Local Protocol #: IRB19-0310

TITLE: A Phase II Trial of Neoadjuvant Pembrolizumab for Resectable Early Stage Gastroesophageal Adenocarcinoma

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This study will be managed by the Personalized Cancer Care Consortium (PCCC).

DOCUMENT HISTORY

Protocol Version Date	Summary of Changes
5/12/21	• Biopsy/Endoscopy language added to Section 8.1.2.6.2 • Standard PCCC Language added to: - Section 5: Registration Procedures - Section 8.2.3.2: UChicago/PCCC SAE reporting language - Section 11.0: Regulatory management language added to

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1.0 TRIAL SUMMARY

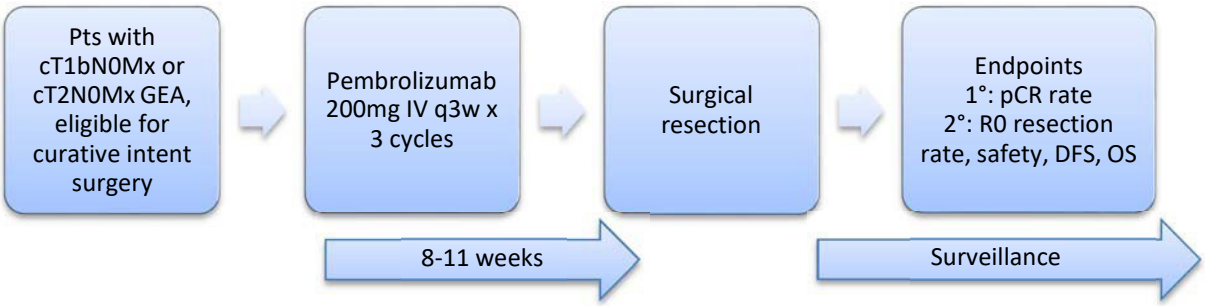
Abbreviated Title	Neoadjuvant pembrolizumab for resectable early stage gastroesophageal adenocarcinoma
Trial Phase	IIa
Clinical Indication	Resectable cT1b-2N0M0 gastroesophageal adenocarcinoma
Trial Type	Prospective clinical trial
Type of control	Historical controls
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	1
Number of trial participants	33
Estimated enrollment period	*24 months*
Estimated duration of trial	*36 months*
Duration of Participation	Until 5 years post-surgical resection, death, or study closure
Estimated average length of treatment per patient	8-11 weeks (pembrolizumab IV q 3 weeks x 3 cycles followed by surgery within 8-11 weeks from first dose of pembrolizumab)

2.0 TRIAL DESIGN

2.1 Trial Design

This is a prospective, single-arm phase IIa study enrolling patients with cT1b-2N0Mx esophageal and gastric adenocarcinoma. Patients will receive 3 cycles of pembrolizumab 200mg intravenously on day 1 of a 3-week cycle. After 3 cycles, patients will undergo surgery. The target surgery date is 8-11 weeks after the first dose of pembrolizumab. Surgical approach will vary based upon tumor location but would entail tumor resection with either D2 or two-field lymphadenectomy. The primary objective is to determine the pathologic complete response (pCR) rate in patients with cT1b-T2N0 gastroesophageal adenocarcinoma (GEA) treated with neoadjuvant pembrolizumab followed by surgical resection. A sample size of 33 patients will provide 90% power with one-sided alpha 0.1 to detect a pCR rate of 15% using neoadjuvant pembrolizumab. Secondary endpoints include R0 resection rate, safety and tolerability, disease-free survival (DFS), and overall survival (OS). Diagnostic tissue biopsy and surgical resection specimen pre- and post-pembrolizumab therapy will be assessed for panel DNA/RNA next generation sequencing (NGS), programmed death-ligand 1 (PD-L1) combined positive score (CPS) testing, Epstein-Barr virus (EBV) testing, microsatellite instability (MSI) testing and tumor mutation burden (TMB). Plasma circulating tumor DNA (ctDNA) samples will be collected for panel NGS (73 gene in real time, and more stored for larger 550 gene and smaller higher depth panels) prior to pembrolizumab therapy, after neoadjuvant pembrolizumab therapy but prior to surgery, after surgery, and upon recurrence if/when this occurs. Stool samples will also be obtained at these time points and subjected to Fecal 16S RNA seq.

2.2 Trial Diagram



3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objective & Hypothesis

Objective:

To determine the pathologic complete response (pCR) rate in patients with cT1b-T2N0 GEA treated with neoadjuvant pembrolizumab followed by surgical resection.

Regression of the primary tumor will be documented by the amount of viable tumor versus the amount of fibrosis (Becker Criteria), ranging from no evidence of any treatment effect to a complete response with no viable tumor identified, as previously described[1] [Grade 1a: complete remission (pCR), no residual tumor/tumor bed; Grade 1b: subtotal remission, <10% residual tumor/tumor bed; Grade 2: partial remission, 10-50% residual tumor/tumor bed; Grade 3: minor/no remission, >50% residual tumor/tumor bed].

Hypothesis:

The pCR rate (Grade 1a+b response) in patients with cT1b-T2N0 GEA who receive neoadjuvant pembrolizumab followed by surgical resection will be 15%, whereas historical patients treated with surgery alone have a pCR rate of virtually 0%.[2, 3]

3.2 Secondary Objectives & Hypotheses

Objectives:

a. To determine the R0 resection rate in patients with cT1b-2N0 GEA treated with neoadjuvant pembrolizumab. Proximal, distal, and circumferential margins will be assessed to determine the completeness of resection. The absence of tumor cells at the proximal and distal margins will be required to be classified as an R0 resection.
b. To determine rates of Grade 1a (complete); 1b (minimal residual disease); 2 (partial response); and 3 (no response) pathologic response grades (PRG).
c. To evaluate the safety/ tolerability of pembrolizumab in patients with cT1b-2N0 GEA
d. To determine the DFS measured from the time of first pembrolizumab dose
e. To determinate OS measured from the time of first pembrolizumab dose

Hypotheses:

a. R0 resection rate will be greater than 95%.
b. A subset of cases will be G1b and G2 responses.
c. Pembrolizumab will be safe and tolerable. Patients will successfully complete neoadjuvant therapy and undergo surgery 8-11 weeks after first dose of pembrolizumab.
d. The DFS rate will be improved compared to historical controls.
e. OS will be improved compared to historical controls.

