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 Product code:	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/S054
Date of FDA Notice of Approval:	November 20, 2024

The original PMA (P990081) for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Antibody was approved for 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. The SSED to support previous indications are available on CDRH website and incorporated by reference here.

The current supplement (S054) was submitted to expand the indication for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Antibody to include testing of FFPE biliary tract cancer (BTC) tissue from patients for whom ZIIHERA® treatment is being considered.

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 and biliary tract cancer (gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, and extrahepatic cholangiocarcinoma) tissue using the ultraView Universal DAB Detection Kit on a BenchMark ULTRA 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	Status	Therapy
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	Herceptin®
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	KADCYLA®
Breast carcinoma	IHC 1+ or IHC 2+/ISH non-amplified	ENHERTU®
Biliary tract cancer	IHC 3+	ZIIHERA®

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

Warnings and precautions can be found in the PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody product labeling.

V. DEVICE DESCRIPTION

The Ventana PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody assay is an automated semi-quantitative immunohistochemical (IHC) staining assay system comprising a pre-dilute, ready-to-use rabbit monoclonal primary antibody (clone 4B5) directed against the internal domain of the transmembrane human epidermal growth factor receptor 2 (HER2), ultraView™ universal Diaminobenzidine (DAB) detection kit (containing Horse Redox Peroxidase (HRP) conjugated secondary antibody), and an automated slide staining platform, BenchMark ULTRA. The reagents and the IHC procedure are optimized for use to detect the primary antibodies bound to the antigen in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The specific antibody-enzyme complex is visualized with a precipitating enzyme reaction product by light microscopy. 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. The staining procedure also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide. The assay is fully automated for use on the BenchMark ULTRA Advanced Staining System utilizing VSS software (Ventana System Software).

It is recommended to 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, which reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting, and manual reagent application.

A. Device Kit Components

PATHWAY anti-HER2 (4B5) antibody contains sufficient reagent for 50 tests. Table 1 and Table 2 below list the PATHWAY anti-HER2 (4B5) antibody kit and assay components respectively. 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 Ethylenediaminetetraacetic acid (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. There is no known irrelevant antibody reactivity observed in this product. 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
ultraView Universal DAB Detection Kit
EZ Prep (10×)
Reaction Buffer (10×)
ULTRA Liquid Coverslip (LCS) (pre-dilute)
ULTRA Cell Conditioning (CC)1
Hematoxylin II counterstain
Bluing Reagent
Staining Instrument/Software
BenchMark ULTRA instrument
Host operating software: VSS 12.3, 12.3.1, 12.5.3 or 12.5.4
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 5 mL 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 6µg/mL.
*ultra*View DAB IHC Detection Kit	Set of 5 dispensers packaged in a kit: 250 tests	***ultra*View DAB Inhibitor** contains 3.0% hydrogen peroxide solution.
		***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 HRP labeled antibodies (goat anti-mouse Immunoglobulin G (IgG), goat anti-mouse Immunoglobulin M (IgM), and goat anti-rabbit IgG) (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 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 of rabbit immunoglobulin (Ig) in sections of FFPE tissue. One 25 mL 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 12.3, 12.3.1, 12.5.3 or 12.5.4.

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. The recommended tissue fixative is 10% neutral buffered formalin. The amount used is 15 to 20 times the volume of tissue. Since no fixative will 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 should be fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25°C).

Tissue sections approximately 4-5 μm thick and mounted on glass slides should be used for the staining. Slides should be stained promptly, as antigenicity of the cut tissue sections may diminish over time.

D. Test Controls

Run controls should be included in each staining run to establish the validity of the test results. The following controls should be run with the assay.

1. Cell Line Controls

PATHWAY anti-HER2 4-in-1 Control Slides include 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 Her-2 4 in 1 Control Slide labeling. If the indicated staining is not evident in the appropriate cores, especially the 1+ and 2+ controls, the staining of the tissues should be repeated. PATHWAY HER2 4-in-1 Control Slides may be useful for a preliminary validation of the instrument used for staining slides with PATHWAY anti-HER2/neu (4B5) antibody, 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 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 staining is a known weak HER2 positive invasive breast carcinoma (for example ductal or lobular). The positive staining tissue components (membrane of neoplastic cells) are used to confirm that the antibody was applied, and the instrument functioned properly.

A known weak HER2 positive invasive breast carcinoma tissue may contain both positive and negative staining cells or tissue components and may serve as both the positive and negative control tissue.

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 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 tissue sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies LCS, 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, with the automated 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 staining protocols and procedures listed in Table 4 are appropriate for use in all HER2 screening of biliary tract cancer cases as indicated in the table. Deviating from the recommended staining protocol may produce invalid results. Decreasing or increasing cell conditioning times are likely to produce HER2-stained samples with altered HER2 scores, which may result in inappropriate treatment decisions for patients.

Table 4. Staining Protocols for PATHWAY anti-HER2 (4B5) antibody for HER2 assessment on a BenchMark ULTRA Instrument

Procedure Type	PATHWAY anti-HER2 (4B5)
Indication	Biliary tract cancer
Protocol step	Parameter input
Staining Procedure	UT PATHWAY HER2 4B5**
Deparaffinization*	Selected, 4 minutes, 72°C
Cell Conditioning*	ULTRA CC1, 36 minutes, Mild (95°C)
Antibody (Primary)*	PATHWAY HER2 4B5 Ab- 12 Min, 36°C Or Neg Ctl Rbt Ig- 12 Min, 36°C
*ultra*View DAB Detection Kit*	*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 H2O2: 8 minutes, 36°C *ultra*View Copper: 4 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes, 36°C
Post Counterstain	Bluing, 4 minutes, 36°C

* These are pre-programmed conditions and are not selectable steps to the user.

** UT PATHWAY HER2 4B5: This is identical in the staining procedure parameters as U PATHWAY HER2 4B5 that is used in breast cancer staining procedure, only difference with this procedure is that it can only be installed on the BenchMark ULTRA instrument while the U PATHWAY HER2 4B5 procedure can be installed on both the instruments, BenchMark ULTRA and BenchMark ULTRA PLUS. The prefix "UT" denotes that this procedure is a temporary staining procedure that has been created for use until BTC is validated and supported on the BenchMark ULTRA PLUS platform.

F. Slide Review and Interpretation of HER2 Staining

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

1. Cell line control

PATHWAY HER-2 4 in 1 Control Slide, the System-level control slide, is useful for a preliminary validation of the instrument used for staining slides with PATHWAY anti-HER2/neu (4B5) antibody on each staining run. See details at Test Controls on Section D under Device Description.

2. 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.

3. 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.

4. 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.

5. 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.

6. PATHWAY anti-HER2 (4B5) Antibody Scoring Method for Biliary Tract Cancer

The HER2 scoring algorithm is based on assessment of percent tumor cell (TC) and stain intensity. Staining must localize to cell membrane but need not be completely circumferential, as basolateral staining is regularly observed and should be considered for scoring (different than scoring of breast tissues). Staining of cytoplasm and/or nucleus may be present but is not included in determination of positivity. In BTC, staining intensity is evaluated using the magnification rule (See section 7) which is different than scoring of breast tissues. The number of viable tumor cells present should be ≥100. Scoring criteria and interpretation for HER2 status in biliary tract cancer is provided in Table 5. Refer to PATHWAY anti-HER2 (4B5) Antibody method sheet and interpretation guide for BTC for additional details.

Table 5. Scoring Criteria for Intensity and Pattern of Cell Membrane Staining with PATHWAY anti-HER2 (4B5) Antibody in Biliary tract cancer

