Phase II trial of pembrolizumab in combination with trastuzumab, Fluoropyrimidine, and Platinum Chemotherapy in first line Stage IV HER2-positive metastatic esophagogastric (EG) cancer

PROTOCOL FACE PAGE FOR MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

OneMSK Sites	Roles
Westchester	All Protocol Activities
Rockville Centre	All Protocol Activities
Commack	All Protocol Activities
Basking Ridge	All Protocol Activities
Monmouth	All Protocol Activities
Manhattan	All Protocol Activities
Bergen	All Protocol Activities
Nassau	All Protocol Activities

Participating Institutions	PI's Name	Site's Role
MD Anderson Cancer Center	Mariela Blum Murphy, MD	Data Collection

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single-arm, open-label, non-randomized multi-institution Phase II study of pembrolizumab in combination with capecitabine/-FU and a platinum and trastuzumab as first line therapy in patients with metastatic HER2 positive esophagogastric (EG) adenocarcinoma. The goal of the study is to determine the efficacy of the drug combination as measured by 6 month progression free survival (PFS). The central hypothesis of this trial is that dual HER2 and PD1/2 blockade will result in enhanced antibody-dependent cell-mediated cytotoxicity (ADCC), NK cell degranulation, and synergistic activity in combination with fluoropyrimidine, and platinum.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary:

To determine the efficacy of pembrolizumab in combination with trastuzumab and capecitabine/5-FU and oxaliplatin/cisplatin and in patients with Stage IV HER2-positive EG adenocarcinoma as measured by 6 month progression free survival (PFS).

Secondary:

• To establish the safety of pembrolizumab in combination with capecitabine/5-FU, oxaliplatin/cisplatin, and trastuzumab in patients with metastatic HER2-positive metastatic EG adenocarcinoma.

• To observe other measures of efficacy of pembrolizumab in combination with capecitabie/5-FU, oxaliplatin/cisplatin, and trastuzumab, including response rate, overall and 1-year survival in patients with Stage IV EG adenocarcinoma.

• Determine overall clinical benefit defined as stable disease (SD), complete response rate (CR), or partial response (PR) safety and tolerability. They will be summarized using binomial proportions along with exact 95% CI.

• Median PFS, overall and 1-year survival in patients with HER2+ Stage IV esophageal, gastric or GEJ adenocarcinoma will be estimated using the Kaplan-Meier method

Exploratory:

- To utilize PMBC and cell-free tumor DNA (cfDNA) from blood specimens collected during the course of treatment to explore mechanism of primary and acquired resistance to pembrolizumab and trastuzumab therapy.
- To explore tumor PD-L1 as predictive biomarker.
- To bank tumor material for future correlative analysis, including but not limited to whole exome analysis to determine mutation load and specific neoantigen landscape with strong association with regimen efficacy and survival.
- To explore changes in ^{89}Zr-trastuzumab PET with trastuzumab and pembrolizumab treatment.
- To explore response rate of pembrolizumab with trastuzumab in patients with HER-2 positive metastatic esophagogastric cancer

3.0 BACKGROUND AND RATIONALE

3.1 HER2-Positive Esophagogastric Cancer

Esophagogastric (EG) cancer is the second most common cause of cancer-related death worldwide, and represents an enormous global health burden.[1] In 2015, an estimated 22,220 people will be diagnosed and 10,990 people will eventually die of their disease in the United States.[2] Depending on tumor characteristics and stage, treatment modalities include combinations of surgery, chemotherapy, and radiation therapy.[1] Even with maximal therapy, prognosis for EG cancer remains poor, with 5-year survival rate of 5%[2,3] and median survival of 10 to 14 months in Stage IV disease.[4-6] The poor prognosis is largely attributed to the fact that the majority of gastric cancer patients have metastatic disease at the time of presentation.[1] Palliative chemotherapy is used to control tumor growth, improve quality of life and has been shown to prolong survival in patients with Stage IV disease.[4-6] Among patients who initially respond to chemotherapy majority ultimately suffer disease progression. In addition, a significant proportion of EG cancer patients have primary chemotherapy refractory disease. For these patients there is a need for development of new therapeutic options.[6]

Human epidermal growth factor receptor 2 (HER2; also known as ERBB2), a member of a family of receptors associated with tumor cell proliferation, apoptosis, adhesion, migration, and differentiation is an established biomarker and key driver of tumorigenesis in gastric cancer.[7] Approximately 20-30% of gastric and GEJ adenocarcinomas harbor HER2 overexpression.[7,8]

Trastuzumab is a humanized monoclonal antibody (IgG1 isotype) directed against the extracellular region of HER2 that induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signaling, and prevents cleavage of the extracellular domain of HER2.[9] The ToGA study is the first randomized, prospective, multicenter, phase III trial to evaluate the efficacy and safety of trastuzumab in HER2-positive EG adenocarcinoma in combination with cisplatin and a fluoropyrimidine.[7] In this study, 594 patients were randomly assigned to study treatment (trastuzumab plus chemotherapy, n=298; chemotherapy alone, n=296). The results of this study established the benefit of trastuzumab in combination with cisplatin and fluoropyrimidine chemotherapy. Median overall survival was 13.8 months (95% CI 12–16) in those assigned to trastuzumab plus chemotherapy compared with 11.1 months (10–13) in those assigned to chemotherapy alone (HR 0.74; 95% CI 0·60–0·91; p=0·0046). Median progression-free survival was 6.7 months (95% CI 6–8) in the trastuzumab plus chemotherapy group compared to 5.5 months (5–6) in the chemotherapy alone group (HR 0.71, 95% CI 0.59–0.85; p=0.0002). Overall tumor response rate, time to progression, and duration of response were significantly improved in the trastuzumab plus chemotherapy group compared to the chemotherapy alone group. Rates of overall grade 3 or 4 adverse events (201 [68%] vs 198 [68%]) and cardiac adverse events (17 [6%] vs 18 [6%]) did not differ between groups.[15] On the basis of these findings, trastuzumab is now considered a standard option for patients with HER2-positive advanced EG cancer when combined with a chemotherapy regimen consisting of a fluoropyrimidine (5-FU or capecitabine) and cisplatin.

3.2 Pembrolizumab Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors. The programmed death (PD)-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-

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

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. KeytrudaTM (pembrolizumab) is approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and metastatic non-small cell lung cancer.

3.3 Rationale for anti-PD-1/2 therapy

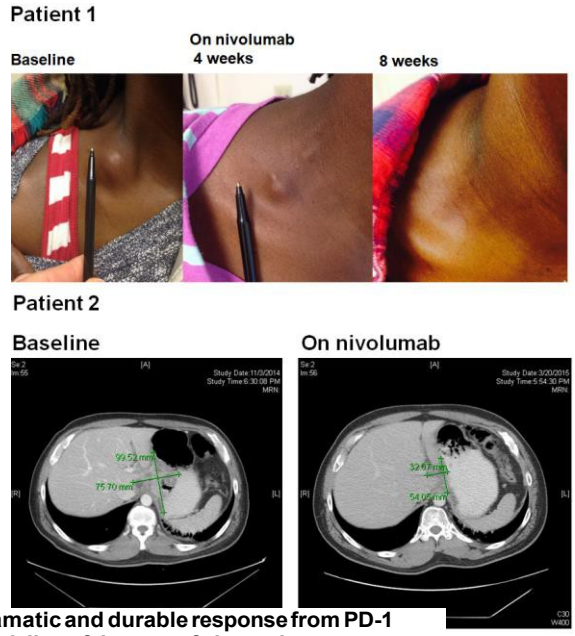

Figure 2 Dramatic and durable response from PD-1 inhibitor in 4th line of therapy of chemotherapy

PD-L1 is expressed in a broad range of cancers with a high frequency, up to 88% in some types of cancer. In a number of these cancers, including lung[10], renal[11], pancreatic[12], and ovarian[13], the expression of PD-L1 is associated with reduced survival and unfavorable prognosis. Several recent studies, however, suggest that high PD-L1 expression is a "positive" prognostic factor (i.e., better prognosis) among metastatic melanoma[14,15], NSCLC[16,17], and gastric cancer patients.[18]

Pembrolizumab is able to achieve a dual blockade (PD-L1 and PD-L2). It shows no cytotoxic (ADCC/CDC) activity. Pharmacokinetics support dosing every 2 weeks (Q2W) or every 3 weeks (Q3W). Pembrolizumab demonstrated a clinical activity in multiple tumor types.The levels of tumor-infiltrating lymphocytes (TILs), and more specifically cytotoxic T cells, have been correlated to improved prognosis in a number of cancers including colorectal, melanoma, and lung[19], suggesting that an antitumor immune response is beneficial to patients. It has been shown in vitro that an antibody that blocks the interaction between PD-L1 and its receptors can relieve PD-L1-dependent immunosuppressive effects and enhance the cytotoxic activity of antitumor T cells.[20] Results of several preclinical studies using mouse tumor models support this hypothesis. In these studies, antibodies directed against PD-L1, or its receptor PD-1, showed antitumor activity.[21-24]

Results from the studies of pembrolizumab in advanced tumors, including gastric, show promising activity and tolerability from this novel monoclonal antibody. The phase I KEYNOTE-012 trial investigated the safety, tolerability, and antitumor activity of pembrolizumab in gastric cancer pts.[25] Only patients with distinctive stromal or ≥1% tumor nest cell PD-L1 staining were included. Of 162 pts screened, 65 (40%) were PD-L1+ and 39 enrolled. Median age was 63 y, and 72% of pts were men. 66% of pts were heavily pretreated (≥2 prior therapies). Median follow-up duration was > 6 mo. The most common AEs deemed treatment related by investigators were hypothyroidism and fatigue (n = 5 each). Grade ≥3 AEs deemed treatment related occurred in 3 pts (n = 1 each for hypoxia, peripheral neuropathy, and pneumonitis). ORR was 30.8%. Responses were ongoing for 11/12 pts (median response duration not reached; range 8+ to 20+ wk). 41% of patients with heavily pretreated disease experienced a decrease in tumor burden. Evidence of an association between PD-L1 expression and PFS (P = 0.032) and ORR (P = 0.071) was observed.While comparison of results between different trials is imprecise, the response rate compared favorable to single-agent irinotecan or paclitaxel in the second-line setting.[26] Keynote 059 is a recently initiated Phase II, 3 cohort trial exploring activity of pembrolizumab alone or in combination with 5-Fu and cisplatin in 1) patients who have progressed on at least 2 prior systemic therapies, 2)first line therapy pembrolizumab + chemotherapy in PD-L1 unselected population, 3) pembrolizumab monotherapy in PD-L1 selected patients. A Phase III trial (NCT02267343), not limiting patient enrollment by PD-L1 biomarker status, was initiated in October 2014 to compare another PD1 directed antibody nivolumab versus placebo in 480 previously treated Japanese patients with Stage IV esophageal, gastric and gastroesophageal junction cancer.