3.3 Exploratory Objectives

a. To evaluate rates of PD-L1 positive expression (by CPS score ≥1 and ≥10), MSI status, TMB and EBV status in pre-treatment biopsy specimens.
b. To evaluate whether PD-L1 expression, MSI status, TMB and/or EBV status in pre-treatment biopsy specimen is a predictive biomarker for pCR/PRG/R0 to pembrolizumab and other clinical outcomes.
c. To evaluate interval change in PD-L1 expression (by CPS score) pre/post pembrolizumab therapy in resected pathological specimens (if residual tumor is present).
d. To determine the somatic genomic changes of actionable cancer related genes (mutation, amplification, indels, and translocation) using targeted deep next-generation sequencing (NGS) oncopanel in pre-treatment specimen and in resected specimen (if tumor is still present) and correlate with clinical outcomes (pCR/PRG, R0 rate, DFS, OS) and other molecular markers.
e. To evaluate ctDNA NGS pre-pembrolizumab, post-pembrolizumab, post-surgery, and if applicable at time of recurrence, and correlate with clinical outcomes (pCR/PRG, R0 rate, DFS, OS) and molecular markers.
f. To determine baseline and interval changes in whole-exome RNAseq expression analysis pre-/post- therapy.
g. To evaluate fecal flora via Fecal 16S RNA seq pre-pembrolizumab, post-pembrolizumab, post-surgery, and if applicable at time of recurrence, and correlate with clinical outcomes.

4.0 BACKGROUND & RATIONALE

4.1 Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of

indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure.

Pembrolizumab is FDA approved for third line treatment of recurrent locally advanced or metastatic CPS ≥1 PD-L1+ GEA [4, 5] and for second line treatment of unresectable or metastatic microsatellite high (MSI-H) or mismatch repair deficient (dMMR) GEA. [5, 6] As therole of pembrolizumab continues to be investigated in the metastatic setting, newer trials are investigating the use of checkpoint blockade in the perioperative setting. KEYNOTE-585 is examining whether the addition of pembrolizumab to chemotherapy improves outcomes in the neoadjuvant or adjuvant setting in untreated patients with cT3 or cN+ GEA (NCT03221426). [7]CheckMate 577 is enrolling patients with stage II or III carcinoma of the esophagus of EGJ (squamous cell carcinoma and AC) treated with chemoradiation followed by surgery, who have an R0 resection yet residual disease, to receive nivolumab or placebo adjuvantly (NCT02743494).[8]

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades. [9] Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells/FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma. [10, 11]

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). [12, 13]

The structure of murine PD-1 has been resolved.[14] PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade.[13, 15-17] The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping

set of signaling proteins. [18, 19] As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in gastroesophageal adenocarcinoma.

4.1.2 Preclinical and Clinical Trial Data

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

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Population

Gastroesophageal cancer is the second leading cause of cancer mortality worldwide. [20] Surgery and endoscopic resection are the only curative options for patients with early stage and/or locally advanced operable GEA. Survival is highly dependent on the extent of disease at presentation, in particular the depth of invasion and nodal status, as well as ability to obtain a complete (R0) resection and pCR. [21] The role of perioperative chemotherapy is clear in ≥cT3 and/or ≥cN1 disease where R0 resection rates are 80-90% and pCR 15-25%. However, the benefit of such therapy in cT2N0 disease is less certain, and in cT1bN0 disease even less well studied. This is in part due to significant heterogeneity that exists in the enrollment criteria for the major clinical trials that have guided the use of neoadjuvant therapy. Trials have varied in their inclusion of cT2N0 disease, use of EUS for staging, use of AJCC staging system, and reporting of outcomes by T and N status. There is thus great heterogeneity across treatment centers regarding the inclusion of cT1b-2N0 patients in perioperative therapy approaches. A recent retrospectiveanalysis of 1704 patients with cT2N0 gastric cancer found that there was no overall survival benefitderived from neoadjuvant and/or perioperative chemotherapy compared to surgery alone.[22]

However, patients with cT2N0 esophageal adenocarcinoma (AC) (clinical stage IIB based on the AJCC 8th edition) have a 5-year risk-adjusted survival of only ~42% [23], a dismal number, and actually comparable to patients with stage III colon cancer where routine adjuvant therapy is implemented. Patients with cT1N0 esophageal AC (clinical stage I), have a 5-year risk-adjusted survival of only approximately 68%. Patients with cT1-2N0M0 gastric AC (clinical stage I) have an estimated 5-year survival of 57%. [24] There is a clear need to improve on outcomes in patients with operable GEA. It is quite evident that the optimal treatment of cT1-2N0 GEA is controversial without widespread implementation of any perioperative therapy, yet with acknowledged relatively poor outcomes. At the University of Chicago and many other centers, the standard of care for cT1-2N0 GEA is surgical or (endoscopic mucosal resection/dissection if early stage I cT1a) followed by observation.

Recently, immune checkpoint blockade has emerged as a promising therapeutic strategy in GEA as discussed above. An unexplored area of research is whether neoadjuvant checkpoint blockade can improve outcomes in patients with cT1-2N0 GEA. In KEYNOTE-012, a phase 1b trial of pembrolizumab in recurrent or metastatic PD-L1+ GEA, the median time to response was 8 weeks.[25] This median time to response has been now observed in other follow up studies of checkpoint inhibition in this disease. Thus, in cT1b-2N0 patients who would ordinarily undergo surgery alone, neoadjuvant pembrolizumab could, in a short amount of time, induce a response and still allow for surgery to be performed in a timely manner. Furthermore, the proportion of

MSI-H tumors (which are more sensitive to PD-1 blockade) appears to be enriched in the early stage setting compared to the metastatic setting in which pembrolizumab is currently approved. Moreover, the rate of PD-L1 positivity has been reported to be generally higher in earlier stage disease. Thus, these early stage patients, who otherwise have a moderate chance of recurrence with surgery alone, may in particular be worth targeting with neoadjuvant pembrolizumab.

A potential concern may be that neoadjuvant pembrolizumab could delay curative surgery, allowing an early stage GEA to progress. However, a non-concurrent long term follow up study of patients with early gastric cancer elucidated the natural history of this disease and found that the median duration of those remaining in the early stage was 44 months. [26] Thus it is unlikely that delaying surgery by 8-11 weeks in order to obtain 3 doses of pembrolizumab would result in a clinically meaningful progression. Furthermore, in practice, surgery is often not scheduled for approximately 2-6 weeks after staging is complete, so the incremental delay attributed to receiving pembrolizumab is likely even less. Finally a recent neoadjuvant study in lung cancer demonstrated feasibility and safety in a similar setting.[27] This pilot study will provide early efficacy of pembrolizumab monotherapy using a pCR surrogate endpoint, and will also lend important pre- and post-pembrolizumab tissue for biomarkers analyses.

