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/S055
Date of Notice of Approval:	January 27, 2025

The original PMA (P990081) for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Antibody was approved for the semiquantitative detection of c-erbB-2 (HER2) antigen in sections of formalin-fixed, paraffin embedded (FFPE) breast cancer tissue from patients for whom Herceptin® treatment is being considered. The SSED to support the indication is available on the CDRH website and is incorporated by reference here.

The current supplement (S055) was submitted to expand the indication for PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Antibody as an aid in the assessment for HER2-ultralow (IHC 0 with membrane staining) breast cancer patients who are eligible for treatment with ENHERTU®.

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 *ultra*View 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:

Indications for Use	HER2 Score	Therapy
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	Herceptin®
Breast carcinoma	IHC 3+ or IHC 2+/ISH amplified	KADCYLA®
Breast carcinoma	IHC 0 with membrane staining, 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-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 30 µg of a rabbit monoclonal antibody directed against the human HER2 antigen. The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. There is trace fetal calf serum, approximately 0.25%, present from the stock solution. Specific antibody concentration is approximately 6 µg/mL. 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 PATHWAY HER2

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 H2O2** 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 rabbit immunoglobulin (Ig) in sections of FFPE tissue. One 25 dispenser of CONFIRM Negative Control Rabbit Ig; contains approximately 250 µg (10 µg/mL) of a rabbit polyclonal immunoglobulin. The immunoglobulin is diluted in Tris buffered saline containing carrier protein and preservative. Total protein concentration of the reagent is approximately 3 mg/mL.

B. Device Instrumentation and Software

The PATHWAY anti-HER2 (4B5) Antibody assay is performed on the BenchMark ULTRA automated staining instruments using VSS Software versions 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 fixatives will not penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period, it is recommended that a 3 mm or smaller section of tissue is fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25°C).

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

D. Test Controls

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

1. Cell Line Controls

PATHWAY anti-HER2 4-in-1 Control Slides 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.

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

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

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

2. Positive Tissue Control

A positive control tissue fixed and processed in the same manner as the patient specimens must be run for each set of test conditions and with every PATHWAY anti-HER2 (4B5) antibody staining procedure performed. This tissue could contain both positive staining cell/tissue components and negative cell/tissue components and serve as both the positive and negative control tissue. Control tissue should be fresh autopsy/biopsy/surgical specimens prepared and fixed as soon as possible in a manner identical to test sections. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue preparation. A tissue with weak positive staining is more suitable than strong positive staining for optimal quality control and to detect minor levels of reagent degradation. Ideally a tissue which is known to have weak but positive staining should be chosen to ensure that the system is sensitive to small amounts of reagent degradation or problems with the IHC methodology. Generally, however, neoplastic tissue that is positive for HER2 is strongly positive due to the nature of the pathology (overexpression). An example of a positive control for PATHWAY anti-HER2 (4B5) antibody is a known weak HER2 positive invasive breast carcinoma (for example ductal or lobular). 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 formalin-fixed, paraffin-embedded (FFPE) tissue sections. The specific antibody is located by a cocktail of enzyme-labeled secondary antibodies that recognize rabbit immunoglobulins followed by the addition of a secondary antibody-HRP conjugate (*ultra*View Universal DAB Detection Kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the BenchMark ULTRA instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide.

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

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

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

Tissue / Indication(s)	Breast carcinoma
Protocol step	**Parameter input**
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 H_2O_2: 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

F. Slide Review and Interpretation of HER2 Staining

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

5. 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.

6. 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.

7. 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.

8. 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. In order to accurately assess the HER2 status, cases (slides) with cell membrane staining in ≤ 5% of tumor cells should be re-read by a second pathologist to confirm the HER2 score or arrive at a consensus score before reporting the results.

