Mayo Clinic Cancer Center

Phase 1b/2 Clinical Trial of Neoadjuvant Pembrolizumab plus Concurrent Chemoradiotherapy with Weekly Carboplatin and Paclitaxel in Adult Patients with Resectable, Locally Advanced Adenocarcinoma of the Gastroesophageal Junction or Gastric Cardia

For any communications regarding this protocol please call the protocol resource person on the following page

Study Chair: Harry H Yoon MD MHS*
Mayo Clinic
200 First Street SW
Rochester MN 55905
507-284-2511
507-284-1803 (FAX)
yoon.harry@mayo.edu

Study Co-Chairs: [redacted]

Statisticians: [redacted]

[redacted]
√Study contributor(s) not responsible for patient care

Drug Availability
Supplied Investigational Agents: Pembrolizumab
Commercial Agents: Carboplatin, paclitaxel

Document History	(Effective Date)	Document History	(Effective Date)
Activation	24Jun2016	MCCC Amendment 9	28Jan2020
MCCCAdd1	23Dec2016		
MCCCAdd2	27Feb2017		
MCCCAdd3	15Jun2017		
MCCCAdd3rev	23Jun2017		
MCCC Amendment 4	06Nov2017		
MCCC Amendment 5	06Aug2018		
MCCC Amendment 6	30May2019		
MCCC Amendment 7	03Jul2019		
MCCC Amendment 8	25Sep2019		

Protocol Resources

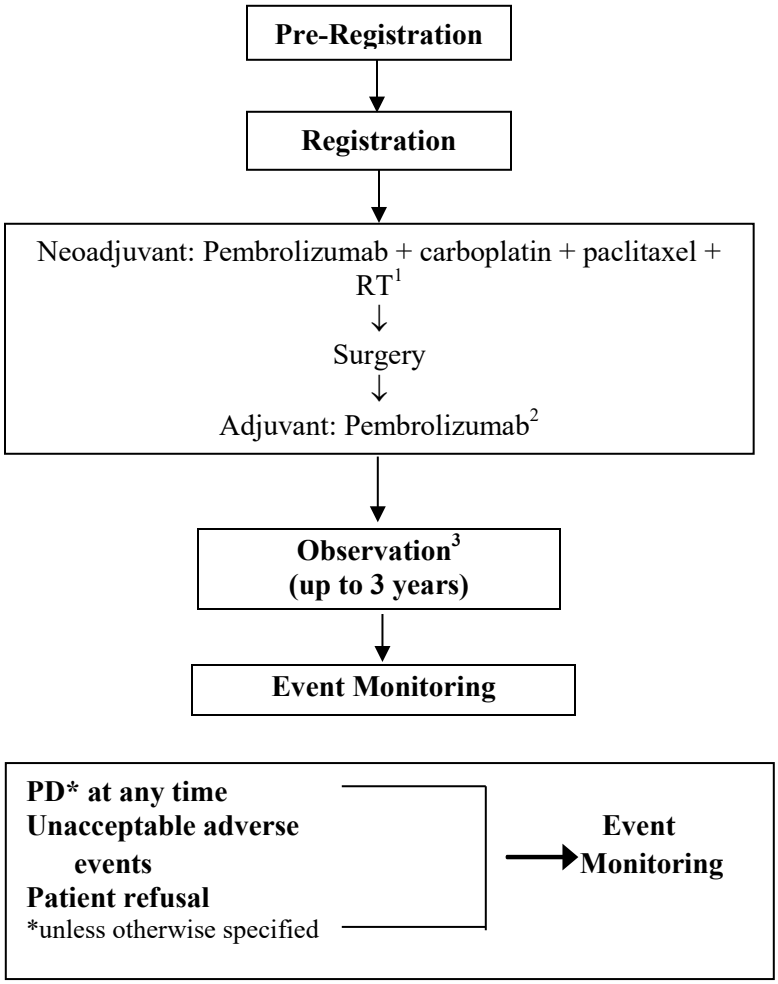
Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/ adjustments, dose modifications, adverse events, forms completion and submission	[redacted] Quality Assurance Specialist Phone: [redacted]
Drug administration, infusion pumps, nursing guidelines	[redacted] Nurse Resource Phone: [redacted]
Forms completion and submission	[redacted] Associate Clinical Research Coordinator Phone: [redacted]
Protocol document, consent form, regulatory issues	[redacted]
Paraffin-embedded tissue pathology	[redacted] Pathology Coordinator Phone: [redacted]
Non-paraffin biospecimens	[redacted] Biospecimen Resource Manager Phone: [redacted]
Serious Adverse Events	[redacted] *Research Base* SAE Coordinator Phone: [redacted]

*No waivers of eligibility

Table of Contents

Schema

Phase 1b and 2



If a patient is deemed ineligible or a cancel, please refer to Section 13.0 for follow-up information.

[1] One induction dose of pembrolizumab will be given on Day 1. Then starting on Day 15, weekly carboplatin plus paclitaxel with concurrent daily RT will be administered for approximately 5 weeks, in combination with 1 dose of pembrolizumab on Day 22.
[2] Administered once every 3 weeks for up to 6 doses.
[3] Every 3 months for 1 year after registration, then every 4 months for 2nd year and every 6 months for 3rd year.

Cycle length = 3 weeks during adjuvant therapy only

Generic name	Pembrolizumab, MK-3475	Carboplatin	Paclitaxel
Brand name	Keytruda®	Paraplatin®	Taxol®
Mayo abbreviation	MK-3475	CBDCA	TAXOL
Availability	Gonda 10 Pharmacy	Commercial	Commercial

1.0 Background

1.1 GEJ/cardia adenocarcinoma poses a worsening public health risk, compared to subcardial gastric cancers, and are distinct from esophageal squamous cancers

In the U.S. and the West, the incidence of esophageal, gastroesophageal junction (GEJ), or gastric cardia adenocarcinoma has risen 6-fold since the early 1970s, driven by obesity and gastroesophageal reflux[1]. By contrast, the incidence of subcardial gastric cancer, in which H Pylori and cadherin gene inactivation play major etiologic roles, has decreased significantly in the US and the West.

In addition, ample epidemiologic and biologic evidence indicates that adenocarcinomas of the GEJ/cardia, as well as of the esophagus, are biologically distinct from esophageal squamous cell carcinomas (SCC).

1.2 Curative-intent treatment for resectable, locally advanced GEJ/cardia cancers involves neoadjuvant chemoradiotherapy plus surgery (ie, trimodality therapy)

Almost half the patients with esophageal/GEJ/cardia cancer present with resectable, locally advanced disease. Standard treatment includes weekly carboplatin (AUC 2) with paclitaxel (50 mg/m2), combined with concurrent radiation (RT; 41.4 Gy to 50.4 Gy) for 5.5 weeks. Within 4-8 weeks of completing chemoradiotherapy (chemoRT), a PET/CT is performed to assess for metastatic disease; and once disseminated disease is excluded, surgical resection is performed.

Though a series of small randomized trials supported the addition of chemoRT to surgery in esophageal/GEJ cancer, the trimodality approach was established as a worldwide standard of care based on the CROSS trial[2]. In CROSS (N = 366), patients with esophageal/GEJ cancer was randomly assigned 1:1 to receive neoadjuvant therapy with weekly carboplatin/paclitaxel plus concurrent RT followed by surgery *vs* surgery alone. By histology, 275 (75%) had adenocarcinoma, 84 (23%) had squamous-cell carcinoma, and 7 (2%) had large-cell undifferentiated carcinoma. Overall survival (OS), the primary endpoint, was significantly improved in the chemoRT arm (HR 0.66; *P* =.003; median 49 *vs* 24 months). Other endpoints also favored the chemoRT arm: Complete resection with no tumor within 1 mm of the resection margins (R0) was achieved in 92% of patients in the chemoRT–surgery group *vs* 69% in the surgery group (*P* <0.001). A pathological complete response (pathCR) was achieved in 47 of 161 patients (29%) who underwent resection after chemoradiotherapy. ChemoRT was well-tolerated.

Utilizing chemoRT plus surgery for GEJ adenocarcinomas is a favored approach among leading cancer centers in the U.S., as demonstrated by the design of most large NCI-sponsored trials (*eg*, RTOG-1010, CALGB 9781), and at other cancer centers in the world.

1.3 Death from GEJ/cardia adenocarcinoma following trimodality therapy usually results from disease occurrence in distant anatomic regions outside the original radiation field

Although the OS observed in the CROSS trial was higher than seen in all prior large trials for patients with locally advanced esophageal/GEJ adenocarcinoma, cure rates remained low: OS rates in the chemoRT arm were 58% at 3 years and 47% at 5 years. These data reflect the aggressive biology of this disease and the need for novel therapies[2]. In one of the largest reported GEJ/esophageal adenocarcinoma cohorts (*N* = 518; 65% had GEJ tumors) that

underwent trimodality therapy at a single institution and where site of recurrence data was collected, 41% (215/518) of patients experienced disease relapse after a median follow up of 29 months. Among relapses, 87% (188/215) included a distant site outside the original radiation field[3]**.** A more recent report from the same institution after longer follow up (median 37 months) described similar results: 40% of patients (144/356) with esophageal/GEJ cancer (85% were GEJ tumors) experienced disease relapse after trimodality therapy, of which 83% (119 /144) included distant metastases outside the radiation field[4]. It is generally accepted that occurrence of disease in distant sites results from growth of micrometastases that were present, but were by definition undetectable, at the time of trimodality therapy.

The sites of recurrence among chemoRT-surgery patients in the CROSS trial were most commonly hematogeneous (29%), followed by mediastinum (7%), para-aortic (7%), supraclavicular (4%), peritoneal (4%), celiac (4%), and anastomosis (3%)[3]. In the single institution series described above of post-trimodality patients, the most common anatomic sites of distant metastases, as the first exclusive event, were lung (20%), distant lymph nodes (13%), liver (14%), peritoneum (10%), bone (6%), brain (8%) and pleura (7%)[4].

1.4 Pembrolizumab

Cancer cells are known to have the ability to evade immunosurveillance through a variety of mechanisms, including reduced expression of tumor antigens, downregulation of MHC class I and II molecules for reduced tumor antigen presentation, secretion of immunosuppressive cytokines such TGF-beta, recruitment or induction of immunosuppressive cells such as regulatory T cells (Treg) or myeloid derived suppressor cells (MDSC), and overexpression of certain ligands (eg, PDL1) that inhibit the host's existing antitumor immunity[5]. Recently, antibodies targeting the PD-1/PD-L1 or PD-L2 pathway have shown clinical benefit in melanoma, with promising activity multiple solid tumors.

The programmed death 1 (PD-1) pathway is a negative feedback system that represses Th1 cytotoxic immune responses and that, if unregulated, can damage the host[6-8]. It is up-regulated in many tumors and in their surrounding microenvironment. Blockade of this pathway with antibodies to PD-1 or its ligands has led to remarkable clinical responses in patients with many different types of cancer, including melanomas, non–small-cell lung cancer, renal-cell carcinoma, bladder cancer, and Hodgkin's lymphoma[9-15]. The expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is an important —but not a definitive— predictive biomarker of response to PD-1 blockade[9,11-13,16].

Immunotherapeutic strategies aimed at overcoming immunotolerance and improving the activation of antitumor T cells represent a new promising therapeutic approach. Among them, 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. It was recently approved in the U.S. for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

In a phase I study, 655 patients with advanced melanoma were treated with pembrolizumab in four treatment cohorts using one of three dose schedules (10 mg/kg every two weeks, 10 mg/kg every three weeks, or 2 mg/kg every three weeks). Approximately three-fourths of patients had received prior systemic therapies for metastatic disease, including 52 percent who had received prior ipilimumab. Results from this study were updated at the 2015 American Society of Clinical Oncology (ASCO) meeting[17]. The overall objective response rate using RECIST criteria and central review was 33%; similar response rates were seen using immune-related response criteria. OS at 12 months was 66% and 49% at 24 months.

Median PFS was 4.4 months, and 35% of patients remained progression free at 12 months. On multivariate analysis, there were no significant differences in outcomes between the three dose schedules. In the randomized cohorts, there were no differences in objective response rates between those treated with either 2 mg/kg every three weeks or 10 mg/kg every three weeks. Preliminary analyses studied PD-L1 expression in the tumor as a predictive marker for responsiveness to pembrolizumab. Although these studies suggested that PD-L1 positivity correlated with increased responsiveness, absence of PD-L1 expression did not preclude a clinical response. Treatment toxicity was manageable; 83 percent of patients experienced one or more treatment-related adverse events. The most common toxicities were fatigue, pruritus, rash, diarrhea, and arthralgia (36%, 24%, 20%, 16%, and 16%, respectively). Overall 14% of patients experienced grade 3 or 4 toxicity, the most common being fatigue (2%), and there were no treatment-related deaths. There were similar safety profiles in those previously treated with ipilimumab and in those who were ipilimumab naïve.

Based upon these results, two phase III trials were conducted, one in patients whose disease was refractory to ipilimumab, and the other in patients who were treatment naïve.

In the KEYNOTE-002 trial, 540 patients with ipilimumab-refractory advanced melanoma were randomly assigned to pembrolizumab (2 mg/kg every three weeks), pembrolizumab (10 mg/kg every three weeks) or chemotherapy (carboplatin plus paclitaxel, paclitaxel alone, dacarbazine, or temozolomide per institutional standard)[18]. Treatment continued until progressive disease. PFS assessed by central review, the primary endpoint of the trial, was significantly improved with both pembrolizumab treatment regimens compared with chemotherapy. The 6-month PFS rates 34%, 38%, and 16% for pembrolizumab 2 mg/kg, pembrolizumab 10 mg/kg, and chemotherapy, respectively (pembrolizumab 2 mg/kg vs chemotherapy HR 0.57, 95% CI 0.45-0.73, and pembrolizumab 10 mg/kg vs chemotherapy HR 0.50, 95% CI 0.39-0.64). The ORRs were 21%, 26%, and 4%, respectively. Treatment was relatively well tolerated, with grade 3-5 adverse events reported in 11% and 14% of the pembrolizumab arms, and 26% of those managed with chemotherapy. The most common pembrolizumab-related adverse events were fatigue, pruritus, and rash. Grade 3 immune related toxicity was reported in two patients treated with pembrolizumab 2 mg/kg (hepatitis, hypophysitis), and in eight patients given pembrolizumab 10 mg/kg (hepatitis, colitis, pneumonitis, and iritis or uveitis). The 2 mg/kg and 10 mg/kg doses also had similar efficacy and toxicity in the three-armed phase III trial when they were compared with ipilimumab[19]. Pembrolizumab at a dose of 2 mg/kg every three weeks was approved by the US Food and Drug Administration (FDA) in September 2014.

The safety and efficacy of pembrolizumab monotherapy was recently assessed in patients with advanced non–small-cell lung cancer (NSCLC)[20]. In this study, 495 patients received pembrolizumab (at a dose of 2 mg/kg or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks) to either a training group (182 patients) or a validation group (313 patients). PD-L1 expression was assessed in tumor samples by immunohistochemistry (IHC), with results reported as the percentage of neoplastic cells with staining for membranous PD-L1 (proportion score). Response was assessed every 9 weeks by central review. Common side effects attributed to pembrolizumab were fatigue, pruritus, and decreased appetite, with no clear difference according to dose or schedule. Among all the patients, the ORR was 19.4%, and the median duration of response (DOR) was 12.5 months. Median PFS was 3.7 months, and median OS was 12.0 months. PD-L1 expression in at least 50% of tumor cells was selected as the cutoff from the training group. Among patients with a proportion score of at least 50% in the validation group, the response rate was 45.2%. Among all the patients with a proportion score of at least 50%, median PFS was 6.3 months; median OS was not reached. Pembrolizumab had an acceptable side-effect profile and showed antitumor activity in

patients with advanced NSCLC. PD-L1 expression in at least 50% of tumor cells correlated with improved efficacy of pembrolizumab.

Pembrolizumab is being compared against chemotherapy in NSCLC. Pembrolizumab has also been studied in clinical trials in recurrent or metastatic adenocarcinoma of the stomach or gastroesophageal junction, classical Hodgkin lymphoma (cHL), urothelial tract carcinoma, and head and neck cancer. Though observed response rates are promising, results are premature, and trials are single-armed with small numbers.

1.5 Pembrolizumab in advanced GEJ/gastric adenocarcinoma: proof of concept.

The safety and efficacy of pembrolizumab in patients with GEJ/gastric cancer was assessed in KEYNOTE-012 (NCT01848834) in a cohort of advanced disease[21]. Archival tumor samples from patients from Asia-Pacific and rest of the world with recurrent or metastatic adenocarcinoma of the stomach or GEJ were screened for PD-L1 expression using a prototype IHC assay with the 22C3 antibody. Only patients with distinctive stromal or ≥1% tumor nest cell PD-L1 staining were eligible. Patients received pembrolizumab 10 mg/kg every 2 weeks for up to 24 months or until complete response, progression, or unacceptable toxicity. Imaging was performed every 8 weeks. Primary efficacy end point was ORR assessed per RECIST v1.1 by independent central review. Secondary end points included DOR, PFS, and OS.

Of the 162 patients screened, 65 (40%) were PD-L1^{+}. Of these 65 patients, 39 enrolled (19 from Asia, 20 from rest of the world; median age, 63 years [range 33-78]). The number of prior therapies for advanced disease ranged from 0 to 5; 67% received ≥2 prior therapies. Median follow-up duration was 8.8 months (range 6.2-12.6); 13 patients (33%) remained on therapy at the time of analysis. Four patients experienced 5 total grade 3-5 drug-related adverse events: peripheral sensory neuropathy, fatigue, decreased appetite, hypoxia, and pneumonitis (n = 1 each). There was 1 drug-related death (hypoxia). ORR was 22% (95% CI 10-39) by central review and 33% (95% CI 19-50) by investigator review. Median time to response was 8 weeks (range 7-16), with a median DOR of 24 weeks (range 8+ to 33+). PD-L1 expression level was associated with ORR (1-sided $P = 0.10$). The 6-month PFS rate was 24%. The 6-month OS rate was 69%. These early results from a single-arm trial demonstrate that pembrolizumab monotherapy has manageable toxicity and promising antitumor activity in advanced GEJ/gastric cancer, and support ongoing development of pembrolizumab for GEJ/gastric cancer.

Currently, pembrolizumab is being tested further in advanced GEJ/gastric cancer in the following planned or ongoing trials, including in combination with chemotherapy.

- KEYNOTE-059 (phase 2): Combination with cisplatin-5FU chemotherapy and as monotherapy in patients with previously untreated GEJ/gastric cancer, and as monotherapy in the 3rd-line setting
- KEYNOTE-061 (phase 3): Combination with paclitaxel as compared to paclitaxel alone in 2nd-line
- KEYNOTE-062 (phase 3): Pembrolizumab as Monotherapy and in Combination with Cisplatin plus 5FU *vs* Placebo plus Cisplatin plus 5FU in 1st-line

In these trials, a fixed dose of pembrolizumab 200 mg IV every 3 weeks is being utilized. The choice of a fixed dose 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 to

be 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. The above data indicate that the 200 mg Q3W fixed dose regimen planned to be used in this trial is likely similar with regard to efficacy and tolerability to the 10 mg/kg Q2W dose regimen used in KEYNOTE-012, and supports its use in this study. A fixed dose regimen will also be more convenient for physicians, reduce potential for dosing errors, reduce complexity in the logistical chain at treatment facilities, and reduce wastage.

1.6 Adding pembrolizumab to neoadjuvant chemoradiotherapy may enhance antitumor activity in the primary tumor and exert greater control on the growth of distant micrometastases, partly through an abscopal effect

The purpose of neoadjuvant radiotherapy in GEJ adenocarcinoma is to enhance locoregional control, and the addition of concurrent chemotherapy to radiotherapy is to enhance radiosensitization and to address micrometastatic disease[22,23]. This combined modality approach is the most effective approach, to date, for treating locally advanced disease. However, the ability of current cytotoxic chemoagents to eradicate micrometastases is poor, yielding a 5-year OS rate of <50%[2]. Clearly, novel approaches are necessary that can build on this combined modality approach.

It has been hypothesized that radiotherapy acts as an “in situ vaccine” to prime the immune response. Despite the known benefit of ionizing radiation (IR) in local tumor control, it also enhances the release of cytokines such TGF-beta, a known inducer of tumor invasion and the epithelial-mesenchymal transition[24-26]. On the other hand, IR can also promote immune responses through the induction of neoantigens and the stimulation of factors such as IFN-gamma that can enhance T cell infiltration[27]. This dual ability to control local tumor progression and to influence metastatic spread and immune response constitutes a compelling argument for including radiation in combination with the current arsenal of immune checkpoint inhibitors.

The abscopal effect refers to the ability of radiation delivered to a local site to minimize or eradicate metastases at distant sites. Although this phenomenon is not often described, it has led to complete regression of metastases at several anatomic sites in patients with lung adenocarcinoma[28] and melanoma[29].

Although radiotherapy causes tumor-cell apoptosis/necrosis, radiation alone is not sufficient to trigger antigenic signals, and a second costimulatory signal is required to elicit systemic antitumor immune responses, especially in poorly immunogenic cancers[30]. Moreover, radiotherapy alone can suppress the growth of primary breast, colon, and lung cancer tumors but not the appearance of lung metastasis in mouse models[30-32]. Thus, the combination of radiotherapy with immune modulators may have the capability to escalate antitumor responses to a level that could suppress or eliminate systemic metastasis.

Although anti–PD-1 and anti–CTLA-4 mAbs may overcome T-cell suppression, T-cell activation depends on the engagement of the antigen receptor and the activating costimulation molecule CD28 expressed by mature APCs[33]. In this regard, ionizing radiation can increase the production and presentation of tumor antigens, not only by immunogenic cancers like melanoma but also by poorly immunogenic tumors; this, in turn, could augment the antitumor immune responses elicited by checkpoint immunomodulators anti–PD-1/PD-L1 and anti–CTLA-4[30,34,35].

Preclinical studies have provided insight on how localized radiotherapy can induce the abscopal effects and have implicated the immune system as a crucial mediator (reviewed by

Frey et al[36]). Local radiotherapy damages DNA within tumor cells, leading to tumor-cell apoptosis/necrosis, increasing cytotoxic T cell activity and the antigenic peptide pool[34,37]. Tumor antigens released from the dying tumor cells potentially can provide antigenic stimulation that induces antitumor-specific immune responses. This hypothesis is supported by the lack of the abscopal effect of radiotherapy in T cell–deficient (nude) mice or in mice with CD8+ T-cell depletion[30,38,39]. New data from Deng and colleagues[40] suggests that the combination of radiation and PD-L1 checkpoint blockade can synergistically reduce MDSCs. Preclinical studies of murine models have demonstrated that various immunomodulators benefit from combinations with radiation through antigen release[41]. Postow and colleagues[29] and Hiniker and colleagues[42] each reported systemic responses in patients with melanoma treated with the combined regimen of anti–CTLA-4 mAb ipilimumab and radiation, suggesting that coupling radiotherapy with immunotherapy may hold promise for inducing powerful, longterm abscopal effects in human patients.

Demaria et al elegantly found that the abscopal effect could be tumor specific by administering the dendritic cell growth factor Flt3-L to mice that were implanted in one flank with mammary 67NR tumor cells alone and in the contralateral flank with both 67NR tumor cells and A20 lymphoma cells[38]. They irradiated the flank with only the 67NR tumor cells, which led to significant regression in the contralateral flank of the nonirradiated 67NR tumors, but did not affect the growth of the antigenically unrelated A20 lymphoma[38]. By contrast, Camphausen et al found that irradiating Lewis lung carcinoma cells restricted the growth of nonirradiated T241 (fibrosarcoma) cells that had been inoculated in a second site, suggesting that the abscopal effect of radiotherapy can also be mediated through other non–tumor-specific mechanisms, or that these two tumors are antigenically related[43]. The systemic increase of many proinflammatory cytokines and chemokines after radiation, from both immune cells and tumor tissues, could account for the nonspecific eradication of distant tumors and metastases[44]. Alternatively, the release of low-affinity tumor antigens prompted by local radiotherapy may also stimulate the release of cross-reactive tumor antigens.

An abscopal effect was observed in animal models of pancreatic cancer, whereby the addition of capecitabine to RT resulted in a synergistic inhibition of growth in lead-shielded distant tumors in the contralateral flank, which was associated with a decrease in proliferation[45]. Similarly, in a preclinical colon adenocarcinoma model, marked inhibition of tumor growth at the non-irradiated site was observed with the addition of a chemokine ECI301 following radiation, as compared to radiation alone or chemokine alone[46].

Effect of RT plus immunomodulation against micro- and macro-metastases. The concept that enhancement of the peptide pool via localized RT can suppress micrometastases was demonstrated by Chakravarty et al[47]. A highly metastatic and poorly immunogenic Lewis lung carcinoma (3LL/D122) was injected into the footpad of mice, and palpable tumors developed. At this time, histologic analysis of lung demonstrated the presence of microscopic pulmonary metastases. If the tumor-bearing leg is amputated, all animals are cured from the primary tumor, but are known to eventually succumb to massive lung metastases. Palpable tumors were given RT (one fraction of 60 Gy, which was previously demonstrated to cure the primary), followed by a 10-day course of Flt3L. Flt3L is a cytokine (naturally occurring glycoprotein) that stimulates the proliferation and differentiation of a variety of hematopoietic cells, including dentritic cells (DCs) *in vivo*, both in mice and in humans, and that stimulates tumor-specific immune responses. DCs can acquire antigen from apoptotic cells and induce cytotoxic T lymphocytes (CTL)[48]. Lung parenchyma of survivors in the RT + Flt3L cohort showed no carcinoma cells but had infiltrates of neutrophils, lymphocytes, and mononuclear leukocytes. By contrast, all animals who received RT alone or Flt3L alone had diffuse metastasis of Lewis lung carcinoma cells. Mean lung weights, measured 21 days after RT (11

days after Flt3L therapy), were significantly lower in the animals that received Flt3L + RT, as compared to RT alone or Flt3L alone. Contrary to these immunocompetent mice, nude mice receiving RT + Flt3L failed to eliminate pulmonary metastatic infiltrates. The combination of RT + Flt3L also improved animal survival. Together, these data indicate that the combination of improved antigen presentation and RT significantly suppressed micrometastases.

The concept that anti-CTLA4 blockade plus RT can decrease lung metastases was demonstrated by Demaria et al[30] in an animal model of mammary carcinoma 4T1. When injected subcutaneously in the mammary fatpad, 4T1 cells metastasize primarily by a hematogenous route and can be found in the lungs within 7 days. Given the poor immunogencity of 4T1 cells, CTLA-4 blockade by the 9H10 mAb alone did not affect the tumor growth or survival of the mice. RT at a single dose of 12 Gy significantly delayed the growth of the primary irradiated tumor in the presence or absence of 9H10. To directly test the effects of treatment on lung metastases, all mice were sacrificed on day 35 post-tumor implantation, and the decrease in lung metastases was significant only when RT and 9H10 were used in combination, consistent with their observation that only this treatment resulted in increased survival. To confirm that the inhibition of lung metastases following RT + 9H10 treatment is mediated by T cells and to identify the population involved, mice were depleted of CD4+ or CD8+ T cells starting 3 days before RT treatment: The median number of metastases in mice treated with RT + 9H10 and depleted of CD8+ T cells was similar to control untreated animals. By contrast, the inhibition of lung metastases observed in mice treated with RT + 9H10 was not affected by CD4+ T-cell depletion. These results indicate that CD8+ T cells play a crucial role in the antimetastatic effect of the combination treatment.

Augmented antitumor effect through the combination of anti-PD-1 blockade plus RT. Anti–PD-1/-PD-L1 mAbs have drawn much interest for their potential use in lung or colon cancer[49]. The mechanism by which radiation augments the therapeutic effects of the anti–PD-1/PD-L1 mAbs was elucidated in a preclinical study of triple-negative breast cancer. In this study, neither anti–PD-1 mAb nor radiation when given alone was effective in a murine model. However, the addition of anti–PD-1 mAbs promoted the rejection of irradiated tumors in the physiologically relevant tissue microenvironment of the mammary fat pad.[50]

Results of a preclinical study of murine intracranial glioma treated with anti–PD-1 mAbs plus radiotherapy showed not only long-term survival of the treated mice, but also robust systemic immunologic memory in the surviving mice, as they were able to reject a secondary challenge of glioma cells injected in the flank[51]. Specifically, median survival periods were similar for control mice (25 days) and mice given only anti–PD-1 mAbs (27 days) or radiation (28 days). However, the combination of radiation plus anti–PD-1 therapy extended the median survival to 53 days ($P < 0.05$), and 15% to 40% of mice survived more than 180 days after treatment[51]. The combination therapy increased tumor infiltration by CD8+ CTLs and decreased the number of CD4+Tregs. Finally, in a test of immunologic memory, naïve and long-term surviving mice were injected in the flanks with GL261-luc cells. All 8 naïve mice died from the growth of the challenged glioma cells, whereas mice that received prior treatment with the combined regimen rejected the glioma challenge[51].

Increased PD-L1 expression in tumor tissue following ionizing radiation
While not definitive, the expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is an important predictive biomarker of response to PD-1 blockade[9,11-13,15,16]. PD-L1 expression in the tumor microenvironment has been associated with poor outcomes following chemoradiotherapy in cancer patients[52,53]. In KEYNOTE-012, the frequency of PDL1 expression was 40% in human GEJ/gastric cancers, and among PD-L1-expressing tumors, a higher degree of expression was non-significantly associated with improved outcome[21]. In the largest study evaluating PDL1 expression in gastric cancer, PD-

L1 expression was evaluated in resected specimens and was defined as ≥5% membrane expression on tumor cells with ≥1+ intensity[54]. In this study, PD-L1 expression was not observed in stroma in cases that lacked expression in tumor cells. The PD-L1+ frequency was 14% (57/398), and PD-L1+ protein expression was correlated with its mRNA expression. These data suggest that a minority of untreated gastric tumors express PD-L1 at baseline.

In an important preclinical report, Deng et al found that ionizing radiation can induce expression of PD-L1 in tumor and their microenvironment[40]. In addition, they found that the addition of PD-L1 blockade to radiation controls primary tumor growth in a CD8+-dependent and tumor antigen–specific manner, leading to prolonged protective T cell immunity. They further found that the PD-L1/radiation combination induces an abscopal effect on non-irradiated distant tumors[40]. These data raise the possibility that exposure to radiation could induce tumors to develop a dependence on the PD-L1/PD-1 pathway as an immune escape mechanism, which could make those tumors more suspectible to PD-1/PD-L1 blockade.

