SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Rabbit monoclonal antibody for detection of HER2 antigen in histological tissue sections

Device Trade Name: PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody

Device Procode: MVC

Applicant's Name and Address: Ventana Medical Systems, Inc.
(Roche Tissue Diagnostics)
1910 E. Innovation Park Drive
Tucson, AZ 85755

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P990081/S060

Date of FDA Notice of Approval: August 25, 2026

The original PMA for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Antibody (P990081) was approved for the semiquantitative detection of c-erbB-2 (HER2) antigen in sections of formalin-fixed, paraffin embedded (FFPE) breast cancer tissue from patients for whom Herceptin® treatment is being considered. Supplement P990081/S054 was approved November 20, 2024 to expand the indication for identifying patients with biliary tract cancer (gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, and extrahepatic cholangiocarcinoma) who are eligible for treatment with Ziihera (zanidatamab-hrii). The SSEDs to support the approved indications are available on the CDRH website and are incorporated by reference here.

The current supplement (P990081/S060) was submitted to expand the indication for identifying patients with gastroesophageal adenocarcinoma (gastric, gastroesophageal junction and esophageal adenocarcinoma) who are eligible for treatment with Ziihera (zanidatamab-hrii).

II. INDICATIONS FOR USE

PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY anti-HER2 (4B5) antibody) is a rabbit monoclonal antibody intended for laboratory use for the semi-quantitative detection of HER2 antigen by immunohistochemistry (IHC) in sections of formalin-fixed, paraffin-embedded breast carcinoma, biliary tract cancer (gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, and extrahepatic cholangiocarcinoma) and gastroesophageal adenocarcinoma (gastric, gastroesophageal

junction and esophageal adenocarcinoma) tissue using the *ultra*View Universal DAB Detection Kit on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

The IHC device is indicated for identifying patients who are eligible for treatment with the following therapies in accordance with the approved therapeutic labeling:

Indication for use	HER2 Score	Therapy
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	Herceptin®
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	KADCYLA®
Breast carcinoma	IHC 3+ or ISH amplified [a]	ENHERTU® [b]
Breast carcinoma	IHC 0 with membrane staining, IHC 1+ or IHC 2+/ISH non-amplified	ENHERTU®
Biliary tract cancer [c]	IHC 3+	ZIIHERA®
Gastroesophageal adenocarcinoma [c]	IHC 3+ or IHC 2+/ISH amplified	ZIIHERA® in combination with fluoropyrimidine- and platinum-containing chemotherapy and tislelizumab-jsgr [d]
Gastroesophageal adenocarcinoma [c]	IHC 3+	ZIIHERA® in combination with fluoropyrimidine- and platinum-containing chemotherapy [d]

[a] See Scoring Convention section for use of ISH in context of the indication for use.
[b] See ENHERTU® product label for specific clinical circumstances guiding HER2 testing.
[c] Biliary tract cancer and gastroesophageal adenocarcinoma indications are only approved for use with BenchMark ULTRA instrument.
[d] See the ZIIHERA® product label for specific clinical circumstances guiding HER2 testing.

Test results should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the PATHWAY anti-HER2 (4B5) antibody labeling.

V. DEVICE DESCRIPTION

A. Device Kit Components

PATHWAY anti-HER2 (4B5) antibody contains sufficient reagent for 50 tests. One 5-mL dispenser of PATHWAY anti-HER2 (4B5) antibody contains approximately 30 µg of a rabbit monoclonal antibody directed against the human HER2 antigen. The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. There is trace fetal calf serum, approximately 0.25%, present from the stock solution. Specific antibody concentration is approximately 6 µg/mL.

PATHWAY anti-HER2 (4B5) antibody is a rabbit IgG diluted from tissue culture supernatants.

Table 1: PATHWAY anti-HER2 (4B5) Antibody Kit Components

Reagent
PATHWAY anti-HER2 (4B5) primary antibody
CONFIRM Negative Control Rabbit Ig (negative reagent control)
*ultra*View Universal DAB Detection Kit
EZ Prep Concentrate (10X)
Reaction Buffer Concentrate (10X)
ULTRA LCS (pre-dilute)
ULTRA Cell Conditioning Solution (ULTRA CC1)
Hematoxylin II counterstain
Bluing Reagent
Staining Instrument/Software
BenchMark ULTRA instrument
Host operating software: VSS 14.3 or earlier
BenchMark ULTRA Software Staining Procedure: UT PATHWAY HER2 4B5

Table 2: Overview of the PATHWAY HER2 (4B5) Antibody Assay Components

Device Components	Packaged Form	Description
PATHWAY HER2 (4B5) Antibody Assay	Dispenser: 50 tests	One 5mL dispenser of PATHWAY HER2 (4B5) Antibody Assay contains approximately 30 µg of a rabbit monoclonal antibody. The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. There is trace fetal calf serum, approximately 0.25 %, present from the stock solution. Specific antibody concentration is approximately 6 µg/mL.
	Set of 5 dispensers	***ultra*View DAB Inhibitor** contains 3.0% hydrogen peroxide solution.

Device Components	Packaged Form	Description
*ultra*View DAB IHC Detection Kit	packaged in a kit: 250 tests	***ultra*View Universal DAB H_2O_2** contains 0.04% hydrogen peroxide in a phosphate buffer solution.
		***ultra*View Universal HRP Multimer** contains a cocktail of horseradish peroxidase (HRP) labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (approximately 55 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		***ultra*View Universal DAB Chromagen** contains 0.2% w/v 3,3′-diaminobenzidine (DAB) tetrahydrochloride in a proprietary stabilizer solution with a proprietary preservative.
		***ultra*View Universal Copper** contains copper sulfate (5.0 g/L) in an acetate buffer with a proprietary preservative.
BenchMark ULTRA automated staining instrument	Instrument installed with the VSS host system software	A PC that runs on Microsoft Windows controls and monitors the BenchMark ULTRA instrument via the host operating software.
CONFIRM Monoclonal Negative Control Ig	1 dispenser packaged as 250 test kit	Intended for laboratory use as a control for nonspecific binding rabbit immunoglobulin (Ig) in sections of FFPE tissue. One 25 dispenser of CONFIRM Negative Control Rabbit Ig; contains approximately 250 µg (10 µg/mL) of a rabbit polyclonal immunoglobulin. The immunoglobulin is diluted in Tris buffered saline containing carrier protein and preservative. Total protein concentration of the reagent is approximately 3 mg/mL.

B. Device Instrumentation and Software

The PATHWAY anti-HER2 (4B5) Antibody assay is performed on the BenchMark ULTRA automated staining instruments using VSS Software versions 14.3 or earlier.

C. Specimen Preparation

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Tissue sections approximately 4 mm thick and mounted on glass slides should be used for staining.

The recommended tissue fixative is 10% neutral buffered formalin. The amount used is 15 to 20 times the volume of tissue. Since fixatives will not penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period, it is recommended that a 3 mm or smaller section of tissue is fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25°C).

D. Test Controls

Run controls are included in each staining run to establish the validity of the test results. Ventana, in the device labeling, instructs that the following controls to be run with the assay.

1. Cell Line Controls

PATHWAY anti-HER2 4-in-1 Control Slides (catalog no. 781-2991) contains four formalin-fixed cell line controls embedded in paraffin, sectioned and placed on a single charged slide and should be stained as part of the staining run. These four cell line controls are characterized by in situ hybridization for gene copy number (Table 3). When processed and stained appropriately, the cell lines should stain as described in the PATHWAY HER2 4-in-1 Control Slide package insert. If the indicated staining is not evident in the appropriate cores, especially the 1+ and 2+ controls, the staining of the tissue should be repeated. However, the PATHWAY anti-HER2 4-in-1 control slides are not intended to be used as a sole system level control for this assay.

Table 3: Characteristics of PATHWAY HER-2 4 in 1 Control Slides

HER2 IHC Score	Cell Line	HER2/Chr17 Ratio*
0	MDA-MB-231	1.11
1+	T47D	1.12
2+	MDA-MB-453	2.66
3+	BT-474	5.53

* HER2/Chr17 ratio is an average of three lots of PATHWAY HER-2 4 in 1 Control Slides determined using fluorescence in situ hybridization (FISH)

2. Positive Tissue Control

A positive control tissue fixed and processed in the same manner as the patient specimens must be run for each set of test conditions and with every PATHWAY anti-HER2 (4B5) antibody staining procedure performed. This tissue could contain both positive staining cell/tissue components and negative cell/tissue components and serve as both the positive and negative control tissue. Control tissue should be fresh autopsy/biopsy/surgical specimens prepared and fixed as soon as possible in a manner identical to test sections. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue preparation.

A tissue with weak positive staining is more suitable than strong positive staining for optimal quality control and to detect minor levels of reagent degradation. Ideally a tissue which is known to have weak but positive staining should be

chosen to ensure that the system is sensitive to small amounts of reagent degradation or problems with the IHC methodology. Generally, however, neoplastic tissue that is positive for HER2 is strongly positive due to the nature of the pathology (overexpression). An example of a positive control for PATHWAY anti-HER2 (4B5) antibody is a known weak HER2 positive invasive breast carcinoma (for example ductal or lobular), which may serve as a positive control tissue for either breast carcinoma, or BTC, or GEA patient samples. The positive staining tissue components (membrane of neoplastic cells) are used to confirm that the antibody was applied and the instrument functioned properly.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, and not as an aid in determining a specific diagnosis of patient samples.

3. Negative Tissue Control

The same tissue used for the positive tissue control (ductal or lobular invasive breast carcinoma) may be used as the negative tissue control. The non-staining components (surrounding stroma, lymphoid cells and blood vessels) should demonstrate absence of specific staining and provide an indication of specific background staining with the primary antibody. Use a tissue known to be fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of PATHWAY anti-HER2 (4B5) antibody for demonstration of HER2, and to provide an indication of specific background staining (false positive staining).

4. Negative Reagent Control

A negative reagent control must be run for every specimen to aid in the interpretation of results. A negative reagent control is used in place of the primary antibody to evaluate nonspecific staining. The slide should be stained with CONFIRM Negative Control Rabbit Ig. The incubation period for the negative reagent control should equal the primary antibody incubation period.

E. Principles of Operation

PATHWAY anti-HER2 (4B5) antibody is a rabbit monoclonal antibody, which binds to HER2 in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The specific antibody is located by a cocktail of enzyme-labeled secondary antibodies that recognize rabbit immunoglobulins followed by the addition of a secondary antibody-HRP conjugate (*ultra*View Universal DAB Detection Kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the BenchMark ULTRA instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide.

The use of pre-diluted PATHWAY anti-HER2 (4B5) antibody and ready-to-use ultraView Universal DAB Detection Kit, in combination with a BenchMark ULTRA instrument, reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting, and manual reagent application.

VENTANA primary antibodies have been developed for use on BenchMark ULTRA instruments in combination with VENTANA detection kits and accessories. The assay/staining protocol is provided in Table 4 below.

Table 4: PATHWAY anti-HER2 (4B5) Antibody Staining Protocol on BenchMark ULTRA Instrument

Tissue / Indication(s):	**Biliary Tract Cancer or Gastroesophageal Adenocarcinoma**
Instrument	**BenchMark ULTRA**
Staining Procedure	**UT PATHWAY HER2 4B5**
Protocol Step	**Protocol Parameters**
Deparaffinization [a]	Selected, 4 minutes, 72°C
Cell Conditioning [a]	ULTRA CC1, 36 minutes, Mild (95°C)
Antibody (Primary) [a]	PATHWAY HER2 4B5 Ab, 12 Min, 36°C Or Neg Ctl Rbt Ig, 12 Min, 36°C
*ultra*View DAB Detection Kit [a]	*ultra*View Inhibitor: 4 minutes, 36°C *ultra*View HRP Multimer: 8 minutes, 36°C *ultra*View DAB: 8 minutes, 36°C *ultra*View DAB H_2O_2: 8 minutes, 36°C *ultra*View Copper: 4 minutes, 36°C
ultraWash [a]	Selected
Counterstain	Hematoxylin II, 4 minutes, 36°C
Post Counterstain	Bluing Reagent, 4 minutes, 36°C

[a] Pre-programmed conditions not selectable for the user.