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

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

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

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

Staining pattern	HER2 (4B5) Score (Report to treating physician)	HER2 Status	Therapy
No membrane staining is observed*	IHC 0 absent membrane staining	HER2-null	None
Any staining of the membrane in greater than 0 and less than or equal to 10% of the cancer cells*, **, ***	IHC 0 with membrane staining	HER2-ultralow expression	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
Faint, partial staining of the membrane in greater than 10% of the cancer cells*	IHC 1+	HER2-low expression	
Weak to moderate complete staining of the membrane in	IHC 2+**** *Reflex test:*	HER2-low expression	

Staining pattern	HER2 (4B5) Score (Report to treating physician)	HER2 Status	Therapy
greater than 10% of the cancer cells	*HER2 Non-Amplified*		
	IHC 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	IHC 3+	HER2 positive / overexpression	

* Review at 40x is recommended to discern the presence or absence of any staining such as faint, partial staining.

** Recommend re-reading by a second pathologist for cases with "faint, partial staining of the membrane" and %Tumor Cells (%TC) ≤ 5%.

*** In the HER2-ultralow "IHC 0 with membrane staining" category, partial membranous staining is usually faint but may exhibit stronger intensities, and such rare cases are scored as HER2-ultralow (IHC 0 with membrane staining) if they do not otherwise qualify for a higher score. Refer to the Interpretation Guide for case examples.

****Recommend reflex test to assess gene amplification.

10. <u>Re-Reading Zone for HER2-Ultralow (IHC 0 with membrane staining) Scoring in Breast Cancer</u>

Cases with HER2 Ultralow (IHC 0 with membrane staining) expression including faint partial staining pattern should be evaluated regarding %TC staining to accurately determine the %TC staining as this assessment is clinically significant. (Patients with staining in zero % tumor cell staining are not eligible for the targeted therapy. However, patients with %TC>0% to 2+/ISH negative are eligible for the targeted therapy, ENHERTU).

To decrease variability in scoring of PATHWAY anti-HER2 (4B5) antibody, cases with HER2 staining in 1% to 5% of tumor cell should be reviewed and adjudicated by one or two additional independent pathologists. The patient's final result with regard to "Eligibility" for the targeted therapy should be obtained by either a majority rule or by consensus among the pathologists.

VI. <u>ALTERNATIVE PRACTICES AND PROCEDURES</u>

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

There are several other FDA-approved IHC tests for the detection of HER2 protein in breast carcinoma. There are also FDA approved in situ hybridization (ISH) tests for the detection of HER2 gene amplification in tissues, which has been correlated to protein overexpression.

Each alternative for HER2 testing in breast carcinoma 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

The intended use prevalence across HER2 categories has been studied through distribution available in commercial tissue banks for analytical studies as well as through review of the clinical trials.

a. Analytical sensitivity was assessed by calculating prevalence of HER2 IHC scores in a panel of 408 breast cancer cases from the commercial tissue bank and is presented in table below.

Table 6. Prevalence of HER2 IHC Scores in Breast Cancer

HER2 IHC Bin	Number of Cases n/N	Prevalence %
IHC 0 absent membrane staining	124 / 408	30.4
IHC 0 with membrane staining	134 / 408	33.1
IHC 1+	40 / 408	9.8
IHC 2+ [b]	24 / 408	5.9
IHC 3+	85 / 408	20.8

[a] In different populations, prevalence of HER2 IHC scores are different from the distribution presented in this table. Note: The commercial cohort used in this study was enriched and not a random population.

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

b. The prevalence was also estimated based on clinical trial screening for inclusion / exclusion criteria in DESTINY-Breast06. A total of 1,940 patients were screened for enrollment in the DB06 clinical trial using the breast scoring algorithm including the HER2-utlralow category. The table below provides the prevalence in the DESTINY-Breast06 clinical trial.

Table 6. HER2 Prevalence [a] in Breast Cancer by IHC Bins/Categories in Clinical Trial DESTINY-Breast06 (DB06)

HER2 IHC Bin	Number of Cases n/N	Prevalence %
IHC 0 absent membrane staining	225 / 1,940	11.6
IHC 0 with membrane staining	400 / 1,904	20.6
IHC 1+	828 / 1,940	42.6
IHC 2+ [b]	423 / 1,940	21.8
IHC 3+	4 / 1,940	0.2
Not evaluable	60 / 1,940	3.1

[a] In different populations, prevalence of HER2 IHC scores are different from the prevalence presented in this table for the DB06 clinical trial population. Note that HER2 positive (IHC 2+/ISH+ and IHC 3+) is underrepresented in this DB06 clinical trial population because the clinical trial design only included participants with a history of HER2-low or negative expression, defined as IHC 2+/ISH- or IHC 1+ (ISH – or untested) or IHC 0 (ISH- or untested) with a validated assay.

