MSK Protocol Cover Sheet

A Single-Arm Pilot Study of Adjuvant Pembrolizumab plus Trastuzumab in HER2+ Esophagogastric Tumors with Persistent Circulating Tumor DNA Following Curative Resection

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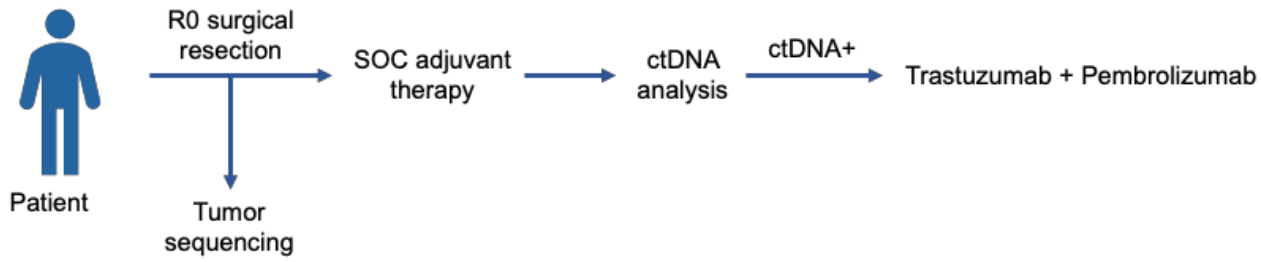
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single arm pilot study of adjuvant trastuzumab and pembrolizumab in patients with HER2-positive gastric, esophageal or gastroesophageal junction (GEJ) tumors with persistent circulating tumor DNA (ctDNA) despite curative surgery and completion of standard perioperative and/or adjuvant therapy. This study will enroll 24 eligible patients over 2 years. The central hypothesis of this trial is that adjuvant trastuzumab + pembrolizumab will improve ctDNA clearance and disease-free survival (DFS) in patients with resected HER2+ tumors with minimal residual disease and very high risk of recurrence. This is based on recent data, which suggest potential synergy of trastuzumab with pembrolizumab. Patients with HER2+ esophagogastric cancer who are ctDNA positive (ctDNA+) will be consented within 8 months after curative surgery and completion of standard of care perioperative and/or adjuvant therapy. Patients with detectable ctDNA will receive 6 months of trastuzumab + pembrolizumab (**Figure 1**). The primary objective of this trial is to determine the ctDNA clearance at 6 months with intensified adjuvant therapy.

Figure 1: Study Schema



2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objective:

To observe the rate of ctDNA clearance at 6 months with pembrolizumab + trastuzumab in patients with HER2+ esophagogastric tumors and persistent ctDNA following curative resection (R0 surgical resection) and standard perioperative and/or adjuvant therapy.

2.2 Secondary Objectives:

- To establish the safety of 6 months of adjuvant pembrolizumab + trastuzumab in patients with resected HER2+ esophagogastric tumors and persistent ctDNA.
- To observe other measures of efficacy including DFS (median, 1-year and 2-year) and overall survival (median, 2-year and 5-year) in ctDNA-positive patients with resected HER2+ esophagogastric tumors.

2.3 Exploratory Objectives:

- To collect archival tumor samples and post-recurrence tumor biopsies for correlative analysis, including TCR rearrangements, transcriptomic analysis and immunohistochemistry.
- To utilize blood and tissue specimens collected during the course of study to explore the mechanisms of primary and acquired resistance to trastuzumab and pembrolizumab and their relationship to response, disease-free survival and overall survival.
- To bank tumor and blood material for future correlative analysis, including but not limited to whole exome analysis to determine mutation load and specific neoantigen landscape.

3.0 BACKGROUND AND RATIONALE

3.1 Introduction

Esophagogastric cancer is a significant global health burden, representing the fifth most common cancer worldwide with over one million new cases each year, and the third leading cause of cancer death[1]. Approximately half of patients present with metastatic disease, but even those that present with resectable disease have a high probability of recurrence. Even with adequate resection and aggressive standard of care perioperative therapy, 3-year overall survival in locoregionally advanced disease is only 57-58%[2,3].

Improving therapy for locally advanced esophagogastric cancer has been challenging. Differing perioperative approaches are utilized, including neoadjuvant chemoradiotherapy[2,4], perioperative chemotherapy[3], adjuvant chemotherapy[5] and adjuvant chemoradiotherapy[6], all followed by guideline-driven radiographic and clinical surveillance. Notably, there are no established biomarkers to guide adjuvant therapy. Therefore, this trial utilizes ctDNA detection at the conclusion of perioperative therapy—regardless of the modality—as a method of identifying patients at highest risk of recurrence to further escalate their therapy and delay recurrence.

Approximately 25% of esophagogastric tumors overexpress the receptor tyrosine kinase human epidermal growth factor receptor 2 (HER2, also known as ERBB2), which has emerged as a critical oncogenic driver and biomarker[7]. In the metastatic setting, targeting this protein with the anti-HER2 antibody trastuzumab demonstrates considerable clinical benefit[8]. Limited data suggest that perioperative trastuzumab is safe and well-tolerated in patients with locally advanced esophagogastric cancer[9,10]. However, results from randomized trials studying perioperative or adjuvant trastuzumab (RTOG-1010, INNOVATION) have not been reported, and a role for adjuvant trastuzumab in locally advanced disease has not been established. Thus, the current recommendation for patients with locally advanced HER2-positive esophagogastric cancer is observation after completion of standard perioperative/adjuvant therapy.

Since development of these trials, a growing body of data suggests potential synergy between trastuzumab and immunotherapy. Trastuzumab activity is enhanced by immune cell recruitment leading to dendritic cell priming, cytotoxic T cell activation and programmed death-ligand 1 (PD-L1) upregulation. Based on these data, a recent phase II study combining pembrolizumab and

trastuzumab in patients with metastatic esophagogastric adenocarcinoma demonstrated a remarkable 89% objective response rate 100% disease control rate[11]. Notably, a decrease in ctDNA was observed after only one dose of pembrolizumab and trastuzumab in 83% of patients. More recently, final results of this phase II trial (N=37) demonstrated that 70% of patients were progression-free at 6 months and the combination of pembrolizumab with trastuzumab and chemotherapy is safe and has promising activity in HER2-positive metastatic esophagogastric cancer. This led to the ongoing KEYNOTE-811 study, a global multicenter, randomized, placebo-controlled phase 3 study in which patients with previously untreated unresectable or metastatic HER2+ gastric/GEJ cancer are randomized 1:1 to chemotherapy + trastuzumab with pembrolizumab or placebo.

Given these promising data, the proposed trial will evaluate the efficacy of adjuvant combined trastuzumab/pembrolizumab in ctDNA+ patients at high risk for recurrence.

3.2 Clinical activity of pembrolizumab in esophagogastric cancer

Programmed death-1 (PD-1) is a surface molecule on T cells that binds its ligand PD-L1 and suppresses antitumor immunity. Pembrolizumab is a humanized monoclonal antibody that inhibits PD-1 and has been shown to have activity in several malignancies, including esophagogastric cancer. In the phase II KEYNOTE-059 study, patients with metastatic esophagogastric cancer who had previously received two or more lines of systemic therapy were given pembrolizumab monotherapy[12]. This trial demonstrated an objective response rate of 12% regardless of PD-L1 status, which improved to 16% in PD-L1-positive patients. Based on these results, pembrolizumab was approved in the metastatic setting for chemotherapy-refractory patients with a combined positive score (CPS) score $\geq$ 1. The KEYNOTE-061 study randomized patients receiving second-line therapy for metastatic esophagogastric cancer to chemotherapy or pembrolizumab. Patients with a PD-L1 CPS score $\geq$1 achieved a 12-month overall survival of 40% with a median duration of response of 18 months[13]. Both of these trials demonstrated that a significant subset of PD-L1-expressing patients derived considerable clinical benefit from pembrolizumab monotherapy.