4.2.2 Justification for Dose

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W) for 3 doses, neoadjuvantly. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg Q3W as the appropriate dose independent of

the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

There is compelling evidence that pathologic complete response rate is an early surrogate endpoint for survival in GEA. In patients with gastric cancer treated with neoadjuvant platinum-based chemotherapy, complete or subtotal tumor regression has been showed to be a prognostic factor independent from ypT and ypN stage.[28] In a large meta-analysis of patients with resectable GEA treated with neoadjuvant chemotherapy followed by surgery, those who achieved a pCR had improved survival than patients who did not achieve a pCR.[29] Thus, histopathologic regression measured as pCR rate is an appropriate endpoint for this phase 2 trial.

4.2.3.2 Biomarker Research

GEA is a heterogeneous group of diseases with variable benefit from pembrolizumab. The identification of biomarkers that predict response to immunotherapy may help guide selection of patients that are most likely to benefit from PD-1 blockade. Assuming adequate tissue and resources, the biomarker assessment outlined in the exploratory objectives (Section 3.3) will be performed.

PD-1 and PD-L1 staining has been studied as predictive response biomarkers in several tumor types. In advanced NSCLC, PD-L1 expression of at least 50% of tumor cells correlates with prolonged PFS and OS with pembrolizumab treatment.[30] In KEYNOTE-059, patients with previously treated advanced GEA received pembrolizumab monotherapy and PD-L1 positive (CPS≥1) patients had a higher ORR compared to PD-L1 negative patients.[31] It is unknown if in the curative neoadjuvant setting, if PD-L1 expression is predictive for response to immunotherapy and whether PD-L1 expression changes pre- and post-pembrolizumab therapy.

The Cancer Genome Atlas research network recently reported the molecular characteristics of untreated gastric adenocarcinomas as part of The Cancer Genome Atlas project.[32] Gastric cancer was classified into the following four subtypes: MSI tumors (22%), EBV+ tumors (9%), tumors exhibiting chromosomal instability (CIN) (50%), and genomically stable (GS) tumors (20%). MSI has also been reported in 6.6% of untreated resected Barrett esophagus-associated EGJ AC.[33] MSI-H tumors and EBV+ tumors are thought to be sensitive to PD-1 blockade. This is due to high neoantigen levels in MSI-H tumors and due to high degree of PD-L1 positive T cell infiltration in both MSI-H and EBV+ tumors. Pembrolizumab is FDA-approved for patients with unresectable or metastatic MSI-H or dMMR of any solid tumor type, including GEA, that has progressed following prior treatment and who have no satisfactory alternatives.[6] The proportion of MSI-H tumors appears to be enriched in the early stage setting. Patients with EBV+ GC have improved survival which has been in part attributed to the associated rich CD8+ T-cell infiltrate present as part of the immune response against EBV[34-36] and increased PD-L1 and PD-L2 expression.[32] Recently reported was a patient with metastatic EBV+ esophagogastric cancer who had a durable complete response to immunotherapy.[37] It is unknown if MSI-H status or EBV positivity is predictive of response to PD-1 blockade in the neoadjuvant curative setting.

TMB quantifies mutations present in tumor cells and is currently under investigation as a potential immunotherapy biomarker in several cancer types. In patients with stage IV NSCLC, an exploratory analysis found patients with high TMB had a improved response rate and progression free survival compared to low/medium TMB, when treated with nivolumab versus platinum-based chemotherapy.[38] There was no association between level of TMB and level of tumor PD-L1 expression, however patients with PD-L1 expressions of <1% were excluded. The role of TMB as a predictive biomarker to PD-1 blockade in the early stage setting remains unknown.

The Gajewski lab has previously demonstrated a correlation between fecal flora composition and response to immunotherapeutics.[39, 40] In this trial, patient fecal microbiomes will be characterized before pembrolizumab therapy, after pembrolizumab therapy, after surgery, and at time of recurrence (if applicable) in order to evaluate the relationship between fecal flora and therapeutic response.

Patients who do not respond to immunotherapy may have various mechanisms of primary and/or acquired resistance. Genomic alterations will be characterized using targeted NGS in the pre-treatment specimen as well as in the resected specimen (if residual tumor is present) to evaluate if non-responders have particular mechanisms of resistance to therapy. Additional ctDNA will be evaluated before pembrolizumab therapy, after pembrolizumab therapy, after surgery, and at time of recurrence (if applicable) to characterize genomic alterations at diagnosis, identify temporal trends, identify ctDNA alterations that may be predictive of outcomes, and determine if presence of residual tumor ctDNA post-operatively is predictive of outcomes.

5.0 REGISTRATION PROCEDURES

5.1 General Guidelines

Prior to registration and any study-specific evaluations being performed, all patients must have given written informed consent for the study and must have completed the pre-treatment evaluations. Patients must meet all of the Participant Inclusion Criteria in Section 6.1.1. Eligible patients will be entered on study centrally by the University of Chicago study coordinator. All sites should contact the study coordinator at PhaseIICRA@medicine.bsd.uchicago.edu to verify availability of a slot.

5.2 Registration Process

When a potential patient has been identified, notify the CRA via email at PhaseIICRA@medicine.bsd.uchicago.edu to ensure a reservation on the study. Reservations for potential subjects will only be held for subjects who have signed consent for that particular study.

When registering a subject, the following must occur:

- Confirm that the institution has a current IRB approval letter for the correct version of protocol/consent and has an annual update on file, if appropriate.
- Submit all required materials (Eligibility Checklist, Source documentation, and signed consent form) to confirm eligibility and required pre-study procedures to the CRA a minimum of 48 hours prior to the subject's scheduled therapy start date.
- Source documentation includes copies of all original documents that support each inclusion/exclusion criteria. The eligibility checklist does not serve as source documentation but rather as a checklist that original source documentation exists for each criterion.
- Communicate with the CRA to ensure all necessary supporting source documents are received and the potential subject is eligible to start treatment on schedule. If there are questions about eligibility, the CRA will discuss it with the Study Lead PI, Dr. Ardaman Shergill . Dr. Shergill may clarify, but not overturn, eligibility criteria.
- Affiliate sites must confirm registration of subjects by obtaining a subject study ID number from the CRA via phone, fax or email.
- If a subject does not start on the scheduled day 1 treatment date, promptly inform the CRA as the delay in start may deem the subject ineligible and/or require further or repeat testing to ensure eligibility.
- If randomization is involved, the date the patient is randomized will be considered the patient's "On Study Date." If randomization is not involved, the first time the patient receives treatment will be considered the patient's "On Study Date." The patient's subject ID will be assigned and a confirmation of registration will be issued by the CRA on this date. Subjects that sign consent and do not go "On Study" will be recorded in the database with the date they signed consent and the reason for not going "OnStudy" (e.g., Ineligible, Screen Failure or Withdrawn Consent).

6.0 METHODOLOGY

6.1 Study Population

6.1.1 Participant Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Male/female participants who are at least 18 years of age on the day of signing informed consent with histologically confirmed diagnosis of untreated esophageal adenocarcinoma (AC) (including Siewert type I and II esophagogastric junction AC) or gastric AC (including Siewert type III esophagogastric junction AC), with clinical stage T1b-T2, N0, and M0. Tumors that are adenosquamous are allowed if they are predominantly adenocarcinoma.

