Official Protocol Title:	A Phase II Study of Pembrolizumab Monotherapy in Third Line, Previously Treated Subjects with Advanced/Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus or Advanced/Metastatic Siewert Type I Adenocarcinoma of the Esophagogastric Junction (KEYNOTE – 180)
NCT number:	NCT02559687
Document Date:	28-Jun-2021

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Protocol-specific Sponsor Contact information can be found in the Investigator Trial File Binder (or equivalent).

TITLE:

A Phase II Study of Pembrolizumab Monotherapy in Third Line, Previously Treated Subjects with Advanced/Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus or Advanced/Metastatic Siewert Type I Adenocarcinoma of the Esophagogastric Junction (KEYNOTE – 180)

IND NUMBER: 123,482

EudraCT NUMBER: 2015-002427-26

DOCUMENT HISTORY

Document	Date of Issue	Overall Rationale
Amendment 6	28-JUN-2021	To update the criteria for early trial termination. To clarify the concomitant use of COVID-19 vaccines.
Amendment 5	30-NOV-2017	To add guidelines for the management of myocarditis based upon health authority feedback.
Amendment 4	29-NOV-2017	To add guidelines for the management of myocarditis, based upon health authority feedback.
Amendment 3	12-JAN-2017	• To indicate prespecified GEP cutoffs. • To clarify requirements for HIV, HBV, and HCV testing for Germany.
Amendment 2	05-DEC-2016	• To indicate prespecified GEP cutoffs.
Amendment 1	21-JUL-2016	• To clarify the inclusion and exclusion criteria.
Original Protocol	24-AUG-2015	Not applicable.

SUMMARY OF CHANGES

PRIMARY REASON(S) FOR THIS AMENDMENT:

Section Number (s)	Section Title(s)	Description of Change (s)	Rationale
5.11	Clinical Criteria for Early Trial Termination	The criteria for early trial termination was updated.	To add flexibility to allow the study to terminate before the planned completion date.

ADDITIONAL CHANGE(S) FOR THIS AMENDMENT:

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
5.1.3, 12.8	Subject Exclusion Criteria, Germany-specific Requirements	To clarify requirements for HIV, HBV, and HCV testing for Germany.	To consolidate the Germany-specific amendment with the global amendment.
1.0, 5.1.2, 5.10, 7.1.1.1.2, 7.1.1.3, 7.1.3.2.2, 7.1.3.3, 7.1.3.5	Trial Summary, Subject Inclusion Criteria, Beginning and End of the Trial, Consent and Collection of Specimens for Future Biomedical Research, Subject Identification Card, Whole Blood Collection for Correlative and Biomarker Studies, Tumor Tissue	Updated informed consent language to provide for documentation of consent.	To align text with legal requirements to obtain informed consent.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
5.5.2	Prohibited Concomitant Medications	Added a note that any COVID-19 vaccine that is licensed in a given country is allowed in the study.	To clarify the concomitant use of COVID-19 vaccines.
Overall	Overall	Minor editorial changes.	For clarity.

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1.0 TRIAL SUMMARY

Abbreviated Title	A Phase II study of Pembrolizumab in Third Line Previously Treated Subjects with Advanced/Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus
Trial Phase	Phase II
Clinical Indication	Advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type I adenocarcinoma of the esophagogastric junction.
Trial Type	Interventional
Type of control	No treatment control
Route of administration	Intravenous (IV)
Trial Blinding	Unblinded Open-label
Treatment Groups	Pembrolizumab (MK-3475) 200 mg IV every 3 weeks
Number of trial subjects	Approximately 100 subjects will be enrolled.
Estimated duration of trial	The Sponsor estimates that the trial will require approximately 24 months from the time the first subject provides documented informed consent until the last subject's last study-related phone call or visit.
Duration of Participation	Each subject will participate in the trial from the time the subject provides the documented Informed Consent Form (ICF) through the final contact. After a screening phase of up to 28 days, each subject will receive pembrolizumab beginning on Day 1 of each 3-week dosing cycle. Treatment will continue until progressive disease, unacceptable adverse events, intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements; subject receives 35 treatments (approximately 2 years) of pembrolizumab, or administrative reasons requiring cessation of treatment. Subjects who stop pembrolizumab as a result of obtaining a centrally confirmed complete response (CR) or those subjects who stop after receiving 35 trial treatments may be eligible, at the discretion of the investigator, for an additional 17 trial treatments (approximately 1 year) after experiencing progressive disease if they meet the criteria for re-treatment (Second Course Phase) and the study is ongoing. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until progressive disease, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed (example; by telephone) for overall survival until death, withdrawal of consent, or the end of the study. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. Serious adverse events will be collected for 90 days after the end of treatment or for 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.
Randomization Ratio	N/A

A list of abbreviations used in this document can be found in Section 12.6.

2.0 TRIAL DESIGN

2.1 Trial Design

This is a single arm, open-label, multi-site trial of pembrolizumab (MK-3475) in subjects with previously treated, advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type I adenocarcinoma of the esophagogastric junction (EGJ). Siewert type I tumors are adenocarcinomas of the lower esophagus with the center located within 1cm to 5cm above the anatomic EGJ.

Subjects will be required to have an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0 or 1, life expectancy greater than 3 months, have at least one measurable lesion by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) for response assessment, and had to have been previously treated with two lines of therapy. Subjects will be required to provide a tumor sample for retrospective analysis of biomarkers which may predict response to pembrolizumab. Biomarkers assessed include an intratumoral immune-related gene expression profile (GEP) and PD-L1 expression assayed by immunohistochemistry (IHC). Approximately 100 subjects will be allocated to receive single agent pembrolizumab 200 mg IV every 3 weeks (Q3W). Additionally, to ensure that an adequate number of adenocarcinoma subjects are included in the study, there will be a cap on enrollment of subjects residing in the Asia Pacific region at approximately 25% of the total number of enrolled subjects.

The primary objective of this trial is to determine the objective response rate (ORR) of pembrolizumab given as monotherapy. Beginning with screening, all imaging assessments will be submitted for central imaging vendor review and will be evaluated using RECIST 1.1 for determining eligibility and assessment of response. On study imaging assessments will be performed every 9 weeks (Q9W) calculated from the date of allocation and independent of treatment delays. RECIST 1.1 will be used by the site for treatment decisions until the first radiologic evidence of progressive disease (PD). Following the first radiologic evidence of PD by RECIST 1.1, treatment decisions may be made by the adaption of RECIST 1.1, as described in Section 7.1.4.1.6 termed immune-related RECIST (irRECIST) to account for the tumor response pattern observed with pembrolizumab treatment (eg, tumor flare). This was first described by Nishino, et al. 2013 [1], but is further modified for the PD1 program. For a clinically stable subject with first radiologic evidence of PD, it is at the discretion of the site investigator to continue treating the subject with pembrolizumab until PD is confirmed at least 4 weeks from the date of the first tumor imaging, suggesting PD per the site investigator. If radiologic PD is confirmed by the subsequent tumor imaging the subject should be discontinued from treatment unless, in the opinion of the investigator, the subject is achieving a clinically meaningful benefit; an exception to continue treatment may be considered, following consultation with the Sponsor.

Subjects will continue to be treated with pembrolizumab until PD is confirmed by irRECIST, unacceptable adverse events (AEs), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, administrative reasons, or the subject has received 35 trial treatments (approximately 2 years) with pembrolizumab. Subjects who discontinue treatment for reasons other than PD will have

post-treatment follow-up for disease status until PD, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed by telephone contact for overall survival (OS) until death, withdrawal of consent or the end of the study, whichever comes first.

Subjects who attain centrally confirmed complete response (CR) by 2 tumor imaging assessments at least 4 weeks apart and who have received at least 8 treatments (approximately 6 months) with pembrolizumab may discontinue treatment at the discretion of the investigator after receiving at least 2 treatments beyond the initial determination of a CR. Subjects who stop pembrolizumab after receiving 35 trial treatments for reasons other than PD or intolerability or who stopped after attaining a CR may be eligible for retreatment with up to an additional 17 treatments (approximately 1 year) after they have experienced radiographic PD. The decision to retreat will be at the discretion of the investigator only if no other cancer treatment was administered since the last dose of pembrolizumab, the subject still meets the parameters listed in the Inclusion and Exclusion Criteria, and the trial remains open.

Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (Section 12.6) After the end of treatment, each subject will be followed for 30 days for AE monitoring. Serious adverse events (SAEs) will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.

This study will be conducted in conformance with Good Clinical Practices (GCP).

The relationship between clinical outcome and an intratumoral immune-related Gene Expression Profile (GEP) and PD-L1 expression assayed by IHC will be assessed retrospectively in this study.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart - Section 6.0. Details of each procedure are provided in Section 7.0 – Trial Procedures.

2.2 Trial Diagram

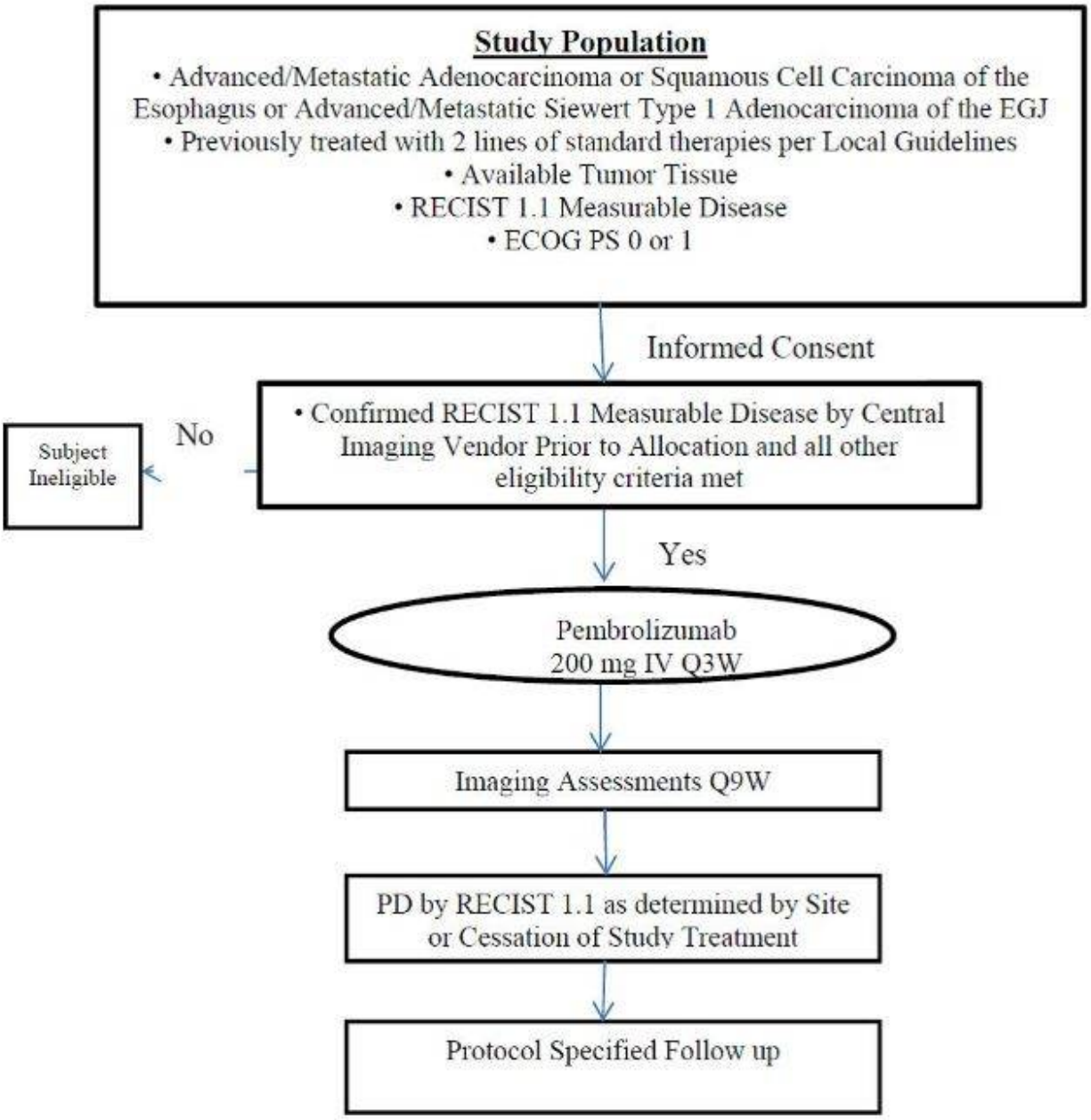

Figure 1 Trial Diagram

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

For subjects previously treated with 2 lines of standard therapy; and with advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus, or advanced/metastatic Siewert type I adenocarcinoma of the EGJ.

3.1 Primary Objective(s) & Hypothesis(es)

Objective: To evaluate the Objective Response Rate (ORR) per RECIST 1.1 assessed by central imaging vendor in all subjects and in subjects whose tumors are classified as GEP intermediate or high and in subjects whose tumors are classified as GEP high.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To evaluate safety and tolerability of pembrolizumab.

(2) **Objective**: To evaluate Duration of Response (DOR), and Progression-free Survival (PFS) per RECIST 1.1 assessed by central imaging vendor and Overall Survival (OS).

(3) **Objective**: To evaluate PD-L1 IHC in esophageal cancer for its utility to predict pembrolizumab efficacy.

3.3 Exploratory Objectives

(1) **Objective:** To explore the relationship between genetic variation and response to the treatment administered. Variation across the human genome will be analyzed for association with clinical data collected in this study.

(2) **Objective**: To evaluate ORR, DOR, and PFS per irRECIST assessed by central imaging vendor.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475.

4.1.1 Pharmaceutical and Therapeutic Background

Pembrolizumab (MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. KEYTRUDA® (pembrolizumab) has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following treatment with ipilumumab, a BRAF inhibitor, if BRAF V600 mutation positive.

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [2]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [3] [4] [5] [6] [7]. In particular, the presence of CD8+ T-cells and the

ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [8] [9]. The structure of murine PD-1 has been resolved [10]. 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 such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [8] [11] [12] [13]. 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 [14] [15]. 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 [16] [17]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells, as well as subsets of macrophages and dendritic cells [18]. 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 [19] [20] [21] [14]. 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 [14]. 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) [22].

4.1.2 Pre-clinical and Clinical Trials

4.1.2.1 Preclinical and Clinical Studies

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a mono-therapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a mono-therapy in models of squamous cell carcinoma, pancreatic carcinoma, MEL and colorectal carcinoma. Blockade of the PD-1 pathway effectively

promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-γ, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [23] [24] [25] [26] [27] [28]. Experiments have confirmed the in vivo efficacy of PD-1 blockade as a mono-therapy as well as in combination with chemotherapy in syngeneic mouse tumor models (see the Investigator's Brochure [IB]).

Clinical trials have demonstrated efficacy in subjects with advanced melanoma, non-small cell lung cancer, head and neck cancer, bladder cancer, Hodgkin's lymphoma, triple-negative breast cancer, and gastric adenocarcinoma. In addition, recent data demonstrates emerging evidence of single agent activity in additional tumor types such as mesothelioma, urothelial cancer, ovarian cancer, neuroendocrine carcinoma, and small cell lung cancer.

4.1.3 Ongoing Clinical Trials

Ongoing clinical trials of pembrolizumab are being conducted in advanced melanoma, non-small cell lung cancer, and a number of other advanced solid tumor indications and hematologic malignancies. For study details please refer to the IB.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Esophageal cancer is the sixth most common cancer in the world and is more prevalent in men than women. However, in the developing countries esophageal cancer is endemic and is the fourth most common cause of cancer deaths. Globally close to 480,000 cases occur annually with 53% of these cases arising in China [29] [30]. In the United States, in 2015, an estimated 15,980 esophageal cancers will be diagnosed and it is estimated that 15,590 people will eventually die of their disease [31]. In Japan, esophageal cancer is the sixth leading cause of cancer deaths, and in 2008, there were 11,746 deaths from esophageal carcinoma with male patients outnumbering female patients 6:1 [32]. Majority of the patients are diagnosed with advanced/metastatic cancer and in this setting, response to chemotherapeutic agents is poor. Given the high incidence and mortality worldwide and lack of good therapeutic options, esophageal cancer patients represent a high unmet need for drug development.

The incidence of esophageal cancer represents one of the widest variations with a 60-fold difference between high and low prevalence regions. High prevalence areas include Asia, Africa, and France where squamous esophageal cancers predominate [33]. A dramatic shift in the histology and location of upper gastrointestinal (GI) tumors has occurred over the past decades. In Western countries, the most common site of esophageal cancer is in the lower third of the esophagus, which often involves the EGJ [34] [35] [36]. Adenocarcinoma has been gradually increasing in men of all ethnic backgrounds and also in women. Squamous cell carcinoma (SCC) seems to be more sensitive to chemotherapy, chemoradiation, and radiation therapy (RT) than adenocarcinoma, but the long-term outcome is the same for both histologies [37] [38], thus emphasizing the need for better improved therapies in both histologies.

Phase III trials specifically designed for metastatic esophageal cancers have not been performed. The survival benefit of second-line chemotherapy compared to best supportive

care has been demonstrated in a small cohort of patients with lower esophageal or EGJ adenocarcinoma included in gastric adenocarcinoma phase III trials [39] [40]. In a randomized phase III study, second-line chemotherapy with irinotecan significantly prolonged OS compared to best supportive in patients with metastatic or locally advanced gastric or EGJ adenocarcinoma (n = 40) [39]. The study was closed prematurely due to poor accrual. Median survival was 4 months in the irinotecan arm compared to 2.4 months in the best supportive care only arm.

In a recent open-label, multicenter, phase III, randomized trial, the addition of docetaxel for active symptom control was associated with a survival benefit for patients with advanced, histologically confirmed adenocarcinoma of the esophagus, EGJ junction, or stomach that had progressed on or within 6 months of treatment with combination chemotherapy with platinum and fluoropyrimidine [40]. In this study, patients (n = 168) with an ECOG PS score of 0-2 were randomly assigned to receive docetaxel plus active symptom control or active symptom control alone. After a median follow-up of 12 months, the median OS was 5.2 months for patients with the docetaxel group compared to 3.6 months for those in the active symptom control group (P = .01). Docetaxel was associated with higher incidence of Grade 3-4 neutropenia, infection, and febrile neutropenia. However, disease-specific, health-related quality of life measures also showed benefits for docetaxel in reducing dysphagia and abdominal pain.

In a phase II study by Ilson et al. [41], one hundred and two patients with advanced esophageal cancer were treated with paclitaxel 80 mg/m^2 weekly. Sixty-six patients had adenocarcinoma (66%) and 65 patients (68%) had no prior chemotherapy. In terms of responses, in patients without prior chemotherapy, partial responses (PR) were seen in 10 patients (15%, 95% confidence interval [CI] 6% to 24%), with comparable response in adenocarcinoma (8/50, 16%) and squamous carcinoma (2/15, 13%). Limited response was seen in patients with prior chemotherapy, in the second line setting (1/21, 5%). Therapy was well tolerated with minimal hematologic or Grade 3 or 4 toxicity.

Docetaxel, paclitaxel, and irinotecan are included as options for second-line therapy for patients with locally advanced or metastatic disease. Other regimens included in the guidelines for patients with locally advanced or metastatic disease are derived from the gastric adenocarcinoma phase III trials that have included patients with lower esophageal and/or EGJ cancer. The use of gefitinib as a second-line treatment for unselected patients does not improve overall survival [42]. There are no standard guidelines for third-line treatment of patients with esophageal cancer as the efficacy of chemotherapy is expected to be very poor in this setting (<5%). In addition, patient PS frequently declines at this stage and patients are managed by addressing symptoms from tumor burden.

KEYNOTE-028, a nonrandomized, multicohort, Phase 1b trial of pembrolizumab for PD-L1$^+$ advanced solid tumors includes esophageal cancer patients. Key eligibility criteria for this cohort included SCC or adenocarcinoma of the esophagus or EGJ junction, measurable disease, PD-L1 expression in ≥1% of cells in tumor nests or PD-L1$^+$ stromal bands determined centrally by IHC, failure of standard therapy, ECOG PS 0-1, and no autoimmune disease. Pembrolizumab 10 mg/kg is being given every 2 weeks for up to 2 years or until confirmed progression. Of the 90 pts with esophageal cancer who were screened, 37 (44.6%)

had PD-L1$^+$ tumors. Of the 23 patients treated between Mar and Dec 2014, 83% were men and median age was 65 years. Histology was squamous in 17 pts (73.9%), adenocarcinoma in 5 pts (21.7%), and mucoepidermoid in 1 patient (4.3%). Eighty-seven percent of pts received ≥2 prior therapies for metastatic disease; all pts received ≥1 platinum-based therapy. Nine patients (39.1%) experienced drug-related adverse events (DRAEs), including 4 (17.4%) who experienced Grade 3 DRAEs. There were no Grade 4 DRAEs, and no pts died or discontinued due to a DRAE. Tumor shrinkage was seen in 52% of patients and ORR was 30.4% (n = 7; 5 squamous (29.4%) and 2 adenocarcinoma (40.0%)); best response was stable disease (SD) in 13% (n = 3; 2 squamous and 1 adenocarcinoma) and progressive disease in 59% (n = 13). Six patients still remain on therapy. Median time to response is 16 weeks (Range: 7.9-36.0 weeks) and median duration of response is 40.0 weeks (Range: 0.1+ to 40.0 weeks) for the patients in the esophageal cohort.