3.3 Clinical activity of trastuzumab in esophagogastric cancer

In the metastatic setting, trastuzumab was the first targeted therapy approved in esophagogastric cancer based on results from the Trastuzumab for Gastric Cancer (ToGA) trial[8]. In this double-blinded phase III study, 594 patients were randomized to receive chemotherapy with or without trastuzumab. The primary endpoint was reached with a superior mOS of 13.8 vs 11.1 months when trastuzumab was added. Of note, the survival benefit was more pronounced in patients who fulfilled current HER2+ criteria (IHC 3+ or IHC 2+ in combination with FISH positivity). Numerous subsequent phase III trials targeting HER2 in esophagogastric cancer using small molecules like lapatinib (LOGiC, TYTAN) or antibodies like pertuzumab or T-DM1 (JACOB, GATSBY) have failed[14–17]. One possibility for the lack of benefit for other HER2-targeted agents is that trastuzumab may induce non-canonical immune stimulation, in addition to its canonical inhibition of HER2 signaling.

3.4 Mechanistic rationale for the combination of trastuzumab and pembrolizumab

Both preclinical and clinical data suggest that trastuzumab modulates antitumor immunity through various mechanisms. For example, trastuzumab promotes immune cell recruitment and activation via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Trastuzumab increases uptake of HER2 by dendritic cells, leading to cytotoxic T cell activation and proliferation[18], and promotes NK cell recruitment via increased MHC class I expression. Both of these mechanisms lead to the production of cytokines such as IFNγ [19], resulting in the upregulation of PD-L1 on tumor cells, which can be inhibited by concurrently blocking PD1[20,21].

These observations have led to clinical studies combining HER2 blockade with anti-PD1 therapy. An investigator-initiated trial conducted at Memorial Sloan Kettering Cancer Center (MSKCC) (NCT2954536) demonstrated that pembrolizumab in combination with trastuzumab and chemotherapy was both safe and efficacious in metastatic esophagogastric cancer patients with an objective response rate of 89% and disease control rate of 100%[11]. Grade 3/4 non-immune toxicities included anemia, transaminitis, diarrhea, rash, mucositis, lymphopenia, and neutropenia, which were all likely due to concurrent chemotherapy. Immune-related toxicities included colitis and transaminitis. These preliminary results demonstrate both efficacy and safety of combined pembrolizumab and trastuzumab in esophagogastric cancer patients.

3.5 Circulating tumor DNA

Circulating tumor DNA (ctDNA) obtained from plasma during a routine blood draw—a technique known as "liquid biopsy"—can be used to detect minimal residual disease (MRD). Whereas histopathologic or molecular characteristics of tumors that are associated with a higher recurrence risk indicate a propensity for metastasis, the presence of circulating DNA molecules containing somatic mutations found in an individual's tumor is a direct indication that occult tumor cells remain after surgery. ctDNA has been detected across diverse tumor types: in patients with localized tumors, ctDNA was detected in 73, 57, 48, and 50% of patients with colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, respectively[22]. Notably, ctDNA was often present in patients without detectable circulating tumor cells, suggesting that these two biomarkers are distinct entities. Given that ctDNA is a relatively new area of investigation, the frequency of positive ctDNA samples after perioperative chemotherapy is not well established, and the ctDNA clearance rate among tumor types is not known. However, several studies have shown that ctDNA can be used to detect MRD and determine those at highest risk for recurrence[22–24].

For example, recently published data suggest that patients with resected stage II colon cancer and detectable ctDNA have residual disease with very high recurrence risk. In this study, 1,046 plasma samples from a prospective cohort of 230 patients with resected stage II colon cancer were used to evaluate the utility of ctDNA to assess the risk of recurrence post-resection[23]. In patients not treated with adjuvant chemotherapy, ctDNA was detected postoperatively in 14 of 178 patients (7.9%), 11 of whom (79%) recurred at a median follow-up of 27 months; by contrast, recurrence occurred in only 16 of 164 patients (9.8%) with negative ctDNA [hazard ratio (HR), 18; 95% confidence interval (CI), 7.9 to 40; $p < 0.001$]. In patients treated with chemotherapy, the presence of ctDNA after completion of chemotherapy was also associated with an inferior recurrence-free survival (HR, 11; 95% CI, 1.8 to 68; $p = 0.001$). Taken together, these studies suggest a positive predictive value (PPV) for

cancer recurrence of >83% in patients who have detectable ctDNA following surgical resection. More recent data also validate that persistent ctDNA after

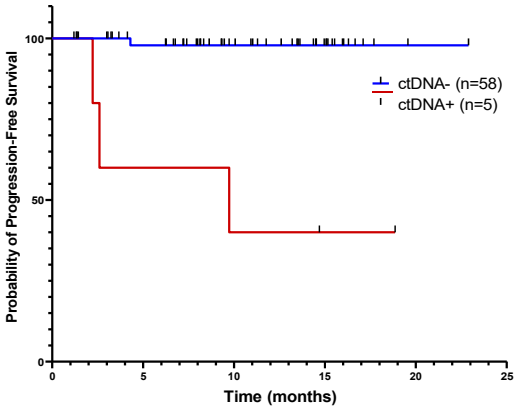


Figure 2. Progression-Free Survival by ctDNA result (N=63). PFS was compared between patients with positive (matched) ctDNA (red) and negative (mismatched or not detected ctDNA (blue) (Protocol 18-399).

prognostic for recurrence. In one study, 10 out of 94 patients with stage I-III colorectal cancer had positive ctDNA at day 30 post-resection, all of whom received standard adjuvant chemotherapy[25]. Of these 10 patients, 7 patients experienced relapse and all had positive ctDNA throughout or shortly after completion of adjuvant therapy. Among the 8 patients with positive ctDNA after surgery and serial ctDNA sampling, 4 of 8 patients (50%) demonstrated ctDNA clearance with adjuvant treatment; two of these patients remained ctDNA negative and did not have disease recurrence within the follow-up period. CtDNA profiling from the 75 patients with serial ctDNA sampling suggest that ctDNA positivity persists in longitudinal samples in the absence of clinical intervention. While data for ctDNA positivity after standard therapy for locoregionally advanced esophagogastric cancer are limited, recent reports suggests that ctDNA is also prognostic for recurrence. For example, in one study of 46 patients with resected gastric cancer, ctDNA was detected in 45% of pretreatment samples[26]. All patients with detectable ctDNA in the post-operative period experienced relapse, and ctDNA positivity during follow-up was associated with worse disease-free survival (HR, 14.78; 95% CI, 7.991 – 61.29; p < 0.0001) and overall survival (HR, 7.664; 95% CI, 2.916 – 21.06, p < 0.002).

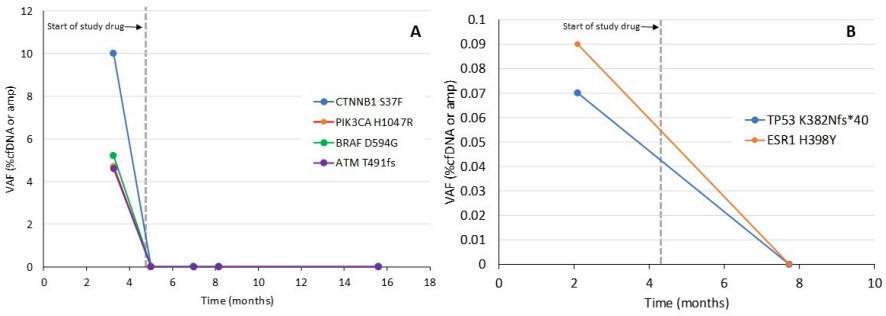


Figure 3. Variant allele frequencies (VAF) in 2 patients with matched ctDNA enrolled on protocol 18-399.

We are conducting an ongoing single arm pilot study of adjuvant pembrolizumab in patients with MSI-H solid tumors and positive ctDNA, similar in design to this protocol (IRB #18-399). From the time of study initiation in 2018, through August 2020, we screened 750 patients and identified 153 with stage II and III MSI-H disease treated with curative intent eligible for this protocol. After exclusion we performed ctDNA testing on 73 patients, of which 7 had positive results, and 5 patients enrolled on study. Among the 63 patients who were ctDNA-negative or unmatched, 2 were lost to follow up, 1 is deceased, and the remainder are alive with no evidence of disease (NED) (**Figure 2**). Preliminary results from two

patients receiving pembrolizumab show a striking decrease in variant allele frequency (VAF) detected in plasma (**Figure 3**), with patients achieving ctDNA clearance after 5 and 8 months of treatment, respectively.