MSK GI oncologists in collaboration with Immunotherapy Group have led accrual of Stage IV esophagogastric cancer patients on immunotherapy trials. PD-1 directed antibody (nivolumab) is well tolerated and has demonstrated dramatic single agent activity in chemotherapy refractory Stage IV esophagogastric cancer not selected based on PD-L1 status. Response images from patients treated by Dr. Janjigian with similar PD1 inhibitor, nivolumab 3 mg/kg shown in Fig 1 (Confidential, unpublished data).

3.4 Rationale for Combination Therapy

Trastuzumab is the standard of care for patients with metastatic HER2+ gastric cancer. However, the response rate in first line setting is only 47% with trastuzumab in combination with chemotherapy and acquired and intrinsic resistance to trastuzumab limits its efficacy. The short duration of response in some patients led to our hypothesis that combined inhibition of multiple oncogenic pathways that drive gastric cancer growth may achieve a greater therapeutic benefit. Some anticancer agents, in addition to their direct cytotoxic effects on tumor cells, feature the ability to promote the activation by inducing hypermutated status in the tumor, activation of the immune system of the host, resulting in enhanced antitumor responses.[27] Capecitabine and cisplatin may have twofold immunogenic effects. Fluropyrimidine demonstrates T-cell-dependent antitumor effects through T-cell activation, reduction of tumor-associated Myeloid-derived suppressor cells (MDSCs) and increase of CD8 tumor-infiltrating lymphocytes as well as target destruction through increased heat shock protein (HSP) production.[27,28] Cisplatin induces immunogenic cell death through pre-apoptotic exposure of calreticulin (CRT) and the post-apoptotic release of high-mobility group box 1 protein (HMGB1), which are required for immunogenic cell death.[29,30]

Treatment with trastuzumab results in beneficial, yet limited, clinical improvement for patients with HER2 positive EG cancer. Antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells contributes to the efficacy of trastuzumab.[31,32] Upon encountering trastuzumab-coated, HER2-overexpressing breast cancer cells, human NK cells become activated and express the costimulatory receptor CD137. Kohry, et al. demonstrated that stimulation of trastuzumab-activated human NK cells with an agonistic mAb specific for CD137 killed breast cancer cells (including an intrinsically trastuzumab-resistant cell line) more efficiently both in vitro and in vivo in xenotransplant models of human breast cancer.[32] The co-stimulatory molecule CD137 (4-1BB) is expressed following NK and memory T cell activation also enhances activity of EGFR monoclonal antibody (cetuximab) and anti-CD137 mAb administration has shown synergism in combination with cetuximab.[31] Junttila et al. demonstrated that combining a trastuzumab-based bispecific antibody HER2-TDB with anti-PD-L1 yielded a combination immunotherapy that enhanced tumor growth inhibition, increasing the rates and durability of therapeutic response.[33]

Phase Ib data from ongoing KN014-PANACEA trial (NCT02129556) indicate that the combination of pembrolizumab and trastuzumab is safe. The Phase Ib part was completed without any safety concerns and the study has entered the Phase II part (confidential data from Merck).

Based on these data showing synergistic effect, we hypothesize that enhanced NK cell degranulation and cytotoxicity using PD-1 blockade will result in synergistic activity of pembrolizumab in combination with trastuzumab. We further hypothesize that using a dual antibody strategy, combining a tumor-targeting antibody with a second antibody that activates the host innate immune system, in combination with standard cytotoxic chemotherapy will improve the therapeutic effects of antibodies against metastatic HER2-expressing EG tumors.

3.5 Rationale for Dose Selection/Regimen/Modification

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

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

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

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

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

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

3.6 Rationale For Correlative Studies

3.6.1 Exploration of Neoantigens

Investigators at Broad/DFCI and MSKCC recently led a comprehensive molecular and genomic characterization of gastric cancer, evaluating the mutations, copy-number, gene expression and DNA methylation across 295 cases through The Cancer Genome Atlas.[34] While therapeutic development for gastric cancer has largely viewed the disease as a single entity, our unbiased informatics approach integrating somatic genomic alterations, methylation and gene expression led us to redefine the disease into four distinct subclasses. 1) Tumors with Epstein-Barr virus (EBV) infection showed profound hypermethylation, and had a novel finding of 80% of tumors harboring a *PIK3CA* mutation. 2) Microsatellite

unstable (MSI) harbored DNA hypermethylation (distinct from that of EBV tumors) and had elevated somatic mutation rates with highly recurrent mutations of *PIK3CA* (42%) and ERBB3 (26%) with 12% of tumor having alterations of both genes. 3) Tumors with chromosomal instability (CIN) showed marked aneuploidy. While lacking common mutations of *PIK3CA/ERBB3* they harbored recurrent amplifications of receptor tyrosine kinases, most notably HER2 (24%). 4) Tumors lacking aneuploidy and elevated rates of mutation or hypermethylation were termed genomically stable (GS), largely those of the diffuse histologic subtype. We identified that 30% of these tumors harbored novel alterations of the Rho signaling pathway, somatic mutations of *RHOA* or fusion genes involving RHO GTPase activating proteins. This classification structure creates a foundation to develop rational therapeutics for distinct groups of patients. At MSKCC next generation sequencing and gene copy number analysis is routinely performed on all advanced gastric tumors. Whole exome sequencing is also routinely done on site at MSK for biomarker development in setting of clinical trials.

Immune checkpoint blockade has led to durable antitumor effects in patients with metastatic melanoma, non–small-cell lung cancer, and other tumor types, but the factors determining whether a patient will have a response remain elusive. The relationship among the genomic landscape of the tumor, the mutational load, and the benefit from treatment has been under investigation at MSK in laboratory of Timothy Chan and recently published in NEJM[40]. Whole-exome sequencing was performed on melanoma tumors and matched blood samples. Somatic mutations and candidate neoantigens generated from these mutations were characterized. Snyder et al reported that mutational load was associated with the degree of clinical benefit (P=0.01) but alone was not sufficient to predict benefit. Using genome wide somatic neoepitope analysis and patient-specific HLA typing, MSK investigators identified candidate tumor neoantigens for each patient and elucidated a neoantigen landscape that is specifically present in tumors with a strong response to CTLA-4 blockade.

We hypothesize that among gastric tumors, MSI and EBV positive subtypes are the most likely to benefit to anti-PD1 blockage. Samples will be banked for future correlative analysis after the trial enrollment is complete. In collaboration with MSKCC immune monitoring core facility we will conduct whole exome and neopeptide analysis on samples banked on the trial to explore a specific signature predictive of strong response to anti-PD1 therapy.

3.6.2 Cell-Free DNA (cfDNA)

Preliminary data obtained in collaboration with MSKCC Laboratory Medicine Core (E. Peerschke and A. Samoila) demonstrates that cf DNA collection is feasible and results in adequate DNA yield from peripheral blood collection of patient with metastatic HER-2 positive GE junction adenocarcinoma. Results from collection of sample from 6 patients treated in Dr. Janjigian's clinic, as summarized in the table below. Cell-free DNA (cfDNA) will be obtained from plasma samples collected at baseline, at week 3, every 9 weeks and at the time of treatment discontinuation. cfDNA will be subject to molecular profiling to identify ERBB2 amplifications and other gene operations related to protocol therapy response and resistance.

		cfDNA results		
EG Cancer Patient	**Tube ID**	**Conc (pg/µl)**	**Elution volume (µl)**	**Total yield (ng)**

1	MJ_901	716	60	43.0
2	AF_203	158.4	60	9.5
3	SF_152	248	60	14.9
4	EH_317	754	60	45.2
5	AD_129	1434	60	86.0
6	EV_233	450	60	27.0

3.6.3 Collection of peripheral blood mononuclear cells (PBMC) and plasma for immunologic analyses

To explore whether therapy will restart T cell activation and function, peripheral blood mononuclear cells will be isolated and cryo preserved analysis may include, but not necessarily be limited to, the proportion of T, B and NK cells, proportion of memory and affect are T cells, and expression levels of PD–1, PD L1, ICOS, and Ki 67. Approximately 32 ml of whole blood per visit (baseline and wks 1, 2, 3, 6, 10, 13 post-dosing.

3.6.4 ^{89}Zr-trastuzumab PETs as PD and predictive biomarker

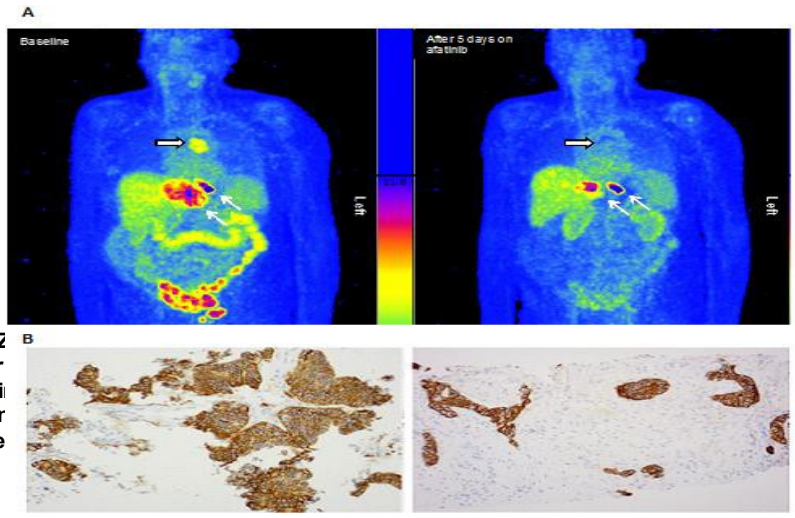

Figure 1 A ^{89}Z cancer befor Correspondi HER2 immu r with persiste

Table 1	Primary	M1	M2	M3
ERBB2 CNV	16.5	8.9	9.2	2.5
^{89}Zr-PET SUV				
Baseline	21	9	16	0
Post afatinib	24	7	16	0