Thus, pembrolizumab has an acceptable safety profile and provides highly promising antitumor activity in patients with heavily pretreated, advanced esophageal carcinoma. The high unmet need, lack of efficacious approved therapies and the above data with pembrolizumab strongly support further development of this drug in patients with esophageal squamous and adenocarcinoma.

4.2.2 Rationale for Evaluating Gene Expression Profile (GEP) in Esophageal Cancer and Implications for Future Studies

Gene expression signatures measuring mRNA for key immune-related genes have been confirmed to be associated with clinical benefit to pembrolizumab treatment in melanoma, head & neck, and gastric cancers [43] [44] [45], as well as in the esophageal cancer cohort in KN028. The predominant pattern indicates that tumors with relatively low expression of these genes have a low probability of response to pembrolizumab. Using data from KN012 and KN028, a GEP combining expression levels of 18 genes into a scalar score has been developed and 2 cut-offs on that score which divide tumors into "low," "intermediate," and "high," were determined using data from KN028, KN012, and KN052. The lower cut-off was defined to favor sensitivity in capturing responders by centrally reviewed RECIST and the higher cut-off was selected to enrich for higher response rates at potentially some cost in sensitivity. In this study, in addition to estimating ORR in all subjects, ORR will be estimated in 2 additional patient subgroups, defined by the GEP categories: (1) subjects whose tumors are classified as "intermediate or high" and (2) subjects whose tumors are classified as "high."

4.2.3 Rationale for Dose Selection/Regimen

The planned dose of pembrolizumab for this trial is 200 mg Q3W. Based on the totality of data generated in the KEYTRUDA® development program, 200 mg Q3W is the appropriate dose of pembrolizumab across all indications, regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W)
- Clinical data showing meaningful improvement in benefit-risk, including overall survival at 200 mg Q3W across multiple indications

- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically based pharmacokinetic [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 subjects were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment-naïve, previously treated, PD-L1 enriched and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 B2, KN001 D, KN002, KN010 and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 B3, KN001 F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5 to 7.5 fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-/exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin lymphoma, confirming 200 mg Q3W as the appropriate dose, independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

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

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

4.2.4 Rationale for Endpoints

4.2.4.1 Efficacy Endpoints

4.2.4.1.1 Primary Efficacy Endpoints

The primary efficacy objective of this study is to evaluate ORR of pembrolizumab in subjects with previously treated advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ. Objective response rate (ORR) will be evaluated per RECIST 1.1 assessed by the central imaging vendor.

4.2.4.1.2 Secondary Efficacy Endpoints

The secondary efficacy objectives of this study are to evaluate DOR, and PFS per RECIST 1.1 assessed by central imaging vendor and OS.

4.2.4.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy objectives of this study are to evaluate ORR, DOR, and PFS per irRECIST assessed by the central imaging vendor and OS. Intratumoral immune-related GEP and PD-L1 IHC in esophageal cancer will also be evaluated for their utility to predict pembrolizumab efficacy.

4.2.4.2 Immune-related RECIST (irRECIST)

RECIST 1.1 will be adapted to account for the unique tumor response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST 1.1 may, thus, not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab.

Based on an analysis of patients with melanoma enrolled in Keynote-001, 7 % of evaluable patients experienced delayed or early tumor pseudoprogression. Of note, patients who had progressive disease by RECIST 1.1 but not by immune related Response Criteria had longer OS than patients with progressive disease by both criteria. Additionally, the data suggest that RECIST 1.1 may underestimate the benefit of pembrolizumab in approximately 15% of patients. These findings support the need to apply a modification to RECIST 1.1 that takes into account the unique patterns of atypical response in immunotherapy and enable treatment beyond initial radiographic progression.

Immune-related RECIST (irRECIST) is RECIST 1.1 adapted to account for the unique tumor response seen with immuno-therapeutics as described in Nishino et al., CCR 2013 [1]. The assessment of unidimensional target lesions and response categories per irRECIST are identical to RECIST 1.1. However, Merck has implemented an adaptation related to new lesions, non-target and tumor burden assessment in order to confirm radiographic progression. irRECIST will be used by local site investigators to assess tumor response and progression, and make treatment decisions, as well as by central imaging vendor in support of exploratory efficacy endpoints.

4.2.4.3 Safety Endpoints

The safety objective of this trial is to characterize the safety and tolerability of pembrolizumab in subjects with advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ who have progressed on 2 lines of previous therapy. The primary safety analysis will be based on subjects who experienced toxicities as defined by CTCAE, v4.0 (Section 12.6).

The attribution to drug, time-of-onset, duration of the event, its resolution, and any concomitant medications administered will be recorded. Adverse event will be analyzed, including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes. Furthermore, specific events will be collected and designated as events of clinical interest (ECIs), as described in Section 7.2.3.2.

4.2.4.4 Biomarker Research

4.2.4.4.1 Biomarker Research for Primary Objectives

Immune-related Gene Expression Profile (GEP)

Intratumoral expression levels of 18 genes will be analyzed and the GEP score determined using the NanoString nCounter Analysis System. Two pre-specified, analytically validated, cut-offs on the GEP will be used to divide tumors into "low," "intermediate," and "high" for the GEP. ORR will be estimated in the set of subjects whose tumors are "intermediate or high" and in the set of subjects whose tumors are "high," and these ORRs will be compared to the All-comers Population.

Tumor PD-L1 Expression

In the pembrolizumab PN001 and PN012 studies, PD-L1 immunohistochemistry (IHC) has successfully been used as a biomarker in the NSCLC and head and neck cancer cohort, respectively, to enrich for a subpopulation with high response to pembrolizumab [46]. Therefore, the relationship between PD-L1 expression in esophageal tumor tissue and response to treatment with pembrolizumab will be evaluated. PD-L1 expression in tumor cells and inflammatory cells within pre-treatment tumor tissue samples will be characterized by immunohistochemistry (IHC) using a combined positive score and a cut-off of 1% (1% CPS) and retrospectively tested for association with response to pembrolizumab.

4.2.4.4.2 Biomarker Research for Exploratory Objectives

Additional biomarker research to identify factors important for pembrolizumab (MK-3475) therapy may also be pursued. For example, tumor and blood samples (including serum and plasma) from this study may undergo proteomic, genomic, metabolomic and transcriptional analyses. Additional research may evaluate factors important for predicting responsiveness or resistance to pembrolizumab therapy and other immunologic targets.

Assays may include but are not be limited to:

Transcriptional Analyses

In addition to examining an immune-related GEP described above, global messenger RNA profiling will also be pursued. Expression of individual genes related to the immune system may also be evaluated such as immune signatures and critical cytokines (eg, IL-10). MicroRNA profiling may also be pursued in serum samples.

Proteomic analysis

In addition to expression on the tumor tissue, PD-L1 can be shed from tumor and released into the blood. Enzyme-linked immunoassay can measure PD-L1 in serum and correlate this expression with response to pembrolizumab (MK-3475) therapy, as well as levels of PD-L1

IHC or protein in the tumor. Blood would be a less invasive compartment compared to tumor from which to measure PD-L1 protein biomarker. In addition to this specific protein biomarker, both tissue and blood derivatives can be subjected to proteomic profiling studies using a variety of platforms that could include and are not limited to immunoassay, liquid chromatography/mass spectrometry. This approach could identify novel protein biomarkers that could aid in patient selection for pembrolizumab (MK-3475) therapy.

Gene Analyses

The application of new technologies, such as next generation sequencing, has provided scientists the opportunity to define certain tumor types at the genetic level as being 'hypermutated' or it can detect the presence of specific T-cell clones within the tumor microenvironment. There is a potential that this hypermutated state and the detection of increased T-cell clonality may correlate with response to pembrolizumab therapy, and/or that the converse, "hypomutated" state or lack of T-cells clones may correlate with non-response.

Planned Genetic Analysis

Understanding genetic determinants of drug response is an important endeavor during medical research. This research will evaluate whether genetic variation within a clinical trial population correlates with response to the treatment(s) under evaluation. If genetic variation is found to predict efficacy or adverse events, the data might inform optimal use of therapies in the patient population. This research contributes to understanding genetic determinants of efficacy and safety associated with the treatments in this study.

4.2.4.5 Future Biomedical Research

The Sponsor will conduct Future Biomedical Research on specimens collected for future biomedical research during this clinical trial. This research may include genetic analyses (DNA), gene expression profiling (RNA), proteomics, metabolomics (serum, plasma) and/or the measurement of other analytes.

Such research is for biomarker testing to address emergent questions not described elsewhere in the protocol (as part of the main trial) and will only be conducted on specimens from appropriately consented subjects. The objective of collecting specimens for Future Biomedical Research is to explore and identify biomarkers that inform the scientific understanding of diseases and/or their therapeutic treatments. The overarching goal is to use such information to develop safer, more effective drugs/vaccines, and/or to ensure that subjects receive the correct dose of the correct drug/vaccine at the correct time. The details of this Future Biomedical Research sub-trial are presented in Section 12.2 - Collection and Management of Specimens for Future Biomedical Research. Additional informational material for institutional review boards/ethics committees (IRBs/ERCs) and investigational site staff is provided in Section 12.3.

4.3 Benefit/Risk

Subjects in clinical trials generally cannot expect to receive direct benefit from treatment during participation, as clinical trials are designed to provide information about the safety and effectiveness of an investigational medicine.

Additional details regarding specific benefits and risks for subjects participating in this clinical trial may be found in the accompanying IB and Informed Consent (ICF) documents.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Male/Female subjects with advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ will be enrolled in this trial.

5.1.2 Subject Inclusion Criteria

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

1. Be willing and able to provide documented informed consent for the trial. The subject may also provide consent for Future Biomedical Research. However, the subject may participate in the main trial without participating in Future Biomedical Research.
2. Be ≥ 18 years of age on the day of providing documented informed consent.
3. Have an ECOG performance status of 0 or 1.
4. Have a life expectancy greater than 3 months.
5. Have histologically proven advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ (defined as adenocarcinomas of the lower esophagus with the center located within 1cm to 5cm above the anatomic EGJ).
    a. Subjects with advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ with HER-2/neu negative tumors are eligible. Subjects with HER2/neu positive tumors or those with an unknown tumor status, need to match the following:
        i. If HER2/neu positive, subject must have documentation of disease progression on treatment containing trastuzumab.
        ii. Subjects with unknown status must have their HER2/neu status determined locally. If HER2/neu negative, the subject will be eligible. If HER2/neu positive, the subject must have documentation of disease progression on treatment containing trastuzumab.
6. Have experienced documented objective radiographic or clinical disease progression on 2 previous lines of standard therapy. This study will only include third-line subjects. Third-line subjects are defined as those who have progressed during or after receiving at least 1 dose of standard therapy given in a second-line setting.
    a. Disease progression should be confirmed by CT scan. In certain situations, clinical evidence of disease progression such as any new or worsening

malignant effusion (documented by ultrasound) and confirmation by pathologic criteria (histology and/or cytology) is acceptable.

b. Treatment with curative intent, including neoadjuvant/adjuvant treatment, given as chemotherapy or chemoradiotherapy, using standard-of-care agents; or definitive chemoradiation, will count as a line of therapy if disease progression occurs during treatment or within 6 months of cessation of treatment.

c. Dose reduction and/or switching of one or more first or second line agents due to toxicity/intolerability as deemed clinically appropriate by the investigator will not constitute a new line of therapy.

7. Have measurable disease based on RECIST 1.1, as determined by central imaging vendor assessment. A lesion(s) situated in a previously irradiated area can be considered a target lesion(s) if progression has been demonstrated and the lesion(s) is considered measurable per RECIST 1.1 criteria.

   Note: The same image acquisition and processing parameters should be used throughout the study for a given subject.

8. Provide either a newly obtained or archival tissue sample for intratumoral immune-related GEP and for PD-L1 by immunohistochemistry analysis. Newly-obtained tissue is preferred. Formalin-fixed, paraffin-embedded (FFPE) block specimens are preferred to slides. Repeat samples will be required if a newly-obtained tissue sample is not adequate and archival tissue is not provided or if a newly obtained tissue sample is not provided and an archival sample is also not adequate. For purposes of this study, newly-obtained tissue refers to tissue that was collected between the last line of therapy and the first dose of study medication.

   a. Central laboratory confirmation of tumor tissue sample adequacy is required prior to subject enrollment in the study. If multiple tumor samples are submitted, at least one of the samples must be confirmed to be adequate by the central laboratory prior to subject being enrolled.

   b. Subjects from whom newly-obtained samples cannot be obtained (eg, inaccessible or subject safety concern) an archived specimen may be submitted.

   c. If newly obtained tissue is provided and an archived tissue sample is available, it should also be provided to support evaluation of the clinical utility of immune-related GEP assessment and PD-L1 analysis by immunohistochemistry analysis in newly obtained versus archived tissue samples; however, a subject will not be excluded from participating if he/she has provided newly obtained tissue and an archived tissue sample is not available or is insufficient for analysis.

9. Female subjects of childbearing potential must have a negative urine or serum pregnancy test 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.

10. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

11. Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception, as outlined in Section 5.7.2 – Contraception and not to donate sperm starting with the first dose of study therapy through 120 days after the last dose of study therapy.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

12. Demonstrated 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 Lab Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /µL
Platelets	≥100,000 / µL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency within 7 days.
Renal	
Creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 × institutional ULN
Hepatic	
Total bilirubin	≤ 1.5 × ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 × ULN **OR** ≤ 5 × ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 × 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 × 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.	

5.1.3 Subject Exclusion Criteria

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

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

   Note: Subjects who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks since the last dose of the previous investigational agent or device.

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

3. 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. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

4. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they have stable brain metastases for at least four weeks prior to the first dose of trial treatment; also, any neurologic symptoms must have returned to baseline See Section 7.1.4.1.1 for additional details.

5. Has received prior anti-cancer mAb, chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to first dose of trial treatment or who has not recovered (ie, ≥ Grade 1 at baseline) from adverse events due to a previously administered agent. The specified 2-week period between last dose of prior therapy and first dose of pembrolizumab is the minimum amount of time allowed. Subjects may not receive pembrolizumab within less than 2 weeks from the last dose of a prior therapy. However, a period of more than 2 weeks may be used if indicated both clinically and due to concern between possible negative interactions between prior therapy and pembrolizumab therapy.

   Note: Subjects with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study

   Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

6. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent, or has previously participated in Merck pembrolizumab (MK-3475) clinical trials.

7. Has a known additional malignancy that progressed or required active treatment within the last 5 years. Exceptions include basal cell carcinoma of the skin, squamous

cell carcinoma of the skin that has undergone potentially curative therapy, in situ cervical cancer, and in situ or intramucosal pharyngeal cancer.

8. Has received a live vaccine within 30 days of the first dose of trial treatment.

   Note: The killed virus vaccines used for seasonal influenza vaccines for injection are allowed; however intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines, and are not allowed.

9. 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.
10. Has a known history of Human Immunodeficiency Virus (HIV) infection. See Germany-specific requirements in Appendix 12.8
11. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). See Germany-specific requirements in Appendix 12.8
12. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
13. Has an active infection requiring systemic therapy.
14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment.

5.2 Trial Treatment(s)

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

Table 2 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen	Use
Pembrolizumab (MK-3475)	200 mg	Q3W	IV Infusion	Day 1 of each 21-day cycle	Experimental

Trial treatment for Cycle 1 should begin within 3 days of allocation. However, every effort should be made to begin trial treatment on the day of allocation

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

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection (Preparation)

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background & Rationale. There are no specific calculations or evaluations required to be performed in order to administer the proper dose to each subject.

5.2.1.2 Dose Modification and Toxicity Management Guidelines for Pembrolizumab

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

Adverse events associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than 1 body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical trial data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

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

General instructions:

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

For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.

For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

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

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	Initiate insulin replacement therapy for participants with T1DM Administer anti-hyperglycemic in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements, as clinically indicated.	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	Monitor for signs and symptoms of thyroid disorders.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Nephritis and Renal dysfunction	Grade 2	Withhold	Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	Based on type and severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and are not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

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

NOTE:

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

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

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

Table 4 Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

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

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Subject is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs, such as medical / surgical events or logistical reasons not related to study therapy. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.2.2 Timing of Dose Administration

Cycle 1 Day 1 treatment with pembrolizumab should begin on the day of allocation, but no later than 3 days from the date the subject is allocated to study treatment. However, every effort should be made to begin trial treatment on the day of allocation.

For all additional cycles of pembrolizumab, treatment may be administered up to 3 days before or 3 days after the scheduled Day 1 of each cycle due to administrative reasons per the investigator’s judgment.

All study treatments will begin on Day 1 of each cycle after all pre-dose study procedures and assessments have been completed as detailed on the Trial Flow Chart –Section 6.0.

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

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

All trial treatments will be administered on an outpatient basis.

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

Treatment allocation will occur centrally using an interactive voice response system/integrated web response system (IVRS/IWRS). All enrolled subjects will be allocated to receive pembrolizumab 200 mg IV Q3W as monotherapy in an unblinded fashion.

5.4 Stratification

No stratification based on age, sex or other characteristics will be used in this trial.

5.5 Concomitant Medications/Vaccinations

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor Clinical Director. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the investigator, the Sponsor and the subject.

5.5.1 Acceptable Concomitant Medications

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

All medications received within 30 days before the first dose of trial treatment and within 30 days after the last dose of trial treatment should be recorded. Medications administered more than 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs, as defined in Section 7.2.3.2.

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy not specified in this protocol
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy for tumor control
  - Note: Radiation therapy to a symptomatic solitary lesion may be allowed after consultation with Sponsor.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chickenpox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. The killed virus vaccines used for seasonal influenza vaccines for injection are allowed; however live attenuated intranasal influenza vaccines (eg, FluMist®) are not allowed.

  Note: Any licensed COVID 19 vaccine (including for Emergency use) in a particular country is allowed in the study as long as they are mRNA vaccines, adenoviral vaccines, or inactivated vaccines. These vaccines will be treated just as any other concomitant therapy.

  Investigational vaccines (i.e., those not licensed or approved for Emergency Use) are not allowed.
- Glucocorticoids (inhaled steroids as part of a stable regimen for the treatment of asthma/chronic obstructive pulmonary disease [COPD] or topical steroids for skin conditions are permitted) for any purpose other than to modulate symptoms from an adverse event. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.
  - Note: Use of prophylactic corticosteroids to avoid allergic reactions (eg, IV contrast dye) is permitted.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be discontinued from study treatment. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describe other medications that are prohibited in this trial.

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

5.6 Rescue Medications & Supportive Care

No rescue or supportive medications are specified to be used in this trial.

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

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

5.7.2 Contraception

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

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

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

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

OR

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

OR

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

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

1) practice abstinence[†] from heterosexual activity;

OR

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

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

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

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

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

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

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

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

Below are the required contraceptions for countries where the health authority requests compliance with the Clinical Trial Facilitation Group (CTFG) Guidance:

Subjects should use birth control methods that can achieve a failure rate of less than 1% per year when used consistently and correctly and are considered as highly effective birth control methods. Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Injectable

- Implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised partner
- Sexual abstinence

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study treatment. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is a serious adverse experience (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to the Sponsor and followed as described in Section 7.2.2 (Reporting of Pregnancy and Lactation to the Sponsor).

5.7.4 Use in Nursing Women

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

5.8 Subject Withdrawal/Discontinuation Criteria

5.8.1 Discontinuation of Treatment

Discontinuation of treatment does not represent withdrawal from the trial.

As certain data on clinical events beyond treatment discontinuation may be important to the study, they must be collected through the subject's last scheduled follow-up, even if the subject has discontinued treatment. Therefore, all subjects who discontinue trial treatment prior to completion of the treatment will still continue to participate in the trial as specified in Withdrawal/Discontinuation.

Subjects may discontinue treatment at any time for any reason or be dropped from treatment at the discretion of the investigator should any untoward effect occur. In addition, a subject may be discontinued from treatment by the investigator or the Sponsor if treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at treatment discontinuation are provided in Section 7.1.5 – Other Procedures.

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

- The subject or subject's legally acceptable representative requests to discontinue treatment.
- Confirmed radiographic PD (irPD) as outlined in Section 7.1.4.1.2 (except if Sponsor approves treatment continuation).
- Unacceptable adverse events as described in Section 7.2.
- Investigator's decision to withdraw the subject
- The subject has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, placed the subject at unnecessary risk from continued administration of study drug.
- Non-compliance with trial treatment or procedure requirements.
- The subject has a confirmed positive pregnancy test
- Administrative reasons.
- Completed 35 cycles of pembrolizumab.
    - Note: 35 treatments (approx. 2 years) are calculated from the first dose. Subjects who stop pembrolizumab after receiving 35 treatments may be eligible for retreatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.6.2.1. Subjects may be retreated in the Second Course Phase with up to 17 (approx. 1 year) additional trial treatments.