F. Slide Review and Interpretation of HER2 Staining

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by the PATHWAY anti-HER2 (4B5) antibody. A qualified pathologist experienced in immunohistochemical procedures must evaluate controls and qualify the stained product before interpreting results.

1. Positive Controls

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the membrane of the target cells is indicative of positive reactivity. Counterstaining with hematoxylin will result in a pale to dark blue

coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

If the positive tissue control fails to demonstrate positive staining, any results with the test specimens should be considered invalid.

2. Negative Tissue Controls

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If the tissue is counterstained, there may be staining around the outside of the cell, i.e., the interstitial spaces. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid.

3. Negative Reagent Controls

Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in tissue sections that are excessively formalin fixed. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically.

4. Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin (H&E) stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

A qualified pathologist who is experienced in immunohistochemical procedures must evaluate positive and negative controls and qualify the stained product before interpreting results.

5. PATHWAY anti-HER2 (4B5) Antibody Scoring Method for GEA

Scoring and interpretation for HER2 status is detailed in Table 5 below. Refer to PATHWAY anti-HER2 (4B5) Antibody package insert and interpretation guide for gastroesophageal adenocarcinoma for additional details.

Table 5: Scoring Criteria for Intensity and Pattern of Cell Membrane Staining with PATHWAY anti-HER2 (4B5) Antibody in Gastroesophageal Adenocarcinoma

Staining Pattern (Resection Specimen)	Staining Pattern (Biopsy Specimen[a])	HER2 (4B5) Score (Report To Requesting Physician)	Recommended Reporting Status	Therapy
No reactivity or membranous reactivity in < 10% of tumor cells	No reactivity or membranous reactivity in any tumor cell	IHC 0	HER2 Negative	None
Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster[b] with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	IHC 1+		
Weak to moderate complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells	Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	IHC 2+[c] *Reflex test: HER2 non-amplified*		
		IHC 2+[c] *Reflex test: HER2 amplified*	HER2 Positive	ZIIHERA (zanidatamab) + HERCEPTIN (trastuzumab) + chemotherapy
Strong complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells	Tumor cell cluster with a strong, complete basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	IHC 3+	HER2 Positive	ZIIHERA (zanidatamab) + chemotherapy Or ZIIHERA (zanidatamab) + HERCEPTIN (trastuzumab) + chemotherapy

[a] Biopsy specimens include endoscopic, pinch, and needle core
[b] ≥ 5 cohesive cells
[c] Recommend reflex test to assess gene amplification

VI. ALTERNATIVE PRACTICES AND PROCEDURES

At present, the recommended practice for HER2 testing in GEA includes IHC staining for HER2 protein expression and in situ hybridization (ISH) testing for determination of HER2 gene copy number.

There is another FDA-approved IHC test for the detection of HER2 protein in gastric or gastroesophageal junction adenocarcinoma. There are also FDA-approved in situ

hybridization (ISH) tests for the detection of HER2 gene amplification in tissues, which has been correlated to protein overexpression.

Each alternative for HER2 testing in GEA has its own advantages and disadvantages. A patient should fully discuss these alternatives with her/his physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

PATHWAY anti-HER2 (4B5) antibody has been marketed in the United States since the approval of P990081/S003 on November 11, 2007 for the breast cancer indication.

PATHWAY anti-HER2 (4B5) antibody is also globally marketed in several countries. The device in the US and ex-US products contain the same reagents.

PATHWAY anti-HER2 (4B5) antibody has not been withdrawn from any market as a result of safety and effectiveness concerns.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

PATHWAY anti-HER2 (4B5) antibody is intended for in vitro diagnostic (IVD) use only. As with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. Failure of the device to perform as expected or failure to correctly interpret results may lead to improper patient management decisions.

IX. SUMMARY OF NON-CLINICAL STUDIES

Nonclinical studies were performed using PATHWAY anti-HER2 (4B5) antibody to establish the analytical performance of the device for the GEA indication. These studies were performed using only the VENTANA BenchMark ULTRA instruments. These studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, evaluate assay precision and robustness, and establish reagent/cut slide stability. The study results detailed below establish the sensitivity, specificity, and precision including reproducibility of the device.

Due to the low prevalence of HER2 protein overexpression among GI-related solid tumors, an analytical validation approach using carcinomas of the digestive system (CDS) tissues, including GEA tumor specimens, was used to support the GEA indication. Some analytical studies (as detailed below) utilized GEA specimens supplemented with other CDS specimens (i.e., biliary tract cancer (BTC) and colorectal carcinomas (CRC) specimens). The CDS tissue indications shared similarities in histomorphology and HER2 (4B5) staining patterns and utilize the same scoring methodology (ASCO/CAP HER2 IHC). Both GEA-specific and combined CDS-specific results were provided for

each applicable analytical validation study. Only GEA specimens were used in the Interlaboratory Reproducibility (ILR) study.

A. Laboratory Studies

1. Analytical Sensitivity

The intended use prevalence across HER2 categories has been studied through distribution available in analytical and clinical studies.

a. Analytical sensitivity was assessed by calculating prevalence of HER2 IHC scores in 300 commercial GEA cases, including 100 each of gastric, gastroesophageal junction (GEJ), and esophageal adenocarcinoma, and the prevalence is presented in Table 6 below. The commercial cohort used in this study may not reflect natural prevalence.

Table 6. Prevalence of HER2 IHC Scores in GEA

HER2 IHC Bin	n/N	%
IHC 0	233/300	77.7%
IHC 1+	29/300	9.7%
IHC 2+[a]	18/300	6.0%
IHC 3+	20/300	6.7%

[a]The IHC 2+ category includes both ISH amplified and non-amplified.

b. The prevalence was also estimated based on clinical trial screening of the intended use population for HERIZON-GEA-01. In different populations, prevalence of HER2 IHC scores can be different from the prevalence presented in Table 7 below.

Table 7. Prevalence of HER2 IHC Scores in Clinical Trial HERIZON-GEA-01

HER2 IHC Bin	n/N	%
IHC 0	775/2487	31.2
IHC 1+	182/2487	7.3
IHC 2+[a]	490/2487	19.7
IHC 3+	980/2487	39.4
Not evaluable	12/2487	0.5

[a]The IHC 2+ category includes both ISH amplified and non-amplified.

2. Analytical Specificity

a. Western Blot

Refer to Summary of Safety and Effectiveness Data for P990081/S054 (section IX.A.2.a) for the Western blot study.

b. Immunoreactivity

Refer to Summary of Safety and Effectiveness Data for P990081/S054 (section IX.A.2.b) for the Immunoreactivity study.

c. Non-Specific Staining Assessment in GEA

i. HER2: Off-target cytoplasmic and nuclear staining was evaluated for all GEA, BTC, and CRC samples included in the study. Cytoplasmic and/or nuclear staining was observed in 16.5% (32/194) CDS cases (GEA: 15.7%, BTC: 28.6%, and CRC: 3.5%). One (1) out of 194 cases stained with HER2 (4B5) displayed cytoplasmic staining that interfered with HER2 interpretation and was deemed non-evaluable.

ii. HER4: A cohort of 194 CDS samples (70 GEA, 70 BTC, and 56 CRC cases) enriched for HER4 expression was screened for HER4 expression using HER4 (EP200) and for HER2 expression using HER2 (4B5). Twenty-seven (27) of the GEA cases, 18 of the BTC cases, and none of the CRC cases demonstrated cytoplasmic staining in tumor area. Three (3) of the 27 GEA cases with HER4 cytoplasmic (mucin) staining also demonstrated cytoplasmic staining with HER2 (4B5). None of the GEA cases stained with HER4 (E200) demonstrated membrane staining. Two (2) of the 18 BTC cases with cytoplasmic HER4 staining in tumor cells also demonstrated cytoplasmic staining with HER2 (4B5). One of the cases demonstrating cytoplasmic staining also had tumor cell membrane staining (2% of tumor cells) staining with an intensity of IHC 1+ and IHC 2+. That case also demonstrated tumor cell membrane staining with HER2 (4B5) (9%, of tumor cells at the same stain intensities).

iii. Zinc Finger and SCAN Domain Containing 18 (ZSCAN18): An IHC analysis of ZSCAN18 on tissue sections from several tissue types including BTC and GEA, showed ZSCAN18 expression in 9 of 49 cases. ZSCAN18 staining was entirely nuclear and was detected primarily in the adjacent stromal tissue rather than the tumor tissue.

iv. Aldo-keto reductase family 1, subfamily B (AKR1B): In a cohort of 96 GEA cases stained with antibodies to AKR1B1, AKR1B10/15, and HER2 (clone 4B5), staining was evaluated for presence, intensity, and localization. Cases exhibiting both 4B5 and AKR1B membrane staining in the same tumor area were further assessed using a second HER2 clone (SP3). AKR1B1 and AKR1B10/15 staining was predominantly cytoplasmic with occasional nuclear localization. The cases demonstrating

cytoplasmic or nuclear staining did not interfere with HER2 interpretation. No membrane staining was observed with AKR1B1. Low-level AKR1B10/15 tumor cell membrane staining was detected at low intensity in 16 of 96 GEA samples, but the %TC fell below a clinically meaningful range (<10% TC).

3. **Robustness**

   a. **Tissue Thickness**

   Tissue thickness was evaluated using a total of 12 single CDS tissue cases (6 negative and 6 positive), including 4 BTC cases (2 gallbladder and 2 bile duct cases), 4 GEA cases (2 GEJ and 2 GA cases), and 4 CRC cases. Duplicate sections at 2, 3, 4, 5, 6, and 7 µm were tested for each case.

   The results for each test sample were compared to the results of its respective reference sample sections at 4 µm and deemed acceptable or unacceptable. Due to change in scoring status of cases for the 2-3 µm and 6-7 µm sections, the recommended tissue thickness range for CDS tissues, including GEA tissues, is 4-5 µm for use with PATHWAY anti-HER2 (4B5) antibody.

   b. **Fixation Study**

   Refer to the Summary of Safety and Effectiveness Data P990081/S047 (section IX.C.1) for the study to evaluate the effects of fixative type and fixation time.

   c. **Ischemia Study (Time to Fixation)**

   Refer to the Summary of Safety and Effectiveness Data P990081/S047 (section IX.C.2) for the study to evaluate the effects of ischemic time.

   d. **Protocol Limitations**

   Refer to the Summary of Safety and Effectiveness Data P990081/S054 (section IX.A.3.d) for the study to evaluate the effects of ischemic time.

4. **Precision**

   For evaluation of the precision of the PATHWAY anti-HER2 (4B5) antibody on BenchMark ULTRA, the following studies were conducted: Intermediate Precision study, Reader (Pathologist) Precision study, and Inter-Laboratory Reproducibility study.

a. Intermediate Precision

Intermediate Precision was evaluated using GEA samples supplemented with samples from other types of carcinomas of the digestive system (CDS). Twenty-eight (28) CDS samples (10 GEA, 10 BTC, and 8 CRC samples) spanning the HER2 IHC staining range were included in this study. The study design for evaluation of staining precision on GEA tissues stained with PATHWAY anti-HER2 (4B5) antibody included:

- Three lots of PATHWAY anti-HER2 (4B5) antibody (between antibody kit lot)
- Three lots of *ultra*View DAB IHC Detection Kits (between detection kit lot)
- Across five non-consecutive days (between day)
- Three BenchMark ULTRA instruments (between instrument)
- One Pathologist, two replicates (within run)

All slides were blinded and randomized and evaluated using the scoring criteria in Table 5 above. There were 22 results for each case, and the majority HER2 score results was assigned based on those 22 results. Results of this analysis are summarized in Table 8 below.