Fractionated (*vs* single-dose) radiotherapy has greater synergy with immune therapy
Dewan et al tested the hypothesis that the type of dose fractionation regimen of RT can determine the ability of RT to synergize with anti-CTLA-4 antibody[31]. In a mouse model where colon or breast cancer cells were injected into both flanks, single-dose or fractionated RT led to growth delay of the primary tumor, regardless of whether anti-CTLA-4 antibody was administered, but had no effect on secondary tumors outside the RT field. However, an abscopal effect on the secondary tumor outside the RT field only occurred in mice treated with the combination of antibody plus fractionated RT, not single-dose RT. The frequency of CD8+ T cells showing tumor-specific IFN-gamma production was proportional to the inhibition of the secondary tumor, suggesting that cell-mediated immunity was responsible for the abscopal effect. In addition, these investigators found that delaying the initiation of anti-CTLA-4 antibody until a few days after the completion of RT reduced the therapeutic effect on the primary and secondary tumor, as compared to initiating antibody treatment on the first or final day of RT (RT was given in 3 fractions of 6 Gy each). Of note, in Deng et al, PD-L1 blockade, administered every 3 days for a total of 4 times, was initiated on the day of IR or 1 day before IR[40].

1.7 Safety for combining chemoradiotherapy with pembrolizumab

In clinical trials completed to date, most immune related adverse events associated with pembrolizumab were mild (grade 1–2), some were severe, and a few were lethal. The most common toxic effects were considered to be immunologic in origin, and the sites most commonly affected were the skin, gastrointestinal system, liver, and lung[10,15,55].

Neoadjuvant carboplatin/paclitaxel chemoradiotherapy in this disease is well tolerated, as reported[2] and based on our high-volume experience. In the CROSS trial, the most common major hematologic toxic effects in the chemoRT–surgery group were leukopenia (6%) and neutropenia (2%). The most common major non-hematologic toxic effects were anorexia (5%) and fatigue (3%). Postoperative complications were similar in the two treatment groups, and in-hospital mortality was 4% in both.

Risk of pneumonitis: Rationale for restrictions on age and pulmonary status

The known toxicities of neoadjuvant carboplatin/paclitaxel/radiotherapy do not generally overlap with pembrolizumab (see below and Section 1.4 and 1.5). One potential exception is toxicity involving the thorax or upper abdomen related to organs within the RT field.

Radiation pneumonitis is thought to reflect an immune-mediated inflammatory reaction to radiation-induced lung damage[56,57]. Severe forms can be lethal. Similarly, radiation treatment in the abdomen that could result in bowel radiation could similarly exacerbate colitis. Radiation pneumonitis has been reported in patients who have undergone mediastinal radiation therapy for lung cancer, Hodgkin's lymphoma (HL), breast cancer, and other cancers that require radiation therapy to the thorax[58]. Radiation pneumonitis is reported in 5% to 15% of patients receiving definitive external-beam radiation therapy for lung cancer[59]. Clinical factors that may increase the risk of radiation pneumonitis include concomitant chemotherapy, previous irradiation, and recent withdrawal of steroids[59], although patients treated with specific chemotherapeutic drugs, such as platinum, etoposide, taxanes, and vinorelbine, and concurrent radiotherapy do not appear to have a higher risk for radiation pneumonitis[60]. A retrospective analysis of 1,911 patients who underwent combined-modality therapy for lung cancer demonstrated that the overall risk of radiation pneumonitis was 7.8%[61]. In a multivariate analysis, the daily fraction dose, number of daily fractions, and total dose were associated with radiation pneumonitis[61]. Both the V20 (ie, the percentage volume of both lungs minus the planning target volume [PTV] receiving 20 Gy) and the mean lung dose (MLD) correlate with the risk for radiation pneumonitis[62]. Although a V20 level of 35% to 37% or an MLD value of 20 to 23 Gy (both calculated with a more advanced algorithm) have been considered safe, ~10%-15% of patients still may develop severe radiation-induced toxicity when they have received much lower doses[63]. Patient factors, such as lung function, age, and sex, do not adequately select patients at high risk for radiation pneumonitis or fibrosis[64].

Radiation pneumonitis is typically a delayed acute reaction, usually occurring 1 to 3 months after completing mediastinal radiation therapy[59]. Patients who experience acute radiation pneumonitis will often have a self-limited course, with complete resolution of this process. However, a minority of patients may develop progressive pulmonary fibrosis, usually 6 to 24 months after treatment. Late complications of pulmonary fibrosis include cor pulmonale and respiratory failure[58]. Although there is a lack of published data, the EORTC panel for radiotherapy use in lung cancer argued that personal experience suggests a high incidence of severe and even lethal radiation pneumonitis in patients with idiopathic interstitial pneumonitis[62].

In esophageal/GEJ cancer, a careful review of the literature reveals that radiation pneumonitis is uncommon in trial settings. In the CROSS trial, during chemoRT, no instance of pneumonitis was reported. The most common toxicities of any grade that could conceivably involve the thorax or upper abdomen were esophagitis (19%), nausea (53%), vomiting (25%), and esophageal perforation (1%). Lung toxicities of any grade were not reported. Grade 3-4 events involving these regions were uncommon: esophagitis (1%), nausea (1%), vomiting (1%), and esophageal perforation (1%). Postoperative events, including death, were not increased in the chemoRT plus surgery group, compared to the surgery alone group. In the chemoRT group, the most common postoperative events were pulmonary complications (46%; pneumonia, serious atelectasis, pneumothorax requiring drainage, pleural effusion requiring drainage, pulmonary embolus, or acute respiratory failure defined as partial pressure of arterial O2 <60 mm Hg while breathing room air), cardiac complications (21%; arrhythmia requiring treatment, MI, left ventricular failure as demonstrated by pulmonary edema on radiograph), chlorothorax (10%; elevated levels of triglycerides in intraothoracic fluid), mediastinitis (3%), anastomotic leak (22%), death in hospital (4%) and death after 30 days (2%).

Other phase 3 trials of patients with esophageal/GEJ cancer, all of which used a cisplatin/5FU chemotherapy backbone, which is considered to have an unfavorable toxicity profile than

carboplatin/paclitaxel, also report low rates of pneumonitis or pulmonary complications (see Table 1).

Table 1

Thoracic toxicities in phase 3 trials of patients with esophageal or GEJ cancer received concurrent chemoRT (CROSS trial not shown)

Study (no. patients receiving chemoRT)	Tumor	Chemo regimen	RT dose	Grade 1 or higher	Grade 3 or higher	Postop
Walsh et al. 1996 (n=58)	Distal esoph AC or cardia AC	Cisplatin + 5FU	40 Gy	Not reported	Cardiac NOS n=2	Respiratory NOS, n=28 Cardiac NOS, n=14 Anast. Leak, n=2 Chlyothorax, n=1
Burmeister et al 2005 (n=128)	Distal, mid, or upper esoph AC or SCC	Cisplatin + 5FU	35 Gy	Esophagitis 80% Pneumonitis 28%	Esophagitis 16% Pneumonitis 2%	Surg-related death 5%[b] Major pulm 20% Cardiac 12% Anast. Leak 5% Anast. Stricture 19%
Conroy et al 2014 (PRODIGE; n=267)	Esoph SCC (85%) or esoph AC (15%)	FOLFOX or cisplatin + 5FU	50 Gy	Esophagitis 22% Cardiac 2% Respiratory, none reported	Esophagitis 8% Cardiac 1% Death due to MI or CVA, 2% Respiratory, none reported	No surgery arm

[a] Herskovic et al 1992 (NEJM) is not shown above, because the trial occurred in an earlier era, and because 84% of patients had esophageal SCC; in this trial, severe respiratory toxicities requiring O2 therapy and initiation of glucocorticoid therapy was reported in 3% of patients.
[b] Causes of death included respiratory (n=2) and MI (n=1).

Among patients treated with agents targeting CTLA-4 and PD-1, rates of severe adverse events (Grade 3–4), including pneumonitis, dyspnea, or cough, have ranged from 2% to 4%[10,15,55,65]. The agents were believed to contribute to 3 deaths in one study[15] and 1 death in another study[10]. In gastric cancer, the only available data for lung toxicity was reported in KEYNOTE-012, which enrolled only patients with advanced stage[21]. Of 39 patients who were evaluable for treatment related AEs, Grade 3-5 treatment related AEs were reported in 1 patient (2.6%) who had Grade 4 pneumonitis and in 1 patient (2.6%) with hypoxia whose death was considered to be treatment related.

In the current trial, as a precaution to reduce risk for thoracic toxicities and/or postoperative complications following chemoradiation plus pembrolizumab, the following measures have been implemented:

- Patients aged 69 or more will be excluded (see Section 3).
  - The most comprehensive evaluation of the impact of age on the benefit of perioperative chemo(radio)therapy was performed in a meta-analysis (14 randomized controlled trials, N = 2,422)[66]. In this analysis, which included individual patient data in the majority of patients, the OS benefit of perioperative therapy was significantly diminished in a stepwise manner as age increased (interaction multivariate P =.0168), with a HR of 1 at ages > 65 years, and HRs >1 observed at ages 70 or more. This lack of benefit in patients with advanced age likely reflects the morbidity associated with trimodality therapy in the setting of subclinical frailty.
- Radiation parameters used in the CROSS trial on tumor length and radius, depth, and total RT dose (41.4 Gy) will be implemented. Restrictions from RTOG-1010 on the size of celiac nodes will be implemented (see section 3).

- Strict limits on normal tissue doses and other aspects of radiation delivery will be mandated per the most updated standard of care and as recommended[5] (see section 7.3). On-study rapid-review quality assurance of radiation planning will be mandated.
- Patients with recent serious pulmonary illness will be excluded (see section 3).
- PFTs will be required pre-chemoRT and pre-surgery (see section 3). PFTs are variably utilized but are a standard of care.
- Monitoring will include late lung toxicities, given that pneumonitis often does not appear until several months after radiation treatment is completed.

The combination of pembrolizumab with trimodality therapy is a promising approach in this highly fatal disease. Clinical data are needed that evaluates the combination of pembrolizumab with concurrent radiation to the esophagogastric region, and with concurrent chemotherapy followed by thoracoabdominal surgery. Outcomes for the combination of pembrolizumab and (chemo)radiation are being collected in ongoing trials in other tumor types.

- **Neoadjuvant pembrolizumab plus concurrent chemoRT:** In a neoadjuvant pancreatic cancer trial, neoadjuvant pembrolizumab 200 mg IV days 1, 22, and 43 will be given during concurrent radiation (50.4 Gy in 28 fractions) and capecitabine (825 mg/m2 BID).
- **Adjuvant pembrolizumab following RT:** In head and neck cancer, adjuvant pembrolizumab will be given once every 3 weeks for a maximum of 6 doses, following completion of adjuvant 60 Gy radiation (over 6 weeks) and risk-based cisplatin on days 1, 22, and 43.

Ongoing or planned studies to assess thoracic RT plus pembrolizumab include:

- MK-3475 and Hypofractionated Stereotactic Radiation Therapy in Patients With Non-Small Cell Lung Cancer (NSCLC) (NCT02444741)
- Phase I Trial of MK-3475 and Concurrent Chemo/Radiation for the Elimination of Small Cell Lung Cancer (NCT02402920)
- Consolidation Pembrolizumab (MK-3475), Following Chemoradiation in Patients With Inoperable/Unresectable Stage III NSCLC (NCT02343952)

In addition, neoadjuvant pembrolizumab with or without chemotherapy (without RT) is currently being studied in multiple solid tumors, including:

- Phase Ib/II Study of Neoadjuvant Pembrolizumab With Gemcitabine-Cisplatin (Cisplatin-Eligible) or Gemcitabine (Cisplatin-Ineligible) in Subjects With T2-4aN0M0 Urothelial Cancer (study start May 2015, end March 2018; NCT02365766)
- Neoadjuvant Pembrolizumab for Unresectable Stage III and Unresectable Stage IV Melanoma (NeoPembroMel; start Jan 2015; end Feb 2017; NCT02306850)
- A Clinical Trial to Evaluate the Effect of Neoadjuvant MK-3475 (Pembrolizumab) Therapy on Intratumoral Immune Infiltrates in Renal Cell Cancer (RCC) Patients Undergoing Surgical Resection (start Dec 2014, end Feb 2016; NCT02212730)
- Immunotherapy With MK-3475 in Locoregionally Advanced, Surgically Resectable Head and Neck Squamous Cell Carcinoma (start March 2015, end March 2022; NCT02296684)
- A Clinical Trial to Evaluate the Effect of Neoadjuvant MK-3475 (Pembrolizumab) Therapy on Intratumoral Immune Infiltrates in Renal Cell Cancer (RCC) Patients Undergoing Surgical Resection (start Dec 2014, end Feb 2016; NCT02212730)

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

1.8 An accepted outcome measure for this trimodality approach is pathCR

A pathologic complete response (pathCR) is defined as the absence of malignant cells in the resected specimen following chemoradiotherapy[67]. Approximately 15%-29% of patients achieve a pathCR, as shown by a large series from Mayo Clinic and randomized clinical trials[2,67-69]. PathCR has become accepted as a primary endpoint in phase 2 trials in the US examining the efficacy of neoadjuvant chemoradiation in this disease, including in NCI-sponsored trials conducted by our group[70] and others[68,71]. This is because the outcome result is known soon (~12 weeks after initiation of therapy) and because it is well-accepted as the strongest prognostic variable for disease-free survival (DFS) or overall survival (OS)[22,23,72]. Pembrolizumab has been shown in preclinical models to enhance antitumor efficacy on primary tumors when added to radiation (see Section 1.6). Together, these considerations support the use of pathCR as an endpoint in the current trial.

1.9a Disease recurrence is an adequate endpoint of long-term outcome after trimodality therapy for GEJ adenocarcinoma

Utilizing pathCR as the sole primary endpoint has limitations, particularly for a drug such as pembrolizumab that may have an abscopal effect on micrometastases that would not be expected for cytotoxic chemoagents. The prior data suggesting pathCR is a surrogate endpoint of long-term survival is based entirely on the use of cytotoxic therapies, not immunotherapies. The benefit from adding pembrolizumab may be demonstrated through its ability to eradicate micrometastases (see Section 1.6); it is possible this effect may not be adequately reflected in the primary tumor in a small single-arm trial. Therefore, assessing disease recurrence is an important endpoint in the current trial.

Disease recurrence is a reasonable surrogate for OS. As described above, ~40% of patients experience disease recurrence after trimodality therapy. The vast majority of recurrences occur at a distant anatomic site, and 90% of distant recurrences occur within 24 months of completing local therapy. Disease recurrence at a distant anatomic site in esophageal/GEJ adenocarcinoma is almost always fatal. The median OS of patients who had distant metastases after trimodality therapy is ~10.2 months[4].

In the CROSS trial, after a minimum follow-up of 24 months and a median follow up of 45 months for surviving patients, 35% of patients in the chemoRT group had disease recurrence. Almost all disease recurrences (91% [32%/35%]) included a distant anatomic site. Every recurrence occurred within 36 months after randomization. Moreover, the KM curves (which include only patients who underwent an R0-R2 resection, of adenoca or SCC histology) reveal a DFS rate of 72% at 24 months and 60% at 36 months. The majority (70% [28%/40%]) of DFS events occurred within 24 months. In a large series from a single institution, 40% of patients (144/356) with esophageal/GEJ cancer (85% were GEJ tumors) experienced disease relapse after trimodality therapy after a median follow up of 37 months. Among patients who relapsed, 83% (119 /144) included distant metastases outside the radiation field[4]. The distant recurrence rate at 24 months after the completion of local therapy was 31% in the overall cohort; among the patient subset who experienced disease recurrence at a distant site after trimodality therapy, 91% of distant metastases were diagnosed within 2 years of local therapy.[4] A separate report from the same institution observed a locoregional recurrence rate at 24 months of 3.4% (17/518), and these 17 recurrences represented 63% (17/27) of all locoregional recurrences from the time of esophagectomy after a median follow up 55 months for surviving patients[3]. Together, these data indicate that 24 months of follow up is sufficient to capture the vast majority of DFS events.

1.9b Perioperative chemotherapy alone (without radiotherapy) is an alternative approach for the definitive treatment of locally advanced GEJ adenocarcinoma

While preoperative chemoRT is a preferred approach at leading cancer centers in the U.S. for the treatment of locally advanced GEJ adenocarcinoma, perioperative chemotherapy alone without radiation is a valid approach. Support for perioperative chemotherapy alone without RT for definitive therapy of GEJ adenocarcinoma is based on multiple phase 3 trials utilizing platinum/fluoropyrimidine-based combination chemotherapy, which have shown an OS benefit for the addition of perioperative chemotherapy to surgery alone for esophagogastric cancer[73-75].

Platin/FP doublet is equal to anthracycline/platin/FP triple (OEO5). In the most recently reported phase 3 trial examining perioperative chemotherapy alone without RT (MRC OEO5, N = 897), patients with adenocarcinoma of the lower esophagus or GEJ were randomized to a standard regimen of 2 cycles of preoperative CF (cisplatin 80 mg/m2, 5FU 1000 mg/m2 D1-4; once every 21 days) *vs* an intensified regimen of 4 cycles of preoperative ECX[76]. The R0 rates were low: 60% in the CF group *vs* 67% in the ECX group. The trial failed to meet its primary endpoint of superior OS in the intensified *vs* standard regimen (HR 0.92, p=.89), and toxicity was increased in the intensified regimen. These data are consistent with cross-trial comparisons suggesting that the OS observed with platinum/FP doublets (ACCORD-07)[75] are comparable to that with platin/FP/anthracycline triplets (MAGIC)[73], and provide support for the use of a platinum/FP doublet as standard therapy for a perioperative approach that utilizes chemotherapy alone without radiation.

Cisplatin/5-FU doublet regimen (ACCORD-07) A preoperative platin/FP doublet was examined in a recent phase 3 trial involving GEJ adenocarcinoma patients (ACCORD-07), which randomized patients to perioperative chemotherapy *vs* surgery alone. In this trial[75], 113 patients were assigned to the chemotherapy arm to receive 2-3 cycles of chemotherapy (cisplatin 100 mg/m2 plus 5FU 800 mg/m2/day Days 1-5; every 28 days), followed by 3-4 cycles of postoperative chemotherapy of the same regimen. While 87% of patients received 2 or more cycles preoperatively, only 12% received 3 preoperative cycles. The R0 rate was 84% (95/113) with a 5 year DFS rate of 34% and 5-year OS rate of 38%. Most common grade 3-4 toxicities were neutropenia 20%, nausea/vomiting 9%, thrombocytopenia 6%, and mucositis 4%. The combination of pembrolizumab 200 mg IV plus cisplatin 80 mg/m2 and 5-FU 800 mg/m2 D1-5, every 21 days, is being examined in patients with previously untreated (KEYNOTE-062) and previously treated (KEYNOTE-059) patients with GEJ/gastric cancer.

Lack of efficacy benefit from the addition of perioperative cisplatin/5FU to surgery alone in US population (INT-0113) However, the combination of cisplatin plus 5-FU is not widely used as preoperative regimen in the U.S. This is partly due to the observed toxicity in this population. In addition, this regimen (cisplatin 100 mg/m2 day 1 plus 5FU 1000 mg/m2 days 1-5, every 28 days) demonstrated a lack of efficacy in the largest U.S. randomized trial examining perioperative chemotherapy plus surgery *vs* surgery alone (INT-0113)[77,78]. In this trial (N = 467), R0 rates were observed in 63% in the perioperative chemotherapy group, comparable to the R0 rates observed in the CF arm of the recent OEO5 trial, *vs* 59% in the surgery alone group. Only patients with an R0 resection experienced substantial long-term survival, which is a well-recognized prognostic factor. After median follow up of 8.8 years, median OS was the same between groups (1.3 years in CF-surgery group *vs* 1.3 years in the surgery alone group).

Combination of oxaliplatin/5FU with PD-L1 inhibitor In immunocompetent mice implanted with CT26 colorectal cancer cells, an anti-mouse PD-L1 antibody was recently shown to significantly improve survival of mice. The antitumor activity of anti-PD-L1 was

enhanced by combination with oxaliplatin, which resulted in increased release of HMGB1 within CT26 tumors[81]. Pembrolizumab is being tested in combination with mFOLFOX6 in a phase 2 trial of advanced CRC (NCT02375672) and in a phase I (dose escalation)/II trial of advanced GI malignancies, including gastroesophageal carcinomas (NCT02268825). In the former trial, pembrolizumab is administered 200 mg IV (flat dose) every 3 weeks, starting with cycle 1, with FOLFOX being given every 2 weeks. In the latter trial, pembrolizumab is administered every 2 weeks, starting with cycle 3, with FOLFOX given every 2 weeks (Level -1: 50mg IV q 2 weeks Level 1: 75mg IV q 2 weeks Level 2: 200mg IV q 2 weeks).

Combination of carboplatin/paclitaxel with pembrolizumab The carboplatin/paclitaxel doublet has known activity in GEJ cancer in locally advanced disease[2] and in advanced disease in the first-line[82-85] and second-line[86,87] settings. Paclitaxel or platinum compounds have demonstrated improved antitumor efficacy when combined with anti-PD-1-based blockade[88]. Carboplatin (AUC 6) plus paclitaxel (200 mg/m2) combined with pembrolizumab 2 mg/kg or 10 mg/kg (all agents given on Day 1 of a 21-day cycle) is currently being tested in a phase I/II trial in metastatic NSCLC (KEYNOTE-021; NCT02039674).

1.9c Rationale for translational studies

1.9c1 Genetic subtypes of GEJ cancer and anti-tumor immunity, including MSI, EBV, and *PDL1* and *PDL2* amplification.

Recent comprehensive molecular characterization of GEJ/gastric adenocarcinomas by the TCGA has revealed 4 molecular subtypes: EBV-associated (10% of cases, which included *PD-L1* and *PD-L2* amplifications), microsatellite unstable (MSI; 20%), chromosomal instability (50%; characterized by intestinal histology, *TP53* mutation, and RTK-RAS activation), and genomic stability (20%; characterized by diffuse histology, *CDH1, RHOA* mutations)[89].

These subtypes may have differential response to immune-modulating agents. EBV-associated gastric adenocarcinomas display recurrent amplification of *PD-L1* and *PD-L2*[89] and are primarily characterized by dysregulation of immune response genes[90]. In addition, EBV ISH-positivity was found to be correlated with PDL1 protein expression in a large series of human gastric cancers from China[54], and EBV infection was strongly associated with intratumoral CD8+ T cell infiltration and moderately correlated to stromal CD8+ T cell accumulation in a small gastric cancer series[91]**.** EBV positivity by ISH was found in 3% of gastric cancers from Caucasians (N>400)[92]**.** This association has not been studied extensively in GEJ adenocarcinomas.

The association between MSI (mismatch repair [MMR] deficiency) and tumor infiltrating lymphocytes (TILs) is well established in multiple epithelial cancers. A recent phase 2 study evaluated the clinical activity of pembrolizumab in 41 patients with progressive metastatic carcinoma with or without deficient MMR. Pembrolizumab was administered at a dose of 10 mg/kg every 14 days in patients with MMR–deficient colorectal cancer (CRC), patients with MMR–proficient CRC, and patients with MMR–deficient non-CRCs. The co-primary end points were the immune-related objective response rate (irORR) and the 20-week immune-related progression-free survival rate (irPFS). The irORR and irPFS rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for MMR–deficient CRCs; and 0% (0 of 18 patients) and 11% (2 of 18 patients) for MMR–proficient CRCs; and 71% (5 of 7) and 67% (4 of 6) for MMR-deficient non-CRC cancers. Median PFS and OS were not reached in MMR-deficient CRCs,

but were 2.2 and 5.0 months, respectively, in MMR-proficient CRCs (HR for disease progression or death, 0.10 [*P* <.001], and HR for death, 0.22 [*P* =.05]). Whole-exome sequencing revealed a mean of 1782 somatic mutations per tumor in MMR–deficient tumors, as compared with 73 in MMR–proficient tumors (*P* =.007), and high somatic mutation loads were associated with prolonged PFS[93].

The largest evaluation of MMR in gastric/GEJ cancer (N = 712) revealed that 9% of tumors from either a European (25 of 264) or Japan cohort (21 of 230) were MMR-deficient[94]. *KRAS* mutation (codons 12, 13, and 61) was detected in ~5% of tumors, and $BRAF^{V600E}$ mutation was detected in <1% of tumors. *KRAS* mutation status was statistically positively correlated with MMR-deficiency. The MMR rate among 446 gastric tumors from Caucasians was reported to be 7%[92].

MSI data are recently available on the Foundation One genomic profile, which also provides information on alterations in hundreds of cancer-related genes and mutational load (FoundationOne.com). This test is commercially available and has been obtained as part of clinical care in Mayo Clinic patients to facilitate treatment options.

1.9c2 Tissue expression of PDL1 and immune markers

PDL1 expression is an important marker that has potential predictive value for pembrolizumab therapy, as described above. Ionizing radiation has been shown to induce PDL1 expression in preclinical models, as described above[39,40].

The density of markers of adaptive immunity such as CD3, CD8, FoxP3, and CD45RO infiltrating intratumoral/stromal tissue compartments have been associated with survival in patients with colorectal cancer, as we[95] and others[96,97] have shown. In gastric cancer, the densities of CD3+, CD8+, and CD45RO+ TILs were independent favorable prognostic factors[98], as were CD8+ and FoxP3+ cells in MSI-high gastric cancers[99]. The percentages of CD8+ T cells that produce IL-17 in gastric tumors are associated with survival times of patients and promote chemotaxis of MDSCs, which might promote tumor progression[100]. It is possible that adaptive immune markers in TILs, apart from PD-L1 expression, can have predictive value for immunomodulating agents[101].

1.9c3 Circulating markers and cells

Soluble PD-L1. Release of inhibitory coregulatory proteins into the circulation may represent one mechanism by which tumors thwart immune responses. A collaborator in the current trial ([redacted] Laboratory) identified a soluble form of PD-L1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma[102]. This lab developed an ELISA for quantification of PD-L1 in biological fluids. Soluble PD-L1 was detected in the cell supernatants of some PD-L1-positive tumor cell lines. Protein sequencing established that the measured soluble PD-L1 retained its receptor-binding domain and could deliver proapoptotic signals to T cells. Higher preoperative soluble PD-L1 levels were associated with larger tumors (*P* < .001), tumors of advanced stage (*P* =.017) and grade (*P* =.044), and tumors with necrosis (*P* =.003). A doubling of soluble PD-L1 levels was associated with a 41% increased risk of death (*P* =.010). These observations suggest that soluble PD-L1 may be detected in the sera of cancer patients and that it may systemically impair host immunity, thereby fostering cancer progression and subsequent poor clinical outcome.

Soluble PD-L1 has not been tested in GEJ cancer patients, nor have changes in levels in response to neoadjuvant chemoRT been adequately studied.

Balance of circulating $CD8^+$ effector cell subpopulation. Given that the antigen spectrum for a given tumor is usually incomplete, it is possible that *neo* antigens could be generated and released from tumor cells following RT, which would not be identified by commercially available tetramers. Since the establishment of antigen specificity for the entire T-cell population is not possible, this study will ascertain whether the antigen burst and/or inflammatory factors released following RT would selectively expand or reduce T cell subpopulation. While RT-induced changes are initiated locally at tumor sites, RT can impact the systemic immune system as observed in the abscopal effect. We hypothesize that tumor antigens and/or inflammatory factors released from RT-induced tumor cell necrosis will trigger an efflux of effector or memory T cells in the peripheral blood since these subpopulation of T cells are antigen-primed and may have a rapid response to altered antigen stimulation in context with inflammatory cytokines. It has been reported that RT increases the serum levels of IL-1beta and IL-6 and reduces the serum levels of TGF-beta in prostate cancer patients after 1-2 weeks of RT. Among the cytokines, IL-6 is responsible for expansion of effector memory $CD8^+$ T cells and IL-1beta and TGF-beta regulate the transmigration or tissue retention of effector and memory $CD8^+$ T cells. In this portion of the project, fluctuations in frequencies among naïve ($CCR7^+CD45RA^+$), central memory ($CCR7^+CD45RA^-$), effector/effector memory ($CCR7^-CD45RA^-$), and terminally differentiated ($CCR7^-CD45RA^+$) $CD8^+$ T cells will be determined and evaluated together with the development of anti-gastric cancer immunity in relation to the timing of RT. PBMCs and plasma will be isolated at (baseline), after RT (day 1, 8, 15, pre-surgery), after adjuvant therapy, and at tumor recurrence. Cells will be co-stained with antibodies for CD3, CD8, CD45RA, CCR7 to identify $CD8^+$ T cells and their phenotype. The percentage of each subset will be determined based on the total $CD8^+$ T cells when analyzed by flow cytometry. We will compare the frequency of each subset of $CD8^+$T cells and their PD-1 expression in relation to the different time points following RT. If we identify any significant changes of T cell subset, we will measure the cytokine profiles of these patients at that time point using multiplex methods.