The End of Treatment and Follow-up visit procedures are listed in Section 7.1.6.3 and the Trial Flow Charts in Section 6.0. After the end of treatment, each subject will be followed for a minimum of 30 days for adverse event monitoring (serious adverse events will be collected for up to 90 days after the end of treatment as described in Section 7.2.3). Subjects who discontinue for reasons other than PD will have post-treatment follow-up for disease status until PD, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented PD each subject will be followed (e.g. by telephone) for OS until death, withdrawal of consent, or the end of the study, whichever occurs.

Discontinuation from treatment is "permanent." Once a subject is discontinued, he/she shall not be allowed to restart treatment unless the conditions in Section 5.8.2 – Discontinuation of Study Therapy after CR are met.

5.8.2 Discontinuation of Study Therapy After Complete Response

Discontinuation of treatment may be considered for subjects who have attained a centrally confirmed CR, and who have received at least 8 cycles (approx. 6 months) of pembrolizumab, and who received at least 2 cycles of pembrolizumab beyond the date when initial CR was declared. Subjects who discontinue pembrolizumab therapy due to CR and

then experience radiographic disease progression may be eligible for up to 17 additional cycles (approx. 1 year) of pembrolizumab in the Second Course Phase, at the discretion of the investigator if:

- No cancer treatment was administered since the last dose of pembrolizumab
- The subject meets the parameters listed in the Inclusion/Exclusion criteria
- The trial is ongoing

Subjects will resume pembrolizumab therapy at the same dose level and on the same schedule as those at the time of initial discontinuation. Additional details are provided in Section 7.1.6.2. Response or progression in this Second Course Phase will not count towards the primary endpoint in this trial.

5.8.3 Withdrawal from the Trial

Subjects may withdraw from the trial at any time for any reason. If a subject withdraws from the trial, they will no longer receive treatment or be followed at scheduled protocol visits.

A subject must be withdrawn from the trial if:

- The subject or subject's legally acceptable representative withdraws consent from the trial.
- The subject is lost to follow-up.

Specific details regarding procedures to be performed at the time of withdrawal from the trial including specific details regarding withdrawal from Future Biomedical Research are outlined in Section 7.1.5 – Other Procedures.

5.9 Subject Replacement Strategy

A subject who discontinues from the trial will not be replaced.

5.10 Beginning and End of the Trial

The overall trial begins when the first subject provides the documented informed consent form. The overall trial ends when the last subject completes the last study-related phone-call or visit, discontinues from the trial or is lost to follow-up (i.e. the subject is unable to be contacted by the investigator).

5.11 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete.
2. Poor adherence to protocol and regulatory requirements.
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects.
4. Plans to modify or discontinue the development of the study drug.

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

5. Any medical, safety, regulatory, administrative, or other reasons consistent with applicable laws, regulations, and GCP.

   In the event the Sponsor prematurely terminates a particular study site, the Sponsor will promptly notify that study site's IRB/IEC.

6.0 TRIAL FLOW CHART

6.1 Initial Treatment Phase with Pembrolizumab

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6	7	8 and Beyond	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up[a]
										At time of Discon	30 Days Post-discon	Every 9 Weeks Post-discon	Every 12 Weeks
Scheduling Window (Days)[b]	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	± 7	± 7
Administrative Procedures													
Informed Consent	X												
Informed Consent for Future Biomedical Research (optional)	X												
Inclusion/Exclusion Criteria	X												
Subject Identification Card	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X		
Post-study Anticancer Therapy Status												X	X
Survival Status		←	-	-	-	-	-	-	-	-	-	-	→ X
Clinical Procedures/Assessments													
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X[q]	
Full Physical Examination	X									X			
Directed Physical Examination		X	X	X	X	X	X	X	X				
Height, Weight, and Vital Signs (T,P,RR,BP)[c]	X	X	X	X	X	X	X	X	X	X			
12-Lead Electrocardiogram	X												
ECOG Performance Status	X[r]	X	X	X	X	X	X	X	X	X			
Pembrolizumab Administration		X[b]	X	X	X	X	X	X	X				
LOCAL Laboratory Assessments													
Pregnancy Test[d]	X[d]		X	X	X	X	X	X	X		X		
PT/INR and aPTT	X[e]												
CBC with Differential[f]	X[e]		X	X	X	X	X		X[f]	X	X		
Chemistry Panel[f]	X[e]		X	X	X	X	X		X[f]	X	X		
Urinalysis[g]	X[e]		X		X		X		X	X			
T3, FT4, and TSH[g]	X[e]		X		X		X		X	X	X		

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6	7	8 and Beyond	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up[a]
										At time of Discon	30 Days Post-discon	Every 9 Weeks Post-discon	Every 12 Weeks
CENTRAL Laboratory Assessments													
Pembrolizumab Pharmacokinetics[h, i]		X[i]	X		X		X		X[h,i]				
Pembrolizumab Anti-Drug Antibodies (ADA)[h]		X	X		X		X		X[h]				
Blood for Genetics[j]		X											
Whole Blood for Biomarker Studies (serum and plasma)[k]		X											
Whole Blood for Correlative Studies (RNA and DNA)[k]		X	X	X						X			
Tumor Tissue Collection													
Newly Obtained Tumor Tissue[m]	X												
Archival Tumor Tissue[n]	X												
Efficacy Measurements													
Tumor Imaging[o]	X[l]	X[o] ← →								X[p]		X	

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6	7	8 and Beyond	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up[a]
										At time of Discon	30 Days Post-discon	Every 9 Weeks Post-discon	Every 12 Weeks

a. After subjects who experience confirmed site-assessed PD or who start a new anti-cancer therapy, each subject will be contacted by telephone for survival approximately every 12 weeks until the subject withdraws consent, is lost to follow-up, death, or the trial ends. In addition, upon Sponsor request, subjects may be contacted for survival status at any time during the course of the trial.
b. Cycle 1 treatment must be given within 3 days of allocation. The window for each visit is ± 3 days unless otherwise noted.
c. Height will be measured at Visit 1 only.
d. For women of reproductive potential, a urine or serum pregnancy test should be performed within 72 hours prior to Day 1 of each treatment cycle and 30 days post treatment. A serum test can be done if urine is not appropriate. Additionally, if urine test is positive or is not evaluable, a serum test is required. Subjects must be excluded/discontinued in the event of a positive test results. Pregnancy tests (serum and/or urine tests) should be repeated if required by local guidelines.
e. Laboratory tests for screening and determining eligibility are to be performed within10 days prior to the first dose of trial treatment.
f. CBC (Hematology) with diff and Chemistry to be performed every cycle.
g. UA and thyroid function tests will be performed every other cycle. T3 is preferred; if not available free T3 may be tested.
h. Both PK and Anti-pembrolizumab Samples: pre-dose (trough) PK and anti-pembrolizumab antibody samples will be collected at within 24 hours before infusion at Cycles 1, 2, 4, 6, 8 and every 4 cycles thereafter.
i. PK Samples: additional post-dose (peak) PK samples will be drawn within 30 minutes after end of pembrolizumab infusion at Cycles 1 and 8. An additional single PK sample should be drawn at; 24 hours (Day 2), between 72 and 168 hours (Day 4-8) and 336 hours (Day 15) after Cycle 1 dosing.
j. Details for collection can be found in Section 7.1.3 Laboratory Procedure/Assessments
k. Whole blood samples for correlative studies (DNA and RNA) should be collected pre-dose on Day 1 of Cycle 1, Cycle 2, and Cycle 3, and at treatment discontinuation if subject discontinues prior to Cycle 3. Whole blood for Biomarker Samples (plasma and serum) to be collected pre-dose on Day 1 of Cycle 1 only.
l. Screening tumor imaging will be performed within 28 days prior to allocation. Confirmation of baseline measurable disease per RECIST 1.1 by the central imaging vendor is required prior to subject allocation. . Confirmation of baseline measurable disease per RECIST 1.1 by the central imaging vendor is required prior to subject allocation.
m. Newly-obtained tissue is preferred; FFPE block specimens are preferred to slides. Newly obtained tissue is defined as no intervening treatment (local or systemic) involving the site of tissue biopsy once tissue biopsy is obtained and time of study enrollment.
n. Archival tumor tissue will also be requested (where available) to assess the clinical utility of PD-L1 and immune-related GEP assessment in newly obtained vs. archived tissue samples.
o. The first on-study imaging time point will be performed at 9 weeks (63 days ± 7 days) calculated from the date of allocation and will continue to be performed Q9W (63 days ± 7 days), or earlier if clinically indicated.
p. In order to follow irRECIST criteria, if a subject is discontinued from study therapy prior to PD being confirmed at the site then that subject should have tumor imaging performed at the time of treatment discontinuation. If previous tumor imaging was obtained within 4 weeks prior to the date of discontinuation, then additional tumor imaging at treatment discontinuation is not required.
q. SAEs will be followed through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier

6.2 Second Course Phase with Pembrolizumab (Retreatment)

Trial Period:	Treatment Cycles								End of Treatment	Post-treatment		
Treatment Cycle/Title:	1	2	3	4	5	6	7	8 and Beyond	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up[a]
									At time of Discon	30 Days Post-discon	Every 9 Weeks Post-discon	Every 12 Weeks
Scheduling Window (Days)[b]		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	± 7	± 7
Administrative Procedures												
Eligibility Criteria	X											
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X		
Clinical Procedures/Assessments												
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X[h]	
Full Physical Examination	X								X			
Directed Physical Examination		X	X	X	X	X	X	X				
Weight, and Vital Signs (T,P,RR,BP)	X	X	X	X	X	X	X	X	X			
ECOG Performance Status	X[d]	X	X	X	X	X	X	X	X			
Post-study anticancer Therapy Status											X	X
Survival Status	←	-	-	-	-	-	-	-	-	-	-	X
Trial Treatment Administration												
Pembrolizumab	X	X	X	X	X	X	X	X				
LOCAL Laboratory Assessments												
Pregnancy Test[c]	X		X	X	X	X	X	X		X		
PT/INR and aPTT	X[d]											
CBC with Differential[e]	X[d]	X	X	X	X	X		X	X	X		
Chemistry Panel[e]	X[d]	X	X	X	X	X		X	X	X		
Urinalysis[e]	X[d]		X		X		X	X	X	X		
T3, FT4, and TSH [e]	X[d]		X		X		X	X	X			

Trial Period:	Treatment Cycles								End of Treatment	Post-treatment		
Treatment Cycle/Title:	1	2	3	4	5	6	7	8 and Beyond	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up[a]
									At time of Discon	30 Days Post-discon	Every 9 Weeks Post-discon	Every 12 Weeks
Efficacy Measurements												
Tumor Imaging	X[f]	X[f] ⟷							X[g]		X	

a. After the start of new anti-cancer treatment or PD, each subject will be contacted by telephone for survival approximately every 12 weeks until the subject withdraws consent, is lost to follow-up, death, or the retreatment phase ends. In addition, upon Sponsor request, subjects may be contacted for survival status at any time during the course of the trial.
b. In general, the window for each visit is ± 3 days unless otherwise noted.
c. For women of reproductive potential, a urine or serum pregnancy test should be performed within 72 hours prior to Day 1 of each treatment cycle and 30 days post treatment. A serum test can be done if urine is not appropriate. Additionally, if urine test is positive or is not evaluable, a serum test is required. Subjects must be excluded/discontinued in the event of a positive test results. Pregnancy tests (serum and/or urine tests) should be repeated if required by local guidelines.
d. Laboratory tests and ECOG PS for determining eligibility are to be performed within 10 days prior to the first retreatment dose of pembrolizumab
e. After Cycle 1, lab samples can be collected up to 72 hours prior to the scheduled time point. CBC (Hematology) and Chemistry, to be performed every cycle. UA and Thyroid function tests to be performed every other cycle. T3 is preferred; if not available free T3 may be tested.
f. Tumor imaging should be performed within 28 days prior to restarting treatment with pembrolizumab and continue to be performed every 9 weeks (63 ± 7 days) after the first dose of retreatment, or more frequently if clinically indicated. At sites where the local regulatory body and/or IRB/ERC will not permit a second tumor imaging within a 28-day period, an already available imaging scan obtained within 28 days prior to first dose may be used with the approval of the Sponsor Clinical Director. For all subjects, already available imaging scans performed as part of routine clinical management are acceptable if they are of diagnostic quality and performed within the acceptable timeframe.
g. Tumor imaging should be performed at the time of treatment discontinuation (ie, date of discontinuation ± 4 week window). If previous tumor imaging was obtained within 4 weeks prior to the date of discontinuation, then additional tumor imaging at treatment discontinuation isn't mandatory.
h. SAEs will be followed through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject or each subject's legally acceptable representative prior to participating in a clinical trial or Future Biomedical Research.

7.1.1.1.1 General Informed Consent

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

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

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

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

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

7.1.1.1.2 Consent and Collection of Specimens for Future Biomedical Research

The investigator or qualified designee will explain the Future Biomedical Research consent to the subject, answer all of his/her questions, and obtain documented informed consent

before performing any procedure related to the Future Biomedical Research sub-trial. A copy of the informed consent will be given to the subject.

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Subject Identification Card

All subjects will be given a Subject Identification Card identifying them as participants in a research trial. The card will contain trial site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the subject with a Subject Identification Card immediately after the subject provides documented informed consent.

The subject identification card also contains contact information for the emergency unblinding call center so that a health care provider can obtain information about trial medication/vaccination in emergency situations where the investigator is not available.

7.1.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the investigator. Disease details regarding the subject's esophageal cancer will be recorded separately and not listed as medical history.

If the subject has lost at least 15 lbs. (6.8 kg) over the 3 months prior to screening, "weight loss" should be entered as an active condition on the Medical History. As well, any autoimmune disorders, regardless of onset date, should be recorded.

Disease details regarding the subject's esophageal carcinoma will be recorded separately and not listed as medical history.

7.1.1.5 Disease Details

The investigator or qualified designee will obtain prior and current details regarding the subject's esophageal carcinoma.

7.1.1.6 Prior and Concomitant Medications Review

7.1.1.6.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 30 days of the first dose of trial treatment.

Prior treatment for esophageal carcinoma will be recorded separately and not listed as a prior medication.

7.1.1.6.1.1 Prior Treatment Details for Esophageal Carcinoma

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

7.1.1.6.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial from the time of signing the informed consent form until the Safety Follow-up Visit. In addition, new medications started during the Second Course Phase through the Second Course Safety Follow-up Visit should be recorded.

All medications related to reportable SAEs and ECIs should be recorded, as defined in Section 7.2.

7.1.1.6.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-cancer therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Safety Follow-up visit must occur before the first dose of the new therapy.

Once new anti-cancer therapy has been initiated the subject will move into survival follow-up. Details regarding survival status follow-up are outlined in 7.1.6.3.3 – Survival Follow-Up.

7.1.1.7 Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to randomization or treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects.

7.1.1.8 Assignment of Treatment/Randomization Number

All eligible subjects will be allocated, by non-random assignment, and will receive an allocation number. The allocation number identifies the subject for all procedures occurring after treatment allocation. Once an allocation number is assigned to a subject, it can never be re-assigned to another subject.

A single subject cannot be assigned more than 1 allocation number.

7.1.1.9 Trial Compliance

7.1.1.9.1 Study Medication

Interruptions from the protocol specified treatment plan for greater than 12 weeks between pembrolizumab doses for non-drug-related or administrative reasons require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on subject management.

The total volume of pembrolizumab infused will be compared to the total volume prepared to determine compliance with each dose of pembrolizumab administered. The instructions for preparing and administering pembrolizumab are provided in the Pharmacy Manual.

Administration of trial medication will be witnessed by the investigator and/or trial staff.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently, if clinically indicated. Adverse events will be graded and recorded throughout the trial and during the Follow-up Period according to NCI CTCAE Version 4.0 (see Section 12.6). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of a potentially immunologic etiology; see Section 5.6.1.

Please refer to Section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Physical Exam

7.1.2.2.1 Full Physical Exam

The investigator or clinical designee will perform a complete physical examination during the screening period. A complete physical examination is a comprehensive inspection of a patient's general appearance, HEENT, neck, chest and lungs, cardiovascular system, abdomen, genitourinary system, musculoskeletal system, lymph nodes, extremities, and neurological system by reviewing history, palpation, percussion, and auscultation. Clinically significant abnormal findings should be recorded as medical history. Additional full physical examinations should be performed as specified in the Trial Flow Chart-Section 6.0. After the first dose of trial treatment new clinically significant abnormal findings should be recorded as AEs.

7.1.2.2.2 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart-Section 6.0, the investigator or qualified designee will perform a directed physical examination, as clinically indicated prior to dosing on Day 1 of each treatment cycle. New clinically significant abnormal findings should be recorded as AEs.

7.1.2.3 Height, Weight, and Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment, and at treatment discontinuation as specified in the Trial Flow Chart- Section 6.0. Height will be measured at Visit 1 only.

Vital signs should include temperature, pulse, respiratory rate, blood pressure, height, and weight.

7.1.2.4 12-Lead Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be performed one time during screening using local standard procedures. Clinically significant abnormal findings should be recorded as medical history. Additional time points may be performed, as clinically necessary.

7.1.2.5 Eastern Cooperative Oncology Group Performance Status

The investigator or qualified designee will assess ECOG Performance Status (see Section 12.5) at screening, prior to dosing on Day 1 of each treatment cycle, and at discontinuation of trial treatment as specified in the Trial Flow Chart – Section 6.0.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in the Procedures Manual.

Refer to the Trial Flow Chart – Section 6.0 for the schedule of laboratory assessments.

7.1.3.1 Local Laboratory Assessments

Local laboratory tests are specified in Table 5.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Alkaline phosphatase	Specific gravity	TSH
Hemoglobin	Blood urea nitrogen/Urea[b]	Microscopic exam, if abnormal results are noted	Pregnancy test[a]
Platelet count	Lactate dehydrogenase (LDH)	Urine pregnancy test[a]	Free thyroxine (FT4)
Red blood count	Alanine aminotransferase (ALT)	Protein	Total triiodothyronine (T3) or Free T3[d]
White blood cell count (total and differential)[e]	Aspartate aminotransferase (AST)	Glucose	aPTT
Absolute neutrophil count[f]	Bicarbonate or Carbon dioxide[c]	Blood	PT(INR)
Absolute lymphocyte count[f]	Calcium		
	Chloride		
	Creatinine		
	Glucose		
	Potassium		
	Sodium		
	Total bilirubin		
	Direct bilirubin, if total bilirubin is elevated above the upper limit of normal		
	Total protein		

a. Perform on women of childbearing potential only 72 hours prior to Day 1 of each cycle and 30 days post treatment.
b. Blood Urea Nitrogen is preferred; if not available urea may be tested.
c. If these tests are not done as part of standard of care in your region then these tests do not need to be performed.
d. T3 is preferred; if not available free T3 may be tested. If the local laboratory is unable to perform either of these tests the site should submit the sample to the central laboratory for testing; details are provided in the procedure manual.
e. Report % or absolute results per standard of practice. Report the results in the same manner throughout the study.
f. Results should be calculated per local standard of practice.

Laboratory tests for screening should be performed within 10 days prior to the first dose of trial treatment. Subjects eligible for retreatment with pembrolizumab should have lab test performed within 10 days prior to the first dose of trial treatment in the Second Course Phase. After Cycle 1, in both the Initial Treatment Phase and the Second Course Phase, pre-dose laboratory safety tests can be conducted up to 72 hours prior to dosing.

Laboratory test results must be reviewed by the investigator or qualified designee and found to be acceptable prior to administration of each dose of trial treatment. Unresolved abnormal labs that are drug related AEs should be followed until resolution. Laboratory tests do not need to be repeated after the end of treatment if lab results are within normal range.

7.1.3.1.1 Pregnancy Tests

All women who are being considered for participation in the trial, and who are not surgically sterilized or postmenopausal, must be tested for pregnancy within 72 hours of each cycle of trial treatment and 30 days post treatment. If a urine test is positive or not evaluable a serum test will be required. Subjects must be excluded/discontinued from the study in the event of a positive or borderline-positive test result.

7.1.3.2 Central laboratory Assessments

Sample collection timing, storage, and shipment instructions for the central laboratory assessments will be provided in the central laboratory manual.

7.1.3.2.1 Pharmacokinetic/Pharmacodynamic Evaluations

To evaluate the immunogenicity and exposure of pembrolizumab in this indication, sample collections for analysis of anti-pembrolizumab antibodies (ADA) and PK are currently planned as shown in the Trial Flowchart (Section 6.1). Blood samples for PK and ADA collected may be stored only at this time. Further sample analysis may be performed, if required. If ongoing PK and/or ADA sampling is deemed to be unnecessary by the Sponsor, it may be reduced or discontinued.

7.1.3.2.1.1 Blood Collection for Serum MK-3475

PK samples should be drawn according to the PK collection schedule.

