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 of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P990081/S047
Date of Notice of Approval:	September 30, 2022

The original PMA (P990081) for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY anti-HER2 (4B5) antibody) was approved for the semi-quantitative detection of c-erbB-2 (HER2) antigen in sections of formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue from patients for whom Herceptin® treatment was being considered. The SSED to support the indication is available on the CDRH website and is incorporated by reference here. The current supplement (S047) was submitted to expand the indication for PATHWAY anti-HER (4B5) antibody to include testing of FFPE breast cancer tissue from patients for whom ENHERTU® 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 normal and neoplastic breast tissue using the *ultra*View Universal DAB Detection Kit on a BenchMark ULTRA instrument.

This IHC device is indicated for identifying breast cancer patients who are eligible for treatment with Herceptin® (IHC 3+ or IHC 2+/ISH amplified), KADCYLA® (IHC 3+ or IHC 2+/ISH amplified) or ENHERTU® (IHC 1+ or IHC 2+/ISH non-amplified).

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

This antibody 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-HER2 (4B5) antibody product labeling.

V. DEVICE DESCRIPTION

A. Device Kit Components

PATHWAY anti-HER2 (4B5) antibody contains sufficient reagent for 50 tests. One 5-mL dispenser of PATHWAY anti-HER2 (4B5) antibody contains approximately 30µg of a rabbit monoclonal antibody directed against the human HER2 antigen. The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. There is trace fetal calf serum, approximately 0.25%, present from the stock solution. Specific antibody concentration is approximately 6µg/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
*ultra*View Universal DAB Detection Kit
EZ Prep (10×)
Reaction Buffer (10×)
ULTRA LCS (pre-dilute)
ULTRA CC1
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: U ultraView 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 IgG, goat anti-mouse IgM, and goat anti-rabbit) (approximately 55 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		***ultra*View Universal DAB Chromagen** contains 0.2% w/v 3,3'-diaminobenzidine 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. The recommended tissue fixative is 10% neutral buffered formalin. The amount used is 15 to 20 times the volume of tissue. Since fixatives will not penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period, it is recommended that a 3 mm or smaller size of tissue be fixed for 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 staining. Slides should be stained promptly, as antigenicity of 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. When processed and stained appropriately, the cell lines should stain as described in the PATHWAY HER2 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 tissue should be repeated. However, the PATHWAY anti-HER2 4-in-1 control slides are not intended to be used as a sole system level control for this assay.

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 for 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. 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 the 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 antibody should equal the primary antibody incubation period.

E. Principles of Operation

PATHWAY anti-HER2 (4B5) antibody is a rabbit monoclonal antibody that 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 sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide.

The use of pre-diluted (ready-to-use) PATHWAY anti-HER2 (4B5) antibody and ready-to-use *ultra*View 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 assay/staining protocol is provided in the table below.

Table 3. PATHWAY anti-HER2 (4B5) Antibody Staining Protocol Using BenchMark ULTRA Instrument

Procedure Type	**PATHWAY anti-HER2 (4B5)**
Protocol step	**Parameter input**
Staining Procedure	U PATHWAY HER2 4B5
Deparaffinization	Selected, 4 minutes, 72°C
Cell Conditioning	ULTRA CC1, 36 minutes, Mild (95°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 H_2O_2: 8 minutes, 36°C *ultra*View Copper: 4 minutes, 36°C
Antibody (Primary)	PATHWAY HER2 4B5 Ab- 12 Min, 36°C Or Neg Ctl Rbt Ig- 12 Min, 36°C
Counterstain	Hematoxylin II, 4 minutes, 36°C
Post Counterstain	Bluing, 4 minutes, 36°C

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 (4B5) antibody. A qualified pathologist experienced in immunohistochemical procedures must evaluate controls and qualify the stained product before interpreting results.

1. Positive Controls

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the membrane of the target cells is indicative of positive reactivity. Counterstaining with hematoxylin will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

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

2. **Negative Tissue Controls**

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

3. **Negative Reagent Controls**

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

4. **Patient Tissue**

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

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

5. **PATHWAY anti-HER2 (4B5) Antibody Scoring Method for Breast Cancer**

Scoring and interpretation for HER2 status is detailed in the table below:

Table 4. PATHWAY anti-HER2 (4B5) Antibody Scoring Method for Breast Cancer

HER2 Score	Staining Pattern
0	No membrane staining is observed or Faint, partial staining of the membrane in 10% or less of the cancer cells
1+	Faint, partial staining of the membrane in greater than 10% of the cancer cells

HER2 Score	Staining Pattern
2+*	Weak to moderate complete staining of the membrane in greater than 10% of the cancer cells
3+	Intense complete staining of the membrane in greater than 10% of the cancer cells

*Results must be reflex tested with a ISH assay to confirm amplified vs non-amplified

Table 5. PATHWAY anti-HER2 (4B5) Antibody Scoring Criteria, Recommended Reporting Status and Associated Therapies

Staining pattern	HER2 Score (Report to treating physician)	Recommended Reporting Status	Therapy
No membrane staining is observed Or Faint, partial staining of the membrane in 10% or less of the cancer cells*	0	HER2 Negative	None
Faint, partial staining of the membrane in greater than 10% of the cancer cells*	1+	HER2-low expression	ENHERTU (fam-trastuzumab deruxtecan-nxki)
Weak to moderate complete staining of the membrane in greater than 10% of the cancer cells	2+** *Reflex test: HER2 Non-Amplified*	HER2-low expression	
	2+** *Reflex test: HER2 Amplified*	HER2 positive/ overexpression	HERCEPTIN (trastuzumab), KADCYLA (trastuzumab emtansine)
Intense complete staining of the membrane in greater than 10% of the cancer cells	3+	HER2 positive / overexpression	

* Recommend re-reading by a second pathologist for cases with "faint, partial staining of the membrane" and %TC near the threshold of 10%, when the %TC ranges from 5%-25%

**Recommend reflex test to assess gene amplification

6. Re-Reading Zone for HER2-Low Scoring

Cases with low HER2 expression and with staining pattern of faint partial staining should be evaluated with regard to %TC, to determine whether the %TC value is below or above 10%, as this assessment is clinically significant (patients with %TC≤10% are not eligible for the targeted therapy. However, patients with %TC>10% are eligible for the targeted therapy, ENHERTU).

To decrease variability of PATHWAY anti-HER2 (4B5) antibody with HER2-low scoring for cases with "Faint incomplete staining" and %TC near the threshold of 10%, a re-read of the slide is performed when the %TC ranges from 5% to 25%. The case result "Faint incomplete staining" and %TC of 5% or larger and 25% or smaller by a pathologist is adjudicated by one or two independent pathologists. The patient's final result with regard to "Eligibility" should be obtained by either a majority rule or by consensus among the pathologists.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

At present, the recommended practice for HER2 testing 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

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

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

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

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Nonclinical studies were performed using PATHWAY anti-HER2 (4B5) antibody to establish the analytical performance of the device. These studies were performed using VENTANA BenchMark ULTRA instruments controlled by the VSS software versions 12.3, 12.3.1, 12.5.3 or 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 of the device.

1. Analytical Sensitivity

Analytical sensitivity was assessed by calculating prevalence of HER2 IHC scores in Clinical Trial DESTINY-Breast04.