Table 8: Median and Range of %TC for Samples in the Intermediate Precision Study for GEA Tissues (Supplemented with CDS)

Case Number	Majority HER2 IHC Score	Median %TC	Range %TC (Min-Max)	Percent Positive at IHC 2+ Cutoff	Percent Positive at IHC 3+ Cutoff
1	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
2*	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
3	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
4	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
5	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
6*	0	0.0	0.0 - 0.0	0.0% (0/22)	0.0% (0/22)
7	0	1.0	0.0 - 1.5	0.0% (0/22)	0.0% (0/22)
8	0	1.5	0.5 - 7.5	0.0% (0/22)	0.0% (0/22)
9*	0	3.0	0.5 - 5.5	0.0% (0/22)	0.0% (0/22)
10	1+	10.5	10.0 - 17.0	0.0% (0/22)	0.0% (0/22)
11*	1+	10.5	10.0 - 23.5	0.0% (0/22)	0.0% (0/22)
12*	1+	12.5	10.0 - 20.0	0.0% (0/22)	0.0% (0/22)
13	1+	17.0	1.0 - 30.0	18.2% (4/22)	0.0% (0/22)
14	1+	20.5	15.0 - 26.0	0.0% (0/22)	0.0% (0/22)
15*	2+	15.0	10.0 - 20.0	100.0% (22/22)	0.0% (0/22)
16	2+	20.0	15.0 - 25.0	100.0% (22/22)	0.0% (0/22)
17	2+	20.0	15.0 - 30.0	100.0% (22/22)	0.0% (0/22)
18	2+	20.0	15.0 - 45.5	100.0% (22/22)	0.0% (0/22)
19*	2+	34.0	25.5 - 57.0	100.0% (22/22)	0.0% (0/22)
20*	2+	35.0	20.0 - 55.0	100.0% (22/22)	0.0% (0/22)
21	2+	37.5	30.0 - 65.0	100.0% (22/22)	0.0% (0/22)
22*	3+	30.0	15.0 - 50.0	100.0% (22/22)	100.0% (22/22)
23*	3+	30.0	18.0 - 45.0	100.0% (22/22)	100.0% (22/22)
24	3+	52.5	30.0 - 80.0	100.0% (22/22)	100.0% (22/22)
25	3+	65.0	60.0 - 70.0	100.0% (22/22)	100.0% (22/22)
26	3+	85.0	70.0 - 95.0	100.0% (22/22)	100.0% (22/22)
27	3+	90.0	85.0 - 95.0	100.0% (22/22)	100.0% (22/22)
28	3+	97.0	93.0 - 100.0	100.0% (22/22)	100.0% (22/22)

* Indicates a GEA case

The variability of %TC values for each of the 28 cases was evaluated, and the following precision components were considered: repeatability (within-run), between-day, between-antibody lot, between-detection kit, between-instrument, and total. Each of the 28 cases had 22 replicates. Results are summarized in Table 9 below.

Table 9: Precision Components for Samples in Intermediate Precision Study for GEA Tissues (Supplemented with CDS)

Case Number	Majority HER2 Score	Median %TC	Standard Deviation of %TC					
			Repeatability (Within-run)	Between-day	Between-antibody lot	Between-detection kit	Between-instrument	Total
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2*	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6*	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0	1.00	0.11	0.36	0.00	0.44	0.00	0.58
8	0	1.50	0.86	2.54	0.00	0.97	0.00	2.85
9*	0	3.00	0.11	1.71	0.00	0.00	0.00	1.71
10	1+	10.50	0.15	0.32	0.00	3.68	2.73	4.60
11*	1+	10.50	0.54	1.15	0.00	0.00	6.89	7.01
12*	1+	12.50	0.32	2.77	0.00	0.00	3.65	4.59
13	1+	17.00	N/A	N/A	N/A	N/A	N/A	N/A
14	1+	20.50	0.30	3.01	1.77	0.00	4.30	5.55
15*	2+	15.00	1.95	2.96	0.00	0.00	0.00	3.55
16	2+	20.00	2.61	0.98	0.00	3.20	0.00	4.24
17	2+	20.00	3.37	0.00	4.63	0.75	1.63	6.00
18	2+	20.00	2.38	1.85	15.29	0.00	0.00	15.59
19*	2+	34.00	2.36	5.95	10.36	0.00	13.32	18.05
20*	2+	35.00	3.20	4.99	0.00	3.10	9.42	11.55
21	2+	37.50	2.82	5.04	10.20	0.00	14.92	18.97
22*	3+	30.00	3.37	9.48	4.81	4.81	0.00	12.15
23*	3+	30.00	3.53	7.63	8.18	0.00	0.00	11.73

Case Number	Majority HER2 Score	Median %TC	Standard Deviation of %TC					
			Repeatability (Within-run)	Between-day	Between-antibody lot	Between-detection kit	Between-instrument	Total
24	3+	52.50	6.91	8.54	0.00	21.63	0.00	24.26
25	3+	65.00	2.13	2.42	2.54	0.00	0.46	4.13
26	3+	85.00	4.13	6.82	3.10	0.00	3.10	9.09
27	3+	90.00	2.82	1.01	0.00	0.00	3.71	4.77
28	3+	97.00	1.09	1.99	0.00	0.00	1.95	2.99

N/A denotes that not all replicates were in the same IHC bin for this case. The ANOVA analyses of %TC were performed only if all replicates were in the same IHC bin (0/1+, 2+, or 3+).

* Indicates a GEA case

In addition, a qualitative analysis of different precision components was performed for both the IHC 2+ and IHC 3+ cutoffs. In this analysis, samples with IHC HER2 scores of "0" and "1+" were grouped together to consider as negative samples. Each precision component was determined with positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all observations. Results are summarized in Table 10 below.

Table 10: Repeatability and Intermediate Precision for GEA Tissues (Supplemented with CDS) on BenchMark ULTRA

Repeatability/Intermediate Precision	Type	Agreement					
		IHC 2+ cutoff			IHC 3+ cutoff		
		n/N	%	95% CI	n/N	%	95% CI
Between-Antibody Lots	PPA	84/84	100.0	(95.6, 100.0)	42/42	100.0	(91.6, 100.0)
	NPA	84/84	100.0	(95.6, 100.0)	126/126	100.0	(97.0, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)	168/168	100.0	(97.8, 100.0)
Between-Detection Kits	PPA	86/86	100.0	(95.6, 100.0)	42/42	100.0	(91.6, 100.0)
	NPA	82/84	97.6	(92.3, 100.0)	126/126	100.0	(97.0, 100.0)
	OPA	166/168	98.8	(96.4, 100.0)	168/168	100.0	(97.8, 100.0)
Between-Instrument (BenchMark ULTRA)	PPA	84/84	100.0	(95.6, 100.0)	42/42	100.0	(91.6, 100.0)
	NPA	84/84	100.0	(95.6, 100.0)	126/126	100.0	(97.0, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)	168/168	100.0	(97.8, 100.0)
Between-Day	PPA	140/140	100.0	(97.3, 100.0)	70/70	100.0	(94.8, 100.0)
	NPA	138/140	98.6	(95.4, 100.0)	210/210	100.0	(98.2, 100.0)
	OPA	278/280	99.3	(97.9, 100.0)	280/280	100.0	(98.6, 100.0)
Within-Run	PPA	154/154	100.0	(97.6, 100.0)	77/77	100.0	(95.2, 100.0)
	NPA	154/154	100.0	(97.6, 100.0)	231/231	100.0	(98.4, 100.0)
	OPA	308/308	100.0	(98.8, 100.0)	308/308	100.0	(98.8, 100.0)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% PPA, NPA, and OPA were calculated using Wilson score method.

The repeatability and intermediate precision data for the subset of 10 GEA cases (not shown) demonstrated 100% agreement at both the IHC 2+ and IHC 3+ cutoffs.

b. Reader Precision

In the Reader Precision study, Between-Reader and Within-Reader components of precision were evaluated. The reader precision study included 28 GEA samples supplemented with 37 BTC samples and 25 CRC samples spanning the HER2 IHC staining range. Seventy-five (75) resection samples and 15 biopsy samples were included. Samples were blinded and randomized prior to evaluation for the GEA HER2 scoring criteria. The study included three readers (pathologists). Each reader

scored all specimens twice, with a minimum of two weeks between reads. Each case had six reads (two reads by each of three readers). Results from the 75 resection samples included in the reader precision study are presented in Table 11 below.

Table 11: Results of the Reader Precision Study in GEA Resection Samples (Supplemented with CDS)

Case Category	HER2 IHC	N of Cases	N of Reads	Results by HER2 IHC Score			
				0	1+	2+	3+
No reactivity < 10%	0	16	96	96	0	0	0
Faint/barely perceptible < 10% / ≥ 10%	0/1+	8	48	21	27	0	0
Faint/barely perceptible ≥ 10% / weak to moderate complete, basolateral or lateral	1+/2+	1	6	0	3	3	0
Weak to moderate complete, basolateral or lateral	2+	25	150	0	0	150	0
Weak to moderate complete, basolateral or lateral / Strong complete, basolateral or lateral	2+/3+	2	12	0	0	6	6
Variable	0/1+/2+	1	6	4	1	1	0
Strong complete, basolateral or lateral	3+	22	132	0	0	0	132

The variability of %TC for cases in each category included in the Reader Precision study was evaluated and the following precision components were considered: within-reader, between-reader, and total. Results from the 75 resection samples are summarized in Table 12 below.

Table 12: Precision Components for Samples in Reader Precision Study for GEA Resection Samples (Supplemented with CDS)

Case Category	HER2 IHC	N of cases	N of reads	Range of median %TC	SD			% Positive for IHC 2+ cutoff	% Positive for IHC 3+ cutoff
					Within-Reader	Between-Reader	Total		
No reactivity < 10%	0	16	96	0.0 - 1.5	1.1	0.2	1.2	0.0% (0/96)	0.0% (0/96)
Faint/barely perceptible < 10% / ≥ 10 %	0/1+	8	48	2.0 - 12.5	4.3	3.9	5.8	0.0% (0/48)	0.0% (0/48)
Faint/barely perceptible ≥ 10% / weak to moderate complete, basolateral or lateral	1+/2+	1	6	17.5 - 17.5	N/A	N/A	N/A	50.0% (3/6)	0.0% (0/6)

Case Category	HER2 IHC	N of cases	N of reads	Range of median %TC	SD			% Positive for IHC 2+ cutoff	% Positive for IHC 3+ cutoff
					Within-Reader	Between-Reader	Total		
Weak to moderate complete, basolateral or lateral	2+	25	150	11.0 - 60.0	11.8	13.0	17.6	100.0% (150/150)	0.0% (0/150)
Weak to moderate complete, basolateral or lateral / Strong complete, basolateral or lateral	2+/3+	2	12	15.0 - 47.5	N/A	N/A	N/A	100.0% (12/12)	50.0% (6/12)
Variable	0/1+/ 2+	1	6	5.0 - 5.0	N/A	N/A	N/A	16.7% (1/6)	0.0% (0/6)
Strong complete, basolateral or lateral	3+	22	132	20.0 - 98.0	12.1	4.4	12.9	100.0% (132/132)	100.0% (132/132)

In addition, a qualitative analysis of within-reader and between-reader precision was determined with average positive agreement (APA), average equivocal agreement (AEA), average negative agreement (ANA), and overall percent agreement across all observations. In this analysis, samples with HER2 IHC scores of "0" and "1+" were grouped together to consider as negative samples, samples with the HER2 IHC score of "2+" were considered equivocal, and samples with the HER2 IHC score of "3+" were considered positive. The results for between-reader and within-reader precision components for CDS cases (including GEA) are summarized in Table 13 below. The results for between-reader and within-reader precision components for GEA cases only are summarized in Table 14 below.