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

2. Analytical Specificity

a. Western Blot

Refer to the Summary of Safety and Effectiveness Data P990081/S047 (Section IX.A.2.a) for the Western Blot Study.

b. Immunoreactivity

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 below. All assessable cores and supplemental tissues had acceptable morphology and acceptable HER2 (4B5) background that did not interfere with interpretation. No unexpected staining was observed, and the study results showed no specific membrane staining for most normal and neoplastic tissues.

Table 8. 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 9. Specificity of PATHWAY anti-HER2 (4B5) Antibody in FFPE Neoplastic Tissues

Pathology	# 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

Pathology	# positive / total cases
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
Renal Cell 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

3. Robustness

a. Tissue Thickness

Refer to the Summary of Safety and Effectiveness Data P990081/S047 (Section IX.A.3.a) for the study to evaluate the effects tissue thickness on staining performance.

b. Fixation Study

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

c. Ischemia Study (Time to Fixation)

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

d. Protocol Limitations

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

4. Precision

Precision of the PATHWAY anti-HER2 (4B5) antibody on BenchMark ULTRA was evaluated in three precision studies which were as follows: 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 (between antibody kit lot)
- Three lots of *ultra*View DAB IHC Detection Kits (between detection kit lot)
- Across three days (between day)
- Three BenchMark ULTRA instruments (between instrument)
- Across all intermediate precision conditions (within run)
- 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 staining specified in Table 5 above. Each case had 18 results and a majority HER2 bin result was assigned based on 18 results. For each case, median %TC and range of %TC of 18 results were calculated. In addition, percent Eligible with regard to HER2-ultralow therapy was calculated. Among 24 cases, there were 6 cases with majority HER2 bin of 0 absent membrane staining, 6 cases with majority HER2 bin of 0 with membrane staining (>0 <1+), 3 cases with majority HER2 bin of 1+, 3 cases with majority HER2 bin of 2+ and 6 cases with majority HER2 bin of 3+. Results of this analysis are presented in the two tables 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% (0/18)
2	0	0.0	0 - 0	0.0% (0/18)
3	0	0.0	0 - 0	0.0% (0/18)
4	0	0.0	0 - 0	0.0% (0/18)
5	0	0.0	0 - 0	0.0% (0/18)
6	0	0.0	0 - 0	0.0% (0/18)
7	>0<1+	2.0	2 - 5	100.0% (18/18)
8	>0<1+	5.0	2 - 5	100.0% (18/18)
9	>0<1+	5.0	5 - 10	100.0% (18/18)
10	>0<1+	5.0	5 - 10	100.0% (18/18)
11	>0<1+	10.0	5 - 10	100.0% (18/18)
12	>0<1+	10.0	5 - 10	100.0% (18/18)
13	1+	20.0	15 - 20	100.0% (18/18)
14	1+	20.0	15 - 30	100.0% (17/17)
15	1+	40.0	30 - 40	100.0% (3/3)
16	2+	15.0	15 - 15	100.0% (18/18)
17	2+	20.0	15 - 70	100.0% (18/18)
18	2+	60.0	50 - 60	100.0% (18/18)
19	3+	20.0	15 - 29	0.0% (0/18)
20	3+	80.0	70 - 80	0.0% (0/18)
21	3+	80.0	80 - 80	0.0% (0/18)
22	3+	90.0	80 - 90	0.0% (0/18)
23	3+	90.0	90 - 90	0.0% (0/18)
24	3+	100.0	90 - 100	0.0% (0/18)