Tumor-reactive $CD11a^{high}PD\text{-}1^{high}$ $CD8^+$ T effector population following RT. T cell receptor (TCR) tetramers can detect tumor-specific immunity but have limitations. Both the down regulation of the TCR induced by persistent tumor antigen encounter and the weak affinity of $CD8^+$ T cells for tumor antigens contribute to the underestimation of anti-tumor responses measured by tetramers. In addition, TCR tetramers are not capable of detecting $CD8^+$ T cell responses to subdominant epitopes. Therefore, a novel method of detecting tumor-related T cell response is necessary in situations where the identity of tumor antigens are unknown or suboptimal to be detected by tetramers. CD11a expression is required in the rejection of tumors[103]**.** A collaborator laboratory recently established that the $CD11a^{high}$ $PD\text{-}1^+$ $CD8^+$ T cell population represent tumor-reactive $CD8^+$ T cells[104]. In addition, work from this lab determined that $CD11a^{high}$CD8+ T cells inside tumors are tumor-specific functional effector cells and that their function is enhanced by stereotactic ablative radiotherapy in PD-1 knockout mice[105]. In the current study, we propose to investigate whether the $CD11a^{high}$ $PD\text{-}1^+$ $CD8^+$ T cell population in GEJ cancer patients following the

initiation of chemoRT plus pembrolizumab can identify tumor-related T cell response. PBMCs and plasma will be isolated at (baseline), after RT (day 1, 8, 15, pre-surgery), after adjuvant therapy, and at tumor recurrence and will be used in the following assays:

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

<u>Circulating MDSCs and immune profile</u>

Recent evidence indicates that circulating myeloid derived suppressor cells (MDSCs) may be increased in gastrointestinal tumors and other cancers, and these cells may correlate with increased response of melanoma patients to ipilumimab. The tumor microenvironment is populated by various types of inhibitory immune cells including Tregs, alternatively activated macrophages, and myeloid-derived suppression cells (MDSCs), which suppress T cell activation and promote tumor outgrowth[106]. Recent studies indicate that MDSCs also play an essential role in chemoresistance and radioresistance[107]. Therapeutic blockade of immune checkpoints has been associated with a reversal in the distribution and proportion of MDSCs[108,109]. In addition, a reduction in circulating MDSCs was associated with regression of metastatic tumors in a melanoma patient treated with ipilimumab and radiotherapy[29]. On day 10 after ionizing radiation, Deng et al observed that MDSCs, defined by CD45+CD11b+Gr1+ expression, were reduced by IR and/or anti–PD-L1[40]. In tumors that received anti–PD-L1 or IR treatment alone, the percentage of MDSCs in the total CD45+ cell population decreased from 19% in untreated tumors to 7% (P =.016) and 5% (P =.0074), respectively. Combination therapy with anti–PD-L1 and IR exhibited the greatest effect on MDSCs and further reduced the percentage to 0.4% of total CD45+ cells (P <.0001). The extent of local reduction in MDSCs was associated with enhanced tumor growth delay and tumor regression, and were dependent on CD8+ T cells. These results raise the possibility that a local reduction in MDSCs is an essential component in the therapeutic efficacy of combination treatment with local IR and anti–PD-L1. Other data from this study suggest that CD8+ cells are directly involved in controlling MDSC cells by inducing apoptosis of MDSCs, and that RT and anti–PD-L1 combination therapy restores the function of CD8+ T cells, which, in turn, results in the direct elimination of MDSCs.

A collaborating laboratory ([redacted]) recently developed a method that allows the analysis of human leukocyte populations, including MDSCs, by the use of eight 10-color flow cytometric protocols in combination with novel software analysis[110]. This method is distinct from, and complementary to, the analysis to be completed by the Dong/Kwon Lab described above, by utilizing un-manipulated biological sample preparation that allows for comprehensive direct quantitation of leukocytes and non-overlapping immunophenotypes. This group specifically designed myeloid protocols that enable the identification of distinct phenotypes that include mature monocytes, granulocytes, circulating dendritic cells, immature myeloid cells, and MDSCs. This method also identified CD123 as an additional distinguishing marker for the phenotypic characterization of immature LIN-CD33+HLADR-MDSCs. This approach permits the comprehensive analysis of all peripheral blood leukocytes and yields data that is highly amenable for standardization across inter-laboratory comparisons for human studies.

1.9d Proposed Trial: Study Overview

This is a single-arm Phase 1b/2 study. Patients with resectable, locally advanced, GEJ/gastric cardia adenocarcinoma will be treated with pembrolizumab plus weekly carboplatin and paclitaxel, and concurrent daily radiotherapy for approximately 5 weeks. Pembrolizumab will be administered once every 3 weeks for up to 3 preoperative doses. Within 4 to 8 weeks following completion of neoadjuvant chemoradiotherapy, a PET/CT scan will be performed to rule out distant disease. If distant disease is not confirmed, patients will undergo curative-intent surgery to remove the tumor within 4 to 8 weeks following completion of neoadjuvant chemoradiotherapy. Following surgery, after all acute toxicities in the postoperative period have resolved to grade 1 or less, patients will receive adjuvant pembrolizumab once every 3 weeks for up to 6 doses.

In the phase 1b portion, 15 evaluable patients will be enrolled. Toxicity and safety will be actively monitored on an ongoing basis. Enrollment will be halted after the 15th patient to assess for safety. If safety parameters fail to be met (see Section 16 for details of safety criteria), data will be analyzed to determine the specific nature and etiologies of safety concerns.

We anticipate that safety parameters will be met, in which case the study will move onto the Phase 2 portion. In the phase 2 portion, 30 evaluable patients will be enrolled and will receive the same neoadjuvant regimen and surgery as in the Phase 1b portion. These 30 patients will include patients who already received this treatment in the Phase 1b portion. It is possible that the neoadjuvant regimen is a modified regimen.

After pembrolizumab is permanently discontinued, patients will be evaluated with history, physical exam, and imaging every 3 months for the 1st year after surgery, then every 4 months for the 2nd year. Diagnostic investigations will be performed only when recurrence is suspected. Patients will be followed for disease recurrence and overall survival up to 2 years after surgery.

If the primary endpoint is met (*ie*, pathCR see Section 16), future plans include expanding to a larger single-arm or randomized Phase 2, or to a Phase 3 trial, that may include a biomarker-selected population.

Potential "pseudoprogression" at the time of repeat PET/CT

In the phase 1b or 2 portion of the trial, repeat PET/CT following the completion of neoadjuvant pembrolizumab/chemoRT may detect new lesions at distant anatomic sites, or demonstrate that the primary has enlarged or that regional lymph nodes have increased in size or number. While these instances are expected to be uncommon, it will be challenging to confirm whether these changes are due to worsening disease *vs* pseudoprogression. This is because 10% or more of patients receiving immunotherapy are characterized as having PD using traditional (WHO) criteria, but then have subsequent evidence of tumor response[111]. In addition, 4-7% of patients (7 of 180 in CROSS[2]; 16 of 128 in Burmeister et al[112]) assigned to standard chemoRT without immunotherapy experience PD prior to surgery, such that surgery is not possible. Symptomatology is often an unreliable indicator of PD due to the confounding effects of chemoRT-induced esophagitis which can worsen swallowing and other symptoms.

Given the complexity and infrequency of these situations, these cases will be addressed on an individual basis, and management will depend on the specific clinical scenario with final decision-making resting in the clinical judgment of the investigative team. The team will undertake multidisciplinary discussions and apply the principles of irRECIST adapted to trimodality therapy in GEJ cancers. According to irRECIST[113-115] or irRC (immune related response criteria)[111], new lesions or lesions that show interval enlargement must be confirmed on repeat imaging that is completed 4 or more weeks later, in order to determine the changes as PD. If new lesions or other radiographic changes are observed during or immediately after chemoRT, or at the time of repeat PET/CT, in the absence of signs or symptoms of definitive unresectable disease, options include: delaying surgery while continuing systemic therapy followed by repeat imaging; proceeding directly to surgery which may be preceded by intraoperative confirmation of resectability; or obtaining tissue confirmation of a new lesion. Given the unreliability of radiographic changes of the primary tumor during or after chemoRT, due to the inflammatory effects of chemoRT on tissue, assessment of PD will not routinely include changes in the primary tumor or regional nodes, as long as the disease remains resectable.

If surgery is delayed, the investigators will have the option of continuing pembrolizumab with or without chemotherapy (without RT), followed by repeat imaging 4 or more weeks later. The recommended chemotherapy backbone will be continuation of carboplatin plus paclitaxel (see Section 1.9b for rationale; see Section 7 for details of therapy) . After completing at least two cycles, repeat imaging will be performed.

2.0 Goals

2.1 Primary objectives

2.11 Phase 1b

- To determine the safety and tolerability of pembrolizumab when combined with radiotherapy plus carboplatin and paclitaxel in locally advanced GEJ/cardia adenocarcinoma.

2.13 Phase 2

2.131 To evaluate the pathCR rate of pembrolizumab when combined with radiotherapy plus carboplatin and paclitaxel in locally advanced GEJ/cardia adenocarcinoma.

2.2 Secondary Objectives

2.21 To determine progression-free survival (PFS), determine time to relapse (TTR), disease-free survival (DFS), R0 rate, and overall survival (OS) of pembrolizumab when combined with radiotherapy plus carboplatin and paclitaxel.

2.3 Translational Research Objectives

2.31 To identify tissue and/or circulating biomarkers that are associated with pathCR, DFS, and other clinical outcomes in patients with locally advanced GEJ/cardia adenocarcinoma treated with neoadjuvant pembrolizumab-based therapy.

2.32 To determine differences in pre-treatment vs post-treatment tissue expression of immune markers, including PDL1 and tumor infiltrating lymphocytes (CD8+, FOXP3+ Tregs, CD45RO, granzyme B), in patients treated with neoadjuvant pembrolizumab-based therapy.

2.33 To identify immune markers in pretreatment tissues that correlate with pathCR and long-term outcome in patients treated with neoadjuvant pembrolizumab-based therapy.

2.34 To explore whether an EBV-associated tumor molecular profile[89] is associated with pathCR and long-term outcome in patients treated with neoadjuvant pembrolizumab-based therapy.

2.35 To explore whether a microsatellite-unstable (MSI) tumor molecular profile[89] is associated with pathCR and long-term outcome in patients treated with neoadjuvant pembrolizumab-based therapy.

3.0 Patient Eligibility

Phase 1b only: Prior to discussing protocol entry with the patient, call the MCCC Registration Office [redacted] to ensure that a place on the protocol is open to the patient.

3.1 Pre-Registration Eligibility - Inclusion

3.11 ≥18 years of age and ≤80 years of age.

3.12 Histologically or cytologically confirmed adenocarcinoma involving the gastroesophageal junction or gastric cardia.

3.13 Central pathology review to determine evaluability of archived EGD/biopsy sample

NOTE: If archived sample was collected >8 weeks prior to Pre-Reg, is not available in a timely manner, or was collected outside of Mayo Clinic and considered unevaluable, then baseline EGD with primary tumor biopsy at Mayo Clinic must be performed unless clinically contraindicated. Patient is allowed to enroll regardless of whether this Mayo Clinic tissue sample is evaluable. (Only 1 EGD with primary tumor biopsy performed at Mayo Clinic ≤8 weeks prior to Pre-Reg is required.)

NOTE:For both archival or newly obtained tissue, only biopsies are adequate (FNA is not adequate).

3.14 Willing to provide mandatory tissue samples for research purposes (Section 4.0, 6.16, and 17.1).

3.2 Registration Eligibility

3.21 Inclusion Criteria

3.211 Baseline imaging with an FDG-PET scan negative for distant metastatic disease must be obtained ≤28 days prior to registration.

3.212 Surgically resectable (T2N0, T3N0, T_{any} with node positivitity, M0), as determined by endoscopic ultrasound (EUS) and the following minimum diagnostic work-up:

- Whole-body PET/CT (PET/CT of skull base to mid-thigh is acceptable)
- EUS ≤35 days prior to registration

NOTE: Patients may have regional adenopathy including para-esophageal, gastric, gastrohepatic and celiac nodes.

NOTE if patient unable to have PET/CT then CT chest/abdomen/pelvis with contrast (preferred) or MRI chest/abdomen/pelvis with contrast.

3.213 Surgical consultation at enrolling site to confirm that patient will be able to undergo curative resection after completion of chemoradiation ≤56 days prior to registration.

- Tumor is amenable to standard resection and reconstruction.

3.214 Must be considered fit candidate currently by treating provider to complete all components of therapy including neoadjuvant chemoradiation and surgery.

NOTE: This assessment must be made ≤7 days prior to registration and ≤3 days prior to anticipated initiation of treatment.

3.215 Radiation oncology consultation at enrolling site to confirm that disease can be encompassed in a radiotherapy field ≤56 days prior to registration.

NOTE: Radiotherapy quality assurance rapid review must be performed before the first fraction of RT is administered (See Section 7.34). If RT constraints cannot be met, the patient will be removed from the protocol prior to treatment.

3.216 Consultation with a medical oncologist at enrolling site ≤56 days prior to registration, with determination that treatment with neoadjuvant

chemoradiotherapy with weekly carboplatin and paclitaxel is considered acceptable.

3.217 ECOG Performance Status 0 or 1 (see Appendix I).

3.218 Adequate oral intake and nutritional status without current or likely need for enteral or parenteral feeding during chemoradiation or the preoperative period.

3.219a Pre-treatment PFTs, collected ≤90 days prior to enrollment, must show FEV1 >60% of predicted.

3.219b Adequate organ function ≤21 days prior to registration:

- Aspartate transaminase (AST) level ≤2.5 x upper limit of normal (ULN) and alanine transaminase (ALT) ≤3 × upper limit of normal (ULN)
- Total bilirubin level of ≤1.5 x ULN
- Creatinine level ≤1.2 x ULN or creatinine clearance >60 mL/min/1.73 m^2 for patients with creatinine levels above or below the institutional normal (as determined by Cockroft-Gault equation*)
- Hemoglobin (Hgb) ≥9 g/dl without transfusion or epoetin dependency (≤7 days prior to assessment)
- Absolute neutrophil count ≥1.5 x 10^9/L
- Platelets ≥100 x 10^9/L
- Albumin ≥2.5 g/dl

*Cockcroft-Gault Equations:

$$\text{Creatinine clearance for males} = \frac{(140 - \text{age})(\text{actual body weight in kg})}{(72)(\text{serum creatinine in mg/dL})}$$

$$\text{Creatinine clearance for females} = \frac{(140 - \text{age})(\text{actual body weight in kg})(0.85)}{(72)(\text{serum creatinine in mg/dL})}$$

3.219c Patients of childbearing potential must 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.

NOTE: Patients of childbearing potential are those who have not been surgically sterilized or have not been free from menses for >1 year.

3.219d Patients of childbearing potential must have a negative urine or serum pregnancy test ≤7 days prior to registration.

NOTE: If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

3.219e Patients able to father a child must 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.

NOTE; Abstinence is acceptable if this is the established and preferred method of contraception for the subject.

3.219f Provide signed written informed consent.

3.219g Willing to return to enrolling institution for follow-up.

3.219h Willing to provide mandatory tissue and blood samples for research purposes.
NOTE: Patients must be willing to provide at the time surgical resection For patients who do not undergo surgery, any *on-study* tumor biopsy obtained for clinical purposes subsequent to the *baseline* biopsy must also be available for analysis.

3.22 Exclusion Criteria

3.221 Tumor characteristics — Any of the following are excluded:
- Evidence of distant metastases
- Tumors whose location is restricted to the tubular esophagus (ie, without involvement of the GEJ or cardia)
- Tumors whose proximal end are at the level of the carina or higher
- Invasion of the tracheobronchial tree or presence of tracheoesophageal fistula
- Palpable supraclavicular nodes, biopsy-proven involvement of supraclavicular nodes, or radiographically involved supraclavicular nodes (>1.5 cm in greatest dimension)
- T1N0M0, T4Nany, or in situ carcinoma
- Tumor must not extend 5 or more cm into the stomach

3.222 Received prior treatment or receiving current treatment for this malignancy.

3.223 Prior radiation to chest or abdomen, or to >30% of the marrow cavity.

3.224 Inadequate caloric or fluid intake whereby there is a current or likely future need for enteral or parenteral feeding during chemoradiation or the preoperative period.

3.225 Major surgery ≤4 weeks prior to registration.

3.226 Active autoimmune disorders, including patients known to be HIV positive, or those requiring chronic steroid administration (excluding inhaled steroids).

3.227 Uncontrolled diabetes (i.e., will interfere with the performance of the FDG PET/CT scans).

3.228 Prior allergic reactions to drugs containing Cremophor, such as teniposide or cyclosporine, or study drugs involved in this protocol, or to a monoclonal antibody or prior hypersensitivity to platinum-containing agents.

3.229a Heart conditions—Any of the following:
- Any atrial fibrillation ≤3 months prior to registration
- Unstable angina ≤12 months prior to registration
- Prior symptomatic congestive heart failure
- Documented myocardial infarction ≤6 months prior to registration (pretreatment ECG evidence of infarct only will not exclude patients)
- Prior significant ventricular arrhythmia requiring medication
- Prior 2nd or 3rd degree heart block or other types of clinically significant conduction delay ≤6 months prior to registration

- Clinically significant pericardial disease (including pericardial effusion, pericarditis) or cardiac valvular disease ≤12 months prior to registration

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

3.229b Prior pancreatitis that was symptomatic or required medical intervention ≤6 months prior to registration (known toxicity of pembrolizumab).

3.229c Prior enteritis that was symptomatic or required medical intervention ≤6 months prior to registration (known toxicity of pembrolizumab).

3.229d Uncontrolled hyper/hypothyroidism or hyper/hypocortism ≤6 months prior to registration (known toxicity of pembrolizumab).

3.229e Pulmonary conditions — any of the following:

- Respiratory condition that required any oxygen supplementation ≤6 months prior to registration
- Prior or current pneumonitis
- Clinically significant pulmonary hypertension ≤12 months prior to registration
- Lung infection requiring treatment ≤3 months prior to registration
- Pulmonary embolism requiring treatment ≤6 months prior to registration
- Pleural effusion requiring drainage ≤12 months prior to registration

3.229f Prior fistula within thorax, including bronchoalveolar or esophageal.

3.229g Pre-existing motor or sensory neurotoxicity greater than CTCAE Grade 1.

3.229h Acute bacterial, viral, or fungal infection requiring treatment at the time of registration.

3.229i Active infection or other serious underlying medical condition which would impair the ability of the patient to receive the planned treatment.

3.229j Uncontrolled intercurrent illness including, but not limited to, psychiatric illness/social situations, or other co-morbid systemic illnesses or severe concurrent diseases which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens.

3.229k Prior malignancy ≤5 years prior to registration if it is believed that ongoing treatment or assessments for prior malignancy could meaningfully interfere with protocol treatment or assessment.
NOTE: Ongoing hormonal therapy is allowed. .

3.229l Dementia or altered mental status that would prohibit the understanding and giving of informed consent.

3.229m Any of the following because this study involves an agent where the genotoxic, mutagenic and teratogenic effects are unknown:

- Pregnant or breastfeeding
- Patient of childbearing potential who is unwilling to employ adequate contraception

3.229n Received live vaccine ≤30 days prior to registration.

4.0 Test Schedules

4.1 Test Schedule[1] - Carboplatin/Paclitaxel/Pembrolizumab/Radiation Therapy

	Active Treatment													Post-Treatment Observation	
	Prior to Registration			Neoadjuvant during RT [2]						Surgical evaluation			End of Treatment		
Tests and procedures	≤56 days prior to pre-registration	≤28 days prior to registration	≤21 days prior to registration	D1	D15	D22	D29	D36	D43	≤2 weeks prior to surgery[3,4]	≤42 days after surgery	During adjuvant pembrolizumab[5]	At the time of discontinuation	Safety follow up: 30 days post-discontinuation	Follow up visits[6]
Clinical Procedures															
History and exam, weight, ECOG PS			X[7]	X[8]			X			X	X	X	X	X	X
Height			X												
Adverse event assessment			X	X	X	X	X	X	X	X	X	X	X	X	X
Consultation with Radiation Oncologist (See Sect. 6.39a)		X[9]													
Surgical consultations (See Sect. 6.39b)		X[7]								X					

[1] All tests and procedures are clinically indicated, unless noted with an R to indicate funding by research. If there is a clinical need for more frequent testing, it may be performed as needed.

[2] Each test or procedure should be obtained within 3 business days of the designated date.

[3] **Timing of surgery:** Surgery to remove the primary cancer should occur no earlier than 24 days after last dose of neoadjuvant pembro. In addition, although clinical judgment is necessary to determine the precise timing of surgery, it is generally encouraged for surgery to occur 5 to 8 weeks after the last dose of RT.

[4] If PET/CT or other post-neoadjuvant assessment shows suspected PD, see Section 11.2 for guidance.

[5] Day 1 of each treatment cycle, unless noted. Cycle length during adjuvant treatment = 21 days.

[6] Starting from registration, every 3 months for 1 year, then every 4 months for 2nd year and every 6 months for 3rd year.

[7] Pre-treatment clinical assessment ≤7 days prior to registration to ensure patient is fit to complete all components of therapy including neoadjuvant chemoradiation and surgery.

[8] Second pre-treatment clinical assessment ≤3 days prior to treatment to ensure patient is fit to complete all components of therapy including neoadjuvant chemoradiation and surgery.

[9] ≤56 days prior to registration

	Active Treatment													Post-Treatment Observation	
	Prior to Registration			Neoadjuvant during RT [2]						Surgical evaluation			End of Treatment	Safety follow up:	
Tests and procedures	≤56 days prior to pre-registration	≤28 days prior to registration	≤21 days prior to registration	D1	D15	D22	D29	D36	D43	≤2 weeks prior to surgery[3,4]	≤42 days after surgery	During adjuvant pembrolizumab[5]	At the time of discontinuation	30 days post-discontinuation	Follow up visits[6]
Consultation with Medical Oncologist (See Section 6.39c)		X[7]													
Hematology: CBC/diff			X		X	X	X	X	X	X	X	X	X	X	X
Chemistry Panel [10]			X		X			X		X	X	X	X	X	X
T3, Free T4, TSH			X							X		X[11]		X	
Urinalysis [12]			X												
Pregnancy test[13]			X												
Bronchoscopy [14]			X												
PFTs [15]			X							X					
FDG PET/CT [16] (trunk="eyes to thighs")		X								X					
CT (chest, abdomen, pelvis)											X	X[17]	X[18]		X[14]
EGD with biopsy	X	X[19]													

[10] AST, ALT, alk phos, total bilirubin (direct if TBili is elevated), BUN, creatinine, calcium, albumin, phos, glucose, Na, K, bicarbonate, uric acid, chloride. If here is a clinical need for more frequent testing, it may be performed as needed.
[11] Thyroid tests are collected every other treatment cycle of adjuvant pembrolizumab, starting with the first adjuvant dose of pembrolizumab
[12] Urinalysis for protein, glucose, blood
[13] For persons of childbearing potential only. Must be done ≤7 days prior to registration
[14] Bronchoscopy required only if primary tumor is adjacent to the trachea or left main stem bronchus and/or patient is experiencing respiratory symptoms (includes transbronchial biopsy)
[15] Including routine spirometry and DLCO
[16] FDG PET/CT imaging is to be used up until the time the patient has had surgery. After surgery, CT imaging must be used.
[17] Starting from the time of surgery, CT scans will be obtained post-surgery as a new baseline, then every 3 months for one year, then every 4 months for 2 years.
[18] During adjuvant pembrolizumab and up to six months after the last dose of adjuvant pembrolizumab: Repeat CT imaging per Section 11.
[19] Only repeat EGD if inadequate tissue is available from pre-registration EGD.

	Active Treatment													Post-Treatment Observation	
	Prior to Registration			Neoadjuvant during RT [2]						Surgical evaluation			End of Treatment	Safety follow up:	
Tests and procedures	≤56 days prior to pre-registration	≤28 days prior to registration	≤21 days prior to registration	D1	D15	D22	D29	D36	D43	≤2 weeks prior to surgery[3,4]	≤42 days after surgery	During adjuvant pembrolizumab[5]	At the time of discontinuation	30 days post-discontinuation	Follow up visits[6]
Endoscopic esophageal ultrasound		X[20]													
ECG/EKG and Troponin T[21]			X		X	X	X	X	X	X	X	X[22]			
Molecular testing[23]		X													
Treatment															
Pembrolizumab[24]				X		X									
Carboplatin + paclitaxel[25]					X	X	X	X	X						
Radiation (daily)[25]					X	X	X	X	X						
Blood or Tissue- Analysis performed by Research Labs															
Mandatory tissue sample (see Section 17.0) [26R]	X										X				

[20] Ultrasound may be performed ≤35 days prior to registration

[21] If troponin or EKG is abnormal, immediately refer patient to Cardio-Oncology, and patient will be seen on the same day or next day. (Page Dr Joerg Herrmann if access is an issue.)

[22] Prior to every adjuvant dose of pembrolizumab.

[23] Study coordinator will notify PI that pretreatment tumor sample has been received, and Investigator may discuss the utility of molecular testing including mismatch repair deficiency by immunohistochemistry and microsatellite instability with patient. Details of specimen requirements are shown in Section 17.0.

[24] First pembrolizumab dose should be given at least 14 days before chemotherapy starts. Exceptions can be considered after discussion with PI and/or study team.

[25] It is strongly encouraged that carboplatin/paclitaxel and radiation be started on the same day, and that initial radiation be administered over five consecutive days (eg, Mo-Fr or Tu-Sa). PI and study team should be contacted if it is being considered to initiate chemotherapy and radiation on different days or to administer initial radiation over five non-consecutive days.

[26] Submission of adequate tissue from the primary tumor (ie, mucosal "pinch" biopsy) is mandatory. A Mayo pathologist must determine that sufficient tumor is evaluable for research purposes. Archived tissue collected ≤8 weeks prior to pre- registration is acceptable. If existing tissue is deemed to be inadequate, repeat EGD with mucosal biopsy of the primary tumor must be performed (see Section 3.13.for details).

	Active Treatment												Post-Treatment Observation	
	Prior to Registration			Neoadjuvant during RT [2]						Surgical evaluation				
Tests and procedures	≤56 days prior to pre-registration	≤28 days prior to registration	≤21 days prior to registration	D1	D15	D22	D29	D36	D43	≤2 weeks prior to surgery[3,4]	≤42 days after surgery	During adjuvant pembrolizumab[5]	End of Treatment At the time of discontinuation	Safety follow up: 30 days post-discontinuation	Follow up visits[6]
Optional follow up tissue sample[27R]													X	X	X
Blood for correlative research (to be sent to [redacted] Lab) [28R]			X		X	X	X			X			X[29]		X[29]
Blood for correlative research (to be sent to [redacted] Lab) [30R]			X												
Blood for DNA sample			X												

[27] An optional newly-obtained core, mucosal ("pinch"), or excisional biopsy (FNA not adequate) is requested at the time of recurrence or progressive disease. Endoscopic biopsies or biopsies of metastatic sites are permitted.

[28] Whole blood samples for correlative research to be sent to [redacted] lab will be collected at the following times points: Baseline, Day 1 (±3 days) of chemoradiotherapy, Day 8 (±3 days) of chemoradiotherapy, Day 15 (±3 days) of chemoradiotherapy, surgical evaluation prior to surgery, at the end of adjuvant therapy (ie, within 4 weeks after final administered dose of adjuvant therapy), and at the time of tumor recurrence. The baseline sample may be collected ≤56 days prior to Day -7 of therapy. The Day 1 sample should be collected prior to administration of chemotherapy and RT.

[29] Sample should be collected if there is disease progression (recurrence), whether it occurs during adjuvant pembrolizumab or during post-treatment observation. To clarify, if a patient completes adjuvant therapy and has disease progression (recurrence) during observation, this patient will undergo two blood collections following definitive therapy (chemoradiation with/without surgery): once at the end of adjuvant therapy and once at the time of disease progression (recurrence) during observation.

[30] Whole blood sample for correlative research to be sent to [redacted] Lab (IMPACT Lab) will be collected only at baseline. Collections will only be performed at Mayo Rochester. Sample must be hand-delivered to [redacted] Lab. The baseline sample may be collected ≤56 days prior to Day 1 of therapy.

R Funded by research

4.2 Test Schedule for patients who, following the completion of neoadjuvant therapy, are not surgical candidates and are still eligible to receive adjuvant pembrolizumab[1]

		Active Treatment			Observation	
Tests and procedures[1]		≤28 days after RT	During adjuvant pembrolizumab[31]	At the time of discontinuation	Safety follow up: 30 days post-discontinuation	Follow up visits[32]
Clinical Procedures						
History and exam, weight, ECOG PS		X	X	X	X	X
Adverse event assessment		X	X	X	X	X
Hematology: CBC/diff		X	X	X	X	X
Chemistry Panel [33]		X	X	X	X	X
T3, Free T4, TSH		X	X[34]		X	
CT (chest, abdomen, pelvis)			X[35]	X[36]		X
ECG/EKG and Troponin T[37]		X	X[38]			
Blood or Tissue- Analysis performed by Research Labs						
Optional follow up tissue sample[39R]				X	X	X
Blood for correlative research (to be sent to Dong/Kwon Lab) [40R]				X	X	X

[31] Day 1 of each treatment cycle, unless noted. Cycle length during adjuvant treatment = 21 days.
[32] Starting from registration, every 3 months for 1 year, then every 4 months for 2nd year and every 6 months for 3rd year.
[33] AST, ALT, alk phos, total bilirubin (direct if TBili is elevated), BUN, creatinine, calcium, albumin, phos, glucose, Na, K, bicarbonate, uric acid, chloride
[34] Thyroid tests are collected every other treatment cycle of adjuvant pembrolizumab, starting with the first adjuvant dose of pembrolizumab.
[35] Starting from the time of registration, CT scans will be obtained every 3 months for one year, then every 4 months for 2 years and every 6 months for the third year for a maximum of 3 years after registration.
[36] During adjuvant pembrolizumab and up to six months after the last dose of adjuvant pembrolizumab: Repeat CT imaging per Section 11.
[37] If troponin or EKG is abnormal, immediately refer patient to Cardio-Oncology, and patient will be seen on the same day or next day. (Page Dr Joerg Herrmann if access is an issue.)
[38] Prior to every adjuvant dose of pembrolizumab.
[39] An optional newly-obtained core, mucosal ("pinch"), or excisional biopsy (FNA not adequate) is requested at the time of recurrence or progressive disease. Endoscopic biopsies or biopsies of metastatic sites are permitted.
[40] Whole blood samples for correlative research to be sent to [redacted] lab will be collected at the following times points: Baseline, Day 15 (±3 days) of chemoradiotherapy, surgical evaluation prior to surgery, at the end of adjuvant therapy (ie, within 4 weeks after final administered dose of adjuvant therapy), and at the time of tumor recurrence. The baseline sample may be collected ≤56 days prior to Day 1 of therapy.

5.0 Grouping Factor

Phase: 1b vs. 2

6.0 Registration/Randomization Procedures

6.1 Pre-Registration (Step 1)

6.11 Pre-registration

To pre-register a patient, fax [redacted] a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

6.12 IRB approval(s) is required for each treating site.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals is no longer necessary.

6.13 Prior to accepting the pre-registration, the registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient pre-registration eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.14 The site has reviewed and understands the process listed in Section*s* 14 amd 17 and must account for sufficient time to complete pre-registration and registration steps.

6.15 Pre-registration tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.16 Correlative Research
A mandatory translational research component is part of this study; the patient will be automatically registered onto this component (Sections 3.14, 4.0, and 17.1*)*.

6.2 Phase 2 (Step 2)

6.21 Registration Procedures

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on

the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted] If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.3 Phase 1b and 2

6.31 Correlative Research

6.311 A mandatory translational research component is part of this study; the patient will be automatically registered onto this component (Sections 3.14, 14.0, 17.0*)*.

6.312 An optional correlative research component is part of this study, there will be an option to select if the patient is to be registered onto this component (see Section 17.1).

- Patient has/has not given permission to give his/her recurrence tissue sample for research testing

6.32 Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.33 Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office [redacted]). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.34 At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research on cancer at Mayo Clinic.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.

- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.35 Treatment on this protocol must commence at Mayo Clinic under the supervision of a Mayo Clinic physician.

6.36 Treatment cannot begin prior to registration and must begin ≤21 days after registration.
Note: It is highly recommended for treatment to be initiated as soon as possible after registration.

6.37 Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified in the test schedule.

6.38 All required baseline symptoms (see Section 10.5) must be documented and graded.

6.39a A radiation oncologist has seen the patient and confirms the patient is a suitable candidate for this study ≤56 days prior to registration.

6.39b A surgeon has seen the patient and confirms the patient is a suitable candidate for this trial ≤56 days prior to registration.

6.39c A medical oncologist has seen this patient and confirms the patient is a suitable candidate for this trial ≤56 days prior to registration.

6.39d Study drug is available on site.

6.39e Blood draw requisition form availability checked (Arizona only).

7.0 Protocol Treatment

7.1 Trial treatments

7.11 Phase 1b and Phase 2

Agents	Route	Starting Dose	Frequency
Neoadjuvant (Provide one cycle prior to surgery)			**(per cycle)**
Pembrolizumab	IV	200 mg	Days 1[a], 22
Carboplatin [b]	IV	AUC 2	Days 15, 22, 29, 36, 43
Paclitaxel [b]	IV	50 mg/m^2	Days 15, 22, 29, 36, 43
Radiotherapy [b]	na	1.8 Gy	Day 15 then once daily for 23 fractions (total 41.4 Gy)
Adjuvant			
Pembrolizumab	IV	200 mg	Every 3 weeks for maximum of 6 cycles

[a] First pembrolizumab dose should be given at least 14 days before chemotherapy starts. Exceptions can be considered after discussion with PI and/or study team.

[b] It is strongly encouraged that carboplatin/paclitaxel and radiation be started on the same day, and that initial radiation be administered over five consecutive days (eg, Mo-Fr or Tu-Sa). PI and study team should be contacted if it is being considered to initiate chemotherapy and radiation on different days or to administer initial radiation over five non-consecutive days.

Neoadjuant Cycle length = 50 days for one cycle
Adjuvant cycle length = 21 days for up to 6 cycles

7.12 Phase 2

Phase 2 will use an updated regimen which involves starting pembrolizumab 14 days prior to other treatments. See Section 7.11.

7.13 Phase 1b or Phase 2: Recommended regimen if repeat imaging after completion of carboplatin/paclitaxel/pembrolizumab/radiation shows concern for progressive disease or toxicity leading to a delay of surgery

(see Section 1.9d)

Agents	Route	Starting Dose*	Frequency	Cycle length
Pembrolizumab	IV	200 mg	Day 1	21 days
Paclitaxel	IV	200 mg/m^2	Day 1	21 days
Carboplatin	IV	AUC 6	Day 1	21 days

* Starting doses of any drug can be modified based on patient toxicity during chemoradiation.

NOTE: A minimum of two cycles, and maximum of 4 cycles, of this regimen (which does not include radiation) will be administered prior to re-imaging. Three cycles is generally encouraged. Further cycles may be considered, but

only after discussion with PI and if PI gives approval. This regimen, or components of the regimen, may also be used if surgery is delayed for other reasons (e.g., adverse events) and if administration of the regimen is believed to provide clinical benefit. Modifications of the dose and schedule are allowed following standard practice according to the clinical judgment of the investigator.

7.14 Phase 2: Alternative regimen if repeat imaging after completion of carboplatin/paclitaxel/pembrolizumab chemotherapy shows concern for progression leading to a delay of surgery, or if carboplatin, paclitaxel, pembrolizumab, and/or RT must be discontinued due to toxicity

(see Section 1.9d)

Agents	Route	Starting Dose [a]	Frequency (one cycle) [b]
Neoadjuvant			
Oxaliplatin	IV	85 mg/m^2	Day 1
Leucovorin	IV	400 mg/m^2	Day 1
5-Fluorouracil	IV (bolus)	400 mg/m^2	Day 1
5-Fluorouracil	IV (over 46-48 hr approx.)	2400 mg/m^2	Days 1

[a] Starting doses of any drug can be modified, or omitted, based on patient toxicity during prior chemotherapy. Clinical judgment of investigator should be applied: for example, if toxicity is due to pembrolizumab or RT alone, carboplatin/paclitaxel can be continued; if toxicity is due to paclitaxel alone, then FOLFOX can be used while continuing RT and pembrolizumab, if clinically indicated.

[b] **Neoadjuvant cycle length = 14 days**

NOTE: If pseudoprogression is being considered, a minimum of 1 cycle will be administered prior to re-imaging.
Four to six cycles are generally encouraged.

7.2 Timing of Dose Administration

All trial treatments will be administered in the order presented below on an outpatient basis.

Treatment with pembrolizumab may be administered up to 3 days before or after the scheduled dose on Day 1 of each cycle due to administrative reasons. See Section 7.11 for details.

Carboplatin or paclitaxel may be administered up to 3 days before or after the scheduled dosing date for administrative reasons per the Investigator's judgment. See Section 7.11 for details.

Note: Dosing of pembrolizumab may be withheld, and dosing of combination chemotherapy regimen may be reduced in the case of medical / surgical events or logistical reasons (i.e. elective surgery, unrelated medical events, subject vacation, holidays) not related to study therapy. Patients should be placed back on study therapy within 3 weeks of the scheduled interruption to remain aligned with the Q3W dosing

interval. The reason for withholding dosing of either pembrolizumab or combination chemotherapy regimen should be documented in the subject's study record.

7.21 Pembrolizumab

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

See Section 15.1 for specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

7.22 Paclitaxel

Administration of paclitaxel should begin following administration of pembrolizumab. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for paclitaxel.

7.23 Carboplatin

Administration of carboplatin should begin following administration of pembrolizumab. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for carboplatin.

Dosed using Calvert Formula with Cockroft & Gault Equation (CBDCA dose [mg] = target AUC x [GFR + 25]). For the purposes of this protocol, the GFR is considered to be equivalent to the creatinine clearance (CrCl). The CrCl is calculated by the method of Cockroft & Gault (CrCl[mL/min] = (140 - age) x actual body weight (kg) divided by plasma Cr [mg/dL] x 72 x [0.85 if female or 1 if male].

GFR = Glomerular filtration rate

7.24 Alternative regimen with FOLFOX

FOLFOX chemotherapy may be administered up to 3 days before or after the scheduled dosing date for administrative reasons per the Investigator’s judgment.

7.241 Oxaliplatin

Administration of oxaliplatin should be initiated with leucovorin. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for oxaliplatin.

7.242 Leucovorin (folinic acid)

Administration of leucovorin should be initiated with oxaliplatin. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for leucovorin.

7.243 Fluourauracil (5FU) bolus

Administration of 5FU bolus should be initiated after oxaliplatin/leucovorin. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for 5FU.

7.244 Fluourauracil (5FU) IV pump

Administration of 5FU IV pump should be initiated after

pembrolizumab. Please refer to the product label for additional guidance on administration procedures (e.g. timing of infusion, premedication) for 5FU.

7.3 Radiation

7.31 Dose specifications and technical factors

Radiation begins on Day 1 of neoadjuvant chemotherapy and continues for approximately 4 ½ weeks. The trial administers 1.8 Gy per day without a break except for weekends and holidays. The total radiation dose is 41.4 Gy in 23 fractions. Dose should be prescribed such that ≥95% of the PTV receives the prescription dose.

Dose (Gy)	Number of Fractions	Fraction Size	Rx Length	Rx Days
41.4	23	1.8 Gy	4 weeks and 3 days	M-F

The energy of the treatment beam will be at least 6 MV and no greater than 18 MV.

The use of intensity-modulated radiation therapy (IMRT) or proton beam radiation is allowed. The use of intraoperative radiotherapy (IORT) is NOT allowed.

Radiation dose prescriptions will be calculated using heterogeneity corrections to take into account tissue inhomogeneities in treatment planning.

7.32 Immobilization, simulation, and localization

Patients should be instructed to be fasting for 2 hours prior to simulation and treatment, with the exception of medications and small sips of clear liquids.

Patients should be positioned head first, supine, with arms up in an immobilization device.

CT simulation should be performed in the treatment position. The planning CT should encompass the chest and abdomen (from the mid-cervical spine to the iliac crests). This should include all of the lungs and all of both kidneys for calculating dose-volume histograms (DVHs) for these organs.

Use of 4 dimensional CT (4DCT) is strongly recommended to characterize organ motion.

Oral or IV contrast (if no contraindications) should be considered to aid in target/normal tissue delineation.

Breath hold treatment can be considered if the tumor involves the stomach.

The planning CT will be registered with the diagnostic PET/CT for aiding in target delineation.

Daily verification of the treatment setup will be performed with kilovoltage xray images or volumetric imaging (kilovoltage or megavoltage CT)

7.33 Treatment planning, target volumes

GTV = gross tumor volume
CTV = clinical target volume
PTV = planning target volume

GTV4140 is defined as the gross primary tumor in the esophagus and involved regional lymph nodes, based on the planning CT scan, registration of the PET/CT with the planning CT, and the endoscopic report.

CTV4140 is defined as a 3.5-4 cm longitudinal expansion of the primary tumor GTV along the esophagus and stomach to cover submucosal tumor spread. This should be further expanded by 1 cm radially. This should be further expanded, as needed, to cover the at risk periesophageal, gastrohepatic ligament, and celiac axis lymph nodes. This should be modified to exclude or minimize uninvolved organs at risk, including the heart, lungs, liver, kidneys, and bowel.

PTV4140 is defined as a 5-10 mm uniform expansion of CTV4140.

7.34 Radiation Compliance Criteria

Target coverage:

PTV4140: V100 ≥ 95% and max dose (0.03 cc) ≤110%

Organs at risk:

All organs at risk constraints ***must*** be met for treatment on this protocol. If these constraints cannot be met, the patient will be removed from the protocol prior to treatment. Therefore, radiotherapy quality assurance rapid review must be performed by [redacted] or [redacted] before the first fraction of RT is administered. This will include review of the plan isodose curves and dose-volume histogram statistics. Email correspondence between Dr. Hallemeier and CRA confirming Rapid Review was performed and that the patient is eligible should be saved for source documentation.

Spinal cord: Max dose (0.03 cc) ≤45 Gy
Lung: mean ≤15 Gy and V20 ≤20%
Heart: mean ≤30 Gy and V40 ≤50%
Bowel (small bowel and large bowel): Max dose (0.03 cc) ≤45 Gy
Liver: mean ≤25 Gy and V30 ≤40%
Kidneys: V20 ≤40%

Recommended (but not required) constraint: Lung: V5 ≤55%

Treatment compliance:

Total administered prescription dose: 41.4 Gy
Total number of fractions: 23
Elapsed days from first fraction to last fraction: ≤38 days

Treatment plans/administration meeting all of the above criteria will be considered "per protocol." Treatment plans/administration failing to meet one or more of the above criteria will be considered an "unacceptable deviation."

7.35 Treatment monitoring requirements

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

performance status (see Section 4.0). See Section 7.37 for recommendations for supportive care during radiation therapy.

7.36 Treatment interruptions

Treatment interruptions should be minimized through the use of supportive care measures (see Section 7.38). Interruptions for life-threatening adverse events (CTCAE Grade 4) are allowed.

7.37 Supportive care during radiation therapy

Significant nutritional compromise may occur during radiation and chemotherapy for esophageal cancer. Esophagitis, nausea, vomiting, and anorexia can all contribute to this issue. Antiemetics such as prochlorpherazine (Compazine®), ondansetron, or granisetron should be used, as needed, to manage treatment related nausea. The use of concurrent lorazepam (0.5 to 1.0 mg) with prochlorperazine can be beneficial. Esophageal pain can be mitigated with liquid acetaminophen and narcotic analgesics. Aggressive management of nutritional problems during treatment should be considered. Consultation with a professional dietitian is appropriate and encouraged. Consideration of a feeding tube should be considered in patients who are unable to maintain adequate nutrition during treatment with radiation and chemotherapy. Some surgeons prefer placement of the feeding tube in the jejunum (PEJ) rather than in the stomach (PEG) with regard to issues of surgical reconstruction of the stomach after resection of the primary lesion. A nasogastric feeding tube is also acceptable.

7.4 Surgery

7.41 Timing of Surgery

Surgery to remove the primary cancer should occur no earlier than 24 days after the last dose of neoadjuvant pembrolizumab. In addition, although clinical judgment is necessary to determine the precise timing of surgery, it is generally encouraged for surgery to occur 5 to 8 weeks after the last dose of RT. For patients assigned to receive a neoadjuvant regimen that consists of chemotherapy alone (ie, no RT), surgery is encouraged to occur 3 to 6 weeks after completion of chemotherapy.

7.42 Surgeon Experience

Individual surgeons must have performed a minimum of 50 surgeries of the surgery type that is planned: ie, ≥50 minimally invasive esophagectomy [MIE] if MIE is planned; ≥50 Ivor-Lewis if Ivor-Lewis is planned; ≥50 transhiatal if transhiatal is planned; ≥50 other [eg, 3-hole or McKweon] if other is planned. Any issues regarding surgeon involvement in the study may be reviewed by the study team.

8.0 Dosage Modification Based on Adverse Events

→ → Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified ← ←

ALERT: ADR reporting may be required for some adverse events (See Section 10.0)

Standard of care should be followed in considering dose modifications based on adverse events. The modifications in this section are considered guidelines. In some cases, a greater or lower level of modification may be indicated or necessary.

- Omit = Treatment is not given for this cycle (missed days are not made up)
- Hold/Delay = Treatment can be made up as part of this cycle
- Discontinue = Treatment is totally stopped

8.1 Dose modification for Radiation Therapy

Radiation modifications should follow standard of care.

8.2 Dosage modifications for pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per the Table below. See Section 9.0 and Appendix VI for supportive care guidelines, including use of corticosteroids.

Table 8.21 Dose Modification Guidelines for Drug-Related Adverse Events for Pembrolizumab

CTCAE System/Organ/Class (SOC)	Adverse Event	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Gastrointestinal disorders	Diarrhea or Colitis	2-3	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue
Investigations	AST, or ALT, or Blood bilirubin	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose.
		3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Metabolism and nutrition disorders	Glucose intolerance (Type 1 diabetes mellitus [if new onset]) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable.
Endocrine disorders	Endocrine disorders – Other, specify: Hypophysitis	2-4	AE resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Endocrine disorders	Hyperthyroidism	3	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue
Endocrine disorders	Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
General disorders and administration site conditions	Infusion related reaction	2[b]	AE resolves to Grade 0-1	Permanently discontinue if AE develops despite adequate premedication
		3-4	Permanently discontinue	Permanently discontinue

CTCAE System/Organ/Class (SOC)	Adverse Event	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Respiratory, thoracic and mediastinal disorders	Pneumonitis	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		3-4	Permanently discontinue	Permanently discontinue
Renal and urinary disorders	Acute kidney injury or Chronic kidney disease (e g Renal failure or Nephritis)	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		3-4	Permanently discontinue	Permanently discontinue
	All Other Drug-Related Adverse Events[c]	3 or severe	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue

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

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

[b] 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.

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

8.22 Other instructions for pembrolizumab

If pembrolizumab-related toxicity does not resolve to Grade 0-1 within 12 weeks after last administration of study drug, study therapy discontinuation is recommended. With Investigator agreement, patients with a laboratory adverse event still at Grade 2 may continue in the study only if asymptomatic and controlled.

In patients who continue on study therapy after experiencing an Adverse Event warranting potential dose modification, if considered drug-related by the investigator, once the patient has recovered to Grade 0-1 the dosing interval in subsequent cycles will be increased by 1 week (e.g., to 3 weeks in patients who were on an every 2-week schedule). Following each such dose delay due to toxicity, the dosing interval should increase by an additional week. For example, patients who began the study on a 3-week dosing schedule, and have stopped

drug twice for due to a drug-related toxicity that meets the above criteria, should now be dosing every 5 weeks.

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

Reduced dose of pembrolizumab dose (ie, below 200 mg) will not be administered.

8.3 Dose modifications for carboplatin and paclitaxel

8.31 Suggested dose levels for carboplatin and paclitaxel*

Dose Level	Carboplatin	Paclitaxel
Starting Dose	AUC2	50 mg/m^2
-1	-20%	-20%
-2	-30%	-30%
-3	-50%	-50%

*These levels are guidelines only

NOTE: If carboplatin or paclitaxel is discontinued, or reduced to dose level -3, the investigator may choose to replace the chemotherapy with FOLFOX, capecitabine (with or without oxaliplatin), or 5FU infusion.

8.32 Guidelines for carboplatin and paclitaxel table of events and modifications

CTCAE System/Organ/Class (SOC)	**ADVERSE EVENT**	**AGENT[a]**	**ACTION**
Blood and lymphatic disorders	Febrile neutropenia (fever without clinically or microbiologically documented infection) [ANC <1.0 X 10^9 C/L] fever ≥38.5^{o}C)	carboplatin paclitaxel	Delay chemotherapy by 1 week until ANC recovers to Grade 0-2 and until fever resolves. Reduce dose by 20% or more for subsequent cycles.
Cardiac Disorders	Ventricular bradycardia (asymptomatic) or isolated and asymptomatic ventricular extrasystoles	paclitaxel	Continue therapy under continuous cardiac monitoring
	Atrioventricular block first degree	paclitaxel	Continue therapy under continuous cardiac monitoring
	Ventricular arrhythmia (symptomatic) or AV block complete or other heart blocks.	paclitaxel	Discontinue paclitaxel. Manage arrhythmia according to standard practice

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT[a]	ACTION
Gastrointestinal disorders	Vomiting ≥Grade 3	carboplatin paclitaxel	If not controlled with optimal medication: Reduce dose by 20% and use reduced level for subsequent cycles
Immune system disorders	Allergic reaction Grade 1 (eg, mild flushing, rash, pruritis)	paclitaxel	Complete infusion Supervise at bedside
Immune system disorders	Allergic reaction Grade 2-3 (e.g. moderate rash, flushing mild dyspnea, chest discomfort, mild hypotension)	paclitaxel	Stop infusion. give IV antihistamine (diphenhydramine 50 mg IV and dexamethasone 10 mg IV) After recovery of symptoms resume paclitaxel infusion at a rate of 20 ml/h for 15 minutes then 50 ml/h for 15 minutes then, if no further symptoms, at full dose rate until infusion is complete.
	Anaphylaxis (e.g. one or more of the following): respiratory distress requiring treatment, generalized urticaria, angioedema, hypotension requiring therapy)	paclitaxel	Stop paclitaxel infusion, give IV antihistamine and steroid as above Add epinephrine or bronchodilators if indicated, report as an adverse event Discontinue therapy.
Investigations	Neutrophil count decreased ≥Grade 3 (ANC 500 to <1000/mm^3)	carboplatin paclitaxel	Delay chemotherapy by 1 week until recovery to Grade 0-2 Consider reducing dose by 20% for subsequent cycles.
	Platelet count decreased ≥Grade 3 (25 to <50 x 10^9/L)	carboplatin paclitaxel	Delay chemotherapy by 1 week until recovery to Grade 0-2. Consider reducing dose by 20% for subsequent cycles.
	Bleeding ≥ Grade 3 or requiring ≥2 platelet transfusions	carboplatin paclitaxel	Delay chemotherapy by 1 week until bleeding recovers to Grade 0-1 Permanently discontinue chemotherapy, or reduce dose by 20% or more for subsequent cycles.
Nervous system disorders	Peripheral motor neuropathy Grade 2	carboplatin paclitaxel	Reduce dose by 20% and use reduced level for subsequent cycles.
	Peripheral motor neuropathy Grade 3 or higher	carboplatin paclitaxel	Discontinue therapy
All other nonhematologic	≥Grade 3	carboplatin paclitaxel	Discontinue therapy, or reduce dose by 20% or more and use reduced dose for subsequent cycles.

[a] If a drug is held, omitted, discontinued or dose-reduced, it is the physician's discretion to apply the modification to one or both cytotoxic drugs, and the decision should depend on the nature and severity of the particular toxicity and its likely causal relation to the agent.

8.4 Dose Modifications for 5-FU and Oxaliplatin

Table 8.41 Dose levels for oxaliplatin and 5-FU

Dose Reduction Steps –Oxaliplatin + 5-fluorouracil/Leucovorin Regimen*				
	Starting Dose	Dose Level -1	Dose Level -2	Dose Level -3**
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m^2	40 mg/m^2
5-fluorouracil infusion****	2400 mg/m^2	1900 mg/m^2	1500 mg/m^2	1200 mg/m^2
* Leucovorin dose remains fixed at 400 mg/m^2 (not adjusted).				
**Further dose levels (-4, -5, etc.) will be 20% dose reductions from the previous level.				
****If the 5-FU infusion dose is decreased, the bolus dose of 5-FU should be discontinued for the current cycle and for all future cycles. Leucovorin will still be given.				

Table 8.42 Guidelines for dose modifications for oxaliplatin and 5-F

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
All adverse events	≤Grade 1	5-FU Oxaliplatin	Maintain dose level
Blood and lymphatic system disorders	Hemolytic uremic syndrome[1] ≥Grade 3	Oxaliplatin	Discontinue oxaliplatin
Gastrointestinal disorders	Anal mucositis Grade 3	5-FU	↓ One 5-FU dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
	Grade 4	5-FU	↓ One 5-FU dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
	Diarrhea Grade 3	5-FU	↓5-FU one dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
	Grade 4	5-FU Oxaliplatin	↓ both 5-FU and oxaliplatin one dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
	Mucositis oral Grade 3	5-FU	↓ One 5-FU dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
	Grade 4	5-FU	↓ One 5-FU dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
Gastrointestinal disorders	Vomiting ≥Grade 3	Oxaliplatin	↓ One oxaliplatin dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
	Grade 4	5-FU Oxaliplatin	↓ both 5-FU and oxaliplatin one dose level If Grade ≥2 at start of new cycle hold and check weekly If Grade ≥2 after 4 weeks, discontinue
Investigations	Neutrophil count decreased ≥Grade 3 (ANC 500 to <1000/mm^3)	Oxaliplatin	↓ One oxaliplatin dose level If ANC<1500 at start of new cycle hold and check weekly If ANC<1500 after 4 weeks, discontinue
	Grade 4	5-FU Oxaliplatin	Omit bolus 5-fluorouracil and ↓ both infusional 5-fluorouracil and oxaliplatin 1 dose level If ANC<1500 at start of new cycle hold and check weekly If ANC<1500 after 4 weeks, discontinue
Investigations	Platelet count decreased ≥Grade 3 (25 to <50 x 10^9/L)	Oxaliplatin	↓ One oxaliplatin dose level If PLT <75,000 at start of cycle, hold and check weekly then treat based on interval adverse event. If PLT <75,000 after 4 weeks, discontinue therapy
	Grade 4	Oxaliplatin	↓ Two oxaliplatin dose levels If PLT <75,000 at start of cycle, hold and check weekly then treat based on interval adverse event. If PLT <75,000 after 4 weeks, discontinue therapy
Respiratory, thoracic and mediastinal disorders	Cough ≥Grade 3	Oxaliplatin	Hold oxaliplatin until interstitial lung disease is ruled out
	Dyspnea ≥Grade 3	Oxaliplatin	Hold oxaliplatin until interstitial lung disease is ruled out
Respiratory, thoracic and mediastinal disorders	Hypoxia ≥Grade 3	Oxaliplatin	Hold oxaliplatin until interstitial lung disease is ruled out
	Pneumonitis ≥Grade 3	Oxaliplatin	Hold oxaliplatin until interstitial lung disease is ruled out

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
All other nonhematologic[2]	≥Grade 3	5-FU Oxaliplatin	↓ One 5-FU dose level **Note:** Dose reduction is not required for hypomagnesemia unless symptoms are present If Grade ≥2 after 4 weeks, discontinue therapy

1. Recommended evaluation of suspected HUS: Evaluation should include CBC differential, platelets, PT, PTT, fibrinogen, FDP (Fibrin degradation products), Anti thrombin III, Von Willebrand factor, anti-nuclear antibody, rheumatoid factor, Compliment Cascade C3, C4, and CH_{50}, anti-platelet antibodies, platelet-associated IgG, and circulating immune complexes. Renal evaluation should include creatinine, BUN, and urinalysis with microscopic examination. Other laboratory and hematological evaluations as appropriate should also be obtained, including peripheral blood smear and free hemoglobin.
2. Exceptions: alopecia, fatigue, anorexia, nausea/vomiting if can be controlled by antiemetics, viral infections.

8.43 Oxaliplatin Dose Modifications for Non-CTCAE Neurologic Adverse Events

Adverse Events	Duration of Adverse Event		Persistent[1] Adverse Events
	1 - 7 Days	>7 Days	
Paresthesias/Dysesthesias			
Paresthesias/dysesthesias[2] of short duration that resolve and do not interfere with function (Grade 1)	no change	no change	no change
Paresthesias/dysesthesias[2] interfering with function, but not activities of daily living (ADL) (Grade 2)	no change	no change	↓1 oxaliplatin dose level
Paresthesias/dysesthesias[2] with pain or with functional impairment that also interfere with ADL (Grade 3)	1st time: ↓1 oxaliplatin dose level 2nd time: ↓1 oxaliplatin dose level	1st time: ↓1 oxaliplatin dose level 2nd time: ↓1 oxaliplatin dose level	Stop
Persistent paresthesias/dysesthesias that are disabling or life-threatening (Grade 4)	Stop	Stop	Stop
Laryngeal Dysesthesias (investigator discretion used for grading):			
Grade 0 = none; Grade 1 = mild	No change	↑ duration of infusion to 6 hours	↑ duration of infusion to 6 hours
Grade ≥2 = moderate (Also recommended is administration of benzodiazepine and patient education Management of patient if ≥Grade 2 laryngeal dysesthesias occurs while treatment is being administered)	Stop oxaliplatin infusion Administer benzodiazepine and give patient reassurance At the discretion of the investigator, the infusion can be restarted at 1/3 the original rate of infusion.		
Grade 3 = severe			

Adverse Events	Duration of Adverse Event		Persistent[1] Adverse Events
	1 - 7 Days	>7 Days	

[1] Not resolved by the beginning of the next cycle.
[2] May be cold-induced.

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

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

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 8.2 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.

9.2 Immunotherapy-related toxicities

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

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

- **Diarrhea/colitis**
  Patients should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
  - All patients who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

- For Grade 2 diarrhea/colitis, administer oral corticosteroids.
- For Grade 3 or 4 diarrhea/colitis, treat with intravenous steroids followed by high dose oral steroids.
- 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.

9.3 Blood products and growth factors

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

9.31 Neutropenia

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

9.32 Anemia

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

9.33 Thrombocytopenia

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

9.4 Anti-infectives

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

9.5 Corticosteroids

Patients requiring chronic steroid administration (excluding inhaled steroids).are excluded from the trial. Patients may continue on inhalation therapy. Corticosteroids are known immunosuppressive agents that can mitigate the effects of pembrolizumab. Steroids should be generally reserved to treat side effects of pembrolizumab. Steroids can be used as primary prevention of nausea per institutional guidelines, but steroid doses should be reduced in subsequent cycles if nausea/vomiting is absent or very mild (see Section 9.5).

9.6 Antiemetics

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

If no or minimal nausea/vomiting is reported after first dose of carboplatin/paclitaxel, investigators are strongly encouraged to reduce the dose of dexamethasone to 10 mg as pre-treatment anti-emetic dosing at time of next dose. In addition, if nausea/vomiting continues to be a minimal issue, investigators are strongly encouraged to progressively decrease the pre-treatment dose of dexamethasone in subsequent doses (ie, 4 mg or zero). If the investigator believes a stronger dose is needed, then s/he may manually increase the dexamethasone dose.

9.7 Anti-diarrheals

Patients should be monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered. (See Section 9.7 for management of treatment-related enterocolitis)

All patients who experience diarrhea should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

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

9.8 Management of treatment related enterocolitis

In patients with severe enterocolitis, pembrolizumab will be held and treatment with systemic corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month.

In patients with moderate enterocolitis, pembrolizumab should be withheld and antidiarrheal treatment should be started. If symptoms are persistent for more than one week, systemic corticosteroids should be initiated (e.g., 0.5 mg/kg/day of prednisone or equivalent). When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month.

9.9a Concomitant Medications/Vaccinations (Allowed & Prohibited)

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

9.9a1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded in the case report forms (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the 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 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 10 and Appendix VI.

9.9a2 Prohibited Concomitant Medications

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

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

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

Patients 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. Patients 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.

9.9b Infusion Reaction

Table 9.9a Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping treatment infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Patients who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of study treatment administration.

9.9c Contraception

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

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

Female patients will be considered of non-reproductive potential if they are either:

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

OR

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

OR

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

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

(1) practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

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

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

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

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

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

9.9d Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with pembrolizumab, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Mayo Clinic and to Merck without delay and within 24 hours to Mayo Clinic 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. If a male subject impregnates his female partner the study personnel at the site must be informed

immediately and the pregnancy reported to Mayo Clinic and to Merck and followed as described above.

9.9e Use in Nursing Women

Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment. Specific additional information follows for individual agents used in this trial.

9.9e1 Pembrolizumab

It is unknown whether pembrolizumab is excreted in human milk.

9.9e2 Cisplatin

Cisplatin has been reported to be found in human milk; patients receiving Cisplatin injection should not breast-feed.

9.9e3 Fluorouracil

It is not known whether 5-fluorouracil is excreted in human milk. Because 5-fluorouracil inhibits DNA, RNA and protein synthesis, mothers should not nurse while receiving this treatment.

10.0 Adverse Event (AE) Reporting and Monitoring

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf	Attach to MCCC Electronic SAE Reporting Form
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form [redacted] AND attach MedWatch 3500A: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf	Fax MedWatch to Merck Global Safety Attn: Worldwide Product Safety Fax [redacted] MCCC SAE form will automatically be sent to [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE version 4.0. Next, determine whether the event is expected or unexpected (see Section 10.2) and if the adverse event is related to the medical treatment or procedure (see Section 10.3). With this information, determine whether the event must be reported as an expedited report (see Section 10.4). Expedited reports are to be completed within the timeframes and via the mechanisms specified in Sections 10.4. All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.5 and 18.0).

10.12 Each CTCAE term in the current version is a unique representation of a specific event used for medical documentation and scientific analysis and is a single MedDRA Lowest Level Term (LLT).

NOTE: A severe AE, as defined by the above grading scale, is **NOT** the same as serious AE, which is defined in the table in Section 10.4.