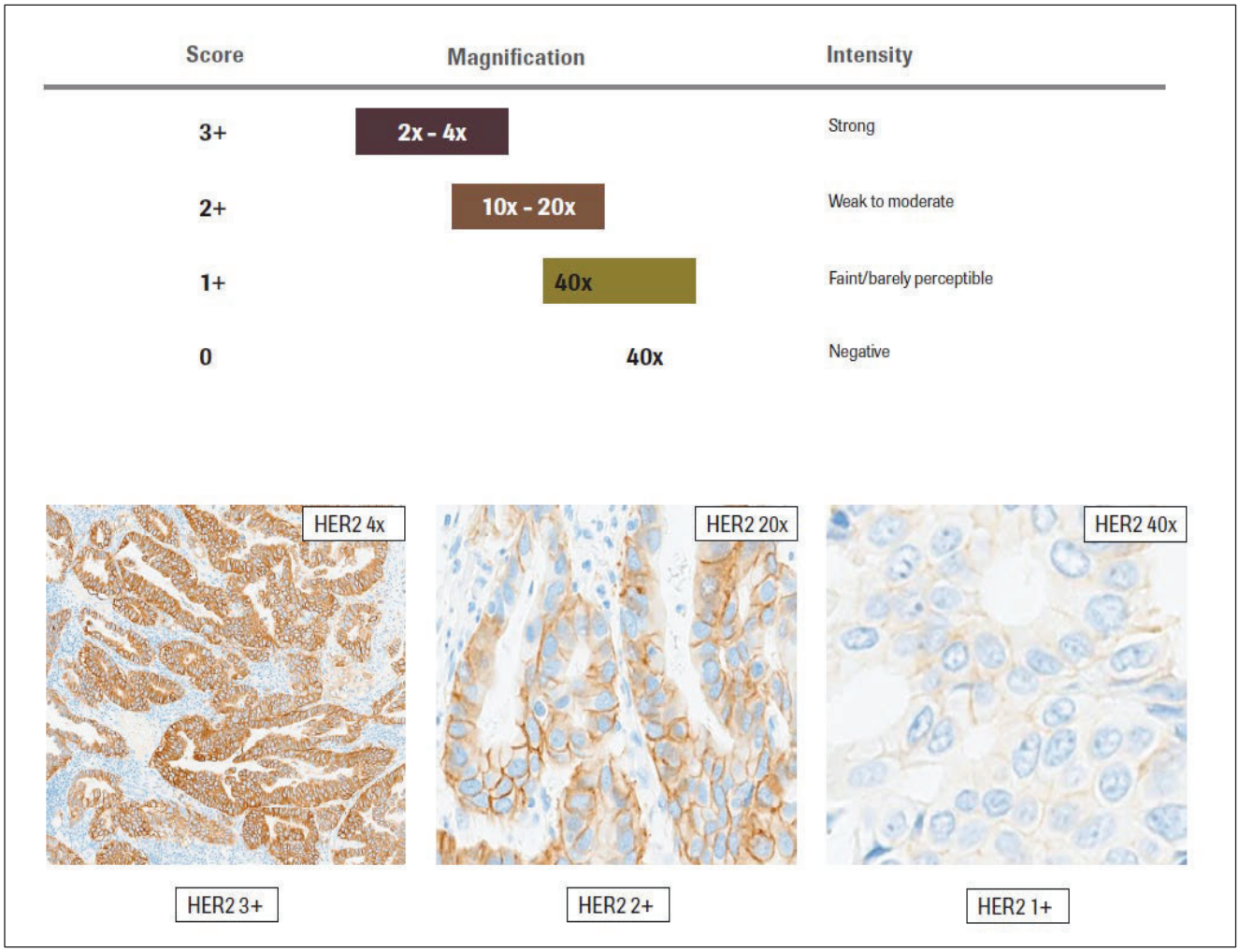
Staining pattern (resection specimen)	Staining pattern (biopsy specimen*)	HER2 (4B5) Score (report to requesting physician)	Recommended Reporting Status	ZIIHERA (zanidatamab)
No reactivity or membranous reactivity in < 10% of tumor cells	No reactivity or membranous reactivity in any tumor cell	0	HER2 Negative	Treatment ineligible
Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster** with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	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***	2+		
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***	3+	HER2 Positive	Treatment eligible

* Biopsy specimens include endoscopic, pinch (forceps) and needle core
** ≥ 5 cohesive cells
*** Recommend re-reading by a second pathologist for cases with "strong complete basolateral or lateral membranous reactivity" and a %TC (resection specimens) or tumor cell cluster (biopsy specimens) near the threshold of 10% (resections) or 5 cohesive cells (biopsies), when the range of %TC is between 1%-20% (resections) or the tumor cell cluster is between 1-20 cohesive cells.

7. Magnification rule for Staining intensity evaluation

BTC staining intensity was evaluated using magnification rule to increase inter-observer reproducibility by removing some subjectivity associated with intensity assessments. For the magnification rule, intensity in BTC tissues was assessed relying on the use of the microscope objectives, specifically the 4x and 20x objectives become very important for distinguishing between HER2 scores. Intensity assessment based on microscope magnification is needed to demonstrate distinct (i.e. complete, basolateral or lateral) intercellular membranous staining. A pictorial representation of the magnification rule is provided in **Figure 1**.

Figure 1: Pictorial Representation of the Magnification Rule



Tumor cells (TC) in slides stained with PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody was evaluated using the approach called Decision Trees for resections or biopsies is noted in the following figures.

Figure 2. Decision tree for scoring Resection using Magnification rule:

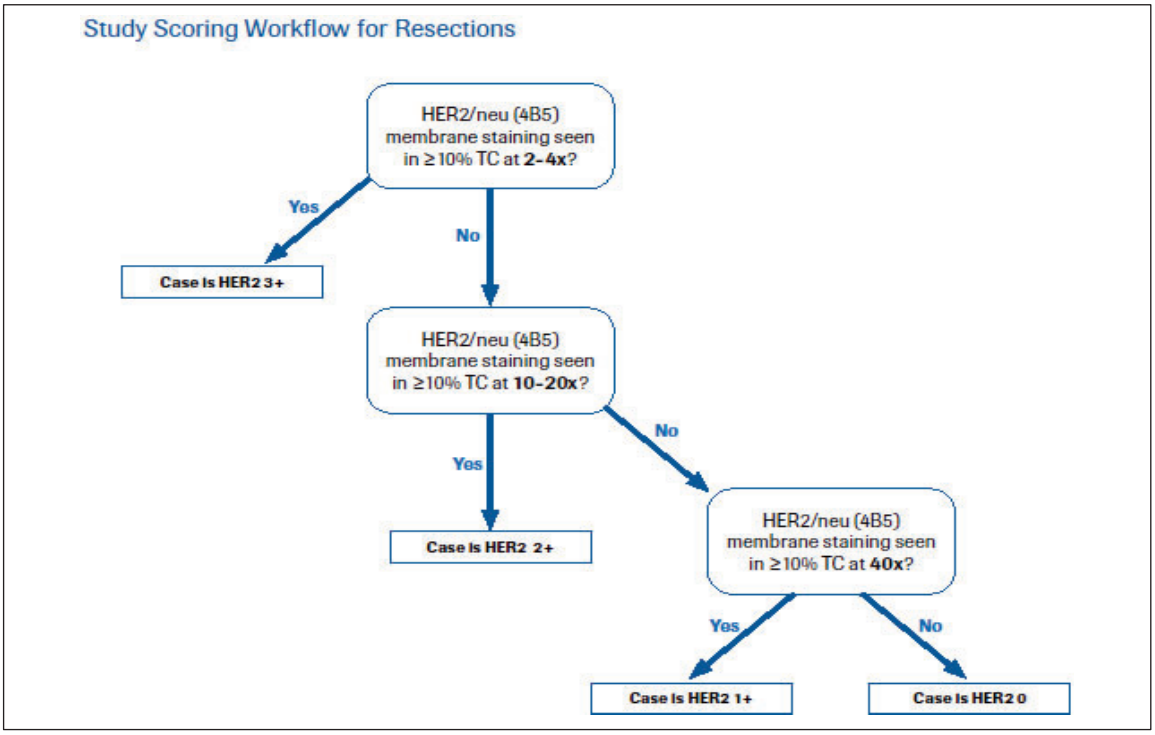
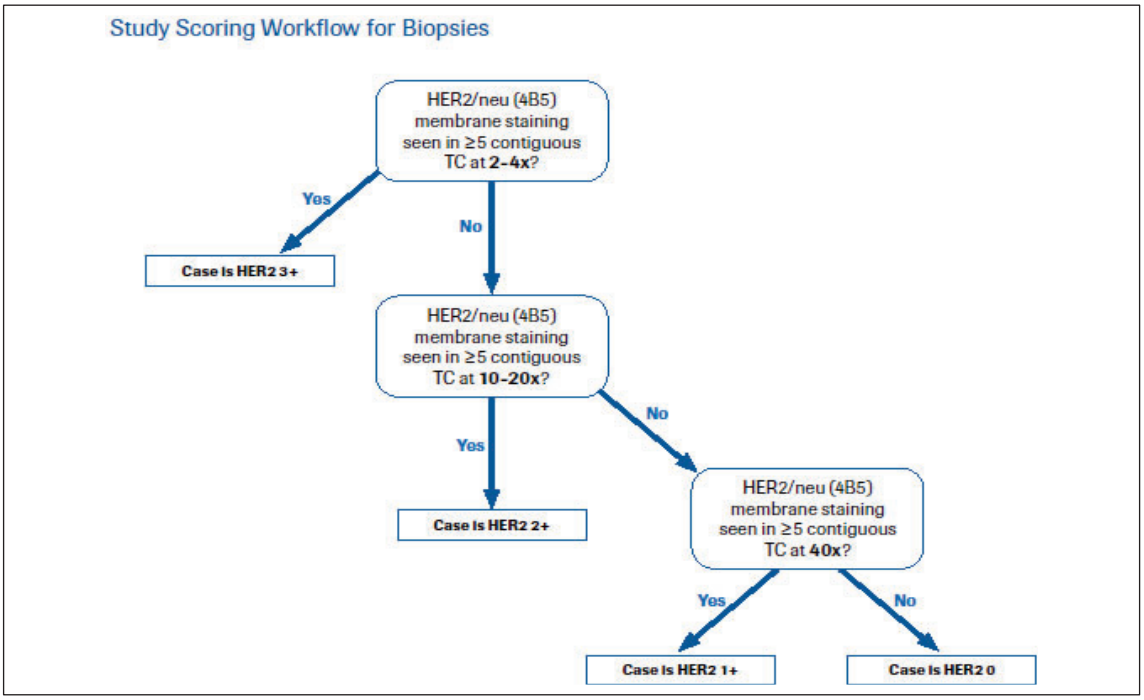


Figure 3. Decision tree for scoring Biopsies using Magnification rule:



8. Re-Reading Zone for HER2-Borderline Scoring in Biliary Tract Cancer

Cases with a staining pattern of strong complete, basolateral or lateral membranous reactivity should be evaluated with regard to %TC (resection specimens) or tumor cell cluster (biopsy specimens), to determine whether the staining is above or below the threshold of 10% (resections) or 5 cohesive cells (biopsies), as this assessment is clinically significant (patients with %TC <10% (resections) or <5 cohesive cells (biopsies) are not eligible for the targeted therapy. However, patients with %TC ≥ 10% (resections) or ≥ 5 cohesive cells (biopsies) are eligible for the targeted therapy, ZIIHERA.