Our group in collaboration with Drs. Lewis and Weber implemented functional imaging with ^{89}Zr-trastuzumab PET to characterize the extent of response heterogeneity to HER2 directed therapy between primary tumor and metastases in HER2+ gastric cancer. ^{89}Zr-trastuzumab PET has a potential advantage over single site biopsies as it can non-invasively assess variation in level of HER2 and target engagement in both the primary tumor and all sites of metastases simultaneously.[41-43] Our preliminary data in 20 patients imaged thus far demonstrates that ^{89}Zr-trastuzumab PET is feasible and provides exceptional image quality in HER2+ gastric cancer patients. ^{89}Zr-trastuzumab was well tolerated with ideal imaging interval of 5 days post injection. **Figure 1** shows preliminary ^{89}Zr-Trastuzumab PET images of a patient with heavily pretreated HER2 + EG cancer which demonstrate the selective and high accumulation ^{89}Zr-trastuzumab in posterior-esophageal lymph nodes and liver metastasis with very low background of uptake in normal organs. **Table 1** shows the estimated ^{89}Zr-trastuzumab PET SUVs and corresponding HER2 copy number variation (CNV) in primary tumor and metastases obtained during rapid autopsy of a patient treated with afatinib (**Figure 1**), where high ^{89}Zr SUV value correlates with HER2 copy number. Heterogeneity in response is noted with dramatic decrease in lymph node uptake after 5 days of afatinib (EGFR/HER2 TKI) and persistently high uptake

noted in liver lesions and primary esophageal mass. Post-afatinib liver biopsy showed persistently high HER2 expression (IHC3+ **Figure 1**).The patient had radiographic tumor response on 8 week CT, 27% reduction in liver and lymph node metastasis. At the time of therapy failure, the disease progressed in liver and primary tumor (sites of persistently high ^{89}Zr-trastuzumab PET), while posterior esophageal mass had durable complete regression. Thus far imaging HER2+ gastric cancer patients with ^{89}Zr-trastuzumab PET (IRB 13-165, co-PI Janjigian) we have been able to demonstrate 1) the differences in degree of HER2 inhibition between afatinib vs. afatinib + trastuzumab therapy and 2) variability in ERBB2 (HER2) amplification between primary tumor and sites of metastasis. The lead in cycle with pembrolizumab + trastuzumab will provide key pilot data on activity on targeted therapy without chemotherapy in first line HER2+ esophagogastric cancer. In select patients baseline and early assessment ^{89}Zr-trastuzumab-PET will be performed under IRB 13-165 (co-PI Janjigian) with CT/MRI scan performed after the initial 3 weeks (1 cycle). All patients will receive capecitabine and cisplatin with subsequent cycles together with pembrolizumab and trastuzumab.

3.6.5 PD-L1 as Biomarker for Anti–PD-1 Immunotherapy

The complexity of PD-L1 expression leads to variations in the thresholds for positivity and differences in the interpretation of test results. Furthermore, narrowing treatment down to PD-L1-positive patients could exclude patients who benefit from these treatments. PD-L1 is a very dynamic marker—it can fluctuate over the course of the disease and treatment. Until more is learned, it remains problematic to use PD-L1 as a biomarker. If PD-L1 is not the best marker, there may be another T-regulatory cell that can be measured. There needs to be a strategy for selecting patients who will not benefit from treatment. An important aspect of development program for pembrolizumab in esophagogastric cancer is identification of a biomarker for patient selection. PD-L1 expression in tumor tissue will be characterized by immunohistochemistry (IHC) to explore the relationship between PD-L1 expression and response to pembrolizumab. Other exploratory biomarkers including but not limited to PD-1 expression, markers of T-cell phenotype may also be evaluated.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is an open-label, non-randomized, single-arm, multi-institution phase II study of pembrolizumab in combination with fluorpyrimidine, platinum, and trastuzumab as first line therapy in patients with metastatic HER2 positive esophagogastric adenocarcinoma. The primary endpoint of the study is to determine the six months progression free survival (PFS) in the first-line treatment of patients with Stage IV HER2 positive esophagogastric adenocarcinoma. With a total of 37 esophagogastric adenocarcinoma patients, we have 80% power to detect an improvement in the 6-month progression free survival (PFS) from a historical control of 55% to 75% with type I error rate of 5%.

4.3 Intervention

Pembrolizumab 200 mg IV every 3 weeks, trastuzumab (8 mg/kg loading dose; 6 mg/kg maintenance) IV every 3 weeks with cisplatin IV every 3 weeks with oral capecitabine 2 weeks on/1 week off. Each cycle consists of 21 days. As an alternative fluoropyrimidine, 5-fluorouracil may be considered for patients who cannot be administered capecitabine for any specific reason. 5-fluorouracil (5-FU) 800mg/m^2/day will be administered on Day 1-Day 5 of every 21 days. If the patient is not a good candidate for induction pembrolizumab/trastuzumab, capecitabine/5-FU and cisplatin/oxaliplatin can be added during cycle 1 of treatment. Oxaliplatin may also be considered for patients who cannot be administered cisplatin for any specific reason. Oxaliplatin 130

mg/m^2/day will be administered on Day 1 beginning with cycle 2. Treatment will be administered on an outpatient basis. In Cycle 1, patients will initiate therapy with trastuzumab 8 mg/kg IV with pembrolizumab 200 mg IV. CT/MRI scan will be performed after the initial 3 weeks (1 cycle) to determine response to pembrolizumab and trastuzumab combination. With subsequent cycles, all patients will begin systemic chemotherapy with capecitabine/5-FU, and cisplatin/oxaliplatin in addition to pembrolizumab 200 mg IV with trastuzumab 6 mg/kg maintenance. Patients will receive cisplatin 80 mg/m^2 IV on Day 1, and capecitabine 850mg/m^2 twice a day on Days 1 through 14, every 3 weeks. Treatment will be performed on the scheduled day ± 7 days. Cisplatin will be given in up to 6 cycles, and may be discontinued before 6 cycles for cumulative toxicity. In case of discontinuation of cisplatin due to cumulative toxicity, patients will be permitted to continue capecitabine maintenance with pembrolizumab 200 mg IV and trastuzumab every 3 weeks. CT scan will be performed at baseline, week 3, week 9 and every 9 weeks thereafter. CT scans occurring every 9 weeks will coincide with the 6 month CT scan. 15 MSK patients only with disease technically amenable to biopsy will be asked to undergo an optional research biopsy for tissue correlative studies during week 3 of treatment.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Pembrolizumab[a]	200 mg X mg/kg	Q3W	IV infusion	Day 1 of each 3 week cycle
Trastuzumab	8 mg/kg loading dose; 6 mg/kg maintenance	Q3W	IV infusion	Day 1 of each 3 week cycle
Cisplatin[b]	80 mg/m^2	Q3W	IV infusion	Day 1 of each 3 week cycle after cycle 1, for up to 6 cycles
Oxaliplatin[c]	130 mg/ m^2	Q3W	IV infusion	Day 1 of each 3 week cycle after cycle 1, for up to 8 cycles
Capecitabine[d]	850mg/m^2	BID	Oral	Days 1 through 14 of each 3 week cycle after cycle 1
5-Fluorouracil[e]	800mg/m^2/day	Q3W	IV infusion	Day 1 to Day 5 of every 21 days

a. Pembrolizumab/trastuzumab will be administered until disease progression or other withdrawal criteria are met. Capecitabine/5-FU and cisplatin/oxaliplatin will be initiated after cycle 1. After 1 year, at the treating investigator's discretion, capecitabine (or 5-FU) treatment can be discontinued.

b. Maintenance of satisfactory GFR and adequate hydration pre- and post-cisplatin administration should be performed per local clinical practices

c. As an alternative, oxaliplatin may be considered for eligible patients who cannot be administered cisplatin for any specific reason. This decision should be made prior to initiation of therapy by the treating investigator, switching from cisplatin to oxaliplatin, or vice versa, will not be allowed during the study. Oxaliplatin will be administered at 130 mg/m^2 continuous IV infusion on Day 1every 21 day cycle, after completion of the first cycle. Patients may begin with reduced dose of oxaliplatin 104

mg/m2 as starting dose if deemed necessary per the treating physician discretion. Oxaliplatin may be stopped after 6 cycles at the treating investigator's discretion

d. Capecitabine should be taken within 30 minutes after a meal. Total daily dose of capecitabine administered will be 1700 mg/m^2. Patients who are unable to take capecitabine on the morning of Day 1 can start their capecitabine on the evening of Day 1, in which case the final dose of capecitabine for that cycle will be administered on the morning of Day 15.
e. As an alternative fluoropyrimidine, 5-FU, may be considered for eligible patients who cannot be administered capecitabine for any specific reason (such as malabsorption, difficulty swallowing, or other conditions that could affect intake of oral capecitabine medication). This decision should be made prior to initiation of therapy by the treating investigator, switching from capecitabine to 5-FU, or vice versa, will not be allowed during the study. 5-FU will be administered at 800 mg/m^2/day continuous IV infusion on Day 1-Day 5 every 21-day cycle, after completion of the cisplatin infusion.

5.1.1. Hydration Schedule for Cisplatin

Maintenance of satisfactory GFR and adequate hydration pre- and post-cisplatin administration should be performed per local clinical practices. Cisplatin administration and hydration will be done per institutional guidelines. It is recommended that a urinary output of 100 mL/h is maintained before, during and after cisplatin administration. If clinically not contraindicated, patients should be advised to drink 3 liters if water or other acceptable fluid over a period of 24 hours before and after administration of cisplatin. The suggested hydration schedule is provided in table 5.1.2.1.

Table 5.1.1.1 Hydration Schedule for Cisplatin Administration

Time	Supportive care and Hydration	Duration
T0[a] + 1h	Antiemetics	15-30min
1h 30min	Prehydration (1L 0.9% saline)	60 min
2h 30min	200mL Mannitol 20%	30min
3h	Cisplatin 80mg/m^2 in500 mL 0.9% saline	60-120min
4-5h	Post hydration (1L 0.9% saline) + 20mmol KCl + 1g $MgSO_4$	2h

Abbreviations: I.V. = intravenously; po = orally
[a] Relative to the start of administration of pembrolizumab

5.1.2. Antiemetic Schedule for Cisplatin

Antiemetic schedule will be done per institutional guidelines. Recommended antiemetic schedule for at-home medication after administration of cisplatin: aprepitant 80 mg PO once daily for 2 days, dexamethasone 4 mg PO (or equivalent) once daily for 3 days, ondansetron 8 mg PO twice daily for 1 day (or equivalent), metoclopramide 10 to 20 mg PO 3 times daily if required. If aprepitant is not given then the dose of dexamethasone should be doubled.

5.2. Pembrolizumab Storage and Accountability

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

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

5.2.1 Dispensing of Pembrolizumab

Pembrolizumab will be provided by Merck at no charge. Pembrolizumab will be shipped by Merck directly to each participating site. 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.

5.2.2 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 three week cycle after all procedures and assessments have been completed as detailed on the Trial Flow Chart (Section 10.0). Pembrolizumab 200mg will be administered as a 30 minute IV infusion every 3 weeks. Variation in infusion time is permitted; a window of -5 minutes and +10 minutes is permitted.

5.3. Preparation and Administration of Trastuzumab

Trastuzumab will be administered on 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.

5.4. Preparation and Administration of Cisplatin

Investigators should consult the manufacturer's instructions for cisplatin for complete prescribing information and follow institutional procedures for the administration of cisplatin. Cisplatin will be administered as an I.V. infusion of 80 mg/m^2 over approximately 60 to 120 minutes on Day 1 every 21-day cycles, up to a maximum of 6 cycles in the absence of disease progression or until other withdrawal criteria are met. Cisplatin will be administered after the completion of the pembrolizumab infusion.

