SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In Vitro diagnostic device for detection of HER-2/neu gene amplification in formalin-fixed, paraffin-embedded (FFPE) Human Breast Tissues using dual chromogenic In Situ Hybridization (Dual ISH)
Device Trade Name:	VENTANA HER2 Dual ISH DNA Probe Cocktail
Device Procode:	NYQ
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: P190031/S016

Date of FDA Notice of Approval: August 25, 2026

The original PMA (P190031) for VENTANA HER2 Dual ISH DNA Probe Cocktail was approved on July 28, 2020 for the determination of HER2 gene amplification status by enumeration of the ratio of the HER2 gene to Chromosome 17 by light microscopy in formalin fixed, paraffin-embedded (FFPE) breast carcinoma tissue specimens stained on a Benchmark ULTRA instrument. It was indicated as an aid in the assessment of patients for whom Herceptin (trastuzumab) is being considered. The SSED to support the approved indications are available on the CDRH website and are incorporated by reference here.

The current supplement (P190031/S016) was submitted to expand the indication for VENTANA HER2 Dual ISH DNA Probe Cocktail as an aid in the assessment for HER2-positive patients with gastroesophageal adenocarcinoma (gastric, gastroesophageal junction and esophageal adenocarcinoma) who are eligible for treatment with ZIIHERA with tislelizumab, and chemotherapy.

II. INDICATIONS FOR USE

The VENTANA HER2 Dual ISH DNA Probe Cocktail is intended to determine the HER2 gene amplification status by enumeration of the ratio of the HER2 gene to Chromosome 17 by light microscopy. The HER2 and Chromosome 17 probes are detected using the VENTANA Silver ISH DNP Detection Kit and the VENTANA Red ISH DIG Detection Kit by a two-color chromogenic in situ hybridization (ISH) in formalin-fixed, paraffin-embedded human breast carcinoma and gastroesophageal adenocarcinoma (gastric, gastroesophageal junction and esophageal adenocarcinoma) tissue specimens following staining on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

The VENTANA HER2 Dual ISH DNA Probe Cocktail is indicated as an aid in the assessment of patients for whom the following therapies are being considered, in accordance with the approved therapeutic labeling:

- Breast carcinoma patients for whom Herceptin (trastuzumab) or ENHERTU® (fam-trastuzumab deruxtecan-nxki) [a] is being considered.
- Gastroesophageal adenocarcinoma[b] patients for whom ZIIHERA® (zanidatamab-hrii) in combination with fluoropyrimidine- and platinum-containing chemotherapy and tislelizumab-jsgr is being considered.

[a] See the ENHERTU® product label for specific clinical circumstances guiding HER2 testing.
[b] Gastroesophageal adenocarcinoma indication is only approved for use with BenchMark ULTRA Instrument.

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 VENTANA HER2 Dual ISH DNA Probe Cocktail labeling.

V. DEVICE DESCRIPTION

A. Device Kit Components

The VENTANA HER2 Dual ISH DNA Probe Cocktail is formulated for use with VENTANA Silver ISH DNP Detection Kit (SISH), VENTANA Red ISH DIG Detection Kit (RISH), and accessory reagents on a BenchMark IHC/ISH instrument to be used on formalin-fixed, paraffin embedded (FFPE) human breast cancer tissue specimens. The components of the VENTANA HER2 Dual ISH DNA Probe Cocktail are provided in Table 1 below. The ancillary reagents required for the BenchMark IHC/ISH instrument are listed in Table 2 below.

Table 1. Components of the VENTANA HER2 Dual ISH DNA Probe Cocktail

Component	Description
VENTANA HER2 Dual ISH DNA Probe Cocktail	HER2 DNP Oligo Probe Mix (0.014 mg/mL); Chr 17 DIG Oligo Probe Mix (0.00024 mg/mL); Hybrisol XX Diluent
VENTANA SILVER ISH DNP HQ	SISH DNP HQ Conjugate (0.0125 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA SILVER ISH DNP HQ HRP	Mouse anti-HQ HRP Conjugate (0.025 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA SILVER ISH DNP CHROMOGEN A	Silver acetate (0.0036 g/mL)
VENTANA SILVER ISH DNP CHROMOGEN B	Hydroquinone (0.0018 g/mL); Citric Acid Monohydrate (0.0136 g/mL); Sodium Citrate Trisodium Salt Dihydrate (0.0103 g/mL)
VENTANA SILVER ISH DNP CHROMOGEN C	Hydrogen peroxide 30% (0.003 mL/mL)
VENTANA RED ISH DIG NP	RISH DIG NP Conjugate (0.0075 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA RED ISH DIG NP AP	RISH DIG NP AP Conjugate (0.010 mg/mL); Enhanced Avidin Diluent
VENTANA RED ISH DIG pH ENHANCER	Magnesium Chloride (2.5% w/v); Tris (0.0121 g/mL); Brij- 35 30% (0.0017 g/mL); Proclin 300 (0.0005 mL/mL)
VENTANA RED ISH DIG NAPHTHOL	NAPHTHOL AS-TR Phosphate, Disodium Salt (0.007 g/mL); Tris (0.061 g/mL); Brij-35 30% (0.0017 g/mL); Proclin 300 (0.0005 mL/mL)
VENTANA RED ISH DIG FAST RED CHROMOGEN	Fast Red KL Salt (0.002 g/mL); Brij-35 30% (0.0123 g/mL); Proclin 300 (0.0005 mL/mL); Glacial Acetic Acid 99.5% (0.0006 mL/mL)

Table 2. Ancillary Reagents

Component	Description
HybReady Solution	Formamide-based buffer that is used to dilute probes and to create appropriate ISH conditions on the slide
ultraView Silver Wash II	Rinse slides between staining steps and provide stable aqueous environment for ISH
ISH Protease 3	Used to remove protein that surrounds target DNA or RNA sequences of interest for ISH
Hematoxylin II Counterstain	Stains a variety of tissue components including nuclei, mitochondria, mucin, hemoglobin, elastic fibers, and collagen
Bluing Reagent	Applied after hematoxylin to change the hue of the hematoxylin to a blue color
Sodium Chloride Sodium Citrate Buffer (SSC) (10×)	Rinse slides between staining steps and provide stable aqueous environment for ISH
EZ Prep Reagent (10×)	Remove paraffin from tissue samples during ISH
ULTRA Cell Conditioning Solution 1	Hydrolyze covalent bonds formed by formalin in tissue to increase target accessibility
ULTRA Cell Conditioning Solution 2	Hydrolyze covalent bonds formed by formalin in tissue to increase target accessibility
Ultra Liquid Coverslip	Serves as a barrier between the aqueous reagents and the air to prevent evaporation and provide a stable aqueous environment for ISH

The staining protocol (provided in Table 3 below) consists of numerous steps in which reagents are incubated for pre-determined times at specific temperatures. At the end of each incubation step, the BenchMark IHC/ISH instrument washes the sections to remove unbound material and applies a liquid coverslip which minimizes the evaporation of the aqueous reagents from the slide.

ISH signals are visualized as single copies, multiple copies and clusters. Single copies in the normal cells are used as a reference to enumerate the signals in the carcinoma nuclei. Signals should be visualized using 20×, 40×, or 60× objectives.

B. Device Instrumentation and Software

The VENTANA HER2 Dual ISH Assay is performed on the BenchMark ULTRA automated staining instrument using the Ventana system software (VSS) software version 14.1 or earlier. Staining procedure is specific for the Ventana HER2 Dual ISH Assay and operates within the VSS environment requiring that all system reagents are used together.