To decrease variability of PATHWAY anti-HER2 (4B5) antibody scoring for cases with "strong complete, basolateral or lateral membranous reactivity" and staining near the above thresholds, a consensus result for the case should be obtained by consulting with other pathologist(s) when the %TC ranges from 1% to 20% (resections) or the tumor cell cluster is between 1 to 20 cohesive cells (biopsies).

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay for testing for HER2 protein expression available for use as an aid in identifying patients with BTC for treatment with ZIIHERA.

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: Argentina, Austria, Belgium, Brazil, Canada, Chile, China, Colombia, Croatia, Denmark, Ecuador, Finland, France, Germany, Greece, Hungary, India, Indonesia, Italy, Japan, Kuwait, Latvia, Lithuania, Mexico, Netherlands, Norway, Pakistan, Peru, Philippines, Poland, Portugal, Romania, Russia, Saudi Arabia, Singapore, Slovakia, Slovenia, South Africa, Spain, Switzerland, Taiwan, Thailand, Turkey, United Arab Emirates, United Kingdom, and Uruguay.

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 NONCLINICAL STUDIES

A. Laboratory Studies

Nonclinical studies were performed using PATHWAY anti-HER2 (4B5) antibody to establish the analytical performance of the device for the BTC indication. These studies were performed using VENTANA BenchMark ULTRA instruments controlled by the VSS software versions 12.3, 12.3.1, 12.5.3 12.5.4. 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, stability, and other performance characteristics of the device. Due to the rarity of BTC tumors and low prevalence of HER2 protein overexpression among GI-related solid tumors as well as limited BTC tissue availability, traditional analytical verification approaches for CDx co-development became challenging. To overcome these challenges an analytical verification approach using carcinomas of the digestive system (CDS) including BTC tumor specimens was used to support the BTC indication. BTC specimens were used in the Interlaboratory Reproducibility (ILR) study while most of the remaining analytical studies in this submission used BTC specimens supplemented with other CDS specimens i.e., with gastro-esophageal adenocarcinoma (GEA) and colorectal carcinomas (CRC) specimens during analytical validation.

1. Analytical Sensitivity

a. Prevalence

Analytical sensitivity was assessed by calculating the prevalence of HER2 biomarker IHC staining scores in tissue cases from the intended use population. Analytical sensitivity for BTC was evaluated by characterizing HER2 prevalence (percent) among BTC specimens in the clinical trial HERIZON-BTC-01.

Among 806 BTC specimens, there were 353 specimens with HER2 0 scores, 108 specimens with HER2 1+ scores, 205 specimens with HER2 2+ scores and 112 specimens with HER2 3+ scores. Also, there were 28 specimens which were not evaluable. In different populations, prevalence of HER2 IHC scores can be different from the prevalence presented in Table 6.

Table 6. Prevalence of HER2 IHC Scores in Clinical Trial HERIZON-BTC-01

HER2 IHC bin	n/N	% Prevalence
0	353/806	43.8
1+	108/806	13.4
2+	205/806	25.4
3+	112/806	13.9
Not evaluable	28/806	3.5

2. Analytical Specificity

a. Western Blot

Western blot analysis was provided in this submission to demonstrate sensitivity and specificity characterization for PATHWAY anti-HER-2/neu (4B5) for its target biomarker protein HER2 and to investigate potential cross-reactivity. The results showed that the HER2 (4B5) antibody can detect recombinant HER4 and recombinant ZSCAN18 proteins in a highly sensitive Western blot assay. The HER2 (4B5) antibody also detects endogenous HER4 when it is overexpressed in a transfected cell line by Western blot. Biological levels of either endogenous HER4 or ZSCAN18 proteins were not detected in whole cell lysates by Western blot or in cell lines by IHC. Together these findings suggest that additional HER2 structural components may contribute to antibody binding interactions and explain why HER2 (4B5) in the IHC assay detects HER2 with an absence of cross-reactivity to other proteins in clinical samples.

b. Immunoreactivity in Human Tissues

The purpose of this study was to assess the analytical specificity on non-neoplastic [Tour of Body (ToB)] and neoplastic tissue [Tour of Tumor (ToT)] samples including non-specific staining, background and cross-reactivity of PATHWAY anti-HER2 (4B5) antibody. Multi-tissue arrays encompassing a range of normal tissues in the ToB, and neoplastic tissues in the ToT, were stained with PATHWAY anti- HER2 (4B5) antibody. No unexpected staining was observed, and the study results showed no specific membrane staining for most normal and neoplastic tissues. This study is leveraged from P990081/S047.The staining results for normal tissue can be found in Summary of Safety and Effectiveness Data for P990081/S047. The staining results for neoplastic tissues from BTC indications including Gallbladder Adenocarcinoma and Cholangiocarcinoma are documented in this submission as shown in the Table 7 below.

Table 7. Specificity of PATHWAY anti-HER2 (4B5) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	No. Positive / Total Cases
Glioblastoma (Cerebrum)	0/2
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous Adenocarcinoma (Ovary)	0/2
Carcinoma Not Otherwise Specified (NOS) (Ovary)	1/2
Neuroendocrine Neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Carcinoma NOS (Pancreas)	0/3
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Carcinoma NOS (Thyroid)	0/3
Microinvasive ductal carcinoma (Breast)	2/2
Invasive ductal carcinoma (Breast)	44/98
Carcinoma NOS (Breast)	1/4
B-cell Lymphoma NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Carcinoma NOS (Lung)	0/2
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/4
Adenocarcinoma (Stomach)	8/88
Signet-ring cell Carcinoma (Stomach)	0/4
Carcinoma NOS (Stomach)	0/3
Adenocarcinoma (Small Intestine)	0/1
Gastrointestinal Stromal Tumor (GIST) (Small Intestine)	0/1
Adenocarcinoma (Colon)	0/32
Gastrointestinal Stromal Tumor (GIST) (Colon)	0/1
Carcinoma NOS (Colon)	1/3
Adenocarcinoma (Rectum)	1/5
Gastrointestinal Stromal Tumor (GIST) (Rectum)	0/1
Mesothelioma (Peritoneum)	0/1
B-Cell Lymphoma NOS (Lymph node)	0/2
Hodgkin lymphoma (Lymph node)	0/1
Lymphoma NOS	0/3
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Pleomorphic rhabdomyosarcoma (Peritoneum)	0/1

Pathology	No. Positive / Total Cases
Hepatocellular carcinoma (Liver)	0/3
Hepatoblastoma (Liver)	0/1
Carcinoma NOS (Liver)	0/3
Clear cell carcinoma (Kidney)	0/1
Carcinoma NOS (Kidney)	0/5
Adenocarcinoma (Prostate)	0/2
Carcinoma NOS (Prostate)	0/3
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Melanoma (Rectum)	0/1
Melanoma NOS	0/2
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Leiomyosarcoma (Smooth muscle)	0/1
Metastatic Adenocarcinoma (from Rectum)	0/1
Metastatic Adenocarcinoma (from Colon)	0/7
Metastatic mucinous adenocarcinoma (from Colon)	0/1
Carcinoid (NOS)	0/2
Leiomyoma NOS	0/2
Sarcoma NOS	0/2
Undifferentiated carcinoma NOS	0/1
Adenocarcinoma (Gallbladder)	33/100
Cholangiocarcinoma (Intra- and Extra-hepatic)	19/100

c. Non-Specific Staining Assessment in BTC

The purpose of this study was to assess non-specific staining including background and potential cross reactivity (specific off-target staining) of the assay. Background assessment was evaluated using two tissue micro-arrays (TMAs; bile duct and gallbladder). All TMA core samples tested had acceptable levels of non-specific background and off-target staining and did not interfere with the HER2 scoring of the samples.