While the recurrence rate for patients with esophagogastric cancer with persistent ctDNA after resection and standard adjuvant therapy is not known, available data suggest that the large majority of these patients will have disease recurrence, and therefore we estimate the null ctDNA clearance rate with surveillance to be ~5% in this population.

MSK has developed our own method of detecting ctDNA in plasma derived from whole blood samples. This method, called MSK-ACCESS, is a next-generation sequencing assay that can accurately detect down to 0.3% mutant allele fraction and will be used in the ctDNA assessments for this study (see Section 10.1.2).

This trial explores adjuvant HER2 and PD-1 blockade patients with HER2+ esophagogastric tumors who have positive ctDNA following completion of standard-of-care surgery and perioperative therapy, a population at extremely high risk for recurrent disease. As administration of trastuzumab and pembrolizumab in the adjuvant setting is not currently approved by the FDA, these drugs will be provided as investigational.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a single arm pilot study of adjuvant pembrolizumab + trastuzumab in patients with HER2+ (IHC 3+ or IHC 2+/FISH ratio >2.0) esophageal, GEJ or gastric cancer and detectable ctDNA after completion of standard perioperative therapy and surgery. Using mutations or amplifications identified in the primary resected tumors (as determined by next-generation sequencing (NGS)), ctDNA levels will be measured in plasma collected up to eight months after curative surgery, and standard perioperative and/or adjuvant therapy and assessed for alterations shared between the tumor and plasma. Eligible patients with at least one shared mutation between tumor and plasma will be enrolled in this study and and receive 6 months of pembrolizumab/trastuzumab. The primary endpoint of the study is to determine ctDNA clearance at 6 months.

4.3 Intervention

This study will be conducted as a single arm pilot study including 24 participants. Treatment will be administered on an outpatient basis as possible to the date of enrollment. Trastuzumab (8mg/kg loading dose; 6mg/kg maintenance) in combination with pembrolizumab 200 mg IV will be administered on day 1 of each 3-week dosing cycle (21 days). One cycle is defined as 21 days. In esophagogastric cancers, standard of care for patients who have completed surgery and perioperative therapy is surveillance. However, in patients with detectable ctDNA, the expected ctDNA clearance rate is close to zero and our data suggest that these patients are essentially metastatic, which justifies our investigation of intensified adjuvant therapy.

Treatment will be performed on the scheduled day (± 7 day treatment window). Adverse events will be monitored and graded in severity according to the guidelines outlined in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Patients will be assessed with a CT scan and ctDNA assessment at baseline and every 3 months for the first 12 months post-enrollment, then a CT scan and ctDNA assessment every 6 months in year 2 and year 3 post-enrollment, and then every 12 months in year 4 and year 5 post-enrollment. Patients will be on this study for up to 5 years. ctDNA assessments for all timepoints will be performed in a CLIA-approved laboratory. Anytime a patient has intolerable toxicity or declines further treatment, they will go to the event monitoring phase and will be followed with a CT scan and ctDNA assessment every 3 months for the first 12 months post-enrollment, then a CT scan and ctDNA assessment every 6 months in years 2 and 3 post-enrollment, and then every 12 months in years 4 and 5 post-enrollment. In the event of disease recurrence during the treatment phase, the patients will be taken off treatment and followed for overall survival. In the event of disease recurrence during the follow-up period, the patients will be followed for overall survival.

We believe that ctDNA detection of MRD after surgery and/or adjuvant therapy portends a high risk of recurrence, and current literature suggests a PPV of > 83% independent of the assay used. Overall, we believe that our proposed approach will result in reduced recurrence and improved overall survival for patients with resected HER2+ esophagogastric tumors with persistent ctDNA.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS & NON-THERAPEUTIC ASSESSMENTS

5.1 Trastuzumab Dose

For this study, a trastuzumab biosimilar, trastuzumab-qyyp, will be provided by Pfizer at no charge. Trastuzumab will be administered on an every 3 week dosing schedule, with initial loading dose of 8 mg/kg as a 90 minute infusion, followed by trastuzumab 6 mg/kg every 3 weeks, administered as a 30 minute infusion if the initial loading dose was well tolerated. Trastuzumab infusion will be prepared and administered in accordance to institutional guidelines.

Trastuzumab-qyyp will be provided and shipped by Pfizer directly to MSKCC. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. The investigator is responsible for keeping accurate records of the clinical supplies received from Pfizer or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

5.2 Pembrolizumab Dose

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks.

5.2.1 Pembrolizumab Storage and Accountability

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.

5.2.2 Dispensing of Pembrolizumab

Pembrolizumab will be provided by Merck at no charge. Pembrolizumab will be provided in 100mg/4mL vials and shipped by Merck directly to the MSKCC. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

MSK will submit a cross reference application to the FDA for the use of pembrolizumab in combination with trastuzumab.

5.2.3 Preparation and Administration of Pembrolizumab

Investigators should consult the manufacturer's instructions for pembrolizumab for complete prescribing information and follow institutional procedures for the administration of pembrolizumab. Pembrolizumab should be administered on Day 1 of each 21 day cycle after all procedures and assessments have been completed as detailed on the Trial Flow Chart (Section 11.0). Pembrolizumab 200mg will be administered as a 30-minute IV infusion every 3 weeks. Variation in infusion time is permitted per institutional standards.

5.2.4. Timing of Administration

Treatment will be administered in the following order: pembrolizumab will be administered first, followed by trastuzumab infusion.

6.1 CRITERIA FOR PARTICIPANT ELIGIBILITY

6.2 Participant Inclusion Criteria

- Age 18 years or older.
- ECOG performance status 0-2.
- Sign informed consent within 8 months after curative surgery and completion of standard of care perioperative and/or adjuvant therapy.
- HER2+ esophageal, GEJ, or gastric adenocarcinoma biopsy or resection specimen as defined by local HER2 IHC3+ or IHC 2+/FISH>2.0 expression.
- Must have genetic testing of DNA from primary tumor for somatic genomic alterations across a minimum of 50 genes.
- Must have undergone a complete curative surgical resection (R0).
- Must have completed standard of care (SOC) surgery, neoadjuvant or adjuvant therapy
- CtDNA will be tested at a minimum of four weeks after completion of surgery and standard perioperative and/or adjuvant therapy. To be eligible for trastuzumab/pembrolizumab therapy, the patients must have positive ctDNA (as defined in section 7.0) within 8 months after completion of appropriate standard of care therapy

(surgery, chemotherapy, radiation as appropriate). For all patients, if the initial ctDNA has a negative result, ctDNA can be re-tested 4 weeks later, within 9 months of completion of standard therapy.

- Demonstrate adequate organ function as defined in Table 1.

Table 1 – Organ Function Requirements for Eligibility

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL
Renal	
Serum creatinine	≤1.5 X upper limit of normal (ULN)
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN. Except patients with Gilbert's disease (≤3xULN)
AST and ALT	≤ 2.5 X ULN
Albumin	≥3 mg/dL

6.3 Participant Exclusion Criteria

- Patients who have not recovered from serious adverse events (as determined by treating MD) related to surgery.
- Presence of metastatic or recurrent disease.
- Had R1 (microscopic residual tumor) or R2 resection (macroscopic residual tumor at resection margin).
- Left ventricular ejection fraction <50% within 1 month of screening by MUGA or echocardiogram.
- Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
- Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
- Patients who have received acute, low dose, systemic immunosuppressant medications (*e.g.*, dexamethasone containing antiemetic regimen or steroids as CT scan contrast premedication) may be enrolled.
- The use of inhaled corticosteroids and mineralocorticoids (*e.g.*, fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed.
- Has a known history of active TB (Bacillus tuberculosis)
- Hypersensitivity to pembrolizumab or any of its excipients.

- Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
- Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis
- Has known history of, or any evidence of active, non-infectious pneumonitis.
- Has an active infection requiring systemic therapy.
- Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
- Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
- Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
- Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-CTLA-4 agent.
- Has received prior therapy with an anti-HER2 agent
- Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease; systemic lupus erythematosus; Wegener syndrome [granulomatosis with polyangiitis]; myasthenia gravis; Graves' disease; rheumatoid arthritis, hypophysitis, uveitis) within the past 3 years prior to the start of treatment. The following are exceptions to this criterion:
    - Subjects with vitiligo or alopecia
    - Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement or psoriasis not requiring systemic treatment.
- Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
- Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
- Has received a live vaccine within 30 days of planned start of study therapy.
    - *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*
- Is unwilling to give written informed consent, unwilling to participate, or unable to comply with the protocol for the duration of the study.