C. Specimen Preparation

Routinely processed tissues that have been fixed in 10% Neutral Buffered Formalin for 6 to 72 hours, paraffin-embedded and cut at 4 µm sections should be used. Specimens fixed < 6 hours in formalin can result in signal loss and nuclear over-digestion, as observed by pale/weak hematoxylin staining. Only fixation in 10% NBF is recommended as some fixatives produce variable staining with ISH-based assays (including Bouin's and Alcohol Formalin-Acetic Acid (AFA).

Slides should be stained immediately, as quality of nucleic acid targets in cut tissue sections may diminish over time.

D. Test Controls

Controls establish the validity of the test results. The following internal positive and negative controls should be assessed:

Normal cells within, or adjacent to, the target area serve as internal controls of the staining. At least 50% of the normal cell nuclei should contain at least one SISH signal and at least 50% should contain at least one Red ISH signal (the SISH and Red ISH signals do not have to be in the same cells) for the target area to be deemed adequate. Failure to detect adequate signal in normal cells on any slide on the run indicates that the particular slide is inadequate for enumeration. Using positive control samples will aid in troubleshooting potential instrument and/or reagent problems.

Additional information about positive and negative controls is available in the product labeling.

E. Principles of Operation

The VENTANA HER2 Dual ISH DNA Probe Cocktail contains HER2 probes (labeled with the hapten dinitrophenyl or DNP) and Chromosome 17 probes (labeled with the hapten digoxigenin or DIG) formulated in a formamide-based buffer. The probes are designed to detect amplification of the HER2 gene in invasive breast carcinoma and gastroesophageal adenocarcinoma (GEA, i.e. gastric adenocarcinoma, gastro-esophageal junction (GEJ) adenocarcinoma, and esophageal adenocarcinoma). The HER2 DNA Probe is a mixture of oligo probes that spans approximately 300 000 base pairs along the genomic region containing the HER2 gene (also known as ERBB2 and NEU), which is located on human Chromosome 17 (17q12). The Chromosome 17 probe is a mixture of oligo probes that target sequences within the centromeric region and serves as a reference for aneusomy. Copy numbers of both probes are enumerated in tumor nuclei and results are reported as a ratio of HER2/Chromosome 17 to determine HER2 amplification status (HER2/Chromosome 17 ratio ≥ 2.0 is amplified, while a ratio < 2.0 is non-amplified). The VENTANA HER2 Dual ISH DNA Probe Cocktail is optimally

formulated for use with VENTANA Silver ISH DNP Detection Kit, VENTANA Red ISH DIG Detection Kit, and accessory reagents on a BenchMark ULTRA instrument.

Patient FFPE tissue specimens are cut 4µm thick and mounted on positively-charged glass slides. These slides are placed in the BenchMark ULTRA instrument. This system first removes the paraffin wax from the tissue and then takes the tissue through the series of hybridization and detection steps that are part of the staining protocol. During the Dual ISH staining process, DNP and DIG labeled probes are co-hybridized to their respective specific target DNA sequences within the cell nuclei. Detection of the DNP-labeled HER2 probe occurs first, using the VENTANA Silver ISH DNP Detection Kit that forms a silver precipitate in the nucleus, and a single copy of the HER2 gene is visualized as a black dot. The DIG-labeled Chr 17 probe is then detected with the VENTANA Red ISH DIG Detection Kit that forms a red precipitate. Tissues are then counterstained with Hematoxylin II for interpretation by light microscopy.

F. Staining Protocol

The VENTANA HER2 Dual ISH DNA Probe Cocktail is designed to be run on the BenchMark ULTRA with VSS software. The staining protocol on the BenchMark ULTRA is provided in Table 3 below.

Table 3. Staining Conditions for VENTANA HER2 Dual ISH DNA Probe Cocktail Assay on BenchMark ULTRA Instrument

Staining Condition	GEA
Baking	Not selected
Deparaffinization	69°C
Cell Conditioning 1	40 mins
Cell Conditioning 2	40 mins
ISH Protease 3	24 mins
Denaturation	80°C, 8 mins
Probe hybridization	44°C, 60 mins
Stringency Wash Temperature	72°C
Stringency Wash Time	24 mins
Silver DNP HQ	16 mins
Silver HRP	24 mins
Silver A	8 mins

Staining Condition	GEA
Silver B	4 mins
Silver C	8 mins
Red DIG NP	16 mins
Red AP	20 mins
Red Enhancer	8 mins
Red Naphthol	4 mins
Red Fast Red	8 mins
Hematoxylin II	8 mins
Bluing Reagent	8 mins

G. Assay Scoring

Once an adequate target area is identified, the reader records the counts for HER2 and Chromosome 17 (Chr 17) copy numbers that are present in 20 representative nuclei. If the resulting HER2/Chr 17 ratio falls within 1.8-2.2 (inclusive), the reader is recommended to count an additional 20 nuclei, and the resulting ratio is calculated from the total 40 nuclei. HER2 gene status is reported as non-amplified (HER2/Chr 17 < 2.0) or amplified (HER2/Chr 17 ≥ 2.0).

Table 4. HER2/Chr17 Amplification Status

Ratio HER2/Chr17	Amplification Status
$\geq$ 2.0	Amplified
< 2.0	Non-amplified
$\geq$ 2.0 and $\leq$ 2.5	Borderline Amplified
$\geq$ 1.5 and < 2.0	Borderline Non-amplified

H. Interpretation of HER2 Staining

A pathologist experienced in the microscopic interpretation of anatomic pathology specimens, ISH procedures and the recognition of single and amplified HER2 and Chromosome 17 copies should evaluate the slides. Slides should be reviewed via light microscopy using 20x, 40x, and/or 60x objectives. Controls must be evaluated before interpreting results. Use of 100x objective is not recommended.

Positive (Internal) Control Tissue Interpretation

Normal cells within, or adjacent to, the target area serve as internal positive controls of the staining. At least 50% of the normal cell nuclei should contain at least one SISH signal and at least 50% should contain at least one Red ISH signal (the SISH and Red ISH signals do not have to be in the same cells) for the target area to be deemed adequate. Failure to detect adequate signal in normal cells on any slide on the run indicates that the particular slide is inadequate for enumeration.

An additional laboratory-specific positive control specimen may be used with every staining procedure performed. Control specimens can be specimens prepared in a manner identical to patient specimens. Such controls are useful to monitor all steps of the procedure, from specimen preparation through staining. Use of a control specimen prepared differently from the test specimens will provide a control for the reagents, instruments and procedures but not for fixation and specimen processing. Results with the test specimens should be analyzed on the same run. Such controls should not replace the proper evaluation of the internal controls in each patient specimen. Interpretation is the same as described above.

Patient Tissue Interpretation

The VENTANA HER2 Dual ISH DNA Probe Cocktail has been designed to enable the HER2 gene and Chr 17 centromere to be co-hybridized and visualized via light microscopy on the same slide. In the slides stained with the VENTANA HER2 Dual ISH DNA Probe Cocktail, HER2 appears as discrete black signals (SISH) and Chr 17 as red signals (Red ISH) in nuclei of normal cells (serving as internal controls for staining adequacy) as well as in nuclei of carcinoma cells. This allows for HER2 gene amplification status determination in the context of its chromosomal state.