2. Patients must undergo EUS, CT chest/abdomen/pelvis with IV/PO contrast (MRI abdomen/pelvis plus noncontrast chest CT is acceptable if patient has a contraindication to iodinated dye), and PET/CT to complete staging and confirm absence of metastatic disease.

3. Patient must agree to a baseline EUS for centralized endoscopic staging to confirm the tumor is uT1b or uT2 and node negative, at which time a research biopsy endoscopically will also be obtained.

4. HER2+ and HER2- patients, and all other known molecular subsets are eligible.

5. Diagnostic laparoscopy is not mandated and can be performed as clinically indicated.

6. Eligible for willing to proceed with surgery with curative intent

7. A female participant is eligible to participate if she is not pregnant (see Appendix 3), not breastfeeding, and at least one of the following conditions applies: a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix 3 OR b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of study treatment.

8. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.

9. Measurable or non-measurable disease by RECIST 1.1 will be allowed.

10. Is willing to provide tissue for correlative studies from the primary tumor lesion at baseline and at time of surgery. Is willing to provide blood and stool samples for all ordered studies.

11. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Evaluation of ECOG is to be performed within 7 days prior to the date of allocation.

12. Have adequate organ function as defined in the following table (Table 1).

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/La
Renal	
Creatinine OR Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

a Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC)transfusion within last 2 weeks.

b Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

6.1.2 Participant Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. A WOCBP who has a positive urine pregnancy test within 72 hours prior to allocation (see Appendix 3). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

2. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX-40, CD137).

3. Previous or concurrent malignancy, except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or any other cancer for which the patient has been previously treated and the lifetime recurrence risk is less than 30%.

4. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

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

6. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.

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

8. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.

9. Has an active infection requiring systemic therapy.

10. Has a known history of Human Immunodeficiency Virus (HIV).

11. Has a known history of active TB (Bacillus Tuberculosis).

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

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

14. Is pregnant or breastfeeding, or expecting to conceive or father children within theprojected duration of the study, starting with the screening visit through 120 days after thelast dose of trial treatment.

15. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.

6.1.3 Lifestyle Restrictions

6.1.3.1 Meals and Dietary Restrictions

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

6.1.3.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Refer to Appendix 3 for approved methods of contraception.

For this study, male participants 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).

6.1.4 Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck within 2 working days if the outcome is a serious adverse experience (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The treating investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck. If a male participant impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to Merck and followed as described in Section 8.2.2.

6.1.5 Use in Nursing Women

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

6.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2

Table 2 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle x 3 cycles	Experimental

6.2.1 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 7.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

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

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

6.2.2 Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

Table 3 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

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

Immune-related AEs	**Toxicity grade or conditions (CTCAEv5.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not
	Grade 4	Permanently discontinue		

				feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
	Grade 2	Withhold		• Monitor changes of renal function

Nephritis and Renal dysfunction	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4 Pembrolizumab Infusion Reaction Dose modification and 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 participant is deemed medically stable in the opinion of the treating investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** 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 participant is deemed medically stable in the opinion of the treating investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusionmay be restarted at 50% of the original infusion rate (e.g. from 100 mL/hrto 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant 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 analgesic).

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: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the treating investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 2 weeks of the scheduled interruption, unless otherwise discussed with the P.I. The reason for interruption should be documented in the patient's study record. If there is any delay in pembrolizumab that would result in patient being unable to undergo surgery within 56-77 days of study initiation (day of first pembrolizumab dose), then patient will go off trial and go directly to surgery.

6.3 Randomization or Treatment Allocation

This is an open-label trial; therefore, the P.I., treating investigator and subject will know the treatmentadministered.

6.4 Concomitant Medications/Vaccinations (allowed & prohibited)

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

6.4.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and Events of Clinical Interest (ECIs) as defined in Section 8.2.

6.4.2 Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase (treatment phase refers to the time between the first dose of pembrolizumab and the date of surgery) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab

- Radiation therapy
- Live vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

- 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 P.I.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the treating investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the treating investigator and/or the participant's primary physician. However, the decision to continue the participant on study treatment requires the mutual agreement of the treating investigator, the P.I.and the participant.

Antibiotic therapies may be required by some participants during the study, but would avoid as safely able as this may affect pembrolizumab response and fecal flora.[41, 42] Antibiotic choice: Different classes of antibiotics have been shown to disrupt the microbiome and potentially interfere with normal host immune function to varying degrees. If equally indicated amoxicillin is the preferred antibiotic as it minimally alters gut flora. Clindamycin is the most detrimental antibiotic and should be avoided if possible.[43] Beta-lactams (other than amoxicillin) and fluoroquinolones broadly should be avoided if possible as well.[44]

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

6.4.3 Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 6.2.2, [Table 3]. Where appropriate, these guidelines include the use of oral or IV 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 treating investigator determines the events to berelated to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the treating investigator does not need to follow the treatment guidance. Refer to [Table 3] in Section 6.2.2 for guidelines regarding dose modification and supportive care.

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

6.5 Participant Withdrawal/Discontinuation Criteria

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the treating investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the treating investigator or the P.I. if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 8.1.4 – Other Procedures.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment
- Confirmed radiographic disease progression outlined in Section 8.1.2.5.1
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Unacceptable adverse experiences as described in Section 6.2.2.
- The participant has a medical condition or personal circumstance which, in the opinion of the treating investigator and/or P.I., placed the participant at unnecessary risk fromcontinued administration of study treatment.
- The participant has a confirmed positive serum pregnancy test
- Noncompliance with study treatment or procedure requirements
- Recurrent Grade 2 pneumonitis
- The participant is lost to follow-up
- Administrative reasons

6.6 Participant Replacement Strategy

Patients that are not treated by intention to treat will be replaced to the total 33 patients intended. All patients who receive the first dose of pembrolizumab will be included in analysis per intention to treat. Patients who receive at least one dose of pembrolizumab and then do not proceed to surgery will be considered grade 3 response.

6.7 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete with ≥10% of data unavailable.

2. Poor adherence to protocol and regulatory requirements

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

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

5. If patient deaths or excessive toxicity attributed to the investigational drug are seen in the 30 days following surgery. A **30-day post-operative** death rate of ≥5% attributed to study agent is considered unacceptable. The relationship of the investigational drug to the mortality will be determined by the treating medical oncologist and surgeon.