7.0 RECRUITMENT PLAN

The sample size will be 24 patients with ctDNA-positive HER2+ EG cancer. Although we initially anticipated that we would need to screen 175 patients to identify 24 HER2+ patients (25% of esophagogastric cancers) with detectable ctDNA after adjuvant therapy (57%, unpublished data),

based on our experience to date, we anticipate that we will need to screen closer to 400 patients to identify 24 patients who meet all enrollment criteria. Given ~240 esophagectomies and gastrectomies annually at MSKCC, an accrual duration of ~2 years is expected.

In order to be eligible, the patient must be ctDNA-positive following standard of care therapy (surgery, chemotherapy, radiation as appropriate). If the initial ctDNA has a negative result, ctDNA can be re-tested 4 weeks later, within 9 months of completion of standard therapy. ctDNA will be defined as positive if at least one mutation is shared between the tumor and ctDNA sequence, as described in a manuscript that is in-preparation for publication. In a review of relatedness among mutations found in ctDNA sequenced with MSK-ACCESS (the novel next-generation sequencing assay designed at MSK to detect and identify ctDNA) and MSI tumors sequenced with MSK-IMPACT, one shared mutation will provide an estimated relatedness of 68.2% [95% CI 46.6% to 98.8%]; if two mutations are shared, the estimated relatedness is 93.3% [95% CI 82.6% to 99.6%]. The mutation(s) of interest can be assessed as changes at the nucleotide or protein level (i.e. KRAS c.35G>A vs. KRAS G12D).

In the event that a patient has multiple concurrent tumors, all resectable tumors identified through imaging will be sequenced with IMPACT (or similar as outlined in this protocol), as per standard of care. Mutations derived from ctDNA data will be interpreted in the context of tumor sequencing data independently. Only ctDNA that has achieved acceptable relatedness with mutations found in tumors (as defined in section 7.0 of this protocol) will used for assessment in this trial.

All eligible patients, regardless of sex and race, will be approached for participation. No additional measures, e.g. advertisement or payment to patients, will be employed to recruit patients.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

Participation in the study is completely voluntary. Patients/LAR will be required to read, agree to, and sign an IRB-approved informed consent form prior to registration on this trial. Patients will not receive payment for their participation on this study. Patients are free to withdraw from the study without consequence at any time.

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at MSKCC. If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and

discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study.

7.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

8.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must *sign* an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

9.1 PRE-TREATMENT/INTERVENTION

Pretreatment evaluation will be performed within 2 weeks of study entry and will include:

- History, concomitant medications, and toxicity assessment.
- Physical exam, vital signs, and performance status (ECOG).
- Serum or urine pregnancy test for women of childbearing potential (WOCBP)

In addition, all WOCBP should be instructed to contact the Principal Investigator immediately if they suspect they might be pregnant (e.g. late or missed period) at any time during study participation.

- Laboratory evaluation including complete blood count, comprehensive chemistry panel (includes BUN, creatinine, sodium, potassium, chloride, CO2, calcium, glucose, bilirubin, total, protein, total, albumin, alkaline phosphatase, AST, ALT), TSH and free T4.
- Research blood tests including whole blood collection for peripheral blood mononuclear cells (PBMC) and peripheral blood lymphocytes (PBLC).
- Tumor Markers: CEA, LDH (per clinically indicated)
- Electrocardiogram (ECG)

The following must be obtained within 28 days prior to starting protocol therapy:

- CT scan of chest, abdomen and pelvis within 4 weeks of study entry
- Collection of archival tumor tissue for correlative testing
- ctDNA collection
- Echocardiogram or MUGA scan

To be completed any time prior to starting therapy:

- Histological confirmation of cancer diagnosis at MSKCC prior to study enrollment.

10.0 TREATMENT/INTERVENTION PLAN

Study treatment in all arms will begin on Day 1 of each 3-week dosing cycle (21 days). This study will be conducted as a single arm pilot study. Trastuzumab (8mg/kg loading dose; 6mg/kg maintenance) will be administered on day one of each 3 week cycle according to standard-of-care. Pembrolizumab will be prepared by the research pharmacy staff and administered intravenously. Pembrolizumab will be given as follows: pembrolizumab 200 mg IV every 3 weeks over 30 minutes. Treatment should begin as close as possible to the date on which the participant is enrolled.

Treatment will be performed on the scheduled day (± 7-day treatment window) on an outpatient basis. Adverse events will be monitored throughout the study and graded in severity according to the guidelines outlined in the NCI CTCAE version 5.

CT scan and ctDNA assessment will be performed at baseline, and every 3 months for the first 12 months post-enrollment. Follow-up will start at the time of disease recurrence, removal from study any reason other than recurrence, or completion of treatment. If the patient is removed for disease recurrence, they will be followed for overall survival every 3 months for the first-year post enrollment, every 6 months for years 2 and 3 post-enrollment, and then every 12 months for years 4 and 5 post-enrollment. If the patient is removed for toxicity or completion of pembrolizumab + trastuzumab therapy, they will be followed with a CT scan and ctDNA assessment every 6 months for years 2 and 3 post-enrollment and then every 12 months for years 4 and 5 post-enrollment. Each subject will be on study from the time of enrollment for up to 5 years.

10.1 Exploratory Correlative Studies

Details for correlative collection are listed in Appendix 1. Patients will be offered the option of consenting to 06-107 and corresponding Biospecimen Research Protocols (optional). For consented patients, upon completion of all exploratory correlative studies, any leftover blood or tissue samples will be re-banked for future use in unspecified research.

10.1.1 Collection of peripheral blood mononuclear cells (PBMC), plasma for peripheral blood lymphocytes (PBLC), and immunologic analyses

Whole blood will be used for isolation of PBMCs. Flow cytometry will be performed on PBMCs at baseline and during treatment to assess changes in composition/activation status of lymphocyte subsets, including CD8+ and CD4+ T-cell subsets (activated; effector/memory; regulatory) and populations of those cells as defined by the expression of activation, exhaustion, or signaling markers such as ICOS, HLA-DR, PD-1, CTLA-4, and/or intracellular IFNγ.

Approximately 16 ml of whole blood per visit (baseline, C1D1, C2D1, C3D1, C4D1, at every imaging timepoint and treatment discontinuation) will be collected at ambient temperature into 2 x 8-ml BD sodium heparin Cell Preparation Tubes (CPT) for density gradient centrifugation and isolation of PBMC and plasma for banking according to institutional procedures at the MSKCC Ludwig Center for Cancer Immunotherapy Immune Monitoring Core Facility. After centrifugation, the plasma supernatant will be collected and then the PBMC monolayer isolated and washed. PBMC will resuspended at ~6-8 million cells per vial in cell freezing medium, frozen at –80 degrees C in controlled rate cooling containers, and then cryopreserved in liquid nitrogen freezers. Plasma will be frozen in 1.5 ml aliquots and stored at –20 degrees C.

10.1.2 Collection of blood for ctDNA

DNA extracted from plasma will be assessed at baseline, every imaging timepoint, and at the time of treatment discontinuation for the presence of mutations. Testing may be performed using digital PCR or next-generation sequencing but must be able to reliably identify genomic alterations (excluding copy number changes or fusions) at or below a minimum allele frequency of 0.25%.
Testing must be performed by a CLIA-certified laboratory or equivalent. We will limit ctDNA testing to the following assays:

- Guardant 360, Guardant Health, Redwood City, CA
- MSKCC Access, MSKCC, New York, NY

Approximately 40-60 ml of whole blood per visit will be collected at ambient temperature into 4-66x 10mL cell free DNA BCT tubes for plasma isolation. Minimum requirements are at least 10cc of plasma or 10ng of isolated cfDNA. Appendix 1 outlines the number of tubes required for ctDNA collection at each timepoint.

The same assay used to detect ctDNA at baseline should be used for all subsequent timepoints.