Among 1,303 breast cancer specimens, there were 267 specimens with HER2 0 scores, 554 specimens with HER2 1+ scores, 440 specimens with HER2 2+ scores and 13 specimens with HER2 3+ scores. Also, there were 29 specimens which were not evaluable.

Table 6. HER2 IHC Scores among Breast Cancer Cases in Clinical Trial DESTINY-Breast04

HER2 IHC Score	n/N	%
0	267/1303	20.5
1+	554/1303	42.5
2+	440/1303	33.8
3+	13/1303	1.0
Not Evaluable	29/1303	2.2

In different populations prevalence of HER2 IHC scores can be different from the prevalence presented in Table 6

2. Analytical Specificity

a. Western Blot

Western blot analysis had been conducted to demonstrate that the rabbit monoclonal anti-HER2/neu (4B5) antibody recognizes the 185KD HER2 protein in cell lysates known to express HER2, to show that the antibody does not cross react with other antigens in the HER family (HER1, HER3, HER4), and that the specificity is similar to the mouse monoclonal anti-HER2 (CB11). Results were based on Pass/Fail acceptance. Blots had visible molecular weight markers, protein bands resulting from the primary antibody corresponded to an approximate molecular weight 185KD, anti-HER2/neu

(4B5) did not react with HER1, HER3 and HER4, and the specificity of anti-HER2/neu (4B5) was similar to anti-HER2 (CB11).

b. Immunoreactivity in Human Tissues

The purpose of this study was to assess the analytical specificity (Tour of Body and Tour of Tumor) including non-specific staining, background and cross-reactivity of PATHWAY anti-HER2 (4B5) antibody on non-neoplastic and neoplastic tissue samples. Multi-tissue arrays encompassing a range of normal tissues in the Tour of Body, and neoplastic tissues in the Tour of Tumor, were stained with PATHWAY anti-HER2 (4B5) antibody. Staining results for normal and neoplastic tissue are listed in Tables 7 and 8 respectively. No unexpected staining was observed, and the study results showed no specific membrane staining for most normal and neoplastic tissues.

Table 7. Specificity of PATHWAY anti-HER2 (4B5) Antibody in FFPE Normal Tissues

Tissue	# Positive/ Total Cases	Tissue	# Positive/ Total Cases
Adrenal Gland	0/6	Ovary	0/6
Bladder	3/3*	Pancreas	0/6
Breast	0/14	Parathyroid	4/6**
Bone Marrow	0/3	Peripheral Nerve	2/6
Cardiac Pericardium	0/3	Prostate	1/6
Cerebrum	0/6	Rectum	0/6
Cerebellum	0/6	Salivary Gland	0/3
Cervix	0/5	Skeletal Muscle	0/6
Colon	0/46	Skin	0/6
Endocervix	0/1	Small Intestine	0/6
Endometrium	0/3	Spleen	0/6
Esophagus	1/6	Stomach	0/11
Heart	0/5	Testis	0/6
Hypophysis	0/5	Thymus Gland	0/5
Kidney	0/6	Thyroid	0/6
Liver	0/6	Tongue	0/3
Lung	0/6	Tonsil	3/6***
Lymph Node	0/12	Uterus	0/3
Mesothelium NOS	0/3		

* Membranous staining of superficial umbrella cells; ** Focal membrane staining; *** Focal staining of surface epithelial cells
NOS = Not otherwise specified

Table 8. Specificity of PATHWAY HER2 (4B5) Antibody in FFPE Neoplastic Tissues

Tumor Type	# 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)	42/99
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

Tumor Type	# Positive/Total Cases
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
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

Tumor Type	# Positive/Total Cases
Metastatic mucinous adenocarcinoma (from Colon)	0/1
Carcinoid (NOS)	0/2
Leiomyoma NOS	0/2
Sarcoma NOS	0/2
Undifferentiated carcinoma NOS	0/1

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 6 FFPE breast carcinoma tissue cases (5 non-borderline, 1 borderline). Duplicate sections at 2, 3, 5, 6, and 7 µm were tested for each case.

The results for each test sample were compared to the results of its respective bracket reference sample sections at 4 µm and deemed acceptable or unacceptable. 2-5 µm thickness demonstrated concordant HER2 expression and acceptable background levels for PATHWAY anti-HER2 antibody staining when compared to the reference of 4 µm. Specimens should be cut at 4-5 µm for staining with PATHWAY anti-HER2 (4B5) antibody.

b. Guard Banding Studies

The purpose of this study was to identify protocol conditions that might lead to a potential false positive, false negative, or unacceptable result and prevent these conditions from affecting the end user. Four breast carcinoma tissue (resection) specimens were tested, and system level controls were evaluated as part of this study to determine if the selected controls could adequately identify some system failures.

Non-optimal parameters (offline baking, bluing incubation time High, Hematoxylin incubation time High, Stacking of Bluing and Hematoxylin conditions High, Cell Conditioning (CC1) incubation times (8, 20, 76, 92 min), Antibody (Ab) incubation times (4, 8, 60, 120 min) that may affect HER2 (4B5) antibody staining performance on breast carcinoma tissue were also tested. The protocol limitations and failure modes identified in this study are listed in the table below:

Table 9. Selected Assay Protocol Deviations and Associated Failures

Condition	Observed Failure
CC1 Incubation (8 min, 76 min, and 92 min)	Discordant for HER2 (4B5) antibody status
Ab Incubation (4 min and 8 min)	Discordant for HER2 (4B5) antibody status

Condition	Observed Failure
Ab Incubation (60 min and 120 min)	NRC Background Unacceptable

Increased and decreased cell conditioning and antibody incubation times may impact HER2 staining and interpretation of samples, especially when the HER2 score is 1+.

Additional "Guard banding" studies around the nominal conditions were also performed to evaluate the impact on HER2 staining and interpretation. Thirteen breast cancer slides (samples) from unique patient cases (2 non-borderline IHC 3+, and 11 borderline) were tested. The 11 borderline cases comprised of 3 IHC with faint partial staining pattern and %TC<10%, 3 IHC 1+, 4 IHC 2+, and 1 IHC 3+.

A sample with the recommended staining protocol on each staining run served as the reference. Various test conditions were tested using combinations of CC1 and Antibody incubation times (CC1 times: 20, 36, 52, 64 minutes, and Ab incubation times: 12, 16, 20, 24, 28, 32, 36, 40, 48, 56 minutes).

Increased antibody incubation time (≥28 minutes) is likely to produce unacceptable staining in the negative reagent control (NRC) slide, which would prevent interpretation and scoring HER2-stained sample. Decreased ≤20 minutes and increased (≥52 minutes) cell conditioning times are likely to produce a change in HER2 score in HER2 stained samples.

Results of the study indicated that deviating from the recommended staining protocol produces unacceptable staining in NRC samples and change 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 (Pathologist) Precision study and Inter-Laboratory and Inter-Reader Precision (Reproducibility) study.

a. Intermediate Precision

Twenty-four breast carcinoma cases (slides) spanning the HER2 IHC staining range were included in the intermediate precision study. The study design for evaluation of staining precision on breast carcinoma tissues stained with PATHWAY anti-HER2 (4B5) antibody included:

- Three lots of PATHWAY anti-HER2 (4B5) antibody
- Three lots of ultraView DAB IHC Detection Kits
- Study performed over three days
- Three BenchMark ULTRA instruments
- One pathologist reader, 2 replicates

All slides were blinded and randomized and evaluated using the scoring method specified in Table 4 above. Each case had 18 results, and a majority HER2 bin results

were assigned based on those 18 results. For each case, a median %TC and range of %TC was calculated for the 18 results. In addition, percent Eligible with regard to HER2-low therapy was calculated. Among 24 cases, there were 3 cases with a majority HER2 bin of 0, 10 cases with a majority HER2 bin of 1+, 6 cases with a majority HER2 bin of 2+ and 5 cases with a majority HER2 bin of 3+. Results of this analysis are presented in the table below.