7.1.3.2.1.2 Blood Collection for Anti-pembrolizumab Antibodies

Anti-pembrolizumab antibody samples should be drawn according to the ADA collection schedule. Simultaneous PK sampling is required for interpretation of ADA analysis.

7.1.3.2.2 Whole Blood Collection for Correlative and Biomarker Studies

Additional biomarker research to identify factors important for pembrolizumab therapy may also be pursued. Analysis to be inclusive of all testing is in Section 4.2.4.4.

Any leftover samples from the correlative and biomarker studies will be stored for future biomedical research if the subject provides documented informed consent for FBR.

7.1.3.3 Planned Genetic Analysis Sample Collection

Sample collection, storage and shipment instructions for Planned Genetic Analysis samples will be provided in the central laboratory manual. This sample should be drawn for planned analysis of the association between genetic variants in DNA and drug response. If there is either a documented law or regulation prohibiting collection, or if the IRB/IEC does not approve the collection of the sample for these purposes, then this sample will not be collected at that site. If the sample is collected, leftover extracted DNA will be stored for future biomedical research if the subject provides the informed consent for FBR. If the planned genetic analysis is not approved, but FBR is approved and consent is given, this sample will be collected for the purpose of FBR.

7.1.3.4 Future Biomedical Research Sample Collection

The following specimens are to be obtained as part of Future Biomedical Research:

- Leftover DNA for future research
- Leftover main study tumor tissue
- Leftover DNA and RNA from correlative studies
- Leftover plasma and serum from biomarker studies

7.1.3.5 Tumor Tissue

Eligibility for this study is dependent upon supplying adequate tumor tissue for biomarker analysis as described under eligibility criteria. Repeat samples may be required if adequate tissue is not provided. If the subject provides informed consent for FBR, any leftover tissue what would ordinarily be discarded at the end of the main study will be retained for FBR.

Newly-obtained tissue is preferred (no intervening treatment [local or systemic] involving the site of tissue biopsy once tissue biopsy is obtained and time of study enrollment). Formalin-fixed, paraffin-embedded (FFPE) block specimens are preferred to slides.

Detailed instructions for tissue collection, processing and shipment are provided in the central laboratory manual.

7.1.4 Efficacy Measurements

7.1.4.1 Tumor Imaging and Assessment of Disease

The process for image collection and transmission to the central imaging vendor can be found in the Site Imaging Manual (SIM). Tumor imaging should be acquired by computed tomography (CT, strongly preferred). Magnetic resonance imaging (MRI) should be used when CT is contraindicated or for imaging in the brain. The same imaging technique regarding modality and use of contrast should be used in a subject throughout the trial to optimize the visualization of existing and new tumor burden. Imaging should include the chest, abdomen, and pelvis at baseline and all subsequent follow up time points; additional details are in the SIM.

Expedited determination of measurable disease based on RECIST 1.1 by central imaging vendor at screening will be used to determine subject eligibility. Confirmation of measurable disease by the central imaging vendor per RECIST 1.1 is required prior to subject enrollment. Although RECIST 1.1 references to maximum of 5 target lesions in total and 2 per organ, Merck allows maximum of 10 target lesions in total and 5 per organ.

All scheduled images for all study subjects from the sites will be submitted to the central imaging vendor. In addition, imaging (including other modalities) that are obtained at an unscheduled time point to determine disease progression, as well as imaging obtained for other reasons but captures radiologic progression, should be submitted to the central imaging vendor, as well.

7.1.4.1.1 Initial Tumor Imaging

Initial tumor imaging (baseline scans) of the chest abdomen and pelvis at screening must be performed within 28 days prior to the date of allocation. The site study team must review baseline images to confirm the subject has measurable disease per RECIST 1.1. The baseline images must be submitted to the central imaging vendor for confirmation of measurable disease per RECIST 1.1; as part of the eligibility determination, prior to allocation.

Scans performed as part of routine clinical management are acceptable for use as baseline tumor imaging if they are of diagnostic quality, performed within 14 days prior to the date of allocation, and can be assessed by the central imaging vendor.

Subjects with previously treated brain metastases may participate provided they have stable brain metastases, i.e. without evidence of progression by imaging [confirmed by magnetic resonance imaging (MRI) if MRI was used at prior imaging, or confirmed by computed tomography (CT) imaging if CT used at prior imaging] for at least 4 weeks prior to the first dose of trial treatment. Any neurologic symptoms must have returned to baseline and subjects must have no evidence of new or enlarging brain metastases, and have not used steroids for brain metastases for at least 7 days prior to trial initiation as per local site assessment. This exception does not include carcinomatous meningitis, as subjects with carcinomatous meningitis are excluded regardless of clinical stability.

7.1.4.1.2 Tumor Imaging During the Trial

The first on study imaging assessment should be performed at 9 weeks (63 days ±7 days) from the date of allocation. Subsequent tumor imaging should be performed Q9W (63 days ±7 days) or more frequently if clinically indicated. Imaging timing should follow calendar days should not be adjusted for delays in cycle starts. Imaging should continue to be performed until disease progression (unless site investigator elects to continue treatment and follow irRECIST), the start of new anti-cancer treatment, withdrawal of consent, death, or notification by the SPONSOR, whichever occurs first. All supplemental imaging must be submitted to the central imaging vendor.

Per RECIST 1.1, partial or complete response should be confirmed by a repeat tumor imaging assessment not less than 4 weeks from the date the response was first documented. The tumor imaging for confirmation of response may be performed at the earliest 4 weeks after the first indication of response, or at the next scheduled tumor imaging (ie, 9 weeks later), whichever is clinically indicated. Subjects will then return to regular scheduled imaging Q9W, starting with the next scheduled imaging time point. Subjects who obtain a confirmation imaging scan do not need to undergo the next scheduled tumor imaging if it is < 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point.

Per irRECIST (Section 7.1.4.1.6) disease progression should be confirmed by the site at least 4 weeks after site-assessed 1st radiologic evidence of PD in clinically stable subjects. Subjects who have unconfirmed disease progression may continue on treatment at the discretion of the site investigator until progression is confirmed by the site provided they have met the conditions detailed in Section 7.1.4.1.6. Subjects who obtain a confirmation scan do not need to undergo the next scheduled tumor imaging if it is < 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point if clinically stable.

Subjects who have confirmed disease progression as assessed by the site will discontinue the treatment. Exception is detailed in Section 7.1.4.1.6.

7.1.4.1.3 End of Treatment and Follow-up Tumor Imaging

In subjects who discontinue trial treatment, tumor imaging should be performed at the time of treatment discontinuation (± 4-week window). If a previous scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation isn't mandatory. In subjects who discontinue trial treatment due to documented disease progression, this is the final required tumor imaging.

In subjects who discontinue trial treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (every 9 weeks) to monitor disease status until (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, or (4) the end of the study, whichever occurs first.

7.1.4.1.4 Second Course Phase Tumor Imaging

A scan must be performed within 28 days prior to restarting treatment with pembrolizumab. Local reading (investigator assessment with site radiology reading) will be used to determine eligibility. Imaging should be submitted to the central imaging vendor for retrospective review.

The first on study imaging assessment should be performed at 9 (63 days ±7 days) after the restart of treatment. Subsequent tumor imaging should be performed Q9W (63 days ±7 days) or more frequently if clinically indicated.

Per irRECIST (Section 7.1.4.1.6), if tumor imaging shows initial PD, tumor assessment should be repeated ≥ 4 weeks later in order to confirm PD with the option of continuing treatment while awaiting radiologic confirmation of progression. Subjects who obtain a confirmation scan do not need to undergo scheduled tumor imaging if it is < 4 weeks later and may wait until the next scheduled imaging time point, if clinically stable.

Imaging should continue to be performed until disease progression, the start of new anti-cancer treatment, withdrawal of consent, death, or notification by the Sponsor, whichever occurs first. Disease progression may be confirmed at least 4 weeks after the first tumor imaging indicating progressive disease in clinically stable subjects.

In subjects who discontinue trial treatment, tumor imaging should be performed at the time of treatment discontinuation (± 4-week window). If a previous scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation isn't mandatory. In subjects who discontinue trial treatment due to documented disease progression, this is the final required tumor imaging.

In subjects who discontinue trial treatment without documented disease progression, every effort should be made to continue monitoring their disease status by radiologic imaging every 9 weeks (63 days ± 7 days) until (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, or (4) the end of the study, whichever occurs first.

7.1.4.1.5 RECIST 1.1 Assessment of Disease

RECIST 1.1 will be applied by the central imaging vendor as the primary measure for assessment of tumor response, date of disease progression, and as a basis for all protocol guidelines related to disease status (eg, discontinuation of study therapy).

7.1.4.1.6 irRECIST Assessment of Disease

irRECIST is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immunotherapeutic drugs. irRECIST will be used by site investigator/local radiology review to assess tumor response and progression, and make treatment decisions. This data will be collected in the clinical database. Treatment efficacy based on irRECIST as assessed by central imaging vendor review will be evaluated retrospectively.

When feasible, subjects should not be discontinued until progression is confirmed by the local site investigator/radiology assessment. This allowance to continue treatment despite initial radiologic progressive disease (PD) takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat tumor imaging for confirmation of PD. Tumor flare includes any of the following scenarios:

- Worsening of existing target lesion(s)
- Worsening of existing non-target lesion(s)
- Development of new lesion(s)

In subjects who have shown initial evidence of radiological PD by RECIST 1.1 as determined by the site, it is at the discretion of the PI whether to continue a subject on study treatment until repeat imaging is obtained (using irRECIST for subject management, see Table 6 and Figure 2). This clinical judgment decision by the site investigator should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive study treatment and tumor assessment should be repeated ≥ 4 weeks later in order to confirm PD by irRECIST per site assessment. Clinical stability is defined as the following:

1) Absence of symptoms and signs indicating clinically significant progression of disease, including worsening of laboratory values
2) No decline in ECOG performance status
3) Absence of rapid progression of disease
4) Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Any subject deemed clinically unstable should be discontinued from trial treatment at site-assessed 1st radiologic evidence of PD and is not required to have repeat imaging for PD confirmation.

In determining whether or not the tumor burden has increased or decreased per irRECIST, the local site investigator should consider all target and non-target lesions as well as any incremental new lesion(s).

Scenarios where PD is not confirmed at repeat imaging if ALL of the following occur by irRECIST:

- Target lesion sum of diameters is < 20 % or < 5 mm absolute increase compared to nadir
- Non-target disease resulting in initial PD is qualitatively stable or improved
- New lesion resulting in initial PD is qualitatively stable or improved
- No incremental new lesion(s) since last evaluation
- No incremental new non-target lesion progression since last evaluation

If repeat imaging does not confirm PD by irRECIST as assessed by the local site investigator and the subject continues to be clinically stable, treatment may continue and follow the regular imaging schedule.

Scenarios where PD is confirmed at repeat imaging if ANY of the following occur by irRECIST:

- target lesion sum of diameters remains ≥ 20% and at least 5 mm absolute increase compared to nadir
- Non-target disease resulting in initial PD is qualitatively worse
- New lesion resulting in initial PD is qualitatively worse
- Additional new lesion(s) since last evaluation
- Additional new non-target lesion progression since last evaluation

If repeat imaging confirms PD due to any of the scenarios listed above, subjects will be discontinued from study therapy.

NOTE: If a subject has confirmed radiographic progression (i.e. 2 scans at least 4 weeks apart demonstrating progressive disease) per irRECIST, but the subject is achieving a clinically meaningful benefit, and there is no further increase in the tumor burden at the confirmatory tumor imaging, an exception to continue treatment may be considered following consultation with the Sponsor. In this case, if treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 6.0 Study Flowchart and be submitted to the central imaging vendor.

Additional details about irRECIST are referenced in Merck TIP Sheet for RECIST 1 1 and irRECIST

Table 6 Imaging and Treatment after First Radiologic Evidence of PD

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD by RECIST 1.1	Repeat imaging at ≥ 4 weeks at site to confirm PD	May continue study treatment at the local site Investigator's discretion while awaiting confirmatory tumor imaging by site by irRECIST.	Repeat imaging at ≥ 4 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat tumor imaging confirms PD by irRECIST at the local site	No additional imaging required	Discontinue treatment (exception is possible upon consultation with sponsor)	No additional imaging required	N/A
Repeat tumor imaging shows SD, PR or CR by irRECIST at the local site	Continue regularly scheduled imaging assessments	Continue study treatment at the local site Investigator's discretion	Continue regularly scheduled imaging assessments	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next tumor image should occur according to the regular imaging schedule outlined in the protocol

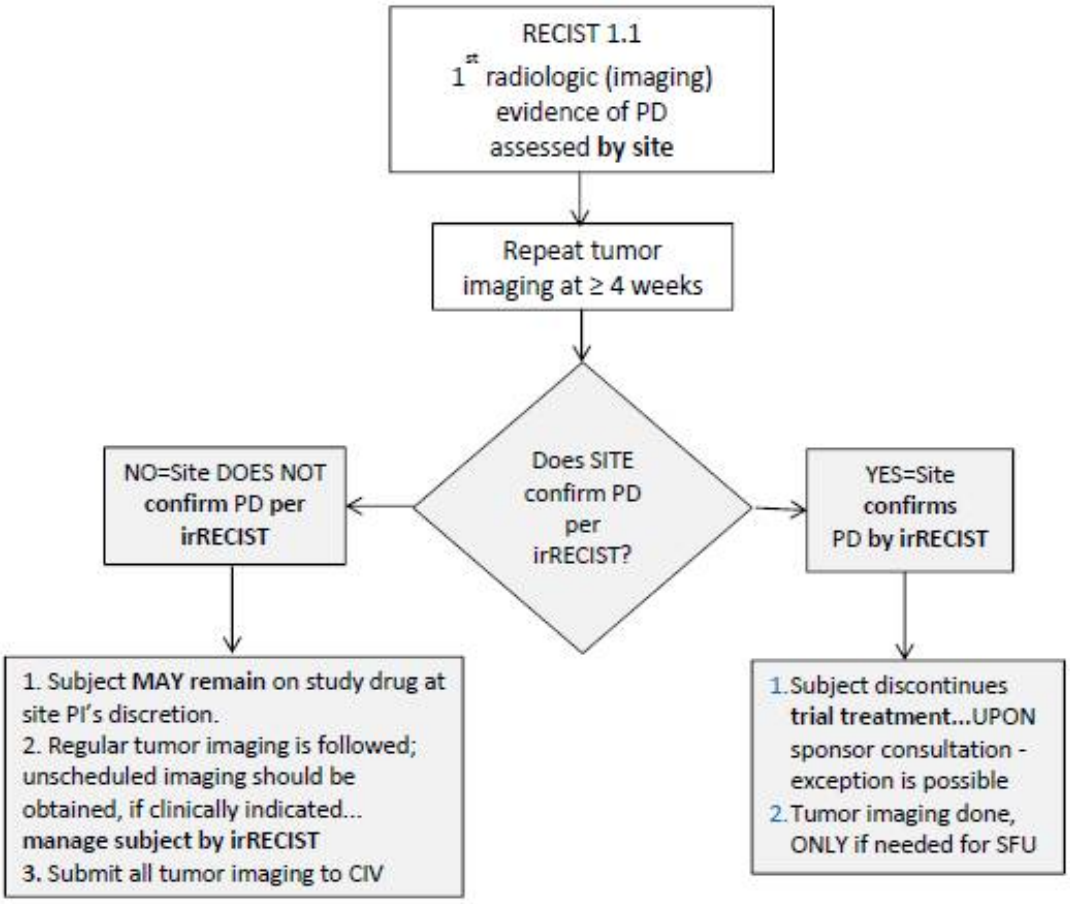

Figure 2 Imaging and Treatment for Clinically Stable Subjects after First Radiologic Evidence of PD Assessed by the Site

7.1.5 Other Procedures

7.1.5.1 Withdrawal/Discontinuation

Subjects who discontinue/withdraw from treatment prior to completion of the treatment should be encouraged to continue to be followed for all remaining study visits.

When a subject discontinues/withdraws from participation in the trial, all applicable activities scheduled for the end of treatment visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who attain a CR or complete 35 trial treatments (approximately 2 years) may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.6.2. After discontinuing treatment following assessment of a CR or the 35 trial treatments, subjects should return to the site for a Safety Follow-up visit (Section 7.1.6.3.1) and then proceed to the Follow-up Period of the study (Section 7.1.6.3.2).

7.1.5.1.1 Withdrawal From Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research and have their specimens and all derivatives destroyed. Subjects may withdraw consent at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com), and a form will be provided by the Sponsor to obtain appropriate information to complete specimen withdrawal. Subsequently, the subject's specimens will be removed from the biorepository and be destroyed. A letter will be sent from the Sponsor to the investigator confirming the destruction. It is the responsibility of the investigator to inform the subject of completion of destruction. Any analyses in progress at the time of request for destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for specimen destruction cannot be processed.

7.1.5.2 Blinding/Unblinding

This is an open label trial; there is no blinding for this trial.

7.1.5.3 Calibration of Critical Equipment

The investigator or qualified designee has the responsibility to ensure that any critical device or instrument used for a clinical evaluation/test during a clinical trial that provides important information about inclusion/exclusion criteria and/or safety or efficacy parameters shall be suitably calibrated and maintained to ensure that the data obtained is reliable and/or reproducible. Documentation of equipment calibration must be retained as source documentation at the trial site.

Critical Equipment for this trial includes:

- Laboratory equipment – as required for inclusion labs and trial assessments
- Imaging equipment – as required for study objectives

See protocol-specified guidance in the Trial Administrative Binder, Procedures Manual, and Site Imaging Manual.

7.1.6 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.6.1 Screening

Approximately 28 days prior to treatment allocation, potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in Section 5.1. Screening procedures may be repeated.

Subjects may not be rescreened in this study.

Screening procedures are to be completed within 28 days prior to the first dose of trial treatment except for the following:

- Laboratory tests and ECOG PS are to be performed within 10 days prior to the first dose of trial treatment.
- For women of reproductive potential, a pregnancy test will be performed within 72 hours prior to the first dose of trial treatment. A urine test may be considered if serum test is not appropriate.

7.1.6.2 Treatment Period

Visit requirements are outlined in the Trial Flow Chart (Section 6.0). Specific procedure-related details are provided above in the Trial Procedures.

Subjects who stop pembrolizumab with SD or better may be eligible for up to 17 additional trial treatments (approximately 1 year) if they progress after stopping study treatments. Retreatment with pembrolizumab is termed the Second Course Phase and is only available if the trial remains open and the subject meets the following conditions:

- **Either**
    - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1
        - Was treated with at least 8 trial treatments (approximately 6 months) with pembrolizumab before discontinuing therapy
        - Received at least 2 treatments with pembrolizumab beyond the date when the initial CR was declared

OR

    - Had SD, PR or CR and stopped pembrolizumab after 35 trial treatments (approximately 2 years) for reasons other than disease progression or intolerability

- **AND**
    - Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
    - Did not receive any anti-cancer treatment since the last dose of pembrolizumab
    - Has a performance status of 0 or 1 on the ECOG Performance Scale
    - Demonstrates adequate organ function as detailed in Section 5.1.2

- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2– Contraception, for the course of the study through 120 days after the last dose of study medication.
    - *Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.*
- Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception and not to donate sperm starting with the first dose of study therapy through 120 days after the last dose of study therapy.
    - *Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.*
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might 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.

Subjects who enter the Second Course Phase will be retreated at the same dose frequency as when they last received pembrolizumab. Pembrolizumab will be administered for up to an additional 17 trial treatments (approximately 1 year).

Visit requirements for the second course phase are outlined in the Second Course Phase Trial Flow Chart (Section 6.2).

7.1.6.3 Post-Treatment Visits

7.1.6.3.1 Safety Follow-up Visits

The mandatory Safety Follow-up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first.

All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

Subjects who are eligible for retreatment with pembrolizumab may have up to 2 safety follow-up visits, one after the Initial Treatment Period and one after the Second Course Treatment Phase.

7.1.6.3.2 Follow-up visits

Subjects who discontinue trial treatment for reasons other than disease progression will move into the Follow-up Phase and should be assessed Q9W by radiologic imaging to monitor disease status. The Sponsor may request survival status to be assessed at additional time points during the course of the study (not to exceed approximately 12 weeks). Every effort should be made to collect information regarding disease status until the start of a new anti-cancer therapy, disease progression, death, or the end of the study.

Information regarding post-study anti-cancer treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.6.2 will move from the Follow-up Phase to the Second Course Phase when they experience disease progression.

7.1.6.3.3 Survival Follow-up

Subjects, who experience disease progression (by site assessment) or start a new anti-cancer therapy, will move into the Survival Follow-Up Phase. Subjects should be contacted by telephone approximately every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.6.3.3.1 Survival Status

To ensure current and complete survival data is available at the time of database locks, updated survival status may be requested during the course of the study by the Sponsor. For example, updated survival status may be requested prior to but not limited to an external Data Monitoring Committee (eDMC) review, interim and/or final analysis. Upon Sponsor notification, all participants who do not/will not have a scheduled study visit or study contact during the Sponsor defined time period will be contacted for their survival status (excluding participants that have a previously recorded death event in the collection tool).