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

7.0 TRIAL FLOW CHART

7.1 Study Flow Chart

	Study Screening	Cycle 1	Cycle 2	Cycle 3	Pre-Surgery	Surgery	Post-Op	Surveillance[e]	Off Study[j]
Scheduling Window (Days)	-28 to -1	Day 1	Day 21 ± 3	Day 42 ± 3	Day 56 ± 3	Day 56-77	42-70 days post-op		
Administrative Procedures									
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Demographics	X								
Medical History	X		X	X	X		X	X	X
Prior and Concomitant Medication Review	X		X	X	X		X	X	X
Clinical Assessments									
Review Adverse Events	X	X	X	X	X		X	X	X
Full Physical Exam	X	X	X	X	X		X	X	X
Vital Signs	X	X	X	X	X		X	X	X
Height	X								
Weight	X	X	X	X	X		X	X	X
ECOG Performance Status	X	X	X	X	X		X	X	X
Diagnostics and Imaging									
Radiologic Evaluation: CT CAP[a]	X				X		X	X[f]	
Radiologic Evaluation: PET/CT	X				X				
Upper Endoscopy[m]	X				X			X[g]	
Endoscopic Ultrasound (EUS)	X				X				
Laboratory Procedures/Assessments									
Pregnancy Test – Urine or Serum -HCG[b]	X	X	X	X	X				
CBC with differential	X		X	X	X		X		
Basic metabolic panel	X		X	X	X		X		
Hepatic function panel	X		X	X	X		X		
CEA	X		X	X	X		X	X[h]	X
CA 19-9	X		X	X	X		X	X[h]	X
Urinalysis	X		X	X	X		X		
TSH, (FT4 and T3)[i]	X		X	X	X		X	X	
PT/INR and aPTT	X								
Intervention									
Pembrolizumab administration		X	X	X					
Surgical Assessment and Resection						X			
Correlative Material Collection									

Pre-treatment research biopsy for pathologist review	X								
Pre-treatment biopsy for biomarker analysis[c]	X								
Resected surgical specimen for pathologist review						X			
Resected surgical specimen for biomarker analysis[c,d]						X			
Foundation One CDx	X					X[d]			
Guardant 360 ctDNA collection and blood banking[l]	X				X		X		X[k]
Stool collection	X				X		X		X[k]

[a]CT scan of chest/abdomen/pelvis with IV and PO contrast, OR MRI of abdomen/pelvis plus non-contrast chest CT if contraindication to iodinated dye.

[b]Only needed in women of childbearing potential

[c]Biomarker analysis includes testing for MSI via immunohistochemistry (IHC), HER2 testing (via IHC and/or fluorescence in situ hybridization (FISH)), Epstein-Barr encoding region in situ hybridization (EBER ISH), PD-L1 CPS testing, and whole-exome RNAseq expression

[d]Will only be able to be performed if there is residual tumor left in surgical specimen

[e]Surveillance visits with physician (or nurse practitioner or physician assistant) will occur every 3 months until year 3 post surgery, and then every 6 months until year 5 post surgery

[f]Surveillance imaging will be performed annually for 2 years post-surgery and then as clinically indicated

[g]Will only be performed as needed

[h]Will be checked every 3 months until year 3 post surgery, and then every 6 months until year 5 post surgery

[i]TSH only be checked, and FT4 and T3 only checked reflexively if TSH is abnormal, per routine care.

[j]Patients will come off study 5 years post-surgery, if cancer recurs, or if patient withdraws consent. However, overall survival follow-up will continue to year 5 if cancer recurs or if patient withdraws consent from treatment but not follow up.

[k]Will only be collected if patient's gastroesophageal cancer recurs

[l]Blood Banking collected and processed per IRB16294A (see Section 8.1.2.6 of this protocol).

[m] Endoscopy must be conducted at enrolling institution (see Section 8.1.2.6.2 of this protocol).

8.0 TRIAL PROCEDURES

8.1 Trial Procedures

The Trial Flow Chart - Section 7.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

8.1.1 Administrative Procedures

8.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential participant prior to participating in a clinical trial.

8.1.1.1.1 General Informed Consent

Consent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

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

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

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

The informed consent will adhere to IRB/ERC requirements and applicable laws and regulations.

8.1.1.2 Inclusion/Exclusion Criteria

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

8.1.1.3 Medical History

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

8.1.1.4 Prior and Concomitant Medications Review

8.1.1.4.1 Prior Medications

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

8.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the participant during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 8.2.

8.1.1.5 Disease Details and Treatments

8.1.1.5.1 Disease Details

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

8.1.1.5.2 Prior Treatment Details

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

8.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a participant initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the safety follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the participant will move into survival follow-up.

8.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects. Any subject who is screened multiple times will retain the original screening number assigned at the initial screening visit. Once a screening number is assigned to a subject, it can never be re-assigned to another subject.

8.1.1.7 Assignment of Randomization Number

No randomization will be performed and no randomization numbers will be used.

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

As discussed above, if there is any delay in pembrolizumab that would result in patient being unable to undergo surgery within 56-77 days of study initiation (day of first pembrolizumab dose), then patient will go off trial and go directly to surgery.

8.1.2 Clinical Procedures/Assessments

8.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each participant to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see Appendix 2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to Section 8.2 for detailed information regarding the assessment and recording of AEs.

8.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period and at all times as indicated on the study flow chart. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

8.1.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at all other times points as specified in the Trial Flow Chart (Section 7.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

8.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

8.1.2.5 Tumor Imaging and Assessment of Disease

CT scans of chest, abdomen, and pelvis with IV and PO contrast will be obtained at baseline during the screening period, after 3 doses of pembrolizumab (day 56 ± 3 days), post-operatively (42-70 days post-operatively) and subsequently according to standard of care surveillance (annually for two years post-surgery and then as clinically indicated). Response will be assessed by institutional radiologists using RECIST 1.1. If a reason exists precluding use of iodinated contrast, MRI abd/pelvis and noncontrast chest CT can be substituted.

8.1.2.5.1 Initial Tumor Imaging

Initial tumor imaging at screening must be performed within 28 days prior to the date of allocation. CT scans of chest, abdomen, and pelvis with IV/PO contrast will be obtained at baseline. If a reason exists precluding use of iodinated contrast, MRI abd/pelvis and noncontrast chest CT can be substituted. PET/CT will also be required at baseline. Finally, EUS will be required at baseline as per this institution's standard of care to confirm staging. Baseline diagnostic laparoscopy is not mandated and can be performed if clinically indicated.

8.1.2.5.2 Tumor Imaging During the Study

The first on-study imaging assessment (CT chest/abdomen/pelvis with IV/PO contrast) should be performed at 8 weeks (56 days ±3 days) from the date of allocation. PET/CT, EUS, and upper endoscopy will also be performed at this time. Subsequent tumor imaging (CT chest/abdomen/pelvis with IV/PO contrast) should be performed 6-10 weeks post-operatively. Thereafter, surveillance imaging will be performed as per standard of care at this institution (annually for 2 years post-surgery and then as clinically indicated). Scans will be evaluated for progression or new lesions as per RECIST 1.1. If a reason exists precluding use of iodinated contrast, MRI abd/pelvis and noncontrast chest CT can be substituted. Surveillance EGD will only be performed as clinically indicated.

