CT. If PET-CT not feasible, CT CA is acceptable.
3. Tumor Imaging pre-surgery should be performed either as PET/CT without diagnostic CT or CT-CA, and as clinically necessary.
4. Tumor Imaging performed after surgery should be contrast-enhanced CT chest and abdomen, only.
5. Tissue sample collection should be performed at screening, week 6/ 7 (if medically feasible), and during surgery.
6. Chemoradiation should start on week 8 +/- 1 week (eg. Treatment can start from week 7-9).
7. Pregnancy test should be performed for women of childbearing potential within 7 days.
8. RECIST 1.1 Assessment is not required postsurgery
9. Pembrolizumab dose is 200 mg flat dose every 3 weeks prior to surgery, and 400 mg flat dose every 6 weeks post surgery. Patient can be switched to low dose pembrolizumab in the adjuvant setting if high dose is not tolerated well.
10. Chemotherapy and pembrolizumab may be administered +/- 3 days to accommodate scheduling (i.e. holidays, travel, etc.)

5.2.1. Overview

This study consist of the screening and enrollment phase, active protocol treatment phase, and then the follow-up phase. Patient's baseline is established during the screening and enrollment. The component of the patient's baseline include the baseline objective (radiologic) disease assessment, tissue samples and the pre-study evaluation, consisting of medical history, physical examination, laboratory assessments, and a review of active symptoms and current medications.

The active protocol treatment phase begins on week 1 and regular evaluations per the schedule of assessment. Continued monitoring occurs with regular evaluations. Tumor assessments are obtained at scheduled times until radiologic disease progression or recurrence is documented.

The active treatment phase closes with an End of Active Treatment visit, to be scheduled at the time of treatment discontinuation at 12 months from resection. The follow –up phase begins after the End of Active Treatment visit.

In general, unless otherwise noted, each study visit should be scheduled on the same day of the weekly treatments with a +/- 3 days window for administrative purposes.

5.2.2. Informed Consent & subject screening

Patients who agree to participate will sign the approved informed and will be provided a copy of the signed document.

Informed consent should be obtained within approximately one month (28 days) prior to week 1 treatment. An appropriate informed consent must be completed prior to undergoing any laboratory or radiologic evaluations that are specifically being performed for the purpose of screening for this study. Assessments or laboratory evaluations performed as part of standard of care (and not specifically for the study) may be used to qualify the patient for enrollment, provided the test result is within the time-frames specified for eligibility. All screening procedures should be performed within the time-frames indicated in the schedule of assessment calendar.

The treating investigator must verify that the patient meets all inclusion/exclusion criteria. The patient is considered enrolled into the study when he or she has been successfully randomized and begins week 1 treatment, though a log will be kept of all patients who sign informed consent. If the patient did not enroll, then the reason the patient did not enroll must be documented.

5.2.3. Pre-Study (Baseline) Evaluation

The following procedures must be completed no more than 28 days from enrollment.

- Informed Consent
- Inclusion/Exclusion Criteria
- Demographics and Medical History
- Prior and Concomitant Medication Review

- Tumor Imaging – PET-CT (with diagnostic quality CT). It can be performed as one procedure (PET/CT with diagnostic CT), or as 2 individual procedures. Diagnostic CT would include CT Chest and Abdomen.

The following tests need to be performed with results within eligibility criteria range within 14 days of Day 1, unless otherwise indicated:

- Physical Exam
- Vitals Signs and Weight
- ECOG Performance Status
- CBC with Differential, Comprehensive Serum Chemistry Panel, Liver Function Tests+ T bili , Comprehensive Serum Chemistry Panel
- Liver Function Tests, including total bilirubin
- Thyroid function panel
- Pregnancy Test (Urine or Serum β-HCG) (done within 7 days of Day 1)
- CEA & CA19-9
- Mouth Wash
- Tissue Biopsy
- Correlative Studies Blood Collection
- Urinalysis
- Coagulation Tests (PT/PTT)

Randomization (section 5.3) – within 72 hours prior to treatment initiation

5.2.4. Active Treatment Period

Week 1

- Prior and Concomitant Medication Review
- Physical Exam
- Vital Signs and Weight
- ECOG performance status
- Review Adverse Events
- Chemotherapy Administration (see Section 5.2)

Week 2

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs+ T bili
- Review Adverse Events
- Pembrolizumab Administration **Cohort 2 only** (see Section 5.2)

Week 4

- Physical Exam
- Vital Signs and Weight
- ECOG performance status
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Coagulation tests (PT/PTT)
- Review Adverse Events
- Chemotherapy Administration (see Section 5.2)

Week 5

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, Liver Function Tests+ T bili
- Review Adverse Events
- Pembrolizumab Administration **Cohort 2 only** (see Section 5.2)

Weeks 6-7

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs+ T bili
- Thyroid function panel
- Review Adverse Events
- Tumor Imaging – PET-CT (CT portion does not have to be of diagnosticquality)
- Correlative Studies Blood Collection
- Urinalysis
- Tissue Biopsy (if medically feasible)

Week 8 (Can occur +/- 7 days)

- Physical Exam
- Vital Signs and Weight
- ECOG performance status
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili

- Review Adverse events
- Chemotherapy administration (see Section 5.2)
- Pembrolizumab administration (see Section 5.2)
- Radiation administration (see Section 5.2)
- Concomitant Medication Review

Weeks 9-10

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Review Adverse Events
- Chemotherapy Administration (see Section 5.2)
- Radiation Administration (see Section5.2)

Week 11

- Physical Exam
- Vital Signs and Weight
- ECOG performance status
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Review Adverse Events
- Chemotherapy Administration (see Section 5.2)
- Pembrolizumab Administration (see Section 5.2)
- Radiation Administration (see Section5.2)

Week 12

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Review Adverse Events
- Chemotherapy Administration (see Section 5.2)
- Radiation Administration (see Section5.2)

Week 14

- Physical Exam
- Vital Signs and Weight

- ECOG performance status
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Thyroid panel
- Pregnancy Test
- Urinalysis
- Review Adverse Events
- Pembrolizumab Administration (week 14) (see Section 5.4)

5.2.5. Surgery Period

Week 15-19

- Review Adverse Events
- Tumor Imaging – PET-CT (without diagnostic quality CT chest, abdomen and pelvis) or CT-CA – must occur after last pembrolizumab dose and before surgical resection. Additional imaging as clinically necessary.
- Correlative Studies Blood Collection
- Tissue Biopsy (tissue to be collected during surgicalresection)

5.2.6. Post-Surgery Period

Pembrolizumab Treatment

Pembrolizumab treatment can start three to fourteen weeks after surgery as standard of care and will be given every 6 weeks (Pembrolizumab 400 mg flat dose) thereafter for 1 year (completion is 1 year from surgery +/- 7 weeks).

Tumor Imaging – computed tomography (CT) of the chest, abdomen and pelvis is performed every 6 months and as clinical indicated.