Table 10. Median and Range of %TC for Cases in the Intermediate Precision Study

Case	Majority HER2 Bin	Median %TC	Range %TC (Min-Max)	Percent Eligible
1	0	0.0	0 - 0	0% (0/18)
2	0	0.0	0 - 0	0% (0/18)
3	0	1.0	1 - 2	0% (0/18)
4	1+	15.0	5 - 20	78% (14/18)
5	1+	15.0	10 - 20	94% (17/18)
6	1+	17.5	8 - 30	94% (17/18)
7	1+	20.0	15 - 20	100% (18/18)
8	1+	20.0	15 - 25	100% (18/18)
9	1+	20.0	15 - 35	100% (18/18)
10	1+	22.5	15 - 25	100% (18/18)
11	1+	25.0	15 - 35	100% (18/18)
12	1+	30.0	20 - 35	100% (18/18)
13	1+	50.0	35 - 50	100% (18/18)
14	2+	20.0	15 - 25	100% (18/18)
15	2+	20.0	15 - 35	100% (18/18)
16	2+	25.0	15 - 35	100% (18/18)
17	2+	35.0	15 - 50	100% (18/18)
18	2+	35.0	25 - 40	100% (18/18)
19	2+	50.0	50 - 50	100% (18/18)
20	3+	60.0	60 - 60	0% (0/18)
21	3+	75.0	75 - 80	0% (0/18)
22	3+	95.0	70 - 100	0% (0/18)
23	3+	100.0	95 - 100	0% (0/18)
24	3+	100.0	100 - 100	0% (0/18)

Twenty one (21) out of 24 cases had 18 results with the same type of staining (‘No staining” or “Faint, partial staining” or “Weak to moderate complete staining” or “Intense complete staining”), variability of %TC values for 21 cases was evaluated and the following precision components were calculated: repeatability (within-pathologist), between-day, between-antibody kit, between-detection kit, between-instrument and total. Results are summarized in the table below:

Table 11. Precision Components for Cases in Intermediate Precision Study

Case	Majority HER2 Bin	Median %TC	Standard Deviation					
			Repeatability (within-run)	Between-day	Between-antibody lot	Between-detection kit	Between-instrument	Total
1	0	0.0	0.00	0.00	0.00	0.00	0.00	0.00
2	0	0.0	0.00	0.00	0.00	0.00	0.00	0.00
3	0	1.0	0.00	0.00	0.00	0.58	0.58	0.82
4	1+	15.0	0.71	7.62	0.00	0.00	0.00	7.65
5	1+	15.0	1.67	0.00	0.00	2.64	2.20	3.82
6	1+	17.5	3.11	1.87	0.00	0.00	4.08	5.46
7	1+	20.0	1.18	1.18	0.00	2.04	0.00	2.64
8	1+	20.0	0.00	0.00	2.89	5.00	2.89	6.45
9	1+	20.0	N/A	N/A	N/A	N/A	N/A	N/A
10	1+	22.5	2.64	3.91	0.00	0.00	0.00	4.71
11	1+	25.0	3.33	0.83	6.77	3.54	2.89	8.86
12	1+	30.0	4.41	0.00	3.91	3.91	4.17	8.21
13	1+	50.0	3.54	5.77	0.00	0.00	0.00	6.77
14	2+	20.0	1.18	0.00	2.76	2.76	1.18	4.25
15	2+	20.0	N/A	N/A	N/A	N/A	N/A	N/A
16	2+	25.0	N/A	N/A	N/A	N/A	N/A	N/A
17	2+	35.0	5.77	9.13	0.00	8.29	0.00	13.62
18	2+	35.0	1.18	0.00	2.36	5.71	2.76	6.87
19	2+	50.0	0.00	0.00	0.00	0.00	0.00	0.00
20	3+	60.0	0.00	0.00	0.00	0.00	0.00	0.00
21	3+	75.0	0.00	0.00	2.89	0.00	2.89	4.08
22	3+	95.0	2.89	0.00	12.16	0.00	2.04	12.67
23	3+	100.0	1.18	0.00	2.76	0.00	2.36	3.82
24	3+	100.0	0.00	0.00	0.00	0.00	0.00	0.00

In addition, a qualitative analysis of different precision components was performed. For the purposes of study analysis, HER2 scores “0” and “3+” were grouped together as negative cases because they were ineligible for HER2-low therapy per the clinical trial design, and HER2 scores of “1+” and “2+” were grouped together as positive cases as they were eligible or potentially eligible for HER2-low targeted therapy per the trial design. Results are summarized in the table below:

Table 12. Repeatability and Intermediate Precision of PATHWAY anti-HER2 (4B5) Antibody on Breast Cancer Tissues with HER2-low Scoring

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	PPA	96/96	100.0	(96.2, 100.0)
	NPA	48/48	100.0	(92.6, 100.0)
	OPA	144/144	100.0	(97.4, 100.0)
Between-Detection Kits	PPA	93/96	96.9	(92.2, 100.0)
	NPA	48/48	100.0	(92.6, 100.0)
	OPA	141/144	97.9	(94.4, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	95/96	99.0	(96.7, 100.0)
	NPA	48/48	100.0	(92.6, 100.0)
	OPA	143/144	99.3	(97.9, 100.0)
Between-Day	PPA	94/96	97.9	(93.3, 100.0)
	NPA	48/48	100.0	(92.6, 100.0)
	OPA	142/144	98.6	(95.8 100.0)
Within-Run	PPA	142/144	98.6	(96.5, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	214/216	99.1	(97.7, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

b. Reader Precision

In the Reader precision study, Between-Reader and Within-Reader components of precision were evaluated. The study included 100 breast carcinoma cases (one slide per case) spanning the HER2 IHC staining range. Samples were blinded and randomized prior to evaluation for HER2-Low status using the scoring method specified in Table 4 above. The study included three readers (pathologist). Readers scored all slides twice, with a minimum of two weeks between reads. Each case had 6 reads (2 reads by each of three readers). Data of the Reader precision study is presented in the table below:

Table 13. Results of the Reader Precision Study

Case Category	HER2 IHC	N of cases	N of reads	Results by HER2 IHC, %TC Category					Percent Results "Eligible"
				0, No staining	0, faint incomplete ≤10%	1+, faint incomplete >10%	2+, Weak to moderate complete	3+, intense complete	
No staining	0	6	36	100% (36/36)					0% (0/36)
No staining/ faint incomplete ≤10%	0	13	78	32% (25/78)	68% (53/78)				0% (0/78)
Faint incomplete ≤10%	0	7	42		100% (42/42)				0% (0/42)
Faint incomplete ≤10%/>10%	0/1+	7	42		79% (33/42)	21% (9/42)			21% (9/42)
Faint incomplete ≤10%/>10%	0/1+	9	54		28% (15/54)	72% (39/54)			72% (39/54)
Faint incomplete >10%	1+	5	30			100% (30/30)			100% (30/30)
Faint incomplete >10%/ weak to moderate complete	1+/2+	13	78			73% (57/78)	27% (21/78)		100% (78/78)
Faint incomplete >10%/ weak to moderate complete	1+/2+	11	66			38% (18/66)	62% (48/66)		100% (66/66)
Weak to moderate complete	2+	15	90				100% (90/90)		100% (90/90)
Weak to moderate complete/ Intense complete	2+/3+	3	18				67% (12/18)	33% (6/18)	67% (12/18)
Variable	0/1+/2+	2	12		25% (3/12)	50% (6/12)	25% (3/12)		75% (9/12)
Intense complete	3+	9	54					100% (54/54)	0% (0/54)