10.2 Expected vs. Unexpected Events

- The determination of whether an AE is expected is based on agent-specific information provided in Section 15.1, 15.2, 15.3, 15.4, Appendix II, and Appendix III of the protocol.
- Unexpected AEs are those not listed in the agent-specific information provided in Section 15.1, 15.2, 15.3, 15.4, Appendix II, and Appendix III of the protocol.

NOTE: "Unexpected adverse experiences" means any adverse experience that is not identified in nature, severity, or frequency of risk in the information provided for IRB review nor mentioned in the consent form.

NOTE: The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure.

10.3 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:
Definite - The adverse event *is clearly related* to the agent(s).
Probable - The adverse event *is likely related* to the agent(s).
Possible - The adverse event *may be related* to the agent(s).
Unlikely - The adverse event *is doubtfully related* to the agent(s).

Unrelated - The adverse event *is clearly NOT related* to the agent(s).

Events determined to be possibly, probably or definitely attributed to a medical treatment suggest there is evidence to indicate a causal relationship between the drug and the adverse event.

10.31 AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME Arm

NOTE: The combination of an investigational agent with a commercial agent is considered investigational.

Routine Reporting

- Routine AE reporting for Phase 1 and Phase 2 clinical studies using an investigational agent /intervention in combination with a commercial agent is stated in the protocol. See Section 10.52.

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the entire combination (arm) is then considered an investigational intervention for reporting.

Expedited Reporting

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for CTEP investigational agents/interventions in Section 10.4, and where indicated, an expedited report must be submitted.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report. Refer to Section 10.4 for specific AE reporting requirements or exceptions.
- Commercial agent expedited reports must be submitted to the FDA via MedWatch.
- An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity, expedited reporting is required. The clinical investigator must determine severity.

10.32 Special Situations for Expedited Reporting and Submission of Notification Forms

Exceptions to Expedited Reporting and Submission of Notification Forms: EXPECTED Serious Adverse Events[1]

An expedited report or notification form may not be required for specific Grade 1, 2, 3 and 4 Serious Adverse Events where the AE is listed in Section 15.0 of the protocol or the consent form* as **EXPECTED**. Any protocol specific reporting procedures MUST BE SPECIFIED BELOW and will **supercede** the standard Expedited Adverse Event Reporting and Notification Form Requirements (Note: These adverse events must still be reported through the routine reporting mechanism [i.e. Nadir/Adverse Events Form]; see Footnote 1):

System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be expeditedly reported[1]
General disorders and administrations site conditions	Fatigue	Grade 3
Gastrointestinal	Vomiting	Grade 3
	Nausea	Grade 3
	Diarrhea	Grade 3
Investigations	Neutrophil count decreased	Grade 3 or 4
	White blood cell count decreased	Grade 3 or 4
	Lymphocyte count decreased	Grade 3 or 4
	Platelet count decreased	Grade 3 or 4
Blood and lymphatic system disorders	Anemia	Grade 3 or 4

[1]These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

NOTE: Hospitalization related to convenience (e.g. transporttation issues, etc.) will *not* be considered an SAE for purposes of reporting to Merck.

Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

Efficacy endpoints as outlined in this section will not be reported to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor (Mayo Clinic) within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study.

10.321 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital abnormities or birth defects, must be reported immediately if they occur at any time following treatment with an agent under an IND/IDE since they are considered to be a serious AE and must be reported to the sponsor as specified in 21 CFR 312.64(b).

10.322 Death

- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

- Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.
- Any death occurring **within 30 days** of the last dose, regardless of attribution to the investigational agent/intervention requires expedited reporting within 24 hours.
- Any death occurring **greater than 30 days** after the last does of the investigational agent/intervention requires expedited reporting within 24 hours **only if** it is possibly, probably, or definitely related to the investigational agent/intervention.

10.323 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE be reported. Three options are available to describe the event:
- Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.324 Second Malignancy

- A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting.

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

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

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in section 10.32 of the protocol.

Expedited AE reporting timelines are defined as:

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

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

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

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

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

Effective Date: May 5, 2011

NOTE: Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered an SAE for purposes of reporting to Merck.

10.42 Additional instructions:

1. Use Mayo Expedited Event Report form [redacted] or investigational agents or commercial/investigational agents on the same arm.

   For commercial agents:

   Submit form MedWatch 3500A to the FDA, 5600 Fishers Lane, Rockville, MD 20852-9787, by fax at [redacted] or online at http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm.

2. Mayo Clinic Cancer Center (MCCC) Institutions:
   Submit to Merck Global Safety (Attn: Worldwide Product Safety Fax [redacted].

   Provide copies, along with the UPIRTSO cover sheet, by fax [redacted]) to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist who will determine and complete IRB reporting. The MCCC SAE form will automatically be sent to [redacted]

10.5 Other Required Reporting

10.51 Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per the CTCAE v4.0 grading unless otherwise stated in the table below:

SYSTEM ORGAN CLASS	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Creatinine increased	X	X
	Neutrophil count decreased	X	X
	Platelet count decreased	X	X
General disorders and administration site conditions	Fatigue	X	X
Gastrointestinal Disorders	Nausea	X	X
	Vomiting	X	X
	# of Stools	X	
	Diarrhea		X
	Constipation		X
Infections and infestations	Sepsis	X	X
Blood and lymphatic system disorders	Febrile neutropenia	X	X
Skin and subcutaneous tissue disorders	Rash, maculo-papular	X	X
Nervous system disorders	Peripheral sensory neuropathy	X	X
	Peripheral motor neuropathy	X	X

10.52 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.5:

10.521 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.522 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.523 Grade 5 AEs (Deaths)

10.5231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.5232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.524 Events of Clinical Interest (ECIs)

ECIs are selected non-serious and serious adverse experiences that must be reported within 24 hours to Mayo Clinic via email [redacted] and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety Fax [redacted]).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 10.55, 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.

10.53 Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

10.54 Procedures for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must fax a completed Pregnancy Form to Regulatory Affairs Unit per instructions below. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Regulatory Affairs Unit per instructions below. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:

Use form available from the CTEP protocol development page: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf

Mayo Clinic Cancer Center (MCCC) Institutions: Attach copy to automated Mayo Clinic Cancer Center Adverse Event Reporting Form [redacted] which will automatically be submitted to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist at [redacted] who will determine and complete IRB reporting. The RAU will submit to the MCCC SAE Coordinator and the MCCC IND Coordinator to determine if FDA submission is needed.

10.541 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.542 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.543 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational

agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.55 Reporting of Overdose of Pembrolizumab

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

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

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

All reports of overdose with and without an adverse event must be reported within 24 hours to Mayo Clinic [redacted] and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX [redacted]).

10.56 Additional Instructions for AE Reporting to Merck

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

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is an other important medical event
- Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Sponsor (Mayo Clinic) and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of

treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

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

SAE reports and any other relevant safety information are to be forwarded to the **Merck Global Safety facsimile number:** [redacted]**.**

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

11.0 Treatment Evaluation

Response and progression will be evaluated in this study using the Immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) guidelines.

11.1 Schedule of Evaluations:

Pre-surgery: Two weeks or less prior to surgery, patients will undergo PETCT imaging to rule out the appearance of new disease that may preclude surgery (see Sections 11.2 and 11.3). The purpose of this repeat PETCT is not to assess response.

At surgery: Patients will be classified with respect to a pathologic tumor response. The evaluation for pathologic tumor response will be determined on the basis of surgical outcome (see Section 11.4).

Post-surgery: Subsequent to surgery, patients will undergo periodic imaging to determine whether disease has recurred (see 11.5).

11.2 Pre-surgery: Evaluation of PET/CT after chemoradiotherapy

11.21 If repeat PET/CT does not show evidence of the development of unresectable disease (eg, metastases in a distant site), the patient will proceed to surgery if he/she remains an operable candidate.

11.22 **If the PET/CT shows lesions that indicate the potential development of unresectable disease, it is strongly encouraged to obtain 3-dimensional imaging within 7 (+/-3) days that includes IV contrast as part of standard clinical practice, so as to further characterize abdominopelvic or other lesions**. Given the unreliability of radiographic changes of the primary tumor during or after chemoRT, due to the inflammatory effects of chemoRT on tissue, assessment of PD will not routinely include changes in the primary tumor or regional nodes, as long as the disease remains resectable. If these images demonstrate unresectable disease in the clinical judgment of the investigator, they will serve as the First Radiologic Evidence of PD (see Table A). RECIST 1.1 will be applied to this radiographic assessment. However, applying the principles of irRECIST, these radiographic changes in themselves will NOT be considered PD in the absence of tissue confirmation of progression or other definitive evidence of progression.

If it is determined that surgery is delayed due to this assessment (see Section 1.9d), the patient is encouraged to undergo further therapy with:

- pembrolizumab combined with chemotherapy, if the patient remains a candidate for chemotherapy or
- pembrolizumab without chemotherapy, if the patient is no longer a candidate for chemotherapy

See Section 1.9b for rationale; see Section 1.9d for study outline; see Section 7 for details of therapy.

To confirm already suspected PD, repeat imaging should occur no earlier than 4 weeks later; repeat imaging is suggested to occur after 6 to 10 weeks of further anticancer therapy. Repeat imaging will generally consist of PETCT with or without contrast CT chest/abdomen/pelvis, as clinically indicated. If repeat imaging confirms PD, then the patient will be designated as having PD and will be taken off protocol treatment.

11.3 Assessment of disease: RECIST 1.1 and irRECIST

This study will apply the principles of irRECIST that is adapted to the management of patients with locally advanced GEJ/cardia cancer undergoing neoadjuvant chemoRT with anticipated surgery. Adapted irRECIST is described below.

RECIST 1.1 will be applied to the First Radiologic Evidence of PD (see Section 11.2).

For subsequent scans following the First Radiologic Evidence of PD, **RECIST 1.1 will be adapted** to account for the unique tumor response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The changes patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an apparent initial increase in tumor volume or even the appearance of new lesions. Standard RECIST may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab. Immune-related RECIST (irRECIST) is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immuno-therapeutics.

irRECIST will be used by Investigators to assess progression after initial radiographic progression per RECIST 1.1, and make treatment decisions accordingly. In this study, there will be no confirmation from central review for irRECIST. Therefore, determination of disease status per irRECIST and subsequent decision of treatment continuation/discontinuation is per the treating physician.

In general, in the absence of surgical resection of the primary and nodes, disease within the RT field (primary or locoregional nodes) will be considered unreliable lesions to be used for designation as radiologic PD. The discussion within this section excludes disease within the RT field.

If radiologic imaging verifies initial disease progression per RECIST 1.1, treatment may continue at the discretion of the Investigator until repeat imaging ≥ 4 weeks later (see Section 7 for systemic therapy regimens). The Investigator's decision to continue treatment while awaiting repeat imaging should be based on the subject's overall clinical condition guided by the following criteria:

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

If repeat imaging shows < 20% increase in tumor burden compared to nadir, stable previous new lesion (if identified as cause for initial disease progression), and stable non-target disease (if identified as cause for initial disease progression), treatment may be continued / resumed, and subsequent tumor imaging follows protocol schedule. If lesions that were identified as cause for initial radiologic disease progression are not deemed to be represent unresectable disease, the patient may proceed to surgery.

If repeat imaging confirms disease progression due to any of the scenarios listed below, patients will be discontinued from study therapy.

- Tumor burden remains ≥20% and at least 5 mm absolute increase compared to nadir
- Non-target disease resulting in initial disease progression is worse (qualitative)

- New lesion resulting in initial disease progression is worse (qualitative)
- Additional new lesion(s) since last evaluation

In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions (with the exception of disease within the RT field) as well as non-target lesions. When feasible, study treatment should not be discontinued until progression is confirmed. Patients that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease. Guidance for treatment continuation/discontinuation and repeat imaging per irRECIST is described in Table A and Table B.

Table A. Prior to surgery

	Clinically stable		Clinically unstable	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD	Repeat imaging at >4 weeks[a] to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at >4 weeks[a] to confirm PD per physician discretion only	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible upon consultation with PI)	No additional imaging required	N/A
Repeat scan shows non-PD	If surgery is not performed, continue further imaging assessments	If disease is resectable, proceeding to surgical resection is strongly encouraged. Continuation of study treatment at the Investigator's discretion	If surgery is not performed, continue further imaging assessments	If disease is resectable and patient is operable, proceeding to surgical resection is strongly encouraged. May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion.

PD = progressive disease

[a]Repeat imaging is suggested to occur after 6 to 10 weeks of further anticancer therapy.

NOTE: If a subject has confirmed radiographic progression (ie, 2 scans at least 4 weeks apart demonstrating progressive disease) per irRECIST, but the subject is achieving a clinically meaningful benefit, an exception to continue treatment may be considered following consultation with the PI.

11.4 At surgery: Measurement of Pathologic Response

Pathologic response will be evaluated after the patient has had surgery and will be based on the pathology review of the submitted surgical specimen according to the following:

- Pathologic Complete Response (pCR): On review of the resected esophageal specimen and accompanying lymph nodes no cancer is recognized by the pathologist and margins are free of tumor.
- Microscopic Cancer: Gross tumor is not seen by the pathologist but tumor remains in the microscopic analysis or any part of the entire specimen submitted for pathology review.
- No Response: Gross cancer is found on pathologic examination of the resected esophageal cancer and draining lymph nodes.

11.5 Completeness of Surgical Resection

All operative and pathology reports from patients in the study must be submitted. Reports must contain information about gross and microscopic contamination of surgical resection margins.

11.51 Curative (R0)

Resections are defined as "curative" (i.e., complete resection; R0 resection) when all gross disease has been removed, and microscopic examination reveals all surgical margins free of tumor (i.e., pathological stage T1-3, NX, MO resected). Resections will still be considered curative if pathologic examination reveals positive lymph nodes as long as the nodes were completely resected.

11.52 Incomplete resection (R1)

"Palliative resection" (i.e., incomplete resection) will be considered to have taken place when microscopic examination reveals surgical margins which are not free of tumor (R1 resection). Positive margins are defined as tumor at or less than 1 mm from the lateral ("deep"), proximal, or distal margins.

11.53 Incomplete resection (R2)

"Palliative resection" (i.e., incomplete resection) will be considered to have taken place when gross disease has been left behind (R2 resection). Positive margins are defined as tumor at or less than 1 mm from the lateral ("deep"), proximal, or distal margins.

11.54 No resection

The primary tumor could not be removed.

11.6 Post-surgery (or after completion of definitive therapy): Evaluation by CT during adjuvant pembrolizumab or during event monitoring

After surgery (or after completion of definitive therapy), CT must be used. If new lesions develop during adjuvant pembrolizumab and up to six months after the last dose of adjuvant pembrolizumab, the appearance of new lesions must be confirmed by repeat imaging performed 4 or more weeks later following the principles outlined in Section 11.3 (see Table B below). Tissue confirmation should be performed if feasible. If new lesions develop subsequent to the period described above, confirmatory imaging is not required.

Table B. Subsequent to surgery

	Clinically stable		Clinically unstable	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD	Repeat imaging at >4 weeks[a] to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at >4 weeks [a] to confirm PD per physician discretion only	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible upon consultation with PI)	No additional imaging required	N/A

PD = progressive disease;
[a] Repeat imaging to confirm suspected PD needs to occur only during adjuvant pembrolizumab or if 1st radiologic evidence of PD occurs within 6 months of completing adjuvant pembrolizumab. The timing of this repeat imaging is suggested to occur 6-10 weeks later. D**uring this period, pembrolizumab may continue to be administered if the patient is in the adjuvant portion of the study.**

11.7 Criteria for Discontinuation of Protocol Treatment

- Progression of disease
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study

If protocol treatment is discontinued, follow-up and data collection will continue as specified in the protocol.

11.8 Guidelines for Evaluation of Measurable Disease

11.81 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up.

11.82 Acceptable imaging modalities for measurable disease:

- FDG PET/CT scan: The PET component needs to be FDG-PET. FDG PET/CT imaging is to be used up until the time the patient has had surgery. After surgery, CT imaging must be used.
- CT and MRI: this guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.
- As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.
- Ultrasound (US) is not acceptable to measure tumor lesions that are clinically not easily accessible.

12.0 Descriptive Factors

12.1 Tumor Location: gastroesophageal junction vs. gastric cardia.

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who are non-PD before surgery will continue treatment per protocol. Patients who are recurrence-free after resection will continue treatment per protocol.

13.2 Progressive disease (PD) or recurrent disease

Patients who develop PD while receiving pre-operative therapy will go to the event-monitoring phase. Patients who develop recurrent disease while receiving adjuvant

therapy after resection will go to event-monitoring phase.

13.3 Off treatment for reasons other than PD or recurrent disease

Patients who go off protocol treatment for reasons other than PD or recurrent disease will go to the event-monitoring phase per Section 18.0.

13.4 Observation

If the patient has undergone surgery, the patient will be observed every 3 months for 1 year and then every 4 months for second year post-surgeryplus every 6 months for third year for up to 3 years post-registration.

13.5 Criteria for Treatment Discontinuation

Patients may discontinue treatment for the following reasons:

- Patient withdraws consent to continue in the trial
- Patient develops an intercurrent illness that precludes further participation, or requires a prohibited concomitant treatment
- The Investigator withdraws the patient in the patient's best interests
- Patient is lost to follow-up (defined as the inability to contact the patient on 3 separate occasions)
- Administrative reasons (e.g., the patient is transferred to hospice care)
- An adverse event, which in the opinion of the Investigator, precludes further trial participation

Patients should go to event monitoring per section 18.0, unless the patient refuses further study participation or is lost to follow-up.

13.6 Criteria for Study Discontinuation

The study may be temporarily or permanently discontinued at any site and at any time. Reasons for study discontinuation may include, but are not limited to, the following:

- Safety concerns
- Poor enrollment
- Non-compliance with the protocol, Good Clinical Practice guidance or other regulatory requirements by the Investigator(s)
- Request to discontinue the trial by a regulatory or health authority or an IRB
- Manufacturing difficulties/concerns

All Investigators and the requisite regulatory authorities will be notified if the study is suspended or terminated for safety reasons. In the case of such termination, the Investigator will notify the IRB.

13.7 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered.

If the patient discontinues treatment, the patient will go directly to the event-monitoring phase of the study (or off study, if applicable):

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 18.0 of the protocol.
- If the patient never received treatment, on-study material must be submitted. No further data submission is necessary.

13.8 Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in Cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. The patient may continue treatment at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. If the physician decides treatment should be discontinued, the patient will go directly to the event monitoring phase per Section 18.0 and all data up until the point of confirmation of a major violation must be submitted.

13.9a Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Body Fluid Biospecimen Submission

14.11 Summary Table of Body Fluid Biospecimens for This Protocol

Type of biospecimen to submit	Mandatory or optional	When to submit	Collection tube description and/or additive (color of tube top)	Volume to collect per tube (number of tubes to be collected)	Blood product being processed and submitted by participating site	Additional processing required at site after blood draw?	Storage/ shipping conditions	Reason for submission (background/ methodology section)	Where to find specific details for specimen submission
Blood for correlative research ([redacted])[a]	Mandatory	Baseline	EDTA (purple top)	6 ml (1)	Whole blood	No	Ambient temperature (DO NOT FREEZE)[a]	Defined translational studies (Section 1.9c)	Section 14.4
Blood for correlative research [redacted][b]	Mandatory	Multiple draws (see Table 14.12 for schedule)	EDTA (purple top)	10 ml (2)	Whole blood	No	Ambient temperature (DO NOT FREEZE)	Defined translational studies (Section 1.9c)	Section 14.4
Blood for DNA[c]	Mandatory	Baseline	EDTA (purple top)	10 ml (1)	Whole blood	No	Refrigerate temperature (DO NOT FREEZE)	Banking for future MSI	Section 14.5

a Specimen for [redacted] will only be collected at Mayo Clinic in Rochester.

b Study coordinator should be contacted. At Mayo Clinic in Rochester, the coordinator will deliver the samples directly to the research lab. At Mayo Clinic in Arizona, samples should be sent by overnight express mail directly to the research lab.

C Specimen will be stored for future testing. At Mayo Clinic in Rochester, the coordinator will deliver the samples directly to BAP. At Mayo Clinic in Arizona, samples should be sent by overnight express mail directly to BAP in Mayo Clinic Rochester.

14.12 Table of Blood Specimens for this Protocol

Indicate if specimen is mandatory or optional	Collection tube description and/or additive (color of tube top)	Volume to collect per tube (number of tubes to be collected)	Blood product being processed and submitted by participating site	Baseline	Day 1, Day 8, and Day 15 of Radiation Tx (Days 15, 22, and 29 of study therapy)	Pre-surgery	End of tx (comp-letion of adj tx)	Recurrence	Additional processing required at site after blood draw?	Storage/ shipping conditions[1]
Mandatory ([redacted])[1]	EDTA (purple top)	6 mL (1)	Whole Blood	X					No	Ambient Temperature (DO NOT FREEZE)
Mandatory [redacted]	EDTA (purple top)	10 mL (2)	Whole Blood	X	X	X	X	X	No	Ambient Temperature (DO NOT FREEZE)
Mandatory[4,5]	EDTA (purple top)	10 mL (1)	Whole Blood	X					No	Refrigerate Temperature (DO NOT FREEZE)

1. MCR only will participate. EDTA tube must be delivered to [redacted] Rochester, MN 55905. Can be collected M-F.
2. MCA: collect M-Th only. Samples will be shipped **Priority Overnight** directly to [redacted], Rochester, MN 55905. **Samples should arrive prior to noon on Fridays otherwise they will be discarded and a repeat sample must be sent. (NOTE:** Please use email notification per Section 14.25.)
3. MCR will collect samples M-F and samples will be delivered directly to [redacted] lab.
4. MCA: collect M-Th only. Samples will be shipped **Priority Overnight** directly to [redacted], Rochester, MN 55902).
5. MCR will collect samples M-F and samples will be delivered directly to BAP.

14.2 Blood/Blood Products Handling

14.21 Kits

14.211 Mayo Clinic in Rochester will use Special Study cards in place of kits

14.212 Fax order form for kits is available in the forms packet for MCA.

14.22 MCA samples must be collected **Monday-Thursday ONLY.**
MCR Samples can be collected Monday-Friday.

14.23 Label specimen tube(s) with protocol number, patient study ID number, and time and date blood is drawn.

14.24 Collect and process all blood/blood products according to the protocol.

14.25 Notification: Use email template to notify [redacted] lab to expect samples:
[redacted]

14.26 Shipping (MCA only)

Specimens must be shipped the same day they are drawn.

14.261 Verify ALL sections of the Blood Specimen Submission Form (see Forms Packet), and specimen collection labels are completed and filled in correctly.

14.262 Specimens will be shipped in an ambient shipping container.

14.263 Ship EDTA whole blood tubes to:

Correlative Research Samples:
[redacted] **Rochester, MN 55905;**
Monday – Thursday Only
Do not send samples on weekends or just prior to federal holidays. NOTE Samples have short viability. They must arrive in the lab prior to noon the next day.

Samples for Future Research:
[redacted]
Rochester, MN 55902
Telephone: [redacted]

14.3 Other Body Fluids Handling – None

14.4 Study Methodology and Storage Information

Blood/blood product samples will be collected for the following research:

14.41 Blood for correlative research (Dietz lab). One 6 ml EDTA tube whole blood specimen will be collected once at baseline for analysis of circulating MDSCs and other circulating immune markers (see Section 1.9c). This specimen will only be collected at Mayo Rochester; other site(s) will not participate in this

portion of the translational study. Whole blood should be sent to the [redacted] Lab, where processing will be completed.

14.43 Blood for correlative research ([redacted]). Two 10 ml EDTA tube whole blood specimens will be collected at the following time points:

- Baseline
- Day 1 of radiation therapy (Day 15 of study therapy, ±3 days)
- Day 8 of radiation therapy (Day 22 of study therapy, ±3 days)
- Day 15 of radiation therapy (Day 29 of study therapy, ±3 days)
- Pre-surgery (≤14 days prior to surgery)
- Following completion of adjuvant therapy (≤4 weeks following final dose of pembrolizumab)
- At the time tumor recurrence (≤14 days of documented tumor recurrence)

Whole blood specimens will be shipped to the [redacted] Lab, where specimens will be processed and specimens will be stored. Specimens will be analyzed for soluble PD-L1, assays for T cell activation, proliferation, differentiation, and function, and for specific effector populations.

14.5 Return of Genetic Testing Research Results

Since the results generated by the genetic testing included in this section are not currently anticipated to have clinical relevance to the patient or their family members, the genetic results will not be disclosed to the patients or their physicians.

For this study, DNA and RNA specimens are only being banked and no specific genetic testing is being performed. If, at any time, genetic results are obtained that may have clinical relevance, IRB review and approval will be sought regarding the most appropriate manner of disclosure and whether or not validation in a CLIA-certified setting will be required. Sharing of research data with individual patients should only occur when data have been validated by multiple studies and testing has been done in CLIA-approved laboratories.

15.0 Drug Information

15.1 Pembrolizumab (MK-3475, SCH 900475, Keytruda®)

15.11 **Background**: Pembrolizumab is a potent humanized IgG4 monoclonal antibody with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1.

15.12 **Formulation**

Pembrolizumab is available as a liquid 25 mg/mL, 100 mg/vial.

15.13 **Preparation and storage**:
Vials should be stored in the refrigerator at temperatures between 2-8°C. Drug concentrate is further diluted with normal saline (or 5% dextrose in the concentration range of 1 to 10 mg/mL) in IV containers made of polyvinyl chloride (PVC) or non-PVC material. The infusion solution in the IV bag should be immediately administered. Pembrolizumab solutions may be stored at room temperature for a cumulative period of up to 4 hours. This includes room temperature storage of reconstituted solution in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion.

In addition,IV bags may be stored at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use.

15.14 **Administration:** Pembrolizumab is administered by intravenous infusion over 30 minutes via a 0.22 micron in-line filter. The final infusion volume must be between 1 and 10 mg/mL. Maximum rate of infusion should not exceed 6.7 mL/minute through a peripheral or indwelling catheter. Flush the line with 0.9% NaCL following the completion of the infusion.

15.15 **Pharmacokinetic information**:

a) Absorption – Because pembrolizumab is administered intravenously, it is immediately and completely bioavailable. Steady-state concentrations of pembrolizumab are reached by 16 weeks of repeated dosing with a Q3W regimen and the systemic accumulation is 2.1-fold. The peak concentration, trough concentration, and area under the plasma concentration versus time curve at steady state of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg Q3W.
b) Distribution – Pembrolizumab has a limited volume of distribution.
c) Excretion - CL is approximately 23% lower after achieving maximal change at steady state compared with the first dose. The terminal elimination half-life (t½) is estimated to be 22 days at steady state.
d) Metabolism - Pembrolizumab is catabolized through non-specific pathways; metabolism does not contribute to its CL.

15.16 **Potential Drug Interactions**: There are no known significant drug interactions.

15.17 **Known potential adverse events**
Very common known potential toxicities, ≥10%:

Gastrointestinal disorders: diarrhea, nausea
Skin and subcutaneous tissue disorders: rash, pruritis
General disorders and administration site conditions: fatigue

Common known potential toxicities, ≥1% to < 10%:
Respiratory, thoracic, and mediastinal disorders: pneumonitis, dyspnea, cough
Gastrointestinal disorders: colitis, vomiting, abdominal pain, constipation, dry mouth
Skin and subcutaneous tissue disorders: severe skin reactions, vitiligo, dry skin, erythema
Musculoskeletal and connective tissue disorders: arthralgia, myositis, musculoskeletal pain, arthritis, pain in extremity
General disorders and administration site conditions: asthenia, edema, pyrexia, influenza like illness, chills
Investigations: alanine aminotransferase increased, aspartate aminotransferase increased, blood alkaline phosphatase increased, blood creatinine increased

Uncommon known potential toxicities, ≥0.1% to <1%:
Blood and lymphatic system disorders: neutropenia, thrombocytopenia, leukopenia, lymphopenia, eosinophilia
Endocrine disorders: hypophysitis, adrenal insufficiency, thyroiditis
Metabolism and nutrition disorders: type I diabetes mellitus, hyponatremia, hypokalemia, hypocalcemia
Psychiatric disorders: insomnia
Nervous system disorders: epilepsy, lethargy, peripheral Neuropathy
Eye disorders: uveitis, dry eye
Cardiac disorders: myocarditis
Vascular disorders: hypertension
Gastrointestinal disorders: pancreatitis
Hepatobiliary disorders: hepatitis
Skin and subcutaneous tissue disorders: lichenoid keratosis, psoriasis, alopecia, dermatitis, dermatitis acneiform, eczema, hair color changes, papule
Musculoskeletal and connective tissue disorders: tenosynovitis
Renal and urinary disorders: nephritis
Investigations: blood bilirubin increased, amylase increased, hypercalcemia

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Blood and lymphatic system disorders: immune thrombocytopenic purpura, hemolytic anemia
Immune system disorders: sarcoidosis
Nervous system disorders: Guillain-Barre syndrome, myasthenic syndrome
Gastrointestinal disorders: small intestinal perforation
Skin and subcutaneous tissue disorders: toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema nodosum

The risk profile for pembrolizumab also includes two important potential

risks: a) myasthenic syndrome, and b) an increased risk of severe complications (such as early severe graft versus host disease and venoocclusive disease) of allogeneic transplant in patients with hematologic malignancies who have previously been treated with PD-1 inhibitors.
Patients with multiple myeloma who were treated with pembrolizumab in combination with either pomalidomide or lenalidomide and dexamethasone, had an increased number of serious side effects and deaths as compared to patients who received only dexamethasone and either pomalidomide or lenalidomide. The benefit-risk profile is unfavorable for the combination of pembrolizumab, pomalidomide, and dexamethasone in relapsed refractory multiple myeloma, and the combination of pembrolizumab, lenalidomide, and dexamethasone in newly diagnosed treatment-naive multiple myeloma.

15.18 **Drug procurement:** Pembrolizumab will be provided free of charge to study participants by Merck.

15.19 **Nursing Guidelines:**

15.191 Pembrolizumab side effects vary greatly from those of traditional chemotherapy and can vary in severity from mild to life threatening. Instruct patients to report any side effects to the study team immediately. Side effects may be immediate or delayed up to months after discontinuation of therapy. Most side effects are reversible with prompt intervention of corticosteroids.

15.192 Diarrhea can be seen however, is less common than that seen with anti-CTLA-4 agents. However it can be severe, leading to colonic perforation. Instruct patients to report ANY increase in the number of stools and/or change in baseline, blood in the stool, abdominal pain to the study team immediately.

15.193 Rash/pruirits/dermatitis is seen. Patients should report any rash to the study team. Treat per Section 9.0 and monitor for effectiveness.