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Pembrolizumab Administration (see Section 5.4)
- Review Adverse Events

At 3 months post surgery

- Physical Exam
- Vital Signs and Weight
- ECOG Performance status
- Correlative blood studies
- Urinalysis
- Thyroid panel

At 6 months post surgery

- Tumor Imaging – computed tomography (CT) of the chest and abdomen only
- Physical Exam
- Vital Signs and Weight
- ECOG Performance status
- Correlative blood studies
- Urinalysis
- Thyroid panel
- Concomitant Medication Review

At 9 months post surgery

- Physical Exam
- Vital Signs and Weight
- ECOG Performance status
- Correlative blood studies
- Urinalysis
- Thyroid panel

At 12 months post surgery

- Tumor Imaging – computed tomography (CT) of chest and abdomen
- Physical Exam
- Vital Signs and Weight
- ECOG Performance status
- Correlative blood studies
- Urinalysis
- Thyroid panel
- Concomitant Medication Review

- An upper Endoscopy is recommended

5.2.7. End of Treatment

(At the time of treatment discontinuation at 12 months from resection)

- Physical Exam
- Vital Signs and Weight
- CBC with Differential, Comprehensive Serum Chemistry Panel, LFTs + T bili
- Review Adverse Events

5.2.8. Surveillance and Follow up

- Patients should be followed for recurrence as per standard practice for 5 years (from surgery)
- Guideline Recommendations include physician evaluation and blood work at 18, 24, 30, 36, 42, 48, 54 and 60 months
    - Physical exam
    - Vital Signs and Weight
    - CBC (absolute neutrophil count, hemoglobin, platelet count)
    - Comprehensive Serum Chemistry Panel
    - Liver Function Tests, including total bilirubin
    - CT (or similar) imaging at 18, 24, 30, 36 months
    - EGD as per symptoms
- After recurrence or the development of metastases, patients will be followed for survival. A phone call every 3 months will be sufficient for this.

5.3 Randomization (within 72 hours prior to treatment initiation)

Stratified and blocked randomization will be performed at all participating sites. Randomization will be stratified by location of cancer (i.e., gastro-esophageal junction vs. esophagus) and participating site. A series of randomized blocks of 2 will be generated for each cancer location stratum within each participating site with a 1:1 allocation ratio. This will provide assurance that after four patients are enrolled in any given cancer location stratum at a participating site, there will be two patients assigned to receive pembrolizumab prior to chemotherapy and two patients assigned to receive pembrolizumab following chemotherapy. This procedure will allow for each cancer location stratum within a participating site to contribute similar numbers of pre-chemotherapy pembrolizumab patients and post-chemotherapy pembrolizumab patients.

5.4 Treatment Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications for Investigational Agent are described in Section 6.

Cohort 1

5. Induction chemotherapy with taxol/carboplatin (q 3 week)

6. Chemoradiation – taxol/carboplatin weekly, with Pembrolizumab
7. Resection
8. Pembrolizumab every 6 weeks for 1 year following surgery (beginning at 3-14 weeks following resection)

Cohort 2

1. Induction chemotherapy with taxol/ carboplatin (q 3 weeks), with Pembrolizumab administered the week after chemotherapy. (i.e. weeks 2 and 5).
2. Chemoradiation – taxol/carboplatin weekly, with Pembrolizumab
3. Resection
4. Pembrolizumab every 6 weeks for 1 year following surgery (beginning at 3-14 weeks following resection)

5.4.1 Induction Chemotherapy

Patients assigned to both Cohort 1 and Cohort 2 will receive the same induction chemotherapy regimen of every 3 weekly dosing of paclitaxel and carboplatin. Paclitaxel is to be given first, over 3 hours, followed by carboplatin over 1 hour. The suggested premedication regimen is as follows:

Dexamethasone 10 mg PO/IV
Pepcid 20 mg PO/IV (or equivalent)
Aloxi 0.25 mg IV (can be substituted for HT3 inhibitors)
Benadryl (diphenhydramine) 25mg IV/po

For patients below the age of 70, the every 3 weekly dosing of paclitaxel and carboplatin, as per the table below:

Agent	Dose	Route	IV Duration	Days	Treatment Frequency
Paclitaxel	175 mg/m^2	IV	3 hours	W1 and W4	every 3 weeks
Carboplatin	AUC 5*	IV	1 hour	W1 and W4	every 3 weeks

For patients 70 years and older, the paclitaxel and carboplatin dosing is as follows:

Agent	Dose	Route	IV Duration	Days	Treatment Frequency
Paclitaxel	150 mg/m^2	IV	3 hours	W1 and W4	every 3 weeks
Carboplatin	AUC 4*	IV	1 hour	W1 and W4	every 3 weeks

*Creatinine clearance (CrCl) will be calculated using the Cockroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{(140\text{-age}) \times \text{actual wt (in kg)}}{72 \times \text{serum creatinine (mg/dl)}}$$

FOR FEMALES, USE 85% OF CALCULATED CrCl VALUE

Dose of carboplatin (mg) = 5 x [CrCl (ml/min) + 25]

Use the serum Cr value reported by the laboratory; do not apply any correction factors to the reported value.
The maximum CrCl that can be used in this calculation, for both women and men, is 125 ml/min. Thus the maximum carboplatin dose is 625mg for patients below the age of 70 and 600 mg for patients 70 years and older. In the case of low creatinine values resulting in high CrCl values that seem to overestimate renal function, a 24-hour urine collection for determination of CrCl is recommended.

Patients Assigned to Cohort 2 will receive additional Pembrolizumab during induction chemotherapy

Pembrolizumab 200 mg IV will be given on W2 and W5 over 30 minutes (-5 or +10min)

5.4.2 Chemoradiation

Chemoradiation will start on week 8 of the protocol, starting on a Monday. If necessary for administrative issues, radiation can start on Tuesday or Wednesday during week 8. A +/- 1week window is permitted to start radiation. If radiation is to start on week 7 or week 9, all of the study procedures will be shifted by 1 week accordingly.

5.4.3 Chemotherapy and Pembrolizumab

Patients assigned to both Cohort 1 and Cohort 2 will receive the same chemotherapy regimen during radiation consisting of weekly paclitaxel and carboplatin. Paclitaxel is to be given first, over 1 hour, followed by carboplatin over 30 minutes. The suggested premedication regimen is as follows:

Dexamethasone 10 mg PO/IV
Pepcid 20 mg PO/IV (or equivalent)
Metacloperamide 10 mg IV/PO at physician discretion
Benadryl (diphenhydramine) 25mg IV/po

Treatment

Paclitaxel 50 mg/m^2 IV over 1 hour, + carboplatin AUC 2 IV over 30 minutes, *weekly x 5 weeks*
Pembrolizumab 200 mg IV is given during weeks 8 and 11 (administered prior to chemotherapy), and 14 (as monotherapy) of the protocol (eg. twice during chemo/RT and once prior to surgery)

Note, if the patient required a dose reduction during the induction chemotherapy treatment, then a dose reduction in weekly taxol/carboplatin is recommended as per the Dose Attenuation Guidelines (section 6).

5.5 Radiation

Radiation therapy will begin on week 8 (+/- 1 week) per the assessment table, combined chemoradiation should start within the first 3 days of radiation therapy preferably on Monday Total dose of RT will be 4140cGy in 180cGy fractions. Subjects will receive radiation dose of 180cGy for 23 days (23 fractions) during week 8 to week 12 as per standard of care. Patients can undergo simulation for radiotherapy planning prior to the second PET scan since the treatment volumes are based on pre-chemotherapy tumor involvement. The simulation should be performed at least 2 weeks prior to the start date for radiotherapy so that submission of dose-volume-histograms can be done and approved centrally without delaying the start date of the radiotherapy.

5.5.1 Technical Factors

Linear accelerators with a minimum energy of 6 MV will be used. A multiple field 3-D conformal technique or IMRT will be used. All fields will be treated each day. The patient will be treated in the supine position. Radiation will be delivered 5 days/week, once per day. 4D planning may be used (described below, Section 5.5.3)

5.5.2 Required Benchmarks and Pre-approval of 3D Treatment Plans

CT-based conformal planning is required on this study. As noted above, 3-D conformal or IMRT techinique will be allowed. Digital submission of treatment plans is required for this portion of study, specifically one axial, cornonal, and sagittal dosimetry plan and the dose volume histogram.

Treatment plans must be reviewed and approved by the radiation therapy monitor (Nicholas Sanfilippo, MD) during the initial week of treatment. Records should be submitted to Weill Cornell Medicine, Joint Clinical Trials Office, Investigator-Initiated Trials and Multicenter Protocol Operations Team: jctoiit@med.cornell.edu.