Fifty-two (52) out of 100 cases had 6 results with the same type of staining ("Faint, partial staining" or "Weak to moderate complete staining" or "Intense complete staining"), variability of %TC values for 52 cases was evaluated and following precision components were calculated: within-reader, between-reader and total. Results are summarized in the table below:

Table 14. Precision Components for Cases in Reader Precision Study

Case Category	HER2 IHC	N of cases	Range of median %TC	SD			Percent Results "Eligible"
				Within-Reader	Between-Reader	Total	
Faint incomplete ≤10%	0	7	3.0-6.5	1.8	1.2	2.2	0% (0/42)
Faint incomplete ≤10%/>10%	0/1+	7	2.5-7.5	3.5	3.4	4.9	21% (9/42)
Faint incomplete ≤10%/>10%	0/1+	9	8.0-25.0	18.5	3.4	18.8	72% (39/54)
Faint incomplete >10%	1+	5	11.5-37.5	17.9	13.5	22.5	100% 930/30)
Weak to moderate complete	2+	15	40.0-92.5	14.7	8.9	17.2	100% (90/90)
Intense complete	3+	9	37.5-99.5	13.8	8.5	16.2	0% (0/54)

In addition, a qualitative analysis of different precision components was performed. For the purposes of study analysis, HER2 scores "0" and "3+" were grouped together as negative cases because they were ineligible for HER2-low therapy per the clinical trial design and HER2 scores of "1+" and "2+" were grouped together as positive cases as they were eligible or potentially eligible for HER2-low targeted therapy per the trial design. The agreement for between-reader and within-reader precision components are summarized in the table below.

Table 15. Within- and Between-Reader Precision of the PATHWAY anti-HER2 (4B5) antibody with HER2-low scoring

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	312/333	93.7	(90.9, 96.4)
	ANA	246/267	92.1	(88.0, 95.6)
	OPA	279/300	93.0	(90.0, 96.0)
Between-Reader	APA	300/332	90.4	(85.8, 94.3)
	ANA	236/268	88.1	(82.1, 93.0)
	OPA	268/300	89.3	(84.7, 94.0)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

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-low status of breast carcinoma cases. The study included 28 archival, FFPE breast carcinoma tissue specimens (one slide per case) from unique patients run across 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 a HER2-low status. Each case had 10 results per site (30 results in total). For each case, median %TC and range of %TC of 30 results were calculated. In addition, percent Eligible with regard to HER2-low therapy was also calculated. Results of this analysis for each case were presented in the table below:

Table 16. Results of the Inter-Laboratory Reproducibility Study

Case	Majority HER2 Bin	N of reads	0, No staining	0, faint incomplete ≤10%	1+, faint incomplete >10%	2+, weak to moderate complete	3+, intense complete	Percent Results "Eligible"			
								Site A	Site B	Site C	Overall
1	0	30	100% (30/30)					0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
2	0	30	100% (30/30)					0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
3	0	30	97% (29/30)	3% (1/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
4	0	30	93% (28/30)	7% (2/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
5	0	30	80% (24/30)	20% (6/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
6	0	30	97% (29/30)	3% (1/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
7	0	28	93% (26/28)	7% (2/28)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
8	0	30	93% (28/30)	7% (2/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
9	0	30	77% (23/30)	23% (7/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
10	0	30	50% (15/30)	50% (15/30)				0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
11	0	30	20% (6/30)	77% (23/30)	3% (1/30)			10% (1/10)	0% (0/10)	0% (0/10)	3% (1/30)
12	1+	30		10% (3/30)	90% (27/30)			70% (7/10)	100% (10/10)	100% (10/10)	90% (27/30)
13	1+	30		7% (2/30)	93% (28/30)			80% (8/10)	100% (10/10)	100% (10/10)	93% (28/30)
14	1+	30			87% (26/30)	13% (4/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
15	1+	30		3% (1/30)	97% (29/30)			100% (10/10)	100% (10/10)	90% (9/10)	97% (29/30)
16	1+	30			93% (28/30)	7% (2/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)

Case	Majority HER2 Bin	N of reads	0, No staining	0, faint incomplete ≤10%	1+, faint incomplete >10%	2+, weak to moderate complete	3+, intense complete	Percent Results "Eligible"			
								Site A	Site B	Site C	Overall
17	1+	30			97% (29/30)	3% (1/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
18	1+	28			57% (16/28)	43% (12/30)		100% (8/8)	100% (10/10)	100% (10/10)	100% (28/28)
19	1+	30		3% (1/30)	80% (24/30)	17% (5/30)		90% (9/10)	100% (10/10)	100% (10/10)	97% (29/30)
20	2+	30			7% (2/30)	93% (28/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
21	2+	30			3% (1/30)	97% (29/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
22	2+	30	3% (1/30)		14% (4/30)	83% (25/30)		100% 10/10)	90% (9/10)	100% (10/10)	97% (30/30)
23	2+	30				100% (30/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
24	2+	30			13% (4/30)	87% (26/30)		100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
25	2+	28			7% (2/28)	89% (25/28)	4% (1/28)	100% (8/8)	90% (9/10)	100% (10/10)	96% (27/28)
26	3+	30				3% (1/30)	97% (29/30)	0% (0/10)	10% (1/10)	0% (0/10)	3% (1/30)
27	3+	30					100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
28	3+	30					100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)

Eight (8) out of 28 cases had 30 results with the same type of staining ("No staining" or "Faint, partial staining" or "Weak to moderate complete staining" or "Intense complete staining"), variability of %TC values for 8 cases was evaluated and following precision components were calculated: between-reader, between-day, between-site and total. Results are summarized in the table below:

Table 17. Precision Components for Cases in the Inter-Laboratory Reproducibility Study

Case	Case category	HER2 Bin	N of reads	Median %TC	Range %TC (Min-Max)	SD			
						Between-reader	Between-day	Between-site	Total
1	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
2	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
12	Faint incomplete ≤10%/>10%	0/1+	30	15.0	5-50	9.0	0.0	0.0	9.0

Case	Case category	HER2 Bin	N of reads	Median %TC	Range %TC (Min-Max)	SD			
						Between-reader	Between-day	Between-site	Total
13	Faint incomplete ≤10%/>10%	0/1+	30	17.5	5-50	11.4	2.8	0.0	11.7
15	Faint incomplete ≤10%/>10%	0/1+	30	25.0	8-50	7.8	6.8	11.2	15.2
23	Weak to moderate complete	2+	30	60.0	20-90	12.1	6.2	19.4	23.7
27	Intense complete	3+	30	95.0	90-100	3.3	0.6	0.0	3.4
28	Intense complete	3+	30	95.0	90-100	2.6	0.0	0.0	2.6