MSK-ACCESS is a next-generation sequencing assay designed to detect mutations in cell free DNA. This assay uses custom DNA probes and unique molecular indexes (Integrated DNA Technologies, US) that were designed to capture selected exons and introns in 129 genes. The selected genes in this panel were chosen to interrogate at least 1 mutation in 84% of >20,000 tumors analyzed with MSK-IMPACT. These libraries are sequenced using the Illumina HiSeq system with paired end reads (2 x 100bp), to achieve >20,000x average total coverage and a collapsed unique coverage of >1,000x. After bioinformatic pipeline filtering the data are pushed through a custom read collapsing algorithm, resulting in a cfDNA consensus read. These consensus reads will be aligned back to the human genome followed by variant calling using VarDict. MSK-ACCESS allows for detection of somatic mutations down to 0.3% mutation allele fractions at 1000x uniquely collapsed coverage per loci. For all time points, ctDNA testing will be performed at the Department of Molecular Pathology at MSK. This is a CLIA-certified laboratory and all testing will be in accordance to the Clinical Laboratory Improvement Amendments of 1988.

10.1.3 T-cell receptor (TCR) Sequencing

DNA extracted from formalin-fixed or frozen primary tumor tissue will need to be sequenced to identify somatic mutations across all or a subset of the coding regions for at least 50 genes. Sequencing must be performed by a CLIA-certified laboratory or equivalent and may be performed using tumor only or tumor with matched normal. Tumor sequencing will be performed in the Memorial Sloan Kettering Molecular Genetics Laboratory.

The presence of tumor infiltrating lymphocytes (TILs) and TCR clonality will be assessed by TCR sequencing of tumor and serially collected PBLC using ImmunoSEQ™ (Adaptive Biotechnologies), or similar assay if updated. This assay identifies unique TCRβ-chains by sequencing the rearranged CDR3 regions in the β-chain gene locus. The relative abundance of unique TCRβ CDR3 regions across the repertoire and interference of T cell quantity within each sample is estimated from sequence data. This will allow us to quantify the presence and relative clonality of specific TCR clones in the tumor and peripheral blood.

The number of antigens on tumors that are recognized by the immune system have been defined at the molecular level, including both tumor-associated antigens and tumor-specific antigens. These antigens can be recognized by the adaptive immune system and serve to direct an immunologic response to cancers. Techniques including intracellular cytokine staining, IFNy release assays and tetramer assays, will be used for quantification and functional characterization of antigen-specific T cells in human peripheral blood and tumors samples. These functional, tumor antigen-specific T cells may be relevant markers of disease response.

Cytokines and chemokines are readily detected in serum samples from patients and may provide evidence of an ongoing immune response. The type(s) of cytokines or chemokines, the kinetics and magnitude of changes, and the corresponding clinical events may be used to characterize the ctDNA clearance and immune response. The MSKCC Immune Monitoring Facility has a specialized

platform, the Meso Scale Delivery System, to detect cytokines and chemokines in patient samples sensitively and accurately.

10.1.4 PD-L1 testing of archival tissue

PD-L1 expression in tumor tissue will be characterized by IHC to explore the relationship between PD-L1 expression and response to pembrolizumab. Other exploratory biomarkers including but not limited to PD-1 expression and markers of T-cell phenotype may also be evaluated. Testing will be done at the Memorial Sloan Kettering Cancer Center Pathology lab.

10.1.5 Tumor microenvironment analysis

FFPE sections of pre-treatment tumors will be analyzed using multi-parameter IHC Vectra platform, focusing on the specific components of the tumor microenvironment (e.g. CD4, CD8, CD68, FoxP3) as well as immune-inhibitory markers (e.g. PD-L1, IDO). RNA will be extracted from archival tumors and analyzed for broad expression of genes related to immune function (e.g. type I and type II IFN signatures) using RNAseq, Affymetrix microarray, or targeted multiparameter PCR (e.g. Nanostring).

11.1 EVALUATION DURING TREATMENT/INTERVENTION

	Screening		Cycle 1 (21 days)	Cycle 2 & thereafter (21 days)	End of Treatment	Follow-up (Every 3 months year 1 post-enrollment, 6 months years 2 and 3 post-enrollment, then every 12 months years 4 and 5 post-enrollment)
	≤28 days	≤14 days	Day 1	Day 1		
Informed consent	X					
Pembrolizumab[1]			X	X		
Trastuzumab			X	X		
AE assessment		X	X	X	X	X
History and exam, vitals, weight, ECOG PS		X	X	X	X	X
CT assessment[2]	X		X- Every 3 months		X	X[2]
Concomitant medications		X	X	X	X	X
CBC with diff, plts		X	X	X	X	X
Chemistry[3]		X	X	X	X	X
TSH, Free T4		X	X	X	X	
Pregnancy test[4]		X				
ECG		X				
ECHO/MUGA[5]	X		X- Every 3 months			
Tissue Collection[6]	X					
PBMC and PBLC[7]		X	X	X[7]	X	

Tumor Markers[8]			X	X	X	
ctDNA[9]	X[9]		X- Every 3 months[9]		X	X[9]
Fresh Biopsy					X[10]	

1. Patients will receive pembrolizumab 200 mg IV every 3 weeks +/-7days off for one year, until disease recurrence, intolerable toxicity or patient refusal.
2. CT scan chest, abdomen and pelvis with IV contrast ≤28 days prior to registration, then once every 3 months for the first 12 months post-enrollment, every 6 months in years 2 and 3 post enrollment, and then every 12 months in years 4 and 5 post enrollment. This CT schedule should occur until documented recurrence of disease. Patients in whom IV contrast is contraindicated are recommended to have MRI abdomen and non-contrast chest CT scan. A given CT or MRI may be +/- 14 days of time points for specific administrative reasons, in particular clinic closure for holidays or patient's preference.
3. Chemistry panel: BUN, creatinine, sodium, potassium, chloride, CO2, calcium, glucose, bilirubin, total protein, albumin, alkaline phosphatase, AST, ALT
4. For women of childbearing potential only, serum or urine pregnancy test within 14 days of starting treatment.
5. Baseline ECHO/MUGA must be obtained within one month prior to starting protocol therapy. ECHO with speckle tracking will be the preferred modality for LVEF assessment. When possible, the same method used to measure LVEF at baseline (either ECHO or MUGA) should be used throughout the study. Repeat LVEF assessments will be performed every 3 months after initiating protocol therapy. Window of +/-14 days for ECHO/MUGA.
6. Tissue collection (Archival) within 28 days of starting treatment.
7. PBMC and PBLC collection should be done at screening, C1D1, C2D1, C3D1, C4D1, each imaging timepoint (every 3 months up to 48 weeks), and end of treatment (EOT)
8. Tumor markers to be drawn as appropriate for tumor type at each visit (CEA, LDH)
9. ctDNA collection should be done at the following frequency if the subject is actively receiving treatment, removed from study for any reason other than recurrence or completes treatment: baseline (at screening or pre-treatment D1), each imaging timepoint, EOT, in follow-up (every 3 months for the first 12 months post-enrollment, every 6 months in years 2 and 3 post enrollment, and then every 12 months in years 4 and 5 post enrollment). ctDNA is not required for collection after a patient is removed from study for disease recurrence.
10. Post recurrence biopsy (optional)

12.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient develops radiographic disease recurrence, defined by radiographic evidence of recurrence by CT imaging, the patient will be taken off study and followed for overall survival. If at any time the patient develops unacceptable toxicity that fails to resolve after a maximum treatment delay of 9 weeks, he/she will be removed from treatment and followed for survival. At the end of treatment visit, patients will be scanned and have ctDNA drawn to assess the response.

A patient will be withdrawn from the study treatment in the following circumstances:

- The patient is no longer able to participate in the study (e.g., AE, surgery, concomitant diagnoses, concomitant therapies or administrative reasons); in such a case the treating investigator's reason for a patient's removal must be recorded in CRDB.
- Patient withdrawal of consents or election to discontinue participation in the trial

- Significant deviation from the protocol or eligibility criteria; such patients will be considered protocol violations and removed from study
- Non-compliance with study or follow-up procedures
- Drug related AE(s) have not resolved after 9 weeks of treatment interruption. Exception to this in patients who derive obvious clinical benefit according to the investigator's judgment could be considered upon discussion with Principal Investigator. The dose reduction scheme provided should be followed in this case.
- Repeated episodes of drug related toxicity appropriate management.
- Documented disease recurrence by CT imaging.