3. Robustness

a. 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 or false

negative result. Tissue thickness was evaluated using a total of 12 single CDS tissue cases (6 negative and 6 positive) which includes 4 BTCs (2 gallbladder and 2 bile duct), 4 GEA including 2 Gastro esophageal junction (GEJ) adenocarcinoma and 2 gastric cancers in addition to 2 CRC with ≥4 borderline (BL) 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. The acceptance criteria were set as concordant staining results in at least 90% of samples with a tissue thickness of 4 µm.

Due to change in scoring status of cases (mainly because of being borderline) for the 2-3µm and 6-7 µm sections, the recommended tissue thickness range for CDS tissue is 4-5µm for use with PATHWAY anti-HER2 (4B5) antibody. Assessment of the section thickness data using the acceptance criteria for biomarker status and stain intensity supports these recommendations and these recommendation are also cited in the device package insert (Method Sheet).

b. Fixation Study

See Summary of Safety and Effectiveness Data for P990081/S047.

c. Ischemia Study (Time to Fixation)

See Summary of Safety and Effectiveness Data for P990081/S047.

d. Protocol Limitations

The purpose of this study was to identify protocol conditions that lead to a potential false positive, false negative, or unacceptable result and prevent these conditions from affecting the end user. Twelve CDS tissues including four BTC tissues (1 gallbladder, 3 bile duct), four GEA (2 GEJ, 1 esophagus, 1 gastric) and four CRC tissues were tested.

Various test conditions were tested using combination of CC1 and antibody incubation times (CC1 times: 20 min, 92 min; antibody incubation times: 4 min, 120 min). Results of the study indicated that deviating from the recommended staining protocol specified in Table 4 above produces unacceptable staining and results in changes in the HER2 score in the PATHWAY anti-HER2 (4B5) antibody-stained samples.

4. Precision

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

a. Intermediate Precision

Intermediate Precision was evaluated using biliary tract cancer (BTC, i.e. gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, and extrahepatic cholangiocarcinoma) samples

supplemented with samples from other types of carcinomas of the digestive system (CDS). Twenty-eight CDS samples (10 BTC, 10 GEA, and 8 CRC samples) with cases with 3 borderline samples (one esophageal adenocarcinoma sample and one GEA sample as the borderline positive samples, and one GEJ sample as the borderline negative sample) spanning the HER2 IHC staining range were included in this study. The study design for evaluation of staining precision with PATHWAY anti-HER2 (4B5) antibody included:

- Three lots of PATHWAY anti-HER2 (4B5) antibody
- Three lots of ultraView DAB IHC Detection Kits
- Across five non-consecutive days
- Three BenchMark ULTRA instruments
- One Pathologist, 2 replicates

All slides were blinded and randomized and evaluated using the Criteria for Intensity and Pattern of Cell Membrane Staining with PATHWAY anti-HER2 (4B5) Antibody in Biliary Tract Cancer specified in Table 5 above. There were 22 results for each case, and the majority HER2 score results was assigned based on those 22 results. In addition, the percent results "Eligible" with regard to HER2-positive therapy for BTC was calculated. Results of this analysis are summarized in the Table 8 below.

Table 8. Median and Range of %TC for Samples in the Intermediate Precision Study for BTC tissues (supplemented with CDS).

Case Number	Majority HER2 Score	Median %TC	Range %TC (Min-Max)	Percent Results "Eligible"
1	0	0	0.0 - 0.0	0.0% (0/22)
2	0	0	0.0 - 0.0	0.0% (0/22)
3	0	0	0.0 - 0.0	0.0% (0/22)
4	0	0	0.0 - 0.0	0.0% (0/22)
5	0	0	0.0 - 0.0	0.0% (0/22)
6	0	0	0.0 - 0.0	0.0% (0/22)
7	0	1	0.0 - 1.5	0.0% (0/22)
8	0	1.5	0.5 - 7.5	0.0% (0/22)
9	0	3	0.5 - 5.5	0.0% (0/22)
10	1+	10.5	10.0 - 17.0	0.0% (0/22)
11	1+	10.5	10.0 - 23.5	0.0% (0/22)
12	1+	12.5	10.0 - 20.0	0.0% (0/22)

13	1+	17	1.0 - 30.0	0.0% (0/22)
14	1+	20.5	15.0 - 26.0	0.0% (0/22)
15	2+	15	10.0 - 20.0	0.0% (0/22)
16	2+	20	15.0 - 25.0	0.0% (0/22)
17	2+	20	15.0 - 30.0	0.0% (0/22)
18	2+	20	15.0 - 45.5	0.0% (0/22)
19	2+	34	25.5 - 57.0	0.0% (0/22)
20	2+	35	20.0 - 55.0	0.0% (0/22)
21	2+	37.5	30.0 - 65.0	0.0% (0/22)
22	3+	30	15.0 - 50.0	100.0% (22/22)
23	3+	30	18.0 - 45.0	100.0% (22/22)
24	3+	52.5	30.0 - 80.0	100.0% (22/22)
25	3+	65	60.0 - 70.0	100.0% (22/22)
26	3+	85	70.0 - 95.0	100.0% (22/22)
27	3+	90	85.0 - 95.0	100.0% (22/22)
28	3+	97	93.0 - 100.0	100.0% (22/22)

The variability of %TC values for each of the twenty-eight 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. Results are summarized in the Table 9 below:

Table 9. Precision Components for Samples in Intermediate Precision Study for BTC tissues (supplemented with CDS)

					Standard Deviation of %TC					
Case Number	Majority HER2 Score	Number of Replicates	Median %TC	Range %TC (Min-Max)	Repeatability (Within-run)	Between-day	Between-antibody lot	Between-detection kit	Between-instrument	Total
Case 1	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 2	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 3	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 4	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 5	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 6	0	22	0.00	0.0 - 0.0	0.00	0.00	0.00	0.00	0.00	0.00
Case 7	0	22	1.00	0.0 - 1.5	0.11	0.36	0.00	0.44	0.00	0.58
Case 8	0	22	1.50	0.5 - 7.5	0.86	2.54	0.00	0.97	0.00	2.85
Case 9	0	22	3.00	0.5 - 5.5	0.11	1.71	0.00	0.00	0.00	1.71
Case 10	1+	22	10.50	10.0 - 17.0	0.15	0.32	0.00	3.68	2.73	4.60
Case 11	1+	22	10.50	10.0 - 23.5	0.54	1.15	0.00	0.00	6.89	7.01
Case 12	1+	22	12.50	10.0 - 20.0	0.32	2.77	0.00	0.00	3.65	4.59
Case 13	1+	22	17.00	1.0 - 30.0	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*
Case 14	1+	22	20.50	15.0 - 26.0	0.30	3.01	1.77	0.00	4.30	5.55
Case 15	2+	22	15.00	10.0 - 20.0	1.95	2.96	0.00	0.00	0.00	3.55
Case 16	2+	22	20.00	15.0 - 25.0	2.61	0.98	0.00	3.20	0.00	4.24
Case 17	2+	22	20.00	15.0 - 30.0	3.37	0.00	4.63	0.75	1.63	6.00
Case 18	2+	22	20.00	15.0 - 45.5	2.38	1.85	15.29	0.00	0.00	15.59
Case 19	2+	22	34.00	25.5 - 57.0	2.36	5.95	10.36	0.00	13.32	18.05
Case 20	2+	22	35.00	20.0 - 55.0	3.20	4.99	0.00	3.10	9.42	11.55
Case 21	2+	22	37.50	30.0 - 65.0	2.82	5.04	10.20	0.00	14.92	18.97
Case 22	3+	22	30.00	15.0 - 50.0	3.37	9.48	4.81	4.81	0.00	12.15
Case 23	3+	22	30.00	18.0 - 45.0	3.53	7.63	8.18	0.00	0.00	11.73
Case 24	3+	22	52.50	30.0 - 80.0	6.91	8.54	0.00	21.63	0.00	24.26
Case 25	3+	22	65.00	60.0 - 70.0	2.13	2.42	2.54	0.00	0.46	4.13
Case 26	3+	22	85.00	70.0 - 95.0	4.13	6.82	3.10	0.00	3.10	9.09
Case 27	3+	22	90.00	85.0 - 95.0	2.82	1.01	0.00	0.00	3.71	4.77
Case 28	3+	22	97.00	93.0 - 100.0	1.09	1.99	0.00	0.00	1.95	2.99

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

In addition, a qualitative analysis of different precision components was performed. In this analysis, samples with HER2 IHC scores of “0”, “1+”, and "2+" were grouped together to consider as negative samples and samples with the HER2 IHC score of “3+” were considered positive 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 the Table 10 below.