In addition, a qualitative analysis of different precision components was performed. For the purposes of study analysis, HER2 scores "0" and "3+" were grouped together as negative cases because they were ineligible for HER2-low therapy per the clinical trial design, and HER2 scores of "1+" and "2+" were grouped together as positive cases as they were eligible or potentially eligible for HER2-low targeted therapy per the trial design. Results of the analysis are presented in the table below:

Table 18. Inter-Laboratory Reproducibility for overall agreement rates for PATHWAY anti-HER2 (4B5)

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	407/416	97.8	(96.2, 99.3)
	NPA	416/418	99.5	(98.8, 100.0)
	OPA	823/834	98.7	(97.7, 99.4)
Within-Site	PPA	407/416	97.8	(96.2, 99.3)
	NPA	416/418	99.5	(98.8, 100.0)
	OPA	823/834	98.7	(97.7, 99.4)
Within-Reader	PPA	407/416	97.8	(96.2, 99.3)
	NPA	416/418	99.5	(98.8, 100.0)
	OPA	823/834	98.7	(97.7, 99.4)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

In addition, pairwise comparisons were made Between-Site, Between-Reader and Between-Day for HER2-low status. A summary of the results can be found in the table below:

Table 19. Inter-Laboratory Reproducibility Pairwise Agreement Rates for the PATHWAY antiHER2 (4B5) antibody

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	APA	7884/8102	97.3	95.4, 98.8
	ANA	8240/8458	97.4	95.7, 98.8
	OPA	8062/8280	97.4	95.5, 98.8
Between-Reader	APA	398/409	97.3	95.4, 98.8
	ANA	414/425	97.4	95.6, 98.8
	OPA	406/417	97.4	95.5, 98.8
Between-Day	APA	1580/1620	97.5	95.9, 98.9
	ANA	1652/1692	97.6	96.2, 98.9
	OPA	1616/1656	97.6	96.1, 98.9

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

5. Stability Studies

a. Cut Slide Stability

The intent of this study was to determine the time point at which degradation of HER2 antigenicity in FFPE tissue sections stored under different storage conditions that may impact the staining performance of the PATHWAY anti-HER2 (4B5) antibody assay on breast carcinoma. The time and storage conditions testes were as follows: 30°C ± 5°C (15% relative humidity (RH) ± 10%), 30°C ± 5°C (85% RH ± 10%), 5°C ± 3°C (15% RH ± 10%), and 5°C ± 3°C (85% RH ± 10%), Cut slide stability was evaluated on a total of 8 breast cancer cases. Tissue samples included 4 FFPE resections (spanning the HER2 (4B5) range of staining, including one borderline case), 2 needle core biopsies, and 2 mock needle core tissue cases encompassing the lower end of the HER2 (4B5) staining range (IHC 1+ to 2+ staining intensity). Slides were sectioned and stained at the day 0 (baseline), day 7, day 45, and months 2, 3, 4, 5, 6, 7, and 9 months.

Based on the study results, the cut slide stability is 45 days when stored at 5 ºC ± 3 ºC (15% Relative Humidity ± 10%).

b. Real Time Stability

The objective of this study was to validate the stability (shelf-life and in-use) and shipping categories of the PATHWAY anti-HER2 (4B5) antibody. Three final 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:

1. Intended Storage (2-8°C)
2. Hot Ship Stress Cat. F (37°C±2ºC 150 hours)

3. Hot Ship Stress Cat. F (37°C±2ºC 192 hours)
4. Hot Ship Stress Cat. J (32°C±2ºC 192 hours)
5. Hot Ship Stress Cat. H (17°C±2ºC 192 hours)
6. Cold Ship Stress Freeze/Thaw (-25°C±5ºC 192 hours)

Each of the 3 lots of PATHWAY anti-HER2 (4B5) antibody was evaluated on 6 breast cancer FFPE resection specimens. Tissue samples included 4 specimens spanning the HER2 (4B5) range of staining from HER2 score 0 to 3+, one specimen for 1+/2+ borderline bin and one specimen for 0/1+ borderline bin. Breast cancer cases were tested in triplicate for each time point.

Based on the data that is provided the reagent stability is 12 months.

B. Animal Studies

None.

C. Additional Studies

1. The Effect of Fixation Type and Time on HER2 Expression

This study evaluated the effect of fixation types and times on the HER2 antigen as detected by the PATHWAY anti-HER2 (4B5) antibody using a xenograft model system with the MDA-MB-361 human breast cancer cell lines. The study evaluated the following fixatives solutions: 10% neutral buffered formalin, Zinc formalin, Z-fix, 10% non-buffered formalin (referred to as 10% UBF for "unbuffered"), 20% formalin, Formagel, Diff Quik, AFA, 95% alcohol, and Prefer (a glyoxal-based fixative). MDA-MB-361 Multi-tissue Blocks (MTBs), 1 block for each fixative was tested at fixation times ranging from one hour to 48 hours. All samples were compared to 24 hours fixation in 10% NBF (baseline slide). This baseline was chosen as the reference standard based on clinical current guidelines for HER2 testing in breast carcinoma. A Staining Intensity Score, which breaks down the dynamic range of expression in 0.25 increments, was used to obtain more granularity in the effect various fixation parameters have on HER2 expression. Based on the study results, it is recommended that 10% neutral buffered formalin (10% NBF) be used to fix samples that are to be stained with the PATHWAY anti-HER2(4B5) antibody with a minimum of 6 hours of fixation time. Zinc formalin, Z-fix, 10% non-buffered formalin (referred to as 10% UBF for "unbuffered"), 20% formalin, Formagel and Diff Quik may also be used because they rendered consistent, homogenous staining compared to the reference. However, AFA, 95% alcohol and Prefer yielded inconsistent, non-uniform weaker staining. Since they did not yield comparable results to the baseline fixation of 10% NBF for 24 hours, AFA, 95% alcohol and Prefer are not recommended for this assay.

2. The Effect of Delay to Fixation (Ischemia) on HER2 Expression

The effect of delay to fixation on the HER2 antigen as detected by the PATHWAY anti-HER2 (4B5) antibody was evaluated in this study. The MDA-MB-361 breast

carcinoma cell line which appropriately expresses the HER2 antigen was used for evaluating staining intensity. Different times ranging from 0-24 hours were selected, and the xenograft tumors were harvested, kept at room temperature without solution until their respective time-point, and then fixed in 10% NBF for 24 hours before processing. A multi-tissue block was created from the samples, and it contained cell lines fixed at each time-point of 0, 30 minutes, 1hour, 3 hours, 5 hours, 12 hours, and 24 hours. Two slides were run in duplicate to ensure that any variances in intensity were the effect of ischemia and not a technical failure. The data from studying the effect of delay to fixation shows that immediate fixation is important to preserve tissue morphology and nuclear detail. It is recommended that samples are immediately fixed in 10% NBF for staining with the PATHWAY anti-HER2 (4B5) antibody, and that samples not be delayed for more than 1 hour before placing in fixative to preserve appropriate nuclear detail.