Table 13: Within- and Between-Reader Precision of the PATHWAY anti-HER2 (4B5) Antibody in GEA (Supplemented with CDS)

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	178/180	98.9	(97.2, 100.0)
	AEA	176/181	97.2	(94.9, 99.4)
	ANA	176/179	98.3	(96.1, 100.0)
	OPA	265/270	98.1	(96.7, 99.6)
Between-Reader	APA	180/180	100	(97.9, 100.0)
	AEA	174/180	96.7	(92.4, 100.0)
	ANA	174/180	96.7	(92.6, 100.0)
	OPA	264/270	97.8	(94.8, 100.0)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% PPA, NPA, and OPA were calculated using Wilson score method.

Table 14: Within- and Between-Reader Precision of the PATHWAY anti-HER2 (4B5) Antibody in GEA Cases Only

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	76/77	98.7	(95.7, 100.0)
	AEA	30/32	93.8	(75.0, 100.0)
	ANA	58/59	98.3	(94.1, 100.0)
	OPA	82/84	97.6	(94.0, 100.0)
Between-Reader	APA	78/78	100	(95.3, 100.0)
	AEA	30/30	100	(88.6, 100.0)
	ANA	60/60	100	(94.0, 100.0)
	OPA	84/84	100	(95.6, 100.0)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% PPA, NPA, and OPA were calculated using Wilson score method.

The relatively low lower bound of the 95% confidence interval for the within-reader AEA for GEA samples were attributed to a combination of low sample size and two (2) cases with borderline, or close to borderline, %TC.

c. Inter-Laboratory Reproducibility

An Inter-Laboratory Reproducibility study of the PATHWAY anti-HER2 (4B5) antibody was conducted to evaluate reproducibility of the assay to determine HER2-status of GEA specimens stained on the BenchMark ULTRA instrument. The study included 28 de-identified FFPE GEA tissue specimens (including borderline cases for both the

IHC 2+ and IHC 3+ cutoffs) stained on three BenchMark ULTRA instruments on each of five non-consecutive days over 20 days at three external laboratories. The specimens represented the range of staining of the PATHWAY anti- HER2 (4B5) antibody. Each set of 5 stained slides per sample per staining day was randomized and evaluated by a total of 6 readers (2 readers/site) for the HER2 status. Each case had 10 results per site (30 results total). For each case, the percent positive with regards to HER2-targeted therapy in GEA was calculated. Results of this analysis for each case were presented in Table 15 below.

Table 15: Results of Inter-Laboratory Reproducibility Study in GEA on the BenchMark ULTRA

Case	Majority HER2 Score[a]	N of reads	HER2 IHC Score				Percent Positive Result (IHC 2+)				Percent Positive Result (IHC 3+)			
			0	1+	2+	3+	Site A	Site B	Site C	Overall	Site A	Site B	Site C	Overall
1	0	30	13 (43.3)	11 (36.7)	6 (20.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	50.0 (5/10)	10.0 (1/10)	0.0 (0/10)	20.0 (6/30)
2	0	30	21 (70.0)	8 (26.7)	1 (3.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	10.0 (1/10)	0.0 (0/10)	0.0 (0/10)	3.3 (1/30)
3	0	30	21 (70.0)	8 (26.7)	1 (3.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	10.0 (1/10)	0.0 (0/10)	0.0 (0/10)	3.3 (1/30)
4	0	30	24 (80.0)	5 (16.7)	1 (3.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	10.0 (1/10)	0.0 (0/10)	0.0 (0/10)	3.3 (1/30)
5	0	28	20 (71.4)	8 (28.6)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/8)	0.0 (0/28)	0.0 (0/10)	0.0 (0/10)	0.0 (0/8)	0.0 (0/28)
6	0	30	24 (80.0)	5 (16.7)	1 (3.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	10.0 (1/10)	0.0 (0/10)	0.0 (0/10)	3.3 (1/30)
7	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
8	0	30	26 (86.7)	4 (13.3)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
9	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
10	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
11	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
12	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
13	0	30	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
14	0	30	20 (66.7)	10 (33.3)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
15	1+	30	7 (23.3)	12 (40.0)	11 (36.7)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	60.0 (6/10)	50.0 (5/10)	0.0 (0/10)	36.7 (11/30)

Case	Majority HER2 Score[a]	N of reads	HER2 IHC Score				Percent Positive Result (IHC 2+)				Percent Positive Result (IHC 3+)			
			0	1+	2+	3+	Site A	Site B	Site C	Overall	Site A	Site B	Site C	Overall
16	2+	30	0 (0.0)	3 (10.0)	27 (90.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	100.0 (10/10)	90.0 (9/10)	80.0 (8/10)	90.0 (27/30)
17	2+	30	2 (6.7)	0 (0.0)	26 (86.7)	2 (6.7)	20.0 (2/10)	0.0 (0/10)	0.0 (0/10)	6.7 (2/30)	100.0 (10/10)	80.0 (8/10)	100.0 (10/10)	93.3 (28/30)
18	2+	30	0 (0.0)	0 (0.0)	30 (100.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
19	2+	30	0 (0.0)	0 (0.0)	30 (100.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
20	2+	30	0 (0.0)	2 (6.7)	28 (93.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)	100.0 (10/10)	90.0 (9/10)	90.0 (9/10)	93.3 (28/30)
21	2+	30	0 (0.0)	0 (0.0)	0 (0.0)	30 (100.0)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
22	3+	30	0 (0.0)	0 (0.0)	1 (3.3)	29 (96.7)	90.0 (9/10)	100.0 (10/10)	100.0 (10/10)	96.7 (29/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
23	3+	30	0 (0.0)	0 (0.0)	0 (0.0)	30 (100.0)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
24	3+	30	0 (0.0)	0 (0.0)	0 (0.0)	30 (100.0)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
25	3+	30	0 (0.0)	0 (0.0)	0 (0.0)	30 (100.0)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
26	3+	30	0 (0.0)	0 (0.0)	3 (10.0)	27 (90.0)	70.0 (7/10)	100.0 (10/10)	100.0 (10/10)	90.0 (27/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
27	3+	30	0 (0.0)	0 (0.0)	2 (6.7)	28 (93.3)	80.0 (8/10)	100.0 (10/10)	100.0 (10/10)	93.3 (28/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)
28	3+	30	0 (0.0)	0 (0.0)	1 (3.3)	29 (96.7)	90.0 (9/10)	100.0 (10/10)	100.0 (10/10)	96.7 (29/30)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (30/30)

[a] Majority HER2 score is based on the majority IHC score of available reads for that case.

Fifteen (15) out of 28 cases had results from all 30 replicates with the same staining intensity and pattern ("No reactivity or membranous reactivity in < 10% of tumor cells",

"Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells", "weak to moderate complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells", or "Strong complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells"). Variability of %TC values for those cases were evaluated and the following precision components were calculated: between-reader, between-day, between-site and total. Results are summarized in the table below.

Table 16. Precision Components for Cases in the Inter-Laboratory Reproducibility Study

Case	Majority HER2 Bin[a]	N of reads	Median %TC	Range %TC (Min-Max)	SD			
					Between-reader	Between-day	Between-site	Total
5	0	28	5	0 - 20	3	3.7	3.9	6.6
7	0	30	0	0 - 0	0	0	0	0
8	0	30	0	0 - 10	1.4	1.6	2.8	3.9
9	0	30	0	0 - 5	0	0.8	0	1
10	0	30	0	0 - 0	0	0	0	0
11	0	30	0	0 - 0	0	0	0	0
12	0	30	0	0 - 1	0	0	0	0.2
13	0	30	0	0 - 0	0	0	0	0
14	0	30	1.5	0 - 30	4.8	0	8.1	10.8
18	2+	30	25	10 - 60	18	0	0	20.8
19	2+	30	30	10 - 50	15.4	3.5	0	16.3
21	3+	30	95	80 - 100	1	0	2.9	5.2
23	3+	30	95	70 - 100	0	3.1	5.9	9.7
24	3+	30	90	65 - 100	0	4.7	8.7	10.9
25	3+	30	80	15 - 95	13	8.4	13.2	22.2

[a] HER2 IHC bin is based on the majority status of available reads for that case.

In addition, a qualitative analysis of different precision components was performed. For the purposes of study analysis, HER2 IHC scores of "0" and "1+" were considered negative. For the IHC 2+ cutoff, HER2 IHC scores of "2+" and "3+" were considered positive. For the IHC 3+ cutoff, a HER2 IHC scores of "2+" was also considered negative, while a HER2 IHC score of "3+" was considered positive. Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all evaluable observations were reported as measures of overall reproducibility. Average Within-Site and Within-Reader precision components are summarized in Table 17 below.

Table 17: Inter-Laboratory Reproducibility for Overall Agreement Rates for PATHWAY anti-HER2 (4B5) Antibody

Inter-Laboratory Reproducibility	Type	Agreement (IHC 2+ cutoff)			Agreement (IHC 3+ cutoff)		
		n/N	%	95% CI	n/N	%	95% CI
Overall	PPA	383/390	98.2	(96.4, 100.0)	233/240	97.1	(94.6, 99.2)
	NPA	427/448	95.3	(91.8, 99.0)	596/598	99.7	(99.0, 100.0)
	OPA	810/838	96.7	(94.5, 98.6)	829/838	98.9	(98.1, 99.6)
Within-Site	PPA	394/410	96.1	(92.4, 99.4)	233/240	97.1	(94.6, 99.2)
	NPA	418/428	97.7	(95.4, 99.5)	596/598	99.7	(99.0, 100.0)
	OPA	812/838	96.9	(95.2, 98.6)	829/838	98.9	(98.1, 99.6)
Within-Reader	PPA	396/405	97.8	(95.8, 99.5)	231/235	98.3	(96.6, 99.6)
	NPA	425/433	98.2	(97.0, 99.2)	599/603	99.3	(98.4, 100.0)
	OPA	821/838	98.0	(96.8, 99.0)	830/838	99.0	(98.3, 99.6)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method.

In addition, pairwise comparisons were made Between-Site, Between-Reader, and Between-Day for HER2 clinical status. These data were analyzed for average positive agreement (APA), average negative agreement (ANA), and OPA and are presented below in Table 18.

Table 18: Inter-Laboratory Reproducibility Pairwise Agreement Rates

Inter-Laboratory Reproducibility	Type	Agreement (IHC 2+ cutoff)			Agreement (IHC 3+ cutoff)		
		n/N	%	95% CI	n/N	%	95% CI
Between-Site	APA	7596/8080	94.0	(90.6, 97.1)	4520/4700	96.2	(93.1, 98.8)
	ANA	8156/8640	94.4	(91.5, 97.3)	11840/12020	98.5	(97.3, 99.5)
	OPA	7876/8360	94.2	(91.2, 97.2)	8180/8360	97.8	(96.2, 99.3)
Between-Reader	APA	382/404	94.6	(90.8, 97.6)	226/235	96.2	(93.1, 98.8)
	ANA	412/434	94.9	(91.6, 97.8)	594/603	98.5	(97.4, 99.5)
	OPA	397/419	94.7	(91.4, 97.6)	410/419	97.9	(96.2, 99.3)
Between-Day	APA	1554/1616	96.2	(93.9, 98.0)	914/940	97.2	(95.3, 99.0)
	ANA	1666/1728	96.4	(94.4, 98.1)	2378/2404	98.9	(98.2, 99.6)
	OPA	1610/1672	96.3	(94.3, 98.1)	1646/1672	98.4	(97.4, 99.4)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method.

