Synopsis

Title	Phase II Study of **Zanidatamab** in Combination with **Pembrolizumab** and **Chemotherapy** in HER2 and PD-L1 Positive Metastatic Gastroesophageal Adenocarcinoma (GEA) Patients
Short title	ZANGEA
Trial type	Prospective, single arm, open-label, multicenter, multinational phase II study
Indication	Patients with previously untreated metastatic HER2 and PD-L1 positive gastroesophageal adenocarcinoma
Investigational medicinal product	Zanidatamab in combination with pembrolizumab and mFOLFOX
Objectives / endpoints	**Primary Objective** To evaluate the efficacy of zanidatamab in combination with pembrolizumab and chemotherapy in previously untreated HER2 and PD-L1 positive metastatic gastroesophageal adenocarcinomas (GEA). Corresponding endpoint • Progression-free survival rate at 12 months (PFS@12), estimated using Kaplan Meier method and defined as proportion of patients alive and progression-free 12 months after start of trial medication **Secondary Objectives** To further determine the efficacy of zanidatamab in combination with pembrolizumab and chemotherapy in metastatic GEA. Corresponding endpoints • Overall response rate (ORR), defined as proportion of patients achieving complete or partial response (CR/PR) acc. to RECIST v1.1. • Disease control rate (DCR), defined as proportion of patients achieving CR, PR, or stable disease (SD) acc. to RECIST v1.1. • Duration of response (DoR), defined as time from response initiation (when either CR or PR is first determined) to disease progression acc. to RECIST v1.1 or death due to any cause. • Progression-free survival (PFS), defined as time from first treatment until date of progression acc. to RECIST v1.1 or death due to any cause. • Overall survival (OS), defined as time from first treatment until date of death due to any cause. To evaluate safety and tolerability of zanidatamab in combination with pembrolizumab and chemotherapy in metastatic GEA. Corresponding endpoints • Assessment of safety of the treatment as determined by the incidence, nature, causality, frequency, timing, and severity of adverse events using NCI CTCAE v5.0

	To assess quality of life (QoL) data Corresponding endpoints • Assessment of QoL during treatment and follow-up using validated EORTC QLQ-C30 [Aaronson NK et al. 1993] and EQ-5D-5L [The EuroQol Group 1990] questionnaires. **Exploratory Objectives** Endpoints • Preplanned matched-pair analyses comparing the study arm to a historical study arm with trastuzumab, chemotherapy and PD-1 inhibitor (AIO INTEGA) in terms of efficacy, tolerability, and translational data • To correlate analysis between selected molecular tumor (such as CPS cut-offs ≤ 5 and >5 – 10, as well as HER2 IHC2+/ISH+ vs. IHC3+), serum and microbiome parameters and clinical data to identify molecular biomarkers predictive for tumor response, toxicity, and survival.
Trial design	Patients eligible for this trial will receive the following treatment: • Zanidatamab 1,200 mg (patients <70 kg at baseline) or 1,600 mg (patients ≥70 kg at baseline), administered i.v. on day 1 of each 2-week cycle (Q2W) • Pembrolizumab 400 mg, administered i.v. on day 1 of every third cycle (Q6W) • Chemotherapy: modified FOLFOX (Oxaliplatin 85 mg/m^2 i.v. on day 1; Folinic Acid 400 mg/m^2 i.v. on day 1; 5-FU 2,400 mg/m^2 i.v. continuous infusion over 48 hours (no bolus!) on days 1 and 2 of each cycle) (Q2W) The treatment will be performed until disease progression, unacceptable toxicity, patients' withdrawal of consent, or end of protocol-defined treatment time (maximum of 24 months). All patients will be followed up for 24 months after last patient in or until death, withdrawal of consent or loss to follow-up, whatever occurs first.
Rationale	On a global scale, there are approximately 1.7 million newly diagnosed cases of gastric and esophageal cancer annually [Sung et al. 2021]. These cancers remain a significant contributor to cancer-related deaths, accounting for over 1.3 million fatalities each year. Around 75% of patients with gastroesophageal adenocarcinoma (GEA) experience recurrent or metastatic disease at some stage, underscoring the challenging nature of these malignancies. Palliative systemic therapy remains the primary approach for treating these patients. For human epidermal growth factor receptor 2 (HER2)-positive GEA identified through immunohistochemistry (IHC) with a score of 3+ or 2+ and amplification via *in situ* hybridization (ISH), the established standard of care entails combining the HER2 antibody trastuzumab with chemotherapy [Bang et al. 2010; Tabernero et al. 2018]. Moreover, for patients exhibiting a programmed death-ligand 1 (PD-L1) Combined Positive Score (CPS) of ≥1, both the EMA and FDA have authorized the incorporation of the PD-1 inhibitor pembrolizumab into this