7.2 Assessing and Recording Adverse Events

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

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

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

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

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure. From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor

In this trial, an overdose is any dose higher than ≥1000 mg (5 times the protocol-defined dose) of pembrolizumab. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, study treatment should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

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

All reports of overdose with and without an adverse event must be reported by the investigator within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor

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

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure. Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.3 Immediate Reporting of Adverse Events to the Sponsor

7.2.3.1 Serious Adverse Events

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

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

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by the Sponsor for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Refer to Table 7 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details), that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details), whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

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

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

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

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

For the time period beginning at treatment allocation/randomization through 30 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to the Sponsor’s product, must be reported within 24 hours to the Sponsor, either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Events of clinical interest for this trial include:

1. an overdose of Sponsor's product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

7.2.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined will not be reported to the Sponsor, as described in Section 7.2.3. - Immediate Reporting of Adverse Events to the Sponsor.

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

The Sponsor will monitor safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to global safety as a SAE within 24 hours of determination that the event is not progression of the cancer under study.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

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

Table 7 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Sponsor's product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Sponsor's product to be discontinued?	
Relationship to Sponsor's Product	Did the Sponsor's product cause the adverse event? The determination of the likelihood that the Sponsor's product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Sponsor's product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Sponsor's product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

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

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

7.3 Trial Governance and Oversight

This trial was developed in collaboration with both Sponsor and non-Sponsor scientific experts who provide input with respect to the trial design, interpretation of trial results and subsequent peer-reviewed scientific publications.

8.0 STATISTICAL ANALYSIS PLAN

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, but prior to any final database lock, changes made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, but prior to final database lock, will be documented in a supplemental SAP (sSAP) and referenced in the Clinical Study Report (CSR) for the study. There will be a separate PK analysis plan. Post hoc exploratory analyses will be clearly identified in the CSR.

8.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below. The comprehensive plan is provided in Sections 8.2 through 8.12.

Study Design Overview	A Phase II Study of Pembrolizumab Monotherapy in Third Line, Previously Treated Subjects with Advanced/Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus or Advanced/Metastatic Siewert Type I Adenocarcinoma of the Esophagogastric Junction (KEYNOTE – 180)
Treatment Assignment	This is a single-arm open-label study.
Analysis Populations	All Subjects as Treated (ASaT)
Primary Endpoint(s)	ORR based on RECIST 1.1 assessed by central imaging vendor
Statistical Methods for Key Efficacy Analyses	95% CI for ORR will be calculated using the exact binomial distribution.
Statistical Methods for Key Safety Analyses	Count and percentage of AE will be provided.
Multiplicity	Not applicable since this is an estimation study.
Sample Size	The planned sample size is approximately 100 subjects. The primary objective of the study is to estimate ORR in all subjects and in subjects whose tumors are classified as GEP intermediate or high and subjects whose tumors are classified as GEP high. Section 8.7 provides the precision of the ORR estimates.

8.2 Responsibility for Analyses/In-House Blinding

The statistical analysis of the data obtained from this study will be the responsibility of the Clinical Biostatistics department of the SPONSOR.

This trial is being conducted as an open-label study, ie, subjects, investigators, and SPONSOR personnel will be aware of subject treatment.

The Clinical Biostatistics department will generate the allocation schedule.

8.3 Estimation

Objectives and estimations of the study are stated in Section 3.0.

8.4 Analysis Endpoints

8.4.1 Efficacy Endpoints

8.4.1.1 Primary Efficacy Endpoint

- **Objective response rate (ORR) - RECIST 1.1 assessed by central imaging vendor**

Objective response rate is defined as the proportion of the subjects in the analysis population who have a complete response (CR) or partial response (PR). Responses are based upon central imaging vendor assessments per RECIST 1.1.

8.4.1.2 Secondary Efficacy Endpoints

- **Duration of Response (DOR) - RECIST 1.1 assessed by central imaging vendor**

For subjects who demonstrated CR or PR, response duration is defined as the time from the date of first response (CR or PR) until the date of disease progression or death.

- **Progression-free Survival (PFS) - RECIST 1.1 assessed by central imaging vendor**

PFS is defined as the time from first day of study treatment to the first documented disease progression or death due to any cause, whichever occurs first.

- **Overall survival (OS)**

OS is defined as the time from first day of study treatment to death due to any cause. Subjects without documented death at the time of the final analysis will be censored at the date of the last follow-up.

8.4.2 Safety Endpoints

Safety measurements are described in Section 7.0 Trial Procedures.

8.5 Analysis Populations

8.5.1 Efficacy Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of ORR, PFS, and OS. The ASaT population consists of all allocated subjects who received at least 1 dose of study treatment.

The analysis population for DOR consists of responders.

Details on the approach to handling missing data are provided in Section 8.6 Statistical Methods.

8.5.2 Safety Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of safety data in this study. The ASaT population consists of all allocated subjects who received at least 1 dose of study treatment.

At least 1 laboratory or vital sign measurement obtained subsequent to at least 1 dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

Details on the approach to handling missing data for safety analyses are provided in Section 8.6 Statistical Methods.

8.6 Statistical Methods

8.6.1 Statistical Methods for Efficacy Analyses

This section describes the statistical methods that address the primary and secondary objectives.

For the primary efficacy endpoint of ORR, the point estimate and 95% confidence interval will be provided using exact binomial method proposed by Clopper and Pearson (1934)[47]. Subjects in the primary analysis population (ASaT) without ORR data will be counted as non-responder.

For PFS, DOR and OS endpoints, Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided as appropriate. The efficacy analysis is summarized in Table 8.

Table 8 Analysis Strategy for Efficacy Variables

Endpoint/Variable‡ (Description, Time Point)	Statistical Method	Analysis Population	Missing Data Approach
Primary Endpoint and Hypothesis			
ORR • RECIST 1.1, Central imaging vendor assessment	Exact method based on binomial distribution	ASaT • All subjects • Subjects whose tumors are classified as GEP intermediate or high • Subjects whose tumors are classified as GEP high	Subjects with missing data are considered non-responders
Secondary Endpoints			
DOR • RECIST 1.1, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	All responders	Non-responders are excluded from analysis
PFS • RECIST 1.1, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	ASaT	Censored at last assessment
OS	Summary statistics using Kaplan-Meier method	ASaT	Censored at last known alive date

8.6.2 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs, laboratory tests, and vital signs. Count and percentage of AEs will be provided.

8.6.3 Summaries of Baseline Characteristics, Demographics, and Other Analyses

The number and percentage of subjects screened, enrolled, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (eg, age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized either by descriptive statistics or categorical tables for all enrolled subjects.

8.7 Interim Analysis

No interim analysis is planned in this study.

8.8 Multiplicity

Multiplicity adjustment is not applicable since this is an estimation study.

8.9 Sample Size

In this study, approximately 100 subjects with previously treated, advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ will be enrolled.

Table 9 shows the two-sided 95% confidence interval of ORR with 100 subjects for different observed response rates.

Table 9 Two-sided 95% Confidence Interval of ORR with 100 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
17	17%	(10.2, 25.8)
25	25%	(16.9, 34.7)
30	30%	(21.2, 40.0)
35	35%	(25.7, 45.2)

For the subjects with tumors classified as GEP intermediate or high, if the prevalence of about 60%, there will be approximately 60 such subjects. Table 10 shows the two-sided 95% CI of ORR with 60 subjects for different response rates. For example, if the observed ORR is 35% in this group, the 95% CI of this estimate of ORR is (23.1%, 48.4%).

Table 10 Two-sided 95% Confidence Interval for ORR with 60 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
15	25%	(14.7, 37.9)
18	30%	(18.8, 43.2)
21	35%	(23.1, 48.4)
24	40%	(27.6, 53.5)

For the subjects with tumors classified as GEP high, if the prevalence of this group is about 40%, there will be approximately 40 such subjects. Table 11 shows the two-sided 95% CI of ORR with 40 subjects for different response rates. For example, if the observed ORR is 35% in this group, the 95% CI of this estimate of ORR is (20.6%, 51.7%).

Table 11 Two-sided 95% Confidence Interval for ORR with 40 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
12	30%	(16.6, 46.5)
14	35%	(20.6, 51.7)
16	40%	(24.9, 56.7)

8.10 Subgroup Analyses and Effect of Baseline Factors

The estimate of the treatment effect for the primary endpoint will be estimated and/or plotted within each category of the following classification variables:

- Age category (≤65 vs. >65 years)
- Sex (Female vs. Male)
- Race (Asian vs. non-Asian)
- Histology (Adenocarcinoma vs. Squamous Cell Carcinoma vs. Siewert type 1 adenocarcinoma of the EGJ)

8.11 Compliance (Medication Adherence)

Drug accountability data for MK-3475 will be collected during the study. Any deviation from protocol-directed administration will be reported.

8.12 Extent of Exposure

Extent of Exposure for a subject is defined as number of cycles in which the subject receives the study medication infusion. Summary statistics will be provided on Extent of Exposure for ASaT population.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Clinical Supplies will be provided by the Sponsor as summarized in Table 12.

Clinical supplies will be packaged to support enrollment and replacement subjects as required. When a replacement subject is required, the Sponsor or designee needs to be contacted prior to dosing the replacement supplies.

Table 12 Product Descriptions

Product Name & Potency	Dosage Form
MK-3475 50 mg/vial	Lyophilized Powder for Injection

All other supplies not indicated in Table 12 above will be provided centrally by the Sponsor or locally by the trial site, subsidiary or designee, depending on local country operational or regulatory requirements.

For any commercially available product that is provided by the trial site, subsidiary or designee every attempt will be made to source these supplies from a single lot/batch number. The trial site is responsible to record the lot number, manufacturer and expiry date for any locally purchased product as per local guidelines unless otherwise instructed by the Sponsor.

9.2 Packaging and Labeling Information

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

Subjects will receive open label vials for each treatment cycle. The MK-3475 will not be kitted.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded. Treatment (name, strength or potency) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

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

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

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

9.5 Discard/Destruction/Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from the Sponsor or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial. For all trial sites, the local country Sponsor personnel or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

9.6 Standard Policies

Trial site personnel will have access to a central electronic treatment allocation/randomization system (IVRS/IWRS system) to allocate subjects, to assign treatment to subjects and to manage the distribution of clinical supplies. Each person accessing the IVRS system must be assigned an individual unique PIN. They must use only their assigned PIN to access the system, and they must not share their assigned PIN with anyone.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

10.1.1 Confidentiality of Data

By signing this protocol, the investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB/ERC) or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

10.1.2 Confidentiality of Subject Records

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), IRB/ERC, or regulatory authority representatives may consult and/or copy trial documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If trial documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor.

By signing this protocol, the investigator agrees to treat all subject data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules and regulations.

10.1.3 Confidentiality of Investigator Information

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all subinvestigators and trial site personnel, may be used and disclosed for trial management purposes, as part of a regulatory submissions, and as required by law. This information may include:

1. name, address, telephone number and e-mail address;
2. hospital or clinic address and telephone number;
3. curriculum vitae or other summary of qualifications and credentials; and
4. other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the Sponsor, and subsidiaries, affiliates and agents of the Sponsor, in your country and other countries, including countries that do not have laws protecting such information. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory authorities or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

If this is a multicenter trial, in order to facilitate contact between investigators, the Sponsor may share an investigator's name and contact information with other participating investigators upon request.

10.1.4 Confidentiality of IRB/IEC Information

The Sponsor is required to record the name and address of each IRB/IEC member that reviews and approves this trial. The Sponsor is also required to document that each IRB/IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies.

10.2 Compliance with Financial Disclosure Requirements

Financial Disclosure requirements are outlined in the US Food and Drug Administration Regulations, Financial Disclosure by Clinical Investigators (21 CFR Part 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/subinvestigator's responsibility to comply with any such request.

The investigator/subinvestigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements. The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor. The investigator/subinvestigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

10.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by Merck, is provided in Section 12.1 - Merck Code of Conduct for Clinical Trials.

The investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator by the Sponsor.

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation and worksheets/case report forms.

The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or subject files.

ICH Good Clinical Practice guidelines recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial.

Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB/IEC.

According to European legislation, a Sponsor must designate an overall coordinating investigator for a multi-center trial (including multinational). When more than one trial site is open in an EU country, Merck, as the Sponsor, will designate, per country, a national principal coordinator (Protocol CI), responsible for coordinating the work of the principal investigators at the different trial sites in that Member State, according to national regulations. For a single-center trial, the Protocol CI is the principal investigator. In addition, the Sponsor must designate a principal or coordinating investigator to review the trial report that summarizes the trial results and confirm that, to the best of his/her knowledge, the report accurately describes the conduct and results of the trial [Clinical Study Report (CSR) CI]. The Sponsor may consider one or more factors in the selection of the individual to serve as the Protocol CI and or CSR CI (e.g., availability of the CI during the anticipated review process, thorough understanding of clinical trial methods, appropriate enrollment of subject cohort, timely achievement of trial milestones). The Protocol CI must be a participating trial investigator.

10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Merck, as Sponsor of this trial, will review this protocol and submit the information necessary to fulfill these requirements. Merck entries are not limited to FDAMA/FDAAA mandated trials. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligations under FDAMA/FDAAA are that of the Sponsor and agrees not to submit any information about this trial or its results to the Clinical Trials Data Bank.

10.5 Quality Management System

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining a quality management system with written development procedures and functional area standard operating procedures (SOPs) to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical trial.

10.6 Data Management

The investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. By signing this protocol, the investigator acknowledges that his/her electronic signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the investigator confirms that all recorded data have been verified as accurate.

Detailed information regarding Data Management procedures for this protocol will be provided separately.

10.7 Publications

This trial is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Sponsor will work with the authors to submit a manuscript describing trial results within 12 months after the last data become available, which may take up to several months after the last subject visit in some cases such as vaccine trials. However, manuscript submission timelines may be extended on OTC trials. For trials intended for pediatric-related regulatory filings, the investigator agrees to delay publication of the trial results until the Sponsor notifies the investigator that all relevant regulatory authority decisions on the trial drug have been made with regard to pediatric-related regulatory filings. Merck will post a synopsis of trial results for approved products on www.clinicaltrials.gov by 12 months after the last subject's last visit for the primary outcome, 12 months after the decision to discontinue development, or product marketing (dispensed, administered, delivered or promoted), whichever is later.

These timelines may be extended for products that are not yet marketed, if additional time is needed for analysis, to protect intellectual property, or to comply with confidentiality agreements with other parties. Authors of the primary results manuscript will be provided the complete results from the Clinical Study Report, subject to the confidentiality agreement. When a manuscript is submitted to a biomedical journal, the Sponsor's policy is to also include the protocol and statistical analysis plan to facilitate the peer and editorial review of the manuscript. If the manuscript is subsequently accepted for publication, the Sponsor will allow the journal, if it so desires, to post on its website the key sections of the protocol that are relevant to evaluating the trial, specifically those sections describing the trial objectives and hypotheses, the subject inclusion and exclusion criteria, the trial design and procedures, the efficacy and safety measures, the statistical analysis plan, and any amendments relating to those sections. The Sponsor reserves the right to redact proprietary information.

For multicenter trials, subsequent to the multicenter publication (or after public disclosure of the results online at www.clinicaltrials.gov if a multicenter manuscript is not planned), an investigator and his/her colleagues may publish their data independently. In most cases, publication of individual trial site data does not add value to complete multicenter results, due to statistical concerns. In rare cases, publication of single trial site data prior to the main paper may be of value. Limitations of single trial site observations in a multicenter trial should always be described in such a manuscript.

Authorship credit should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors must meet conditions 1, 2 and 3. Significant contributions to trial execution may also be taken into account to determine authorship, provided that contributions have also been made to all three of the preceding authorship criteria. Although publication planning may begin before conducting the trial, final decisions on authorship and the order of authors' names will be made based on participation and actual contributions to the trial and writing, as discussed above. The first author is responsible for defending the integrity of the data, method(s) of data analysis and the scientific content of the manuscript.

The Sponsor must have the opportunity to review all proposed abstracts, manuscripts or presentations regarding this trial 45 days prior to submission for publication/presentation. Any information identified by the Sponsor as confidential must be deleted prior to submission; this confidentiality does not include efficacy and safety results. Sponsor review can be expedited to meet publication timelines.

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12.0 APPENDICES

12.1 Merck Code of Conduct for Clinical Trials

Merck*
Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of subject safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (e.g., contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine subject preferences, etc.

The design (i.e., subject population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research subjects must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate subjects, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-non-Compliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Subject Protection

A. IRB/ERC review

All clinical trials will be reviewed and approved by an independent IRB/ERC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/ERC prior to implementation, except that changes required urgently to protect subject safety and well-being may be enacted in anticipation of IRB/ERC approval. For each site, the IRB/ERC and Merck will approve the subject informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that subject welfare is of primary importance. Potential subjects will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Subjects are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Subjects are enrolled only after providing informed consent for participation. Subjects may withdraw from a Merck trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding subject confidentiality, to the greatest extent possible. Unless required by law, only the investigator, sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research subject by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll subjects in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for subject referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible subjects.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/ERC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by investigators and support staff (e.g., to scientific meetings, investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc."

12.2 Collection and Management of Specimens for Future Biomedical Research

1. Definitions

a. Biomarker: A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.[1]

b. Pharmacogenomics: The investigation of variations of DNA and RNA characteristics as related to drug/vaccine response.[2]

c. Pharmacogenetics: A subset of pharmacogenomics, pharmacogenetics is the influence of variations in DNA sequence on drug/vaccine response.[2]

d. DNA: Deoxyribonucleic acid.

e. RNA: Ribonucleic acid.

2. Scope of Future Biomedical Research

The specimens collected in this trial as outlined in Section 7.1.3.4 – Future Biomedical Research Sample Collection will be used to study various causes for how subjects may respond to a drug/vaccine. Future biomedical research specimen(s) will be stored to provide a resource for future trials conducted by the Sponsor focused on the study of biomarkers responsible for how a drug/vaccine enters and is removed by the body, how a drug/vaccine works, other pathways a drug/vaccine may interact with, or other aspects of disease. The specimen(s) may be used for future assay development and/or drug/vaccine development.

It is now well recognized that information obtained from studying and testing clinical specimens offers unique opportunities to enhance our understanding of how individuals respond to drugs/vaccines, enhance our understanding of human disease and ultimately improve public health through development of novel treatments targeted to populations with the greatest need. All specimens will be used by the Sponsor or those working for or with the Sponsor.

3. Summary of Procedures for Future Biomedical Research

a. Subjects for Enrollment

All subjects enrolled in the clinical trial will be considered for enrollment in the Future Biomedical Research sub-trial.

b. Informed Consent

Informed consent for specimens (i.e., DNA, RNA, protein, etc.) will be obtained during screening for protocol enrollment from all subjects or legal guardians, at a trial visit by the investigator or his or her designate. Informed consent for Future Biomedical Research should be presented to the subjects on Visit 1. If delayed, present consent at next possible Subject Visit. Informed consent must be obtained prior to collection of all Future Biomedical Research specimens. Consent forms signed by the subject will be kept at the clinical trial site under secure storage for regulatory reasons.

A template of each trial site's approved informed consent will be stored in the Sponsor's clinical document repository. Each consent will be assessed for appropriate specimen permissions.

c. eCRF Documentation for Future Biomedical Research Specimens

Documentation of patient consent for Future Biomedical Research will be captured in the electronic Case Report Forms (eCRFs). Any specimens for which such an informed consent cannot be verified will be destroyed.

d. Future Biomedical Research Specimen Collections

Collection of specimens for Future Biomedical Research will be performed as outlined in the trial flow chart. In general, if additional blood specimens are being collected for Future Biomedical Research, these will usually be obtained at a time when the subject is having blood drawn for other trial purposes.

4. Confidential Subject Information for Future Biomedical Research

In order to optimize the research that can be conducted with Future Biomedical Research specimens, it is critical to link subject' clinical information with future test results. In fact little or no research can be conducted without connecting the clinical trial data to the specimen. The clinical data allow specific analyses to be conducted. Knowing subject characteristics like gender, age, medical history and treatment outcomes are critical to understanding clinical context of analytical results.

To maintain privacy of information collected from specimens obtained for Future Biomedical Research, the Sponsor has developed secure policies and procedures. All specimens will be single-coded per ICH E15 guidelines as described below.

At the clinical trial site, unique codes will be placed on the Future Biomedical Research specimens for transfer to the storage facility. This first code is a random number which does not contain any personally identifying information embedded within it. The link (or key) between subject identifiers and this first unique code will be held at the trial site. No personal identifiers will appear on the specimen tube.

5. Biorepository Specimen Usage

Specimens obtained for the Merck Biorepository will be used for analyses using good scientific practices. Analyses utilizing the Future Biomedical Research specimens may be performed by the Sponsor, or an additional third party (e.g., a university investigator) designated by the Sponsor. The investigator conducting the analysis will follow the Sponsor's privacy and confidentiality requirements. Any contracted third party analyses will conform to the specific scope of analysis outlined in this sub-trial. Future Biomedical Research specimens remaining with the third party after specific analysis is performed will be reported to the Sponsor.