Table 10. Repeatability and Intermediate Precision for BTC tissues (supplemented with CDS) on BenchMark ULTRA

Repeatability/Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	PPA	42/42	100.0	(91.6, 100.0)
	NPA	126/126	100.0	(97.0, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between-Detection Kits	PPA	42/42	100.0	(91.6, 100.0)
	NPA	126/126	100.0	(97.0, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between- Instrument (BenchMark ULTRA)	PPA	42/42	100.0	(91.6, 100.0)
	NPA	126/126	100.0	(97.0, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between-Day	PPA	70/70	100.0	(94.8, 100.0)
	NPA	210/210	100.0	(98.2, 100.0)
	OPA	280/280	100.0	(98.6, 100.0)
Within-Run	PPA	77/77	100.0	(95.2, 100.0)
	NPA	231/231	100.0	(98.4, 100.0)
	OPA	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.

b. Reader Precision

In the Reader Precision study, Between-Reader and Within-Reader components of precision were evaluated. The Reader Precision study included 37 BTC samples supplemented with 28 GEA 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 HER2 score using the Criteria for Intensity and Pattern of Cell Membrane Staining with PATHWAY anti-HER2 (4B5) Antibody in Biliary Tract Cancer. 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 BTC 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 BTC resection samples (supplemented with CDS)

					Standard Deviation			
Case Category	HER2 IHC	N of cases	N of reads	Range of median %TC	Within-Reader	Between-Reader	Total	Percent Results "Eligible"
No reactivity / Faint/barely perceptible <10%	0	16	96	0.0 - 1.5	1.1	0.2	1.2	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)
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	0.0% (0/6)
Weak to moderate complete, basolateral or lateral	2+	25	150	11.0 - 60.0	11.8	13.0	17.6	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	50.0% (6/12)
Variable	0/1+/2+	1	6	5.0 - 5.0	N/A	N/A	N/A	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)

In addition, a qualitative analysis of different precision components was performed. Within-reader and between-reader precision was determined with average positive agreement (APA) and average negative agreement (ANA) across all observations. In this analysis, samples with HER2 IHC scores of "0", "1+", and "2+" were grouped together to consider as negative samples, and samples

with the HER2 IHC score of "3+" were considered positive samples. The results for between-reader and within-reader precision components are summarized in the Table 13 below.

Table 13. Within- and Between-Reader Precision of the PATHWAY anti-HER2 (4B5) Antibody in BTC tissues (supplemented with CDS)

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	178/180	98.9	(97.2, 100.0)
	ANA	358/360	99.4	(98.6, 100.0)
	OPA	268/270	99.3	(98.1, 100.0)
Between Reader	APA	180/180	100	(97.9, 100.0)
	ANA	360/360	100	(98.9, 100.0)
	OPA	270/270	100	(98.6, 100.0)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

c. Inter-Laboratory Reproducibility Study

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 BTC specimens stained on the BenchMark ULTRA instrument. The study included 28 de-identified FFPE BTC tissue specimens with two borderline positive and two borderline negative cases 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 regard to HER2-positive therapy in BTC was calculated. Results of this analysis for each case were presented in the Table 14 below:

Table 14. Results of Inter-Laboratory Reproducibility study in BTC

			HER2 IHC Score				Percent Positive Result			
Case Number	Majority HER2 Score	No of Reads	0	1+	2+	3+	Site A	Site B	Site C	Overall
Case 1	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)
Case 2	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)
Case 3	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)
Case 4	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)

Case 5	0	28	28 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/8)	0.0 (0/10)	0.0 (0/28)
Case 6	0	30	14 (46.7)	10 (33.3)	6 (20.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 7	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)
Case 8	0	30	29 (96.7)	1 (3.3)	0 (0.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 9	0	30	20 (66.7)	7 (23.3)	3 (10.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 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)
Case 11	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)
Case 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)
Case 13	1+	30	12 (40.0)	17 (56.7)	1 (3.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 14	1+	30	7 (23.3)	19 (63.3)	4 (13.3)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 15	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)
Case 16	2+	30	4 (13.3)	8 (26.7)	18 (60.0)	0 (0.0)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/30)
Case 17	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)
Case 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)
Case 19	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)
Case 20	2+	30	1 (3.3)	1 (3.3)	25 (83.3)	3 (10.0)	0.0 (0/10)	0.0 (0/10)	30.0 (3/10)	10.0 (3/30)
Case 21	2+	30	0 (0.0)	0 (0.0)	21 (70.0)	9 (30.0)	20.0 (2/10)	30.0 (3/10)	40.0 (4/10)	30.0 (9/30)
Case 22	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)
Case 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)
Case 24	3+	30	0 (0.0)	0 (0.0)	6 (20.0)	24 (80.0)	80.0 (8/10)	60.0 (6/10)	100.0 (10/10)	80.0 (24/30)
Case 25	3+	30	0 (0.0)	0 (0.0)	5 (16.7)	25 (83.3)	60.0 (6/10)	100.0 (10/10)	90.0 (9/10)	83.3 (25/30)
Case 26	3+	30	0 (0.0)	0 (0.0)	5 (16.7)	25 (83.3)	50.0 (5/10)	100.0 (10/10)	100.0 (10/10)	83.3 (25/30)
Case 27	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)
Case 28	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)

In addition, a qualitative analysis of different precision components was performed. Based on the observed clinically meaningful efficacy in clinical trial, Inter-Laboratory Reproducibility study was reviewed and the data were re-analyzed by treating samples with the HER2 IHC score of "3+" as positive samples and the samples with HER2 IHC score of "0", "1+", and "2+" as negative samples. Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all evaluable observations were reported for the overall reproducibility. Within-Site and Within-Reader precision components and a summary is presented in the Table 15 below.

Table 15. Inter-Laboratory Reproducibility for Overall Agreement Rates for PATHWAY anti-HER2 (4B5) Antibody

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	194/210	92.4	(85.2, 97.6)
	NPA	616/628	98.1	(95.2, 100.0)
	OPA	810/838	96.7	(94.0, 99.0)
Within-Site	PPA	194/210	92.4	(85.2, 97.6)
	NPA	616/628	98.1	(95.2, 100.0)
	OPA	810/838	96.7	(94.0, 99.0)
Within-Reader	PPA	200/210	95.2	(89.5, 99.5)
	NPA	622/628	99.0	(97.3, 100.0)
	OPA	822/838	98.1	(96.2, 99.8)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

In addition, pairwise comparisons were performed for Between-Site, Between-Reader, and Between-Day precision components for HER2 clinical status. The data was analyzed for average positive agreement (APA), average negative agreement (ANA), and overall percent agreement (OPA) and are presented below in Table 16.

Table 16. Inter-Laboratory Reproducibility Pairwise Agreement Rates

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	APA	3636/4120	88.3	(79.7, 96.3)
	ANA	12116/12600	96.2	(93.3, 98.8)
	OPA	7876/8360	94.2	(90.0, 98.2)
Between-Reader	APA	182/206	88.3	(78.6, 96.5)
	ANA	608/632	96.2	(92.7, 98.9)
	OPA	395/419	94.3	(89.0, 98.3)
Between-Day	APA	772/824	93.7	(87.5, 99.0)
	ANA	2468/2520	97.9	(95.7, 99.7)
	OPA	1620/1672	96.9	(93.7, 99.5)

Note: Two-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method with 2000 replicates.

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 was evaluated on a total of 13 single tissues of CDS (4 BTC, 4 GEA, 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 2 desiccant packets with and without humidity control to assess the stability of the epitope. Six single tissues including 2 BTC (1 gallbladder, 1 bile duct), 2 GEA and 2 CRC tissues were used for this purpose of the study.

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 17. Cut Slide Stability results based on HER2 clinical status with IHC 3+ cut off:

Cut-off	5°C ± 3°C, 15% R.H.	5°C ± 3°C, 85% R.H.
BTC	180 days	90 days
GEA	180 days	180 days
CRC	150 days	60 days

R.H.: Relative Humidity

Cut slide stability dating results at high temperature and high humidity condition are presented in the Table 18 below.

Table 18. Cut slide stability results at high temperature and high humidity condition:

Indication	30°C ± 3°C, 85% R.H.	30°C ± 3°C, 85% R.H. in Mylar Bag with desiccant
BTC	15 days	30 days
GEA	15 days	30 days
CRC	15 days	30 days

In summary, based on the study results, cut slide stability is 45 days when stored at 5 ºC ± 3 ºC (15% Relative Humidity ± 10%) which is also documented in the device Package Insert.

b. Real Time Stability

The objective of this study was to evaluate the stability (shelf-life and in-use) of three (3) production lots and shipping category of PATHWAY anti-HER2 (4B5) antibody on CDS FFPE tissues. These 3 productions lots of PATHWAY anti-HER2 (4B5) antibody were subjected to different stress conditions and then placed at the intended storage condition (2-8°C). The conditions tested were as follows:

- Intended Storage 2-8°C.
- Hot Ship Stress Category F 37±2ºC (192 hours)
- Hot Ship Stress Category J 32±2ºC (192 hours)
- Hot Ship Stress Category H 17±2ºC (192 hours)
- Hot Ship Stress Category H 27±2ºC (192 hours)
- Cold Ship Stress (Freeze/Thaw Cycles) -25±5ºC (192 hours)

Time points tested for shelf-life storage were at T_0, 6, 10, 14, 20, 24, and 26 months and for ship stress [hot ship stress (Category F): 37±2°C for 192 hours and cold ship stress (Freeze/Thaw Cycles): 3 cycles at -25±5˚C] at 6, 10, 14, 20, 24, and 26 months. Each of the 3 lots of PATHWAY anti-HER2/neu (4B5) antibody was assigned a sample set of BTC, CRC, GEA FFPE tissues with HER2 staining expression ranging from 0 to 3+. Real time and ship stress stability testing for all three lots have met the acceptance criteria through Month 26.