8.1.2.5.3 End of Treatment and Follow-up Tumor Imaging

In participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (6-10 weeks after surgery, then annually to year 2, and then as needed) to monitor disease status until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

8.1.2.5.4 RECIST 1.1 Assessment of Disease

RECIST 1.1 will be used as the primary measure for assessment of tumor response, date of disease progression/recurrence, and as a basis for all protocol guidelines related to disease status (eg, discontinuation of study treatment).

8.1.2.5.5 iRECIST Assessment of Disease

iRECIST is based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs. When clinically stable, participants should not be discontinued until progression is confirmed by the Investigator, working with local radiology, according to the rules below. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some participants can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response.

A description of the adaptations and iRECIST process is provided in Appendix 4, with additional detail in the iRECIST publication. [45] iRECIST will be used by the Investigator to assess tumor response and progression/recurrence, and make treatment decisions.

8.1.2.6 Tumor Tissue Collection and Correlative Studies Sampling

Patients will undergo tumor and blood collection as per routine clinical practice and will be consented to IRB 16294A prospective protocol for all indicated correlatives.

Tissue specimens collected at diagnosis and at time of surgical resection will be obtained per standard-of-care (SOC) practices. As per SOC at this institution, tissue at diagnosis and/or at time of surgical resection (if tumor is still present) will undergo testing for MSI via immunohistochemistry (IHC), HER2 testing (via IHC and/or fluorescence in situ hybridization (FISH)), and Epstein-Barr encoding region in situ hybridization (EBER ISH). These specimens will also undergo PD-L1 CPS testing, whole-exome RNAseq expression, as well as NGS testing via Foundation One CDx™ (Foundation Medicine, Cambridge, MA). ctDNA using Guardant360 (Guardant Health, Redwood, CA) will be evaluated before pembrolizumab therapy, after pembrolizumab therapy, after surgery, and at time of recurrence (if applicable) as per SOC.

Finally, patient fecal microbiomes will be characterized using fecal 16S RNA sequencing before pembrolizumab therapy, after pembrolizumab therapy, after surgery, and at time of recurrence (if applicable) as discussed below.

8.1.2.6.1 Fecal 16S RNA Sequencing

At least 3 aliquots of stool will be stored at -80ºC in tubes from the UltraClean® Fecal DNA Isolation Kits (MoBio). This technique uses a bead-beating method and removes inhibitors of PCR enzymatic reactions. Backup samples will be stored in a 50ml tubes. We will characterize the intestinal microbiota by MiSeq deep sequencing of bacterial 16S rRNA. Following assessment of genomic DNA quality and concentration, 16S rRNA-based tag sequence

libraries will be generated from an evolutionarily conserved gene marker to obtain deep surveys of the microbial communities present using next generation sequencing. The 16S rRNA-based Illumina library preparation and data analysis PCR primers to be used are specific for the 515-806 base pair V3-V4 region of the 16S rRNA encoding gene (338F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3') and contain Illumina 3' adapter sequences as well as a 12-base pair barcode. This barcode-based primer approach allows sequencing of multiple samples in a single sequencing run without the need for physical partitioning. Sequencing will be performed by the Next Generation Sequencing Core at Argonne National Laboratory using an Illumina MiSeq. Sequences will then be trimmed and classified with the QIIME toolkit. Using the QIIME wrappers, OTUs are picked at 97% sequence identity using uclust and a representative sequence is then chosen for each OTU by selecting the most abundant sequence in that OTU. These representative sequences are classified and assigned a taxonomic string using the RDP Classifier. PyNAST-aligned sequences are used to build a phylogenetic tree with FastTree for calculation of unweighted/weighted UniFrac distances (as well as other measures of diversity) for ecological analyses, including visualization via ordination (e.g. principal coordinates analysis). Furthermore, the transformation of sequence data into a numerical format (abundance of each OTU) allows the application of a variety of ecologic metrics to characterize the samples individually (measures of α-diversity) as well as between sample comparisons (measures of β-diversity). A comparison of frequencies of specific sequences in patients having a T cell-inflamed versus non-T cell-inflamed tumor microenvironment will be performed, and also comparisons between clinical responders versus non-responders. Rarefaction curves will be used to evaluate and compare OTU richness between libraries. Diversity indices, including the Shannon diversity index, and richness estimators (Chao1 and ACE) will be calculated.

8.1.2.6.2 Initial Screening Endoscopy

All subjects must receive an initial screening endoscopy at the site enrolling the subject. If the subject has received an endoscopy per standard of care prior to joining the study and the endoscopy was conducted at the enrolling study site, the subject does not have to repeat the procedure. Tissue samples collected from standard of care endoscopies can be accepted at screening only if the endoscopy was conducted at the enrolling institution.

If the subject received an endoscopy prior to joining the study at a non-enrolling site, a new endoscopy must be conducted at the enrolling site and will be considered part of clinical research.

8.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

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

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	Total thriiodothyronine (T3)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Free thyroxine (T4)
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Thyroid stimulating hormone (TSH)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Carcinoembryonic antigen (CEA)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Carbohydrate antigen 19-9 (CA 19-9)
	Uric Acid		Blood for correlative studies
	Calcium		Stool for correlative studies
	Chloride		PT/INR
	Glucose		aPTT
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

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

‡ If considered standard of care in your region.

8.1.4 Other Procedures

8.1.4.1 Withdrawal/Discontinuation

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 8.2 - Assessing and Recording Adverse Events.

8.1.4.2 Blinding/Unblinding

Not applicable as this is an open label study.

8.1.5 Visit Requirements

Visit requirements are outlined in Section 7.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 8.1 - Trial Procedures.

8.1.5.1 Screening

8.1.5.1.1 Screening Period

The screening period is 28 days during which laboratory and imaging studies prior to treatment must be completed. See flow chart, Section 7.0 for further details.

8.1.5.2 Treatment Period

8.1.5.3 Post-Treatment Visits

8.1.5.3.1 Safety Follow-Up Visit

Safety assessments will be performed at each physician visit. This includes on day 21 ± 3, day 42 ± 3, day 56 ± 3, and 42-70 days post-operatively.

The mandatory Safety Follow-Up Visit will be conducted approximately 14 days after the last dose of study treatment (on day 56 ± 3). All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment (end of treatment is the day of last dose of pembrolizumab) or before initiation of a new anti-cancer treatment should also be followed and recorded.