5.5 Preparation and Administration of Oxaliplatin

Investigators should consult the manufacturer's instructions for Oxaliplatin for complete prescribing information and follow institutional procedures for the administration of Oxaliplatin.

Oxaliplatin may be administered instead, at a dose of 130 mg/m^2/day as an I.V.over approximately 2 hours on Day 1 every 21-day cycles, up to a maximum of 8 cycles in the absence of disease progression or until other withdrawl criteria are met. Patients may begin with reduced dose of oxaliplatin 104 mg/m2 as starting dose if deemed necessary per the treating physician discretion. Oxaliplatin will be administered after the completion of the pembrolizumab infusion.

Oxaliplatin will continue until progression of disease, intolerable toxicity, or other withdrawal criteria are observed. After 1 year, at the treating physician's discretion, oxaliplatin treatment can be discontinued.

5.6. Administration of Capecitabine

Investigators should consult the manufacturer's instructions for capecitabine for complete prescribing information and follow institutional procedures for the administration of capecitabine.Capecitabine will be orally self-administered at 850 mg/m^2 twice daily on Day 1 through Day 14, followed by a 7-day non-dosing interval in each 21-day cycle in the absence of disease progression or until other withdrawal criteria are met. Patients who are unable to take capecitabine on the morning of Day 1 can start their capecitabine on the evening of

Day 1 in which case the final dose of capecitabine for that cycle will be administered on the morning of Day 15. Capecitabine should be taken within 30 minutes after a meal. Capecitabine will continue until PD, intolerable toxicity, or other withdrawal criteria are observed. After 1 year, at the treating investigator's discretion, capecitabine treatment can be discontinued.

5.7 Preparation and Administration of 5-Fluorouracil

Investigators should consult the manufacturer's instructions for 5-FU for complete prescribing information and follow institutional procedures for the administration of 5-FU.

Only for patients unable to take oral medications (because of certain circumstances such as malabsorption, difficulty swallowing, or other conditions that could affect intake of oral capecitabine medication), 5-FU may be administered instead, at a dose of 800 mg/m^2/day as a continuous infusion over 5 days (Day 1 to Day 5 of each cycle), every 21 days. This decision should be made prior to registration by the treating physician; switching from capecitabine to 5-FU, or vice versa, will not be allowed during the study. 5-FU will be administered after completion of the cisplatin infusion.

5-FU will continue until progression of disease, intolerable toxicity, or other withdrawal criteria are observed. After 1 year, at the treating physician's discretion, 5-FU treatment can be discontinued.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- Patient must have pathologically or cytologically MSKCC confirmed esophageal, gastric or gastroesophageal junction (GEJ) adenocarcinoma by the enrolling institution.
- Patients must have esophageal, gastric or gastroesophageal adenocarcinoma with HER2 overexpression and/or amplification as determined by next generation sequencing assay, immunohistochemistry (IHC 3+) or fluorescent in situ hybridization (FISH+ is defined as HER2:CEP17 ratio ≥ 2.0). MSKCC or enrolling institution confirmation of HER2 status is not mandatory prior to enrollment and treatment on study. For patients with outside HER2 testing, if sufficient tissue is available HER2 testing will be repeated at MSKCC or the enrolling institution for purpose of analysis and will not impact the patient's eligibility.
- Additional available archival tumor tissue in the form of 15-20 unstained slides should be submitted to MSKCC for future correlative analysis, but will not be required prior registration. Note: if tissue is depleted, patient will still be eligible after discussion with the MSK PI.
- Patients may have received no prior chemotherapy for Stage IV disease. Patients may have received prior adjuvant therapy (chemotherapy and/or chemoradiation) if more than 6 months have elapsed between the end of adjuvant therapy and registration
- Patients must have disease that can be evaluated radiographically. This may be measurable disease or non-measurable disease per RECIST 1.1.
- Patient must have a normal LVEF (>/= 53%). If a patient has a borderline LVEF (40-52%) they may be considered after consultation with cardiology and study PI and treated per the guidelines in section 11.2.2.
- Age 18 years or older.
- ECOG performance status 0-2.

- Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** (measured via 24-hour urine collection) ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN (1.5 mg/dL or 25.65 µmol/L) **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN. Except patients with Gilbert's disease (≤3xULN)
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥3 mg/dL
Coagulation	
Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

- Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

- Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

- Peripheral neuropathy ≤grade 1

6.3 Subject Exclusion Criteria

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

- 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.
- 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-PD-L2 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, unwillingness to participate, or inability to comply with the protocol for the duration of the study.
- Has active or clinically significant cardiac disease including:
    - Congestive heart failure – New York Heart Association (NYHA) > Class II.
    - Active coronary artery disease.
    - Cardiac arrhythmias requiring anti-arrhythmic therapy other than beta blockers or digoxin.
    - Unstable angina (anginal symptoms at rest), new-onset angina within 3 months before initiation, or myocardial infarction within 6 months before initiation.

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by members of the patient's treatment team, the protocol investigator or research team at participating institutions. If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants. The investigator will use information provided by the patient and/or medical record to confirm that the patient is eligible and contact the patient regarding study enrollment. All eligible patients, regardless of sex and race, will be approached for participation. No additional measures, e.g. advertisement, 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. Participation in the study is completely voluntary. Patients 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.

Inclusion of women and minorities

The investigators take due notice of the NIH policy concerning inclusion of women and minorities in clinical research populations. There will be no limitation with regards to race or gender.

Our institutional demographics for accrual of patients on esophageal, gastric and GE junction cancer trials reflect the national incidence of this disease: 10-15% of our patients have been women; African-American males comprise 3-5% of patients treated on protocol. Given that our protocol accrual closely reflects the national incidence of this disease, no specific strategy will be undertaken to recruit women or persons of color on this trial. None of the eligibility requirements (section 6) will make it difficult for diverse populations to participate in this trial.

This protocol does not include children because the number of children with esophageal, gastric and GE junction cancer is very small and because the majority are already accessed by a nation-wide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

8.1 PRETREATMENT EVALUATION

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 (WOCP)
  In addition, all WOCP should be instructed to contact the MSK Prinicipal 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, magnesium, comprehensive chemistry panel (includes BUN, creatinine, ALT, AST, albumin, glucose, total protein, calcium, bilirubin, bicarbonate, sodium, chloride, potassium, alkaline phosphatase), and coagulation studies (PT/PTT)

- Research blood tests including plasma collection for cfDNA analysis and whole blood collection for PBMC.

The following must be obtained within one month prior to starting protocol therapy:

- CT scan of chest, abdomen and pelvis within 4 weeks of study entry
- Electrocardiogram
- Assessment of Left Ventricular Ejection Fraction (LVEF) as measured by echocardiography or MUGA scan. The same method of measurement has to be used throughout the study.

To be completed any time prior to starting therapy:

- Histological confirmation of esophageal, gastric or GE junction adenocarcinoma at MSKCC prior to study enrollment.

9.1 TREATMENT/INTERVENTION PLAN

Treatment will be administered on an outpatient basis. Pembrolizumab 200 mg IV every 3 weeks, trastuzumab (8 mg/kg loading dose; 6 mg/kg maintenance) IV every 3 weeks with cisplatin IV every 3 weeks with oral capecitabine 2 weeks on/1 week off. Each cycle consists of 21 days. Treatment will be administered on an outpatient basis. In Cycle 1, patients will initiate therapy with trastuzumab 8 mg/kg IV with pembrolizumab 200 mg IV. CT/MRI scan will be performed after the initial 3 weeks (1 cycle) to determine response to pembrolizumab and trastuzumab combination. With subsequent cycles, all patients will begin systemic chemotherapy with the capecitabine/5-FU, and a cisplatin/oxaliplatin regimen in addition to pembrolizumab 200 mg IV with trastuzumab 6 mg/kg maintenance. Patients will receive cisplatin 80 mg/m^2 IV on Day 1, and capecitabine 850mg/m^2 twice a day on Days 1 through 14, every 3 weeks. Treatment will be performed on the scheduled day ± 7 days. Cisplatin will be given in up to 6 cycles, and may be discontinued before 6 cycles for cumulative toxicity. In case of discontinuation of cisplatin due to cumulative toxicity and administration as a single agent during the study, patients will be permitted to continue capecitabine maintenance with pembrolizumab 200 mg IV and trastuzumabevery 3 weeks. CT scan will be performed at baseline, week 3, week 9 and every 9 weeks thereafter. CT scans occurring every 9 weeks will coincide with the 6 month CT scan.

Grade 1 and 2 toxicities will be managed with medical therapy specific to the particular adverse reaction. Intrapatient dose reduction will be allowed depending on the type and severity of toxicity encountered provided that criteria for patient withdrawal from study treatment have not been met. Evaluation in person will be made when there is concern for a drug-related toxicity irrespective of grade.

9.2 Correlative Studies Research

9.2.1 Sample Acquisition and DNA Preparation

Available archival tumor samples will be obtained and stored for future correlative analysis including but not limited to the below. For the discovery set, we will plan conduct whole-exome sequencing of DNA from tumors and matched normal blood from patients that responded to trial therapy (PR or prolonged SD) and compare to the analysis of 'non-responders'.. DNA will be extracted, and exon capture will be performed with the use of the SureSelect Human All Exon 50-Mb kit (Agilent Technologies). Enriched exome libraries were sequenced on the HiSeq 2000 platform (Illumina) to provide mean exome coverage of more than 100× (Memorial Sloan Kettering Cancer Center Genomics Core and Broad Institute).

Immunogenicity Analysis of Somatic Mutations

MSK investigators (Chan and Snyder) created a bioinformatic tool to translate all mutations in exomes and then evaluate binding with major histocompatibility complex (MHC) class I molecules. The neoantigen signature was generated from the nonamers containing four amino acid strings of peptides that are common to tumors from patients with a long-term benefit from therapy.

Intracellular Cytokine Staining

Candidate neoantigen peptides will be synthesized (GenScript), cultured with autologous peripheral-blood mononuclear cells (PBMCs), and then analyzed by means of intracellular cytokine staining for interleukin-2, CD107a, macrophage inflammatory protein 1β, tumor necrosis factor α, and interferon-γ on restimulation of cells with the candidate peptides.