5. Stability Studies

a. Cut-Slide Stability

The intent of this study was to assess the stability of the HER2 epitope in FFPE tissue sections stored for an extended duration of time at various environmental conditions by evaluating staining performance. The cut slide study was tested at the storage conditions

at low temperature with low humidity [5°C ± 3°C at 15% relative humidity (R.H.)] and low temperature with high humidity [5°C ± 3°C at 85% R.H.]. The study evaluated a total of 13 CDS single tissue cases: 4 GEA (1 gastric, 1 GEJ, 1 esophageal), 4 BTC, and 5 CRC cases. Slides were stained at specific time points at T0 (day 0), 7, 14, 30, 60, 90, 120, 150, 180 days comparing staining results to time T0.

Additionally, the study was also tested at high temperature with high humidity condition [30°C ± 5°C at 85% R.H.] with modified time points (at T0, 7, 10, 15, 20, 25, 30 days) to capture data points between 7 and 30 days for 30°C ± 5°C at 85% R.H. in slide boxes with two desiccant packets with and without humidity control to assess the stability of the epitope. The study evaluated six single tissue cases including 2 GEA (1 gastric, 1 esophageal), 2 BTC, and 2 CRC cases.

The cut slide stability study showed the slides containing BTC tissues are stable up to 180 days when stored at low (5°C ± 3°C) temperature with low humidity and 90 days at the same low temperature with high humidity. The following table summarizes the cut slide stability results for each condition tested.

Table 19. Cut Slide Stability results for CDS Tissue based on HER2 clinical status

Cut Slide Stability for CDS Tissues								
Condition:	**5°C ± 3°C, 15% R.H.**		**5°C ± 3°C, 85% R.H.**		**30°C ± 5°C, 15% R.H.**		**30°C ± 5°C, 85% R.H.**	
Cutoff:	**IHC 2+**	**IHC 3+**	**IHC 2+**	**IHC 3+**	**IHC 2+**	**IHC 3+**	**IHC 2+**	**IHC 3+**
GEA	180 days	180 days	180 days	180 days	180 days	180 days	7 days	180 days
CRC	150 days	180 days	60 days	180 days	60 days	180 days	7 days	90 days
BTC	180 days	180 days	180 days	180 days	120 days	180 days	7 days	180 days

Based on the study results, a cut slide stability of 45 days is acceptable when stored at 5 ºC ± 3 ºC (15% Relative Humidity ± 10%), as stated in the device Package Insert.

b. Real-Time and Ship Stress Stability

See Summary of Safety and Effectiveness Data for P990081/S054 (section IX.A.5.b) for the study details and results. See Summary of Safety and Effectiveness Data for P990081/S055 (section IX.A.6.a) for the most current product dating (18 months).

B. Animal Studies

Not applicable.

C. Additional Studies

1. Primary vs. Metastatic Tumor

This study evaluated the staining performance of the assay on primary tumor versus patient-matched metastatic tumor samples from the intended use population. The 46 CDS patient cases consisted of 18 GEA (7 gastric, 8 GEJ, 4 esophagus), 13 BTC, and 15 CRC cases.

At the IHC 2+ cutoff, 95.7% (44/46) of the CDS cases [94.4% (17/18) GEA, 92.3% (12/13) BTC, and 100% (15/15) CRC cases] demonstrated concordant HER2 status between the primary tumor block and the patient-matched metastatic tumor block. At the IHC 3+ cutoff, 97.8% (45/46) of the CDS cases [94.4% (17/18) GEA, 100% (13/13) BTC, and 100% (15/15) CRC cases] demonstrated concordant HER2 status between the primary tumor block and the patient-matched metastatic tumor block. Two cases were excluded from analysis due to no tumor cells being observed in one of the patient-matched blocks. The results from this study demonstrate that HER2 status can be discordant between the primary tumor and metastatic tumor block within individual patient cases.

2. Tissue Heterogeneity

a. Case Heterogeneity

This study characterized case heterogeneity by evaluating staining performance of VENTANA/PATHWAY anti-HER2/neu (4B5) on multiple blocks from the same patient case. Case heterogeneity compares biomarker status between multiple blocks from one patient case. A total of 50 CDS patient cases encompassing 17 GEA cases (including 5 gastric, 7 esophagus, 5 GEJ cases), 14 BTC cases, and 19 CRC cases, were used in this study. Each patient case had between 2-4 individual tissue blocks, yielding a total of 141 CDS tissue blocks (55 GEA, 34 BTC, and 52 tissue blocks). Of the 141 CDS blocks tested, the percentage of blocks that demonstrated concordant HER2 status with the case-level mode for HER2 status was 97.2% (137/141) based on an IHC 2+ cutoff and 98.6% (139/141) based on an IHC 3+ cutoff. Of the 55 GEA blocks tested, the percentage of blocks that demonstrated concordant HER2 status with the case-level mode for HER2 status was 98.2% (54/55) based on an IHC 2+ cutoff and 100% (55/55) based on an IHC 3+ cutoff. The results from this study demonstrate that heterogeneity among tumor blocks from the same patient case may exist in a low proportion of CDS cases, which may lead to discordant staining results.

b. Block Heterogeneity

This study characterized within-block heterogeneity by evaluating staining outcome from multiple samples from the same tissue block. Twelve (12) individual CDS tissue cases including 6 GEA cases (2 each gastric, esophagus, and GEJ), 3 BTC cases, and 3 CRC cases were evaluated.

At the IHC 2+ cutoff, three cases (1 GEJ, 1 CRC and 1 esophagus) exhibited heterogeneity. Two of the heterogeneous cases, including the GEJ case, had sections that alternated between IHC 1+ and 2+ scores. The third case (esophageal) that exhibited heterogeneity had one section that changed status to negative while all other sections for that case were positive. Based on the status IHC 2+ and 3+ as IHC positive vs IHC 0 and 1+ as IHC negative, the concordance observed in this study was OPA 90.4% (161/178) for CDS tissues and 87.6% (78/89) for GEA tissues.

At the IHC 3+ cutoff, two cases (1 BTC and 1 esophagus) exhibited heterogeneity. The heterogeneous BTC case had sections that alternated between IHC 2+ and 3+ scores, and the heterogeneous esophageal case had one section with an IHC 2+ score while all other sections for that case were IHC 3+. Based on the status IHC 3+ as IHC positive vs IHC 0/1+/2+ as IHC negative, the concordance observed in this study was OPA 97.2% (173/178) for CDS tissues and 98.9% (88/89) for GEA tissues.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of PATHWAY anti-HER2 (4B5) antibody as a companion diagnostic (CDx) device to identify patients with gastroesophageal adenocarcinoma (GEA, i.e. gastric, gastroesophageal junction, and esophageal adenocarcinoma) who may be eligible for treatment with ZIIHERA (zanidatamab) was evaluated in the Phase 3 clinical trial HERIZON-GEA-01 (ZWI-ZW25-301).

A. Study Design

The HERIZON-GEA-01 study (A randomized, Multicenter, Phase 3 Study of Zanidatamab in Combination with Chemotherapy with or without Tislelizumab in Subjects with HER2-positive Unresectable Locally Advanced or Metastatic Gastroesophageal Adenocarcinoma (GEA), NCT05152147) enrolled adult patients with HER2-positive GEA. HER2-positivity was defined as IHC 3+ or IHC 2+/ISH-amplified per central testing. Tumor samples from patients undergoing screening were centrally tested with the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody to assess HER2 protein expression by IHC and determine HER2

IHC status. The samples were also tested with the VENTANA HER2 Dual ISH DNA Probe Cocktail for HER2 ISH status determination (see P190031/S016 SSED).

A total of 914 GEA participants (of which 638, 195, and 81 had gastric, gastroesophageal junction, and esophageal adenocarcinomas, respectively) were randomized 1:1:1 to one of three treatment arms, each including investigator's choice of a fluoropyrimidine- and platinum-based chemotherapy (CAPOX: capecitabine plus oxaliplatin, or FP: 5-Fluorouracil + cisplatin): trastuzumab plus chemotherapy (Arm A, n = 308), zanidatamab plus chemotherapy (Arm B, n = 304), or zanidatamab and tislelizumab plus chemotherapy (Arm C, n = 302). Participants were stratified by geographic region, HER2 status (IHC3+ or IHC 2+/ISH+), and Eastern Cooperative Oncology Group (ECOG) performance status (0 or 1). Treatment continued in each arm until disease progression, unacceptable toxicity, or other discontinuation criteria were met.

1. Clinical Inclusion and Exclusion Criteria

Key Inclusion Criteria

Subjects were required to fulfill the criteria in the HERIZON-GEA-01 clinical protocol, including but not limited to the following:

a. Histologically confirmed unresectable locally advanced, recurrent or metastatic HER2-positive gastroesophageal adenocarcinoma (adenocarcinomas of the stomach or esophagus, including the gastroesophageal junction), defined as 3+ HER2 expression by IHC or 2+ HER2 expression by IHC with ISH-positivity per central assessment. Subjects with esophageal adenocarcinoma must not be eligible for combined chemoradiotherapy at the time of enrollment.
b. New formalin-fixed, paraffin-embedded (FFPE) tumor sample or archival tumor tissues must be tested at a central laboratory to confirm HER2 status prior to randomization.
c. Male or female, ≥ 18 years of age (or the legal age of adulthood per country-specific regulations).

To be included in the study a specimen had to meet all the following criteria:

1. It was a tumor specimen submitted for patient enrollment screening for Study ZWIZW25-301.
2. It was an FFPE specimen or fresh tissue fixed in formalin.
3. It contained sufficient tumor tissue for interpretation at the discretion of the reviewing pathologist.
4. If submitted as unstained FFPE slides rather than as fixed tissue or an FFPE tissue block, at least 4 slides were available for testing with HER2 (4B5) IHC and HER2 Dual ISH assays.

Key Exclusion Criteria

Patients were not permitted to enroll in the clinical study if they met any of the exclusion criteria in the HERIZON-GEA-01 clinical protocol, including but not limited to the following:

1. Prior treatment with a HER2-targeted agent, with the exception of subjects who received HER2-targeted treatment for breast cancer >5 years prior to initial diagnosis of GEA.
2. Prior treatment with systemic antineoplastic therapy or intraperitoneal chemotherapy for unresectable locally advanced, recurrent or metastatic GEA. Subjects who have received treatment with adjuvant or neoadjuvant chemotherapy or chemoradiotherapy must not have cancer recurrence or progression within 6 months of completing that therapy. Subjects who have received prior palliative local therapy (e.g., radiation therapy) are eligible.
3. Received radiation therapy within 14 days prior to randomization.
4. Major surgery within 28 days prior to randomization.
5. Clinically significant cardiac disease, such as ventricular arrhythmia requiring therapy, uncontrolled hypertension or any history of symptomatic congestive heart failure (CHF). Subjects with known myocardial infarction or unstable angina within 6 months prior to randomization are also excluded. Previous anticancer therapy-related CHF must have been ≤ Grade 1 at the time of occurrence and must have completely resolved.
6. Symptomatic pulmonary embolism ≤ 28 days prior to randomization.
7. Any history of cerebrovascular accident ≤ 6 months prior to randomization.
8. Administered a live vaccine ≤ 4 weeks prior to randomization.
9. Treated with another investigational product within 28 days of randomization.

Specimens were excluded from the study if they met any of the following criteria:

1. It was a fine needle aspirate or cytology specimen.
2. It consisted of tissue that had been decalcified. Evidence of decalcification should have been obtained from the pathology report. If the pathology report did not specify whether the sample was decalcified or not, the processing lab should have been contacted for confirmation.
3. It was known to be fixed in 95% alcohol, AFA, PREFER, ZF, or Bouin's.