8.1.5.3.2 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should continue surveillance per standard of care. This includes

physician visits with labs (CEA and CA 19-9) every 3 months until year 3 post surgery and then every 6 months until year 5 post surgery as well as CT scan of chest/abdomen/pelvis with IV/PO contrast (or MRI abdomen/pelvis plus noncontrast chest CT if iodinated dye cannot be administered) annually for 2 years and then on an as needed basis.

Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

8.1.5.3.3 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

8.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event caused the

participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

● All AEs from the time of treatment allocation/randomization through 30 days following cessation of study treatment must be reported by the investigator.

● All AEs meeting serious criteria, from the time of treatment allocation/randomization through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.

● All pregnancies and exposure during breastfeeding, from the time of treatment allocation/randomization through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.

● Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify Merck.

8.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Principal Investigator and to Merck

For purposes of this study, 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 participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

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

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

8.2.2 Reporting of Pregnancy and Lactation to the Principal Investigator and to Merck

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

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

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

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

8.2.3 Immediate Reporting of Adverse Events to the Principal Investigator and to Merck

8.2.3.1 Serious Adverse Events

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

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another 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.

Refer to Table 6 for additional details regarding each of the above criteria.

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 that occurs to any participant must be reported within 24 hours to the P.I. and within 2 working days to Merck Global Safety if it causes the participant 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 participant 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 whether or not related to the Merck product, must be reported within 24 hours to the P.I. 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 Merck Global Safety.

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

8.2.3.2 Serious Adverse Event Reporting to the Coordinating Center

Use the University of Chicago Comprehensive Cancer Center protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

All serious adverse events (as defined in Section 8.2.3.1) and all adverse events that have been specified to require expedited reporting occurring on this study require expedited reporting to the University of Chicago Comprehensive Cancer Center (UC CCC). The responsible Research Nurse or other designated individual at the treating site should report the SAE to the Study Lead Principal Investigator, the University of Chicago CRA and the CCTO by the end of the business day when s/he becomes aware of the event. Events occurring after business hours should be reported to the CCTO by 12 p.m. (noon) the next business day. Reports should be made using the 'Serious Event Report' Form. Please scan and send via email (preferred) or fax to the following:

University of Chicago Phase II CRA General:
PhaseIICRA@medicine.bsd.uchicago.edu
Phone: 773-834-1746
Fax: 773-702-4889

UC CCC Cancer Clinical Trials Office Quality Assurance:
qaccto@bsd.uchicago.edu

8.2.3.3 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest

(ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

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 participant must be reported within 2 working days to Merck Global Safety if it causes the participant 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 participant 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 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 8.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the P.I., 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.

8.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 6 Evaluating Adverse Events

An investigator who is a qualified physician (or nurse practitioner or physician assistant), will evaluate all adverse events as to:

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, lasting >24 hours, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of participant taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the P.I. within 24 hours or 1 business day and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the P.I. and to Merck within 2 working days...	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the participant was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the P.I.'s product; or (3) the trial is a single-dose drug trial); or (4) P.I.'s product(s) is/are only used one time.)
	Rechallenge	Was the participant re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) P.I.'s product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PARTICIPANT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE P.I. AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.
No, there is not a reasonable possibility of Merck product relationship		Participant did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a participant with overdose without an associated AE.)

8.2.5 Principal Investigator Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.2.6 Additional Safety Considerations

Should a patient's surgery be delayed beyond the surgical window due to pembrolizumab-associated toxicity, the study would be held for a safety review by the lead investigators in order to evaluate if this event was attributable to the study intervention. If not, accrual would resume.

Safety will be assessed throughout the trial at weekly data safety monitoring meetings (DSM). Assessments will be based upon medical review of adverse events reports and the result of vital sign measurements, physical examinations, and clinical laboratory tests. The trial will be discontinued if any pembrolizumab-related deaths occur or if grade 3-4 toxicity exceeds tolerable limits by DSM review.

9.0 STATISTICAL ANALYSIS PLAN

9.1 Statistical Analysis Plan Summary

9.2 Statistical Analysis Plan

At our institution, patients with cT1b-2N0M0 GEA typically undergo surgery alone without additional therapy. Thus, we expect 100% of resected specimens to have evidence of cancer in historical controls. We hypothesize that neoadjuvant pembrolizumab will result in a pCR rate (Grade 1a+1b) of 15%. A sample size of 33 patients will provide 90% power with one-sided alpha 0.1 to detect this pCR rate using neoadjuvant pembrolizumab. More specifically, we will use an optimal, Simon two-stage design to test the null hypothesis that the Grade 1 response rate is 1% vs. the alternative that it is 15%. Fifteen patients will be enrolled in the first stage and if no complete pathological responses are observed the trial will be terminated for futility. If one or more responses are seen, an additional 18 patients will be studied for a total of 33. Two or more responses in 33 patients will indicate that the treatment is promising, whereas 0/15 or 1/33 will be considered a negative result. Patients who receive pembrolizumab but do not go to surgery will be counted as not having achieved a pCR.

We will also assess activity in the subset of patients who are PD-L1 positive. Assuming an approximate PD-L1 positivity incidence (CPS score $\geq$1) of 50% of all patients enrolled, this sample size of 16 expected PD-L1 positive patients will provide over 95% power with one-sided alpha 0.05 to detect a pCR rate (Grade 1a+1b) of 30% vs. 1% using neoadjuvant pembrolizumab in this preplanned subset. Using a higher CPS cutoff of $\geq$10 we assume 20% of patients will be PD-L1 positive based on recent data, and the sample size of 7 expected PD-

L1 positive patients will provide 67% power with one-sided alpha 0.05 to detect a pCR rate (Grade 1a+1b) of 30% vs. 1%

Complete responses (R0) will be tabulated along with an exact 90% confidence interval. DFS and OS will be estimated using the Kaplan-Meier method. Adverse events will be summarized by type, grade, and attribution to the study drug.

Chisquare tests and Spearman or Pearson correlation coefficients will be calculated to assess associations among the correlative endpoints. Comparisons of pre-treatment PD-L1 expression and other markers between responders and non-responders will be performed usingtwo-sample t or Wilcoxon rank-sum tests. Changes in tumor markers will be analyzed using paired t or Wilcoxon signed rank tests. If the number of events is sufficient, Cox regression models will be fit to determine whether tumor markers are predictive of DFS and OS.

10.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

10.1 Investigational Product

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

Pembrolizumab will be provided by Merck as summarized in Table 7.