As soon as a patient is removed from the study treatment, the End of Treatment (EOT) visit has to be performed within 1-14 days after off treatment date. Every effort should be made to follow up patients in case an adverse event is still ongoing at the time of withdrawal. Patients with radiologically documented disease recurrence should be removed from the study.

13.0 CRITERIA FOR OUTCOME ASSESSMENT AND ENDPOINT EVALUABILITY

13.1 Criteria for Therapeutic Response/Outcome Assessment

The primary endpoint of the study is the proportion of patients with ctDNA clearance at 6 months with trastuzumab/pembrolizumab in patients with HER2+ esophagogastric cancer with persistent ctDNA despite curative surgery and standard perioperative/adjuvant therapy. CtDNA clearance is the conversion of detectable to undetectable ctDNA (as defined in section 7.0). The secondary endpoints include safety, DFS and OS. The patients will be assessed with a CT scan and ctDNA assessment at baseline and every 3 months for first 12 months post-enrollment, every 6 months in years 2 and 3 post-enrollment, and then in years 4 and 5 post-enrollment. CtDNA recurrence is defined as the detection of ctDNA after prior non-detectable ctDNA; however, only recurrence by standard radiographic criteria will be used for removal from study and secondary endpoints of DFS. If the scan shows disease recurrence, the patient will enter the event monitoring phase and will be followed for overall survival. Anytime a patient has intolerable toxicity, or declines further treatment they will go to event monitoring phase and will be followed with a CT scan and ctDNA assessment every 3 months for first 12 months post-enrollment, every 6 months in years 2 and 3 post-enrollment, and then every 12 months in years 4 and 5 post-enrollment. Patients will be considered as responders if they have ctDNA clearance at 6 months. Patients will be considered as non- responders if they do not experience ctDNA clearance at 6 months or If imaging shows disease recurrence before or at 6 month assessment.

13.2 Criteria for Study Endpoint Evaluability

Patients who were enrolled but not treated with trastuzumab + pembrolizumab will be replaced by a new patient. Patients are evaluable for the primary endpoint if they receive at least one dose of trastuzumab/pembrolizumab and will be assessed for therapeutic response as per Section 13.1. All patients who receive at least one dose of trastuzumab + pembrolizumab or will be evaluable for the secondary endpoints of safety, DFS and OS, as well as the exploratory endpoints. OS is defined as

the time from treatment start until death or last follow up, whichever comes first, while DFS is defined as the time from treatment start until recurrence or death, whichever comes first.

14.0 BIOSTATISTICS

This is a single arm pilot study in which HER2+ patients with MRD (defined by detection of ctDNA in their plasma after completion of curative surgery with R0 resection and standard perioperative therapy) receive trastuzumab + pembrolizumab. Patients with HER2+ esophagogastric cancer will be consented within 8 months after curative surgery and completion of standard of care adjuvant therapy (if appropriate), and will undergo ctDNA testing at a minimum of 4 weeks after completion of definitive therapy. Patients with positive ctDNA (as defined per Section 7.0) will be enrolled to receive 6 months of trastuzumab in combination with pembrolizumab.

The statistical analysis of the data obtained will be the responsibility of the MSKCC Biostatistics department. The official, final database will not be analyzed until medical/scientific review has been performed, protocol deviations have been identified, and data have been declared final and complete. The primary endpoint is clearance of ctDNA at 6 months post-enrollment. CtDNA clearance is defined as the conversion of detectable to undetectable ctDNA (as per in section 7.0). Patients who have ctDNA clearance at 6 months will be considered responders, and patients who do not have ctDNA clearance at 6 months or have disease recurrence by CT scan before or at 6 months will be considered non-responders. Patients are evaluable for the primary endpoint if they receive at least one dose of trastuzumab + pembrolizumab. Patients will receive therapy for 6 months and will be followed on study for 5 years after enrollment for survival. The patients will be assessed with a CT scan and ctDNA assessment at baseline and every 3 months for first 12 months post-enrollment, every 6 months in years 2 and 3 post enrollment, and then every 12 months in years 4 and year 5 post enrollment. Patients who received at least one dose of study drug and come off therapy before 6 months for reasons other than disease recurrence will be followed with scans and ctDNA levels. Anytime a patient has intolerable toxicity or declines further treatment, they will go to event monitoring phase and will be followed with a CT scan and ctDNA assessment every 3 months for the first 12 months post-enrollment, every 6 months in years 2 and year 3 post enrollment, and then every 12 months in year 4 and year 5 post enrollment. In the event of disease recurrence by CT imaging, the patient will be followed for overall survival. Patients that are lost to follow up without a 6-month evaluation will be treated as events (non-responder).

A Simon's two-stage optimal design will be used to differentiate 6-month clearance rates of 5% vs 25%. We are using a null ctDNA clearance rate of 5% because the expected ctDNA clearance rate with observation alone is extremely low, as nearly all patients with positive ctDNA after surgery and adjuvant chemotherapy relapse. The chosen design has a type I and type II error rate of 0.1 each. We plan to treat 9 patients in the first stage. If one or more patients demonstrate ctDNA clearance at 6 months, then we would enroll 15 more patients (total of 24 patients). We will not hold accrual after the first 9 patients, but we will monitor these patients for 6-month ctDNA clearance to assess for futility and early termination of the arm based on the decision rule. If we observe three or more

patients with ctDNA clearance among the 24 patients, then the treatment will be considered worthy for further investigation.

Clearance rate of ctDNA at 6 months will be evaluated using binomial proportions, and exact 95% confidence interval will be provided. With 24 patients we can estimate the 6-month clearance rate to within +/- 20% margins of error with 95% confidence. As we will need to screen approximately 400 patients to accrue this study, we anticipate enrollment will take 2 years.

Secondary endpoints of the study include:

- Safety of 6 months of adjuvant trastuzumab in combination with pembrolizumab in HER2+ patients. AEs of any grade considered related to trastuzumab and pembrolizumab will be summarized descriptively using percentages separately for each treatment arm.
- DFS (median, 1-year and 2-year) and OS (median, 2-year and 5-year) will be analyzed separately in each arm. DFS is defined as the time from treatment start till recurrence or death, whichever comes first. OS is defined as the time from treatment start until death or last follow up, whichever comes first. Survival curves will be estimated using the Kaplan-Meier method.

Exploratory endpoints of the study include:

- Perform correlative analyses including whole exome analysis in order to determine mutation load and specific neoantigen landscape with strong association in patients with ctDNA clearance. The Mann–Whitney test will be used to compare mutational loads of tumors among responders and non-responders.
- Plasma samples taken at sequential time points during this trial will be used to evaluate the role of trastuzumab and pembrolizumab in reducing or delaying disease recurrence. Multiple plasma collection timepoints to monitor ctDNA clearance is integral to generate sufficient data to establish the effect of HER2-targeted therapy and immunotherapy in the adjuvant setting for samples with MRD as determined with ctDNA. PBMC and ctDNA levels will be summarized at each timepoint using descriptive statistics including median and interquartile range. Due to the limited sample size, these analyses are exploratory.
- To describe the independent and inter-dependent associations between other assays such as gene expression, TCR sequencing, immunophenotyping, as well as their association with ctDNA clearance. Gene set enrichment analysis will explore pathways associated with response as determined by ctDNA clearance. Deconvolution of immune gene expression to estimate the relative proportion of immune cell subtypes in the tumor microenvironment will be explored using CIBERSORT.
- Descriptive statistics of the longitudinal ctDNA levels and changes in the immune landscape using PBMC from timepoints taken from enrollment to end-of-study will be used to explore the mechanisms of primary and acquired resistance.

- Perform correlative analyses on archival tumor samples and post-recurrence tumor biopsies, including TCR rearrangements and whole exome sequencing to compare any changes in tumor mutational burden in HER2+ tumors. Changes in tumor mutational burden between the pre- and post- recurrence will be assessed using Wilcoxon sign rank test.
- DFS and OS for ctDNA responders and non-responders will be assessed using the Kaplan Meier method and log-rank test.