HER2 gene amplification status is reported as the ratio of the number of HER2 gene copies to the number of Chr 17 copies in nuclei of cells within an invasive breast carcinoma and gastroesophageal adenocarcinoma (GEA, i.e. gastric adenocarcinoma, gastro-esophageal junction (GEJ) adenocarcinoma, and esophageal adenocarcinoma). Twenty nuclei, each containing red (Chr 17) and black (HER2) signals, should be enumerated. The final results for the HER2 status are reported based on the ratio calculated by dividing the sum of HER2 signals for all 20 nuclei divided by the sum of Chromosome 17 signals for all 20 nuclei. The amplification status is defined as Amplified if the HER2/Chr 17 ratio ≥ 2.0 and as Non- Amplified if the HER2/Chr 17 ratio < 2.0. If the HER2/Chr 17 ratio falls between 1.8 to 2.2, an additional 20 nuclei should be enumerated. A new ratio should then be calculated on the basis of all 40 nuclei, and the amplification status reported as described above. Refer to the VENTANA HER2 Dual ISH DNA Probe Cocktail Interpretation Guide for more information about interpretation of HER2 staining.

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 are multiple other FDA-approved IHC tests for the detection of HER2 protein. 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 has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

VENTANA HER2 Dual ISH DNA Probe Cocktail has been marketed in the United States since the approval of P190031 on July 28, 2020, for the breast cancer indication.

VENTANA HER2 Dual ISH DNA Probe Cocktail is also globally marketed in several countries. The device in the US and ex-US products contain the same reagents.

VENTANA HER2 Dual ISH DNA Probe Cocktail has not been withdrawn from any market as a result of safety and effectiveness concerns.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

VENTANA HER2 Dual ISH DNA Probe Cocktail is intended for in vitro diagnostic (IVD) use only. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently improper patient management decisions in GEA.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

Nonclinical studies were performed using VENTANA HER2 Dual ISH DNA Probe Cocktail to establish the analytical performance of the device. These studies were performed using VENTANA BenchMark ULTRA instruments only. 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, precision including reproducibility of the device.

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

1. Analytical Sensitivity

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

The Carcinoma of the Digestive System (CDS) database included 4,577 previously screened cases. The cases included biliary tract carcinoma (BTC) tissues (bile duct and gallbladder), gastroesophageal adenocarcinoma (GEA) tissues (gastric, gastroesophageal junction (GEJ) and esophagus) and colorectal (CRC) tissue. Totally, 945 CDS cases were randomly selected. Case Positive Prevalence for each indication and for CDS indications overall for 945 randomly selected CDS cases in the Carcinoma of the Digestive System database is shown in Table 5. From the selected CDS case population, prevalence was 16.3% (120 amplified cases out of 735 total enumerable cases) and the associated confidence interval (CI) was 13.8% to 19.2%.

Table 5: Case Positive Prevalence for each indication

Indication Tissue	Number of Cases				Prevalence [a] % (95% CI [b])
	Cases Evaluated	Enumerable	Non-enumerable	Amplified	
Bile Duct	160	143	17	6	4.2 (1.9, 8.9)
Gallbladder	160	144	16	46	31.9 (24.9, 39.9)
Gastric	158	116	42	20	17.2 (11.4, 25.1)
GEJ	161	123	37	20	16.3 (10.8, 23.8)
Esophagus	148	97	51	21	21.6 (14.6, 30.8)
CRC	159	112	47	7	6.3 (3.1, 12.3)
Overall CDS Cases	945	735	210	120	16.3 (13.8, 19.2)

[a]: Case Positive Prevalence was calculated as the proportion of positive (amplification status ≥ 2.0) cases among all of the cases used in this study.
[b]: 95% Confidence Interval (95% CI) was calculated using the Wilson score method.

2. Analytical Specificity

Refer to the Summary of Safety and Effectiveness Data P190031 (Section IX.A.1b) for the study to evaluate the sensitivity and specificity by assessing hybridization to Chr 17.

3. Precision

Repeatability and Intermediate Precision

Within-Run Repeatability was evaluated using GEA (gastric, gastroesophageal junction and esophageal adenocarcinoma) samples supplemented with samples from carcinomas of the digestive system (CDS). Thirty CDS samples (10 BTC samples, 15 GEA samples, and 5 CRC samples were included in this study. Two replicate slides from each of the CDS specimens were stained with VENTANA HER2 Dual ISH DNA Probe Cocktail on a single BenchMark ULTRA instrument.

Between-Day Intermediate Precision was evaluated using 30 CDS specimens, including 15 GEA samples. Replicate slides from each of 30 specimens were stained with VENTANA HER2 Dual ISH DNA Probe Cocktail on a single BenchMark ULTRA instrument on 5 nonconsecutive days.

Within-Run Repeatability and Between-Day Intermediate Precision were determined with positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all the observations from the evaluable population. A summary of the results of repeatability and intermediate precision are presented in Table 6 (CDS) and Table 7 (GEA-specific) below.

Table 6. BenchMark ULTRA Instrument Within-Run Repeatability and Between-Day Intermediate Precision

Repeatability/ Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Run Repeatability	Amplified	PPA	157/157	100.0	(97.6, 100.0)
	Non-amplified	NPA	159/159	100.0	(98.8, 100.0)
	Total	OPA	316/316	100.0	(98.8, 100.0)
Between-Day Intermediate Precision	Amplified	PPA	147/147	100.0	(97.5, 100.0)
	Non-amplified	NPA	145/145	100.0	(97.4, 100.0)
	Total	OPA	292/292	100.0	(98.7, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used. Four cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

Table 7. BenchMark ULTRA Instrument Within-Run Repeatability and Between-Day Intermediate Precision in GEA samples

Repeatability/Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Run Repeatability	Amplified	PPA	73/73	100.0	(95.0, 100.0)
	Non-amplified	NPA	84/84	100.0	(95.6, 100.0)
	Total	OPA	157/157	100.0	(97.6, 100.0)
Between-Day Intermediate Precision	Amplified	PPA	68/68	100.0	(94.7, 100.0)
	Non-amplified	NPA	77/77	100.0	(95.2, 100.0)
	Total	OPA	145/145	100.0	(97.4, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used.

Between-Instrument Intermediate Precision

BenchMark ULTRA instrument between-instrument intermediate precision of the VENTANA HER2 Dual ISH DNA Probe Cocktail was determined by staining replicate slides of 15 GEA specimens supplemented with additional CDS specimens (10 BTC samples and 5 CRC samples) on three BenchMark ULTRA instruments with the VENTANA HER2 Dual ISH DNA Probe Cocktail using the VENTANA Silver ISH DNP Detection Kit and VENTANA Red ISH DIG Detection Kit. The between-instrument intermediate precision was determined with PPA, NPA, and OPA across all the observations from the evaluable population. A summary of the results of this study is presented in Table 8 (CDS) and Table 9 (GEA specific) below.

Table 8. BenchMark ULTRA Between-Instrument Intermediate Precision

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Between-Instrument Intermediate Precision	Amplified	PPA	88/88	100.0	(95.8, 100.0)
	Non-amplified	NPA	87/87	100.0	(95.8, 100.0)
	Total	OPA	175/175	100.0	(97.9, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used. Four cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

Table 9. BenchMark ULTRA Between-Instrument Intermediate Precision in GEA samples

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Between- Instrument Intermediate Precision	Amplified	PPA	41/41	100.0	(91.4, 100.0)
	Non-amplified	NPA	47/47	100.0	(92.4, 100.0)
	Total	OPA	88/88	100.0	(95.8, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used.

Lot-to-Lot Precision

Lot-to-lot Precision was determined by testing 3 production lots of the VENTANA HER2 Dual ISH DNA Probe Cocktail, VENTANA Silver ISH DNP Detection Kit, and VENTANA Red ISH DIG Detection Kit on BenchMark ULTRA instruments. 15 GEA specimens supplemented with additional CDS specimens (10 BTC samples and 5 CRC samples) were stained with each probe and detection kit. A summary of the results for Lot-to-Lot Precision of the assay is shown in Table 10 (CDS) and Table 11 (GEA specific) below.