9.2.2 Functional Imaging with ^{89}Zr-trastuzumab PET

In select patients, ^{89}Zr-trastuzumab PETs will be performed on IRB 13 -165. The patients willing to undergo additional imaging on protocol 13-165 and that meet eligibility criteria for both protocols will be approached for IRB 13-165. We will assess ^{89}Zr-trastuzumab PET uptake as a predictor of tumor response. We will compare the proportion of patients with a drop in ^{89}Zr-trastuzumab tumor uptake on PET (pre-therapy and at 3-wk) of 30% or greater in the responder versus in the nonresponder groups. Fisher's exact test will be used to determine the significance of the association. ^{89}Zr-trastuzumab PET imaging will be an exploratory study, with only a select subset of MSK patients on this trial with measurable disease undergoing imaging with ^{89}Zr-trastuzumab PET. If deemed appropriate, MSK patients will be approached for participation in the Pilot imaging trial with ^{89}Zr-trastuzumab PET in HER2+ EG tumors, MSKCC protocol IRB13 -165.

9.2.3 Collection of peripheral blood mononuclear cells (PBMC) and plasma for immunologic analyses

Whole blood will be used for isolation of peripheral blood mononuclear cells (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 32 ml of whole blood per visit (baseline and wks 1, 2, 3, 6, 10, 13, and EOT post-dosing) will be collected at ambient temperature into 4 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 Memorial Sloan-Kettering Cancer Center 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.

9.2.4 Collection of blood of cell-free DNA(cfDNA)

Cell-free DNA (cfDNA) be obtained from plasma samples collected at baseline, week 3, every 9 weeks, and at the time of treatment discontinuation

Approximately 20 ml of whole blood per visit (baseline, every 9 weeks, and treatment discontinuation) will be collected at ambient temperature into 2x 10mL cell free DNA BCT tubes for plasma isolation.

9.2.5 PD-L1 testing of archival tissue

An important aspect of development program for pembrolizumab in esophagogastric cancer is identification of a biomarker for patient selection. PD-L1 expression in tumor tissue will be characterized by immunohistochemistry (IHC) to explore the relationship between PD-L1 expression and response to pembrolizumab. Other exploratory biomarkers including but not limited to PD-1 expression, markers of T-cell phenotype may also be evaluated. Testing will be done at QualTek Laboratories, the recommended laboratory to perform this work. MSKCC will contract with QualTek directly for this work. Tumor tissue for biomarker analysis from archival tissue samples will be batched and shipped to QualTek for testing.

9.2.6 Biopsies of tumor

15 MSK patients only with disease technically amenable to biopsy will be asked to undergo an optional research biopsy at week 3 after the start of therapy for correlative studies evaluating the biologic effects of pembrolizumab and trastuzumab. In addition, patients will be asked to consent to tumor banking such that both frozen and fixed tumor cores can be utilized for future investigations.

Pre- and post-treatment tumor biopsies will be used to evaluate biomarker expression and target inhibition (described below). In addition, archived tissue from prior tumor biopsies obtained at the time of cancer diagnosis, and prior to initiation of trastuzumab will be analyzed, when available. Our primary aims are to identify changes in expression of specific biomarkers or in the genomic HER2 sequence as a result of pembrolizumab and trastuzumab therapy in patients with HER2-overexpressed esophagogastric cancer. After obtaining informed consent, a diagnostic biopsy will be performed either by radiologic guidance (sonogram guided core biopsy or stereotactic biopsy) or endoscopy. The appropriate type of diagnostic biopsy will be determined by the treating physician based on the tumor location most amenable to biopsy. The biopsy specimen will be accessioned and sent to pathology for routine analysis (histology, tumor, grade, HER2 by immunohistochemistry (IHC), and HER2 by fluorescence in situ hybridization (FISH)). Where possible, multiple cores will be obtained. When only one core is obtained, half the core will be snap frozen using liquid nitrogen with a selection evaluated for the presence of invasive carcinoma by a participating pathologist and stored for later analysis. The second and larger sample will be formalin fixed and used for IHC studies.

- The number of biopsies taken will be based on the clinical judgement of the endoscopist, sugergeon, or intervential radiologist, but ideally will be less than or equal to four.
- Tumor DNA (post-treatment) will be isolated for whole exome sequencing and compared to genomic DNA from PBMC in order to identify tumor-specific mutations. Candidate neo-epitopes will be predicted using epitope-prediction algorithms, such as SYFPEITHI. Candidate neo-epitopes will be validated by RNA transcription and protein expression, if possible.
- Changes in the tumor microenvironment will be evaluated comparing baseline and on-treatment biopsies. We will perform immunohistochemistry for PD-L1 expression, IHC for immune cell subsets (including CD4, CD8, regulatory T-cells, macrophages and myeloid

derived suppressor cell populations) and RNA expression analyses (RNAseq) for immune-related genes.

10.1 EVALUATION DURING TREATMENT/INTERVENTION

Procedure	Screen[a, p]	Cycle 1 (21 days)[p]			Cycle 2[g, p]			Cycle 3-[p] subsequent cycles			EOT[c, p]	F/U[d, p]
		D 1	D8	D15	D 1	D8	D15	D1	D8	D15		
Medical Hx/Demographics	X	X	X	X	X	X	X	X			X	X
Serum or urine Pregnancy Test	X											
Archival tumor samples	X											
Optional research biopsy[s]				X								
Physical exam[b]	X	X[f]	X	X	X	X	X	X			X	X
Performance status	X	X[f]	X	X	X	X	X	X			X	X
Toxicities/AE assessment[h]	X	Reported from screening throughout all subsequent cycles.									X	X
Hematology[b]	X	X[f]	X	X	X	X	X	X			X	
Chemistry[b]	X	X[f]	X	X	X	X	X	X			X	
Magnesium	X	X	X[f]	X	X	X	X	X			X	
TSH, Free T3, Free T4	X	X	X	X	X	X	X	X			X	
INR&APTT[m]	X	Will be monitored per treating investigator discretion										
MRI/CT assessment[i]	X			X				Every 9 weeks (3 cycles) after initial CT			X	X
Concomitant medications[j]	X	All medications taken during the study will be documented										X
12-lead ECG	X											
ECHO/ MUGA[0]	X						X	Every 12 weeks (4 cycles) [o]				
^{89}Zr- trastuzumab PET[n]	X			X								
Research Test: PMBC[l]	X	X	X	X			X	X (weeks 10 and 13)			X	
Research Test: cfDNA[k]	X			X				X Every 9 weeks (3 cycles)			X	
Pembrolizumab		X			X			X				
Trastuzumab		X			X			X				
Cisplatin or Oxaliplatin[q]					X			X				
Capecitabine or 5FU					2 weeks on/ 1 week off after cycle 1 completion							
Capecitabine compliance[r]		Compliance will be assessed at every visit										

ECG = electrocardiogram; ECHO = echocardiogram; EOT = end of treatment; FU = follow-up; LVEF = left ventricular ejection fraction; h = hour(s); hx = history; MUGA = multiple gated acquisition scan
Radiological imaging studies <28 days prior to start of study treatment, other screening evaluation within 2 weeks prior to start of study treatment.

a Radiological imaging studies and electrocardiogram <28 days prior to start of study treatment, other screening evaluation within 2 weeks prior to start of study treatment.

b Physical exam (vital signs [BP, heart rate, temperature], weight, and a review of body systems), and chemistry (includes BUN, creatinine, ALT, AST, albumin, glucose, total protein, calcium, bilirubin, bicarbonate, sodium, chloride, potassium, alkaline phosphatase) on intended days 1 and 15 of each cycle prior to chemotherapy or ≤ 72 h prior to dosing. If chemotherapy is delayed then the physical exam must be repeated weekly until retreatment or ≤ 72 h prior to dosing. Hematology and physical exam will be done weekly for the first 6 weeks and on the intended days 1 and days 15 of each cycle prior to the chemotherapy or ≤ 72 h prior to dosing thereafter.

c Within 14 days of tumor progression or study treatment discontinuation. If permanent discontinuation of the study drug falls on a scheduled visit, examinations as defined for EOT should be performed instead of the examinations of the scheduled visit. If patient is unable to come in to see MD, a nurse phone call to evaluate toxicities can be substituted.

d Assessment by treating medical oncologist as clinically indicated. For those patients no longer followed by an MD at MSK or the participating site, a nurse will make a follow up phone call every 3 months to assess patient's vital status.

e A sample of archival tumor biopsy, or other available tumor biopsy, should be submitted for biomarker analysis during screening. Tumor biopsy samples may also be submitted for biomarker analysis at any other time during the course of the clinical study.

f Physical exam and laboratory assessments are not required if screening assessments were done within 72 hours of first dose. ECG is not required if screening ECG was done within 7 days of first dose.

g In cycle 2 procedures will be performed identical to cycle 1. Radiologic tumor assessment will be performed after the initial three weeks (only applicable to patients receiving induction pembrolizumab and trastuzumab for cycle 1) to determine response and every 9 weeks thereafter. If patients experience new lesions or progression at the initial three week CT scan, they will remain on study and be followed by subsequent CT scans. Decision to keep the patient on study will be at the discretion of the treating investigator.

h AE monitoring should continue for at least 4 weeks following the last dose of study treatment. AEs that occur within 30 days following the last study treatment will be followed until resolution.

i The baseline and subsequent scans to assess response must be performed using identical technique. Radiological imaging studies to evaluate tumor status will be performed after the initial three weeks (1 cycle) to determine response, and then at week 9 and every 9 weeks thereafter while on study regardless of treatment delays. Patients who received chemotherapy upfront and did not have an induction period with pembrolizumab/trastuzumab, do not need the 3 week scan. CT scans occurring every 9 weeks will coincide with the 6 month CT scan. For subjects who have entered the survival follow-up period and have not yet experienced PD, every effort should be made to follow-up for tumor evaluation (by CT or MRI) until progression of their malignancy every 9 weeks, or until a new anticancer treatment is started. Window of +/- 7 days during the first cycle, and +/-14 days for all subsequent cycles for radiological imaging studies. Patient may have CT or MRI scan on an earlier or later date than allowed by the window of +/-14 days, if clinically indicated at the MSK Prinicipal Investigator's discretion.

j All medication given within 2 weeks prior to the start of study treatment.

k Cell-free DNA (cfDNA) will be obtained from plasma samples collected at baseline, at week 3, every 9 weeks and at the time of treatment discontinuation, as the same time as the MRI/CT evaluations. Research tests may vary by up to one to fourteen (1-14 days).

l PBMC will be obtained at baseline and wks 1, 2, 3, 6, 10, 13, and EOT post-dosing. Research tests will coincide with physician visits that may vary by up to one to fourteen (1-14 days).

m PT/INR assessments will be continued per treating investigator's discretion

n For MSK only, some patients will have 89Zr--trastuzumab PET at baseline and week 3 only if deemed appropriate by MSK Principal Investigator. Patients will have to sign a written informed consent for MSK IRB#13-165.

o 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 after 6 and 12 weeks of initiating protocol therapy, and thereafter every 12 weeks (4 cycles). Window of +/-14 days for ECHO/MUGA.

p To avoid deviations from the protocol, blood tests, CT/MRI scans, optional research biopsies, and physician visits (including physical exam, vitals, blood pressure, weight, performance status, and concomitant medications) may vary by up to one to fourteen (1-14) days to allow flexibility of scheduling.

q Upon treating investigator discretion, Oxaliplatin (130 mg/m^2 infusion on Day 1 of Cycle 2 and on) may be considered. Patients may begin with reduced dose of oxaliplatin 104 mg/m2 as starting dose if deemed necessary per the treating physician discretion. The decision to treat with oxaliplatin or cisplatin needs to be made prior to treatment initiation by the treating investigator. Switching between oxaliplatin and cisplatin is not allowed.

r Only for patients unable to take oral medications (because of certain circumstances, such as malabsorption, difficulty swallowing, or other conditions that could affect intake of oral capecitabine medication), 5 -FU (800 mg/$m^{2/}$day continuous infusion on Day 1 to Day 5 of Cycle 2 and on) may be considered. The decision to t reat with capecitabine or 5-FU needs to be made prior to initiation by the treating investigator. Switching between 5-FU and capecitabine is not allowed. Patients who received chemotherapy upfront and did not have an induction period with pembrolizumab/trastuzumab, do not need the 3 week scan.

s For 15 MSK patients only.