2. Follow-up Schedule

Study participants were to be followed for survival after discontinuation of study treatment approximately every 3 months (± 14 days) after the last follow-up visit or as directed by the sponsor until death, lost to follow-up, withdrawal of consent, or study completion.

3. Clinical Endpoints

The primary efficacy endpoints for the HERIZON-GEA-01 study were Progression-free survival (PFS) by the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1), assessed by blinded independent central review (BICR) and overall survival (OS).

Key secondary endpoints included confirmed objective response rate (ORR) by RECIST 1.1, assessed by BICR, and duration of response (DOR) by RECIST 1.1, assessed by BICR. An additional secondary endpoint specific to the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody was the final staining acceptability rate in the intended use population, at the patient level.

B. Accountability of PMA Cohort

In total, 2493 prescreened and/or screened participants were tested with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody for the HERIZON-GEA-01 study and comprise the IHC Intend-to-Diagnose (ITD) Population. Six participants from the IHC ITD population were associated with exclusionary diagnostic protocol deviations/incidents. The remaining participants (2487) constitute the IHC IU population. Of these, 490 participants were HER2 IHC 2+ and 980 participants were HER2 IHC 3+ (See Table 20).

Finally, 914 participants were enrolled and randomized across the three treatment arms: 308 in Arm A (trastuzumab + chemotherapy), 304 in Arm B (zanidatamab + chemotherapy), and 302 in Arm C (zanidatamab + tislelizumab + chemotherapy).

Table 20: Accountability of the PMA Cohort for Study HERIZON-GEA-01

Patients Disposition for Study ZWI-ZW25-301 (IHC PMA cohort)	N
Total number of patients pre-screened and/or screened	2743
IHC Intent-to-Diagnose (ITD) Population	2493
Excluded from IHC ITD population (No Sample Submitted/Test Cancelled)	250
IHC Intended Use (IU) population	2487
HER2 IHC 3+	980
HER2 IHC 2+	490
HER2 IHC 1+	182
HER2 IHC 0	775
HER2 IHC Not Evaluable	12
HER2 IHC Not Evaluated	48
Excluded from IHC IU (Exclusionary RD005953 Protocol Deviation/Incident)	6

C. Study Population Demographics and Baseline Parameters

The patients' demographics and sample characteristics in IHC IU population are presented by HER2 IHC status (IHC positive, negative, or equivocal) in Table 21 and Table 22, respectively. The IHC Intended Use (IU) population comprises all patients in the IHC ITD population for whom the final staining attempt was performed according to the requirements of study Dx protocol.

In the IHC IU population, approximately 73.5% were male, and the median age was 63 years with approximately 53.8% <65 years old. Of the patients in the IHC IU population with reported characteristics, the majority were White (46.3%) or Asian (42.8%).

Table 21: Patient Demographic and Baseline Characteristics by Treatment Arm – IU Population

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
	(N=306)	(N=303)	(N=301)	(N=1577)	(N=2487)
Age (years)					
N	306	303	301	1577	2487
Mean ± SD	62.1 ± 11.85	61.2 ± 11.15	61.9 ± 10.40	61.7 ± 12.09	61.7 ± 11.75
Median	64.0	62.0	63.0	63.0	63.0
Min, Max	21.0, 84.0	25.0, 87.0	22.0, 81.0	23.0, 92.0	21.0, 92.0
Age Group, n (%)					
<65	160 (52.3%)	174 (57.4%)	162 (53.8%)	843 (53.5%)	1339 (53.8%)
>=65	146 (47.7%)	129 (42.6%)	139 (46.2%)	734 (46.5%)	1148 (46.2%)
Sex, n (%)					
Female	70 (22.9%)	60 (19.8%)	57 (18.9%)	473 (30.0%)	660 (26.5%)
Male	236 (77.1%)	243 (80.2%)	244 (81.1%)	1104 (70.0%)	1827 (73.5%)
Ethnicity, n (%)					
Hispanic or Latino	47 (15.4%)	37 (12.2%)	36 (12.0%)	480 (30.4%)	600 (24.1%)
Not Hispanic or Latino	251 (82.0%)	250 (82.5%)	260 (86.4%)	1052 (66.7%)	1813 (72.9%)
Not Reported	7 (2.3%)	15 (5.0%)	5 (1.7%)	35 (2.2%)	62 (2.5%)
Unknown	1 (0.3%)	1 (0.3%)	0 (0.0%)	10 (0.6%)	12 (0.5%)

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
Race, n (%)					
American Indian or Alaska Native	9 (2.9%)	5 (1.7%)	3 (1.0%)	118 (7.5%)	135 (5.4%)
Asian	164 (53.6%)	167 (55.1%)	166 (55.1%)	567 (36.0%)	1064 (42.8%)
Black or African American	1 (0.3%)	3 (1.0%)	1 (0.3%)	20 (1.3%)	25 (1.0%)
White	120 (39.2%)	115 (38.0%)	122 (40.5%)	795 (50.4%)	1152 (46.3%)
Multiple	1 (0.3%)	1 (0.3%)	1 (0.3%)	23 (1.5%)	26 (1.0%)
Not Reported	5 (1.6%)	10 (3.3%)	4 (1.3%)	25 (1.6%)	44 (1.8%)
Other	5 (1.6%)	2 (0.7%)	3 (1.0%)	16 (1.0%)	26 (1.0%)
Unknown	1 (0.3%)	0 (0.0%)	1 (0.3%)	13 (0.8%)	15 (0.6%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Country, n (%)					
Argentina	5 (1.6%)	6 (2.0%)	6 (2.0%)	88 (5.6%)	105 (4.2%)
Australia	9 (2.9%)	5 (1.7%)	1 (0.3%)	5 (0.3%)	20 (0.8%)
Belgium	7 (2.3%)	0 (0.0%)	6 (2.0%)	4 (0.3%)	17 (0.7%)
Brazil	7 (2.3%)	8 (2.6%)	10 (3.3%)	111 (7.0%)	136 (5.5%)
Canada	4 (1.3%)	2 (0.7%)	4 (1.3%)	9 (0.6%)	19 (0.8%)
Chile	12 (3.9%)	10 (3.3%)	12 (4.0%)	145 (9.2%)	179 (7.2%)
China	89 (29.1%)	88 (29.0%)	87 (28.9%)	178 (11.3%)	442 (17.8%)
Estonia	0 (0.0%)	2 (0.7%)	0 (0.0%)	8 (0.5%)	10 (0.4%)
France	14 (4.6%)	15 (5.0%)	11 (3.7%)	30 (1.9%)	70 (2.8%)
Georgia	2 (0.7%)	1 (0.3%)	2 (0.7%)	19 (1.2%)	24 (1.0%)
Germany	2 (0.7%)	1 (0.3%)	0 (0.0%)	3 (0.2%)	6 (0.2%)
Greece	1 (0.3%)	5 (1.7%)	2 (0.7%)	16 (1.0%)	24 (1.0%)
Guatemala	4 (1.3%)	1 (0.3%)	0 (0.0%)	49 (3.1%)	54 (2.2%)
India	0 (0.0%)	4 (1.3%)	3 (1.0%)	37 (2.3%)	44 (1.8%)
Ireland	1 (0.3%)	2 (0.7%)	2 (0.7%)	4 (0.3%)	9 (0.4%)
Italy	11 (3.6%)	10 (3.3%)	13 (4.3%)	39 (2.5%)	73 (2.9%)
Japan	22 (7.2%)	20 (6.6%)	21 (7.0%)	101 (6.4%)	164 (6.6%)

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
Malaysia	1 (0.3%)	2 (0.7%)	3 (1.0%)	74 (4.7%)	80 (3.2%)
Mexico	4 (1.3%)	3 (1.0%)	1 (0.3%)	75 (4.8%)	83 (3.3%)
Netherlands	1 (0.3%)	8 (2.6%)	1 (0.3%)	10 (0.6%)	20 (0.8%)
Poland	3 (1.0%)	1 (0.3%)	7 (2.3%)	11 (0.7%)	22 (0.9%)
Portugal	7 (2.3%)	3 (1.0%)	4 (1.3%)	24 (1.5%)	38 (1.5%)
Romania	12 (3.9%)	10 (3.3%)	15 (5.0%)	134 (8.5%)	171 (6.9%)
Serbia	3 (1.0%)	8 (2.6%)	7 (2.3%)	103 (6.5%)	121 (4.9%)
Singapore	0 (0.0%)	1 (0.3%)	1 (0.3%)	6 (0.4%)	8 (0.3%)
South Africa	1 (0.3%)	1 (0.3%)	0 (0.0%)	4 (0.3%)	6 (0.2%)
South Korea	48 (15.7%)	47 (15.5%)	44 (14.6%)	109 (6.9%)	248 (10.0%)
Spain	23 (7.5%)	25 (8.3%)	24 (8.0%)	62 (3.9%)	134 (5.4%)
Taiwan	2 (0.7%)	2 (0.7%)	0 (0.0%)	7 (0.4%)	11 (0.4%)
Thailand	1 (0.3%)	2 (0.7%)	2 (0.7%)	54 (3.4%)	59 (2.4%)
Turkey	3 (1.0%)	3 (1.0%)	5 (1.7%)	26 (1.6%)	37 (1.5%)
Ukraine	0 (0.0%)	0 (0.0%)	1 (0.3%)	11 (0.7%)	12 (0.5%)
United Kingdom	7 (2.3%)	7 (2.3%)	6 (2.0%)	21 (1.3%)	41 (1.6%)
Primary Diagnosis, n (%)					
Esophageal Adenocarcinoma	22 (7.2%)	39 (12.9%)	20 (6.6%)	103 (6.5%)	184 (7.4%)
GEJ Adenocarcinoma	60 (19.6%)	61 (20.1%)	74 (24.6%)	246 (15.6%)	441 (17.7%)
Gastric Adenocarcinoma	224 (73.2%)	203 (67.0%)	207 (68.8%)	1101 (69.8%)	1735 (69.8%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	127 (8.1%)	127 (5.1%)
Presence of Brain Metastases, n (%)					
Yes	3 (1.0%)	2 (0.7%)	1 (0.3%)	11 (0.7%)	17 (0.7%)
No	303 (99.0%)	301 (99.3%)	300 (99.7%)	1429 (90.6%)	2333 (93.8%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	137 (8.7%)	137 (5.5%)
Stage at Initial Diagnosis, n (%)					
Stage Ia	4 (1.3%)	1 (0.3%)	4 (1.3%)	9 (0.6%)	18 (0.7%)
Stage Ib	2 (0.7%)	3 (1.0%)	1 (0.3%)	8 (0.5%)	14 (0.6%)

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
Stage Ic	1 (0.3%)	0 (0.0%)	0 (0.0%)	3 (0.2%)	4 (0.2%)
Stage Iia	6 (2.0%)	4 (1.3%)	6 (2.0%)	24 (1.5%)	40 (1.6%)
Stage Iib	2 (0.7%)	8 (2.6%)	5 (1.7%)	38 (2.4%)	53 (2.1%)
Stage Iic	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Stage Iiia	17 (5.6%)	14 (4.6%)	16 (5.3%)	85 (5.4%)	132 (5.3%)
Stage Iiib	15 (4.9%)	10 (3.3%)	12 (4.0%)	71 (4.5%)	108 (4.3%)
Stage Iiic	7 (2.3%)	5 (1.7%)	10 (3.3%)	34 (2.2%)	56 (2.3%)
Stage Iv	159 (52.0%)	150 (49.5%)	157 (52.2%)	690 (43.8%)	1156 (46.5%)
Stage v	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Stage Iva	6 (2.0%)	12 (4.0%)	13 (4.3%)	75 (4.8%)	106 (4.3%)
Stage va	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Stage Ivb	66 (21.6%)	76 (25.1%)	62 (20.6%)	311 (19.7%)	515 (20.7%)
Stage vb	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Stage vc	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	21 (6.9%)	20 (6.6%)	15 (5.0%)	91 (5.8%)	147 (5.9%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	138 (8.8%)	138 (5.5%)
ECOG Performance Status at Enrollment, n (%)					
0	118 (38.6%)	134 (44.2%)	121 (40.2%)	0 (0.0%)	373 (15.0%)
1	188 (61.4%)	169 (55.8%)	179 (59.5%)	0 (0.0%)	536 (21.6%)
2	0 (0.0%)	0 (0.0%)	1 (0.3%)	0 (0.0%)	1 (0.0%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	1577 (100.0%)	1577 (63.4%)
IHC Score, n (%)					
0	0 (0.0%)	1* (0.3%)	0 (0.0%)	774 (49.1%)	775 (31.2%)
1+	0 (0.0%)	1* (0.3%)	0 (0.0%)	181 (11.5%)	182 (7.3%)
2+	51 (16.7%)	52 (17.2%)	51 (16.9%)	336 (21.3%)	490 (19.7%)

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
3+	254 (83.0%)	249 (82.2%)	250 (83.1%)	227 (14.4%)	980 (39.4%)
Missing	1* (0.3%)	0 (0.0%)	0 (0.0%)	59 (3.7%)	60 (2.4%)

* Three (3) patients without HER2-positive test results (IHC 2+/ISH-positive or IHC 3+) were randomized in error.