Table 7 Product Descriptions

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

10.2 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

10.3 Clinical Supplies Disclosure

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

10.4 Storage and Handling Requirements

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

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

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

10.5 Returns and Reconciliation

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

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

11.0 STUDY MANAGEMENT AND REGULATORY AFFAIRS

11.1 Multicenter Guidelines

The specific responsibilities of the Study Lead Principal Investigator and the Coordinating Center are presented in Appendix B. Clinical studies coordinated by The University of Chicago must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements

The Study Lead PI/Coordinating Center is responsible for distributing all official protocols, amendments, and IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

11.2 Institutional Review Board (IRB) Approval and Consent

Unless otherwise specified, each participating institution must obtain its own IRB approval. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the treating investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the treating investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.3 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Cancer Clinical Trials Office (CCTO) at the University of Chicago Comprehensive Cancer Center. All documents must be sent to CCTO prior to study activation.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the Study Lead Principal Investigator and any sub-investigators who will be involved in the study.
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Investigational drug accountability standard operating procedures
- Additionally, before the study can be initiated at any site, the required executed research contract/subcontract must be on file with the University of Chicago.

11.4 Data and Safety Monitoring

This study will be remotely monitored by the University of Chicago PCCC Monitoring Staff in accordance with the University of Chicago, Section of Hematology/Oncology standard operating procedure titled Monitoring of Multi-Institutional Investigator Initiated Clinical Trials.

Prior to subject recruitment, and unless otherwise specified, a participating site will undergo a Site Initiation Teleconference to be conducted by the designated University of Chicago research team. The site's Study Lead Principal Investigator and his or her study staff must attend the site initiation meeting.

Monitoring will be conducted to verify the following:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Compliance with regulations
- Submission of required source documents

Participating sites will also undergo a site close-out teleconference upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and to ensure that the site Investigator is aware of his/her ongoing responsibilities.

Unless otherwise specified, this protocol will undergo weekly review at the multi-institutional data and safety monitoring teleconference as per procedures specified by the UC CCC NCI-approved Data and Safety Monitoring Plan. The conference will review:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

Protocol deviations are to be documented using the Protocol Deviation Form and sent via email to PhaseIICRA@medicine.bsd.uchicago.edu. Deviations that are considered major because they impact subject safety or alter the risk/benefit ratio, compromise the integrity of the study data, and/or affect subjects' willingness to participate in the study must be reported within 7 days. Please contact the University of Chicago CRA (PhaseIICRA@medicine.bsd.uchicago.edu) if you have questions about how to report deviations. All major protocol deviations should also be reported to the local IRB of record according to their policies and procedures.

11.5 Auditing

In addition to the clinical monitoring procedures, the University of Chicago Comprehensive Cancer Center will perform routine Quality Assurance Audits of investigator-initiated clinical trials as described in the NCI-approved UC CCC DSM Plan. Audits provide assurance that trials are conducted and study data are collected, documented and reported in compliance with the protocol. Further, quality assurance audits ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements. The audit will review subjects enrolled at the University of Chicago in accordance with audit procedures specified in the UC CCC Data and Safety Monitoring plan. For institutions who are formal members of the Personalized Cancer Care Consortium (PCCC), the UC CCC will conduct on site quality assurance audits on average every two years during the enrollment and treatment phase of the study.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator

must immediately inform the University of Chicago Cancer Clinical Trials Office and Regulatory Manager that such a request has been made.

11.6 Amendments to the Protocol

All modifications to the protocol, consent form, and/or questionnaires will be submitted to the University of Chicago IRB for review and approval. A list of the proposed modifications or amendments to the protocol and/or an explanation of the need of these modifications will be submitted, along with a revised protocol incorporating the modifications. **Only the Study Lead PI can authorize any modifications, amendments, or termination of the protocol.** Once a protocol amendment has been approved by the University of Chicago IRB, the UCCCC regulatory contact or their designee will send the amended protocol and consent form (if applicable) to the affiliate institutions electronically.

For external sites utilizing a local IRB:

- Upon receipt of the amendment documents the affiliate institution is expected to submit the amendment documents to their local IRB for approval as soon as possible.

- A copy of the IRB approval letter and approved consent document(s) should be sent to the UCCCC regulatory contract as soon as possible.
    - IRB approval should be obtained within 90 calendar days of the document distribution date. If approval cannot be obtained within this window, the reason for the delay should be provided to the designated UCCCC regulatory contact.

For external sites utilizing the BSD IRB as the IRB of record:

- Upon receipt of the amendment documents the affiliate institution is expected to implement the revised documents as soon as possible and no later than 30 calendar days from the document distribution date.
    - The date of local implementation should be documented in the local study records and provided upon request at time of monitoring and/or auditing.
- **No changes to the provided documents may be made at the local site without prior approval from the BSD IRB.**

11.7 Annual IRB Renewals, Continuing Review and Final Reports

A continuing review of the protocol will be completed by the University of Chicago IRB and the participating institutions' IRBs at least once a year for the duration of the study. The annual IRB renewal approvals for participating institutions should be forwarded promptly to the Regulatory Manager. If the institution's IRB requires a new version of the consent form with the annual renewal, the consent form should be included with the renewal letter.

11.8 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Study Lead Principal Investigator/treating Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all

other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.9 Obligations of Study Site Investigators

The Study Site Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Study Site Principal Investigator is responsible for personally overseeing the treatment of all study patients. He/she must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Study Site Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the CRFs. Periodically, monitoring visits or audits will be conducted and he/she must provide access to original records to permit verification of proper entry of data.

12.0 APPENDICES

Appendix 1: ECOG Performance Status

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

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

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)

Appendix 2: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)

Appendix 3: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

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

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

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

Contraception Requirements

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 8 during the treatment period and for at least 120 days after the last dose of study treatment

Table 8 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception [b, c] ○ Oral ○ Intravaginal ○ Transdermal

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

Pregnancy Testing

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

Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected; at the time points specified in the Schedule of Activities, and as required locally.

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

Appendix 4: Description of the iRECIST Process for Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

In participants who show evidence of radiological PD by RECIST 1.1 the treating investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained(using iRECIST for participant management). This decision by the treating investigator should be based on the participant's overall clinical condition.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

Any participant deemed clinically unstable should be discontinued from study treatment at site-assessed first radiologic evidence of PD, and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the treating investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirmPD by iRECIST, per treating investigator assessment.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥ 20% and ≥ 5 mm from nadir
    - Please note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment,

the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated, and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
    - For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point
    - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
    - For new lesions, worsening is any of these:
        - An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point
        - Visible growth of new non-target lesions
        - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND

- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the scan on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation scan proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the treating investigator, and theparticipant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

NOTE: If a participant has confirmed radiographic progression (iCPD) as defined above, but the participant is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 7.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (ie, achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
    - Sum of diameters reaches the PD threshold (≥ 20% and ≥ 5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.

- Non-target lesions
    - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
    - If non-target lesions had shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.
- New lesions
    - New lesions appear for the first time
    - Additional new lesions appear
    - Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
    - Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, except in one respect. If new lesions occurred at a prior instance of iUPD, and at the confirmatory scan the burden of new lesions has increased from its smallest value (for new target lesions, their sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication.[45]

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