  These analyses are exploratory in nature and hypothesis generating due to limited sample size. Exploratory endpoints will be evaluated in each treatment arm separately.

15.0 TOXICITIES/RISKS/SIDE EFFECTS

The treating investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart (Section 11.0) and more frequently if clinically indicated. AEs will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs).

15.1 Trastuzumab

Principal AEs of trastuzumab include the following:

Cardiomyopathy

Trastuzumab can cause left ventricular cardiac dysfunction, arrhythmias, hypertension, cardiomyopathy, cardiac death or asymptomatic decline in left ventricular ejection fraction (LVEF). Caution should be exercised in treating patients with increased cardiac risk (e.g., hypertension, documented coronary artery disease, CHF, diastolic dysfunction, older age). Monitoring and management is described below in 15.1.2.

Infusion reactions

Infusion/administration-related reactions (ARRs) and hypersensitivity are known to occur with the administration of trastuzumab. Pre-medication may be used to reduce risk of occurrence of infusion reactions.

Pulmonary toxicity

Serious and fatal pulmonary toxicity has been reported with trastuzumab. Pulmonary toxicity includes dyspnea, interstitial pneumonitis, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, acute respiratory distress syndrome, and pulmonary fibrosis.

Embryo-fetal toxicity

Trastuzumab can cause fetal harm when administered to a pregnant woman. In post-marketing reports, use of trastuzumab during pregnancy resulted in cases of oligohydramnios.

Other toxicities

Most common adverse reactions (≥ 10%) include neutropenia, diarrhea, fatigue, anemia, stomatitis, weight loss, upper respiratory tract infections, fever, thrombocytopenia, mucosal inflammation, nasopharyngitis, and dysgeusia.

15.1.1 Trastuzumab Dose Delays or Modifications

There will be no dose modifications of trastuzumab. Trastuzumab dose delays are permitted for Grade 3/4 clinical toxicity or at the treating investigator's discretion. Dose delays are not required for laboratory abnormalities unless associated with clinical symptoms. Omitted doses of trastuzumab are not replaced or restored; instead, the patient should resume the planned treatment cycles. Patients who develop signs and symptoms of congestive heart failure (CHF) should have trastuzumab held and should receive treatment for CHF. Patients with an asymptomatic absolute decrease in LVEF of >16 percentage points or an absolute decrease in LVEF of 10 to 15 percentage points to below the lower limit of normal should have trastuzumab held as outlined below.

15.1.2 Congestive Heart Failure and other Cardiac Dysfunction Associated with Trastuzumab

All patients must have a baseline evaluation of cardiac function including a measurement of LVEF by ECHO (with speckle tracking) prior to entry into the study. If an ECHO cannot be performed or is technically limited, a MUGA scan can alternatively be performed. Patients with a normal LVEF (>50%) are eligible for entry into the study.

All patients will undergo regular cardiac monitoring throughout the study, including at baseline and every 12 weeks after initiating trastuzumab. During the course of trastuzumab therapy, patients should be monitored for signs and symptoms of CHF (i.e., dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). Patients who develop signs or symptoms of CHF will be further evaluated with a repeat LVEF assessment using the same method selected at baseline (either ECHO or MUGA) if possible.

Management of Symptomatic Cardiac Changes

Patients who develop signs and symptoms of CHF should have trastuzumab held and should receive treatment for CHF as recommended by the American Heart Association (AHA)/American College of Cardiology (ACC) (e.g., ACE inhibitors, angiotensin-II receptor blockers, beta-blockers, diuretics, and cardiac glycosides, as needed) with referral to cardiology for consultation. If the symptoms of CHF resolve with treatment, and/or cardiac function improves to baseline, reinitiation of trastuzumab can be considered at the discretion of the treating investigator after discussion with the patient concerning the risks and benefits of continued therapy and in consultation with a cardiologist. If trastuzumab is restarted, continued surveillance with noninvasive measures of LVEF (MUGA or ECHO) will resume as regularly scheduled. Additional LVEF assessments prior to the next regularly scheduled LVEF measurement may be performed at the treating investigator's discretion.

Management of asymptomatic Decreases in LVEF

Trastuzumab can be continued in patients experiencing an asymptomatic absolute decrease in LVEF of <16 percentage points from baseline, when the LVEF remains within the imaging center's range of normal limits. Repeat measures of LVEF should be obtained using the methodology selected at baseline if possible. Close follow-up of such patients is recommended. Patients with an asymptomatic absolute decrease in LVEF of >16 percentage points or an absolute decrease in LVEF of 10 to 15 percentage points to below the lower limit of normal should have trastuzumab held. Referral to cardiology should be considered for evaluation and management of left ventricular systolic dysfunction with adherence to ACC/AHA guidelines. In light of the variability inherent in the assessment of ejection fraction, consideration should be given to repeating the study within 4-7 days to confirm an observed decline. Repeat measures of LVEF should be obtained using the same methodology selected at baseline if possible, but at the discretion of the treating investigator or consulting cardiologist. If trastuzumab has been held for an asymptomatic decline in LVEF, a repeat measure of LVEF will be obtained within 1 month to evaluate for recovery of LVEF. If LVEF does not improve after repeat assessment within 1 month, the patient should be monitored with monthly or as clinically indicated ECHOs/MUGAs until LVEF is improved. If cardiac function improves and LVEF no longer meets hold criteria as defined above, trastuzumab may be restarted. If trastuzumab is restarted, continued surveillance with noninvasive measures of LVEF (MUGA or ECHO), using the optimal methodology as determined by the treating investigator or consulting cardiologist, will resume per the standard schedule.

15.2 Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per table 2 below. Most common adverse reactions (≥20%) are fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain.

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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue	Permanently discontinue

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

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[1] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at treating physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

15.2.1 Supportive Care

- **Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

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

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

  - For **Type 1 DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

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

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

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.

  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - Treat with IV methylprednisone 1-4 mg/kg at investigator's discretion
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

15.2.2 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 adverse events (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one 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. Please refer to the IB for a complete list of reported AEs. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3.

Table 3: Dose Modification and Toxicity Management Guidelines for Immune-Related AEs Associated With Pembrolizumab

General instructions:

Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.

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.

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 (CTCAE v5.0)**	**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 1-2mg/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 1-2mg/kg prednisone or equivalent) followed by taper	Monitor participants for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., 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 feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or	Grade 2	Withhold	Administer corticosteroids (initial dose of 0.5- 1mg/kg	Monitor with liver function tests (consider weekly or more frequently until liver

General instructions:

Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.

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.

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 (CTCAE v5.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
increased Bilirubin			prednisone or equivalent) followed by taper	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	
T1DM or hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of b-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 (e.g., propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or Permanently discontinue[1]		

General instructions:

Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.

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.

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 (CTCAE v5.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (e.g., levothyroxine or liothyroinine) per standard of care	Monitor for signs and symptoms of thyroid disorders.
Nephritis and renal dysfunction	Grade 2	Withhold	Administer corticosteroids (prednisone 1-2mg/kg or equivalent) followed by taper.	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome and encephalitis		
	Grade 4 or	Permanently discontinue		

General instructions:

Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.

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.

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 (CTCAE v5.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
	recurrent Grade 3			

AE=adverse event; ALT=alanine aminotransferase; AST= aspartate aminotransferase; CTCAE= Common Toxicity Criteria for Adverse Events; GI=gastrointestinal; irAE=immune related adverse event; IV=intravenous ; T1DM=Type 1 diabetes mellitus.

[1] Withhold or permanentlydiscontinue pembrolizumab is at the discretion of the investigator or treating physician;

NOTE: For participants with Grade 3 or 4 immune-related endocrinopathywhere withhold of pembrolizumab is required, pembrolizumab maybe resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapyor achieved metabolic control (in case of T1DM).

15.2.3 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 investigator.	None
Grade 2 Requires therapy or infusion interruption but	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to:	Participant may be premedicated 1.5 h (±30 minutes) prior to

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	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 investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the 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.	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 investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately.	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
	Participant is permanently discontinued from further study drug treatment.	