Table 22: Sample Characteristics by Treatment Arm - IU Population

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
	(N=307)	(N=305)	(N=306)	(N=1625)	(N=2543)
Sample Collection Method, n (%)					
Biopsy	252 (82.1%)	269 (88.2%)	263 (85.9%)	1335 (82.2%)	2119 (83.3%)
Resection	55 (17.9%)	36 (11.8%)	43 (14.1%)	290 (17.8%)	424 (16.7%)
Specimen Type, n (%)					
Archive FFPE block	106 (34.5%)	115 (37.7%)	120 (39.2%)	994 (61.2%)	1335 (52.5%)
Archive slides	189 (61.6%)	182 (59.7%)	174 (56.9%)	551 (33.9%)	1096 (43.1%)
Archive slides, Archive FFPE block	0 (0.0%)	1 (0.3%)	0 (0.0%)	7 (0.4%)	8 (0.3%)
Fresh slides	8 (2.6%)	4 (1.3%)	8 (2.6%)	30 (1.8%)	50 (2.0%)
Fresh tissue block	4 (1.3%)	3 (1.0%)	4 (1.3%)	43 (2.6%)	54 (2.1%)
Fixative Used, n (%)					
10% Neutral Buffered Formalin (NBF)	307 (100.0%)	300 (98.4%)	305 (99.7%)	1589 (97.8%)	2501 (98.3%)
Other formalin-based fixatives	0 (0.0%)	5 (1.6%)	1 (0.3%)	35 (2.2%)	41 (1.6%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	1 (0.0%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Characteristics	Treatment Arm				
	Arm A	Arm B	Arm C	Not Enrolled	Overall
Time to Fixation, n (%)					
<= 1 hour	202 (65.8%)	211 (69.2%)	201 (65.7%)	948 (58.3%)	1562 (61.4%)
> 1 hour	32 (10.4%)	28 (9.2%)	28 (9.2%)	163 (10.0%)	251 (9.9%)
Unknown	73 (23.8%)	66 (21.6%)	77 (25.2%)	514 (31.6%)	730 (28.7%)
Tumor Type, n (%)					
Primary	283 (92.2%)	271 (88.9%)	270 (88.2%)	1447 (89.0%)	2271 (89.3%)
Metastatic	24 (7.8%)	34 (11.1%)	36 (11.8%)	178 (11.0%)	272 (10.7%)

D. Safety and Effectiveness Results

1. Safety Results

   No adverse events associated with use of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody were reported during the course of the HERIZON-GEA-01 clinical study. One Serious Adverse Event (SAE) was reported in the conduct of the clinical performance study. The participant experienced hemorrhage and subsequent anemia causally related to sample collection procedure; however, specimens from this participant were never tested with the investigational devices.

   For the complete safety evaluation of ZIIHERA® (zanidatamab) and specific adverse events that occurred in the HERIZON-GEA-01 study, please see the ZIIHERA® (zanidatamab) PI available at Drugs@FDA.

2. Effectiveness Results

ZIIHERA in combination with tislelizumab-jsgr and chemotherapy

Efficacy results comparing Arm C and Arm A in the overall population (including both IHC 2+/ISH-amplified and IHC 3+ patients) are summarized in Table 23, Figure 1 and Figure 2. Statistically significant improvements in PFS and OS were demonstrated for the comparison of Arm C to Arm A.

Table 23: Efficacy Results in HERIZON-GEA-01 Comparing Arm C and Arm A in the overall population

	Arm C ZIIHERA with chemotherapy and tislelizumab-jsgr N=302	Arm A Trastuzumab with chemotherapy N=308
Overall Survival		
Deaths (%)	134 (44%)	170 (55%)
Median, months[a]	26.4	19.2
(95% CI)[a]	(21.5, 30.3)	(16.8, 21.8)
Hazard ratio (95% CI)[b]	0.72 (0.57, 0.90)	
p-value[c]	0.0043	
Progression-Free Survival		
Number of events (%)	154 (51%)	196 (64%)
Median, months[a]	12.4	8.1
(95% CI) [a]	(9.8, 18.5)	(7.0, 8.9)
Hazard ratio (95% CI)[b]	0.63 (0.51, 0.78)	
p-value[c]	< 0.0001	
Objective Response Rate (ORR)		
ORR (95% CI[d])	67% (61, 72)	63% (57, 68)
Complete response rate	20%	12%
Partial response rate	47%	50%
Duration of Response (DOR)	N=202	N=193
Median in months (95% CI)[a]	18.9 (121, 37.7)	8.3 (6.8, 10.1)

[a] Estimates per Kaplan-Meier method; CIs based on Brookmeyer and Crowley method with log-log transformation.
[b] Hazard ratio and 95% CIs were calculated in comparison with treatment arm Trastuzumab + Chemotherapy stratified by geographic region, HER2 status, and ECOG performance status.
[c] The p-values were calculated by stratified log-rank test.
[d] Two-sided 95% exact confidence interval using the Clopper-Pearson method.
CI=confidence interval

In pre-specified exploratory analyses comparing Arm C and Arm A in the HER2 IHC3+ population, the PFS HR was 0.54 (95% CI: 0.43, 0.69), with a median 16.1 months (95% CI: 10.9, 22.0) in Arm C and 7.6 months (95% CI: 6.9, 8.5) in Arm A; the OS HR was 0.70 (95% CI: 0.55, 0.91), with a median 27.3 months (95% CI: 22.3, NE) in Arm C and 19.8 months (95% CI: 16.2, 22.8) in Arm A. In the HER2 IHC2+/ISH+ population, the PFS HR was 1.08 (95% CI: 0.65, 1.78), with a median 8.6 months (95% CI: 6.7, 11.1) in Arm C and 10.0 months (95% CI:

6.9, 18.4) in Arm A; the OS HR was 0.83 (95% CI: 0.50, 1.39), with a median 21.5 months (95% CI: 16.0, 26.4) in Arm C and 18.7 months (95% CI: 13.6, 23.1) in Arm A.

Figure 1: Kaplan-Meier Plot of Overall Survival for Overall ITT Patients in Arm A and Arm C in HERIZON-GEA-01

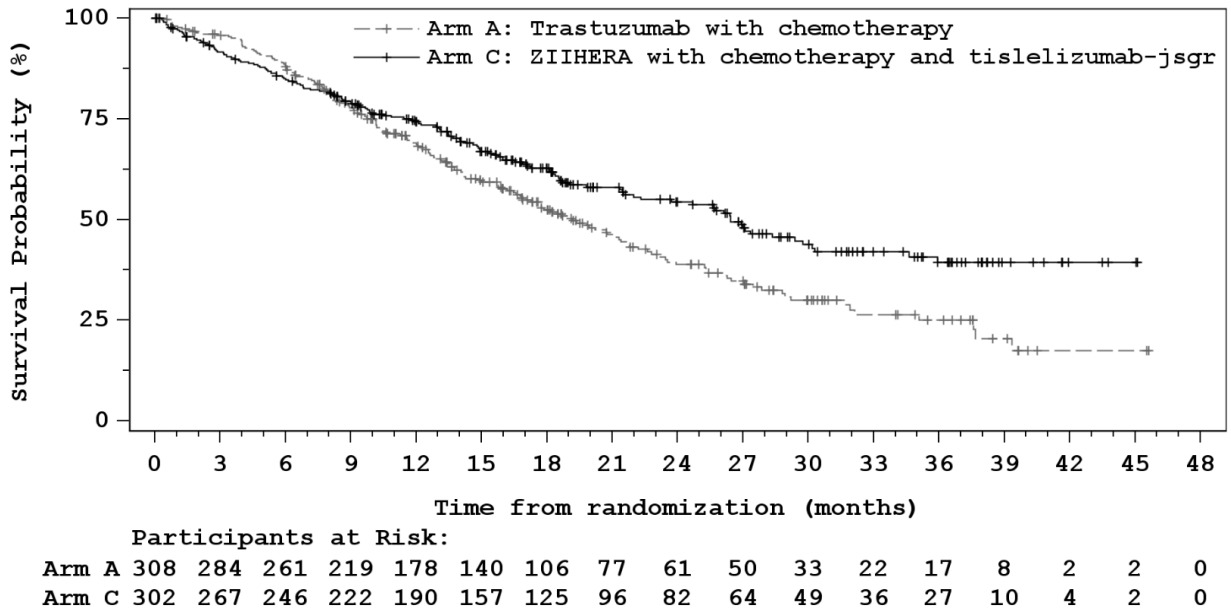
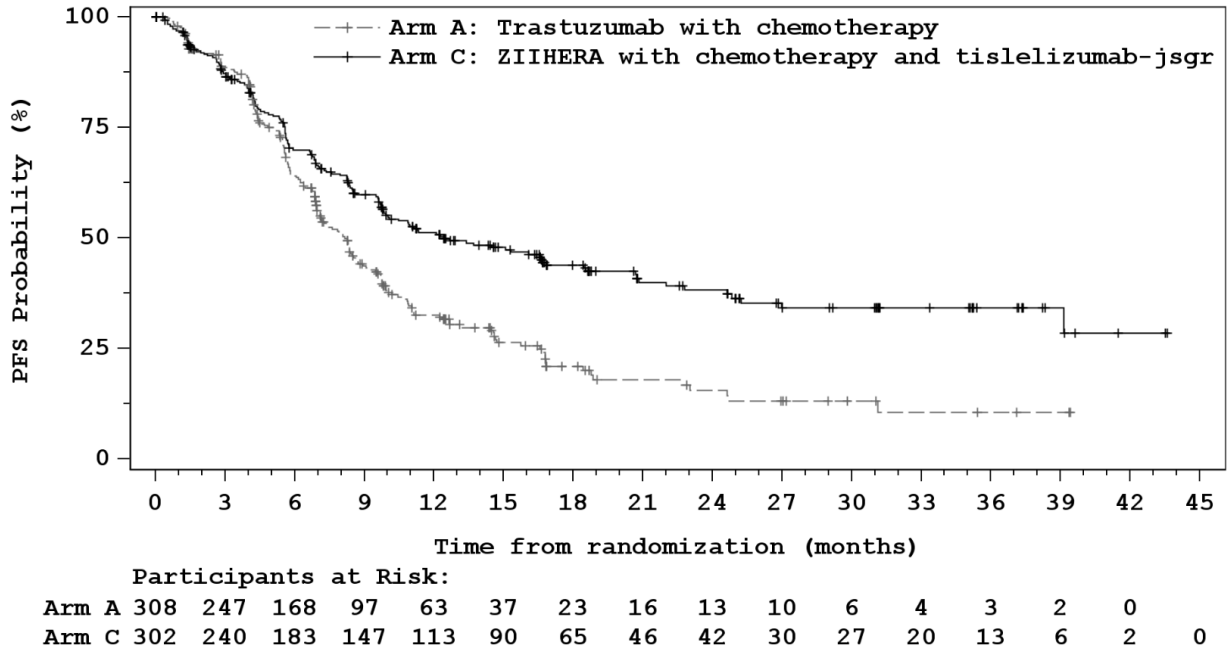


Figure 2: Kaplan-Meier Plot of Progression-Free-Survival for Overall ITT Patients in Arm A and Arm C in HERIZON-GEA-01



ZIIHERA in combination with chemotherapy

Efficacy results comparing Arm B and Arm A in the HER2 IHC3+ population are summarized in Table 24 and Figure 3. A statistically significant improvement in PFS was demonstrated in the overall population (including both HER2 IHC2+/ISH-amplified and IHC 3+) comparing Arm B and Arm A; however, an exploratory analysis of PFS in the HER2 IHC2+/ISH-amplified population showed a HR of 1.73 (95% CI: 1.06, 2.83), with a median of 6.2 months (95% CI: 3.0, 8.5) for Arm B and 10.0 months (95% CI: 6.9, 18.4) for Arm A, indicating that the improvement in the overall population was primarily attributed to the results observed in the subgroup of patients with HER2 IHC3+. OS in the overall population did not achieve statistical significance at the current interim analysis.