15.194 Monitor LFT's closely as elevations in these levels could indicate early onset autoimmune hepatitis. Patients should also be instructed to report any jaundice, or right upper quadrant pain to the study team immediately.

15.195 Pneumonitis can be seen and may be mild (only seen on imaging) to severe. Patients should be instructed to report any SOB, dyspnea, cough, chest pain, etc. to the study team immediately. Patients reporting these symptoms should have a pulse ox checked and consider immediate imaging per the treating MD.

15.196 Endocrinopathies (including hypopituitarism, hypothyroidism, hypophysistis, and adrenal insufficiency) are seen with this agent. Patients may present only with the vague sense of fatigue and "not feeling well." Additional symptoms may be that of nausea, sweating and decreased activity tolerance. Instruct patients to report these signs or symptoms immediately and obtain appropriate labs as ordered by MD.

15.197 Patients who are started on steroid therapy for any side effects of pembrolizimab toxicity should be instructed to take the steroids as ordered, and not to discontinue abruptly as symptoms may return and be severe. Patients may be on steroid therapy for weeks. Instruct patients to report any increase or change in side effects with any dosage decrease as patients may need a slower taper.

15.198 Fatigue is common and may or may not be associated with immune related side effects. Assess patient's fatigue level prior to each cycle of therapy and report any changes to the study team.

15.199a Patients should avoid receiving live vaccines within 30 days of study drug administration or per other study guidelines.

15.199b Patients who have undergone an allogenic bone marrow transplant, have an increased risk of severe complications including early GVHD, and venoocclusive disease, if they have previously been treated with pembrolizumab

15.199c Myocarditis has been reported and associated with pembrolizumab. Instruct patients to report chest pain, SOB, or dyspnea to study team immediately and/or seek emergency medical attention.

15.199d Autoimmune hematologic disorders including ITP and hemolytic anemia have been reported. Monitor blood counts closely and report any abnormalities to the study team.

15.199e Rare neurologic disorders including Guillian-Barre syndrome and myasthenia gravis have been reported. Instruct patients to report any neurologic symptoms including weakness, parasthesias or numbness, tingling to the study team immedicately.

15.2 Carboplatin (Paraplatin®, CBDCA)

15.21 Background

Carboplatin is an alkylating agent which covalently binds to DNA;interferes with the function of DNA by producing intrerstrand DNA cross-links.

15.22 Formulation

Commercially available for injection as:
Solution Reconstituted: 150 mg.
Solution: 10 mg/mL (5 mL, 15 mL, 45 mL, 60 mL)

15.23 Preparation, storage, and stability

Refer to package insert for complete preparation and dispensing instructions. Store intact vials at room temperature at 25ºC (77F); excursions permitted to 15ºC to 30ºC (59ºF to 86ºF). Protect from light. Further dilution to a concentration as low as 0.5 mg/mL is stable at room temperature (25ºC) for 8 hours in 0.9% NaCl or D5W; Stability has also been demonstrated for dilutions in D5W in PVC bags at room temperature for 8 days; however the manufacturer states to use within 8 hours due to lack of preservative.

15.24 Administration

Refer to the treatment section for specific administration instructions. When administered as a part of a combination chemotherapy regimen, sequence of administration may vary by regimen; refer to specific protocol for sequence recommendation. Needles or IV administration sets that contain aluminum should not be used in the preparation or administration of carboplatin; aluminum can react with carboplatin resulting in precipitate formation and loss of potency.

15.25 Pharmacokinetic information:

Distribution: $V_{d:}$ 16 L/kg; into liver, kidney, skin, and tumor tissue.
Protein binding: 0%; however the platinum from carboplatin becomes irreversibly bound to plasma proteins.
Metabolism: Minimally hepatic to aquated and hydroxylated compounds.
Half-life elimination: CrCl >60 mL/min: Carboplatin: 2.6-5.9 hours (based on dose of 300-500 mg/m^2); Platinum (from carboplatin): $\geq$5 days.
Excretion: Urine (~70% as carboplatin within 24 hours; 3% to 5% as platinum within 1-4 days).

15.26 Potential Drug Interactions:

Increased Effect/Toxicity: Aminoglycosides increase risk of ototoxicity and/or nephrotoxicity. When administered as sequential infusions, observational studies indicate a potential for increased toxicity when platinum derivatives (carboplatin, cisplatin) are administered before taxane derivatives (docetaxel, paclitaxel).
Herb/Nutraceutical Interactions: Avoid black cohosh, dong quai in estrogen-dependent tumors.

15.27 Known potential adverse events

Consult the package insert for the most current and complete information. Percentages reported with single-agent therapy. **Note**: Myelosuppression is dose related, schedule related, and infusion-rate dependent (increased

incidences with higher doses, more frequent doses, and longer infusion times) and, in general, rapidly reversible upon discontinuation.

Common known potential toxicities, >10%:
Central nervous system: Pain
Endocrine & metabolic: Hyponatremia, hypomagnesemia, hypocalcemia, hypokalemia
Gastrointestinal: Vomiting, abdominal pain, nausea
Hematologic: Myelosuppression (dose related and dose limiting; nadir at ~21 days; recovery by ~28 days), leukopenia, anemia, neutropenia, thrombocytopenia
Hepatic: Alkaline phosphatase increased, AST increased
Hypersensitivity: Hypersensitivity
Neuromuscular & skeletal: Weakness
Renal: Creatinine clearance decreased, BUN increased

Less common known potential toxicities, 1% - 10%:
Central nervous system: Peripheral neuropathy, neurotoxicity
Dermatologic: Alopecia
Gastrointestinal: Constipation, diarrhea, stomatitis/mucositis, taste dysgeusia
Hematologic: Hemorrhagic/bleeding complications
Hepatic: Bilirubin increased
Infection: Infection
Local: Pain at the injection site
Neuromuscular & skeletal: Peripheral neuropathy
Ocular: Visual disturbance
Otic: Ototoxicity
Renal: Creatinine increased

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Anaphylaxis, anorexia, bronchospasm, cardiac failure, cerebrovascular accident, dehydration, embolism, erythema, febrile neutropenia, hemolytic anemia (acute), hemolytic uremic syndrome, hyper-/hypotension, injection site reaction (pain, redness, swelling), limb ischemia (acute), malaise, metastases, pruritus, skin rash, tissue necrosis (associated with extravasation), urticaria, vision loss

15.28 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.29 Nursing Guidelines

15.291 Monitor CBC and PLTs. Watch for profound neutropenia and give low count precautions and instructions as necessary. Nadir occurs at approximately day 21 with recovery at day 28-30. Thrombo-neutro-leukopenia may be cumulative. Thrombocytopenia can be dose-limiting and is more pronounced than with cisplatin. It can be more severe in patients with previous chemotherapy, concurrent radiation therapy, or patients with impaired renal function. Instruct patient to immediately report any unusual bruising or bleeding. Anemia (70-90% of patients) may be symptomatic with asthenia being the most common complaint. Instruct patient in energy saving lifestyle.

15.292 Assess baseline renal function (creatinine clearance). Reduced renal function can contribute to an increased risk of thrombocytopenia.

15.293 Monitor fluid status - encourage hydration.

15.294 Advise patient of probable taste alterations. Frequent oral hygiene is helpful. Instruct patient in appropriate interventions to achieve and maintain optimal nutritional status.

15.295 Older patients (>65) may experience some peripheral neuropathy with paresthesias. Instruct patients to report any tingling, burning, loss of sensation.

15.296 Mild nausea and vomiting occur in up to 94% of patients, 6-12 hours after treatment and may persist for 24 hours or longer. Diarrhea/cramping/constipation has been experienced by approximately 17%. Premedicate with antiemetics/antidiarrheals—evaluate effectiveness.

15.197 Administer following paclitaxel (in regimens that contain both drugs) to maximize cell kill.

15.196 Patients have experienced allergic reactions while receiving carboplatin. Watch for signs and symptoms of hypersensitivity reactions. If these occur, stop drug immediately, notify MD, and treat appropriately.

15.3 Paclitaxel (Taxol)

15.31 Background

Antineoplastic Agent, Antimicrotubular, Taxane derivative. Paclitaxel promotes microtubule assembly by enhancing the action of tubulin dimers, stabilizing existing microtubules, and inhibiting their disassembly, interfering with the late G_2 mitotic phase, and inhibiting cell replication. In addition, the drug can distort mitotic spindles, resulting in the breakage of chromosomes. Paclitaxel may also suppress cell proliferation and modulate immune response.

15.32 Formulation

Commercially available for injection 6 mg/mL (5 mL, 16.7 mL, 25 mL, and 50 mL) [contains alcohol and purified Cremophor EL (polyoxyethylated castor oil)].

15.33 Preparation, storage, and stability

Refer to package insert for complete preparation and dispensing instructions. Store intact vials at room temperature and protect from light. Dilute in 250-1000 mL D_5W or 0.9% NaCL to a concentration of 0.3 – 1.2 mg/mL. Solutions in D_5W and 0.9% NaCl are stable for up to 3 days at room temperature. Chemotherapy dispensing devices (e.g., Chemo Dispensing Pin) should not be used to withdraw paclitaxel from the vial.

Paclitaxel should be dispensed in either glass or non-PVC containers (e.g., Excel/PAB). Use nonpolyvinyl (non-PVC) tubing (e.g., polyethylene) to minimize leaching.

15.34 Administration

Infuse IV over 1-96 hours. When administered as sequential infusions, taxane derivatives should be administered before platinum derivatives (cisplatin, carboplatin) to limit myelosuppression and to enhance efficacy. Infuse through a 0.22 micron in-line filter and nonsorbing administration set.

15.35 Pharmacokinetic information:

Distribution: $V_{d:}$ Widely distributed into body fluids and tissues; affected by dose and duration of infusion
V_{dss}: 1- to 6-hour infusion: 67.1 L/m^2
V_{dss}: 24-hour infusion: 227-688 L/m^2
Metabolism: Hepatic via CYP2C8 and 3A4; forms metabolites (primarily 6α-hydroxypaclitaxel).
Half-life elimination: 1- to 6-hour infusion: Mean (beta): 6.4 hours,
3-hour infusion: Mean (terminal): 13.1-20.2 hours
24-hour infusion: Mean (terminal): 15.7-52.7 hours
Excretion: Feces (~70%, 5% as unchanged drug); Urine (14%)
Clearance: Mean: Total body: After 1- and 6-hour infusions: 5.8-16.3 L/hour/m^2; after 24-hour infusions: 14.2-17.2 L/hour/m^2

15.36 Potential Drug Interactions:

Cytochrome P450 Effect: Substrate (major) of CYP2C8, CYP3A4;
Induces CYP3A4 (weak).
Increased Effect/Toxicity: CYP2C8 inhibitors may increase the levels/effects of paclitaxel. Refer to the package insert or LexiComp[1] for example inhibitors.
Decreased Effect: CYP2C8 inducers may decrease the levels/effects of paclitaxel. Refer to the package insert or LexiComp[1] for example inducers.
Herb/Nutraceutical Interactions: Avoid black cohosh, dong quai in estrogen-dependent tumors. Avoid valerian, St John's wort (may decrease paclitaxel levels), kava kava, gotu kola (may increase CNS depression).

15.37 Known potential adverse events

Consult the package insert for the most current and complete information. Percentages reported with single-agent therapy.

U.S. Boxed Warning: Bone marrow suppression is the dose-limiting toxicity; do not administer if baseline absolute neutrophil count (ANC) is <1500 cells/mm^3 (1000 cells/mm^3 for patients with AIDS-related KS); reduce future doses by 20% for severe neutropenia.

U.S. Boxed Warning: Severe hypersensitivity reactions have been reported.

Common known potential toxicities, >10%:
Cardiovascular: Flushing, ECG abnormal, edema, hypotension.
Dermatologic: Alopecia, rash.
Gastrointestinal: Nausea/vomiting, diarrhea, mucositis, stomatitis, abdominal pain (with intraperitoneal paclitaxel)
Hematologic: Neutropenia, leukopenia, anemia, thrombocytopenia, bleeding.
Hepatic: Alkaline phosphatase increased, AST increased.
Local: Injection site reaction (Erythema, tenderness, skin discoloration, swelling).
Neuromuscular & skeletal: Peripheral neuropathy, arthralgia, myalgia, weakness.
Renal: Creatinine increased.
Miscellaneous: Hypersensitivity reaction, infection.

Less common known potential toxicities, 1% - 10%:
Cardiovascular: Bradycardia, tachycardia, hypertension, rhythm abnormalities, syncope, venous thrombosis.
Dermatologic: Nail changes.

Hematologic: Febrile neutropenia.
Hepatic: Bilirubin increased.
Respiratory: Dyspnea.

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Anaphylaxis, ataxia, atrial fibrillation, AV block, back pain, cardiac conduction abnormalities, cellulitis, CHF, chills, conjunctivitis, dehydration, enterocolitis, extravasation recall, hepatic encephalopathy, hepatic necrosis, induration, intestinal obstruction, intestinal perforation, interstitial pneumonia, ischemic colitis, lacrimation increased, maculopapular rash, malaise, MI, necrotic changes and ulceration following extravasation, neuroencephalopathy, neutropenic enterocolitis, ototoxicity, pancreatitis, paralytic ileus, phlebitis, pruritus, pulmonary embolism, pulmonary fibrosis, radiation recall, radiation pneumonitis, pruritus, renal insufficiency, seizure, skin exfoliation, skin fibrosis, skin necrosis, Stevens-Johnson syndrome, supraventricular tachycardia, toxic epidermal necrolysis, ventricular tachycardia (asymptomatic), visual disturbances.

15.38 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.39 Nursing Guidelines:

15.391 Premedicate with steroids, antihistamines, and H2 blockers as per institutional guidelines.

15.392 Mix the infusion bag well. Thorough admixture of this drug often prevents a hypersensitivity reaction. An inline filter of <0.22 micron must be used distal to the infusion pump. Filter may need to be changed if infusion is to be prolonged >12 hours. Inspect solution for excessive particulate matter, if present do not use.

15.393 Caution patients that the alcohol contained in the infusion may cause impairment in operating heavy equipment or in driving a vehicle and to assess their ability before trying either. Advise avoidance of any alcohol or depressants such as sedatives and opiates if not necessary.

15.394 Assess the patient frequently for the first 30 minutes. Taxol® hypersensitivity reactions, which may include chest pain, back pain, flushing, diaphoresis, dyspnea, pruritus, hypotension, hypertension, bronchospasm and/or urticaria, usually occur early in the infusion. Have the anaphylaxis tray available.

15.395 If a reaction occurs, stop the infusion immediately. Epinephrine, IV fluids, diphenhydramine, and methylprednisolone may be used as per MD's order.

15.396 Approximately 60% of patients experience peripheral sensory neuropathy (numbness, tingling, burning pain, fine motor skills impairment, paresthesias, distal sensory loss). Patients receiving higher doses at shorter infusion times are at greater risk. Most cases have been reported at doses >170 mg/m^2/day and with cumulative doses over multiple courses of therapy. The nerve damage may take months to resolve. Nonsteroidal anti-inflammatory agents and opiates have not

been effective in treating neuropathic pain. Consult MD about trying tricyclic antidepressants or possibly Neurontin.

15.397 Increased risk of cardiotoxicity when given in combination with doxorubicin, with a sharp increase in risk of CHF once cumulative dose of doxorubicin is > 380 mg/m^2. At this point taxol should be continued as a single agent only. Monitor for sign/symptoms of CHF. Instruct patient to report any swelling in the hands, arms, feet, or legs, and any chest pain.

15.398 Mucositis can usually be managed with a salt and soda mouthwash (1 tsp. Salt, 1 tsp. Soda and 1 quart boiled water) or try OTC oral Lysine or Vitamin E.

15.399a Narcotics and nonsteroidal anti-inflammatory drugs may be used to manage the myalgias.

15.399b Monitor CBC closely. Neutropenia is most severe in patients who have had previous chemotherapy. Instruct patient to report signs or symptoms of infection, unusual bruising or bleeding to the health care team.

15.399c There is an increased risk of neutropenia and stomatitis when given prior to doxorubicin. Therefore Taxol should always be given after doxorubicin administration.

15.399d Monitor IV site closely and establish patency before administration. Paclitaxel is an irritant, however rarely rash, radiation recall, and ulceration have occurred with infiltration of drug.

15.399e Monitor liver function tests

15.399f Inform patient about total alopecia.

15.399g If given on the same day as a platinum agent, paclitaxel should be administered first to limit myelosuppression and enhance efficacy of agent.

15.4 Regimen if carboplatin with paclitaxel not tolerated

15.41 **Oxaliplatin** (Eloxatin®, OXAL)

15.411 Background

Oxaliplatin, a platinum derivative, is an alkylating agent. Following intracellular hydrolysis, the platinum compound binds to DNA forming cross-links which inhibit DNA replication and transcription, resulting in cell death. Cytotoxicity is cell-cycle nonspecific.

15.412 Formulation

Commercially available for injection as:
Solution [preservative free]: 5 mg/mL (10 mL, 20 mL, 40 mL)

15.413 Preparation, storage, and stability

Refer to package insert for complete preparation and dispensing instructions. Store intact vials in original outer carton at room temperature and; do not freeze. According to the manufacturer, solutions diluted for infusion are stable up to 6 hours at room temperature or up to 24 hours under refrigeration. Oxaliplatin solution diluted with D_5W to a final concentration of 0.7 mg/mL (polyolefin container) has been shown to retain >90% of it's original concentration for up to 30 days when stored at room temperature or refrigerated; artificial light did not affect the concentration (Andre, 2007). As this study did not examine sterility, refrigeration would be preferred to limit microbial growth. Do not prepare using a chloride-containing solution (e.g., NaCl). Dilution with D_5W (250 or 500 mL) is required prior to administration. Infusion solutions do not require protection from light.

15.414 Administration

Refer to the treatment section for specific administration instructions. Administer as I.V. infusion over 2-6 hours. Flush infusion line with D5W prior to administration of any concomitant medication. Patients should receive an antiemetic premedication regimen. Cold temperature may exacerbate acute neuropathy. Avoid mucositis prophylaxis with ice chips during Oxaliplatin infusion.

15.415 Pharmacokinetic information

Distribution: V_d: 440 L
Protein binding: >90% primarily albumin and gamma globulin (irreversible binding to platinum)
Metabolism: Nonenzymatic (rapid and extensive), forms active and inactive derivatives
Half-life elimination: Terminal: 391 hours; Distribution: Alpha phase: 0.4 hours, Beta phase: 16.8 hours
Excretion: Primarily urine (~54%); feces (~2%)

15.416 Potential Drug Interactions

Increased Effect/Toxicity: Nephrotoxic agents may increase Oxaliplatin toxicity. When administered as sequential infusions, observational studies indicate a potential for increased toxicity when platinum derivatives (carboplatin, cisplatin, oxaliplatin) are administered before taxane derivatives (docetaxel, paclitaxel).
Decreased Effect: Oxaliplatin may decrease plasma levels of digoxin.

15.417 Known potential adverse events

Consult the package insert for the most current and complete information. Percentages reported with monotherapy.

Common known potential toxicities, > 10%:

Central nervous system: Fatigue, fever, pain, headache, insomnia

Gastrointestinal: Nausea, diarrhea, vomiting, abdominal pain, constipation, anorexia, stomatitis

Hematologic: Anemia, thrombocytopenia, leukopenia

Hepatic: Liver enzymes increased

Neuromuscular & skeletal: Back pain, peripheral neuropathy (may be dose limiting). The most commonly observed oxaliplatin toxicity is acute and cumulative neurotoxicity, observed in patients treated at doses above 100 mg/m^2/cycle. This neurotoxicity has included paresthesias and dysesthesias of the hands, feet, and perioral region as well as unusual laryngopharyngeal dysesthesias characterized by a loss of sensation of breathing without any objective evidence of respiratory distress (hypoxia, laryngospasm, or bronchospasm). OXAL neurotoxicity appears to be exacerbated by exposure to cold. Patients on this study will be counseled to avoid cold drinks and exposure to cold water or air. Should a patient develop laryngopharyngeal dysesthesia, their oxygen saturation should be evaluated via a pulse oximeter; if normal, an anxiolytic agent should be given and the patient observed in the clinic until the episode has resolved. Because this syndrome may be associated with the rapidity of OXAL infusion, subsequent doses of OXAL should be administered as a 6-hour infusion (instead of the normal 2-hour infusion).

Acute and cumulative neurotoxicities are dose limiting for OXAL. The acute neurotoxicity is characterized by paresthesias and dysesthesias that may be triggered or exacerbated by exposure to cold. These symptoms occur within hours of exposure and are usually reversible over the following hours or days. Cumulative doses of OXAL above 680 mg/m^2 may produce functional impairment characterized by difficulty performing activities requiring fine sensory-motor coordination; impairment is caused by sensory rather than motor changes.
The likelihood of experiencing neurotoxicity is directly related to the total cumulative dose of OXAL administered. The relative risk of developing neurotoxicity was 10%, 50%, and 75% in patients who received total cumulative OXAL doses of 780 mg/m^2, 1,170 mg/m^2, and 1,560 mg/m^2, respectively. Both acute and cumulative neurotoxicities due to OXAL have lessened in 82% of patients within 4 to 6 months, and have completely disappeared by 6 to 8 months in 41% of patients. In addition, the likelihood that neurologic symptoms will regress has been shown to correlate inversely with cumulative dose.
Respiratory: Dyspnea, cough

Less common known potential toxicities, 1% - 10%:
Cardiovascular: Edema, chest pain, peripheral edema, flushing, thromboembolism
Central nervous system: Dizziness
Dermatologic: Rash, alopecia, hand-foot syndrome

Endocrine & metabolic: Dehydration, hypokalemia
Gastrointestinal: Dyspepsia, taste perversion, flatulence, mucositis, gastroesophageal reflux, dysphagia
Genitourinary: Dysuria
Hematologic: Neutropenia
Local: Injection site reaction
Neuromuscular & skeletal: Rigors, arthralgia
Ocular: Abnormal lacrimation
Renal: Serum creatinine increased
Respiratory: URI, rhinitis, epistaxis, pharyngitis, pharyngolaryngeal dysesthesia
Miscellaneous: Allergic reactions, hypersensitivity (includes urticaria, pruritus, facial flushing, shortness of breath, bronchospasm, diaphoresis, hypotension, syncope, hiccup

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Gastrointestinal: Life threatening enteric sepsis secondary to neutropenia and diarrhea.
Hepatic: Veno-occlusive disease of the liver is a rare serious adverse event that has occurred in association with administration of oxaliplatin and fluorouracil.
Otic: Clinical ototoxicity occurs in less than 1% of patients following oxaliplatin administration, and sever ototoxicity has not been reported.

15.418 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.42 Leucovorin Calcium (CF)

15.421 Background

A reduced form of folic acid, leucovorin supplies the necessary cofactor blocked by methotrexate, enters the cells via the same active transport system as methotrexate. Stabilizes the binding of 5-dUMP and thymidylate synthetase, enhancing the activity of fluorouracil.

15.422 Formulation

Commercially available as:
Injection, powder for reconstitution: 50 mg, 100 mg, 200 mg, 350 mg
Injection, solution: 10 mg/mL (50 mL)

15.423 Preparation, storage, and stability

Powder for injection: Store at room temperature, protect from light. Reconstitute with sterile water for injection or bacteriostatic water for injection; dilute in 100-1000 mL 0.9% NaCl or D5W. When doses > 10 mg/m2 are required, reconstitute using sterile water for injection, not a solution containing benzyl alcohol. Solutions reconstituted with bacteriostatic water for injection must be used within 7 days. Solutions reconstituted with sterile water for injection must be used immediately. Parenteral admixture is stable for 24 hours stored at room temperature and for 4 days when stored under refrigeration.

Solution for injection: Prior to dilution, store vials under refrigeration, protect from light.

15.424 Administration

Due to calcium content, do not administer I.V. solutions at a rate > 160 mg/minute. Refer to individual protocols for specific administration instructions.

Leucovorin should be administered I.V. push or I.V. infusion (15 minutes to 2 hours). Leucovorin should not be administered concurrently with methotrexate. It is commonly initiated 24 hours after the start of methotrexate. Toxicity to normal tissues may be irreversible of leucovorin is not initiated by ~40 hours after the start of methotrexate.

In combination with fluorouracil: The fluorouracil is usually given after the leucovorin infusion. Leucovorin is usually administered by I.V. bolus injection or short (10-120 minutes) I.V. infusion. Other administration schedules have been used; refer to the treatment section of the protocol for specific directions.

In combination with oxaliplatin: Leucovorin is compatible with oxaliplatin and may be administered concurrently via Y-connector at normal doses. Oxaliplatin is incompatible with 0.9% NaCl. Leucovorin must be diluted in D5W when administered with oxaliplatin.

In combination with irinotecan: Leucovorin is compatible with irinotecan via Y-site injection.3

15.425 Pharmacokinetic information:

Metabolism: Intestinal mucosa and hepatically to 5-methyl-tetrahydrofolate (5MTHF; active)

Half-life elimination: ~4-8 hours

Time to peak: I.V.: Total folates: 10 minutes; 5MTHF: ~1 hour

Excretion: Urine (primarily); feces

15.426 Potential Drug Interactions:

Decreased Effect: May decrease efficacy of trimethoprim/ sulfamethoxazole against Pneumocystis carinii pneumonia.

15.427 Known potential adverse events

Consult the package insert for the most current and complete information.

Common known potential toxicities, >10%:
None

Less common known potential toxicities, 1% - 10%:
Dermatologic: Rash, pruritus, erythema, urticaria
Gastrointestinal: Nausea, vomiting

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Allergic reactions, anaphylactoid reactions, dyspnea, thrombocytosis

15.428 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.43 Fluorouracil (Adrucil, Efudex, [5FU])

15.431 Background

Antineoplastic Agent, Antimetabolite (Pyrimidine Analog). Fluorouracil is a fluorinated Pyrimidine Antimetabolite that inhibits thymidylate synthetase, blocking the methylation of deoxyuridylic acid to thymidylic acid, interfering with DNA, and to a lesser degree, RNA synthesis. Fluorouracil appears to be phase specific for the G1 and S phases of the cell cycle.

15.432 Formulation

Commercially available for injection 50 mg/mL (10 mL, 20 mL, 50 mL, and 100 mL).

15.433 Preparation, storage, and stability

Store intact vials at room temperature and protect from light. A slight discoloration may occur with storage but usually does not denote decomposition. Dilute in 50 – 1000 mL of 0.9% NaCl or D5W. If exposed to cold, a precipitate may form; gentle heating to 60ºC will dissolve the precipitate without impairing the potency. Solutions in 50 – 1000 mL 0.9% NaCl or D5W or undiluted solutions in syringes are stable for 72 hours at room temperature. Fluorouracil should not be coadministered with either diazepam, doxorubicin, daunorubicin, idarubicin, cisplatin, or cytarabine. However, fluorouracil and leucovorin are compatible for 14 days at room temperature. Fluorouracil is compatible with vincristine, methotrexate, and cyclophosphamide.

15.434 Administration

Fluorouracil may be given IV push, IV infusion.
Refer to Section 7.0 (treatment) administration instructions specific to the protocol.

15.435 Pharmacokinetic information:

Distribution: Vd ~ 22% of total body water; penetrates extracellular fluid, CSF, and third space fluids (e.g., pleural effusions and ascitic fluid)

Metabolism: Hepatic (90%); via a dehydrogenase enzyme; Fluorouracil must be metabolized to be active.

Half-life elimination: Biphasic: Initial: 6-20 minutes; two metabolites, FdUMP and FUTP, have prolonged half-lives depending on the type of tissue.

Excretion: Lung (large amounts as CO2); urine (5% as unchanged drug) in 6 hours.

15.436 Potential Drug Interactions

Fluorouracil may increase effects of warfarin. Avoid ethanol (due to GI irritation). Avoid black cohosh.

15.437 Known potential adverse events

Consult the package insert for the most current and complete information.

Common known potential toxicities, >10%:

Dermatologic: Dermatitis, pruritic maculopapular rash, alopecia.
Gastrointestinal (route and schedule dependent): Heartburn, nausea,

vomiting, anorexia, stomatitis, esophagitis, anorexia, diarrhea. GI toxicity (anorexia, nausea, and vomiting) is generally more severe with continuous-infusion schedules.
Emetic potential: <1000 mg: Moderately low (10% to 30%) ≥ 1000 mg: Moderate (30% to 60%)
Hematologic: Leukopenia; Myelosuppressive (tends to be more pronounced in patients receiving bolus dosing of FU). Decreased white blood cell count with increased risk of infection; decreased platelet count with increased risk of bleeding.
Local: Irritant chemotherapy.

Less common known potential toxicities, 1% - 10%:
Dermatologic: Dry skin
Gastrointestinal: GI ulceration

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Cardiac enzyme abnormalities, chest pain, coagulopathy, dyspnea, ECG changes similar to ischemic changes, hepatotoxicity; hyperpigmentation of nail beds, face, hands, and veins used in infusion; hypotension, palmar-plantar syndrome (hand-foot syndrome), photosensitization. Cerebellar ataxia, headache, somnolence, ataxia are seen primarily in intracarotid arterial infusions for head and neck tumors.

15.438 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

16.0 Statistical Considerations and Methodology

16.1 Study Overview / Rationale

This single-arm Phase 1b/2 study is designed to evaluate the safety and anti-tumor effect of patients with resectable, locally advanced, GEJ/gastric cardia adenocarcinoma, who are treated with pembrolizumab plus weekly carboplatin and paclitaxel, and concurrent daily radiotherapy for approximately 5 weeks, followed by surgery and post-operative pembrolizumab. Fifteen patients will be enrolled for phase 1b portion of the study to conduct a thorough review of adverse event data of pre-operative pembrolizumab. The phase II portion of the study implements a single-arm design based on the primary endpoint of pathological complete response (pathCR) rate. Other endpoints include toxicity profile of pre-operative and post-operative treatment and surgical complications, progression-free survival (PFS), disease free survival (DFS), R0 rate, time-to-relapse (TTR), and overall survival (OS).

16.2 Sample size and accrual time:

A maximum of 34 patients (17-phase I, 17-phase II) will be enrolled to yield a total of 30 evaluable patients (15-phase I, 15-phase II), assuming 10% of patients who are deemed ineligible after registration, withdraw consent before any treatment or are lost-to-follow up prior to resection. If more than the targeted evaluable patients are accrued, the additional patients will not be used in any decision making process; however, they will be included in final estimations of rates and associated confidence interval of primary and secondary endpoints. We estimate that we will need to accrue an additional 11 patients during the pre-registration phase to end up with 34 patients for the registration phase of the trial (17-phase I, 17-phase II). So in summary, we anticipate pre-registering 45 patients to register a total of 34 patients necessary for the study design and allotted over accrual.