CTCAE=Common Toxicity Criteria for Adverse Events; IV=intravenous; NCI= National Cancer Institute; NSAID=non-steroidal anti-inflammatory drug; po=per OS (orally)
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 CTCAE v5.0 at http://ctep.cancer.gov

15.2.4 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 3 weeks of the scheduled interruption, unless otherwise discussed with the study PI. The reason for interruption should be documented in the participant's study record.

Interruptions from the protocol-specified treatment plan for greater than 12 weeks between pembrolizumab doses for non-study medication-related or administrative reasons require consultation between the investigator and the study PI.

15.3 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described in Table 5) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

For this trial, male subjects 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).

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). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - 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.

Male Participants:

Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 9 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that has a low user dependency consistently and correctly as described in Table 4 during the protocol-defined time frame.

Table 5. Contraceptive Methods

Acceptable Contraceptive Methods *Failure rate of >1% per year when used consistently and correctly.*
• Male or female condom with or without spermicide

• Cervical cap, diaphragm or sponge with spermicide
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]
  • Oral
  • Intravaginal
  • Transdermal
  • Injectable

• Progestogen-only hormonal contraception [b]
  • 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.)

Use in Pregnancy

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

Use in Nursing Women

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

15.4 Serious Adverse Event (SAE) Reporting

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

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form

- If the SAE is an Unanticipated Problem

15.5 Merck Global Safety Reporting

For the time period beginning when the consent form is signed until treatment allocation/enrollment, any AE, or follow up to a serious AE, including death due to any cause that occurs to any participant must be reported within 24 hours to the Sponsor 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 or a procedure.

For the time period beginning at treatment allocation/enrollment 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 Sponsor and within 2 working days to Merck Global Safety.

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

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

15.6 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/enrollment, 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, or a procedure.

For the time period beginning at treatment allocation/enrollment 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 15.7 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

15.7 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor 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 Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

15.8 Pfizer Global Safety Reporting

16.1 PROTECTION OF HUMAN PARTICIPANTS

The responsible principal investigator will ensure that this study is conducted in agreement with the declaration of Helsinki. The study will seek to protect the rights of human subjects in every way. The potential risks, including adverse drug reactions and potential benefits in terms of pain control will be discussed in detail with the patients.

Potential side effects as outlined above will be discussed with the patients. No patient will be required to participate in the study and participation, or refusal to do so, will not affect the patient's care or treatment.

The patient will not incur any financial cost as a result of participation in the study. Patients will be given up to $100 per treatment visit (for up to 1 year) for travel related expenses incurred while on study.

Participation will be purely voluntary, patient confidentiality will be maintained. No results of the study will be presented or discussed in a fashion that will allow identification of a particular patient in the study. All adverse events will be fully reported to the IRB in a timely fashion as required.

16.1 Privacy

MSK's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals/entities described in the Research Authorization form. A Research Authorization form must be approved by the IRB and Privacy Board (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with others at the time of study publication.

16.2 Data Management

Data to be collected include documentation from all outpatient visits, laboratory, pharmacy, and treatment records. Data will be collected and stored in Medidata.

At MSKCC a Clinical Research Associate (CRA) and/or Clinical Research Coordinator (CRC) will be assigned to the study. The responsibilities of the CRA and/or CRC include confirmation of patient eligibility, project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team. Source documentation will be available to support computerized patient records.

Final data sets for publication are required to be locked and stored centrally for potential future access requests from outside entities.

16.3 Quality Assurance

Data and project enrollment will be monitored on an ongoing basis by the Principal Investigator. The CRA or CRC will inform the PI about the number of patients enrolled, the number of patients in follow-up, and any other outstanding issues. A log will be maintained of eligible vs. enrolled patients. The study data will be assessed for completeness. Random-sample data quality and protocol compliance audits will be conducted by the study team at minimum of two times per year or more frequently if problems are encountered.

16.4 Data and Safety Monitoring

The Data and Safety Monitoring Plan utilized for this study must align with the MSK DSM Plan, where applicable.

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering were approved by the National Cancer Institute in August 2018. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials."

There are several different mechanisms by which clinical studies are monitored for data, safety and quality. At a departmental/PI level there exists procedures for quality control by the research team(s). Institutional processes in place for quality assurance include protocol monitoring, compliance and data verification audits, staff education on clinical research QA and two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Deputy Physician-in-Chief, Clinical Research.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required.

The MSK DSMB monitors phase III trials and the DSMC monitors non-phase III trials. The DSMB/C have oversight over the following trials:

- MSK Investigator Initiated Trials (IITs; MSK as sponsor)
- External studies where MSK is the data coordinating center
- Low risk studies identified as requiring DSMB/C review

The DSMC will initiate review following the enrollment of the first participant/or by the end of the year one if no accruals and will continue for the study lifecycle until there are no participants under active therapy and the protocol has closed to accrual. The DSMB will initiate review once the protocol is open to accrual.

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18.0 APPENDICES

Appendix 1. Correlative Collection Table

Appendix 2. Investigational Scan Considerations

Specimen	Collection Time Points	Mandatory or Optional?	SOC or Research?	Collection Amount	Specimen Processing Location	Shipping Details	Analysis (reason for specimen collection)	Storage
Tumor tissue	SCR, Progression	Screening (Mandatory), Recurrence (optional)	RNB (screening), SOC at recurrence	**TISSUE SECTIONING REQUESTS ARE TO BE MADE IN BATCH ONLY** TCR: 10 x 5µm FFPE tissue curls are to be collected from each biopsy. Curls are to be cut serially and kept in a DNAse/RNAse free screw-top microtube. PD-L1, IHC Vectra: 10 x 5µm serial sections are to be cut from each biopsy. Sections are to be mounted on charged slides, one section per slide. Slides should be numbered to differentiate section sequence (e.g. 1/5, 2/5, etc).	TCR sequencing: MSK IMF lab PD-L1: MSK Pathology Lab Tumor environment analysis via IHC Vectra: Dr. Taha Merghoub/Wolchok lab	TCR sequencing: FFPE tissue samples ship ambient, Fresh-frozen tissue shipped on dry ice. PD-L1, IHC Vectra: FFPE tissue samples ship ambient, Fresh-frozen tissue shipped on dry ice	TCR sequencing PD-L1 via IHC Tumor environment analysis via IHC Vectra	Yes to future use
Blood for SOC labs	SCR, all D1, EOT	Mandatory	SOC	Variable	MSK	N/A	CBC, chemistry, thyroid function, pregnancy test (as required)	N/A
Plasma for ctDNA	SCR, imaging timepoint, EOT, Follow-up	Mandatory	RNB	4x 10mL cell free DNA BCT tubes (screening) 6x 10mL cell free DNA BCT tubes (all other time points)	Department of Molecular Pathology at MSK	Ambient, shipped immediately by courier to Department of Molecular Pathology at MSK	ctDNA	Yes to future use

Specimen	Collection Time Points	Mandatory or Optional?	SOC or Research?	Collection Amount	Specimen Processing Location	Shipping Details	Analysis (reason for specimen collection)	Storage
Whole blood for PBMC PBLC	SCR, C1D1, C2D1, C3D1, C4D1, Each imaging timepoint(up to 48 weeks), EOT	Mandatory	RNB	2 x 8-ml BD sodium heparin Cell Preparation Tubes	MSK: Center Ludwig Center for Cancer Immunotherapy Immune Monitoring Core Facility	Ambient, shipped immediately by courier to IMC Facility at MSK Ludwig Cancer for processing	Density gradient centrifugation and isolation of PBMC/PBLC and plasma PBMC/PBLC will resuspended at ~6-8 million cells per vial in cell freezing medium, frozen at –80 degrees C in controlled rate cooling containers, and then cryopreserved in liquid nitrogen freezers Plasma will be frozen in 1.5 ml aliquots and stored at –20 degrees C.	Yes to future use

Appendix 2: Investigational Scan Considerations

All disease assessment scans will be performed per standard of care.

Patients on study will have a biopsy at time of recurrence. Patients undergoing investigational CT-guided biopsies are exposed to an additional 0.626 Gy (+/- 0.132) dermatologically, an additional 29.9 mSv in for scan done in helical mode and an additional 18.9 mSv for scans done in axial mode.

It is estimated that each patient will have up to 1 additional scan for CT-guided biopsies.

The informed consent will include the necessary risk language on contrast use and low-level radiation.