Table 10. Lot-to-Lot Precision

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Lot-to-Lot (Probe)	Amplified	PPA	88/88	100.0	(95.8, 100.0)
	Non-amplified	NPA	89/89	100.0	(95.9, 100.0)
	Total	OPA	177/177	100.0	(88.3, 100.0)
Lot-to-Lot (Detection kit)	Amplified	PPA	86/86	100.0	(95.7, 100.0)
	Non-amplified	NPA	90/90	100.0	(95.9, 100.0)
	Total	OPA	176/176	100.0	(97.7, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used. Four cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

Table 11. Lot-to-Lot Precision in GEA samples

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Lot-to-Lot (Probe)	Amplified	PPA	41/41	100.0	(91.4, 100.0)
	Non-amplified	NPA	47/47	100.0	(92.4, 100.0)
	Total	OPA	88/88	100.0	(95.8, 100.0)
	Amplified	PPA	40/40	100.0	(91.2, 100.0)

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Lot-to-Lot (Detection Kit)	Non-amplified	NPA	48/48	100.0	(92.6, 100.0)
	Total	OPA	88/88	100.0	(95.8, 100.0)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates; in instances where the point estimate was 100%, Wilson Score method was used.

Within-Reader and Between-Reader Precision

Within-Reader and Between-Reader Precision of the VENTANA HER2 Dual ISH DNA Probe Cocktail was determined by having three readers evaluate 80 CDS specimens (32 BTC samples, 32 GEA samples, and 16 CRC samples) stained with the VENTANA HER2 Dual ISH DNA Probe Cocktail using the VENTANA Silver ISH DNP Detection Kit and VENTANA Red ISH DIG Detection Kit on BenchMark ULTRA instrument. For within-reader precision, the same set of slides were read twice after a minimum of two weeks between reads. The within-reader and between-reader precision was determined with PPA, NPA, and OPA across all the observations from the evaluable population. A summary of the results of this study can be found in Table 12 (CDS) and Table 13 (GEA specific) below.

Table 12. Within-Reader and Between-Reader Precision

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Overall	Amplified	PPA	230/240	95.8	(92.5, 98.3)
	Non-amplified	NPA	233/240	97.1	(94.6, 99.2)
	Total	OPA	463/480	96.5	(94.4, 98.3)
Within-Reader	Amplified	PPA	230/236	97.5	(95.7, 99.2)
	Non-amplified	NPA	237/244	97.1	(94.6, 99.2)
	Total	OPA	467/480	97.3	(95.8, 98.8)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates. Eight cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

Table 13. Within-Reader and Between-Reader Precision in GEA samples

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Overall	Amplified	PPA	89/96	92.7	(84.4, 100.0)
	Non-amplified	NPA	95/96	99.0	(96.7, 100.0)
	Total	OPA	184/192	95.8	(91.4, 99.5)
Within-Reader	Amplified	PPA	89/92	96.7	(92.9, 100.0)
	Non-amplified	NPA	99/100	99.0	(96.7, 100.0)
	Total	OPA	188/192	97.9	(96.0, 99.5)

Note: 95% CIs were calculated using the percentile bootstrap method with 2000 replicates.

Inter-Laboratory Reproducibility Study

An inter-laboratory reproducibility study of the VENTANA HER2 Dual ISH DNA Probe Cocktail was conducted to evaluate reproducibility of the assay in determining HER2 gene status in GEA tissues stained on the BenchMark ULTRA instrument in combination with the VENTANA Silver ISH DNP Detection Kit and VENTANA Red ISH DIG Detection Kit.

The study included 28 de-identified FFPE GEA tissue specimens stained on three BenchMark ULTRA instruments on each of five non-consecutive days over 20 days at three external laboratories. The specimens included 14 HER2 amplified and 14 HER2 non-amplified cases. Following staining, two qualified readers at each site independently evaluated each case slide to assign a HER2 amplification status.

The data were analyzed for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all evaluable observations, and a summary is presented in Table 14 below.

Table 14. Inter-Laboratory Reproducibility for Overall Agreement Rates for VENTANA HER2 Dual ISH Assay in GEA on BenchMark ULTRA

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	373/386	96.6	(92.9, 100.0)
	NPA	434/448	96.9	(92.5, 99.8)
	OPA	807/834	96.8	(93.5, 99.4)
Within-Site	PPA	377/386	97.7	(95.4, 99.5)
	NPA	438/448	97.8	(95.7, 99.3)
	OPA	815/834	97.7	(95.8, 99.4)
Within-Reader	PPA	384/395	97.2	(94.1, 99.5)
	NPA	436/439	99.3	(98.6, 100.0)
	OPA	820/834	98.3	(96.9, 99.5)

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates. Four cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

In addition, pairwise comparisons were made Between-Site, Between-Reader, and Between-Day for HER2 amplification status. These data were analyzed for average positive agreement (APA), average negative agreement (ANA), and OPA and are presented in Table 15 below.

Table 15. Inter-Laboratory Reproducibility Pairwise Agreement Rates for VENTANA HER2 Dual ISH Assay in GEA

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	APA	7212/7644	94.3	(88.1, 98.7)
	ANA	8484/8916	95.2	(90.6, 98.9)
	OPA	7848/8280	94.8	(89.9, 98.8)
Between- Reader	APA	368/387	95.1	(90.6, 98.7)
	ANA	428/447	95.7	(91.9, 98.9)
	OPA	398/417	95.4	(91.5, 98.8)
Between- Day	APA	1480/1529	96.8	(94.4, 98.8)
	ANA	1734/1783	97.3	(95.1, 98.9)
	OPA	1607/1656	97.0	(94.9, 98.8)

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method using 2000 replicates. Four cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

4. Robustness

Tissue thickness

The purpose of this study was to determine the impact of section thickness on assay staining performance and identify those section thickness(es) that may contribute to a false positive for false negative result. Tissue thickness was evaluated using a total of 10 uniblock including 4 GEA samples in addition to BTC and CRC samples (5 HER2 amplified and 5 HER2 non-amplified). Each single tissue case was sectioned at 2 µm, 3 µm, 4 µm, 5 µm, 6 µm, and 7 µm, compared to evaluable tissue cut at 4 µm.

VENTANA HER2 Dual ISH-stained test samples sectioned at 3, 5, 6, and 7 µm demonstrated 100% concordance for HER2 amplification status when compared to the respective reference sample for each case. Tissue thickness of 2 µm resulted in less than 90% OPA. Background acceptability rate for all evaluable observations was 100%.

Fixation Study

Refer to the Summary of Safety and Effectiveness Data P190031 (Section 1X.A.2c) for the study to evaluate the effects of fixative type and fixation time.

Protocol Limitations

The purpose of this study was to identify protocol conditions that lead to a potential false positive, false negative, or unacceptable result. Twenty individual CDS tissue cases including 8 GEA samples in addition to BTC and CRC samples were tested.

Results for each of the protocol limitations and failure modes are summarized below in Table 16.