16.3 Statistical design for phase 1b portion

16.31 Definition of dose limiting toxicities (DLTs)

We anticipate neoadjuvant carboplatin/paclitaxel/radiotherapy will be well-tolerated when combined with pembrolizumab. However, theoretically, toxicities may arise from an interaction between radiotherapy and pembrolizumab, so as a precaution, safety will be assessed in the phase 1b portion of the study. When the first 15 patients who become evaluable for phase 1b dose limiting toxicities (DLTs), accrual will be halted. A patient is evaluable for phase 1b safety assessment if the patient initiates pre-operative treatment per protocol. We anticipate accruing 17 eligible patients to yield 15 evaluable patients, based on 93% rate of undergoing surgery reported for the chemoRT arm in the CROSS trial. The DLTs during the phase 1b portion are defined as the following:

1. <u>Type 1 DLT:</u>
   Grade 4 (life-threatening) non-hematologic adverse event (AE) during pre-operative chemoradiotherapy that is possibly, probably, or definitely related to pembrolizumab-based chemo(radio)therapy; OR
   A delay in the initiation of neoadjuvant chemotherapy or neoadjuvant radiation by >14 days that is at least possibly due to pembrolizumab toxicity during the neoadjuvant portion of the study; OR
   A delay in surgery such that surgery occurs >12 weeks after the last dose of RT due to toxicities that are at least possibly related to pembrolizumab will

be considered a DLT during the neoadjuvant portion of the study; OR
Any Grade 5 adverse event during neoadjuvant therapy.
NOTE: Patients will be followed for up to 90 days after last dose of neoadjuvant therapy or until surgery, whichever occurs first.

2. Type 2 DLT:
Grade 5 event within 30 days after surgery; OR Grade 4 non-hematologic AE within 30 days after surgery at least possibly related to pembrolizumab, excluding AEs that resolve to Grade 2 or less within 7 days.

3. Type 3 DLT:
Grade 4 non-hematologic adverse event during adjuvant pembrolizumab that is possibly, probably, or definitely related to pembrolizumab; OR
Any Grade 5 adverse event during adjuvant pembrolizumab
NOTE: Patients who received at least one dose of pembrolizumab after surgery will be considered evaluable for a Type 3 DLT.
NOTE: Patients will be followed for a Type 3 DLT for up to 90 days after last dose of adjuvant pembrolizumab.

16.32 Decision rules for continuation of accrual for phase 2 portion

With 15 evaluable patients, the analyses of DLTs will be conducted. Consideration will be made to proceed to the Phase 2 portion if all of the following criteria are met (see above for definition of DLT):

1. ≤4 patients (out of 15 evaluable patients) have a Type 1 DLT
2. ≤2 patients (out of 15 evaluable patients) have a Grade 5 Type 2 DLT
3. ≤6 patients (out of 15 evaluable patients) have a Grade 4 non-hematologic Type 2 DLT
4. ≤3 patients (out of 15 evaluable patients) have a Grade 4 non-hematologic Type 3 DLT
5. ≤1 patients (out of 15 evaluable patients) have a Grade 5 Type 3 DLT

In addition, dose delays and interruptions of chemotherapy and radiation that are at least possibly related to toxicity of the treatment regimen will be considered in the evaluation of safety.

After this evaluation, we will consider resuming accrual, for the phase 2 portion. Patients enrolled for the phase 1b portion who are evaluable for phase 2 primary endpoint evaluation will be included in the phase 2 efficacy decision-making processes, and final endpoint rates estimations.

If the results from safety analysis indicate that safety parameters were not met, data will be analyzed to determine the specific nature and etiologies of safety concerns. After consideration by the study team (study chair[s], statistician, etc.) and the primary IRB, a decision will be made as to whether accrual can be resumed, or modifications to the protocol should be adapted.

We acknowledge that the final decision to move to the Phase 2 portion of the study will rest in the clinical judgment of the investigative team and that mitigating factors such as a patient's decision not to undergo surgery, or the occurrence of severe unexpected complications during neoadjuvant therapy may potentially factor into the decision-making process.

If more than 2 patients experience a Grade 5 event that is at least possibly related to study treatment, the study will not proceed to Phase 2.

16.33 Phase 1b portion study operating characteristics:

Assuming that the occurrence of two types of DLTs are independently distributed with binomial distribution, the probabilities of failing to meet the phase 1b decision rules can be tabulated according to various true rate of the DLTs. See table below:

True rate of type 1 DLT	True rate of type 2 DLT	Probability of failing to meet the phase 1b safety criteria
0.1	0.05	0.0485
0.2	0.05	0.1945
0.3	0.05	0.5032
0.1	0.1	0.1944
0.2	0.1	0.3181
0.3	0.1	0.5794
0.1	0.2	0.6070
0.2	0.2	0.6673
0.3	0.2	0.7948
0.1	0.3	0.8748
0.2	0.3	0.8940
0.3	0.3	0.9346

16.4 Statistical design for phase 2 portion

16.41 Overview

A single-arm design will be implemented for the phase 2 portion. Phase 2 portion patients will receive the same neoadjuvant regimen and surgery as in the Phase 1b portion (pembrolizumab plus weekly carboplatin and paclitaxel, and concurrent daily radiotherapy for approximately 5 weeks, followed by surgery and post-operative pembrolizumab).

The CROSS trial was the largest trial, to date, examining neoadjuvant carboplatin/paclitaxel/RT therapy in esophageal/GEJ adenocarcinoma patients2. This trial established this regimen as a new standard of care in this indication. In CROSS, of 180 patients assigned to chemoRT/surgery, 161 patients underwent resection, and 148 patients had an R0 resection. Primary analysis included only those patients who were assigned to the chemoRT arm and who did not withdraw consent. A total of 178 patients were included primary analysis (2 patients of the 180 patients total withdrew consent prior to initiating chemoRT), among which 134 patients had adenocarcinoma histology. Among the 134 adenocarcinoma patients assigned to the chemoRT/surgery arm included in primary analysis, 21% (28/134) had a pathCR. (It is important to distinguish histologic subtypes, since the pathCR rate among SCC patients was significantly higher [~49%].) The number of adenocarcinoma patients who initiated chemoRT, underwent surgery, or underwent resection, was not reported. As a second reference, in Burmeister et al (see below), 80 eligible patients with esophageal/GEJ adenocarcinoma were assigned to cisplatin/5FU-based chemoRT (35 Gy), of whom 6 patients had a pathCR, yielding a pathCR rate of 7.5% (6/80) with the denominator being all eligible patients assigned to chemoRT regardless of whether treatment was initiated.

Based on these data from CROSS, we conservatively selected a pathCR rate of 21% as our historical control, with the denominator being eligible patients who

do not withdraw consent, as it is the most reliable available estimate of the reference pathCR rate in this patient population. The addition of neoadjuvant pembrolizumab is expected to improve the pathCR rate by 27% or more in this population, to yield a pathCR rate of 48% or more.

16.42 Primary endpoint

Pathological complete responses (pathCR): is defined as number of patients with pathologic complete responses (see definition in section 11.6) divided by total evaluable patients.

All phase II patients meeting the eligibility criteria that have signed a consent form and have begun protocol treatment will be considered evaluable for pathCR.

16.43 Study design and decision rules

The phase 2 portion is powered to detect an improvement in the pathCR rate of 27% (i.e. achieving pathCR rate of at least 48% for the alternative hypothesis) compared to historical control of 21%, which is the null hypothesis. A total of 15 evaluable patients provides a power of 81% to claim the proposed regimen warrants further study at one-sided significance level of 0.08, if at least 6 patients achieve pathCR (40% observed pathCR rate). We acknowledge the limitations of pathCR in reflecting the anti-tumor efficacy of pembrolizumab. If fewer than 6 patients achieve pathCR, the decision for further study will be discussed by the study team, after analysis of safety and toxicity data, PFS and other endpoints.

16.44 Power and significance level

Assuming the number of patients with pathCR is binomially distributed, the operating characteristics of current design can be tabulated according to various true rates of pathCR (see table below).

True pathCR rate	**Probability of declaring that the proposed regimen warrants further study is…**
0.21	0.08
0.30	0.28
0.40	0.60
0.45	0.74
0.48	0.81

16.45 Analysis plan

Pathological complete responses (pathCR) rate: The pathCR rate will be estimated by the number of patients who achieve pathCR divided by the total evaluable number of patients. Ninety percent two-sided confidence interval will be calculated according to the exact binomial method.

16.5 Analyses plan for secondary endpoints:

16.51 Progression-free survival (PFS)

Progression-free survival is defined as the time from the date of study registration to the date of death due to all causes, recurrences if R0 resections are achieved, progression disease before undergoing surgery, or R1/R2 resection at surgery, whichever occurs first, among evaluable patients. The distribution of PFS will be estimated using the method of Kaplan-Meier. Two-year PFS rate and confidence interval will be estimated based on Kaplan-Meier curve.

16.52 Overall survival (OS)

Overall survival is defined as the time from the date of study registration to the date of death due to all causes, among evaluable patients. The distribution of OS will be estimated using the method of Kaplan-Meier. Two-year OS rate and confidence interval will be estimated based on Kaplan-Meier curve.

16.53 Disease free survival (DFS)

DFS is defined as the time from the date of study registration to the date of death due to all causes or recurrence, whichever occurs first, among patients who achieved an R0 resection. The distribution of DFS will be estimated using the method of Kaplan-Meier. Two-year DFS rate and confidence interval will be estimated based on Kaplan-Meier curve.

16.54 Time-to-relapse (TTR)

TTR is defined as the time from the date of study registration to the date of 1st documented relapse/recurrence among patients who achieve R0 resection. The distribution of TTR will be estimated using the method of Kaplan-Meier. Two-year relapse-free rate and confidence interval will be estimated based on Kaplan-Meier curve.

16.55 R0 rate

R0 rate is defined as the number of patients who achieve R0 resection divided by total number of evaluable patients.

16.56 Adverse events

The maximum grade for each type of adverse events that are possibly, probably or definitely related to study treatments will be recorded for each patient. The frequency tables will be reviewed to determine the patterns.

16.6 Data & Safety Monitoring

16.61 Review

The principal investigator(s) and the study statistician will review the study at least twice a year to identify accrual, adverse event, and any endpoint problems that might be developing. The trial is monitored continually by the study team who are notified of every Grade 4 and 5 event in real time. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office.

16.62 Adverse Event Stopping Rules

The stopping rules specified below are based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the intervention(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below. CTCAE v4.0 will be used to determine grading for these stopping rules.

Accrual will be temporarily suspended to this study if at any time we observe adverse events that satisfy any of the following during the Phase II portion of the trial:

- If at any time 33% or more of patients, including the patients from the phase 1b, experience a Grade 4 or 5 non-hematologic adverse event (AE) during neoadjuvant therapy that is possibly, probably, or definitely related to pembrolizumab-based chemo(radio)therapy. (Type 1 DLT, where patients will be followed for a Type 1 DLT (ie, during neoadjuvant therapy) for up to 90 days after last dose of neoadjuvant therapy or until surgery, whichever occurs first)
- If at any time 14% or more of patients, including the patients from the phase 1b, experience a Grade 5 event within 30 days after surgery that is possibly, probably, or definitely related pembrolizumab-based chemo(radio)therapy. (Type 2 DLT)
- If at any time 54% or more of patients, including the patients from the phase 1b, experience a grade 4 non-hematologic AE within 30 days after surgery at least possibly related to pembrolizumab, excluding AEs that resolve to grade 2 or less within 7 days. (Type 2 DLT)
- If at any time 25% or more of patients, including the patients from the phase 1b, experience a Grade 4 non-hematologic adverse event during adjuvant pembrolizumab that is possibly, probably, or definitely related pembrolizumab. (Grade 4 non-hematologic Type 3 DLT)
- If at any time 10% or more of patients, including the patients from the phase 1b, experience a Grade 5 adverse event during adjuvant pembrolizumab that is possibly, probably, or definitely related pembrolizumab. (Grade 5 Type 3 DLT)

We note that we will review all Grade 5 adverse events on a case-by-case basis as well (regardless of attribution), and may suspend accrual after just one Grade 5 event, if we feel it is necessary for patient safety.

16.7 Inclusion of Women and Minorities

16.71 Study availability

This study will be available to all eligible patients, regardless of race, gender, or ethnic origin.

16.72 Statistical analysis by subset

There is no information currently available regarding differential effects of study regimens in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.73 Regional population

Based on prior studies involving similar disease sites, we expect about 10% patients will be classified as minorities by race and about 12% of patients to be women. Expected size of racial by gender subsets are shown in the following table:

	Sex/Gender			
Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino	0	3	0	3
Not Hispanic or Latino	4	27	0	31
Ethnic Category: Total of all subjects	**4**	**30**	**0**	**34**
Racial Category				
American Indian or Alaskan Native	0	2	0	2
Asian	0	0	0	0
Black or African American	0	1	0	1
Native Hawaiian or other Pacific Islander	0	0	0	0
White	4	27	0	31
Racial Category: Total of all subjects	**4**	**30**	**0**	**34**

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Tissue Biospecimen Submission

17.11 Summary Table of Tissue Biospecimens for This Protocol

Type of tissue biospecimen to submit	Mandatory or optional	When to submit	Reason for submission (background/methodology section)	Where to find specific details for biospecimen submission
ALL prior to treatment diagnostic slides from original biopsy or cytologic tissue	Mandatory	As soon as possible after biopsy, prior to enrollment	Confirmation of tissue evaluability through central review (Section 17.21)	Section 17.21
FFPE tissue blocks with corresponding H&E, OR unstained slides with corresponding H&E, from original biopsy	Mandatory*	As soon as possible after biopsy, prior to enrollment	Correlative studies (Section 17.5)	Section 17.321
ALL diagnostic slides from surgically resected tissue post-treatment	Mandatory	≤42 days after surgical resection	Confirmation of determination of pathCR through central review and (Section 17.21)	Section 17.23
FFPE tissue blocks with corresponding H&E, OR unstained slides with corresponding H&E, from surgical resection	Mandatory	≤42 days after surgical resection	Correlative studies (Section 17.5)	Section 17.322
Recurrence/PD –any type of tissue available (See Section 17.31)	Optional	≤30 days after biopsy	Correlative studies (Section 17.5)	Section 17.32

*If an outside institution is not able to provide the mandatory tissue, it is mandatory that a new attempt be made to collect adequate tissue at Mayo Clinic (see Section 3). However, if adequate tissue is not obtained after an additional attempt, then the patient will be allowed to enroll. (Only one EGD with primary tumor biopsy performed at Mayo Clinic ≤8 weeks prior to pre-registration is required).

FFPE = Formalin-fixed paraffin-embedded

17.2 Required materials for confirmation of eligibility (Section 17.21) **AND** from surgical resection (Section 17.22).

17.21 **Original biopsy or cytological specimen** - ALL diagnostic slides used to make the diagnosis of adenocarcinoma of the gastroesophageal junction, or gastric cardia should be clearly labeled and forwarded as soon as possible after surgery for pre-registrationcentral review. If the original slides cannot be released, slides from the same tumor biopsy block used to make the diagnosis are acceptable.

The following materials below are mandatory (unless indicated otherwise) and required for shipment:

- All diagnostic slides from original biopsy or cytologic specimen
- Pathology Reporting Form
- Tissue Submission Form
- Surgical Pathology Report
- Operative Report *(optional)*

Note: Please include the patient study ID number on all materials listed above.

17.22 New EGD and biopsy - For pinch biopsies from new EGD, a minimum of eight (8) tissue pieces from the primary tumor must be submitted. Please attempt to submit all tissue in one (1) container.

17.23 **Surgical resection** - ALL diagnostic slides used to make the diagnosis of adenocarcinoma of the gastroesophageal junction, or gastric cardia should be clearly labeled and forwarded ≤42 days after surgery for central review and assessment of pathological response. If the original slides cannot be released, slides from the same tumor block used to make the diagnosis are acceptable.

The following materials below are mandatory (unless indicated otherwise) and required for shipment:

- All diagnostic slides from surgical resection
- Pathology Reporting Form
- Tissue Submission Form
- Surgical Pathology Report
- Operative Report *(optional)*

Note: Please include the patient study ID number on all materials listed above.

17.24 The diagnostic slide(s) must be appropriately packed to prevent damage (e.g., slides should be placed in appropriate slide container) and placed in an individual plastic bag. Label the bag with the protocol number, patient study ID number, and patient initials.

17.25 Verify that Section 1 of the appropriate Pathology Reporting Form is completed and filled in correctly.

17.26 Review is being performed at Mayo Clinic in Rochester. Please ship specimens and appropriate materials as indicated below:

17.261 Mayo Clinic Rochester patients only – Please forward pathology materials to [redacted] for review.
NOTE: Notify [redacted] using email template found in Cancer Center Protocol Catalog and CC: [redacted] Copy template into email body – **DO NOT ATTACH.**
[redacted]

17.262 For Mayo Clinic FL and Mayo Clinic AZ – Ship all specimens and accompanying materials to the MCCC Research Base at the following address: **MCCC Operations Office Attn: PC Office (Study MC1541)** [redacted] **Rochester, MN 55905.**

17.3 Paraffin embedded tissue blocks/slides

17.31 The FFPE tumor tissue block is preferred. However, **if an institution is unable to provide the biopsy or surgical resection tissue block**, cut sections of five-micron thickness and mount sections on charged glass slides. The precise number of sections depends on the type of block; see Section 17.5 for details. Label the slides with **patient study ID number, accession number, order of sections cut,**

date sections were cut, and thickness of section. The H&E stain should be labeled as such and will count as Slide 1. This H&E slide will be reviewed centrally under the research base's protocol for assessing tissue quality. The remaining unstained slides will be processed as described in Section 17.5. For samples containing less than 7 square millimeters of tumor tissue, multiple sections should be mounted onto each slide to ensure that the appropriate amount of tumor tissue is available. Ideally, each slide must have a minimum of 75% tumor tissue on the slide to be deemed adequate for study. **Do not bake or place cover slips on the slides**.

Note: For the biopsy specimen, the participating institution may provide fewer sections from the biopsy block but only if insufficient tissue will remain for clinical purposes. **A minimum of eighteen (18) unstained slides of 5-micron thickness.**

17.32 The following materials at the indicated time points below are mandatory (unless indicated otherwise) and required for shipment.

17.321 Baseline Biopsy Tissue (mandatory):

- Paraffin embedded tissue blocks with corresponding H&E slide (OR unstained sections with corresponding H&E as outlined in Section 17.31)
- Research Tissue Submission Form
- Surgical Pathology Report
- Operative Report *(optional)*

Note: Please include the patient study ID number on all materials listed above.

17.322 Surgical Resection Tissue (mandatory):

- Paraffin embedded tissue block with corresponding H&E slide (OR unstained slides with corresponding H&E as outlined in Section 17.31)
- Research Tissue Submission Form
- Surgical Pathology Report
- Operative Report *(optional)*

Note: Please include the patient study ID number on all materials listed above.

17.323 Recurrence Biopsy Tissue (optional):

- Paraffin embedded tissue blocks with corresponding H&E slide (OR unstained sections with corresponding H&E as outlined in Section 17.31)
- Research Tissue Submission Form
- Surgical Pathology Report
- Operative Report *(optional)*

Note: Please include the patient study ID number on all materials listed above.

17.33 The block/slides must be appropriately packed to prevent damage (e.g., slides should be placed in appropriate slide container) and placed in an individual plastic bag. Label the bag with the protocol number, patient study ID number, and patient initials.

17.34 Baseline tissue specimens must be shipped ≤30 days after registration and surgical resection tissue specimens must be shipped ≤42 days after surgery.

17.35 Verify that the appropriate sections of the Tissue Specimen Submission Form are completed and filled in correctly. Enter information from the Tissue Specimen Submission Form into the remote data entry system on the same day the specimen is submitted (see Forms Packet). A hard copy of the form must be submitted with the tissue.

17.37 Ship all block/slide tissue specimens and accompanying materials to the Mayo Clinic Research Base:

Mayo Clinic Cancer Center Operations Office
Attn: [redacted]
Rochester, MN 55905

If a corresponding H&E wasn't submitted with the block/slides, the Mayo Clinic Cancer Center Operations Office will request a slide to be processed (i.e., cut and H&E stained) from the tumor tissue block and forwarded to [redacted], to be reviewed under the research base's protocol for assessing tissue quality for the proposed correlative studies, unless the tumor size is too small. If the tumor tissue is too small, assessment of tissue quality will occur at the time the translational studies are performed.

17.4 Frozen Tumor Tissue – None

17.5 Study Methodology and Storage Information

It is understood that pre-operative biopsy material quantity may be limited and post-operative material quantity/quality may not be optimal; therefore, the following correlative studies will be prioritized after accrual is complete and based on the current state of the science at that time. Tissue samples will be analyzed as follows:

17.51 Pre-treatment biopsy (total 25 sections: Slides 1-25): Order of priority for utilizing the unstained slides.

1) Microsatellite instability (MSI), mutational load, genomic profile (e.g., Foundation One assay) (8 unstained slides, each of 5 mm thickness, +1 slide for H&E) – Testing will be performed by Foundation Medicine or TEMPUS
2) PDL1 IHC (4 unstained slides +1 slide for H&E) – Testing will be performed in PRC or Merck-associated lab
3) BIM/PD-1 expression in tumor infiltrating T cells using double-stained IHC (1 unstained slide +1 slide for H&E). Testing will be performed in [redacted] lab.
4) EBV ISH (4 unstained slides [1 of which includes H&E]) – Samples will be sent to Immunostains Lab (supervisor [redacted] Building)
5) CD3, CD8, CD45, granzyme B by IHC (4 unstained slide + 1 H&E) - Testing will be performed in [redacted]

17.52 Post-treatment surgical resection (total 12 sections: Slides 1-12):

1) PDL1 IHC (4 unstained slides +1 slide for H&E) – Testing will be performed in PRC or Merck-associated lab

2) BIM/PD-1 expression in tumor infiltrating T cells using double-stained IHC (1 unstained slide +1 slide for H&E). Testing will be performed in [redacted] lab.
3) CD3, CD8, CD45, granzyme B by IHC (4 unstained slide + 1 H&E) - Testing will be performed in [redacted] lab.

17.53 Recurrent tissue (if collected; total 12 sections: Slides 1-12):

1) PDL1 IHC (4 unstained slides +1 slide for H&E) – Testing will be performed in PRC or Merck-associated lab
2) BIM/PD-1 expression in tumor infiltrating T cells using double-stained IHC (1 unstained slide +1 slide for H&E). Testing will be performed in [redacted] lab.
3) CD3, CD8, CD45, granzyme B by IHC (4 unstained slide + 1 H&E) - Testing will be performed in [redacted] lab.

17.54 The institutional pathologist will be notified by the Operations Office (Pathology Coordinator) if the block may be depleted.

17.55 Blocks requested to accommodate individual patient management will be returned promptly upon request.

17.6 Return of Genetic Testing Research Results

No genetic specimens will be collected from tissue biospecimens for this study. If future genetic testing is being requested for stored tissue, patient reconsent is required.

18.0 Records and Data Collection Procedures

18.1 Submission Timetables

18.11 Pre-Registration Material(s)

Case Report Form (CRF)	**Pre-Screen Phase** (No active testing)
Pre-Registration Screening Failure	Complete only if patient is NOT registered after he/she is pre-registered
Pathology Materials – (See Section 17.0)	X

18.12 Initial Material(s)

Case Report Form (CRF)	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)
On-Study	≤2 weeks after registration
Adverse Event - Baseline	
Reports	
Research Blood Submission – Baseline (see Section 14.0)	
Research Tissue Submission (see Section 17.0)	
Laboratory - Chemistry	
Laboratory – Thyroid Function	
End of Active Treatment/Cancel Notification	Submit ≤2 weeks after registration if withdrawal/refusal occurs prior to beginning protocol therapy

18.13 Test Schedule Material(s)

CRF	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)		**Observation**
	At each evaluation during treatment	At end of treatment	Follow-up visits
Evaluation/Treatment Phase 1b/Phase 2[1] (Neoadjuvant)	X	X	
Evaluation/Treatment Phase 1b/Phase 2[2] (Adjuvant)	X	X	
Adverse Event	X	X	X
Pre-Surgery Measurement	X	X	
Pre-Surgery Confirmation Measurement	X	X	
Laboratory - Chemistry	X	X	X
Laboratory – Thyroid Function[2]	X	X	X
Measurement	X	X	X
Surgical	X		
Pathology Materials (See Section 17)	X		
RT Material	X		

CRF	Active-Monitoring Phase (Compliance with Test Schedule Section 4.0)		Observation
	At each evaluation during treatment	At end of treatment	Follow-up visits
Evaluation/Observation[3]			X
Reports	X[4]	X[4]	
Research Blood Submission (see Section 14.0)	X[5]	X[5]	
Research Tissue Submission Mandatory (see Section 17.0)	X[5]		
Research Tissue Submission - Optional - At PD (see Section 17.0)	X[5]	X[5]	
End of Active Treatment/Cancel Notification		X	
ADR/AER (see Section 10.0)	At each occurrence		

1. Complete at each evaluation during Neoadjuvant Active Treatment (see Section 4.0).
2. Complete at each evaluation during Adjuvant Active Treatment (see Section 4.0).
3. Complete at each evaluation during Observation (see Section 4.0).
4. Submission of these reports is only required for documentation of recurrence. For documentation of recurrence, submit one report for one of the measures where recurrence was seen. Attention: QAS for MC1541.
5. Only when required by the Test Schedule (see Section 4.0).

18.14 Follow-up Material(s)

CRF	Event Monitoring Phase[1]				
	q. 6 months until PD or subsequent treatment[2]	At PD or subsequent treatment	q. 6 months after PD or subsequent treatment	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

1. If a patient is still alive 3 years after registration, no further follow-up is required.
2. Submit copy of documentation of progression to Attention: [redacted] Rochester, MN 55905; Fax [redacted]

19.0 Budget

19.1 Costs charged to patient

Routine clinical care costs will be the responsibility of the patient and/or the patient's insurance company. This responsibility includes costs associated with surgery and with the administration of standard chemotherapy (carboplatin/paclitaxel or FOLFOX), and radiotherapy. These drugs are commercially available and will be the responsibility of the patient and/or the patient's insurance company.

19.2 Costs to be research funded

- Correlative studies
- Pembrolizumab, including its administration
- Collection, processing and storage of blood and tissue samples for future, unspecified research.