5.5.3 Protocol Treatment Volumes

A volumetric treatment planning CT study will be required for this study. Each patient will be positioned in an individualized immobilization device in the treatment position on a flat table. Contiguous CT slices, 3-5 mm thickness of the regions harboring gross tumor and grossly enlarged nodes and 8-10 mm thickness of the remaining regions, are to be obtained starting from the level of the cricoid cartilage and extending inferiorly through the liver.

4-D CT planning is permitted: 4-D CT may be employed through the respiratory cycle for the purpose of forming an internal target volume (ITV). The ITV is the union of the gross tumor volumes (GTV) of primary tumor and pathlogic nodes contoured on the maximum intensity projection image (MIP) to form the envelope that encompasses the motion of the GTV for a complete respiratory cycle.

Gross Tumor Volume (GTV): The GTV is based on the pre-chemotherapy extent of disease using the initial PET/CT scan, endoscopy report, and CT scan. The entire esophageal wall, including any disease that has extended through the wall should be contoured as the GTV as well as any PET/CT-avid or enlarged lymph nodes.

Clinical Target Volume (CTV): The intent of pre-operative treatment is to include the tumor bed plus the nodal groups at risk (whether clinically positive or negative). The clinical target volume (CTV) should encompass the peri-esophageal lymph nodes, mediastinal lymph nodes for mid- and upper- thoracic esophageal tumors, and the submucosal spread longitudinally along theesophagus. This is generally a 3.5-4 cm expansion on the GTV (or ITV when 4D planning is employed) superiorly and inferiorly and 1.0 cm expansion radially. For distal esophageal tumors and GE junction tumors, the CTV should include the celiac lymph nodes. For tumors above the carina, the supraclavicular lymph nodes should be included in the CTV.

Planning Target Volume: The PTV is established by expanding the CTV by 0.5 cm in all directions. This will result in a margin of up to 5 cm superior and inferior and approximately 1.0-2 cm radially to the extent of tumor (GTV or ITV). For distal esophageal and GE junction tumors where 4D planning is not being used, the superior-inferior expansion can be 0.7-1.0 cm to account for the respiratory motion at the discretion of the attending physician while radial expansions remain at 0.5 cm.

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5.5.4 Target Dose and Normal Tissue Dose Constraints

Dose Prescription

The prescribed dose to the PTV is 4140 cGy delivered in 180 cGy/day over 23 fractions (for 23 days).

Dose Uniformity

The dose to 99% of the PTV must be at least 93% of the prescribed dose, and a contiguous volume of no more than 2cc inside the PTV may exceed 20% of the prescribed dose.

Tissue Heterogeneity

Calculation shall take into account the effects of tissue heterogeneities. Planning must be performed using an approved dose calculation algorithm. Approved algorithms include: convolution superposition, collapsed cone convolution, and Monte Carlo.

5.5.5 Normal tissue Dose Constraints

The normal structures to be contoured will depend on the level of the esophagus involved, but can include left and right lungs, heart, esophagus, brachial plexus, left and right kidneys, liver, stomach, small intestine, and spinal canal. The dose to normal tissues should be kept within the parameters described below.

Normal Tissue Constraints:

1. Lungs
   a. $V_{20Gy} \leq 20\%$
   b. and $V_{30Gy} \leq 15\%$

c. and $V_{40Gy} \leq 10\%$

d. $V_{10Gy} \leq 40\%$

2. Cord
   a. Max ≤ 4500 cGy
3. Bowel
   a. Max bowel dose < Max PTV dose
   b. and $D_{05} \leq 4500$ cGy
4. Heart
   a. $V_{30Gy} \leq 30\%$ (closer to 20% preferred)
   b. Mean < 3000 cGy
5. Lt_Kidney, Rt_Kidney (evaluate each one separately):
   a. No more than 33% of the volume can receive 1800 cGy
6. Liver
   a. $V_{20Gy} \leq 30\%$
   b. $V_{30Gy} \leq 20$
   c. mean < 2500 cGy
7. Stomach
   a. Mean < 3000 cGy (if not within PTV)
   b. Max dose < 54Gy

5.5.6 Treatment Planning

Simulation: Patients will be positioned supine with arms above the head, immobilized in an aquaplast mold. A CT simulation should be performed using oral contrast, when possible, using 3-5 mm slice thickness. Intravenous contrast is permitted but not required.

Motion Management: As noted above, 4D planning is permitted, but not required. This is especially useful for distal esophageal and G-E junction tumors. This scan will be performed throughout the breathing cycle so that a MIP scan can be used for formation of and ITV, as described above.. Treatment delivery can be done using the motion management technique available at the institution and can include treatment during free-breathing as long as an internal target volume (ITV) has been designed based on the motion of the tumor on the 4DCT.

Beam Arrangements:

5.5.6.1 3D Conformal Beam Arrangements

Beam arrangement selection for 3D conformal treatment will vary based on the shape, size, and location of the CTV and the resulting PTV in relation to normal organs. The most common arrangement is an AP/PA with Right and Left Lateral beams.

5.5.6.2 IMRT - Beam Arrangement

A five-field beam arrangement is preferred to minimize the low dose distributed to the lungs. Suggested beam arrangements are:

a. For distal esophagus the following beam arrangement is useful for minimizing dose to the heart and lungs:

LPO (155), LAO(70-80), AP(0), RAO(280-290), RPO(205)

Note that the range of gantry angles for the LAO and RAO fields is due to the fact that one needs to find the best compromise between the amount of heart and lung in the field.

b. For GE-junction esophagus, the following beam arrangement may be substituted if it is better for minimizing dose to the kidney. All beams are 15 MVx:

PA (180º), close to LL (90º+/-10º), LAO (30-35º), RAO (325-330º), close to RL (270º+/-10º)

These recommended beam arrangements may be changed to one more fitting for the patient's particular anatomy. Volumetric modulated arc therapy (VMAT) is also permitted. The treating physician may select whichever technique results in the optimal dose distribution

Field verification: As a minimum requirement, institutions are required to obtain verification images at the start of treatment and each week thereafter. Prior to the first treatment images that verify the position of the isocenter placement must be obtained. For 3D-CRT this imaging can include individual portal views. Weekly imaging can consist of portal views for 3D-CRT. For IMRT orthogonal images (KV or MV) verifying isocenter position are perferred. More frequent (daily) imaging is allowed, but not required. Cone-beam verification is allowed.

5.5.7 Verification Film Review

All verification images should be reviewed promptly, as is customary. First day images should be reviewed prior to the first fraction and subsequent images should be reviewed before the next treatment.

5.5.8 Definitions of Deviations in Protocol Performance

Prescription Dose

- **No Deviation:** ≥ 99% of the PTV receives ≥ 93% of the prescribed dose, and a contiguous volume of no more than 2cc inside PTV exceeds 20% of the prescribed dose.
- **Minor Deviation:** Deviations of this magnitude are not desirable, but are acceptable. Coverage that is equal to 93% of the prescribed dose and falls between 99% and 95% of the PTV, or a contiguous volume of no more than 2cc inside the PTV exceeds 20-25% of the prescribed dose.
- **Major Deviation:** Doses in this region are not acceptable. More than 1 cm^3 of tissue outside the PTV receives ≥ 120% of the prescribed dose, or 93% of the prescribed dose falls below 95% of the PTV, or a contiguous volume of no more than 2cc inside the PTV exceeds 25% of the prescribed dose.

Volume

- **Minor Deviation:** Margins less than specified, or field(s) 1-3 cm greater than specified.
- **Major Deviation:** Fields transect tumor or specified target volume(s), or fields are more than 3 cm greater than specified.