	treatment regimen, based on data derived from the KEYNOTE 811 trial [Janjigian et al. 2021; Janjigian et al. 2023]. Zanidatamab is a novel humanized, immunoglobulin G isotype 1 (IgG1)-like bispecific antibody targeting HER2, which has shown encouraging antitumor activity in early phase studies in multiple cancers, including GEA. Zanidatamab binds to the juxtamembrane extracellular domain (ECD4) and the dimerization domain (ECD2) of HER2 and induces formation of receptor clusters and receptor internalization resulting in downregulation of HER2 on the cell surface [Weisser et al. 2023]. The currently ongoing HERIZON-GEA-01 trial evaluates zanidatamab in combination with chemotherapy and with or without PD-1 inhibition by tislelizumab compared to chemotherapy and trastuzumab [Tabernero et al. 2022]. The ZANGEA trial will evaluate pembrolizumab and chemotherapy in combination with zanidatamab instead of trastuzumab in terms of efficacy and tolerability. In addition, preplanned matched-pair analyses comparing the study arm to a historical study arm with trastuzumab, chemotherapy and PD-1 inhibitor (AIO INTEGA) in terms of efficacy, tolerability and translational data is planned. For the patient group under evaluation in this trial, standard of care would be chemotherapy and pembrolizumab in combination with trastuzumab. The only adaptation within this standard regimen would be the replacement of trastuzumab by zanidatamab, which has an improved HER2 blocking mode of action [Weisser et al. 2023]. In addition, results from the randomized phase III HERIZON-GEA-01 trial will be available upon start of this trial, which will inform about the comparative tolerability and efficacy of chemotherapy in combination with trastuzumab or zanidatamab with or without PD-1 inhibitor.
Inclusion criteria	The following criteria must be met to be eligible for the study: 1. Patient* has signed and dated a written informed consent form in accordance with regulatory and institutional guidelines and approved by an institutional Review Board / Independent Ethics Committee. This must be obtained before the performance of any protocol-related procedures that are not part of normal subject care. 2. Patient is, in the investigator's judgement, willing and able to comply with scheduled visits, treatment schedule, laboratory tests and other requirements of the study. *3.* Patient is ≥ 18 years of age at time of signing the written informed consent. 4. Patient has been diagnosed with histologically confirmed unresectable advanced/metastatic HER2-positive (defined as IHC 3+ or IHC 2+ with ISH+) and PD-L1-positive (combined positive score CPS ≥ 1) gastroesophageal adenocarcinoma per local standard assessment of new or archival tumor tissue. Results of local HER2 and PD-L1 assessment will be retrospectively confirmed by central pathological re-assessment.

	Note: In case of metachronous metastases, particularly in case of prior treatment with PD-(L)1-antibodies, a fresh re-biopsy should be performed for immunohistochemistry testing (local pathology), if feasible. 5. Patient has assessable disease (measurable or non-measurable) per RECIST v1.1. 6. Patient did not receive previous palliative treatment. Prior adjuvant or neoadjuvant chemotherapy, immunotherapy, radiotherapy and/or chemoradiotherapy (but not anti HER2-targeted treatment) are permitted as long as the last administration of the last regimen (whichever was given last) occurred at least 6 months prior to enrolment. 7. Patient has ECOG performance status ≤ 1. 8. Patient has adequate hepatic, renal and hematologic functions: a) Absolute number of neutrophils (ANC) ≥ 1.5 x 10^9/L b) Platelets ≥ 100x10^3/µL c) Serum creatinine ≤ 1.5 x ULN or creatinine clearance (measured by 24 h urine) ≥ 30 mL/min (i.e., if serum creatinine level is > 1.5 x upper limit of normal (ULN), then a 24-hour urine test must be performed to check the creatinine clearance to be determined. d) AST (SGOT) and ALT (SGPT) ≤ 3.0 x ULN (or ≤ 5.0 x ULN if liver metastases are present) e) Total Bilirubin ≤ 1.5 x ULN (or < 3.0 x ULN in case of prior liver involvement or Gilbert's Syndrome) 9. Patient has adequate coagulation function as defined by International Normalized Ratio (INR) ≤ 1.5, and a partial thromboplastin time (PTT) ≤ 5 seconds above the ULN (unless receiving anticoagulation therapy). 10. Women of childbearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotrophin [hCG]) within 7 days prior to the start of study drug. Women must not be breastfeeding. WOCBP must use a highly effective method(s) of contraception during the treatment period and for 4 months after last dose of zanidatamab and/or pembrolizumab, or 6 months after the last dose of chemotherapy, whichever occurs last. Males who are sexually active with WOCBP must agree to remain abstinent or follow instructions for method(s) of contraception during the treatment and for 4 months after the last dose of zanidatamab and/or pembrolizumab, or 6 months after the last dose of chemotherapy, whichever occurs last. In addition, male subjects must be willing to refrain from sperm donation during this time. * There are no data that indicate special gender distribution. Therefore, patients will be enrolled in the study gender-independently.
Exclusion criteria	Patients who meet at least one of the following criteria are not eligible for trial participation:

	1. Patient has any known contraindication including allergy or hypersensitivity to the trial drugs or any constituent of the products as well as a known history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies. 2. Patient received prior anti HER2-targeted treatment for GEA. 3. Patient has malignancies other than the disease under study within 5 years prior to inclusion, except for those with a negligible risk of metastasis or death (e.g., expected 5-year OS > 90%) treated with expected curative outcome (such as adequately treated carcinoma in situ of the cervix, basal or squamous cell skin cancer, localized prostate cancer treated surgically with curative intent, ductal carcinoma in situ treated surgically with curative intent). 4. Patient has untreated known CNS metastases. Patient is eligible, if previous CNS metastases are adequately treated and patient has neurologically returned to baseline (except for residual signs or symptoms related to the CNS treatment) for ≥ 2 weeks prior to enrolment, and did not receive corticosteroids, or is on a stable or decreasing dose of < 10 mg daily prednisone (or equivalent) for ≥ 2 weeks prior to inclusion. 5. Patient has abnormal baseline left ventricular ejection fraction (LVEF < 50 %), assessed by echocardiogram, multigated acquisition (MUGA) scan, or cardiac magnetic resonance imaging (MRI) scan. 6. Patient has active, known, or suspected autoimmune disease. Exception: Type I diabetes mellitus, residual hypothyroidism due to autoimmune thyroiditis only requiring hormone replacement, or skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment are permitted to enroll. For any cases of uncertainty, it is recommended that the medical expert/sponsor be consulted prior to signing informed consent. 7. Patient has a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of trial drug administration. Inhaled or topical steroids, and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. 8. Patient has persisting toxicity related to prior therapy (NCI CTCAE v.5.0 Grade > 1); however, alopecia, sensory neuropathy Grade ≤ 2, or other Grade ≤ 2 not constituting a safety risk based on investigator's judgment are acceptable. 9. Patient has any serious or uncontrolled medical disorder or active infection that, in the opinion of the investigator, may increase the risk associated with trial participation, trial drug administration, or would impair the ability of the patient to receive trial drug. 10. Patient has significant acute or chronic infections including, among others: - Any positive test for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS). - Any positive test result for hepatitis B virus or hepatitis C virus indicating acute or chronic infection.

	11. Patient has history of allogeneic tissue / solid organ transplant. 12. Patient has been incarcerated or involuntarily institutionalized by court order or by the authorities [§ 40 Abs. 1 S. 3 Nr. 4 AMG]. 13. Patient is unable to consent because he/she does not understand the nature, significance and implications of the clinical trial and therefore cannot form a rational intention in the light of the facts [§ 40 Abs. 1 S. 3 Nr. 3a AMG]. 14. Patient has evidence of any other disease, neurologic or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of any of the trial medications, puts the patient at higher risk for treatment-related complications or may affect the interpretation of trial results. 15. Patient currently participates in any other interventional clinical study within 30 days before the first administration of the investigational product or at any time during the trial, unless it is an observational (non-interventional) study, or during the follow-up period of an interventional study with last dose of investigational product ≥28 days prior to enrolment in this trial. 16. Patient has a known complete absence of dihydropyrimidine dehydrogenase (DPD) activity or use of any medications known to inhibit DPD (including brivudine, sorivudine and analogs) within 4 weeks prior to enrolment. 17. Female patients, who are pregnant or breast feeding or planning to become pregnant within and 6 months after the end of treatment. Female patients of childbearing potential must have a negative serum pregnancy test result within 7 days prior to initiation of trial treatment.
Sample size	A total of N=80 patients will be included in the trial. The present trial is designed as a single arm phase II study, which aims to estimate the therapeutic efficacy of the experimental regimen (zanidatamab in combination with pembrolizumab and FOLFOX) in HER2 and PD-L1 positive metastatic gastroesophageal adenocarcinomas to complement the results of the randomized phase 3 HERIZON-GEA-01 trial with a different PD-1 inhibitor and chemotherapy backbone. The PFS rate at 12 months (PFS@12), based on the ITT population, is the primary efficacy endpoint. PFS@12 will be estimated using Kaplan Meier analysis and the corresponding 95% CI calculated using Greenwood method will be presented. Based on historical data from KEYNOTE-811 (PFS@12 ~46% with pembrolizumab plus trastuzumab and chemotherapy), a PFS@12 of ~55% is anticipated. With 80 patients, the 95% confidence interval for PFS@12 is expected to have a width of approximately 22 percentage points, providing sufficient precision to allow descriptive comparison with historical results.
Efficacy evaluations/ criteria	On study, tumor assessment (CT or MRI) will be performed at baseline (≤ 4 weeks before treatment start) and every 8 weeks (Q8W ± 7 days) during trial treatment for up to 12 months calculated from the date of treatment initiation according to