Table 16. Protocol Limitations and Failure Mode Analysis of VENTANA HER2 Dual ISH DNA Probe Cocktail in Carcinomas of the Digestive System (CDS)

Condition	Protocol Step	Parameters	Result
Target Retrieval	Baking	Offline: oven at 60°C for 20 min	Limitation (19/20 passing; 95%)
		Online: Instrument Bake	No Limitation (20/20 passing; 100%)
		Double Bake: oven at 60°C for 20 min followed by baking on the ULTRA	Limitation (19/20 passing; 95%)
	Cell Conditioning (CC1)	Low 16 minutes	No Limitation (20/20 passing; 100%)
		Medium 36 minutes	No Limitation (20/20 passing; 100%)
		High 64 minutes (Not available to customers)	Limitation (19/20 passing; 95%)
	Cell Conditioning (CC2)	Low 16 minutes	Limitation (16/20 passing; 80%)
		Medium 36 minutes	No Limitation (20/20 passing; 100%)
		High 64 minutes (Not available to customers)	Limitation (15/20 passing; 75%)
	ISH Protease 3	Low 4 minutes (Not available to customers)	Limitation (5/20 passing; 25%)
		Medium 16 minutes	Limitation (15/20 passing; 75%)
		High 32 minutes (Not available to customers)	No Limitation (20/20 passing; 100%)
	Stringency Wash Temperature	Low 74°C	Limitation (19/20 passing; 95%)
		Medium 76°C	Limitation (17/20 passing; 85%)

Condition	Protocol Step	Parameters	Result
		High 78°C	Limitation (14/20 passing; 70%)
	Double Stacking (CC1, CC2, ISH Protease 3 and Stringency Wash Temperature)	Low: CC1 16 min, CC2 16 min, Protease 3 4 min, Stringency Wash Temp 74°C	Limitation (1/20 passing; 5%)
		High: CC1 64 min, CC2 64 min, Protease 3 32 min, Stringency Wash Temp 78°C	Limitation (8/20 passing; 40%)
Target Probe and Detection	HER2 Dual ISH Probe Cocktail	Partial: 1 Dispenser Drop	Limitation (18/20 passing; 90%)
		Over Dispense: 3 Dispenser Drops	Limitation (18/20 passing; 90%)
	VENTANA Silver ISH DNP Detection Kit Component (Chromogen C); VENTANA Red ISH DIG Detection Kit Component (pH Enhancer)	Partial: Chromogen C ½ Drop; pH Enhancer 1 drop	Limitation (18/20 passing; 90%)
		Over dispense: Chromogen C 2 Drops; pH Enhancer 3 drops	Limitation (19/20 passing; 95%)

5. Tissue Heterogeneity

Case Heterogeneity

This study characterized case heterogeneity of VENTANA HER2 Dual ISH DNA Probe Cocktail by evaluating staining outcome from 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, including 11 GEA samples in addition to BTC and CRC samples, with a total of 141 individual tissue blocks were evaluated. Of these, 97.2% of blocks (137/141) demonstrated concordant HER2 status with the case-level mode for HER2 status. 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.

Block Heterogeneity

This study characterized within-block heterogeneity by evaluating staining outcome from multiple samples from the same tissue block. Block heterogeneity compares biomarker status between multiple slides sectioned from one block. Twelve (12) individual CDS tissue cases including 6 GEA samples in addition to BTC and CRC samples, were evaluated. HER2 amplification status did not change within a block; therefore, none of the blocks exhibited heterogeneity. Although none of the tissue blocks enrolled for this particular study exhibited heterogeneity of HER2 amplification, other blocks could present heterogeneity.

6. Primary vs Metastatic Tumor

This study characterized the staining performance of the VENTANA HER2 Dual ISH DNA Probe assay (hereafter, HER2 Dual ISH) on the primary tumor versus patient-matched metastatic tumor sample. Of the 28 CDS patient cases included in analysis, 85.7% of cases (24/28) demonstrated concordant HER2 status between the primary tumor block and the patient-matched metastatic tumor block. 88.5% of GEA cases (17/19) demonstrated concordant status between the primary and metastatic tumor blocks. Twenty (20) cases had blocks with non-enumerable results and were therefore excluded from analysis. For prevalence analysis of unmatched cholangiocarcinoma blocks, metastatic tumor blocks had a sample positive prevalence of 0.0% (0/15) and primary tumor blocks had a sample positive prevalence of 4.4% (7/160). The results from this study demonstrate that HER2 amplification status can be discordant between the primary tumor and metastatic tumor block within individual patient cases.

7. Stability Studies

Cut Slide Stability

The intent of this study was to assess the stability of nucleic acids in FFPE tissue that has been sectioned onto glass microscope slides and stored for an extended duration of time at various environmental conditions by evaluating staining performance. A total of 12 CDS patient cases, including 4 GEA samples in addition to BTC and CRC samples, were evaluated. The time and storage conditions tested were as follows: 30°C ± 5°C at 15% R.H. and 5°C ± 3°C at 15% R.H. Cut slide stability was evaluated on a total of five single tissues of CDS. Slides were stained at specific time points and staining results were compared to time 0 (day 0). For the expanded study, time points were modified to capture data points between 7 and 60 days for the 30°C and 85% R.H. storage condition and data points between 7 and 180 days for the 5°C and 85% R.H. condition. In addition to these time points, two test conditions were added that consisted of cut slides sealed in a mylar bag with desiccant and stored at low temperature/high humidity and high temperature/high

humidity. Seven single tissues of CDS were evaluated and the results from this study are presented in Table 17 below.

Table 17. Summary of Results for CDS – Overall Percent Agreement for HER2 Clinical Status

Time Point	Agreement % (n/N)					
	5°C ± 3°C, 15% R.H.	**30°C ± 5°C, 15% R.H.**	**5°C ± 3°C, 85% R.H.**	**5°C ± 3°C, 85% R.H. in Mylar Bag**	**30°C ± 5°C, 85% R.H.**	**30°C ± 5°C, 85% R.H. in Mylar Bag**
Day 7	100 % (10/10)	100 % (10/10)	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 10	N/A	N/A	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 15	N/A	N/A	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 20	N/A	N/A	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 25	N/A	N/A	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 30	100 % (10/10)	100 % (10/10)	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 45	100 % (10/10)	100 % (10/10)	N/A	N/A	N/A	N/A
Day 60	100 % (10/10)	100 % (10/10)	100 % (6/6)	100 % (6/6)	100 % (6/6)	100 % (6/6)
Day 73*	N/A	N/A	100 % (1/1)	100 % (1/1)	N/A	N/A
Day 90	100 % (10/10)	100 % (10/10)	N/A	N/A	N/A	N/A
Day 180	100 % (10/10)	100 % (10/10)	100 % (6/6)	100 % (6/6)	N/A	N/A

Real Time Stability

The objective of this study was to verify the stability (shelf-life and in-use) and shipping category of VENTANA HER2 Dual ISH DNA Probe Cocktail and the detection kits. Three production lots of VENTANA HER2 Dual ISH DNA Probe Cocktail and the detection kits were subjected to different condition:

- Intended Storage 2-8°C

- Hot Ship Stress Category F 37±2ºC (192 hours)
- Hot Ship Stress Cat. F 37±2ºC (150 hours)
- Hot Ship Stress Cat. J 32±2ºC (192 hours)
- Hot Ship Stress Cat. H 17±2ºC (192 hours)
- Hot Ship Stress Cat. H 27±2ºC (192 hours)
- Cold Ship Stress (Freeze/Thaw Cycles) -25±5ºC (192 hours)

Each of the 3 lots of VENTANA HER2 Dual ISH DNA Probe Cocktail was assigned a sample set of breast and CDS tissues containing amplified and non-amplified cases. HER2/Chr 17 ratio ≥2.0 is amplified, while a ratio <2.0 is non-amplified.

The data supports the current product dating of 18 months.

B. Animal Studies

Not applicable

C. Additional Studies

Not applicable

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The clinical performance of VENTANA HER2 Dual ISH DNA Probe Cocktail 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 summary of the clinical study is presented below.