The data supports product dating of 24 Months and shipping Category F (>-20 & ≤35°C).

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. This was a characterization study to compare the staining performance of PATHWAY anti-HER2 (4B5) antibody on the primary tumor versus patient-matched metastatic tumor sample. Of the 46 CDS patient cases (13 BTC, 18 GEA and 15 CRC cases) included in analysis, 97.8% (45/46) of cases demonstrated concordant HER2 status [92% (13/13) BTC with all gallbladder cases, 94% (17/18) GEA, 100% (15/15) CRC cases] between the primary tumor block and the patient-matched metastatic tumor

block. Two cases were excluded from analysis due to no tumor cells in one of the patient-matched blocks. For prevalence analysis of unmatched cholangiocarcinoma blocks (due to unavailability of the matched cases), metastatic tumor blocks had a sample positive prevalence of 6.7% (1/15) and primary tumor blocks had a sample positive prevalence of 10.0% (10/100). 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 encompassing14 BTC cases (including 10 Gallbladder and 4 Bile duct), 17 GEA cases (including 5 gastric, 7 Esophagus, 5 GEJ cases) and 19 CRC cases, were used in this study. Each patient case had between 2-4 individual tissue blocks. Out of 50 CDS patient cases, 47 cases had 100% concordance of all tissue blocks for the case, while three cases had one tissue block that was discordant for HER2 status compared to the case-level mode. Of the 141 CDS blocks tested, 98.6% (139/141) of blocks demonstrated concordant HER2 status with the case-level mode for HER2 status where OPA for BTC was 94.1% (32/34). 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 is 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. 12 individual CDS tissue cases including 3 BTC cases (2 gallbladder, 1 bile duct), 6 GEA cases (2 each gastric, esophagus, GEJ) and 3 CRC cases were evaluated. Three cases (1 GEJ, 1 CRC and 1 esophagus) exhibited heterogeneity. Two of the cases that exhibited heterogeneity had sections that alternated between IHC1+ and 2+ scores. The third case 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 3+ as IHC positive vs IHC 0/1+/2+ as IHC negative, OPA 97.2% (173/178) concordance was observed in this study. Four out of five blocks with discordant scores were noted as borderline.

c. BTC Heterogeneity

This study is characterized to inform the appropriate number of biopsies that would yield the same results as a resection in BTC tumor samples. The study simulated biopsy samplings of BTC tumors in clinical practice by placing tissue sections from BTC resection samples on glass slides containing 2x2 mm grids and scoring each grid using the biopsy scoring criteria for HER2. Of the 30 cases studied for the HER2 (4B5 assay), 18 were cholangiocarcinoma and 12 were gallbladder adenocarcinoma. A total of 9 cases out of 30 cases (9/30, 30%) which were observed to be

heterogeneous, 22.2% (4/18) cases were cholangiocarcinoma, while 41.66% (5/12) cases were gallbladder adenocarcinoma.

VI. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of PATHWAY anti-HER2/neu (4B5) antibody as a companion diagnostic (CDx) device to identify patients with BTC, i.e. gallbladder adenocarcinoma [GBC], intrahepatic cholangiocarcinoma [ICC], or extrahepatic cholangiocarcinoma [ECC] who may be eligible for treatment with ZIIHERA® (zanidatamab) was evaluated in the Phase 2b clinical trial HERIZON-BTC-01 (ZWI-ZW25-203).

VII. Clinical Performance in Biliary Tract Cancer

The clinical performance of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody and the efficacy of ZIIHERA® (zanidatamab) were evaluated in 62 patients with HER2-positive (IHC 3+ by central assessment) BTC (gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, or extrahepatic cholangiocarcinoma) in Cohort 1 of HERIZON-BTC-01 (NCT04466891), an open label, multicenter, single-arm, trial in patients with unresectable or metastatic BTC disease. Patients were required to have HER2-amplified BTC, to have received at least one prior gemcitabine-containing systemic chemotherapy regimen in the advanced disease setting.
Tumor samples from patients undergoing screening were tested with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody to assess HER2 protein expression by IHC.

1. Clinical Inclusion and Exclusion Criteria

a. Key Trial Inclusion Criteria

Subjects were required to fulfill each of the following criteria:

1. Histologically or cytologically confirmed BTC, including ICC, ECC, or GBC.
2. Metastatic or unresectable HER2-positive (IHC 3+) BTC which is not eligible for curative resection, transplantation, or ablative therapies.
3. Received at least 1 prior gemcitabine-containing systemic chemotherapy regimen in advanced disease setting.
4. Subjects must have HER2 positive (IHC 3+) tumors by a central laboratory assessment and adequate cardiac function (defined as LVEF ≥ 50%).
5. Male or female, ≥ 18 years of age (or the legal age of adulthood per country-specific regulations).
6. Females of childbearing potential must have had a negative serum or urine beta-human chorionic gonadotropin (β-hCG) pregnancy test result within 3 days prior to the first dose of ZIIHERA® (zanidatamab). Females with false positive urine test results could be enrolled if subsequent serum testing was negative.

b. Key Trial Exclusion Criteria

Subjects were excluded if they met any of the following exclusion criteria:

1. Received systemic anticancer therapy within 3 weeks of the first dose of ZIIHERA® (zanidatamab).
2. Received radiotherapy within 2 weeks of the first dose of ZIIHERA® (zanidatamab).
3. Had major surgery within 4 weeks of the first dose of ZIIHERA® (zanidatamab).
4. Prior treatment with HER2-targeted agents.
5. Subjects with uncontrolled or significant cardiovascular disease or any history of myocardial infarction, uncontrolled hypertension, clinically severe pulmonary compromise, spinal cord compression, or clinically active central nervous system (CNS) metastases.

VIII. Follow-up Schedule

Patients received ZIIHERA® (zanidatamab) 20 mg/kg intravenously every 2 weeks and was administered until disease progression or unacceptable toxicity. Adverse events and complications were recorded at all visits.

IX. Clinical Endpoints

Primary Efficacy and Key Secondary Endpoints of the HERIZON-BTC-01 study

The major efficacy outcome measures were objective response rate (ORR) and duration of response (DOR) as determined by an independent central review (ICR) according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

An important secondary endpoint was duration of response (DOR) by RECIST v1.1 assessed by ICR.

Other secondary endpoints included progression free survival (PFS) by RECIST v1.1 assessed by ICR, and overall survival (OS).

A. Accountability of PMA Cohort

A total of 956 patients signed informed consent for HERIZON-BTC-01 study of which 910 went through the optional pre-screening for HER2 testing. In total, 850 of the prescreened and/or screened patients were tested with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody, which constitutes the IHC Intend-to-Diagnose (ITD) population. From the IHC ITD population of 850 patients, 44 patient samples/testing were associated with exclusionary diagnostic protocol deviations/incidents and were therefore excluded from the Intended Use (IU) population. The remaining 806 patients constitute the IU population. From these 806 patients, 112 patients (13.9%) showed HER2 score IHC3+ (See Table 19). Finally, 85 of the pre-screened participants with *HER2* gene amplified BTC advanced to screening, and another 46 proceeded directly to screening for enrollment. From the screen population, 87 participants with HER2- amplified tumors were enrolled in the HERIZON-BTC-01 study. Eighty enrolled patients in Cohort 1 with HER2 IHC score of 3+ or 2+ received study treatment. Among the patients enrolled in Cohort 1, only 62 patients with IHC 3+score showed clinically meaningful efficacy (See Figure 4 for accountability of PMA Cohort).