3. Tissue Heterogeneity

a. Case Heterogeneity

Heterogeneity that may exist between breast cancer tissue blocks from the same patient was evaluated in this study. Twenty FFPE breast cancer resections (10 cases, 2 blocks per case) were used for the study. Out of these, there were 18 non-borderline cases (2 HER2 IHC Bin 3+, 2 HER2 IHC Bin 1+, and 14 HER2 IHC Bin 0), and 2 HER2 IHC Bin 0 negative borderline designated as <0-10%TC. The overall percent agreement (OPA) calculated for all cases, as well as for each of the HER2 IHC bins (0, 1+ and 3+) was 100%. The PATHWAY anti-HER2 (4B5) antibody stained across breast cancer tissues did not significantly impact the HER2 status.

b. Block Heterogeneity

Heterogeneity of HER2 expression that may exist throughout different tissue sections of the same breast cancer tissue blocks was evaluated in this study. A slide from approximately every 10th section was stained with PATHWAY anti-HER2 (4B5) antibody. Fifteen FFPE breast cancer resections and biopsy tissue cases (10 resection cases and 3 core needle biopsy cases) were used for the study. Out of these, there were 13 non-borderline cases (2 HER2 IHC Bin 3+, 4 HER2 IHC Bin 2+, 3 HER2 IHC Bin 1+, and 4 HER2 IHC Bin 0), and 2 HER2 IHC Bin 0 negative borderline designated as <0-10%TC. Twelve out of fifteen cases maintained concordant HER2-low status throughout the block. In two of the 3 discordant cases, heterogeneity was due to a significant reduction in viable tumor expressing HER2. In the final case, heterogeneity was due to the block having an extreme borderline HER2 status.

4. Primary versus Metastatic

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 HER2-low expression level of the patient matched primary and metastatic breast tissue tumors stained with the PATHWAY anti-

HER2 (4B5) antibody assay. Out of 27 pairs of matched cases of breast cancer evaluated, 20 pairs demonstrated concordant HER2-low expression status between primary and metastatic tumors with the OPA rate of 74.1% (20/27) for all cases (OPA of 80% for HER2 IHC bin 0, 50% for HER2 IHC bin 1+, 50% for HER2 IHC bin 2+, and OPA 100% for HER2 IHC bin 3+). Seven paired cases were discordant changing HER2-low expression status negative to positive or positive to negative. These data indicate that HER2-low expression level can be different in the patient's primary and metastatic tumors stained with the PATHWAY anti-HER2 (4B5) antibody assay.

X. SUMMARY OF CLINICAL STUDIES

The clinical performance of PATHWAY anti-HER2 (4B5) antibody assay as a CDx device for the breast cancer (BC) indication for ENHERTU (fam-trastuzumab deruxtecan-nxki; DS-8201a) was based on the clinical trial DESTINY-Breast04 (Study U303).

A. Study Design

DESTINY-Breast04 was a Phase 3 randomized, open-label, multicenter study sponsored by Daiichi Sankyo to compare the efficacy and safety of ENHERTU to those of the "treatment of physician's choice" (TPC; chemotherapy with capecitabine, eribulin, gemcitabine, paclitaxel, or nab-paclitaxel) in patients with unresectable/metastatic BC that expresses low levels of HER2 (HER2 IHC 1+ or HER2 IHC 2+/ISH-negative). Potential participants in study DESTINY-Breast04 were screened for their tumor HER2 status using the PATHWAY anti-HER2 (4B5) antibody. Clinical performance of the PATHWAY anti-HER2 (4B5) antibody as a companion diagnostic (CDx) device for ENHERTU therapy in HER2-low unresectable and/or metastatic BC was evaluated in study DESTINY-Breast04.

A total of 557 subjects were randomized 2:1 to the ENHERTU (n = 375) and TPC (n = 184) treatment arms. Randomization was stratified by the following:

- HER2-low status of tissue samples assessed by a central laboratory: HER2 IHC 1+ or HER2 IHC 2+/ISH-non-amplified
- Number of prior lines of chemotherapy: 1 vs. 2
- Hormone receptor (HR) (estrogen and/or progesterone receptor) / cyclin dependent kinase (CDK) status: HR-positive BC with prior CDK4/6 inhibitor treatment vs. HR-positive BC without prior CDK4/6 inhibitor treatment vs. HR-negative BC.

First patient was treated on December 27, 2018. The database for the DESTINY-Breast04 trial reflected data collected through January 11, 2022 and included 557 randomized patients. There were 211 investigational sites.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in study DESTINY-Breast04 was limited to patients who met the following inclusion criteria:

Key Trial Inclusion Criteria

- Pathologically documented, HR-positive or HR-negative unresectable or metastatic BC with centrally confirmed HER2 low expression.
- All subjects were to have received at least 1 or at most 2 prior lines of chemotherapy in the recurrent or metastatic setting.
- For subjects with HR+ tumors, BC was to be refractory to endocrine therapy (ET), where refractory was defined as progression on at least one ET and determination by the investigator that the subject would no longer benefit from further treatment with ET).
- Radiological progression during or after the most recent treatment had to be documented.
- If recurrence occurred within 6 months of (neo) adjuvant chemotherapy, (neo) adjuvant therapy was counted as 1 line of chemotherapy.
- Targeted agents, immunotherapies, CDK4/6 inhibitors, and ET on their own did not contribute to the count of prior lines of chemotherapy, although regimens with such agents in combination with chemotherapy still counted as 1 line of chemotherapy.
- In additions, subjects had to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and at least 1 measurable lesion per Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1).

Key Trial Exclusion Criteria

- Subjects who had previously been treated with anti-HER2 therapy
- Had tumors that previously tested positive for HER2 (i.e., scoring as IHC 3+ or IHC 2+/ISH-positive).
- Subjects with uncontrolled or significant cardiovascular disease, noninfectious interstitial lung disease (ILD), clinically severe pulmonary compromise, spinal cord compression, or clinically active central nervous system (CNS) metastases.

2. Follow-up Schedule

For each subject there was a 40-day (+7 days) follow-up after the last study drug treatment administration or before starting new anticancer treatment, whichever comes first, followed by Long-term/Survival Follow-up every 3 months (±14 days) from the date of 40-day (+7 days) follow-up, until death, withdrawal of consent, loss to follow-up, or study closure, whichever occurred first.

3. Clinical Endpoints

The primary efficacy endpoint was progression free survival (PFS) based on blinded independent central review (BICR), in subjects with hormone receptor (HR)-positive BC. PFS was defined as the time from the date of randomization to the earliest date of first objective documentation of radiographic disease progression per Response

Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) or death by any cause, whichever occurred first.

The key secondary efficacy endpoints were as follows:

- PFS, based on BICR, in all randomized subjects irrespective of HR status (the Full Analysis Set)
- Overall survival (OS) in subjects with HR-positive BC
- OS in all randomized subjects, regardless of HR status.

OS was defined as the time from the date of randomization to the date of death due to any cause. If death was not reported for a subject before the data cutoff for the OS analysis, OS was censored at the last contact date at which the subject was known to be alive.