Critical Organ

- **Major Deviation:** The maximum dose to the spinal cord exceeds 4500cGy; the heart mean dose exceeds 3000cGy; the lung V20 exceeds 30% or the V10 exceeds 50% (see dose limits in section 8.3.5).
- **Minor Deviation:** The lung V20 exceeds 20% or the V10 exceeds 40% (see dose limits in section 8.3.5).
- These deviation will be assessed with the initial submission of dosimetry information as noted in section 5.5.2.

Treatment Interruption

- **Minor Deviation:** Treatment interruptions between five and nine normally scheduled treatment days with documentation of the reason for interruption..
- **Major Deviations:** Treatment interruptions totalling more than nine normal scheduled treatment days with documentation of the reason for interruption.

  At the conclusion of treatment, the treating physician must send information of any treatment interruption to Nicholas Sanfilippo, MD.

5.6 General Concomitant Medication and Supportive Care Guidelines for Pembrolizumab

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 berequired. The investigator should discuss any questions regarding this with the Principal Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.6.1 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 7.2.

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
- 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.

5.6.2 Rescue Medications & Supportive Care

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

Table 4 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1	Increase monitoring of vital signs as	None

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Mild reaction; infusion interruption not indicated; intervention not indicated	medically indicated until the subject is deemed medically stable in the opinion of the investigator.	
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available		

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
during the period of drug administration.		

5.7 Surgery

All patients will proceed to surgical resection regardless of local tumor response. Treatment will be interrupted and surgical resection performed if at any time the patient develops 1) unacceptable adverse events, 2) intercurrent illness precluding further treatment or 3) withdraws consent from the study. Surgical resection will be performed 3-7 weeks after the last dose of chemotherapy. Surgical resection will be performed using standard surgical practices. All post-operative adverse events will be recorded using NCI common toxicity criteria version 4.

Any of the following surgical approaches are deemed acceptable, as long the approach includes the surgical removal of any lymph nodes felt to be suspicious:

- Ivor Lewis esophagogastrectomy
- Thoracoabdominal esophagectomy
- 3-hole esophagectomy (Mckeown procedure)
- Transhiatal esophagectomy
- Minimally invasive esophagectomy

A gross surgical margin of at least 5 cm both proximally and distallyare recommended. A frozen section of both the proximal and distal surgical margin should be obtained to confirm a negative microscopic surgical margin.

Pathologic specimens will undergo the standard histopathologic and immunohistochemical evaluation for esophageal cancer. The pathologic response will be assessed based on the estimated percentage of vital residual tumor cells (VRTCs) (Schneider, Ann Surg 2005). The degree of histomorphologic regression will be classified into the following categories:

No response: > 50% residual tumor cells (or < 50% treatment effect)

Partial response; 10-50% VRTCs (or $\geq$ 50% treatment effect, but < 90%)

Near complete response: < 10% VRTCs (or > 90% treatment effect)

Complete response: 0% VRTCs (or 100% treatment effect), ypCR, ypT0

Fresh frozen samples of tumor and non-tumor adjacent esophagus will be harvested from waste material immediately after resection. Tissue specimens will be stored at -80˚ C for correlative studies. Similarly paraffin embedded tissue will be obtained from resected tumor andnon-tumor adjacent tissue for correlative studies.

5.8 Post-operative Pembrolizumab

Post-operative pembrolizumab will start around week 6 following resection (and may begin anywhere from 3-14 weeks following surgery). To initiate therapy, patients must be in stable clinical condition, without having an active infection, the need of intravenous antibiotics, or the need of total parenteral nutrition.

In both cohorts, Pembrolizumab 400 mg IV will be administered every 6 weeks for up to 1 year (end date will be up to 1 year from the time of resection). If Pembrolizumab 400 mg dose is not well tolerated, patient may be switched to 200 mg dose at the discretion of the principle investigator.

5.9 Diet/ Activity/ Other Considerations

Diet

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

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 7.2.2-Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

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 7.2.2.

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.

5.10 Duration of Therapy and Criteria for Removal From Study

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.11 Duration of Follow up

All patients will be followed for up to 5 years following resection or disease recurrence, whichever occurs first. Subjects with recurrence or progression prior to surgery will receive a follow up phone call every 3 months for survival. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.1 DOSING DELAYS/ DOSE MODIFICATIONS

6.2 Timing of Chemotherapy Administration

Trial treatment should be administered on Day 1 of each treatment week after all procedures/assessments have been completed as detailed on the study assessment chart (Section 5.2). Trial treatment may be administered up to 3 days before or after the scheduled treatmentday due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Prior to resection, pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Following resection, pembrolizumab 400 mg IV infusion over 30 minutes will be administered every 6 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: 25min or up to 40min). If the subject is not tolerating the 400 mg flat dose following resection, the subject can continue to receive 200 mg flat dose of pembrolizumab every three weeks at the discretion of the principle investigator.

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

6.3 Chemotherapy Dose Levels and Attenuation Guidelines

General guidelines and Chemotherapy Dose Levels

- If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions apply to treatment given in the preceding week and are based on adverse events observed since the prior dose.
- Neither paclitaxel nor carboplatin will be re-escalated once reduced.
- If both paclitaxel and carboplatin are held for one week during induction chemotherapy, it is not to be made up. Proceed with chemoradiation as scheduled.
- Depending on the specific toxicity, a single chemotherapy drug (i.e. either carboplatin or paclitaxel) may be modified without alteration of the other drug.

Dose levels of chemotherapy during Induction Therapy

Induction period for Age < 70

Dose Level	Drug Name	Dose (mg/m^2)
0*	Paclitaxel	175
-1	Paclitaxel	140

* Dose level 0 refers to the starting dose.

Induction period for age ≥ 70

Dose Level	Drug Name	Dose (mg/m^2)
0*	Paclitaxel	150
-1	Paclitaxel	110

* Dose level 0 refers to the starting dose.

Induction period for Age < 70

Dose Level	Drug Name	AUC
0*	Carboplatin	5
-1	Carboplatin	3.5

* Dose level 0 refers to the starting dose.

Induction period for age ≥ 70

Dose Level	Drug Name	AUC
0*	Carboplatin	4
-1	Carboplatin	2.5

* Dose level 0 refers to the starting dose.

Dose levels of chemotherapy during Chemoradiation

* If a dose reduction was recommended during induction therapy, the patient should initiate the corresponding chemotherapy at dose level -1 during chemoradiation.

Dose Level	Drug Name	Dose (mg/m^2)
0*	Paclitaxel	50
-1	Paclitaxel	40
-2	Paclitaxel	30

* Dose level 0 refers to the starting dose of no dose reduction was required during induction therapy.

Dose Level	Drug Name	AUC
0*	Carboplatin	2
-1	Carboplatin	1.5
-2	Carboplatin	1.2

* Dose level 0 refers to the starting dose if no dose reduction was required during induction therapy.