	the standard of care. Response criteria according to RECIST v1.1 will be used by the site for treatment decisions until evidence of tumor progression. Subjects who completed 12 months of treatment or discontinued trial intervention for reasons other than progressive disease will have further follow-up for disease status performed Q12W ± 14 days (which is in line with the routine follow-up tumor assessment) until progression, initiation of another anti-cancer treatment, withdrawing consent, loss to follow-up death, or end of the trial, whichever occurs first. A change from CT into MRI in the follow-up period is possible at any time. All subjects will be followed for survival until death, withdrawing consent, loss to follow-up or end of the study, whichever occurs first.
Translational research (optional)	Tumor tissue, CT scans (obtained at tumor assessments), as well as blood and stool samples will be collected for analysis in an concomitant translational research (TR) project if patients give their consent. Archival tumor biopsy samples will be collected for concomitant translational research whenever possible. Re-biopsy should be performed at time of progression if performed according to investigator's clinical judgment and local guidelines and standards and as far as considered feasible and safe for the patient. In addition, the following will be collected: • 20 mL blood samples in STRECK's cell-free DNA BCT tubes collected at baseline, after 4 weeks, as well as 6 months after initiation of treatment or upon progression (whatever occurs first). and • Stool samples (using OMNIgene self-collection kits [OM-200 and ME-200] and a toilet accessory kit [OM-AC-1] at home), collected at baseline, at 4 weeks, as well as 6 months after initiation of treatment or upon progression (whatever occurs first). In addition, stool samples will be obtained in case of first event of grade 3/4 diarrhea. Blood samples will be used for ctDNA, cytokine/chemokine analysis, microbiota-derived metabolites, and T-cell receptor (TCR) sequencing. Additionally, tumor tissue will be used to identify cancer-specific mutations and analyze the tumor microenvironment (TME); stool samples will be used for microbiota analysis. In addition, dietary patterns will be assessed at baseline, at 4 weeks, as well as 6 months after initiation of treatment or upon progression (whatever occurs first), through questionnaires, which participants can complete either at study centers or at home.
Safety evaluation	Safety assessments including physical examinations with vital signs (blood pressure, heart rate, respiratory rate, oxygen saturation and body temperature), performance status (ECOG), clinical laboratory profile and continuous assessment of adverse events will be performed at every visit. Adverse events (AEs) will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI CTCAE v5.0 Each subject will be followed during the treatment and for 30 days after last

	administration of study medication for AEs, and for 90 days after last administration for serious adverse events (SAE), adverse events of special interest (AESIs), or immune-mediated AEs, or until initiation of new anti-cancer therapy, whichever occurs first. SAEs and AESIs which are characterized as related to the treatment will be collected even after expiration of the 90 days after the last administration of study medication There will be monitoring of safety parameters (e.g., SAEs reported, any potential unexpected adverse events, accumulation of certain non-serious AEs) by an independent safety monitoring board (SMB) to identify any risks for patient safety. The SMB will meet every 3-6 months.
Number of trial sites	This trial will be conducted at approximately 30 centers in Germany and Austria.
Duration of the trial (planned)	The estimated trial duration is 54 months (18 months recruitment plus 24 months' time span from LPI to LPLV plus 12 months study set up and closure activities). The global end of this trial is defined as the date of database closure to ensure the collection of survival data of patients and the active involvement of sites in the data cleaning process (e.g., additional source data may be requested, or an additional monitoring visit may be necessary). The Sponsor may decide to terminate the trial at any time (refer to protocol section 5.5.2 for criteria for early trial termination).