6. Withdrawal From Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research and have their specimens and all derivatives destroyed. Subjects may withdraw consent at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated

mailbox (clinical.specimen.management@merck.com) and a form will be provided to obtain appropriate information to complete specimen withdrawal. Subsequently, the subject's specimens will be removed from the biorepository and be destroyed. Documentation will be sent to the investigator confirming the destruction. It is the responsibility of the investigator to inform the subject of completion of destruction. Any analyses in progress at the time of request for destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for specimen destruction can not be processed.

7. Retention of Specimens

Future Biomedical Research specimens will be stored in the biorepository for potential analysis for up to 20 years from the end of the main study. Specimens may be stored for longer if a regulatory or governmental authority has active questions that are being answered. In this special circumstance, specimens will be stored until these questions have been adequately addressed.

Specimens from the trial site will be shipped to a central laboratory and then shipped to the Sponsor-designated biorepository. If a central laboratory is not utilized in a particular trial, the trial site will ship directly to the Sponsor-designated biorepository. The specimens will be stored under strict supervision in a limited access facility which operates to assure the integrity of the specimens. Specimens will be destroyed according to Sponsor policies and procedures and this destruction will be documented in the biorepository database.

8. Data Security

Databases containing specimen information and test results are accessible only to the authorized Sponsor representatives and the designated trial administrator research personnel and/or collaborators. Database user authentication is highly secure, and is accomplished using network security policies and practices based on international standards (e.g., ISO17799) to protect against unauthorized access.

9. Reporting of Future Biomedical Research Data to Subjects

No information obtained from exploratory laboratory studies will be reported to the subject, family, or physicians. Principle reasons not to inform or return results to the subject include: Lack of relevance to subject health, limitations of predictive capability, and concerns regarding misinterpretation.

If any exploratory results are definitively associated with clinical significance for subjects while the clinical trial is still ongoing, investigators will be contacted with information. After the clinical trial has completed, if any exploratory results are definitively associated with clinical significance, the Sponsor will endeavor to make such results available

through appropriate mechanisms (e.g., scientific publications and/or presentations). Subjects will not be identified by name in any published reports about this study or in any other scientific publication or presentation.

10. Future Biomedical Research Study Population

Every effort will be made to recruit all subjects diagnosed and treated on Sponsor clinical trials for Future Biomedical Research.

11. Risks Versus Benefits of Future Biomedical Research

For future biomedical research, risks to the subject have been minimized. No additional risks to the subject have been identified as no additional specimens are being collected for Future Biomedical Research (ie, only leftover samples are being retained).

The Sponsor has developed strict security, policies and procedures to address subject data privacy concerns. Data privacy risks are largely limited to rare situations involving possible breach of confidentiality. In this highly unlikely situation there is risk that the information, like all medical information, may be misused.

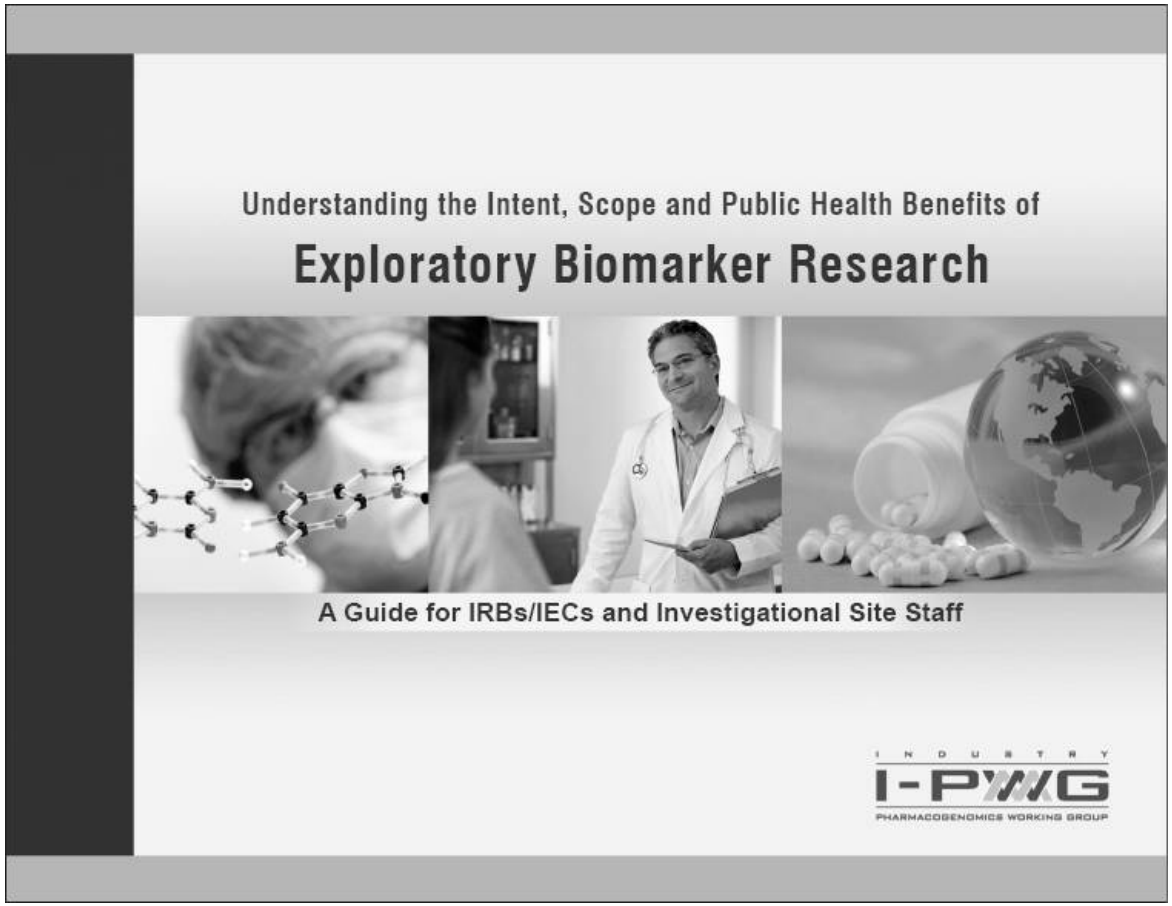
12. Questions

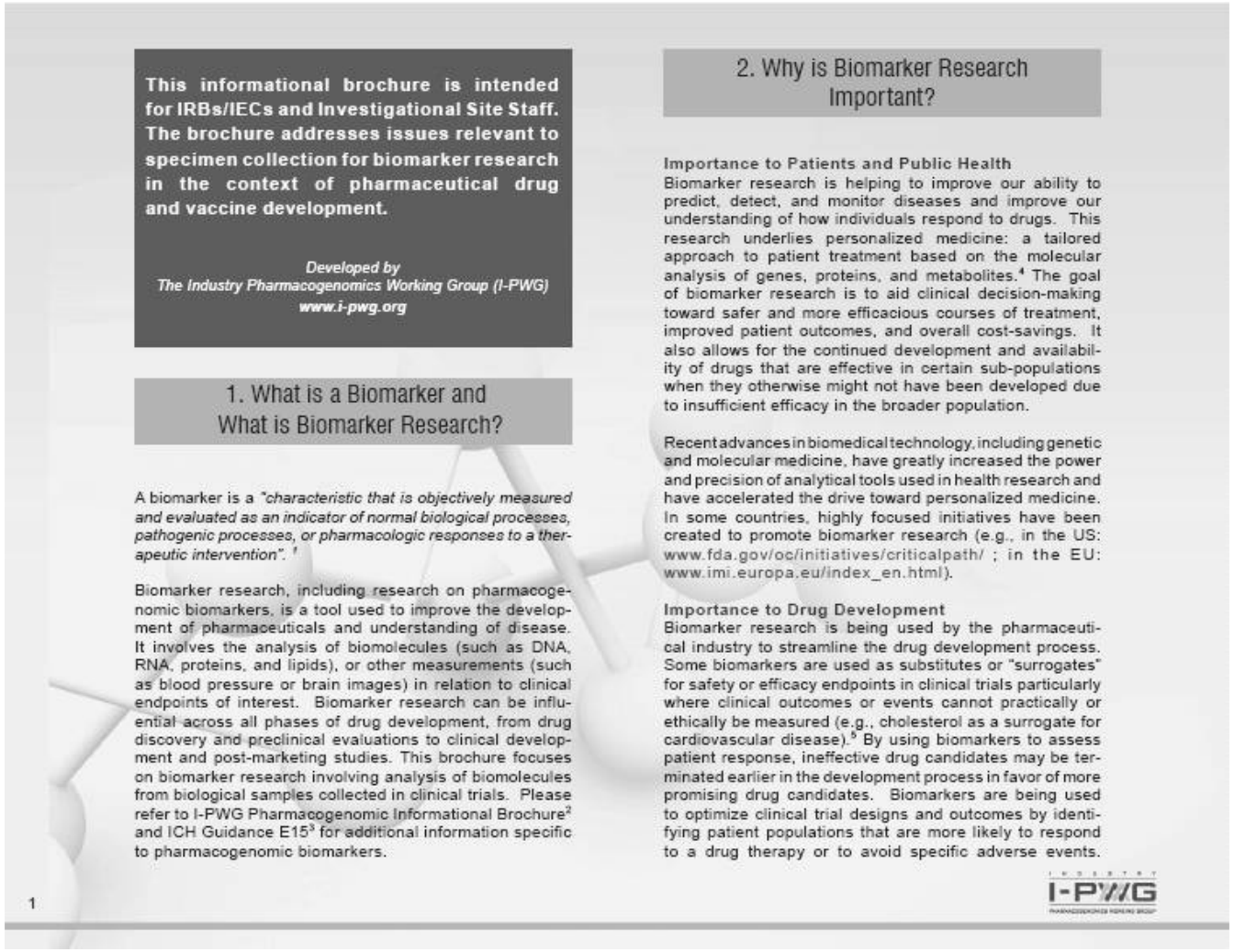
Any questions related to the future biomedical research should be e-mailed directly to clinical.specimen.management@merck.com.

13. References

1. National Cancer Institute: http://www.cancer.gov/dictionary/?searchTxt=biomarker
2. International Conference on Harmonization: DEFINITIONS FOR GENOMIC BIOMARKERS, PHARMACOGENOMICS, PHARMACOGENETICS, GENOMIC DATA AND SAMPLE CODING CATEGORIES - E15; http://www.ich.org/LOB/media/MEDIA3383.pdf

12.3 Understanding the Intent, Scope and Public Health Benefits of Exploratory Biomarker Research: A Guide for IRBs/IECs and Investigational Site Staff





This informational brochure is intended for IRBs/IECs and Investigational Site Staff. The brochure addresses issues relevant to specimen collection for biomarker research in the context of pharmaceutical drug and vaccine development.

Developed by
The Industry Pharmacogenomics Working Group (I-PWG)
www.i-pwg.org

1. What is a Biomarker and What is Biomarker Research?

A biomarker is a *"characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".* [1]

Biomarker research, including research on pharmacogenomic biomarkers, is a tool used to improve the development of pharmaceuticals and understanding of disease. It involves the analysis of biomolecules (such as DNA, RNA, proteins, and lipids), or other measurements (such as blood pressure or brain images) in relation to clinical endpoints of interest. Biomarker research can be influential across all phases of drug development, from drug discovery and preclinical evaluations to clinical development and post-marketing studies. This brochure focuses on biomarker research involving analysis of biomolecules from biological samples collected in clinical trials. Please refer to I-PWG Pharmacogenomic Informational Brochure[2] and ICH Guidance E15[3] for additional information specific to pharmacogenomic biomarkers.

2. Why is Biomarker Research Important?

Importance to Patients and Public Health
Biomarker research is helping to improve our ability to predict, detect, and monitor diseases and improve our understanding of how individuals respond to drugs. This research underlies personalized medicine: a tailored approach to patient treatment based on the molecular analysis of genes, proteins, and metabolites.[4] The goal of biomarker research is to aid clinical decision-making toward safer and more efficacious courses of treatment, improved patient outcomes, and overall cost-savings. It also allows for the continued development and availability of drugs that are effective in certain sub-populations when they otherwise might not have been developed due to insufficient efficacy in the broader population.

Recent advances in biomedical technology, including genetic and molecular medicine, have greatly increased the power and precision of analytical tools used in health research and have accelerated the drive toward personalized medicine. In some countries, highly focused initiatives have been created to promote biomarker research (e.g., in the US: www.fda.gov/oc/initiatives/criticalpath/ ; in the EU: www.imi.europa.eu/index_en.html).

Importance to Drug Development
Biomarker research is being used by the pharmaceutical industry to streamline the drug development process. Some biomarkers are used as substitutes or "surrogates" for safety or efficacy endpoints in clinical trials particularly where clinical outcomes or events cannot practically or ethically be measured (e.g., cholesterol as a surrogate for cardiovascular disease).[5] By using biomarkers to assess patient response, ineffective drug candidates may be terminated earlier in the development process in favor of more promising drug candidates. Biomarkers are being used to optimize clinical trial designs and outcomes by identifying patient populations that are more likely to respond to a drug therapy or to avoid specific adverse events.

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Biomarker research is also being used to enhance scientific understanding of the mechanisms of both treatment response and disease processes, which can help to identify future targets for drug development. Depending on the clinical endpoints in a clinical trial, biomarker sample collection may either be a required or optional component of the trial. However, both mandatory and optional sample collections are important for drug development.

3. Importance of Biomarkers to Regulatory Authorities

Regulatory health authorities are increasingly aware of the benefits of biomarkers and how they may be used for drug approval, clinical trial design, and clinical care. Biomarkers have been used to establish risk:benefit profiles. For example, the FDA has modified the US warfarin (Coumadin®) label to include the analysis of *CYP2C9* and *VKORC1* genes to guide dosing regimens. Health authorities such as the FDA (USA), EMEA (European Union), MHLW (Japan), and ICH (International) are playing a key role in advancing this scientific field as it applies to pharmaceutical development by creating the regulatory infrastructure to facilitate this research. Numerous regulatory guidances and concept papers have already been issued, many of which are available through www.i-pwg.org. Global regulatory authorities have highlighted the importance of biomarker research and the need for the pharmaceutical industry to take the lead in this arena.[3, 6-24]

4. How are Biomarkers Being Used in Drug/Vaccine Development?

Biomarker research is currently being used in drug/vaccine development to:

- Explain variability in response among participants in clinical trials
- Better understand the mechanism of action or metabolism of investigational drugs
- Obtain evidence of pharmacodynamic activity (i.e., how the drug affects the body) at the molecular level
- Address emerging clinical issues such as unexpected adverse events
- Determine eligibility for clinical trials to optimize trial design
- Optimize dosing regimens to minimize adverse reactions and maximize efficacy
- Develop drug-linked diagnostic tests to identify patients who are more likely or less likely to benefit from treatment or who may be at risk of experiencing adverse events
- Provide better understanding of mechanisms of disease
- Monitor clinical trial participant response to medical interventions

Biomarker research, including research on banked samples, should be recognized as an important public health endeavor for the overall benefit of society, whether by means of advancement of medical science or by development of safer and more effective therapies.[7] Since the value of collected samples may increase over time as scientific discoveries are made, investment in long-term sample repositories is a key component of biomarker research.

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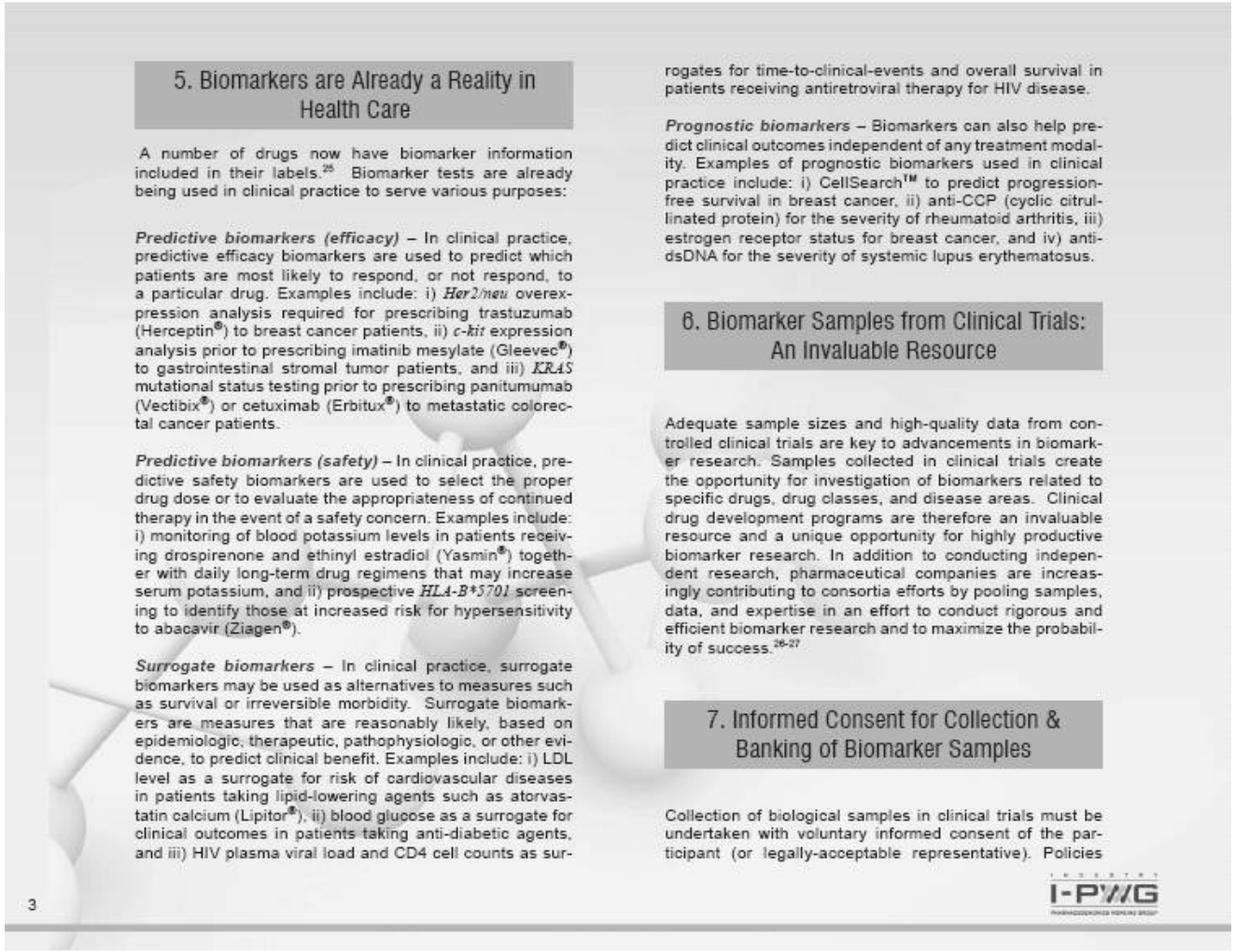

5. Biomarkers are Already a Reality in Health Care

A number of drugs now have biomarker information included in their labels.[25] Biomarker tests are already being used in clinical practice to serve various purposes:

Predictive biomarkers (efficacy) – In clinical practice, predictive efficacy biomarkers are used to predict which patients are most likely to respond, or not respond, to a particular drug. Examples include: i) *Her2/neu* overexpression analysis required for prescribing trastuzumab (Herceptin®) to breast cancer patients, ii) *c-kit* expression analysis prior to prescribing imatinib mesylate (Gleevec®) to gastrointestinal stromal tumor patients, and iii) *KRAS* mutational status testing prior to prescribing panitumumab (Vectibix®) or cetuximab (Erbitux®) to metastatic colorectal cancer patients.

Predictive biomarkers (safety) – In clinical practice, predictive safety biomarkers are used to select the proper drug dose or to evaluate the appropriateness of continued therapy in the event of a safety concern. Examples include: i) monitoring of blood potassium levels in patients receiving drospirenone and ethinyl estradiol (Yasmin®) together with daily long-term drug regimens that may increase serum potassium, and ii) prospective *HLA-B*5701* screening to identify those at increased risk for hypersensitivity to abacavir (Ziagen®).

Surrogate biomarkers – In clinical practice, surrogate biomarkers may be used as alternatives to measures such as survival or irreversible morbidity. Surrogate biomarkers are measures that are reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit. Examples include: i) LDL level as a surrogate for risk of cardiovascular diseases in patients taking lipid-lowering agents such as atorvastatin calcium (Lipitor®), ii) blood glucose as a surrogate for clinical outcomes in patients taking anti-diabetic agents, and iii) HIV plasma viral load and CD4 cell counts as surrogates for time-to-clinical-events and overall survival in patients receiving antiretroviral therapy for HIV disease.

Prognostic biomarkers – Biomarkers can also help predict clinical outcomes independent of any treatment modality. Examples of prognostic biomarkers used in clinical practice include: i) CellSearch™ to predict progression-free survival in breast cancer, ii) anti-CCP (cyclic citrullinated protein) for the severity of rheumatoid arthritis, iii) estrogen receptor status for breast cancer, and iv) anti-dsDNA for the severity of systemic lupus erythematosus.