6.4 Chemotherapy Dose Modifications

Hematologic Toxicities

For **grade 3 neutrophil count decreased on day 1**, delay paclitaxel and carboplatin until grade ≤ 2, then resume paclitaxel at 1 dose level decreased, and carboplatin at same dose

For **grade 4 neutrophil count decreased or grade 3 or 4 febrile neutropenia**, delay paclitaxel and carboplatin until grade ≤ 2, then resume paclitaxel and carboplatin with one dose level decreased

For **grade 2 platelet count decreased**, delay paclitaxel and carboplatin until grade ≤ 1, then resume paclitaxel at one dose level decreased, and carboplatin at same dose

For grade 3 or 4 platelet count decreased, delay paclitaxel and carboplatin until grade ≤ 1, then resume paclitaxel and carboplatin with one dose level decreased

Neurotoxicity

For **grade 2 neuropathy**, continue paclitaxel at one dose level decreased, and carboplatin at the same dose

For **grade 3 or 4 neuropathy**, discontinue paclitaxel

Gastrointestinal Toxicity

For **grade 3 nausea or vomiting**, delay paclitaxel and carboplatin until grade ≤ 2, then restart carboplatin at 1 dose level decreased, and paclitaxel at the same dose

For **grade 4 vomiting**, delay paclitaxel and carboplatin until grade ≤ 2, then restart paclitaxel and carboplatin with one dose decreased

For **grade 2 diarrhea**, delay paclitaxel and carboplatin until grade ≤ 1, then restart paclitaxel and carboplatin at same dose

For **grade 3 or 4 diarrhea**, delay paclitaxel and carboplatin until grade ≤ 1, then restart paclitaxel and carboplatin at one dose decreased

For **grade 3 or 4 anorexia**, delay paclitaxel and carboplatin until grade ≤ 2, then restart paclitaxel and carboplatin at 1 dose level reduced

For **grade 2 stomatitis**, treat with paclitaxel at 1 dose level reduced and carboplatin at same dose level

For **grade 3 or 4 stomatitis**, delay paclitaxel and carboplatin until grade ≤ 1, then restart carboplatin and paclitaxel at 1 dose level reduced

Hepatobiliary Toxicity

For **grade 2 or 3 AST or ALT elevation,** delay paclitaxel and carboplatin until grade ≤ 1, then restart paclitaxel and carboplatin at one dose level decreased

For **grade 4 AST or ALT elevation**, discontinue paclitaxel and carboplatin

For **grade 2 total bilirubin elevation**, delay paclitaxel and carboplatin until grade ≤ 1, then restart paclitaxel and carboplatin at 1 dose level decreased

For **grade 3 or 4 total bilirubin elevation**, discontinue paclitaxel and carboplatin

Renal Insufficiency:

For grade 3 or 4 creatinine increased and NOT attributed to study treatment, delay paclitaxel and carboplatin until grade ≤ 1 then restart paclitaxel and carboplatin at same dose

Opthalmalogic Toxicities:

For **grade 3 watering eyes,** delay paclitaxel until grade ≤ 1, then restart paclitaxel at 1 dose level decreased

For **any grade cystoid macular edema,** discontinue paclitaxel

General disorders

For **grade 3 or 4 edema**, omit paclitaxel until grade < 2, then resume paclitaxel at one dose level decreased

For **grade 3 or 4 fatigue,** delay paclitaxel and carboplatin until grade ≤ 2, then resume paclitaxel and carboplatin at 1 dose level decreased

For **grade 4 hypersensitivity reaction,** discontinue offending study agent.

Non-hematologic Toxicities

For all other **grade 3 or 4 non-hematologic toxicities** likely related to paclitaxel and/or carboplatin, omit paclitaxel and/or carboplatin until resolved to < grade 1, then resume paclitaxel and/or carboplatin at one dose decreased

Dose Modifications for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by actual weight without any modification unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.

6.5 Pembrolizumab Related Toxicity

Pembrolizumab dose is 200 mg flat dose every 3 weeks prior to surgery, and 400 mg flat dose every 6 weeks post surgery. There are no dose modifications to this treatment.

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 3 below. See Section 5.5 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

Table 3
Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with

Pembrolizumab

General instructions:

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

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signsand ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of ϕ3-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
NOTE: For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

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.

9.0 ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

7.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

7.1.1 Investigational Agent Risks

Please refer to the Investigational Brochure for Risks.

7.1.2 Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Attribution of the AE:

- Definite – The AE *is clearly related* to the study treatment.

- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – the AE is *clearly* not to the study treatment.

7.1.3 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart.

7.1.4 Reporting of AE to WCMC IRB

All AEs occurring on this study will be reported to the IRB according to the WCMC IRB policy, which can be accessed via the following link:

http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

7.2 Definition of SAE

SAE's include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other serious events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.3 Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the WCMC IRB policy

7.4 Reporting of SAE to FDA (where WCMC is the Sponsor-Investigator)

If an SAE occurs on this study and meets the FDA reporting criteria, the event will be filed on a MedWatch form with the FDA. The investigator must notify the FDA of any SAE's as soon as possible but no later than 7 calendar days after the initial receipt of the information.Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biologic Products Document Room

5901-B Ammendale Road
Beltsville, MD 20705-1266

7.5 Reporting of SAE to MERCK

Institution will send Merck copies of any and all serious adverse event reports filed with the FDA or other applicable regulatory authorities, as well as copies of any correspondence with the FDA or other applicable regulatory authorities, regarding any and all serious adverse events, irrespective of association with the Study Drug(s) in the course of the Clinical Trial, within two (2) working days of such report or correspondence being sent to the FDA or other applicable regulatory authorities. Copies should be faxed directly to *Merck Global* Safety Department at **1-215-993-1220.**

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

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

1. Additional adverse events:

A separate guidance document has been provided entitled “Event of Clinical Interest Guidance Document” (previously entitled, “Event of Clinical Interest and Immune-Related Adverse Event Guidance Document”). This document can be found in Appendix 4 and provides guidance regarding identification, evaluation and management of ECIs and irAEs.

ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

7.6 AE/ SAE Follow up

All SAEs and AEs reported during treatment on this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required. This requirement indicates that follow-up may be required for some events after the patient discontinues participation from the study.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with *Investigational Agent* (Pembrolizumab) can be found in the Investigator's Brochure. Paclitaxel and Carboplatin are commercially available

8.1 Investigational Agent

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

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

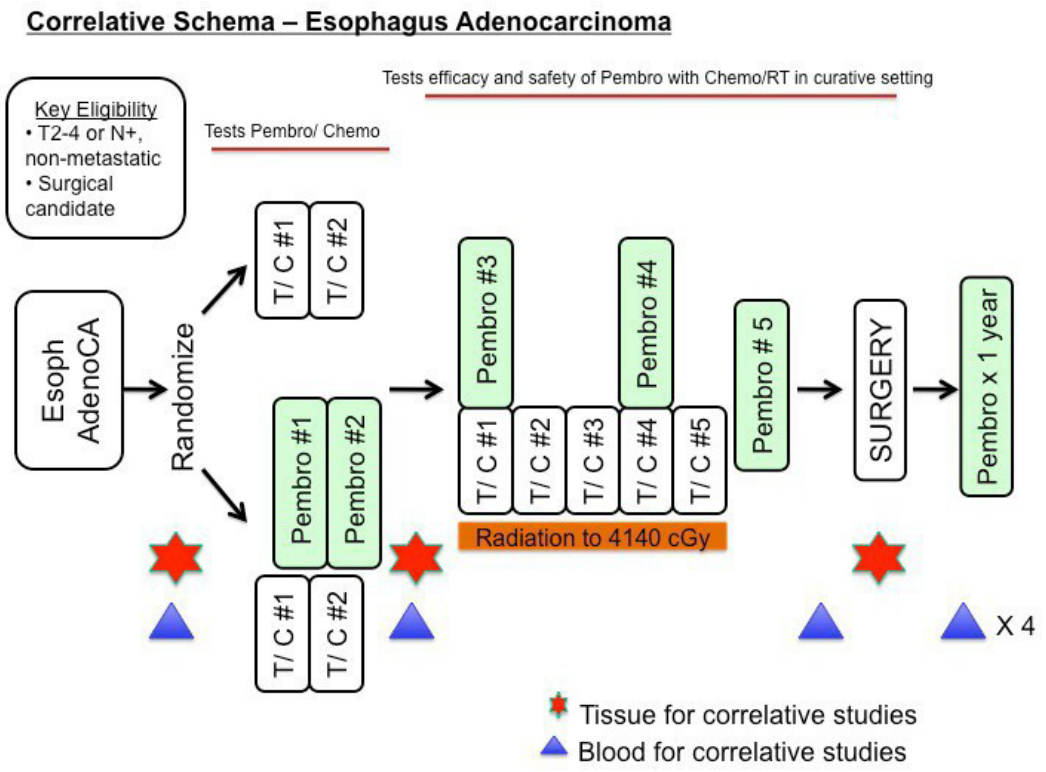
Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

8.2 Agent Accountability

Pembrolizumab Inventory Records – The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents received from *Sponsor* on a Drug Accountability Record Form (DARF).