20.0 Reference

1. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst 2005;97:142-6.
2. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. The New England journal of medicine 2012;366:2074-84.
3. Sudo K, Taketa T, Correa AM, et al. Locoregional failure rate after preoperative chemoradiation of esophageal adenocarcinoma and the outcomes of salvage strategies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:4306-10.
4. Shiozaki H, Sudo K, Xiao L, et al. Distribution and timing of distant metastasis after local therapy in a large cohort of patients with esophageal and esophagogastric junction cancer. Oncology 2014;86:336-9.
5. Tang C, Wang X, Soh H, et al. Combining radiation and immunotherapy: a new systemic therapy for solid tumors? Cancer Immunol Res 2014;2:831-8.
6. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 1999;11:141-51.
7. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science 2001;291:319-22.
8. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol 2004;4:336-47.
9. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. The New England journal of medicine 2015;372:311-9.
10. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. The New England journal of medicine 2013;369:134-44.
11. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563-7.
12. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515:558-62.
13. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:1020-30.
14. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine 2012;366:2455-65.
15. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443-54.
16. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2014;20:5064-74.
17. Duad A, Ribas A, Robert C, al e. Long-term efficacy of pembrolizumab (pembro; MK-3475) in a pooled analysis of 655 patients (pts) with advanced melanoma (MEL) enrolled in KEYNOTE-001. . J Clin Oncol 2015;33:Abst 9005.
18. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. The Lancet Oncology 2015.
19. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. The New England journal of medicine 2015;372:2521-32.
20. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung

cancer. The New England journal of medicine 2015;372:2018-28.
21. Muro K, Bang Y-J, Shankaran V, et al. Relationship between PD-L1 expression and clinical outcomes in patients (Pts) with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (Pembro; MK-3475) in KEYNOTE-012. J Clin Oncol 2015;33:Abstract 3.
22. Yoon HH, Gibson MK. Combined-modality therapy for esophageal and gastroesophageal junction cancers. Curr Oncol Rep 2007;9:184-92.
23. Kleinberg L, Forastiere AA. Chemoradiation in the management of esophageal cancer. J Clin Oncol 2007;25:4110-7.
24. Morrison CD, Parvani JG, Schiemann WP. The relevance of the TGF-beta Paradox to EMT-MET programs. Cancer Lett 2013;341:30-40.
25. Andarawewa KL, Erickson AC, Chou WS, et al. Ionizing radiation predisposes nonmalignant human mammary epithelial cells to undergo transforming growth factor beta induced epithelial to mesenchymal transition. Cancer Res 2007;67:8662-70.
26. Jung JW, Hwang SY, Hwang JS, Oh ES, Park S, Han IO. Ionising radiation induces changes associated with epithelial-mesenchymal transdifferentiation and increased cell motility of A549 lung epithelial cells. Eur J Cancer 2007;43:1214-24.
27. Baschnagel AM, Williams L, Hanna A, et al. c-Met expression is a marker of poor prognosis in patients with locally advanced head and neck squamous cell carcinoma treated with chemoradiation. Int J Radiat Oncol Biol Phys 2014;88:701-7.
28. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 2013;1:365-72.
29. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. The New England journal of medicine 2012;366:925-31.
30. Demaria S, Kawashima N, Yang AM, et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2005;11:728-34.
31. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:5379-88.
32. Chakravarty PK, Guha C, Alfieri A, et al. Flt3L therapy following localized tumor irradiation generates long-term protective immune response in metastatic lung cancer: its implication in designing a vaccination strategy. Oncology 2006;70:245-54.
33. Lu P, Wang YL, Linsley PS. Regulation of self-tolerance by CD80/CD86 interactions. Curr Opin Immunol 1997;9:858-62.
34. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. The Journal of experimental medicine 2006;203:1259-71.
35. Zhang B, Bowerman NA, Salama JK, et al. Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. The Journal of experimental medicine 2007;204:49-55.
36. Frey B, Rubner Y, Wunderlich R, et al. Induction of abscopal anti-tumor immunity and immunogenic tumor cell death by ionizing irradiation - implications for cancer therapies. Curr Med Chem 2012;19:1751-64.
37. Brody JD, Ai WZ, Czerwinski DK, et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:4324-32.
38. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors

(abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 2004;58:862-70.
39. Liang H, Deng L, Chmura S, et al. Radiation-induced equilibrium is a balance between tumor cell proliferation and T cell-mediated killing. J Immunol 2013;190:5874-81.
40. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. The Journal of clinical investigation 2014;124:687-95.
41. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 1999;163:5211-8.
42. Hiniker SM, Chen DS, Reddy S, et al. A systemic complete response of metastatic melanoma to local radiation and immunotherapy. Transl Oncol 2012;5:404-7.
43. Camphausen K, Moses MA, Menard C, et al. Radiation abscopal antitumor effect is mediated through p53. Cancer Res 2003;63:1990-3.
44. Demaria S, Formenti SC. Radiation as an immunological adjuvant: current evidence on dose and fractionation. Front Oncol 2012;2:153.
45. Blanquicett C, Saif MW, Buchsbaum DJ, et al. Antitumor efficacy of capecitabine and celecoxib in irradiated and lead-shielded, contralateral human BxPC-3 pancreatic cancer xenografts: clinical implications of abscopal effects. Clinical cancer research : an official journal of the American Association for Cancer Research 2005;11:8773-81.
46. Shiraishi K, Ishiwata Y, Nakagawa K, et al. Enhancement of antitumor radiation efficacy and consistent induction of the abscopal effect in mice by ECI301, an active variant of macrophage inflammatory protein-1alpha. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:1159-66.
47. Chakravarty PK, Alfieri A, Thomas EK, et al. Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer. Cancer Res 1999;59:6028-32.
48. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature 1998;392:86-9.
49. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clinical cancer research : an official journal of the American Association for Cancer Research 2012;18:6580-7.
50. Verbrugge I, Hagekyriakou J, Sharp LL, et al. Radiotherapy increases the permissiveness of established mammary tumors to rejection by immunomodulatory antibodies. Cancer Res 2012;72:3163-74.
51. Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 2013;86:343-9.
52. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A 2004;101:17174-9.
53. Ghebeh H, Mohammed S, Al-Omair A, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 2006;8:190-8.
54. Geng R, Dai C, Wong A, et al. Prognostic significance of tumor infiltrating immune cells and PD-L1 expression in gastric carcinoma in Chinese patients. J Clin Oncol 2015;33:abstract 4042.
55. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. The New England journal of medicine 2013;369:122-33.
56. Morgan GW, Breit SN. Radiation and the lung: a reevaluation of the mechanisms mediating pulmonary injury. Int J Radiat Oncol Biol Phys 1995;31:361-9.
57. Roberts CM, Foulcher E, Zaunders JJ, et al. Radiation pneumonitis: a possible lymphocyte-mediated hypersensitivity reaction. Ann Intern Med 1993;118:696-700.
58. Carver JR, Shapiro CL, Ng A, et al. American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. Journal of clinical oncology : official journal of the American Society of Clinical Oncology

2007;25:3991-4008.
59. McDonald S, Rubin P, Phillips TL, Marks LB. Injury to the lung from cancer therapy: clinical syndromes, measurable endpoints, and potential scoring systems. Int J Radiat Oncol Biol Phys 1995;31:1187-203.
60. Auperin A, Le Pechoux C, Rolland E, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:2181-90.
61. Roach M, 3rd, Gandara DR, Yuo HS, et al. Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1995;13:2606-12.
62. De Ruysscher D, Faivre-Finn C, Nestle U, et al. European Organisation for Research and Treatment of Cancer recommendations for planning and delivery of high-dose, high-precision radiotherapy for lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:5301-10.
63. van Baardwijk A, Wanders S, Boersma L, et al. Mature results of an individualized radiation dose prescription study based on normal tissue constraints in stages I to III non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1380-6.
64. Dehing-Oberije C, De Ruysscher D, van Baardwijk A, Yu S, Rao B, Lambin P. The importance of patient characteristics for the prediction of radiation-induced lung toxicity. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 2009;91:421-6.
65. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. The New England journal of medicine 2010;363:711-23.
66. Ronellenfitsch U, Schwarzbach M, Hofheinz R, et al. Perioperative chemo(radio)therapy versus primary surgery for resectable adenocarcinoma of the stomach, gastroesophageal junction, and lower esophagus. The Cochrane database of systematic reviews 2013;5:CD008107.
67. Donahue JM, Nichols FC, Li Z, et al. Complete pathologic response after neoadjuvant chemoradiotherapy for esophageal cancer is associated with enhanced survival. Ann Thorac Surg 2009;87:392-8; discussion 8-9.
68. Kleinberg L, Powell ME, Forastiere A, Keller S, Anne P, Benson AB. E1201: An Eastern Cooperative Oncology Group (ECOG) randomized phase II trial of neoadjuvant preoperative paclitaxel/cisplatin/RT or irinotecan/cisplatin/RT in endoscopy with ultrasound (EUS) staged adenocarcinoma of the esophagus. *Journal of Clinical Oncology* ASCO Annual Meeting Proceedings Part I 2007;25:4533.
69. Burmeister BH, Smithers BM, Gebski V, et al. Surgery alone versus chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: a randomised controlled phase III trial.[see comment]. Lancet Oncology 2005;6:659-68.
70. Alberts SR, Soori GS, Shi Q, et al. Randomized phase II trial of extended versus standard neoadjuvant therapy for esophageal cancer, NCCTG (Alliance) trial N0849. J Clin Oncol 2013;31.
71. Ajani JA, Xiao L, Roth JA, et al. A phase II randomized trial of induction chemotherapy versus no induction chemotherapy followed by preoperative chemoradiation in patients with esophageal cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2013;24:2844-9.
72. Yoon HH, Forastiere AA. Locally advanced esophageal adenocarcinoma: current standards and molecular predictors of outcome. Future Oncol 2008;4:413-25.
73. Cunningham D, Allum WH, Stenning SP, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006;355:11-20.
74. MRC G. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet 2002;359:1727-33.

75. Ychou M, Boige V, Pignon JP, et al. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:1715-21.
76. Alderson D, Langley RE, Nankivell MG, et al. Neoadjuvant chemotherapy for resectable oesophageal and junctional adenocarcinoma: results from the UK Medical Research Council randomised OEO5 trial (ISRCTN 01852072). J Clin Oncol 2015;33:abstr 4002.
77. Kelsen DP, Ginsberg R, Pajak TF, et al. Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. N Engl J Med 1998;339:1979-84.
78. Kelsen DP, Winter KA, Gunderson LL, et al. Long-term results of RTOG trial 8911 (USA Intergroup 113): a random assignment trial comparison of chemotherapy followed by surgery compared with surgery alone for esophageal cancer. J Clin Oncol 2007;25:3719-25.
79. Cunningham D, Okines AF, Ashley S. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2010;362:858-9.
80. Conroy T, Galais MP, Raoul JL, et al. Definitive chemoradiotherapy with FOLFOX versus fluorouracil and cisplatin in patients with oesophageal cancer (PRODIGE5/ACCORD17): final results of a randomised, phase 2/3 trial. The Lancet Oncology 2014;15:305-14.
81. Stewart R, Morrow M, Hammond SA, et al. Identification and Pre-clinical Characterization of MEDI4736, an Antagonistic anti-PD-L1 Monoclonal Antibody. Cancer Immunol Res 2015.
82. El-Rayes BF, Shields A, Zalupski M, et al. A phase II study of carboplatin and paclitaxel in esophageal cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2004;15:960-5.
83. Polee MB, Sparreboom A, Eskens FA, et al. A phase I and pharmacokinetic study of weekly paclitaxel and carboplatin in patients with metastatic esophageal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2004;10:1928-34.
84. Gadgeel SM, Shields AF, Heilbrun LK, et al. Phase II study of paclitaxel and carboplatin in patients with advanced gastric cancer. Am J Clin Oncol 2003;26:37-41.
85. Philip PA, Zalupski MM, Gadgeel S, Hussain M, Shields A. A phase II study of carboplatin and paclitaxel in the treatment of patients with advanced esophageal and gastric cancer. Semin Oncol 1997;24:S19-86-S19-88.
86. Stathopoulos GP, Rigatos SK, Fountzilas G, Polyzos A, Stathopoulos JG. Paclitaxel and carboplatin in pretreated advanced gastric cancer: a phase II study. Oncol Rep 2002;9:89-92.
87. Chang HM, Kim TW, Ryu BY, et al. Phase II study of paclitaxel and carboplatin in advanced gastric cancer previously treated with 5-fluorouracil and platinum. Jpn J Clin Oncol 2005;35:251-5.
88. Lu L, Xu X, Zhang B, Zhang R, Ji H, Wang X. Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. J Transl Med 2014;12:36.
89. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-9.
90. Kim SY, Park C, Kim HJ, et al. Deregulation of immune response genes in patients with Epstein-Barr virus-associated gastric cancer and outcomes. Gastroenterology 2015;148:137-47 e9.
91. Haas M, Buttner M, Rau TT, Fietkau R, Grabenbauer GG, Distel LV. Inflammation in gastric adenocarcinoma of the cardia: how do EBV infection, Her2 amplification and cancer progression influence tumor-infiltrating lymphocytes? Virchows Archiv : an international journal of pathology 2011;458:403-11.
92. Lin SJ, Gagnon-Bartsch JA, Tan IB, et al. Signatures of tumour immunity distinguish Asian and non-Asian gastric adenocarcinomas. Gut 2014.
93. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England journal of medicine 2015;372:2509-20.

94. van Grieken NC, Aoyama T, Chambers PA, et al. KRAS and BRAF mutations are rare and related to DNA mismatch repair deficiency in gastric cancer from the East and the West: results from a large international multicentre study. Br J Cancer 2013;108:1495-501.
95. Yoon HH, Orrock JM, Foster NR, Sargent DJ, Smyrk TC, Sinicrope FA. Prognostic impact of FoxP3+ regulatory T cells in relation to CD8+ T lymphocyte density in human colon carcinomas. PLoS One 2012;7:e42274.
96. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.
97. Pages F, Berger A, Camus M, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005;353:2654-66.
98. Lee HE, Chae SW, Lee YJ, et al. Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 2008;99:1704-11.
99. Kim KJ, Lee KS, Cho HJ, et al. Prognostic implications of tumor-infiltrating FoxP3+ regulatory T cells and CD8+ cytotoxic T cells in microsatellite-unstable gastric cancers. Hum Pathol 2014;45:285-93.
100. Zhuang Y, Peng LS, Zhao YL, et al. CD8(+) T cells that produce interleukin-17 regulate myeloid-derived suppressor cells and are associated with survival time of patients with gastric cancer. Gastroenterology 2012;143:951-62 e8.
101. Kluger HM, Zito CR, Barr ML, et al. Characterization of PD-L1 Expression and Associated T-cell Infiltrates in Metastatic Melanoma Samples from Variable Anatomic Sites. Clinical cancer research : an official journal of the American Association for Cancer Research 2015;21:3052-60.
102. Frigola X, Inman BA, Lohse CM, et al. Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2011;17:1915-23.
103. Schmits R, Kundig TM, Baker DM, et al. LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. The Journal of experimental medicine 1996;183:1415-26.
104. Liu X, Gibbons RM, Harrington SM, et al. Endogenous tumor-reactive CD8 T cells are differentiated effector cells expressing high levels of CD11a and PD-1 but are unable to control tumor growth. Oncoimmunology 2013;2:e23972.
105. Park SS, Dong H, Liu X, et al. PD-1 Restrains Radiotherapy-Induced Abscopal Effect. Cancer Immunol Res 2015;3:610-9.
106. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012;12:298-306.
107. Acharyya S, Oskarsson T, Vanharanta S, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell 2012;150:165-78.
108. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.
109. Duraiswamy J, Freeman GJ, Coukos G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. Cancer Res 2013;73:6900-12.
110. Gustafson MP, Lin Y, Maas ML, et al. A method for identification and analysis of non-overlapping myeloid immunophenotypes in humans. PLoS One 2015;10:e0121546.
111. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:7412-20.
112. Burmeister BH, Smithers BM, Gebski V, et al. Surgery alone versus chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: a randomised controlled phase III

trial. The lancet oncology 2005;6:659-68.
113. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. Clinical cancer research : an official journal of the American Association for Cancer Research 2013;19:3936-43.
114. Nishino M, Gargano M, Suda M, Ramaiya NH, Hodi FS. Optimizing immune-related tumor response assessment: does reducing the number of lesions impact response assessment in melanoma patients treated with ipilimumab? J Immunother Cancer 2014;2:17.
115. Bohnsack O, Ludajic K, Hoos A. Adaptation of the immune-related response criteria: irRECIST. ESMO 2014:Abstr 4958; Accessed on 7/10/2015: https://www.parexel.com/files/9214/1449/9078/Adaptation_of_the_Immune_Related_Response_Criteria_irRECIST.pdf.

Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

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

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

Appendix II Surgical Guidelines for Esophagectomy

Procedure Type: Esophageal malignancies located in the upper esophagus (above azygous vein) will be removed by either an "Extended Ivor Lewis" approach utilizing a combined abdominal, right chest, and neck incision; or transhiatal resection. For lower mid-esophageal and gastroesophageal malignancies, either an "Ivor-Lewis" procedure (abdominal incision and right thoracotomy) or transhiatal resection should be performed. Gastroesophageal tumors also may be removed via a left thoracoabdominal incision. Lesions located with the cervical esophagus thus requiring concurrent laryngectomy should not be included. Minimally invasive resections involving either a laparoscopic or combined laparoscopic/ thoracoscopic resection are permissible provided lymph node dissection guidelines are followed. Robotic dissections in either the chest, or abdomen, or both are also permissible under the same criteria. Any questions regarding eligibility of surgical technique can be referred to the surgical PI for review.

It is strongly recommended that the mediastinal pleura overlying the esophageal cancer and the periesophageal fat 5-cm proximal and distal to the primary lesion be removed *en bloc* with the tumor during the esophagectomy. Ligation of the thoracic duct is encouraged if this structure is seen at the time of surgery or that if injury to the duct may have occurred to prevent postoperative chylothorax. Also any indeterminate lesions with in the lung should be removed to determine if metastatic disease is present.

Surgical Margins: Lateral surgical margins between the primary tumor and mediastinal structures should be marked by both the surgeon (metal clips) and pathologist (inked). Microscopic or deep margins <1mm will be considered positive for malignancy and will be dually noted.

All margins should be at least 5 cm beyond gross tumor if possible after removal of the esophagus. It is recommended that the length of the margin be measured with the specimen stretched out by the pathologist before surgical closure. Frozen sections should be obtained to ensure negative surgical margins for cancer as well as Barrett's esophagus.

Lymph Node Dissections: All patients should undergo complete mediastinal lymph node and perigastric (lesser curvature and left gastric artery) dissection regardless of whether the nodes appeared positive or negative during clinical staging. If a patient had positive lymph nodes on clinical staging, the stations that had been positive should be removed at the time of surgery to confirm nodal status. At the time of esophagectomy, lymph node stations should be should be individually sampled and sent as separate specimens to the pathologist. However, lymph nodes densely adherent to the tumor should not be removed separately, but sent *en bloc* with the specimen. All lymph nodes that are technically accessible should be removed. After careful labeling, the status of these nodes should be conveyed to the central review site. Simple sampling of representative nodes should be discouraged.

Lymph nodes from levels 2R, 3p, 4R, 7,8,9,15,16,17,18,19,20 should be submitted for review if feasible. All submitted nodes should be resected and labeled for each individual station. Lesser curve nodes and celiac axis nodes should be marked by the surgeon and evaluated for levels 15,16,17,20. If one of the levels sited above is not sampled, an explanation should be submitted to the Central Office.

Exceptions: Exceptions to any of the above are acceptable if documentation of rationale is provided. If the patient is found to have evidence of metastatic disease intraoperatively, a palliative resection may be pursued.

Known potential adverse events from esophageal/gastric surgery or postoperative period (usually up to 60 days, but can be longer)

Common potential toxicities, >10%:

- Central nervous system: Pain. Pain at the wound site may be chronic.
- Endocrine & metabolic: Hyponatremia, hypomagnesemia, hypocalcemia, hypokalemia, abnormal glucose levels
- Gastrointestinal: Flatulence; cramping; bloating; nausea; vomiting; diarrhea; constipation; abdominal pain; trouble and/or pain with swallowing; delayed gastric emptying or other digestive dysfunction (e.g., dumping); reflux; decreased movement of the bowels (ileus) that could lead to complications that include but are not limited to small bowel dilatation, delay in conduit emptying, distension, pain, delay in reaching enteral nutrition goals, prolonged nasogastric tube requirement, wound complications, bacteremia (gut translocation), and abscess formation. Symptoms of dumping syndrome include nausea, diarrhea, weakness, sweating, dizziness or feeling faint.
- Hematologic: High white count, anemia, thrombocytopenia; deep venous thrombosis
- Cardiovascular: Abnormal heart rhythm which can cause irregular and/or forceful beating of the heart (palpitations, tachycardia), lightheadedness, fainting, and/or decreased blood pressure and be associated with EKG, cardiac imaging (including depressed ejection fraction), and/or biochemical abnormalities; edema of the extremities
- Respiratory, thoracic, or mediastinal: : Incomplete expansion of the lungs (atelectasis) with retention of secretions and shortness of breath that may require procedural intervention; pulmonary embolism; edema, effusion, shortness of breath, injury to diaphragm, lung damage, ventilator dependence or prolonged ventilation, respiratory failure, coughing, aspiration; laryngopharyngeal dysfunction including but not limited to issues with swallowing, airway protection, and speech.
- Nutrition: Poor nutritional status requiring enteral or parenteral feeding; poor appetite; weight loss
- Infection: pneumonia or thoracic, wound, urinary, intra-abdominal, bowel (e.g., c dif)
- Constitutional: Fatigue, fever

Less common potential toxicities, rare to 10%:

- Blood and lymphatic: Bleeding during or after the operation, which may require a blood transfusion; thrombosis with/without embolic event
- Respiratory, thoracic, or mediastinal: Laryngopharyngeal dysfunction including but not limited to issues with recurrent laryngeal nerve injury, voice/pharyngeal fatigue, vocal cord paralysis, sensory or motor dysfunction; thoracic dissection including but not limited to dissection of lymphatic, vascular, or other tissues and blood loss
- Cardiac: Myocardial infarction
- Other: A leak from the thoracic duct (a lymph vessel) that can be damaged during surgery, which could result in fluid around the lungs, shortness of breath, and loss of protein leading to malnutrition. Anastomotic leak which could result in fever, increased white cell count, and low blood pressure. Leaks could lead to increased fluid in the abdominal cavity. Fistula formation including but not limited to esophagus and tracheobronchial tree, or between bowel and other organs
- Gastrointestinal: esophageal/anastomotic stricture, fibrosis, or perforation; serious ileus or small bowel dilatation can lead to bacteremia, sepsis, perforation, peritoneal infection, abscess; bowel necrosis or ischemia
- Death

Any of the above postoperative complications can lead to prolonged hospitalization, permanent injury, or other complications including death

Appendix III Radiation Therapy Quality Control Guidelines

1) Prescription dose and fractionation:

   Total administered prescription dose: 41.4 Gy
   Total number of fractions: 23

2) Target coverage

   PTV4140: V100 ≥ 95% and max dose (0.03 cc) ≤ 110%

3) Normal tissue constraints

   Spinal cord: Max dose (0.03 cc) ≤45 Gy
   Lung: mean ≤15 Gy and V20 ≤20%
   Heart: mean ≤30 Gy and V40 ≤50%
   Bowel (small bowel and large bowel): Max dose (0.03 cc) ≤45 Gy
   Liver: mean ≤25 Gy and V30 ≤40%
   Kidneys: V20 ≤40%

4) Treatment duration/interruptions

   Elapsed days from first fraction to last fraction: ≤38 days

Treatment plans/administration meeting all of the above criteria will be considered "per protocol." Treatment plans/administration failing to meet one or more of the above criteria will be considered an "unacceptable deviation."

Known Potential Adverse Events of Radiation Therapy

Common (>20%)

- Inflammation of the esophagus
- Narrowing or scarring of the esophagus, which can cause problems with swallowing
- Fatigue
- Decrease in blood counts, which can cause infection, bleeding, and bruising
- Tanning and redness of the skin in the treatment area
- Nausea/vomiting

Occasional (4-20%)

- Growth of fibrous tissues underneath your skin
- Diarrhea
- Weight loss

Rare (1-3%)

- Inflammation of the muscle tissue of the heart
- Inflammation and/or scarring of the lung tissue or the lining around the lung
- Inflammation of the spinal cord
- Bleeding from the esophagus and stomach

Appendix IV Guidelines for Histopathological Examination of Esophagogastrectomy Specimens

Gross examination

The esophagogastrectomy specimen should be opened along the longest side of stomach after inking the esophageal adventitia.

Example of Gross Description:

1. A segment of (distal, proximal, mid) esophagus, __ cm in length and __ cm in circumference, with portion of proximal stomach, __ cm in length.

2. An ulcerated/fungating tan friable/firm tumor, __x__x__ cm, has its epicenter in the distal esophagus, __ cm proximal to the EGJ/in the esophagogastric junction and focally extends to the adventitial margin. The tumor is __ cm from the proximal and __ cm from the distal resection margins.

OR

2. A shallow ulcer/smooth depressed area, __x_x_ cm, overlying fibrotic wall is present, has its epicenter in the distal esophagus, __ cm proximal to the EGJ/in the esophagogastric junction and focally extends to the adventitial margin. The ulcer is __ cm from the proximal and __ cm from the distal resection margins.
   (Note: Post treatment resection specimens may have little grossly evident tumor. The location of the tumor should be verified by referring to previous path reports and/or clinic notes. The entire abnormal area must be submitted.)

3. The uninvolved esophagus is unremarkable/dull, granular/a segment of Barrett's mucosa, __ cm in length is evident proximal/distal/surrounding the tumor. (Describe any nodular area within the Barrett's mucosa.)

The stomach is unremarkable, (OR comment on any gross abnormalities)

Sampling for esophagogastrectomy specimen

Typically, esophageal carcinomas show extensive ulceration after pre-operative chemoradiation.

1. If residual tumors are grossly identifiable, tumor and ulcerated area (this includes the whole circumference of esophagus if ulceration is focal or patchy) should be sampled for histological evaluation at 2-3 mm intervals. Each section should include whole thickness of esophageal wall including inked adventitia for evaluation of radial margin. At least 1 cm of normal appearing esophagus or stomach should be sampled proximal and distal to tumor and ulcerated area.

2. If no residual tumors are grossly identified, areas with ulceration (this includes the whole circumference of esophagus if ulceration is focal or patchy) indicating of treatment field should be completely sample at 2-3 mm intervals and submitted for histological examinations. Each section should include whole thickness of esophageal wall including inked adventitia for evaluation of radial margin. At least 1 cm of normal appearing esophagus or stomach should be sampled proximal and distal to tumor and ulcerated area.

3. If no ulceration indicating of treatment field is identified. The entire esophagus including gastroesophageal junction should be completely sampled at 2-3 mm intervals and submitted for

histological examinations. Each section should include whole thickness of esophageal wall including inked adventitia for evaluation of radial margin.

4. Proximal esophageal resection margin and distal gastric resection margin should be completely submitted for histological evaluation.

5. All grossly identifiable lymph nodes should be completely submitted for histological evaluation. If less than 10 lymph nodes are identified, the whole periesophageal and perigastric soft tissue should be completely submitted for histological evaluation for the presence of lymph nodes.

6. Representative sections of normal esophagus and normal stomach sections should also be sampled.

Sections:

- proximal margin en face (frequently submitted for frozen section)- **open the specimen first before submitting blocks for frozen section**
- distal margin en face (frequently submitted for frozen section)- **open the specimen first before submitting blocks for frozen section**
- tumor with deepest extent and adventitial margin
- tumor with adjacent non-neoplastic tissue
- Barrett's, if any
- When tumor is not grossly evident as in post-chemoradiated esophagus, the entire abnormal segment must be submitted.
- **Lymph nodes should be clearly labeled as paraesophageal and paragastric lymph nodes indicating location (greater or lesser curvature, etc.)**
- Representative GE junction, normal esophagus and normal stomach

Histological examination for esophagogastrectomy specimen

Immunohistochemical stain for Cytokeratin (AE1/AE3) should be performed if microscopic foci of suspicious residual tumor are identified on routine H&E sections (tumor, lymph node, or margin sections) to accurately stage the tumor. This stain will not be performed routinely if no histological evidence of residual tumor is seen on H&E sections.

Appendix V Pembrolizumab ECI Drug-Induced Liver Injury (DILI)

Hepatotoxicity is injury or damage to the liver that may be associated with impaired liver function (Navarro and Senior 2006). Drug-induced hepatotoxicity is one of the most common causes of termination of drug development, a major reason for refusal of market authorization and for restricted use, and the single most important cause of the withdrawal of market authorization for products (Björnsson 2006). Thus, drug-induced hepatotoxicity is a major concern during the discovery, development to postauthorization phases of the product life cycle (excerpted from Draft Guidance Document, Hepatotoxicity of Health Products, Ministry of Public Health, Canada, December 2010).

As stated in the United States Food and Drug Administration (FDA) Guidance for Industry - Drug-Induced Liver Injury: Premarketing Clinical Evaluation; hepatocellular injury (usually detected by serum aminotransferase elevations [AT]) can be caused by drugs that rarely, if ever, cause severe DILI (e.g. aspirin, tacrine, statins, and heparin) as well as by drugs that do cause such injury. The frequency of serum AT elevations also is not a good indicator of a potential for severe DILI, because drugs such as tacrine (not a cause of severe DILI) can cause AT elevations in as many as 50 percent of patients. Very high levels of observed ATs may be a somewhat better indicator of potential for severe DILI, but the most specific indicator is evidence of altered liver function accompanying or promptly following evidence of hepatocellular injury.

The single clearest (most specific) predictor found to date of a drug's potential for severe hepatotoxicity, is the occurrence of hepatocellular injury (AT elevation) accompanied by increased serum total bilirubin (TBL), not explained by any other cause, such as viral hepatitis or exposure to other hepatotoxins, and without evidence of cholestasis, together with an increased incidence of AT elevations in the overall trial population compared to control. Increased plasma prothrombin time, or its international normalized ratio (INR), a consequence of reduced hepatic production of Vitamin K dependent clotting factors, is another potentially useful measure of liver function that might suggest the potential for severe liver injury.

Recognition of the importance of altered liver function, in addition to liver injury, began with Hyman Zimmerman's observation that drug-induced hepatocellular injury (i.e. AT elevation) accompanied by jaundice (i.e. TBL elevation) had a poor prognosis, with a 10 to 50 percent mortality from acute liver failure (in pretransplantation days) (Zimmerman 1978, 1999). This became known as "Hy's Law". This document describes the recommended process for monitoring and evaluation of subjects meeting the laboratory criteria for potential DILI defined as:

- an elevated alanine transaminase (ALT) or aspartate transaminase (AST) lab value that is greater than or equal to three times (3X) the upper limit of normal (ULN)
  and
- an elevated total bilirubin (TBL) lab value that is greater than or equal to two times (2X) ULN
  and
- at the same time, an alkaline phosphatase (ALP) lab value that is less than 2X ULN,

as a result of within-protocol-specific testing or unscheduled testing.

The protocol identifies these laboratory criteria for potential DILI as ECIs. ECIs are selected adverse experiences that must be reported within 24 hours.

Initiate **close observation** as defined below and continue follow-up until resolution.

- Repeat liver enzyme and serum bilirubin tests two (2) or three (3) times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or study drug has been discontinued and the subject is asymptomatic.
- Obtain a more detailed history of symptoms and prior or concurrent diseases.

- Obtain a history of concomitant medication use (including prescription and nonprescription medications, herbal and other dietary supplements), alcohol use, recreational drug use and special diets. (See Section 5 for details.)
- Obtain a history of exposure to chemical agents or other environmental toxins.
- Obtain additional history and complete Stage 1 workup to attempt to rule out other potential causes of the transaminase elevation, including but not limited to the following: acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease
- Consider gastroenterology or hepatology consultation

Appendix VI Presentation of Dermatologic Event

Collect information on clinical presentation and potential contributing factors. Key information should be summarized and entered on the Adverse Experience CRF. Any treatments administered should be entered on the Concomitant Medication CRF. ***Scan and forward this form to Principal Investigator***.

1. What is the onset time of the skin reaction, skin eruption, or rash relative to dose of study drug?

   _____

2. Has the subject contacted any known allergens? ☐ Yes ☐ No

   If so what kind? _____

3. Has the subject contacted new, special, or unusual substances (e.g., new laundry detergents, soap, personal care product, poison ivy, etc.)? ☐ Yes ☐ No

   If so what kind? _____

4. Has the subject taken any other medication (over the counter, prescription, vitamins, and supplement)? ☐ Yes ☐ No

   If so what kind? _____

5. Has the subject consumed unaccustomed, special or unusual foods? ☐ Yes ☐ No

   If so what kind? _____

6. Does the subject have or had in the last few days any illness? ☐ Yes ☐ No

   If so what kind? _____

7. Has the subject come into contact with any family or house members who are ill? ☐ Yes ☐ No

   If so what kind? _____

8. Has the subject recently been near children who have a skin reaction, skin eruption, or rash (e.g. *Molluscum Contagiosum*)? ☐ Yes ☐ No

9. Has the subject had recent sun exposure? ☐ Yes ☐ No

10. For the current rash, have there been any systemic clinical signs? ☐ Yes ☐ No

    If so what kind? _____

    i. Anaphylaxis? ☐ Yes ☐ No

    ii. Signs of hypotension? ☐ Yes ☐ No

    iii. Signs of dyspnea? ☐ Yes ☐ No

iv. Fever, night sweats, chills? ☐ Yes ☐ No

11. For the current rash, has the subject needed subcutaneous epinephrine or other systemic catecholamine therapy? ☐ Yes ☐ No

If so what kind? _____

12. For the current rash, has the subject used any other medication, such as inhaled bronchodilators, antihistaminic medication, topical corticosteroid, and/or systemic corticosteroid? ☐ Yes ☐ No

List medication(s) and dose(s): _____

13. Is the rash pruritic (itchy)? ☐ Yes ☐ No

Focused Skin Examination

Focused Skin Examination:

Key information should be summarized and entered on the Adverse Experience CRF.

Primary Skin Lesions Description

Color: _____

General description:

_____

_____

Describe the distribution of skin reaction, skin eruption, or rash on the body:

_____

Is skin reaction, skin eruption, or rash resolving or continuing to spread?

_____

Any associated signs on physical examination?

_____

Scan this form and forward by email to the Principal Investigator listed on the cover page of the protocol.