A. Study Design

The HERIZON-GEA-01 (NCT05152147) 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)] enrolled adult patients with HER2-positive GEA (defined as IHC 3+ or IHC 2+/ISH amplified per central testing). Tumor samples from patients undergoing screening were centrally tested with the VENTANA HER2 Dual ISH DNA Probe Cocktail to assess HER2 gene amplification by ISH and determine HER2 ISH status (in addition to being tested with the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody for HER2 IHC status determination – refer to P990081/S060 SSED).

A total of 914 GEA patients (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

Enrollment in the HERIZON-GEA-01 clinical study was limited to patients who met the following inclusion criteria:

- 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.
- 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.
- Male or female, ≥ 18 years of age (or the legal age of adulthood per country-specific regulations).
- Female subjects of childbearing potential must have a negative serum/plasma or urine beta human chorionic gonadotropin (β-hCG) pregnancy test result prior to randomization. Females with false positive urine test results can be enrolled if subsequent serum/plasma testing is negative.

Specimen Inclusion Criteria

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

- It was a tumor specimen submitted for patient enrollment screening for Study
- ZWI-ZW25-301.
- It was an FFPE specimen or fresh tissue fixed in formalin.

- It contained sufficient tumor tissue for interpretation at the discretion of the reviewing pathologist.
- 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 ZWI-ZW25-301 study if they met any of the following exclusion criteria:

- 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.
- 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.
- Received radiation therapy within 14 days prior to randomization.
- Major surgery within 28 days prior to randomization.
- 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 $\leq$ Grade 1 at the time of occurrence and must have completely resolved.
- Symptomatic pulmonary embolism $\leq$ 28 days prior to randomization.
- Any history of cerebrovascular accident $\leq$ 6 months prior to randomization.
- Administered a live vaccine $\leq$ 4 weeks prior to randomization.
- Treated with another investigational product within 28 days of randomization.

Specimen Exclusion Criteria

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

- It was a fine needle aspirate or cytology specimen.
- It consisted of tissue that had been decalcified.
- It was known to be fixed in 95% alcohol, AFA, PREFER, ZF, or Bouin's.
- The unstained slides were prepared:
  - More than 15 days prior to staining if not kept under refrigeration (5 °C ± 3 °C); or

- More than 180 days prior to staining only if kept under refrigeration (5 °C ± 3 °C)

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 stud 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). Additional efficacy outcome measures included Objective response rate (ORR) and Duration of Response (DOR) per BICR.

B. Accountability of PMA Cohort

In total of 2743 pre-screened and/or screened participants, 2493 participants were tested with VENTANA HER2 Dual ISH DNA Probe Cocktail for the HERIZON-GEA-01 study. Of these, 1182 participants were HER2 ISH-amplified, and 1149 participants were HER2 ISH-nonamplified (See Table 18). No protocol deviations resulted in exclusions from the ISH population.

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 18. Accountability of the PMA Cohort for Study HERIZON-GEA-01

Patients Disposition for Study ZWI-ZW25-301 (ISH PMA cohort)	**N**
Total number of patients pre-screened and/or screened	2743
ISH tested Population	2493
Excluded Due to No Sample Submitted or Test Cancelled	250
HER2 ISH Amp	1182
HER2 ISH Non-Amp	1149
HER2 ISH Not Evaluable	112
HER2 ISH Not Evaluated	50
Excluded from ISH testing	0

C. Study Population Demographics and Baseline Parameters

The patients' demographics and sample characteristics in ISH population are presented by HER2 ISH status (amplified or nonamplified) in Table 19 and Table 20, respectively.

In the ISH 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 (46.2%) or Asian (42.9%).

Table 19: Patient Characteristics by HER2 ISH Status

HER2 ISH Status					
Lab	**Characteristics**	**Amplified**	**No-Amplified**	**Not Evaluable/Not Evaluated**	**Overall**
		(N=1182)	**(N=1149)**	**(N=162)**	**(N=2493)**
All	**Age** (years)				
	N	1182	1149	162	2493
	Mean ± SD	61.9 ± 11.30	61.6 ± 12.13	61.4 ± 12.17	61.7 ± 11.74
	Median	63.0	63.0	63.0	63.0
	Min, Max	21.0, 90.0	23.0, 92.0	29.0, 84.0	21.0, 92.0
	Age Group, n (%)				
	< 65	637 (53.9%)	620 (54.0%)	86 (53.1%)	1343 (53.9%)
	>= 65	545 (46.1%)	529 (46.0%)	76 (46.9%)	1150 (46.1%)
	Sex, n (%)				
	Female	259 (21.9%)	368 (32.0%)	34 (21.0%)	661 (26.5%)
	Male	923 (78.1%)	781 (68.0%)	128 (79.0%)	1832 (73.5%)
	Ethnicity, n (%)				
	Hispanic or Latino	161 (13.6%)	379 (33.0%)	61 (37.7%)	601 (24.1%)
	Not Hispanic or Latino	973 (82.3%)	746 (64.9%)	99 (61.1%)	1818 (72.9%)
	Not Reported	41 (3.5%)	19 (1.7%)	2 (1.2%)	62 (2.5%)
	Unknown	7 (0.6%)	5 (0.4%)	0 (0.0%)	12 (0.5%)
	Race, n (%)				
	American Indian or Alaska Native	22 (1.9%)	96 (8.4%)	17 (10.5%)	135 (5.4%)
	Asian	649 (54.9%)	394 (34.3%)	26 (16.0%)	1069 (42.9%)
	Black or African American	7 (0.6%)	13 (1.1%)	5 (3.1%)	25 (1.0%)
	White	447 (37.8%)	598 (52.0%)	108 (66.7%)	1153 (46.2%)
	Multiple	4 (0.3%)	22 (1.9%)	0 (0.0%)	26 (1.0%)
	Not Reported	33 (2.8%)	1 (1.0%)	0 (0.0%)	44 (1.8%)

HER2 ISH Status					
Lab	**Characteristics**	**Amplified**	**No-Amplified**	**Not Evaluable/Not Evaluated**	**Overall**
	Other	12 (1.0%)	8 (0.7%)	6 (3.7%)	26 (1.0%)
	Unknow	8 (0.7%)	7 (0.6%)	0 (0.0%)	15 (0.6%)
	Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Country, n (%)				
	Argentina	23 (1.9%)	66 (5.7%)	16 (9.9%)	105 (4.2%)
	Australia	17 (1.4%)	3 (0.3%)	0 (0.0%)	20 (0.8%)
	Belgium	15 (1.3%)	2 (0.2%)	0 (0.0%)	17 (0.7%)
	Brazil	36 (3.0%)	80 (7.0%)	21 (13.0%)	137 (5.5%)
	Canada	15 (1.3%)	4 (0.3%)	0 (0.0%)	19 (0.8%)
	Estonia	3 (0.3%)	4 (0.3%)	3 (1.9%)	10 (0.4%)
	France	48 (4.1%)	19 (1.7%)	3 (1.9%)	70 (2.8%)
	Georgia	7 (0.6%)	15 (1.3%)	2 (1.2%)	24 (1.0%)
	Germany	3 (0.3%)	2 (0.2%)	1 (0.6%)	6 (0.2%)
	Greece	11 (0.9%)	13 (1.1%)	0 (0.0%)	24 (1.0%)
	Guatemala	6 (0.5%)	43 (3.7%)	5 (3.1%)	54 (2.2%)
	India	15 (1.3%)	24 (2.1%)	5 (3.1%)	44 (1.8%)
	Ireland	7 (0.6%)	2 (0.2%)	0 (0.0%)	9 (0.4%)
	Italy	48 (4.1%)	22 (1.9%)	3 (1.9%)	73 (2.9%)
	Japan	76 (6.4%)	88 (7.7%)	0 (0.0%)	164 (6.6%)
	Malaysia	14 (1.2%)	65 (5.7%)	1 (0.6%)	80 (3.2%)
	Mexico	14 (1.2%)	57 (5.0%)	12 (7.4%)	83 (3.3%)
	Netherlands	16 (1.4%)	3 (0.3%)	1 (0.6%)	20 (0.8%)
	Poland	14 (1.2%)	8 (0.7%)	0 (0.0%)	22 (0.9%)
	Portugal	17 (1.4%)	20 (1.7%)	1 (0.6%)	38 (1.5%)
	Romania	42 (3.6%)	101 (8.8%)	28 (17.3%)	171 (6.9%)
	Serbia	25 (2.1%)	83 (7.2%)	13 (8.0%)	121 (4.9%)
	Singapore	4 (0.3%)	4 (0.3%)	0 (0.0%)	8 (0.3%)
	South Africa	3 (0.3%)	2 (0.2%)	1 (0.6%)	6 (0.2%)
	South Korea	172 (14.6%)	72 (6.3%)	5 (3.1%)	249 (10.0%)
	Spain	84 (7.1%)	37 (3.2%)	13 (8.0%)	134 (5.4%)
	Taiwan	5 (0.4%)	5 (0.4%)	1 (0.6%)	11 (0.4%)
	Thailand	9 (0.8%)	49 (4.3%)	1 (0.6%)	59 (2.4%)
	Turkey	12 (1.0%)	21 (1.8%)	4 (2.5%)	37 (1.5%)
	Ukraine	3 (0.3%)	9 (0.8%)	0 (0.0%)	12 (0.5%)
	United Kingdom	28 (2.4%)	11 (1.0%)	2 (1.2%)	41 (1.6%)
	Primary Diagnosis, n (%)				
	Esophageal Adenocarcinoma	111 (9.4%)	59 (5.1%)	14 (8.6%)	184 (7.4%)