9. CORRELATIVE/ SPECIAL STUDIES

This study will provide a unique opportunity to evaluate the impact of pembrolizumab on the quality and character of the immune infiltrate in patient tumor samples. The primary aims of the correlative studies is to use IHC to determine PD-L1 expression in tumors before and after treatment, utilize flow cytometry to characterize immune infiltrate profiles before and after therapy with pembrolizumab, and, perform RNA Sequencing to determine any correlation between transciptomic signatures of intra-tumoral immune activation and response/survival. We will plan to collect tumor and non-tumor adjacent and tissue (4-6 tissue biopsies for each per time point), blood for plasma biomarkers throughout the study, and mouth wash at screening. The protocol schema is provided below that highlights blood and tissue ascertainment as it relates to this study.



9.1 To examine whether anti PD1 therapy is associated with a transcriptomic signature of intra-tumoral immune activation predictive of response and improved 1 year survival.

From fresh biopsy samples, we will perform RNA Sequence analysis of tumors at baseline and following induction chemotherapy or induction chemotherapy with pembrolizumab to examine the T cell signature of the immune infiltrate from these tissue biopsysamples.

We have previously performed an analysis on the TCGA involving the T cell gene signature in gastric cancer. The large sets of genomic data collected by the TCGA project provide an opportunity to examine the tumor-immune interactions. One such approach is to examine the expression levels of immune-specific genes to quantify the levels of tumor-infiltrating T cells in samples[21,22]. The method is based simply on selecting a small list of T cell specific genes and generating a "meta gene" expression signature by eigenvalue decomposition of the expression matrix. We applied this algorithm on the stomach TCGA RNA-sequence data to generate a measure of T cell infiltration in the TCGA samples (Figure 8). We found that the majority of the chromosomal instability (CIN) subtype samples (as defined by the TCGA study) have a distinct T cell signature ($p \leq 1e\text{-}6$, Fisher exact test). As expected, EBV samples have elevated levels of T cell infiltration (Figure 8) which is also consistent with previous studies demonstrating increased levels of cytolytic markers in gastric EBV subtypes[21].

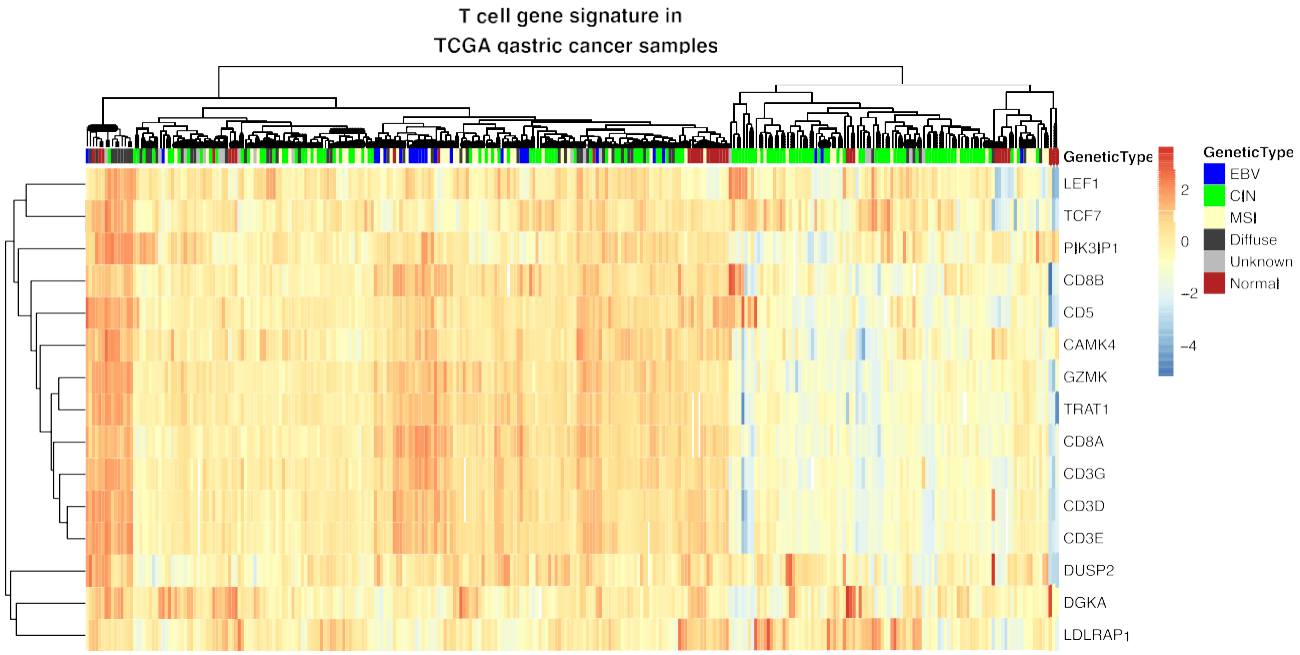


Figure 8. T cell signature of TCGA gastric samples. Hierarchical clustering of the samples by 15 genes characteristic of T cell signature identified two predominant clusters one of which is enriched with CIN tumor subtype.

9.2 To examine whether chemotherapy is associated with induction of PD-L1 expression in tumors.

9.2.1 PD-L1 Immunohistochemistry

The PD-1/PD-L1 pathway is thought to be involved in tumor immune evasion and is a prospective therapeutic target. Pembrolizumab directly blocks the interaction between PD-1 and PD-L1 (as well as PD-L2) and current clinical data indicates a potential additive effect of immune checkpoint inhibitors when combined with cytotoxic therapy. Chemotherapy as well as radiotherapy is known to stimulate PD-L1 expression and we hypothesis this will enhance the efficacy of pembrolizumab. We will assess changes in PD-L1 expression in patients by performing IHC staining in tumor tissue taken prior to and after treatment with pembrolizumab.

9.3 To examine whether anti PD1 therapy is associated with increased intra-tumoral immune cell infiltration.

We will examine the intra-tumoral response to chemotherapy and anti-PD1 therapy in by both flow cytometery and immunohistochemistry. Tissue will be obtained at baseline, following induction chemotherapy, and at the time of surgical resection.

Phenotypic and functional characterization of T cells isolated from human endoscopic biopsies. We have optimized a 16-color flow cytometry panel with the following antibodies of interest: CD3+ (Total T cells), CD8+ (Cytotoxic T cells), CD4+ (T helper cells), CD45+ (total hematopoietic cells), FOXP3+ (Treg cells), CD33+ (myeloid precursor cells), CD20+ (B cells),

CD16+ (neutrophils), CD62L (isotype control), and viability dye. Flow cytometric analysis will be performed in collaboration with Dr. Gregory Sonnenberg at Weill Cornell Medical College. We will comprehensively interrogate the immunologic parameters of the innate and adaptive immune system including analyses of CD4+ T cell subsets and their associated cytokines including Th1 cells (IL-12, TNFα and IFN7), Th2 cells (IL-4, IL-5 and IL-13), regulatory T cells (IL-10) and Th17 cells (IL-17A, IL-17F, IL-21, IL-22).

Further we can profile ILCs and examine for expression of the associated cytokines IL-4, IL-5, IL-13, IL-17A, IFNψ and IL-22. We developed a new protocol to enable the isolation, processing and storage of live immune cells from tissue biopsies.