- Other secondary endpoints were as follows: Confirmed objective response rate (ORR), based on BICR and Investigator assessment in HR-positive subjects
- Duration of response (DoR), based on BICR in HR-positive subjects

B. Accountability of PMA Cohort

A total of 1,340 patients were screened for enrollment in the study. Of these patients, 1,308 patients were tested with the PATHWAY anti-HER2 (4B5) antibody assay because no sample was submitted for n=28 patients, or the submitted samples did not meet the study eligibility criteria for n=4 patients. For the 1308 patients tested with the PATHWAY anti-HER2 (4B5) antibody device, 968 (74.0%) were biopsy samples, 339 (25.9%) were resections, and collection method for 1 (0.1%) sample was missing. Among 1,308 patients, 5 patients had protocol deviations (3 patients were excluded due to improper sample types and 2 patients were finally included in the study), 29 patients had samples which were not evaluable, 267 patients had IHC 0 (HER2 negative), 554 patients had IHC 1+ (HER2-low), 440 patients had IHC 2+ (reflex to ISH), and 13 patients had IHC 3+ (HER2 positive). Out of 554 IHC 1+ (HER2-low) patients, only 318 patients were enrolled in the study. 440 patients with IHC 2+ were further assessed for ISH status using INFORM HER2 Dual ISH assay. Among 440 patients who were tested with the ISH assay, 14 patients had samples which were not evaluable, 401 patients had ISH negative (non-amplified) status, and 25 had ISH positive (amplified) status. Out of 401 IHC 2+/ISH negative patients, only 237 patients were enrolled in the study. The final number of enrolled patients was 557 patients, consisting of 318 IHC 1+, 237 IHC 2+/ISH negative, and two patients from protocol deviations but enrolled in the study based on the final IHC score 1+.

Table 20. Accountability of the PMA Cohort for Study DESTINY-Breast04

Patients Disposition for study **DESTINY-Breast04**	N
Total number of patients screened	1,340
No sample accessioned	28
Samples did not meet the study eligibility criteria	4
Number tested with the PATHWAY anti-HER2 (4B5) antibody assay	1,308
Protocol Deviation for IHC testing	5
Not evaluable	29
IHC 0 (HER2-negative)	267
IHC 1+ (HER2-Low)	554
IHC 3+ (IHC-positive)	13
IHC 2+ (reflex to ISH) – ISH tested samples	440
IHC 2+ / ISH Not evaluable	14
IHC 2+ /ISH negative (HER2 Non-amplified)	401
IHC 2+ ISH positive (HER2 Amplified)	25
Final number of enrolled patients	557
IHC 1+	318*
IHC 2+/ISH negative (HER2 Non-amplified)	237*
Protocol deviations but enrolled in the study based on the final IHC score of 1+	2

*Among IHC 1+ (N=554) or IHC 2+/ISH negative (N=401) samples, 318 IHC 1+ and 237 IHC 2+/ISH negative were enrolled in study DESTINY-Breast04. Remaining 236 IHC 1+ and 164 IHC2+/ISH negative samples were excluded due to: 1) Patients decided not to move forward with the study screening procedures after HER2 testing. The patients were either no longer willing to participate in the trial or experiencing a disease status change (responding to current treatment, or death of the patient). Also, some patients with HR- (minus) disease became ineligible to continue screening procedures as the HR- (minus) cohort closed; 2) Patients entered the main study screening procedures, however, they did not meet the rest of the inclusion/exclusion criteria for the study; 3) Study enrollment closed, and no more patients were included in the trial.

C. Study Population Demographics and Baseline Parameters

In the IHC and ISH populations (n = 1303 and 440, respectively), 99% of patients were female, the mean age was approximately 56 years, and approximately 2/3 were 45 to < 65 years old. Of the IHC patients with reported characteristics, the majority had an ECOG performance status of 0 and/or had received at least 1 prior line of therapy for their disease.

Table 21. Patient Demographic and Baseline Clinical Characteristics for the IHC Intended Use Population [a], by HER2 Status

Characteristic	HER2 Clinical Status			
	HER2-Low (N=955)	Not HER2-Low (N=305)	Not Evaluable (N=43)	Overall (N=1303)
Age (year)				
Mean (SD)	56.1 (11.3)	55.3 (12.45)	59.8 (10.60)	56.0 (11.6)
Median	57	55	57	56
Min - Max	25 - 84	29 - 90	39 - 87	25 - 90
Age Group 1 (years), n (%)				
>=18 - 40	76 (8.0)	33 (10.8)	1 (2.3)	110 (8.4)

>=40 - 65	652 (68.3)	194 (63.6)	29 (67.4)	875 (67.2)
>=65 - 75	176 (18.4)	53 (17.4)	8 (18.6)	237 (18.2)
>=75	51 (5.3)	25 (8.2)	5 (11.6)	81 (6.2)
Age Group 2 (years), n (%)				
<65	728 (76.2)	227 (74.4)	30 (69.8)	985 (75.6)
>=65	227 (23.8)	78 (25.6)	13 (30.2)	318 (24.4)
Sex, n (%)				
Female	947 (99.2)	302 (99.0)	43 (100.0)	1292 (99.2)
Male	8 (0.8)	3 (1.0)	0	11 (0.8)
Ethnicity, n (%)				
Hispanic or Latino	25 (2.6)	0	0	25 (1.9)
Not Hispanic or Latino	586 (61.4)	0	1 (2.3)	587 (45.0)
Unknown	22 (2.3)	0	0	22 (1.7)
Missing	322 (33.7)	305 (100.0)	42 (97.7)	669 (51.3)
Race, n (%)				
American Indian or Alaska Native	1 (0.1)	0	0	1 (0.1)
Asian	281 (29.4)	0	1 (2.3)	282 (21.6)
Black or African American	14 (1.5)	0	0	14 (1.1)
Native Hawaiian or Other Pacific Islander	1 (0.1)	0	0	1 (0.1)
White	344 (36.0)	0	0	344 (26.4)
Other	67 (7.0)	0	0	67 (5.1)
Missing	247 (25.9)	305 (100.0)	42 (97.7)	594 (45.6)
Region, n (%)				
Asia	271 (28.4)	0	1 (2.3)	272 (20.9)
Europe + Israel	310 (32.5)	0	0	310 (23.8)
North America	128 (13.4)	0	0	128 (9.8)
Missing	246 (25.8)	305 (100.0)	42 (97.7)	593 (45.5)
Hormone Receptor/CDK Status, n (%)				
Hormone receptor-negative	63 (6.6)	0	0	63 (4.8)
Hormone receptor-positive with prior CDK4/6 inhibitor treatment	347 (36.3)	0	0	347 (26.6)
Hormone receptor-positive without prior CDK4/6 inhibitor treatment	145 (15.2)	0	0	145 (11.1)
Missing	400 (41.9)	305 (100.0)	43 (100.0)	748 (57.4)
VENTANA HER2 (4B5) IHC Score, n (%)				
0 (HER2-negative) [b]	0	267 (87.5)	0	267 (20.5)
1+ (HER2-low) [c]	554 (58.0)	0	0	554 (42.5)
2+ (IHC-equivocal) [d]	401 (42.0)	25 (8.2)	14 (32.6) [e]	440 (33.8)
3+ (HER2-positive) [b]	0	13 (4.3)	0	13 (1.0)
Not Evaluable [b, e]	0	0	29 (67.4)	29 (2.2)
INFORM HER2 Dual ISH Result, n (%)				
Non-amplified (ISH-; *HER2*:Chr17 < 2.0) [d, f]	401 (42.0)	0	0	401 (30.8)
Amplified (ISH+; *HER2*:Chr17 ≥ 2.0) [b]	0	25 (8.2)	0	25 (1.9)

Not Evaluable	0	0	14 (32.6)	14 (1.1)
Not Evaluated	554 (58.0)	280 (91.8)	29 (67.4)	863 (66.2)
Prior Lines of Chemotherapy, n (%)				
1	313 (32.8)	0	0	313 (24.0)
2	242 (25.3)	0	0	242 (18.6)
Missing	400 (41.9)	305 (100.0)	43 (100.0)	748 (57.4)
ECOG Performance Status at Enrollment, (%)				
0	304 (31.8)	0	0	304 (23.3)
1	251 (26.3)	0	0	251 (19.3)
Missing	400 (41.9)	305 (100.0)	43 (100.0)	748 (57.4)