Twenty-four 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 24 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 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	0.00	0.00	0.00
4	0	15.0	0.00	0.00	0.00	0.00	0.00	0.00
5	0	15.0	0.00	0.00	0.00	0.00	0.00	0.00
6	0	17.5	0.00	0.00	0.00	0.00	0.00	0.00
7	>0<1+	20.0	0.00	0.00	1.73	1.73	1.73	3.00
8	>0<1+	20.0	0.00	1.73	0.00	0.00	0.00	1.73
9	>0<1+	20.0	0.00	2.89	0.00	0.00	0.00	2.89
10	>0<1+	22.5	0.00	2.89	0.00	0.00	0.00	2.89
11	>0<1+	25.0	2.04	2.50	0.00	0.00	0.00	3.23
12	>0<1+	30.0	0.00	0.00	2.89	2.89	2.89	5.00
13	1+	50.0	0.00	2.89	0.00	0.00	0.00	2.89
14	1+	20.0	0.00	5.77	6.45	0.00	6.45	10.80
15	1+	20.0	0.00	7.07	0.00	0.00	0.00	7.07
16	2+	25.0	0.00	0.00	0.00	0.00	0.00	0.00
17	2+	35.0	0.00	2.89	28.72	0.00	5.00	29.30
18	2+	35.0	0.00	0.00	0.00	0.00	5.77	5.77
19	3+	50.0	2.12	0.00	2.12	2.47	0.00	3.88
20	3+	60.0	0.00	0.00	0.00	5.77	0.00	5.77
21	3+	75.0	0.00	0.00	0.00	0.00	0.00	0.00
22	3+	95.0	0.00	0.00	5.77	5.77	0.00	8.16
23	3+	100.0	0.00	0.00	0.00	0.00	0.00	0.00
24	3+	100.0	3.33	1.67	0.00	0.00	0.00	3.73

In addition, a qualitative analysis of different precision components was performed for HER2-ultralow on BenchMark ULTRA instrument. For the purposes of study analysis, HER2 scores "0 absent membrane staining" and "3+" were grouped together as negative cases because they were ineligible for HER2-ultralow therapy per the clinical trial design, and HER2 scores of "0 with membrane staining", "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 table below.

Table 12. Repeatability and Intermediate Precision of PATHWAY anti-HER2 (4B5) Antibody

Repeatability / Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	PPA	67/67	100.0	(94.6, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	139/139	100.0	(97.3, 100.0)
Between-Detection Kits	PPA	67/67	100.0	(94.6, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	139/139	100.0	(97.3, 100.0)
Between- Instrument (BenchMark ULTRA)	PPA	67/67	100.0	(94.6, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	139/139	100.0	(97.3, 100.0)
Between-Day	PPA	68/68	100.0	(94.7, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	140/140	100.0	(97.3, 100.0)
Within-Run	PPA	99/99	100.0	(96.3, 100.0)
	NPA	108/108	100.0	(96.6, 100.0)
	OPA	207/207	100.0	(98.2, 100.0)

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

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 by assessing concordance of HER2 status between 3 readers and within 3 individual readers. The study included 100 breast carcinoma cases spanning the HER2 IHC staining range. Eighty (80) were non-borderline and twenty (20) cases were

enrolled as borderline/challenging for HER2 ultralow status. Slides 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 Breast Cancer incorporating the HER2-ultralow category for IHC 0 with membrane staining specified in Table 5 above. Readers scored all specimens twice, with a minimum of 2 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 two tables below.

Within-reader and between-reader precision was determined with average positive agreement (APA), average negative agreement (ANA), and overall percent agreement (OPA) across all observations.