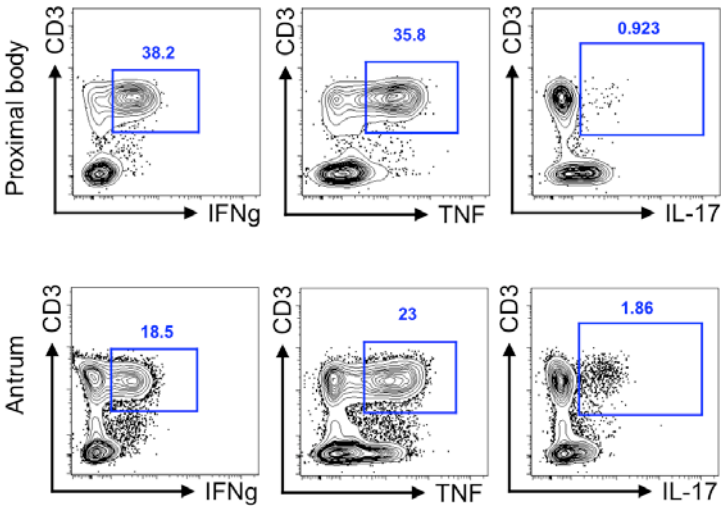


Figure 2. Phenotypic and functional characterization of T cells isolated from endoscopic biopsies. Immune cells were isolated from enzymatically digested tissue, and stained with mAbs for flow cytometry, Gated on live CD45+ cells.

Our preliminary analysis (performed on a gastric biopsy) identified an accumulation of CD4+ T cells in the tumor that were positive for intracellular IFNg, TNF and IL-17A (Figure 2). Employing this approach, we propose to examine the phenotype and functional potential of CD4+ and CD8+ T cells, as well as ILCs isolated from the tumor before and while on STAT3 inhibition therapy to assess whether there is an augmentation of the immune infiltrate and to evaluate the character of that infiltrate.

IHC and Flow

The immune cell subsets within the tumor tissue will also be evaluated by IHC using cell-specific surface markers. This will include CD45+ hematopoietic cells including T cells ($CD3^+$), $CD3^+CD4^+$ T helper cells and $CD3^+CD8^+$ effector T cells, CD4+ CD25+ FoxP3+ T regs. IHC analysis will be done on formalin-fixed, OCT embedded tissue sections (5-10 µm thickness) that will be stained with the fluorescence-labeled antibodies described above. Fluorescent images will be obtained using an Axiovert 200M fluorescent microscope (Carl Zeiss Inc.).

9.4 To explore baseline tumor tissue expression as a predictor of pembrolizumab therapy.

9.4.1 RNA Expression Analysis

Anti PD-1 therapy may be related to a transcriptomic signature of intra-tumoral immune activation and could be predictive of both response and survival. Through RNA Sequencing we will evaluate and monitor the expression levels of immune-specific genes over the various time points for each patient. RNA Sequencing will be performed on tissue biopsies acquired

at baseline (prior to treatment), on-treatment (within the first week of the 2nd treatment), and at time of surgical resection. We will also sequence germline DNA from the whole blood of each patient to ensure any gene alterations/mutations we find in the tumor tissue is somatic and not germline.

9.6 Collection of Specimen(s)

Fresh tissue will be collected during study specified upper endoscopy performed to assess response. Biopsies will be collected prior to initiation of the study and again prior to radiation therapy (optional). A third collection of tissue specimens will be taken at the time of surgical resection.

9.7 Handling of Specimens(s)

Biopsies will be collected in the endoscopic suite or surgical pathology, processed and shipped according to the study Lab Manual.

9.8 Mouth Biome

Mouthwash samples will be collected for all subjects during screening to understand how alternation of regional mucosal microbiome alters the tumor microenvironment, thus creating conditions conducive to tumorigenesis. (All samples will be collected, processed, handled and shipped according to the study Lab Manual)

9.9 To collect blood as a biomarker for future study (All samples will be processed, handled and shipped according to the study Lab Manual)

Whole exome sequencing (WES) will be collected in the study for the purpose of a normal DNA comparison. Blood for WES will be collected at the same time points as tissue procurement (baseline, on-treatment and surgical resection). We will also consider further examining the whole blood to identify polymorphisms thought to be important in drug metabolism and correlate these findings with toxicity and efficacy (as an exploratory sub-aim of the correlative aim).

RNAseq will be collected in the study for the purpose of a normal RNA comparison. Blood for RNAseq will be collected at the same time points as tissue procurement (baseline, on-treatment and surgical resection).

Blood tubes at various time points in patients enrolled in this study for the purpose of banking plasma. Blood will be drawn at baseline, on-treatment following chemotherapy, prior to surgical resection and every 3 months post surgery for up to a year. Plasma will be stored at -80C, in the future, plasma samples will be used to explore biomarkers that may be predictive of response and resistance to pembrolizumab. Also, in the future, we will use

these plasma samples to assess circulating tumor DNA in the patients at the various time points.

Blood tubes will also be collected at various time points in patients enrolled in this study for the purpose of banking serum. Blood will be drawn at baseline, on-treatment following chemotherapy, prior to surgical resection and every 3 months post surgery for up to a year. Serum will be stored at -80C, in the future, serum will be used to explore biomarkers that may be predictive of response and resistance to pembrolizumab.

10 MEASUREMENT OF EFFECT

For the purposes of this study, patients should be evaluated for response every 6 weeks of chemotherapy.

10.1 Response Criteria

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. We will be using RECIST v1.1 for this study [Eisenhauer EA et al. New response evaluation criteria in solid tumor:revised RECIST guideline (version1.1). Eur J Cancer 2009;45:228-47). Selected sections of RECIST 1.1. are provided below.

Definitions

At baseline, tumor lesions/lymph nodes will be categorized as measurable or non-measurable as described below. For the purposes of this study, stomach, GEJ, or esophageal wall thickening will be considered non-measurable.

a. Measurable Tumor Lesions

Tumor Lesions. Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size as follows:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval no greater than 5 mm)
- 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and follow-up, only the short axis will be measured and followed. See also notes below on "Baseline Documentation of Target and Non-Target Lesions" for information on lymph node measurement.

b. Non-Measurable Tumor Lesions

Non-measurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological

lymph nodes with short axis ≥ 10 but < 15 mm) as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal mass/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

c. Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

Bone Lesions:

• Bone scan, positron emission tomography (PET) scan, or plain films are not considered adequate imaging techniques for measuring bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic–blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

• Blastic bone lesions are non-measurable.

Cystic Lesions:

• Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

• Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

10.2 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Endoscopy must be without evidence of tumor with negative cytologic brushings and esophageal biopsies. The patient must be free of all symptoms of cancer.

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD. Positive washing, brushing or biopsy and/or residual tumor may still be evident on endoscopy and/or CT scan. No lesion may increase in size and no new lesion may appear.

Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

10.3 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.
Non-Complete Response (non CR): Persistence of one or more non-target lesions or/ and maintenance of tumor marker level above the normal limits. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. (Although a clear progression of "non-target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the reference radiologist (or studychair).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

1. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment. All subjects with progression should be followed and assessed every 3 months for survival.
2. In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

10.4 Guidelines for Evaluation of Measurable Disease

All measurements should be recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.
Note: Tumor lesions in a previously irradiated area are not optimally considered measurable disease.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

- Clinical lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes). In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.
- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to the chest, abdomen, and pelvis. Head & neck and extremities usually require specific protocols.
- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions that are clinically not easily accessible. It is a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

10.5 Confirmatory Measurement/Duration of Response

Confirmation: The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed. To be assigned a status of PR or CR changes in tumor measurements must be confirmed by repeat studies that should be performed 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 8 weeks.