Table 24: Efficacy Results in HERIZON-GEA-01 Comparing Arm B and Arm A in Patients with HER2 IHC3+

	Arm B ZIIHERA with chemotherapy N=251	**Arm A Trastuzumab with chemotherapy N=255**
Overall Survival*		
Deaths (%)	116 (46%)	138 (54%)
Median, months[a]	25.5	19.8
(95% CI)[a]	(21.9, 30.3)	(16.2, 22.8)
Hazard ratio (95% CI)[b]	0.74 (0.58, 0.95)	
Progression-Free Survival		
Number of events (%)	125 (50%)	167 (65%)
Median, months[a]	14.2	7.6
(95% CI)[a]	(11.7, 16.7)	(6.9, 8.5)
Hazard ratio (95% CI)[b]	0.55 (0.43, 0.69)	
Objective Response Rate (ORR)		
ORR (95% CI)[c]	71% (64, 76)	64% (57, 69)
Complete response rate	19%	12%
Partial response rate	51%	52%
Duration of Response (DOR)	N=177	N=162
Median in months (95% CI)[a]	16.9 (12.8, 25.3)	8.1 (6.5, 9.8)

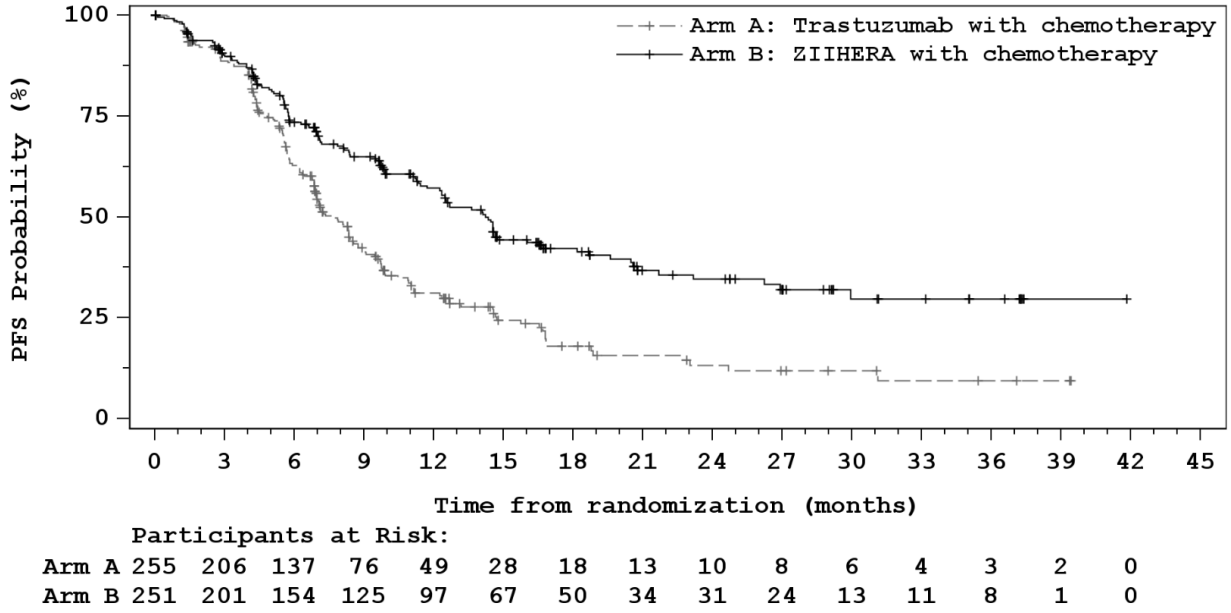
[a] Estimates per Kaplan-Meier method; CIs based on Brookmeyer and Crowley method with log-log transformation.

[b] Hazard ratio and 95% CIs were calculated in comparison with treatment arm Trastuzumab + Chemotherapy stratified by geographic region, HER2 status, and ECOG performance status.

[c] Two-sided 95% exact confidence interval using the Clopper-Pearson method.

* Overall survival data was immature at the time of this analysis.

Figure 3: Kaplan-Meier Plot of Progression-Free-Survival for HER2 IHC3+ patients in Arm A and Arm B in HERIZON-GEA-01



3. Relevant Subgroup Analyses

Subgroup analyses were performed on patients in the ITT population according to their HER2 IHC score (IHC 2+/ISH-amplified versus IHC 3+) and are summarized in the Effectiveness Results section above.

4. Pediatric Extrapolation

In this premarket application, existing clinical data from HERIZON-GEA-01 was leveraged to support approval of 18-21 years age pediatric patient population. Patients that were 18 years or older were eligible for enrollment into HERIZON-GEA-01 study if they fulfilled other inclusion criteria. The youngest age enrolled in the study was 21 years. For the HERIZON-GEA-01 study, data from patients 21 years or older is generalizable to the pediatric population (18-21 years) and can be relied on to establish safety and effectiveness within the 18-21 years age group.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 27 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XIII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The data from the analytical validation of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay and the assessment of clinical performance based on the clinical outcome data from HERIZON-GEA-01 study support the reasonable assurance of safety and effectiveness of the use of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody as a companion diagnostic device for identifying GEA patients eligible for treatment with ZIIHERA® (zanidatamab) with or without tislelizumab, and chemotherapy. Overall, data showed statistically robust and clinically meaningful efficacy of the ZIIHERA combination regimens in the patient population identified by PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody, validating its intended use.

B. Safety Conclusions

The risks of the device are based on analytical study data as well as data collected in the clinical study conducted to support PMA approval as described above. The PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody is an in vitro diagnostic device, which is used to test FFPE tumor specimens collected from patients with GEA, and no adverse events associated with the diagnostic testing procedure were reported during the HERIZON-GEA-01 study. In the context of an in vitro diagnostic test, there are no directly harmful events from testing FFPE GEA tissue sections. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely collected for diagnosis.

As PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody is intended to identify GEA patients for treatment with ZIIHERA® (zanidatamab) with or without tislelizumab, and chemotherapy, failure of the device to perform as expected may lead to incorrect or false results and GEA patients may not receive the proper treatment. Patients with false positive results may undergo treatment without much clinical benefit and may experience adverse reactions associated with the

ZIIHERA® (zanidatamab) combination regimen/s. Patients with false negative results may not be considered for treatment and therefore may receive other treatment options that do not offer the same clinical benefit.

C. Benefit-Risk Determination

The probable benefits and risks of the device are based on data collected in the HERIZON-GEA-01 clinical study conducted to support the supplemental PMA approval as described above. The clinical performance of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody was demonstrated in the HERIZON-GEA-01 study.

As shown in Table 20, ZIIHERA® (zanidatamab) with tislelizumab, in combination with chemotherapy demonstrated anticancer activity in patients with HER2 IHC 3+ or HER2 IHC 2+/ISH amplified) GEA, where HER2 protein expression was determined by the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (and reflexed to ISH as needed). In the primary analysis of PFS, zanidatamab with tislelizumab, in combination with chemotherapy demonstrated clinically meaningful and statistically significant improvements in PFS compared to trastuzumab plus chemotherapy. In the first interim analysis of OS, zanidatamab and tislelizumab plus chemotherapy demonstrated a clinically meaningful and statistically significant improvement in OS compared to trastuzumab plus chemotherapy.

As shown in Table 21, zanidatamab (without tislelizumab) in combination with chemotherapy demonstrated anticancer activity in patients with HER2 IHC 3+ GEA, where HER2 protein expression was determined by the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody. In the primary analysis of PFS, zanidatamab in combination with chemotherapy demonstrated clinically meaningful improvements in PFS compared to trastuzumab plus chemotherapy. Overall survival data in the zanidatamab plus chemotherapy arm was immature at the time of the first interim analysis of OS.

The data from that study, thus support the use of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody as an aid to identify patients with HER2 positive GEA and, who may be eligible for treatment with ZIIHERA® (zanidatamab) with or without tislelizumab, and chemotherapy in accordance with approved therapeutic labeling.

The main risk of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody is obtaining a false result. A false positive result could lead to treatment with a reduced probability of benefit and unnecessarily expose the patient to potential toxicity from the drug combination/s, while a false negative result could deprive a patient of its potential benefit. The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with acceptable accuracy, mitigating the potential risk for false results.

1. Patient Perspective
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the potential for clinical benefit outweighs the clinical risk for GEA patients tested with the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody to determine eligibility for ZIIHERA® (zanidatamab) with or without tislelizumab and chemotherapy.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the HERIZON-GEA-01 study support the use of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody as a companion diagnostic to identify patients with GEA who may be eligible for treatment with ZIIHERA® (zanidatamab) with or without tislelizumab, and chemotherapy in accordance with approved therapeutic labeling as the probable benefits outweigh the probable risks.

XV. CDRH DECISION

CDRH issued an approval order on August 25, 2026. The final non-clinical conditions of approval cited in the approval order are described below.

1. Ventana Medical Systems, Inc. must provide supplementary data from a between-day (3 days) precision study for the PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody assay utilizing intended use specimens that reflect current clinical practice for the gastroesophageal adenocarcinoma (GEA) indication. The study must include an adequate number of biopsy specimens representing all three GEA tissue subtypes (gastric, gastroesophageal junction, and esophageal adenocarcinoma), evaluated at both the IHC 2+ and IHC 3+ cutoffs. The data from this study must be provided to confirm the precision of the assay across the specimen types commonly encountered in clinical practice.

2. Ventana Medical Systems, Inc. must provide data from re-reading the slides stained in the original interlaboratory reproducibility study to confirm the reproducibility of PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody assay for the gastroesophageal adenocarcinoma (GEA) indication at both the IHC 2+ and IHC 3+ cutoffs when a formal washout period between reading sets has been maintained. The slides must be read by a minimum of 3 pathologists not involved in the original interlaboratory reproducibility study, including case qualification and/or post-study evaluation. There must be an adequate washout period between reading sets (≥ 14 days).

The data from this study must be provided to confirm the reproducibility of the assay across readers, sites, and days in the GEA intended use population.

The final study protocols, data, study conclusions, and any related labeling revisions should be submitted within 12 months of the PMA approval date.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality Management System (QMSR) regulation (21 CFR 820).

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVII. REFERENCES

None