6. Biomarker Samples from Clinical Trials: An Invaluable Resource

Adequate sample sizes and high-quality data from controlled clinical trials are key to advancements in biomarker research. Samples collected in clinical trials create the opportunity for investigation of biomarkers related to specific drugs, drug classes, and disease areas. Clinical drug development programs are therefore an invaluable resource and a unique opportunity for highly productive biomarker research. In addition to conducting independent research, pharmaceutical companies are increasingly contributing to consortia efforts by pooling samples, data, and expertise in an effort to conduct rigorous and efficient biomarker research and to maximize the probability of success.[26-27]

7. Informed Consent for Collection & Banking of Biomarker Samples

Collection of biological samples in clinical trials must be undertaken with voluntary informed consent of the participant (or legally-acceptable representative). Policies

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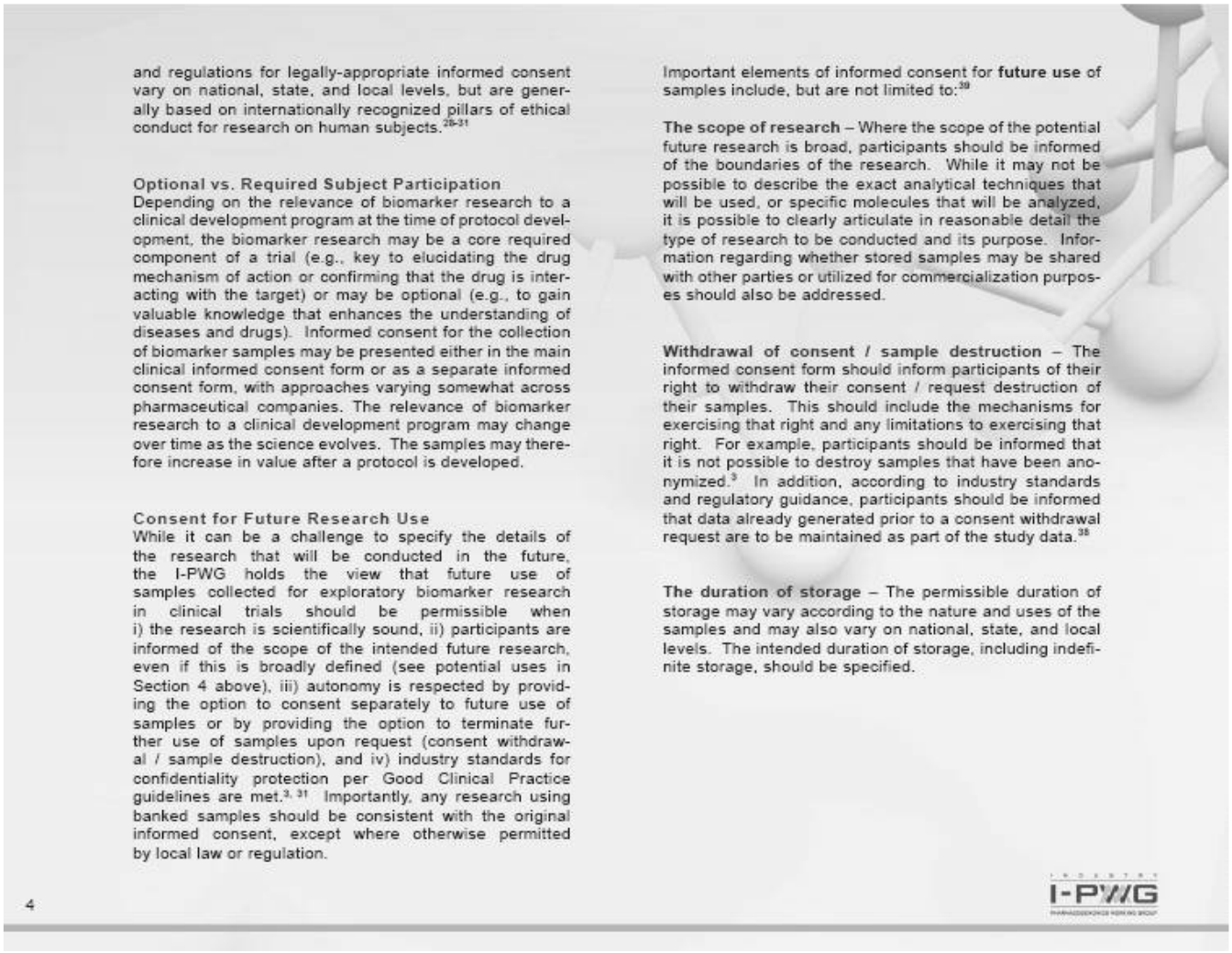

and regulations for legally-appropriate informed consent vary on national, state, and local levels, but are generally based on internationally recognized pillars of ethical conduct for research on human subjects.[28-31]

Optional vs. Required Subject Participation
Depending on the relevance of biomarker research to a clinical development program at the time of protocol development, the biomarker research may be a core required component of a trial (e.g., key to elucidating the drug mechanism of action or confirming that the drug is interacting with the target) or may be optional (e.g., to gain valuable knowledge that enhances the understanding of diseases and drugs). Informed consent for the collection of biomarker samples may be presented either in the main clinical informed consent form or as a separate informed consent form, with approaches varying somewhat across pharmaceutical companies. The relevance of biomarker research to a clinical development program may change over time as the science evolves. The samples may therefore increase in value after a protocol is developed.

Consent for Future Research Use
While it can be a challenge to specify the details of the research that will be conducted in the future, the I-PWG holds the view that future use of samples collected for exploratory biomarker research in clinical trials should be permissible when i) the research is scientifically sound, ii) participants are informed of the scope of the intended future research, even if this is broadly defined (see potential uses in Section 4 above), iii) autonomy is respected by providing the option to consent separately to future use of samples or by providing the option to terminate further use of samples upon request (consent withdrawal / sample destruction), and iv) industry standards for confidentiality protection per Good Clinical Practice guidelines are met.[3, 31] Importantly, any research using banked samples should be consistent with the original informed consent, except where otherwise permitted by local law or regulation.

Important elements of informed consent for **future use** of samples include, but are not limited to:[39]

The scope of research – Where the scope of the potential future research is broad, participants should be informed of the boundaries of the research. While it may not be possible to describe the exact analytical techniques that will be used, or specific molecules that will be analyzed, it is possible to clearly articulate in reasonable detail the type of research to be conducted and its purpose. Information regarding whether stored samples may be shared with other parties or utilized for commercialization purposes should also be addressed.

Withdrawal of consent / sample destruction – The informed consent form should inform participants of their right to withdraw their consent / request destruction of their samples. This should include the mechanisms for exercising that right and any limitations to exercising that right. For example, participants should be informed that it is not possible to destroy samples that have been anonymized.[3] In addition, according to industry standards and regulatory guidance, participants should be informed that data already generated prior to a consent withdrawal request are to be maintained as part of the study data.[38]

The duration of storage – The permissible duration of storage may vary according to the nature and uses of the samples and may also vary on national, state, and local levels. The intended duration of storage, including indefinite storage, should be specified.

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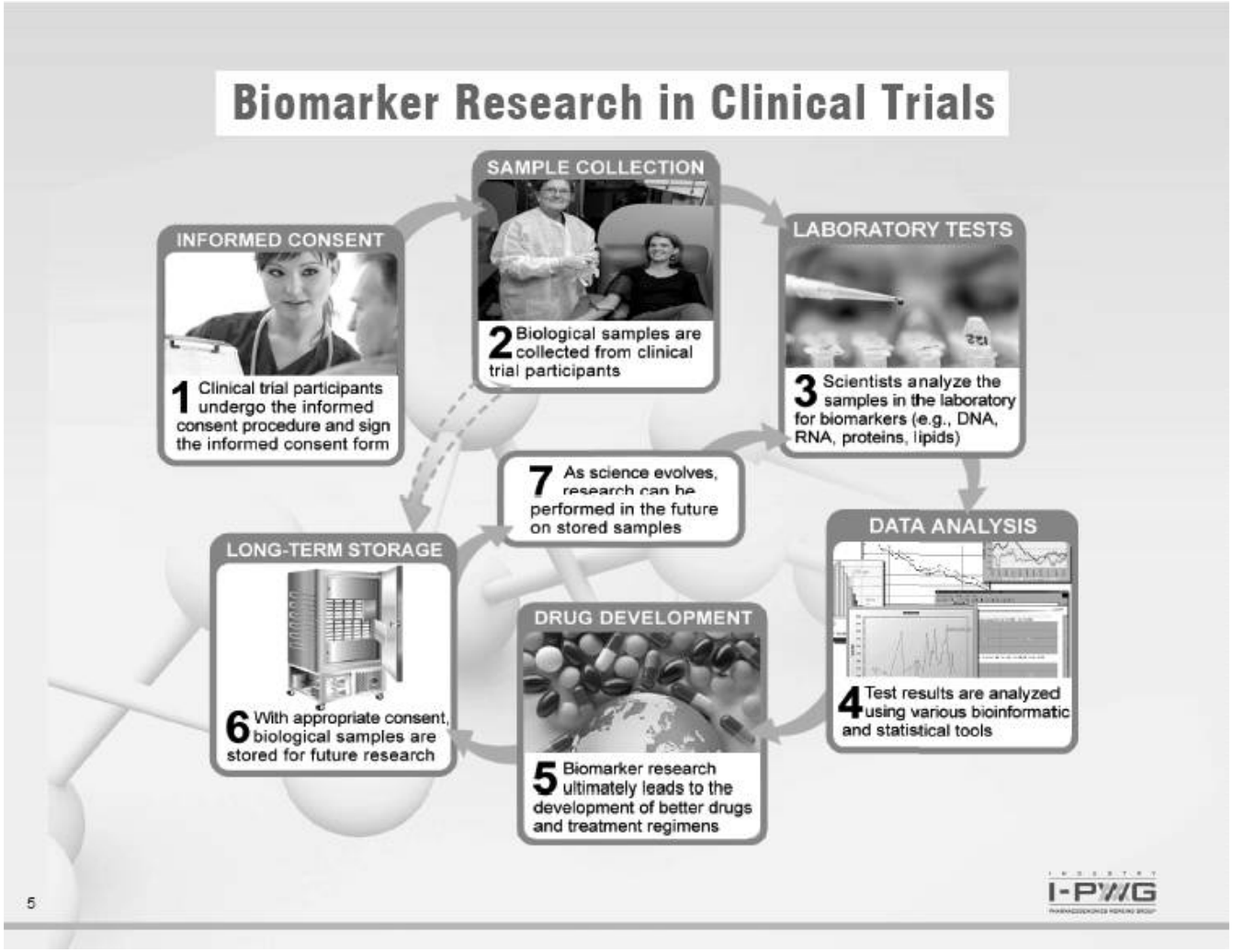
Biomarker Research in Clinical Trials
SAMPLE COLLECTION
2 Biological samples are collected from clinical trial participants
INFORMED CONSENT
1 Clinical trial participants undergo the informed consent procedure and sign the informed consent form
LABORATORY TESTS
3 Scientists analyze the samples in the laboratory for biomarkers (e.g., DNA, RNA, proteins, lipids)
7 As science evolves, research can be performed in the future on stored samples
DATA ANALYSIS
4 Test results are analyzed using various bioinformatic and statistical tools
LONG-TERM STORAGE
6 With appropriate consent, biological samples are stored for future research
DRUG DEVELOPMENT
5 Biomarker research ultimately leads to the development of better drugs and treatment regimens
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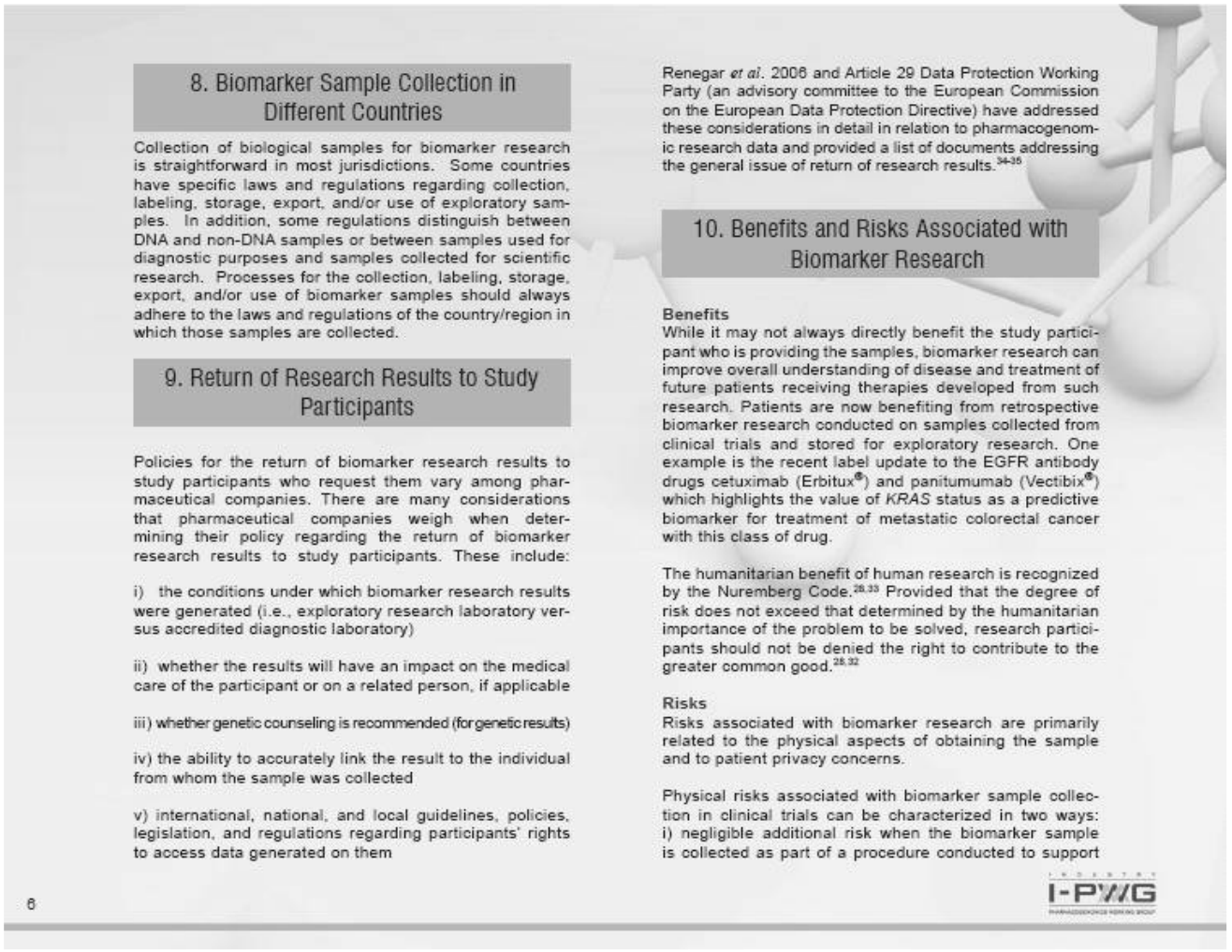

8. Biomarker Sample Collection in Different Countries

Collection of biological samples for biomarker research is straightforward in most jurisdictions. Some countries have specific laws and regulations regarding collection, labeling, storage, export, and/or use of exploratory samples. In addition, some regulations distinguish between DNA and non-DNA samples or between samples used for diagnostic purposes and samples collected for scientific research. Processes for the collection, labeling, storage, export, and/or use of biomarker samples should always adhere to the laws and regulations of the country/region in which those samples are collected.

9. Return of Research Results to Study Participants

Policies for the return of biomarker research results to study participants who request them vary among pharmaceutical companies. There are many considerations that pharmaceutical companies weigh when determining their policy regarding the return of biomarker research results to study participants. These include:

i) the conditions under which biomarker research results were generated (i.e., exploratory research laboratory versus accredited diagnostic laboratory)

ii) whether the results will have an impact on the medical care of the participant or on a related person, if applicable

iii) whether genetic counseling is recommended (for genetic results)

iv) the ability to accurately link the result to the individual from whom the sample was collected

v) international, national, and local guidelines, policies, legislation, and regulations regarding participants' rights to access data generated on them

Renegar *et al*. 2008 and Article 29 Data Protection Working Party (an advisory committee to the European Commission on the European Data Protection Directive) have addressed these considerations in detail in relation to pharmacogenomic research data and provided a list of documents addressing the general issue of return of research results.[34-35]

10. Benefits and Risks Associated with Biomarker Research

Benefits
While it may not always directly benefit the study participant who is providing the samples, biomarker research can improve overall understanding of disease and treatment of future patients receiving therapies developed from such research. Patients are now benefiting from retrospective biomarker research conducted on samples collected from clinical trials and stored for exploratory research. One example is the recent label update to the EGFR antibody drugs cetuximab (Erbitux®) and panitumumab (Vectibix®) which highlights the value of *KRAS* status as a predictive biomarker for treatment of metastatic colorectal cancer with this class of drug.

The humanitarian benefit of human research is recognized by the Nuremberg Code.[28,33] Provided that the degree of risk does not exceed that determined by the humanitarian importance of the problem to be solved, research participants should not be denied the right to contribute to the greater common good.[28,32]

Risks
Risks associated with biomarker research are primarily related to the physical aspects of obtaining the sample and to patient privacy concerns.

Physical risks associated with biomarker sample collection in clinical trials can be characterized in two ways: i) negligible additional risk when the biomarker sample is collected as part of a procedure conducted to support

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other core trial objectives, and ii) some added risk where the sampling procedure would otherwise have not been performed as a core component of a trial. Risks are also determined by the invasiveness of the sample collection procedure.

Privacy risks are generally those associated with the inappropriate disclosure and misuse of data. Pharmaceutical companies have policies and procedures for confidentiality protection to minimize this risk for all data collected and generated in clinical trials. These may vary across companies, but are based on industry standards of confidentiality and privacy protection highlighted in the following section. Importantly, privacy risks inherent to biomarker data are no greater than other data collected in a clinical trial.

11. Privacy, Confidentiality, and Patient Rights

Maintaining the privacy of study participants and the confidentiality of information relating to them is of paramount concern to industry researchers, regulators, and patients. Good Clinical Practice (GCP), the standard adhered to in pharmaceutical clinical research, is a standard that

"...provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected",

where confidentiality is defined as, *"The prevention of disclosure, to other than authorized individuals, of a sponsor's proprietary information or of a subject's identity."*

This standard dictates that *"the confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with applicable regulatory requirements."* [31]

Exploratory biomarker research in pharmaceutical development is commonly conducted in research laboratories that are not accredited to perform diagnostic tests used for healthcare decision-making. Therefore, results from exploratory biomarker research usually are not appropriate for use in making decisions about a trial participant's health. In addition, exploratory research data should not be included as part of a participant's medical record accessible for use by insurance companies. Legislation and policies to protect individuals against discrimination based on genetic information continually evolve based on social, ethical, and legal considerations. Examples of such legislation include the Human Tissue Act 2004 (UK) and the Genetic Information Nondiscrimination Act (GINA) 2008 (USA).[36-37]

12.Where to Get More Information?

Educational resources related to biomarker and pharmacogenomic research that caters to health care professionals, IRBs/IECs, scientists, and patients are continually being created and are publicly available. Links to many of these resources are available through the I-PWG website: www.i-pwg.org.