HER2 ISH Status					
Lab	**Characteristics**	**Amplified**	**No-Amplified**	**Not Evaluable/Not Evaluated**	**Overall**
	GEJ Adenocarcinoma	237 (20.1%)	176 (15.3%)	28 (17.3%)	441 (17.7%)
	Gastric Adenocarcinoma	817 (69.1%)	810 (70.5%)	114 (70.4%)	1741 (69.8%)
	Missing	17 (1.4%)	104 (9.1%)	6 (3.7%)	127 (5.1%)
	Presence of Brain Metastases, n (%)				
	Yes	14 (1.2%)	2 (0.2%)	1 (0.6%)	17 (0.7%)
	No	1150 (97.3%)	1035 (90.1%)	154 (95.1%)	2339 (93.8%)
	Missing	18 (1.5%)	112 (9.7%)	7 (4.3%)	137 (5.5%)
	Stage at Initial Diagnosis, n (%)				
	Stage Ia	11 (0.9%)	6 (0.5%)	1 (0.6%)	18 (0.7%)
	Stage Ib	6 (0.5%)	6 (0.5%)	2 (1.2%)	14 (0.6%)
	Stage Ic	1 (0.1%)	2 (0.2%)	1 (0.6%)	4 (0.2%)
	Stage Iia	15 (1.3%)	21 (1.8%)	4 (2.5%)	40 (1.6%)
	Stage Iib	18 (1.5%)	32 (2.8%)	3 (1.9%)	53 (2.1%)
	Stage Iic	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Stage Iiia	58 (4.9%)	64 (5.6%)	10 (6.2%)	132 (5.3%)
	Stage Iiib	48 (4.1%)	55 (4.8%)	5 (3.1%)	108 (4.3%)
	Stage Iiic	25 (2.1%)	26 (2.3%)	5 (3.1%)	56 (2.2%)
	Stage Iv	590 (49.9%)	494 (43.0%)	77 (47.5%)	1161 (46.6%)
	Stage v	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Stage Iva	41 (3.5%)	53 (4.6%)	12 (7.4%)	106 (4.3%)
	Stage va	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Stage Ivb	266 (22.5%)	226 (19.7%)	23 (14.2%)	515 (20.7%)
	Stage vb	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%)
	Other	87 (7.4%)	49 (4.3%)	12 (7.4%)	148 (5.9%)
	Missing	16 (1.4%)	115 (10.0%)	7 (4.3%)	138 (5.5%)
	ECOG Performance Status at Enrollment, n (%)				
	0	363 (30.7%)	5 (0.4%)	7 (4.3%)	375 (15.0%)
	1	516 (43.7%)	8 (0.7%)	14 (8.6%)	538 (21.6%)
	2	1 (0.1%)	0 (0.0%)	0 (0.0%)	1 (0.0%)
	Missing	302 (25.5%)	1136 (98.9%)	141 (87.0%)	1579 (63.3%)

Table 20. Sample Characteristics by HER2 ISH Status

		HER2 ISH Status			
Lab	**Characteristics**	**Amplified**	**No-Amplified**	**Not Evaluable/Not Evaluated**	**Overall**
		(N=1183)	**(N=1168)**	**(N=197)**	**(N=2548)**
All	**Sample Collection Method**, n (%)				
	Biopsy	1014 (85.7%)	939 (80.4%)	170 (86.3%)	2123 (83.3%)
	Resection	169 (14.3%)	229 (19.6%)	27 (13.7%)	425 (16.7%)
	Specimen Type, n (%)				
	Archive FFPE block	444 (37.5%)	744 (63.7%)	146 (74.1%)	1334 (52.4%)
	Archive Slides	700 (59.2%)	366 (31.3%)	36 (18.3%)	1102 (43.2%)
	Archive slides, Archive FFPE block	2 (0.2%)	4 (0.3%)	2 (1.0%)	8 (0.3%)
	Fresh slides	25 (2.1%)	23 (2.0%)	2 (1.0%)	50 (2.0%)
	Fresh tissue block	12 (1.0%)	31 (2.7%)	11 (5.6%)	54 (2.1%)
	Fixative Used, n (%)				
	10% Neutral Buffered Formalin (NBF)	1170 (98.9%)	1144 (97.9%)	192 (97.5%)	2506 (98.4%)
	Other formalin-based fixatives	13 (1.1%)	24 (2.1%)	4 (2.0%)	41 (1.6%)
	Other	0 (0.0%)	0 (0.0%)	1 (0.5%)	1 (0.0%)
	Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Time to Fixation, n (%)				
	<= 1 hour	790 (66.8%)	661 (56.6%)	115 (58.4%)	1566 (61.5%)
	> 1 hour	108 (9.1%)	118 (10.1%)	25 (12.7%)	251 (9.9%)
	Unknown	285 (24.1%)	389 (33.3%)	57 (28.9%)	731 (28.7%)
	Tumor Type, n (%)				
	Primary	1080 (91.3%)	1027 (87.9%)	169 (85.8%)	2276 (89.3%)
	Metastatic	103 (8.7%)	141 (12.1%)	28 (14.2%)	272 (10.7%)

D. Safety and Effectiveness Results

1. Safety Results

No adverse events associated with use of VENTANA HER2 Dual ISH DNA Probe Cocktail were reported during the course of the HERIZON-GEA-01 clinical study.

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 21, Figure 1 and Figure 2.