10.1 Future Unspecified Use of Biospecimens

The protocol includes an informed consent document and research authorization that meets statutory guidelines. Each participating site will have its own consent form meeting the requirements described in this section. The consent form will inform patients of the purpose of the bank, their rights in relation to it, and the safeguards in place to protect the confidentiality of their health information. The consent will state that some of the biospecimens will be saved to use for future research.

Type of future use
The consent specifically describes the types of future research that may be performed, including use of tissues to develop new drugs with cancer-associated molecular targets, development of cell lines, future use of cell lines to define cancer phenotype and (somatic) genotype, DNA sequence analysis of tumor compared to normal and identification of tumor-associated proteins as diagnostic or prognostic markers. It will be stated that researchers at MSK may either keep indefinitely or dispose of any leftover blood or tissues or other samples, including DNA that the samples contain. Blood and tissues will be stored with identifiers in secure tissue or fluid banks. It is stated that the samples could be lost or ruined because of mechanical failure, and that MSK cannot guarantee that samples will be stored indefinitely. The samples will be stored for as long as deemed useful for research purposes.

Consent for future use and re-contact
Patients are asked in a series of check boxes at the end of the consent if 1) they permit their biospecimen samples to be stored and used in future research to learn about or prevent cancer or side effects of treatment, or to develop new treatments; 2) if they permit their samples to be stored and used in future research to learn about, prevent, or treat diseases other than cancer; or 3) if they permit their samples, with personal identifiers protected, to be used for research about inherited genetic factors, 4) if they permit their samples to be used for genetic analysis of the tumor and normal tissue to learn about the causes of cancer, 5) participants are asked if they agree to be contacted in the future as part of research studies for additional health information or to be asked to participate in future biospecimen research studies and 6) if they consent to be contacted to discuss research findings which may come from their sample. Finally, if not available (e.g. deceased), if they wish to have their designee designated on the consent to be contacted.

Participants will not be provided with specific results of research tests performed on their collected human biologic specimens.

Use of identifiable information for genetic studies
In the course of this research it is possible that some patients whose tumors are analyzed through investigational “next-generation” profiling in a research (non-CLIA) environment will be found to have somatic or germline mutations in genes that are known to be associated with an increased risk of cancer or other diseases. It will be stated in the consent that the participants will not receive any specific results from research tests. The consent will tell participants that if they wish to have genetic testing done for personal reasons than they should make an appointment with the MSK Clinical Genetics Service or Clinical Genetics Service at their site.

If in the course of this research a research finding is obtained that, in the opinion of the investigator, may be critical to the preventive care of the participant or their family, the investigator can communicate that finding to the MSK IRB Genomic Advisory Panel (GAP). The finding will be reviewed by the GAP to determine whether the incidental finding should be discussed with the participant. For MSK patients, in the event that the GAP determines that the finding should be discussed with the participant, and the participant has consented to be re-contacted, then the

treating/consenting physician shall be contacted by the panel and asked to refer the participant to the Clinical Genetics Service for further discussion of the research finding.

The following information must be provided to GAP for review:

- Participant Name/MRN #
- Type of Biospecimen (tissue, blood, saliva)
- Incidental Finding
- Collection Protocol #
- Contact: ocrgapirb@mskcc.org

For non-MSK patients being treated at one of the participating institutions, if the GAP determines the finding to be reportable to the participant and the participant has consented to be re-contacted, results will be returned to the Site Principal Investigator via the study team. Site policies on returning these research findings to the patient should be followed.

We anticipate that other research assays may be incorporated into this protocol as technology evolves.

Voluntariness of research participation

It is stated that taking part in this tissue and blood bank is voluntary and patients have the right to withdraw at any time. Participation in the study will not impact on the clinical care patients receive.

Withdrawal

Participants may decide at a later date that they do not want identified blood and tissue samples to be stored in the tissue bank and /or used for future research. If participants decide to withdraw from the study, specimens that have not yet left the specimen archive will not be used in new studies and any remaining portions of samples that have not been used for research will be used only for clinical purposes or, if requested by the patient, destroyed. For specimens already shipped out from the archive, it may not be possible to locate the samples or stop already ongoing research. When a participant withdraws from the protocol, MSK's Protocol Participant Registration (PPR) Office should be notified immediately. If a non-MSK participant withdraws from the study, the MSK study staff member will notify PPR. The withdrawal request will be documented in CRDB and the system updated accordingly. In addition, a note-to-file documenting the patient withdrew must be filed in his/her medical records.

Rights after death

The consent states that if the research participant dies or is unable to make his/her wishes known, all of their rights to decide about future uses of the blood or tissues will pass to the authorized representative of the estate. If there is no representative of the estate, the rights pass to the next of kin.

Risks of research participation

The greatest risk is release of information from health or research records in a way that violates privacy rights. MSK and any participating sites will protect records so that name, address, phone number, and any other information that identifies the participant will be kept private. It will be stated to the participant that the chance that this information will be given to an unauthorized individual without the participant's permission is very small.

Costs/compensation

There is no cost to the participant to enroll in this research. Tissue or blood obtained in this research may be used to make a cell line, and these may be patented or licensed and thus may have significant commercial value. The participant is informed that there are no plans to provide financial

compensation for use of their human biologic specimens, nor are there plans for the participant to receive money for any new products, tests, and discoveries that might come from this research.

Biospecimen Privacy
Medical information is confidential. The participant's personal identity will not be used in reports that are written about the research. The MSK IRB/PB will review all requests for research performed involving biospecimens ascertained through this protocol. Blood and tissue samples may be stored with a code linked to the patient's medical record. The results of any research using blood or tissues will not be placed in the medical record.

The consent indicates that samples and genetic information collected may be shared with other qualified researchers and placed in online databases. An example of an online database is the NIH dbGAP database, which is monitored by the National Institutes of Health, and may be made accessible to investigators approved by the U.S. government. Such information will not include identifying information such as name. It is also stated in the Research Authorization (HIPAA Authorization) that research data (e.g. genomic sequence) may be shared with regulators. The requirements for submission of genotype/phenotype data into the NIH dbGAP or any other public database will be followed as per the IRB SOP for Genomic Data Sharing.

Use of banked samples (at MSK)
When samples are to be analyzed, the individual investigator needs to write an IRB biospecimen protocol. This protocol is fast-tracked through MSK Research Council review and is reviewed at the MSK IRB by the expedited review process. This protocol is only for research that will be done on biospecimens obtained under identified protocols and their informed consent and research authorization that include the institutional future use questions. The consent and research authorization for the use of the biospecimens will be waived as per 45 CFR 46.116(d) and 45 CFR 164.512(i)(2)(ii).

11.1 TOXICITIES/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 (see Section 10.0) and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs).

11.2 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.

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

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

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

[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at 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.

[3] For patients with history of gilberts disease and there is an isolated grade 2 elevation of increased bilirubin, patients do not need to be held and can be treated.

11.1.1 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.

Table 3: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the treating investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the treating investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose.	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	Subjects who develop Grade 2 toxicity despite adequate premedications should be permanently discontinued from further trial treatment administration.	
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the treating investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

11.2 Trastuzumab

Principal adverse effects include cardiac dysfunction, infusion associated symptoms, potentiation of chemotherapy related hematologic side effects. A complete listing of toxicities can be found in the trastuzumab package insert.

11.2.1 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 treating investigator 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 CHF should have trastuzumab held and should receive treatment for CHF. Patients with an asymptomatic absolute decrease in LVEF of $\geq$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.

11.2.2 Congestive Heart Failure and other Cardiac Dysfunction

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 (>/= 53% are elgible for entry into the study. Patients with a borderline decreased LVEF (40-52%) and with no signs of clinical heart failure may still be considered for inclusion in the study after consultation with cardiology and MSK Principal Investigator. Patients with decreased LVEF should be optimized on heart failure medical therapy (E.g ACE inhibitors, β-blockers, diuretics, and/or cardiac glycosides), as recommended by the cardiology consultant prior to entry in the study. All patients will undergo regular

cardiac monitoring throughout the study, including at baseline, 6 and 12 weeks after initiation of trastuzumab therapy, and every 12 weeks (4 cycles) thereafter. 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, β-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 the patient is benefiting clinically from trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction or heart failure. 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 maybe 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 ejection fraction 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. Additional LVEF

Assessments prior to the next regularly scheduled LVEF measurement may be performed at the treating investigator's discretion. If the patient is benefiting clinically from trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction even in the setting of an asymptomatic LVEF decline, and reinitiating of trastuzumab can be considered at the discretion of the treating investigator after consultation with a cardiologist and discussion with the patient concerning the risks and benefits of continued therapy.

Caution should be exercised when treating patients with clinically significant cardiovascular disease such as pre-existing coronary artery disease or CHF. Patients with symptomatic CHF, unstable angina pectoris, or symptomatic or poorly controlled cardiac arrhythmia should not be enrolled in clinical trials with pembrolizumab. Pembrolizumab should be discontinued in the event of any Grade 3 or 4 events consistent with CHF.

11.2.3 Infusion-Related Reactions

Any treatment-related infusion-related reactions are defined according to the NCI-CTCAE Version 4.0 definition (General disorders and administration site conditions). Symptoms occurring during or following infusion of investigational therapy may also be defined according to AE categories such as allergic reaction, anaphylaxis, or cytokine release syndrome (Immune system disorders). In the setting of symptoms occurring during or following infusion of investigational therapy, investigators are encouraged to use the AE term "infusion-related reaction" and any additional terms (including those not listed here) that best describe the event. Consistent with usual medical practice, the patient should be clinically monitored and selected parenteral medications may be utilized for Grade 2 allergic/hypersensitivity reaction as detailed below. Clinical and laboratory monitoring will be performed per institutional guidelines.