Figure 4. Patient Disposition associated with HER2 IHC for HERIZON-BTC-01 study

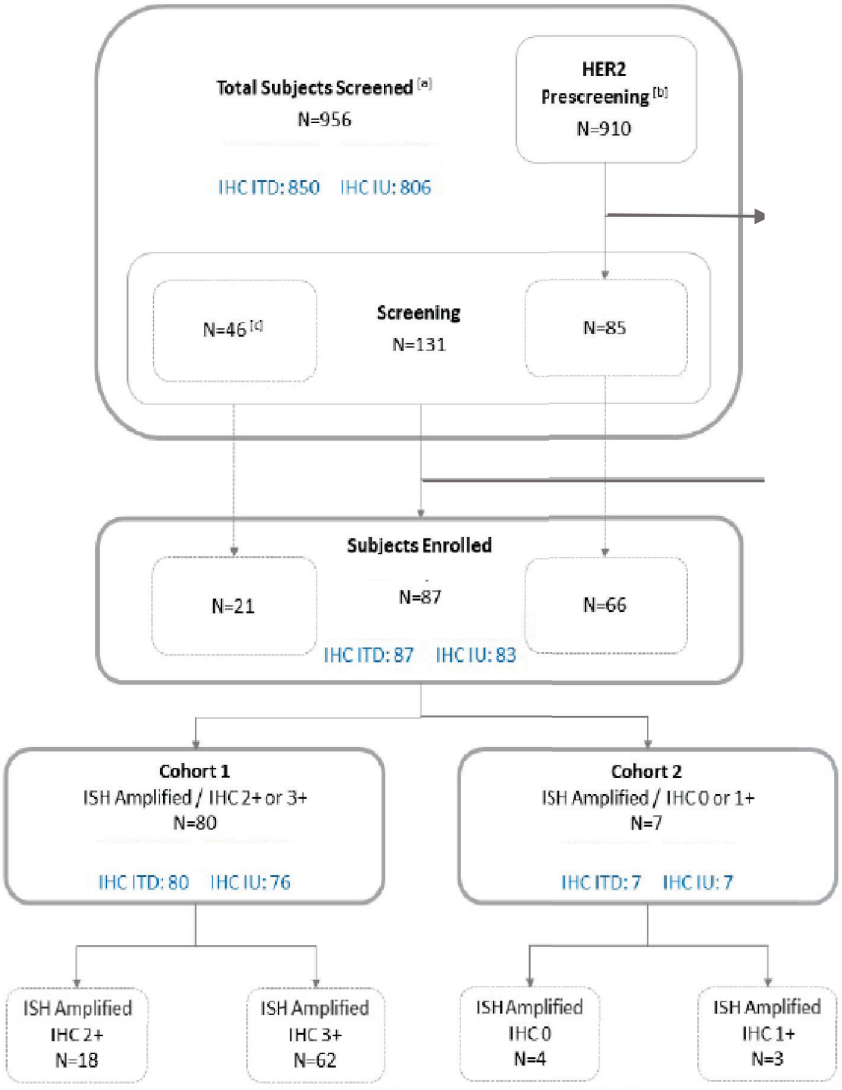


Table 19. VENTANA HER2 (4B5) IHC Staining Results for the IHC IU Population [a] (Patient Level) based on HER2 (4B5) IHC Score (HER2 Expression Status)

0	353 (353/806, 43.8%)
1+	108 (108/806, 13.4%)
2+	205 (205/806, 25.4%)
3+	112 (112/806, 13.9%)
Not evaluated	28 (28/806, 3.5%)

Note: [a] 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.

[b] Final staining attempt is defined as the attempt that was used for the cohort assignment in Study ZWI-ZW25-203 or with the latest date of staining.

B. Study Population Demographics and Baseline Parameters

The patients' demographics and sample characteristics in IHC IU population are presented by HER2 IHC score (IHC 0, 1+, 2+ or 3+) in Table 20 and Table 21 below. 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 Diagnostics study, Dx protocol.
In the HER2 positively expressed (IHC3+) IU population, approximately 46% were male, and the median age was 64 years with approximately 52% <65 years old. Of the patients in the IHC IU population with reported characteristics, the majority (55%) were of Asian origin.

Table 20: Patient Characteristics by HER2 IHC Score – IU Population

	HER2 Expression Status			
Characteristic	**Positive[a] (N=112)**	**Negative[b] (N=666)**	**Not Evaluated (N=28)**	**Overall (N=806)**
Age (years)				
Mean (SD)	63.0 ± 9.27	61.4 ± 10.88	61.0 ± 10.21	61.6 ± 10.65
Median	64.0	63.0	62.0	63.0
Range	38.0 - 85.0	22.0 - 87.0	29.0 - 77.0	22.0 - 87.0
Age Group, n (%)				
<65	58 (51.8%)	374 (56.2%)	17 (60.7%)	449 (55.7%)
≥65	54 (48.2%)	292 (43.8%)	11 (39.3%)	357 (44.3%)
Sex, n (%)				
Female	61 (54.5%)	301 (45.2%)	12 (42.9%)	374 (46.4%)
Male	51 (45.5%)	365 (54.8%)	16 (57.1%)	432 (53.6%)
Ethnicity, n (%)				
Hispanic or Latino	9 (8.0%)	39 (5.9%)	1 (3.6%)	49 (6.1%)
Not Hispanic or Latino	87 (77.7%)	330 (49.5%)	18 (64.3%)	435 (54.0%)
Not Reported	3 (2.7%)	7 (1.1%)	0 (0.0%)	10 (1.2%)
Unknown	2 (1.8%)	5 (0.8%)	0 (0.0%)	7 (0.9%)
Missing	11 (9.8%)	285 (42.8%)	9 (32.1%)	305 (37.8%)
Race, n (%)				
American Indian or Alaska Native	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Asian	61 (54.5%)	225 (33.8%)	12 (42.9%)	298 (37.0%)
Black or African American	1 (0.9%)	6 (0.9%)	0 (0.0%)	7 (0.9%)
Not Reportable	3 (2.7%)	6 (0.9%)	0 (0.0%)	9 (1.1%)
White	30 (26.8%)	130 (19.5%)	6 (21.4%)	166 (20.6%)
Multiple	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)
Other	1 (0.9%)	9 (1.4%)	1 (3.6%)	11 (1.4%)
Unknown	5 (4.5%)	4 (0.6%)	0 (0.0%)	9 (1.1%)
Missing	11 (9.8%)	285 (42.8%)	9 (32.1%)	305 (37.8%)
Country, n (%)				
Canada	2 (1.8%)	0 (0.0%)	0 (0.0%)	2 (0.2%)
Chile	6 (1.8%)	15 (2.3%)	1 (3.6%)	18 (2.2%)
China	32 (28.6%)	188 (28.2%)	10 (35.7%)	230 (28.5%)
France	5 (4.5%)	25 (3.8%)	0 (0.0%)	30 (3.7%)
Italy	5 (4.5%)	22 (3.3%)	2 (7.1%)	29 (3.6%)
South Korea	26 (23.2%)	32 (4.8%)	1 (3.6%)	59 (7.3%)
Spain	7 (6.3%)	60 (9.0%)	2 (7.1%)	69 (8.6%)
United Kingdom	6 (5.4%)	23 (3.5%)	0 (0.0%)	29 (3.6%)
United States	16 (14.3%)	16 (2.4%)	3 (10.7%)	35 (4.3%)
Missing	11 (9.8%)	285 (42.8%)	9 (32.1%)	305 (37.8%)
Primary Diagnosis, n (%)				
Extrahepatic Cholangiocarcinoma	16 (14.3%)	56 (8.4%)	1 (3.6%)	73 (9.1%)
Gallbladder Cancer	55 (49.1%)	63 (9.5%)	2 (7.1%)	120 (14.9%)
Intrahepatic Cholangiocarcinoma	21 (18.8%)	153 (23.0%)	8 (28.6%)	182 (22.6%)
Missing	20 (17.9%)	394 (59.2%)	17 (60.7%)	431 (53.5%)
Presence of Brain Metastases, n (%)				
Yes	3 (2.7%)	1 (0.2%)	0 (0.0%)	4 (0.5%)
No	89 (79.5%)	271 (40.7%)	11 (39.3%)	371 (46.0%)
Missing	20 (17.9%)	394 (59.2%)	17 (60.7%)	431 (53.5%)
Stage at Initial Diagnosis, n (%)				
Stage I	5 (4.5%)	10 (1.5%)	0 (0.0%)	15 (1.9%)
Stage II	13 (11.6%)	48 (7.2%)	0 (0.0%)	61 (7.6%)
Stage III	25 (22.3%)	85 (12.8%)	1 (3.6%)	111 (13.8%)
Stage IV	47 (42.0%)	121 (18.2%)	8 (28.6%)	176 (21.8%)
Missing	22 (19.6%)	400 (61.6%)	17 (60.7%)	439 (54.5%)
Baseline Performance Status, n (%)				
0	19 (17.0%)	3 (0.5%)	0 (0.0%)	22 (2.7%)
1	39 (34.8%)	22 (3.3%)	0 (0.0%)	61 (7.6%)
Missing	54 (48.2%)	641 (96.2%)	28 (100.0%)	723 (89.7%)

Note: 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.

[a]: Positive = IHC score of 3+; [b]: Negative = IHC score of 0, 1+ or 2+

Table 21. Sample characteristics by HER2 status IHC intended use (IU) population.