Table 21. 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

	ARM C ZIIHERA with chemotherapy and tislelizumab-jsgr N=302	**ARM A Trastuzumab with chemotherapy N=308**
(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	10%	12%
Partial response rate	47%	50%
Duration of Response (DOR)	N=202	N=193
Median in months (95% CI) [a]	18.9 (12.1, 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

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

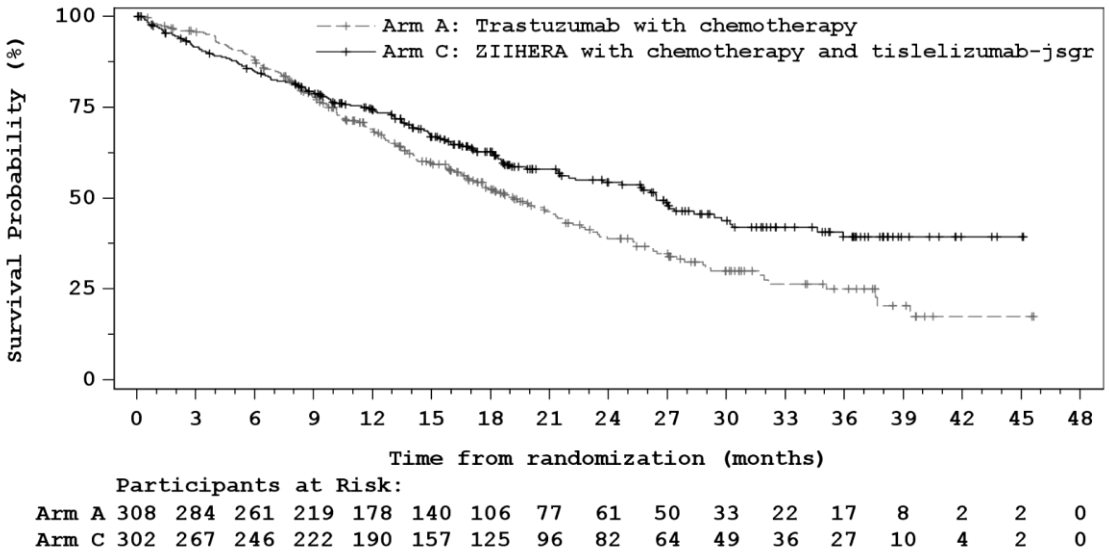
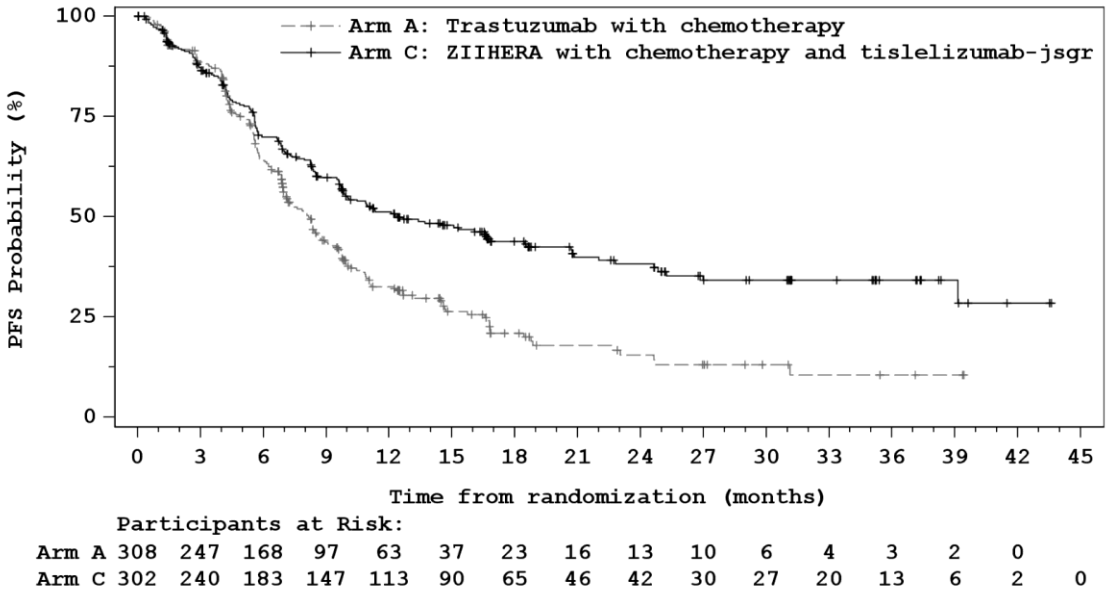


Figure 2. Kaplan-Meier Plot of Progression Free Survival for Arm A and Arm C in in HERIZON-GEA-01



PFS and OS for the IHC 2+/ISH-Positive population

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 and 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.

3. 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 two (2) 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 VENTANA HER2 Dual ISH DNA Probe Cocktail 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 VENTANA HER2 Dual ISH DNA Probe Cocktail as a companion diagnostic device for identifying GEA patients eligible for treatment with ZIIHERA (zanidatamab) with tislelizumab, and chemotherapy. Overall, data showed clinically meaningful efficacy of the ZIIHERA combination regimen in the patient population identified by VENTANA HER2 Dual ISH DNA Probe Cocktail, 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. VENTANA HER2 Dual ISH DNA Probe Cocktail 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 VENTANA HER2 Dual ISH DNA Probe Cocktail is intended to identify GEA patients for treatment with ZIIHERA (zanidatamab) with 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. 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 of the device are based on data collected in the HERIZON-GEA-01 clinical study conducted to support the supplemental PMA approval.

The only meaningful benefit expected for patients with GEA who have been classified as HER2-positive (IHC2+ISH+ subgroup) as per the IU of this device, and treated with zanidatamab (ZIIHERA) plus tislelizumab and chemotherapy, which includes studies based on both IHC and ISH, are as follows:

Improved overall survival with adjuvant treatment: In the HER2 IHC2+/ISH+ population, 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 (zanidatamab + tislelizumab + chemotherapy) and 18.7 months (95% CI: 13.6, 23.1) in control Arm (trastuzumab + chemotherapy).

The data may support the use of VENTANA HER2 Dual ISH DNA Probe Cocktail as an aid to identify patients with HER2 positive GEA and who may be eligible for treatment with ZIIHERA (zanidatamab) with tislelizumab, and chemotherapy in accordance with approved therapeutic labeling.

The main risk of the VENTANA HER2 Dual ISH DNA Probe Cocktail 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 combinations, as well as the probability of delaying or forgoing other effective treatments, 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.

Given the available information above, the potential for clinical benefit outweighs the clinical risk for unresectable locally advanced, recurrent or metastatic HER2- positive GEA patients tested with the VENTANA HER2 Dual ISH DNA Probe Cocktail to determine eligibility for ZIIHERA (zanidatamab) with tislelizumab and chemotherapy.

The risks of a false positive test include drug toxicities, which can at times be severe, while receiving therapy which may not be effective. The risks of a false negative include forgoing therapy which may be effective, as noted. On balance, in the opinion of this consultant, there is a reasonable assurance that the benefits exceed the risks when this device and the referent drug are used according to the FDA-approved intended use statements, and therefore approval of this PMA is recommended.

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

D. Overall Conclusions

The data in this application supports the reasonable assurance of safety and effectiveness of this device when used in accordance with the instructions for use. The data from the HERIZON-GEA-01 study support the use of the VENTANA HER2 Dual ISH DNA Probe Cocktail as a companion diagnostic to identify patients with GEA who may be eligible for treatment with ZIIHERA (zanidatamab) with 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 condition of approval cited in the approval order are described below:

Ventana Medical Systems, Inc. must provide data from a single-site precision study with three pathologists to confirm the performance of the VENTANA HER2 Dual ISH DNA Probe Cocktail in the IHC 2+ population. The majority of tested samples must be HER 2 IHC 2+ gastroesophageal adenocarcinoma (GEA) tissue samples to reflect the intended target population, with a balanced representation of amplified and non-amplified cases and include adequate borderline GEA cases.

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

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