Grade 1 IRR

- Slow the infusion rate by 50%.
- Monitor the patient for worsening of condition.
- For subsequent infusions, premedicate with diphenhydramine hydrochloride 50 mg I.V. (or equivalent); additional premedication may be administered at the treating investigator's discretion.

Grade 2 IRR

- Stop the infusion
- Administer diphenhydramine hydrochloride 50 mg I.V. (or equivalent), acetaminophen 650 mg orally for fever, and oxygen.
- Resume the infusion at 50% of the prior rate once the IRR has resolved or decreased to Grade 1; the infusion duration should not exceed 2 hours.
- Monitor for worsening of condition.
- For subsequent infusions, premedicate with diphenhydramine hydrochloride 50 mg I.V. (or equivalent); additional premedication may be administered at the treating investigator's discretion.

For a second Grade 1 or 2 IRR, administer dexamethasone 8-20 mg I.V. (or equivalent); then, for subsequent infusions, premedicate with diphenhydramine hydrochloride 50 mg I.V. (or equivalent), acetaminophen 650 mg orally, and dexamethasone 8-20 mg I.V. (or equivalent).

11.3 Capecitabine, 5-FU, Cisplatin, and Oxaliplatin Dose Delays and Modifications

For hematologic laboratory abnormalities as an initial step, the dose of capecitabine/5-FU/cisplatin/oxaliplatin should be reduced first before any dose reductions for pembrolizumab are considered. capecitabine/5-FU/cisplatin/oxaliplatin will be held for grade 3 or 4 non-hematologic toxicity (with the exception of grade 3 electrolyte abnormalities) or for not meeting treatment parameters as described in the tables below on the day of treatment. If the toxicity has resolved and the patient meets treatment parameters but experienced interval toxicity, then for the purposes of determining dose reductions, the grade of toxicity should be that seen despite maximal medical management (i.e. intensive loperamide or tincture of

opium for diarrhea). If multiple toxicities are seen the dose administered for a particular drug should be based on the most severe toxicity noted. In general, when multiple toxicities are experienced that can result in the dose reduction of multiple drugs, reducing multiple drugs at one time is the preferred approach. Treatment may resume when the toxicity has resolved to $\leq$ grade 2, except as indicated below.

Generally, during the first six cycles of therapy when treatment is held for chemotherapy related toxicity, all drugs (cisplatin or oxaliplatin and capecitabine or 5-FU, Pembrolizumab/Trastuzumab) will be held for coordinated scheduling. Re-evaluation visit should occur in no more than 7 days. For an elevated creatinine or for ototoxicity, cisplatin or oxaliplatin alone may be held at the treating investigator's discretion and the patient may continue with capecitabine or 5-FU without treatment delay. If capecitabine is held for more than 4 weeks for toxicity, patients will be taken off study, unless there is a clinical benefit. If there is a clinical benefit, patients may be retreated at a lower dose after resolution of toxicity to $\leq$ NCI- CTCAE v4.0 grade 2, except as indicated below. Cisplatin or oxaliplatin and capecitabine or 5-FU may each be dose attenuated, either in combination, or individually. For all toxicities, appropriate clinical judgment should be applied to manage the symptoms.

Tables **11.4.1, 11.4.2**, **11.4.3, 11.4.4, 11.4.5, 11.4.6, and 11.4.7** address specific chemotherapy dose delays and modifications instructions. Colony stimulating factors: Patients should not routinely receive prophylactic colony stimulating factors (e.g., G-CSF, GM-CSF) during cycle 1. Subsequent use will be at the discretion of the treating physician.

Table 11.4.1 Capecitabine dose levels

Starting dose 0	Capecitabine 850 mg/m^2 orally twice daily
Dose Level -1	Capecitabine 640 mg/m^2 orally twice daily
Dose Level -2	Capecitabine 425 mg/m^2 orally twice daily

Table 11.4.2 Cisplatin dose levels

Starting dose 0	Cisplatin 80 mg/m^2
Dose Level -1	Cisplatin 60 mg/m^2
Dose Level -2	Cisplatin 40 mg/m^2

Table 11.4.3 Oxaliplatin dose levels

Starting dose 0	Oxaliplatin 130 mg/m^2*
Dose Level -1	Oxaliplatin 104 mg/m^2
Dose Level -2	Oxaliplatin 65 mg/m^2

*Patients may begin with reduced dose of oxaliplatin 104 mg/m2 as starting dose if deemed necessary per the treating physician discretion.

Table 11.4.4 Dose-modification guidelines for cisplatin, oxaliplatin,capecitabine or 5-FU for thrombocytopenia, neutropenia, febrile neutropenia, and infection[a].

ANC Count (x 10^9/L)	Platelet Count (x 10^9/L)	Dose adjustment for cisplatin, oxaliplatin, 5-FU or capecitabine
>1.5 and	>75	Maintain dose level without interruption

<1.0	and/or	<75	Wait for counts to recover[b]; if within 42 days of interruption, ANC ≥1.0 x 10^9/L and platelets ≥75 x 10^9/L, reduce by 1 dose level if permissible[c].
<1.0	and	Fever ≥38.5°C (101°F) or infection of any duration	Reduce by 1 level on recovery of ANC ≥1.0 x 10^9/L

Abbreviations: ANC = Absolute Neutrophil Count.

a. No chemotherapy should be administered if ANC <1.0 x 10^9/L and platelet count <75 x 10^9/L
b. If unscheduled laboratory assessments during a treatment cycle show that the neutrophil count drops <1.0 x 10^9/L or that the platelet count drops <75 x 10^9/L, treatment with capecitabine should be interrupted
c. Dose reductions of capecitabine for ANC <1.5 x 10^9/L but ≥1.0 x 10^9/L may be determined at the discretion of the treating investigator or institutional guidelines. In such situations, if administration of chemotherapy is prohibited, then capecitabine must be held until the ANC recovers to ≥1.5 x 10^9/L

Table 11.4.5 Dose-modification guidelines for nonhematologic adverse events thought to be related to capecitabine[a]

Toxicity CTCAE Grades	During a Course of Therapy	Dose adjustment for next dose (% of starting dose)
Grade 1	Maintain dose level	Maintain dose level
Grade 2		
- First appearance	Interrupt until resolved to Grade 0-1 or pretreatment baseline	75%
- Second appearance		50%
- Third appearance	Discontinue treatment permanently	Not applicable
Grade 3		
- First appearance	Interrupt until resolved to Grade 0-1 or pretreatment baseline	75%
- Second appearance		50%
- Third appearance	Discontinue treatment permanently	Not applicable
Grade 4		
- First appearance	Discontinue treatment permanently OR If the treating investigator deems it to be in the patient's best interest to continue, interrupt until resolved to Grade 0-1 or pretreatment baseline	Not applicable OR 50%
- Second appearance	Discontinue treatment permanently	Not applicable

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; SmPC = Summary of Product Characteristics; USPI = United States Package Insert

a. Adapted from Xeloda USPI and SmPC

Table 11.4.6 Dose-modification guidelines for cisplatin for reduced glomerular filtration rate

Creatinine clearance requirement	Dose adjustment for cisplatin
>60 mL/min prior to Day 1 of Cycle 1-6	Maintain dose level
51-59 mL/min prior to Day 1 of Cycle 2-6	Reduce 1 dose level, if permissible
41-50 mL/min prior to Day 1 of Cycle 2-6	Reduce 2 dose levels, if permissible
<40 mL/min prior to Day 1 of Cycle 2-6	Stop cisplatin permanently

Table 11.4.7 Dose-modification guidelines for cisplatin and oxaliplatin related for nonrenal and nonhematologic adverse events

Toxicity	Grade	Dose adjustment for cisplatin
Nausea/vomiting/diarrhea (either on its own or in combination)	≥3	Reduce 1 dose level, if permissible, on recovery to Grade 1 or below
Ototoxicity	>2	Discontinue
Neurotoxicity	0-1	No dose adjustment required
Neurotoxicity	2	Reduce 2 dose levels, if permissible, on recovery to Grade 1 or below
Neurotoxicity	>3	Discontinue
Any other toxicity thought to be due to cisplatin	4	Discontinue

For toxicities not listed in table 11.4.4, appropriate clinical judgment should be applied to manage any symptoms arising out of these AEs. For any AE which is Grade ≥3 or if the AE is considered clinically significant by the treating investigator, temporarily stop administering cisplatin to allow for recovery of the toxicity to Grade 1 or baseline, for a maximum period of 42 days. Once the toxicity recovers, reduce the dose of cisplatin by 1 dose level, if permissible. If the toxicity does not recover within the 42-day period, permanently discontinue cisplatin. Discontinue cisplatin if a third dose reduction is required.

Table 11.4.8 Dose modification guidelines for 5-FU related to nonhematologic adverse events

Toxicity	**Grade**	**Dose adjustment for 5-FU**
Diarrhea/stomatitis	3	Reduce 1 dose level, if permissible, on recovery to Grade 1 or below
Diarrhea/stomatitis	4	Reduce 2 dose levels, if permissible, on recovery to Grade 1 or below
Cardiac toxicity (related to 5-FU)	≥2	Discontinue
Skin toxicities	≥3	Reduce 2 dose levels, if permissible, on recovery to Grade 1 or below

Table 11.4.9 5-FU dose levels

Starting dose 0	5-FU 800 mg/m^2 /day, Day 1-Day 5
Dose Level -1	5-FU 600 mg/m^2 /day, Day 1-Day 5
Dose Level -2	5-FU 400 mg/m^2 /day, Day 1-Day 5

11.4 General Dose-Modification Guidelines for Chemotherapy

- Treatment for the first cycle should only commence if all the inclusion and exclusion criteria are met and patient has been enrolled. For subsequent cycles, dose delay/modification is permitted as described in sections specific for pembrolizumab, trastuzumab, capecitabine, 5-FU, cisplatin, and oxaliplatin. All study treatment will be discontinued in case of disease progression.
- The dose of capecitabine or 5-FU should be determined at the start of each treatment cycle. Based on treating investigator's discretion, full dose of capecitabine can be re-administered after 1 dose reduction (25% dose reduction). Re-escalation is not allowed after the second dose reduction. The maximum number of dose reductions for capecitabine is 2 (50% of the original dose).
- Doses of any study drug omitted for toxicity are not replaced or restored; instead, the patient should resume the planned treatment cycles. Supportive care (for example, colony-stimulating factors, blood and blood products, etc. can be administered in accordance with the latest ASCO or other equivalent guidelines.
- Dose modification, for non-serious and non-life-threatening toxicities like alopecia, altered taste or nail changes may not be required and the final decision is left to the discretion of the treating investigator.
- In situations where concomitant toxicities of varying severity exist, dose modification will be tailored for the toxicity with highest CTCAE grading.
- If there is a delay or modification in administration of study drug(s) due to toxicity, treatment with the other study agent(s) should also be held. If clinically appropriate, the treating investigator can delay all treatment components up to a maximum of 7 days to allow synchronized administration of all agents.
- If a toxicity related to any component of chemotherapy does not resolve in the same treatment cycle, the administration of that component can be delayed up to 63 days (9 weeks). If the toxicity does not resolve within 63 days (9 weeks), that component will be discontinued unless it is determined by the treating investigator that the patient might benefit from continuation of the component.