[a] All PATHWAY anti-HER2 (4B5) antibody assay-tested patients whose final patient-level PATHWAY anti-HER2 (4B5) antibody staining attempt was performed according to the requirements of the diagnostic protocol.
[b] These patients were ineligible for enrollment in study DESTINY-Breast04.
[c] Tumors with a final result of IHC 1+ were considered HER2-low. Patients with HER2-low tumors were eligible for further screening for study DESTINY-Breast04.
[d] If sufficient sample remained, IHC 2+ samples were reflexed to HER2 ISH testing to determine *HER2* gene amplification status [positive (ISH+) or negative (ISH-)].
[e] Reasons for non-evaluability were provided.
[f] IHC 2+ tumors with a HER2+/ISH negative (HER2–non-amplified) status were considered HER2-low. Patients with HER2-low tumors were eligible for further screening for study DESTINY-Breast04 enrollment.

D. Safety and Effectiveness Results

1. Safety Results

Adverse events (AEs) in study DESTINY-Breast04 were specific to the toxicity of the investigational agent. There were no device-specific adverse events in the diagnostic study. Based on the treatment-emergent adverse events (TEAEs) in this trial, the product labeling for ENHERTU indicates that serious adverse reactions occurred in 19% of patients receiving ENHERTU and was permanently discontinued in 14% of patients. Please refer to Drugs@FDA for complete safety information on ENHERTU.

2. Effectiveness Results

The analysis of effectiveness was based on the efficacy population in clinical trial DESTINY-Breast04. In the primary analysis, progression-free survival (PFS) based on BICR assessment was analyzed in the subset of randomized patients with endocrine therapy (ET)-refractory, HR-positive unresectable/metastatic BC with stratification by centrally assessed HER2-low score (IHC 1+ or IHC 2+/ISH-), number of prior lines of chemotherapy (1 or 2), and prior CDK4/6 inhibitor treatment (yes or no). Compared to treatment of physician's choice (TPC), ENHERTU treatment was associated with statistically significant and clinically meaningful increases in both PFS and OS in this population as seen in table below:

. **Table 22. Efficacy Results in Clinical Trial DESTINY-Breast04**

Efficacy Parameter	HR+ Cohort		Overall Population (HR+ and HR- Cohorts)	
	ENHERTU (N=331)	Chemotherapy (N=163)	ENHERTU (N=373)	Chemotherapy (N=184)
Overall Survival				
Number of events (%)	126 (38.1)	73 (44.8)	149 (39.9)	90 (48.9)
Median, months (95% CI)	23.9 (20.8, 24.8)	17.5 (15.2, 22.4)	23.4 (20.0, 24.8)	16.8 (14.5, 20.0)
Hazard ratio (95% CI)	0.64 (0.48, 0.86)		0.64 (0.49, 0.84)	
p-value	0.0028		0.001	
Progression-Free Survival per BICR				
Number of events (%)	211 (63.7)	110 (67.5)	243 (65.1)	127 (69.0)
Median, months (95% CI)	10.1 (9.5, 11.5)	5.4 (4.4, 7.1)	9.9 (9.0, 11.3)	5.1 (4.2, 6.8)
Hazard ratio (95% CI)	0.51 (0.40, 0.64)		0.50 (0.40, 0.63)	
p-value	<0.0001		<0.0001	
Confirmed Objective Response Rate per BICR				
n (%)	175 (52.9)	27 (16.6)	195 (52.3)	30 (16.3)
95% CI	47.3, 58.4	11.2, 23.2	47.1, 57.4	11.3, 22.5
Complete Response n (%)	12 (3.6)	1 (0.6)	13 (3.5)	2 (1.1)
Partial Response n (%)	164 (49.5)	26 (16.0)	183 (49.1)	28 (15.2)

CI = confidence interval

3. Subgroup Analyses

There was no subgroup analysis performed.

4. Pediatric Extrapolation

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

E. 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. None of the investigators had disclosable financial interests/arrangements as defined in Section 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 THE STUDIES

A. Effectiveness Conclusions

The effectiveness of PATHWAY anti-HER2 (4B5) antibody assay for identifying HER2-low in patients eligible for treatment with ENHERTU® is based on the clinical performance and benefit to patients with unresectable or metastatic breast cancer as assessed in the study DESTINY-Breast04 study. Overall, data show clinically meaningful efficacy for this patient population.

The performance of the PATHWAY anti-HER2 (4B5) antibody assay was also supported by the analytical validation studies.

B. Safety Conclusions

The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above.

The PATHWAY anti-HER2 (4B5) antibody assay is an in vitro diagnostic device, which tests FFPE tumor specimens collected from patients with BC. No adverse events associated with the diagnostic testing procedure were reported during this study. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely collected for diagnosis. Failure of the device to perform as expected may lead to a failure to correctly interpret test results. As PATHWAY anti-HER2 (4B5) antibody assay is intended for use to identify patients for ENHERTU therapy, if incorrect, or false, results are reported, then BC patients may not receive the proper treatment. Patients with false positive (HER2-low) results may undergo treatment with ENHERTU without much clinical benefit and may experience adverse reactions associated with ENHERTU therapy. Patients with false negative (not HER2-low) results may not be considered for treatment with ENHERTU, and therefore, may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with ENHERTU.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in the clinical study DESTINY-Breast04 conducted to support the supplemental PMA approval as described above.

The probable risks of the device are also based on data collected in the clinical study conducted to support the supplemental PMA approval as described above.

The clinical performance of the PATHWAY anti-HER2 (4B5) antibody assay was demonstrated in the clinical validation studies. The studies demonstrated that PATHWAY anti-HER2 (4B5) antibody assay appropriately and reproducibly detects HER2 antigen in breast carcinoma tissue and can aid in the assessment of breast cancer patients being considered for Herceptin®, KADCYLA®, or ENHERTU®.

The main risk of the PATHWAY anti-HER2 (4B5) antibody assay is obtaining a false result. A false positive result could lead to the treatment with 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 HER2-targeted treatment.

Patient Perspective

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

The clinical performance of the PATHWAY anti-HER2 (4B5) antibody assay was demonstrated in the DESTINY-Breast04 clinical trial. Data from this trial demonstrated that PATHWAY anti-HER2 (4B5) antibody assay appropriately and reproducibly detects HER2 antigen (HER2-low) in breast carcinoma tissue and can aid in the assessment of breast cancer patients being considered for ENHERTU®. When compared to chemotherapy, there was a statistically significant and clinically meaningful increase in both PFS and OS in the BC patients.

In the primary analysis, PFS based on BICR assessment was analyzed in the subset of randomized patients with ET-refractory, HR-positive unresectable/metastatic BC with stratification by centrally assessed HER2-low score (IHC 1+ or IHC 2+/ISH negative), number of prior lines of chemotherapy (1 or 2), and prior CDK4/6 inhibitor treatment (yes or no). Compared to TPC, ENHERTU treatment was associated with statistically significant and clinically meaningful increases in both PFS and OS in this population.

XIV. CDRH DECISION

CDRH issued an approval order on September 30, 2022.

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.