13. What is I-PWG?

The Industry Pharmacogenomics Working Group (I-PWG) (formerly the Pharmacogenetics Working Group) is a voluntary association of pharmaceutical companies engaged in pharmacogenomic research. The Group's activities focus on non-competitive educational, informational, ethical, legal, and regulatory topics. The Group provides information and expert opinions on these topics and sponsors educational/informational programs to promote better understanding of pharmacogenomic and other biomarker research for key stakeholders. The I-PWG interacts with regulatory author-

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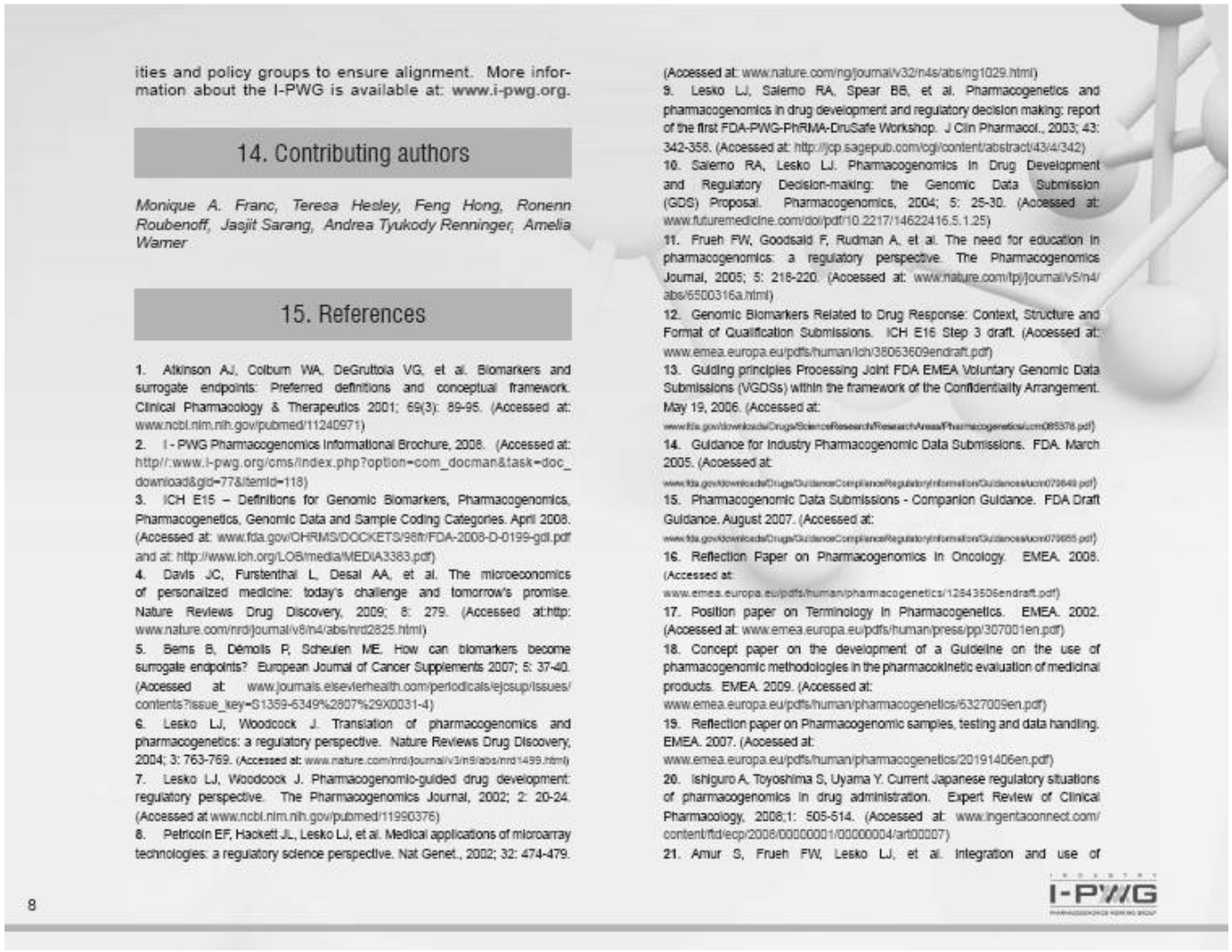

ities and policy groups to ensure alignment. More information about the I-PWG is available at: www.i-pwg.org.

14. Contributing authors

Monique A. Franc, Teresa Hesley, Feng Hong, Ronenn Roubenoff, Jaajit Sarang, Andrea Tyukody Renninger, Amelia Warner

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www.i-pwg.org

12.4 Response Evaluation Criteria in Solid Tumors

RECIST 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer

12.5 ECOG Performance Status

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

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

12.6 Common Terminology Criteria for Adverse Events

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

12.7 List of Abbreviations

Abbreviation/Term	Definition
1L	First Line
2L	Second Line
AE	Adverse Event
ADA	Anti-Drug Antibodies
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
APaT	All Patients as Treated
aPTT	Activated Partial Thromboplastin Time
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
bid	Twice a Day
ß-HCG	Beta Human Chorionic Gonadotropin
BSA	Body Surface Area
CBC	Complete Blood Count
CI	Confidence Interval
CIMP	CPG Island Methylator Phenotype
CIN	Chromosomal Instability
COPD	Chronic Obstructive Pulmonary Disease
CNS	Central Nervous System
CR	Complete Response
CRC	Colorectal Carcinoma
CrCl	Calculated Creatinine Clearance
CRF	Case Report Form
CSR	Clinical Study Report
CT	Computed Tomography
CTCAE	Common Toxicity Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte-Associated Antigen-4
dMMR	Deficient Mismatch Repair
DNA	Deoxyribonucleic acid
DOR	Duration of Response
DR	Drug Related
ECI	Events of Clinical Interest
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
EGJ	Esophagogastric Junction
eDMC	external Data Monitoring Committee
EOC	Executive Oversight Committee
EORTC	European Organisation for Research and Treatment of Cancer
ePRO	Electronic Patient Reported Outcomes
ERC	Ethics Review Committee
ESMO	European Society for Medical Oncology
FBR	Future Biomedical Research
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FDAMA	Food and Drug Administration Modernization Act
FNA	Fine Needle Aspirate

Abbreviation/Term	Definition
GCP	Good Clinical Practice
GEP	Gene Expression Profile
GI	Gastrointestinal
GFR	Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HEA	Health Economic Assessment
HIV	Human Immunodeficiency Virus
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human Papillomavirus
HR	Hazard Ratio
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
INR	International Normalized Ratio
irAE	immune-related adverse event
irRECIST	Immune related RECIST (Modification of RECIST 1.1)
irRC	Immune related Response Criteria
IRB	Institutional Review Board
ITIM	Immunoreceptor Tyrosine-based Inhibition Motif
ITSM	Immunoreceptor Tyrosine-based Switch Motif
ITT	Intention To Treat
IV	Intravenous
IVRS	Interactive Voice Response System
IWRS	Integrated Web Response System
Kg	Kilogram
LDH	Lactate Dehydrogenase
mAb	Monoclonal Antibody
mCRC	Metastatic Colorectal Carcinoma
mcL	Microliters
MEL	Melanoma
Mg	Milligram
Mg/kg	Milligram per Kilogram
mL	milliliter
MRI	Magnetic Resonance Imaging
MSD	Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.
MSI-H	Microsatellite Instability High
MTD	Maximum Tolerated Dose
NA or N/A	Not Applicable
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NSAID	Non-Steroidal Anti-inflammatory Drug
NSCLC	Non-Small Cell Lung Cancer
ORR	Objective Response Rate
OS	Overall Survival
OTC	Over-the-counter
PBPK	Physiologically based pharmacokinetic
PD	Progressive Disease

Abbreviation/Term	Definition
PFS	Progression Free Survival
PGt	Pharmacogenetic
PIN	Personal Identification Number
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/Pharmacodynamic
PO	Oral Administration
PR	Partial Response
PRO	Patient Reported Outcomes
PT	Prothrombin Time
PS	Performance Status
QoL	Quality of Life
R/M	Recurrent or Metastatic
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic Acid
RR	Response Rate
QoL	Quality of Life
Q2W	Every 2 Weeks
Q3W	Every 3 Weeks
Q9W	Every 9 Weeks
RT	Radiation Therapy
SAE	Serious Adverse Events
SAP	Statistical Analysis Plan
SCC	Squamous Cell Carcinoma
SD	Stable Disease
SFU	Survival Follow-Up
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SIM	Site Imaging Manual
SOP	Standard Operating Procedures
TIL	Tumor Infiltrating Lymphocytes
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
WBC	White Blood Cell

12.8 Germany-specific Requirements

Section 5.1.3 Exclusion Criteria

Exclusion Criterion: HIV 1/2 antibodies testing is required for participants when the investigator has reason to suspect the patient has Human Immunodeficiency Virus infection or is otherwise mandated per local guidance.

Exclusion Criterion: Hepatitis B surface Antigen reactive and HCV RNA [qualitative] testing is only required when the investigator has reason to suspect the patient has an Hepatitis B, Hepatitis C infection or testing is mandated per local guidance.

13.0 SIGNATURES

13.1 Sponsor's Representative

TYPED NAME	
TITLE	
SIGNATURE	
DATE SIGNED	

13.2 Investigator

I agree to conduct this clinical trial in accordance with the design outlined in this protocol and to abide by all provisions of this protocol (including other manuals and documents referenced from this protocol). I agree to conduct the trial in accordance with generally accepted standards of Good Clinical Practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse events as defined in Section 7.0 – Assessing and Recording Adverse Events. I also agree to handle all clinical supplies provided by the Sponsor and collect and handle all clinical specimens in accordance with the protocol. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information. Since the information in this protocol and the referenced Investigator's Brochure is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the trial is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure or access by third parties.

TYPED NAME	
TITLE	
SIGNATURE	
DATE SIGNED	

Supplemental Statistical Analysis Plan (sSAP)

Table of Contents

List of Tables

1 INTRODUCTION

This supplemental SAP (sSAP) is a companion document to the protocol. In addition to the information presented in the protocol SAP which provides the principal features of confirmatory analyses for this trial, this supplemental SAP provides additional statistical analysis details/data derivations and documents modifications or additions to the analysis plan that are not "principal" in nature and result from information that was not available at the time of protocol finalization.

2 SUMMARY OF CHANGES

Section Number (s)	Section Title(s)	Description of Change (s)	Rationale
3.4.1.1	Primary Efficacy Endpoint	DOR definition updated	Using the most up to date DOR definition in oncology SAP template.
3.6.1	Statistical Methods for Efficacy Analyses	The Table 1: censoring rules for DOR; and Table 2: Censoring rules for PFS analysis are updated.	Updates are based on the changes of censoring rules in the latest SAP template.
3.6.1	Statistical Methods for Efficacy Analyses	Added the sentence: "The secondary and exploratory endpoints may be analyzed by populations as appropriate."	The secondary and exploratory endpoints may also be analyzed by all three populations.
3.6.3	Summaries of Baseline Characteristics, Demographics, and Other Analyses	Paragraphs related to biomarker analysis are deleted.	The cutoffs of GEP "low", "intermediate" and "high" have been pre-specified at -1.540 and -0.945.
3.6.4	Sample size	The sample size descriptions based on the different assumptions of "GEP intermediate or high"	The cutoffs of GEP "low", "intermediate" and "high" have been pre-specified at -1.540

Section Number (s)	Section Title(s)	Description of Change (s)	Rationale
		and "GEP high", respectively, are added.	and -0.945.

3 ANALYTICAL AND METHODOLOGICAL DETAILS

3.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below; the comprehensive plan is provided in Sections 3.2 to Section 3.9. There will be a separate PK analysis plan. Post hoc exploratory analyses will be clearly identified in the CSR.

Study Design Overview	A Phase II Study of Pembrolizumab Monotherapy in Third Line, Previously Treated Subjects with Advanced/Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus or Advanced/Metastatic Siewert Type I Adenocarcinoma of the Esophagogastric Junction (KEYNOTE – 180)
Treatment Assignment	This is a single arm open-label study.
Analysis Populations	All Subjects as Treated (ASaT)
Primary Endpoint(s)	ORR based on RECIST 1.1 assessed by central imaging vendor
Statistical Methods for Key Efficacy Analyses	95% CI for ORR will be calculated using the exact binomial distribution.
Statistical Methods for Key Safety Analyses	Count and percentage of AE will be provided.
Multiplicity	Not applicable since this is an estimation study.
Sample Size	The planned sample size is approximately 100 subjects. The primary objective of the study is to estimate ORR in all subjects, in subjects whose tumors classified as "GEP intermediate or high" (GEP $\geq$ -1.540) and subjects whose tumors classified as "GEP high" (GEP $\geq$ -0.945).

3.2 Responsibility for Analyses/In-House Blinding

The statistical analysis of the data obtained from this study will be the responsibility of the Clinical Biostatistics department of the SPONSOR.

This trial is being conducted as an open-label study, i.e., subjects, investigators, and SPONSOR personnel will be aware of subject treatment.

The Clinical Biostatistics department will generate the allocation schedule.

3.3 Estimation

Objectives of the study and estimation to be performed are stated in Section 3.0 in the protocol.

3.4 Analysis Endpoints

3.4.1 Efficacy Endpoints

3.4.1.1 Primary Efficacy Endpoint

- **Objective response rate (ORR) - RECIST 1.1 assessed by central imaging vendor**

Objective response rate is defined as the proportion of the subjects in the analysis population who have a complete response (CR) or partial response (PR). Responses are based upon blinded central imaging vendor assessments per RECIST 1.1.

3.4.1.2 Secondary Efficacy Endpoints

- **Disease Control Rate (DCR) - RECIST 1.1 assessed by central imaging vendor**

Disease control rate (DCR) is defined as the percentage of subjects who have achieved confirmed CR or PR or have demonstrated SD prior to any evidence of progression.

- **Duration of Response (DOR) - RECIST 1.1 assessed by central imaging vendor**

For subjects who demonstrate CR or PR, duration of response is defined as the time from first documented evidence of CR or PR until disease progression or death due to any cause, whichever occurs first.

- **Progression-free Survival (PFS) - RECIST 1.1 assessed by central imaging vendor**

PFS is defined as the time from first day of study treatment to the first documented disease progression or death due to any cause, whichever occurs first.

- **Overall survival (OS)**

OS is defined as the time from first day of study treatment to death due to any cause. Subjects without documented death at the time of the final analysis will be censored at the date of the last follow-up.

3.4.1.3 Exploratory Efficacy Endpoints

- Progression-free Survival (PFS) - irRECIST assessed by central imaging vendor

Progression-free-survival (PFS) is defined as the time from first day of study treatment to the first confirmed disease progression or death due to any cause, whichever occurs first.

- ORR – - irRECIST assessed by central imaging vendor

Overall response rate is defined as the proportion of the subjects in the analysis population who have a complete response (CR) or partial response (PR). Responses are based upon blinded central imaging vendor assessments per irRECIST.

- Duration of Response (DOR) - irRECIST assessed by central imaging vendor

For subjects who demonstrated CR or PR, response duration is defined as the time from the date of first response (CR or PR) until the date of confirmed disease progression or death.

3.4.2 Safety Endpoints

Safety measurements are described in Section 7 Trial Procedures in the protocol.

3.5 Analysis Populations

3.5.1 Efficacy Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of ORR, DCR, PFS, and OS. The ASaT population consists of all allocated subjects who received at least one dose of study treatment.

The analysis population for DOR consists of responders.

Details on the approach to handling missing data are provided in Section 3.6 Statistical Methods.

3.5.2 Safety Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of safety data in this study. The ASaT population consists of all allocated subjects who received at least one dose of study treatment.

At least one laboratory or vital sign measurement obtained subsequent to at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

Details on the approach to handling missing data for safety analyses are provided in Section 3.6 Statistical Methods.

3.6 Statistical Methods

3.6.1 Statistical Methods for Efficacy Analyses

This section describes the statistical methods that address the primary and secondary objectives.

For the primary efficacy endpoint of ORR, the point estimate and 95% confidence interval will be provided using exact binomial method proposed by Clopper and Pearson (1934)[Ref. 5.4: 03RMKM]. Subjects in the primary analysis population (ASaT) without ORR data will be counted as non-responders.

For DCR, the point estimate, 95% confidence interval will be provided using the exact binomial method proposed by Clopper and Pearson (1934)[Ref. 5.4: 03RMKM]. Subjects in the analysis population (ASaT) with missing DCR data are considered as disease not under control.

For DOR, Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided, as appropriate. Subjects who are alive, have not progressed, have not initiated new anti-cancer treatment, and have not been determined to be lost to follow-up are considered ongoing responders at the time of analysis. Censoring rules for DOR are summarized in Table 1.

Table 1. Censoring Rules for DOR

Situation	Date of Progression or Censoring	Outcome
No progression nor death, no new anti-cancer therapy initiated	Last adequate disease assessment	Censored (non-event)
No progression nor death, new anti-cancer therapy initiated	Last adequate disease assessment before new anti-cancer therapy initiated	Censored (non-event)
≥ 2 consecutive missed disease assessments at any time prior to progression or death	Last adequate disease assessment prior to ≥ 2 missed adequate disease assessments	Censored (non-event)
Death or progression after ≤ 1 missed adequate disease assessments	PD or death	End of response (Event)
A missed disease assessment includes any assessment that is not obtained or is considered inadequate for evaluation of response. Subjects are considered to have an ongoing response if censored, alive, have not progressed, have not started a new anti-cancer therapy, have not had ≥ 2 consecutive missed disease assessments, and have not been determined to be lost to follow-up.		

For PFS and OS endpoints, Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided, as appropriate. The censoring rules for PFS are summarized in Table **2**. The efficacy analysis is summarized in Table **3**. The secondary and exploratory endpoints may be analyzed by populations as appropriate.

Table 2 Censoring rules for PFS analysis

Situation	Primary Analysis
No PD and no death; new anticancer treatment is not initiated	Censored at last disease assessment
No PD and no death; new anticancer treatment is initiated	Censored at last disease assessment before new anticancer treatment

Table 3 Analysis Strategy for Efficacy Variables

Endpoint/Variable‡ **(Description, Time Point)**	**Statistical Method**	**Analysis Population**	**Missing Data Approach**
Primary Endpoint and Hypothesis			
ORR • RECIST 1.1, Central imaging vendor assessment	Exact method based on binomial distribution	ASaT • All subjects • Subjects whose tumors classified as "GEP intermediate or high" (GEP ≥ -1.540) • Subjects whose tumors classified as "GEP high" (GEP ≥ -0.945)	Subjects with missing data are considered non-responders

Endpoint/Variable‡ (Description, Time Point)	**Statistical Method**	**Analysis Population**	**Missing Data Approach**
Secondary Endpoints			
DCR • RECIST 1.1, Central imaging vendor assessment	Exact method based on binomial distribution	ASaT	Subjects with missing data are considered as disease not under control
DOR • RECIST 1.1, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	All responders	Non-responders are excluded from analysis
PFS • RECIST 1.1, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	ASaT	Censoring rules follow Table 2.
OS	Summary statistics using Kaplan-Meier method	ASaT	Censored at last known alive date
Exploratory Endpoints			
ORR • irRECIST, Central imaging vendor assessment	Exact method based on binomial distribution	ASaT • All subjects • Subjects whose tumors classified as "GEP intermediate or high" (GEP ≥ -1.540) • Subjects whose tumors classified as "GEP high" (GEP ≥ -0.945)	Subjects with missing data are considered non-responders

Endpoint/Variable‡ (Description, Time Point)	**Statistical Method**	**Analysis Population**	**Missing Data Approach**
PFS • irRECIST, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	ASaT	Censoring rules per irRECIST also follow Table 2. If there is no confirmation scan available after the initial PD scan, then it is considered as a PFS event at the initial PD scan time point.
DOR • irRECIST, Central imaging vendor assessment	Summary statistics using Kaplan-Meier method	All responders	Non-responders are excluded from analysis; If there is no confirmation scan available after the initial PD scan, then it is considered as a PFS event at the initial PD scan time point.

3.6.2 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs, laboratory tests, and vital signs. Count and percentage of AEs will be provided.

3.6.3 Summaries of Baseline Characteristics, Demographics, and Other Analyses

The number and percentage of subjects screened, enrolled, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized either by descriptive statistics or categorical tables for all enrolled subjects.

3.6.4 Sample Size

In this study, approximately 100 subjects with previously treated, advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ will be enrolled.

Table 4 shows the two-sided 95% confidence interval of ORR with 100 subjects for different observed response rates.

Table 4 Two-sided 95% Confidence Interval of ORR with 100 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
17	17%	(10.2, 25.8)
25	25%	(16.9, 34.7)
30	30%	(21.2, 40.0)
35	35%	(25.7, 45.2)

For the subjects with tumors classified as "GEP intermediate or high" (GEP ≥ -1.540), if the prevalence is about 60%, there will be approximately 60 such subjects. Table **5** shows the two-sided 95% CI of ORR with 60 subjects for different response rates. For example, if the observed ORR is 35% in this group, the 95% CI of this estimate of ORR is (23.1%, 48.4%).

Table 5 Two-sided 95% Confidence Interval for ORR with 60 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
15	25%	(14.7, 37.9)
18	30%	(18.8, 43.2)
21	35%	(23.1, 48.4)
24	40%	(27.6, 53.5)

For the subjects whose tumors classified as "GEP high" (GEP ≥ -0.945), if the prevalence of this group is about 40%, there will be approximately 40 such subjects. Table 6 shows the two-sided 95% CI of ORR with 40 subjects for different response rates. For example, if the observed ORR is 35% in this group, the 95% CI of this estimate of ORR is (20.6%, 51.7%).

Table 6 Two-sided 95% Confidence Interval for ORR with 40 Subjects

Number of Observed Responders	ORR Estimates	95% CI of ORR (%)
12	30%	(16.6, 46.5)
14	35%	(20.6, 51.7)
16	40%	(24.9, 56.7)

3.7 Subgroup Analyses and Effect of Baseline Factors

The estimate of the treatment effect for the primary endpoint will be estimated and/or plotted within each category of the following classification variables:

- Age category (≤65 vs. >65 years)
- Sex (Female vs. Male)
- Race (Asian vs. non-Asian)
- Histology (Adenocarcinoma vs. Squamous Cell Carcinoma vs. Siewert type 1 adenocarcinoma of the EGJ)

3.8 Compliance (Medication Adherence)

Drug accountability data for MK-3475 will be collected during the study. Any deviation from protocol-directed administration will be reported.

3.9 Extent of Exposure

Extent of Exposure for a subject is defined as number of cycles in which the subject receives the study medication infusion. Summary statistics will be provided on Extent of Exposure for ASaT population.

LIST OF REFERENCES

[Ref. 5.4: 03RMKM] Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika 1934;26(4):404-13.