If at least six months have elapsed since oxaliplatin discontinuation, oxaliplatin may be reinitiated at the last dose level for this patient at the investigator's discretion for clinical progression concern.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

All patients who receive at least one dose of pembrolizumab and began trastuzumab therapy will be evaluable for toxicity and response. Patients who are did not begin pembrolizumab therapy will not be evaluated for toxicity or response and will be replaced by a new patient.

The primary endpoint of this study is to determine the six months progression free survival (PFS) in the first-line treatment of patients with Stage IV HER2 positive esophagogastric adenocarcinoma. The same imaging modality performed at baseline (CT or MRI) will be repeated at subsequent imaging.

Secondary endpoints include toxicity, overall survival, median PFS, response to pembrolizumab + trastuzumab, best overall RECIST 1.1 response (CR+PR) to capecitabine/5-FU, cisplatin/oxaliplatin, pembrolizumab and trastuzumab, overall clinical benefit defined as stable disease (SD), complete response rate (CR), or partial response (PR) safety and tolerability. The type, frequency, severity, timing, and relationship of each adverse event will be determined as per the NCI Common Toxicity Criteria, version 4.0. Toxicity during cycle 1 and subsequent cycles will be reported.

13.0 CRITERIA FOR REMOVAL FROM STUDY

- If at any time the patient develops progressive disease on combination of trastuzumab, pembrolizumab, and chemotherapy he/she will be taken off study unless the treating investigator determines that it is of clinical benefit, defined as improvement in quality of life, function, or general well-being, for the patient to continue on trial therapy. If at least six months have elapsed since oxaliplatin discontinuation, oxaliplatin may be reinitiated at the last dose level for this patient at the investigator's discretion for clinical progression concern.

If after nine weeks of continued treatment a second scan shows additional disease progression, treatment will be discontinued and the patient will be taken off of study. 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 study. Before being removed from the study, patients will be scanned to assess the response. If the off study scan shows progression of disease then the patient will be considered as a non responder, while a CR or PR will be considered as 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 despite dose reduction as indicated in Section 11.
● Documented progressive disease

As soon as a patient is withdrawn 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 who show a clinical benefit (i.e., with either an objective tumor response or the absence of disease progression), may continue to receive additional treatment courses. Patients with radiologically documented progressive disease should be removed from the study unless the treating investigator judges it to be of clinical benefit for the patient to continue on trial therapy.

14.0 BIOSTATISTICS

The phase III ToGA study established the benefit of trastuzumab in combination with cisplatin and fluoropyrimidine (CF) chemotherapy in HER2-positive metastatic EG adenocarcinoma. The trastuzumab/CF cohort from the ToGA will be used as historic control for this study. In ToGA patients assigned to receive trastuzumab + CF had a significant improvement in progression free survival (5.5 mos vs 6.7 mos, HR 0.71 [0.59-0.85], p=0.0002), overall survival (13.8 mo vs 11.1 mo, HR 0.74 [0.6-0.91], p = 0.0046). Approximately 55% of the capecitabine/cisplatin/trastuzumab-treated patients were progression free at 6 months. The hypothesis is that addition of pembrolizumab to fluorpyrimidine and platinum and trastuzumab will improve outcome in previously untreated patients with HER2 gastric, esophageal or GE junction gastric adenocarcinoma.

The primary endpoint is 6-month progression free survival (PFS), as measured from the start of the treatment with trastuzumab and pembrolizumab to the date of either documentation of disease progression on fluoropyrimidine and platinum with trastuzumab and pembrolizumab or death. Patients with disease progression on pembrolizumab and trastuzumab will be removed from study per the rules described in section 13.0.. We will define progression of disease to capecitabine/5-FU, and a cisplatin/oxaliplatin with trastuzumab and pembrolizumab per RECIST 1.1 criteria. Patients with measurable disease and with evaluable radiographically but non-measurable disease will be eligible for study entry. As per RECIST 1.1 criteria, any evidence of progression in non-measurable lesions, measurable lesions, or the development of new lesions, would qualify as disease progression. Using an exact single stage binomial design, we will accrue 37 Stage IV esophageal, gastric and GE junction adenocarcinoma patients to differentiate between 6-month PFS of 55% and 75% with type I of 5% and power of 80%. After week 9, CT/MRI scans occurring every 9 weeks will coincide with the 6 month CT/MRI scan, and if necessary, an additional scan will be done at 6 months. If there is a delay in treatment for a patient the 6 month scan will not be delayed. If 26 or more patients are progression free at 6 months, capecitabine/5-FU, cisplatin/oxaliplatin and trastuzumab + pembrolizumab will be considered worthy of further investigation. Patients who come off study before 6 months without documented progression will be considered as events for the primary endpoint of 6 months PFS. We anticipate enrollment to be 1-2 patients/months with completion of accrual in approximately 36 months. Patients that come off study due to toxicity before 6 months without documented progression will continue to be scanned to obtain 6 months assessment of progression. Patients that were lost to follow up or withdrew consent before 6 months without documented progression will be counted as events for the primary endpoint; however this is expected to be a rare occurrence.

The patients who completed at least one cycle of trastuzumab + pembrolizumab will be considered evaluable.

Secondary and exploratory endpoints include:

- Calculation of the rate of best RECIST 1.1. response (CR+PR) estimated using binomial proportions along with 95% confidence intervals; median PFS, overall and 1-year survival in patients with HER2+ Stage IV esophageal, gastric or GEJ adenocarcinoma will be measured from the start of treatment to death or last follow-up.
- Perform correlative analyses including whole exome analysis in order to determine mutation load and specific neoantigen landscape with strong association in patients with regimen efficacy (PR, CR or prolonged SD). The Mann–Whitney test will be used to compare mutational loads.

- Correlate the change in uptake on ^{89}Zr-trastuzumab PET with changes in tumor size on CT in patients receiving study therapy. We will compare the proportion of patients with a drop in ^{89}Zr-trastuzumab tumor uptake on PET (pre-therapy and at 3-wk) of 30% or greater in the responder versus in the nonresponder groups. Fisher's exact test will be used to determine the significance of the association. This will be conducted for MSK patients only.
- Determine overall clinical benefit defined as stable disease (SD), complete response rate (CR), or partial response (PR) safety and tolerability. They will be summarized using binomial proportions along with exact 95% CI.

- Generalized estimating equations (GEE) will be used to look at associations between response and PBMC and cfDNA while Cox regression with time dependent covariate will be used to associate survival with PBMC and cfDNA.

- PD-L1 will be associated with response using Fisher's exact test while log-rank test will be used to compare survival curves between PD-L1 positive and negative patients.

Previous clinical trials have reported the frequency of adverse events attributable to treatment with capecitabine, cisplatin, and trastuzumab. A similar frequency of adverse events will be considered attributable to the capecitabine, cisplatin and trastuzumab plus pembrolizumab used in this trial and therefore acceptable. In order to reduce patient risk, the study design includes early termination of the trial in the event of excessive grade 4+ neutropenia, grade 3+ diarrhea despite adequate antidiarrheal management (loperamide and diphenoxylate/atropine) or grade 3+ neuropathy. In addition, the safety analysis will assess the toxicity rates that may arise related to pembrolizumab persistent grade 3+ hypertension despite adequate medical management. Furthermore, presence of grade 3+ events which in the clinical judgment of the MSK Principal Investigator are felt to be serious, unexpected, and a side effect likely due to pembrolizumab , will give evidence to reduce the dose as well. Adverse events (possibly, probably, or definitely) related to the study treatment during all treatment cycles will count towards the excessive toxicity boundaries below. The stopping rules are derived using repeated significance testing are given in the table below.

Toxicity	# of toxicities needed to stop the study	Toxicity rate	Probability boundary is crossed
Gr 4+ Neutropenia	**5 within the first 10 patients 7 within the first 20 patients 11 within 37 patients**	**.19**	**.11**
		.45	**.98**
Gr 3+ Diarrhea	**4 within the first 10 patients 6 within the first 20 patients 8 within 37 patients**	**.12**	**.08**
		.35	**.97**
Gr 3+ Neuropathy	**2 within the first 10 patients 3 within the first 20 patients 5 within 37 patients**	**.05**	**.13**
		.25	**.98**

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 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 or not 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).

15.3 Randomization

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA), or equivalent at each participating site, will be assigned to the study. The responsibilities of the MSK RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database, Clinical Research Database (CRDB) Source documentation will be available to support the computerized patient record. Anonymization will take place at the point of entry into the database. Subsequent laboratory analysis will take place on the anonymized samples.

MSK will be the data coordinating center under the guidance of the Multicenter Protocol Executive Committee (MPEC). MSKCC will be responsible for reporting to the funding source (as applicable) and governing agencies.

Tumor slides from MSK and the participating sites will be stored in the MSK pathology laboratory. Results from laboratory studies will include computer files of sequencing data, and analyses. These files will be stored on the Department of Medicine server. Documentation linking patient identifiers and patient samples results will be securely maintained in the secure Clinical Research Database (CRDB) with access limited to study investigators.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. 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" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are 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 Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Alternative, non-protocol, treatment options will be discussed with the patient. It will be reviewed that participation in this clinical trial is voluntary and that the patient may withdraw consent at any time. The study is designed with careful safety monitoring for toxicity including physician visits and serial cardiac monitoring. Specific guidelines for symptom management are in place to protect the study participant. The financial costs of the study will be discussed; pembrolizumab will be provided free of charge. Biopsy cost may be covered by the patient's insurance if it is required for confirmation of metastatic disease, if not covered will be covered by research funds. The patient will be responsible for the cost of standard medical care and all hospitalizations, even for complications of treatment.

No incentives will be offered to patients/subjects to participate in this study.

17.2 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 described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 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 subject 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 signs consent ***and*** is registered. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

An SAE must be reported to the IRB/PB within 5 calendar days of the event. The IRB/PB requires a Clinical Research Database (CRDB) SAE report will be submitted electronically to the SAE Office as follows:.

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSK)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study

- If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.
Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

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

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

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

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

18.1 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.

19.0 REFERENCES

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20.0 APPENDICES

Appendix 1: Multicenter Addendum

Appendix 2: Laboratory Manual

Appendix 3: Pill Diary