Characteristic	HER2 Expression Status			
	Positive[a] (N=112)	Negative[b] (N=666)	Not Evaluated (N=28)	Overall (N=806)
Sample collection method, n%				
Biopsy	55 (49.1%)	312 (66.4%)	28 (70%)	395 (47.9%)
Excision/Resection	57 (50.9%)	361 (53.6%)	12 (30%)	430 (52.1%)
Specimen type, n (%)				
Archive Block	11 (9.8%)	117 (17.4%)	4 (10.0%)	132 (16.0%)
Archive Slides	64 (57.1%)	194 (28.8%)	13 (32.5%)	271 (32.8%)
Fresh Block	4 (3.6%)	8 (1.2%)	3 (7.5%)	15 (1.8%)
Fresh Slides	33 (29.5%)	354 (52.6%)	20 (50.0%)	407 (49.3%)
Fixative used, n (%)				
10% Neutral Buffered Formalin	111 (99.1%)	672 (99.1%)	40 (100.0%)	823 (99.8%)
Unknown	1 (0.9%)	1 (0.1%)	0 (0.0%)	2 (0.2%)
Time to Fixation, n (%)				
0-<6 hr	9 (8.0%)	21 (3.1%)	3 (7.5%)	33 (4.0%)
24-<48 hr	9 (8.0%)	60 (8.9%)	4 (10.0%)	73 (8.8%)
48-<72 hr	6 (5.4%)	62 (9.2%)	0 (0.0%)	68 (8.2%)
6-72 hr	23 (20.5%)	140 (20.8%)	8 (0.0%)	171 (20.7%)
6-<24 hr	35 (31.3%)	241 (35.8%)	15 (37.5%)	291 (35.3%)
6-<24 hr and 48<72 hr	0 (0.0%)	1 (0.1%)	0 (0.0%)	1 (0.1%)
>72 hr	3 (2.7%)	3 (0.4%)	0 (0.0%)	6 (0.7%)
Unknown	27 (24.1%)	145 (21.5%)	10 (25.0%)	182 (22.1%)
Tumor Type, n (%)				
Primary	38 (33.9%)	335 (49.8%)	18 (45.0%)	391 (47.4%)
Metastatic	64 (57.1%)	276 (41.0%)	18 (45.0%)	358 (43.4%)
Advanced	9 (8.0%)	61 (9.1%)	3 (7.5%)	73 (8.8%)
Information not available	1 (0.9%)	1 (0.1%)	1 (2.5%)	3 (0.4%)

Note: 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.
[a]: Positive = IHC score of 3+; [b]: Negative = IHC score of 0, 1+ or 2+

Among the enrolled patients in Cohort 1, only 62 patients were with IHC 3+score who showed clinically meaningful efficacy. The patients' demographics and sample characteristics in IHC Drug efficacy population are presented by HER2 IHC score (IHC 3+) in Table 22 and Table 23 below.

Table 22: Patient Characteristics by HER2 IHC Score – Drug efficacy Population

Zanidatamab 20 mg/kg (Cohort 1)	IHC 3+ N=62
Age	
Median (min, max) years	64 (38, 79)
< 65 years (%)	33 (53)

≥ 65 years (%)	29 (47)
Gender	
Male (%)	28 (55)
Female (%)	34 (45)
Region (%)	
North America	15 (24)
Asia	36 (58)
Europe	11 (18)
Race (%)	
American Indian or Alaska Native	1 (2)
Asian	38 (61)
White	19 (31)
Not reportable/Unknown	4 (6)
Ethnicity (%)	
Hispanic or Latino	5 (8)
Non-Hispanic or Latino	55 (88)
Unknown/ Not Reported	2 (3)
ECOG PS (%)	
0	20 (32)
1	42 (68)

Table 23: Sample characteristics by HER2 IHC Score – Drug efficacy Population

Zanidatamab 20 mg/kg (Cohort 1)	**Central IHC 3+**
	N=62
Primary Tumor Classification (%)	
Gallbladder Cancer	33 (53)
IHCC	17 (27)
EHCC	12 (19)
Local HER2 IHC results (%)	
IHC 1+	1 (2)
IHC 2+	9 (15)
IHC 3+	20 (32)
Unknown/Not reported	32 (52)
Prior Curative Surgery (%)	17 (27)
Prior Radiotherapy (%)	9 (15)
Stage at Study Enrollment (%)	
III	8 (13)
IV	21 (34)
IVB	33 (53)
Number of prior systemic treatment (median, range)	1 (1, 7)
Gemcitabine based treatment	
Any Gemcitabine	62 (100)
Gemcitabine/cisplatin	47 (76)

Gemcitabine/oxaliplatin	19 (31)
Prior Anti PD(L)-1 treatment	16 (26)

C. 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-BTC-01 clinical study.

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

2. Effectiveness Results

The efficacy of ZIIHERA® (zanidatamab) was evaluated in 62 patients who were HER2-positive (IHC3+ by central assessment) BTC patients having either gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, or extrahepatic cholangiocarcinoma in Cohort 1 of HERIZON-BTC-01.

Tumor samples from patients undergoing screening were tested with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody to assess HER2 IHC 3+ protein expression.

The major efficacy outcome measures were objective response rate (ORR) and duration of response (DOR) as determined by an independent central review (ICR) according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Efficacy results (data cutoff date - DCO: 28 July 2023) are summarized in the Table 24 below. The cORR and mDOR for HER2 positive IHC 3+ tumors were 52% (n=32 of 62) and 15 months, respectively.

Table 24. Efficacy Results in HERIZON-BTC-01 (ZWI-ZW25-203)

Efficacy Parameter*	ZIIHERA (N=62)
Objective Response Rate (95% CI)	**52% (39, 65)**
Complete response, n (%)	2 (3.2)
Partial response, n (%)	30 (48)
Duration of Response (DOR) [b]	**N=32**
Median †, months (95% CI)	14.9 (7.4, NE)
DOR ≥ 6 months (95% CI) ‡ DOR ≥12 months (95% CI) ‡	19 (59) 14 (44)

*Assessed by independent central review

† Based on Kaplan-Meier estimate

‡ Based on observed duration of response

NE = not estimable

3. Relevant Subgroup Analyses

There was no subgroup analysis performed.

4. Pediatric Extrapolation

In this premarket application, existing clinical data from HERIZON-BTC-01 was not leveraged to support approval of a pediatric patient population.

X. 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.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XII. 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 did not raise any new safety and effectiveness questions compared with information previously reviewed by this panel.

XIII. 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 clinical outcome data from HERIZON-BTC-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 BTC patients eligible for treatment with ZIIHERA® (zanidatamab) based on the clinical performance assessed in the HERIZON-BTC-01 study.

Overall, data show clinically meaningful efficacy of ZIIHERA® (zanidatamab) monotherapy in the patient population identified by PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody and thus supports the clinical validation of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody per 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 BTC, and no adverse events associated with the diagnostic testing procedure were reported during the HERIZON-BTC-01 study. In the context of an in vitro diagnostic test, there are no directly harmful events from testing FFPE BTC tissue sections. These tissue sections are routinely removed as part of the diagnosis of BTC by pathologists. 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 BTC patients for treatment with ZIIHERA® (zanidatamab), failure of the device to perform as expected may lead to incorrect or false results and BTC patients may not receive the proper treatment. Patients with false positive results may undergo treatment with ZIIHERA® (zanidatamab) without much clinical benefit and may experience adverse reactions associated with ZIIHERA® (zanidatamab) therapy. Patients with false negative results may not be considered for treatment with ZIIHERA® (zanidatamab), 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-BTC-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-BTC-01 study.

As shown in Table 24, ZIIHERA® (zanidatamab) demonstrated anticancer activity in patients with HER2 positive BTC (HER2 IHC 3+protein expression), where HER2 protein expression was determined by the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody. Patients were required to have HER2-amplified BTC and to have received at least one prior gemcitabine-containing systemic chemotherapy regimen in the advanced disease setting and to have adequate cardiac function (defined as LVEF $\geq$ 50%).

The confirmed ORR by ICR in Cohort 1 was 41.3% (95% CI: 30.4, 52.8). Responses were durable, with a median DOR per ICR for Cohort 1 of 14.9 months (95% CI: 7.39, NE). Results of PFS and OS support the primary endpoint and demonstrate the persistence of the ZIIHERA® (zanidatamab) treatment effect observed in Cohort 1.

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 BTC who may be eligible for treatment with ZIIHERA® (zanidatamab) 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 ZIIHERA® (zanidatamab) treatment

with a reduced probability of benefit. This could unnecessarily expose the patient to toxicity of the drug. A false negative result could deprive a patient of the potential benefit of ZIIHERA® (zanidatamab). 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.

In conclusion, given the available information above, the potential for clinical benefit outweighs the clinical risk for BTC patients tested with the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody to determine eligibility for ZIIHERA® (Zanidatamab) therapy.

D. Patient Perspective

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

E. 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-BTC-01 study support the use of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody as a companion diagnostic to identify patients with BTC who may be eligible for treatment with ZIIHERA® (zanidatamab) in accordance with approved therapeutic labeling as the probable benefits outweigh the probable risks.

XIV. CDRH DECISION

CDRH issued an approval order on November 20, 2024.
The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. 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.