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

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectivelydocumented.

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

11 DATA REPORTING / REGULATORY CONSIDERATIONS

11.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

11.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

11.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator. Merck & Co., Inc. will have the opportunity to review and approve the changes prior to submission of these changes to the local IRB and distribution to participating sites.

12 STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The primary aim of this study is to demonstrate that the use of the experimental therapy (pembrolizumab + standard pre-operative chemotherapy and chemoradiation) is associated with an improvement in the major pathological response proportion from 30% (historical control) to approximately 47% with the use of the new regimen. With a sample size of 36 patients receiving the experimental therapy, a one-sided chi-square test will have 80% power to detect the difference between a major pathological response proportion of 30% (i.e., historical control) and an alternative hypothesis major pathological response proportion of 47% (new regimen), with a 0.10 one-sided significance level. As well, a sample size of 36 patients will allow a two-sided 95% confidence interval for the expected major pathological response proportion (47%) to be constructed to be within ± 16.3% of the observed major pathological response proportion. We anticipate that up to 15% of patients may not proceed to surgery, and will therefore enroll up to a total of 42.

Primary Endpoint: The primary endpoint of the major pathological response proportion will be calculated, and a 95% confidence interval will be estimated via binomial proportions. The primary endpoint of major pathological response proportion will be compared to the historical major pathological response proportion by an exact one-sample chi-square test. Patients will be considered evaluable for the primary endpoint if they undergo resection of their primary tumor, or if they are found to have disease progression prior to resection.

12.2 Sample Size/Accrual

The sample size is 42 patients.

12.3 Stratification Factors

There are no stratification factors for the primary endpoint. The randomization is performed primarily for the correlative studies to evaluate the impact of pembrolizumab on the immune infiltration following chemotherapy. Stratified and blocked randomization will be performed at all participating sites. Randomization will be stratified by location of cancer (i.e., gastro-esophageal junction vs. esophagus) and participating site. A series of randomized blocks of 2 will be generated for each cancer location stratum within each participating site with a 1:1 allocation ratio. This will provide assurance that after four patients are enrolled in any given cancer location stratum at a participating site, there will be two patients assigned to receive pembrolizumab prior to chemotherapy and two patients assigned to receive pembrolizumab following chemotherapy. This procedure will allow for each cancer location stratum within a participating site to contribute similar numbers of pre-chemotherapy pembrolizumab patients and post-chemotherapy pembrolizumab patients.

12.4 Analysis of Secondary Endpoints

Secondary Clinical Endpoints: Disease-free and overall survival (DFS) will also be estimated using the Kaplan-Meier method, and 95% confidence intervals for DFS estimates will be calculated using Greenwood's formula. Median DFS will also be estimated along with a 95% confidence interval.

23 patients will be randomly assigned to receive pembrolizumab prior to or following chemotherapy in the induction period. Only a descriptive (non-inferential) comparison will be made between the twoarms in this cohort (i.e. pembrolizumab prior to chemotherapy vs. following chemotherapy). Endpoints to be descriptively compared include pathologic complete response rate and the character and extent of immune infiltration in the biopsy specimen following induction chemotherapy, and the resection specimen following chemoradiation. Ninety-five percent confidence intervals for differences in outcome proportions between the two sub-arms will be calculated to assess the precision of the obtained estimates.

Correlative endpoints: Descriptive statistics (including mean, standard deviation, median, range, frequency, and percent) will be calculated to 1) evaluate the prevalence of specific immune related gene-expression signatures among pembrolizumab-treated patients with improved clinical outcomes, 2) evaluate the prevalence of PD-L1 expression in chemotherapy and/or radiation-treated patients, and 3) evaluate the prevalence of tumor infiltration by CD8+ lymphocytes in pembrolizumab-treated patients. All correlative endpoints will include an associated 95% confidence interval to assess the precision of the obtained estimates.

All analyses will be performed in SAS Version 9.4 (SAS Institute, Inc., Cary, North Carolina) and STATA Version 14.0 (StataCorp, College Station, Texas).

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12.5 Reporting and Exclusions

12.5.1 **Evaluation of toxicity.** All patients will be evaluable for toxicity from the time of their first treatment with *Investigational Agent.*

12.5.2 **Evaluation of response.** All patients included in the study will be assessed for response to treatment if they have completed chemoradiation therapy. A separate analysis will be performed for patients who are able to complete chemoradiation therapy and undergo resection.

Approximately 5-10% of patients are unable to proceed with surgery due to known toxicity of chemoradiation. If greater than 25% of the first 20 enrolled patients in the study are unable to proceed to resection (i.e., > 5 patients out of the first 20 enrolled patients, regardless of study arm), the study will be stopped due to excessive toxicity. This rule does not require that 20 patients be enrolled before we assess the toxicity proportion. If at any point up to the enrollment of the 20th patient, 6 patients have been determined not to be able to proceed to resection, the study will be stopped due to excessive toxicity.

13 Data and Safety Monitoring Plan (DSMP)

This study will utilize the Weill Cornell Medical College (WCMC) Institutional Data Safety Monitoring Board (DSMB) and follow its policies and procedures for monitoring this study for safety concerns, with ongoing updates from the Study Chair on an ongoing basis. The interim analysis will be performed by the DSMB and the decision to proceed or stop the trial will be provided to the Study Chair.

The Weill Cornell DSMB is comprised of medical specialists and advisors on human rights issues in human subjects research. The DSMB currently has 9 members, meets at quarterly intervals during the year, and carries out ongoing review of protocols submitted throughout the year. Once a protocol has been submitted and approved by the Institutional Review Board (IRB) and is recommended for oversight by the DSMB, the Board determines if the protocol will be reviewed quarterly, semi-annually, or annually.

The DSMB evaluates the accumulated data from the study in order to monitor the safety of subjects throughout the trial and reviews the risks and benefits, as well as the efficacy, of the study. The DSMB will also evaluate the overall trial conduct and progress. Ultimately, the DSMB validates the continuation of the trial or determines if a study needs modification or termination.

Reports to the DSMB will include the following items for review:
1. Completed DSMB Periodic Review Form.
2. Synopsis of the study to date.
3. IRB approved consent form.
4. IRB current protocol.
5. Summary table of study results.
6. Adverse event table.

7. Data safety monitoring plan.

Safety monitoring is carried out to ensure and maintain the scientific integrity of human subject research projects and to protect the safety of human subjects. Safety monitoring can be viewed as any process during a clinical trial that involves the review of accumulated outcome data for groups of patient-subjects to determine if any of the treatment procedures practiced should be altered or stopped. NIH Guidelines (1998, 2000) specify that all clinical trials should have a system in place for appropriate oversight and monitoring to ensure the safety of participants and the validity of the data.

Monitoring activities will be commensurate with the nature, size, and complexity of the trial in accordance with institutional policies and will be determined after IRB and DSMB review of the protocol immediately prior to study activation. For a small, single-center study, the monitoring is usually performed by a statistician in conjunction with a Safety Officer. For those single-site, high risk trials, a DSMB may be appropriate. For larger, single or multi-site studies, the monitoring is usually performed by a committee, often called a Data Safety Monitoring Board (DSMB). Ongoing review of the data by an independent individual or committee assures the investigators, the IRB, the study's sponsor, and the funding agency that the trial can continue without jeopardizing subjects' safety.

Weill Cornell Medical College requires that all research approved by the WCMC IRB include an appropriate plan for the monitoring of data to ensure the safety of human subjects. Research supported by Federal agencies will be monitored according to all regulations and guidelines of the relevant Federal agency.

13.1 DSMB Reporting

Reports will be made to the DSMB at the following timepoints:

- After the first 5 patients are treated
- After the first 23 patients are treated
- Semi-annual basis from then on

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