Table 13. Results of the Reader Precision Study

Case Category	HER2 IHC	N of cases	N of reads	Results by HER2 IHC, %TC Category				
				0, No staining	>0 <1+, faint incomplete ≤10%	1+, faint incomplete >10%	2+, Weak to moderate complete	3+, intense complete
No staining	0	32	192	192	0	0	0	0
No staining/faint incomplete <=10%	0/>0<1+	3	18	9	9	0	0	0
Faint incomplete <=10%	>0<1+	9	54	0	54	0	0	0
Faint incomplete <=10%/>10%	>0<1+/1+	3	18	0	15	3	0	0
Faint incomplete <=10%/>10%	>0<1+/1+	4	24	0	12	12	0	0
Faint incomplete >10%	1+	11	66	0	0	66	0	0
Faint incomplete >10%/weak to moderate complete	1+/2+	4	24	0	0	18	6	0
Faint incomplete >10%/weak to moderate complete	1+/2+	3	18	0	0	5	13	0
Weak to moderate complete	2+	14	84	0	0	0	84	0
Weak to moderate complete /Intense complete	2+/3+	5	30	0	0	0	12	18
Very variable	0/3+	1	6	1	0	0	0	5
Intense complete	3+	11	66	0	0	0	0	66

Table 14. Precision Components for Cases in Reader Precision Study

Case Category	HER2 IHC	N of cases	Range of median %TC	Standard Deviation			Percent Results "Eligible"
				Within-Reader	Between-Reader	Total	
No staining	0	32	0.0 - 0.0	0.0	0.0	0.0	0.0% (0/192)
No staining/faint incomplete <=10%	0/>0<1+	3	0.0 - 1.0	0.5	0.4	0.5	50.0% (9/18)
Faint incomplete <=10%	>0<1+	8	2.0 - 9.5	1.4	1.4	1.4	100.0% (54/54)
Faint incomplete <=10%/>10%	>0<1+/1+	3	5.0 - 10.0	2.9	0.0	2.9	100.0% (18/18)
Faint incomplete <=10%/>10%	>0<1+/1+	4	8.0 - 12.5	6.0	3.9	6.0	100.0% (24/24)
Faint incomplete >10%	1+	11	15.0 - 55.0	14.9	5.1	14.9	100.0% (66/66)
Faint incomplete >10%/weak to moderate complete	1+/2+	4	N/A	N/A	N/A	N/A	100.0% (24/24)
Faint incomplete >10%/weak to moderate complete	1+/2+	3	N/A	N/A	N/A	N/A	100.0% (18/18)
Weak to moderate complete	2+	14	15.0 - 77.5	13.7	16.6	13.7	100.0% (84/84)
Weak to moderate complete /Intense complete	2+/3+	5	N/A	N/A	N/A	N/A	40.0% (12/30)
Very variable	0/3+	1	N/A	N/A	N/A	N/A	0.0% (0/6)
Intense complete	3+	11	30.0 - 100.0	8.2	16.3	8.2	0.0% (0/66)

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

Table 15. Between- and Within-Reader Precision of the PATHWAY anti-HER2 (4B5) Antibody in Breast Cancer, Incorporating HER2-ultralow Scoring

Precision	Pairwise Comparison	Agreement			
		Type	n/N	%	95% CI
Between Reader	IHC 0* & IHC 3+ (ENHERTU® ineligible, considered negative) vs. IHC >0 <1+**, IHC 1+ & IHC 2+ (ENHERTU® eligible or potentially eligible, considered positive)	APA	296/310	95.5	(91.7, 98.4)
		ANA	276/290	95.2	(91.1, 98.2)
		OPA	286/300	95.3	(92.0, 98.0)
Within-Reader	IHC 0* & IHC 3+ (ENHERTU® ineligible, considered negative) vs. IHC >0 <1+**, IHC 1+ & IHC 2+ (ENHERTU® eligible or potentially eligible, considered positive)	APA	302/309	97.7	(95.9, 99.3)
		ANA	284/291	97.6	(95.6, 99.3)
		OPA	293/300	97.7	(95.7, 99.3)

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

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

Note: For the purposes of study analysis, HER2 scores 0 absent membrane staining and 3+ were grouped together as negative cases because they were ineligible for the clinical trial investigating HER2-low and HER2-ultralow breast cancer. HER2 scores of 0 with membrane staining, 1+ and 2+ were grouped together as positive cases as they were eligible or potentially eligible for the clinical trial.

* IHC 0 in this study represented "IHC 0 absent membrane staining" (HER2-null).

** IHC >0<1+ in this study represented "IHC 0 with membrane staining" (HER2-ultralow), eligible for ENHERTU.

5. Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility study of the VENTANA HER2 (4B5) antibody was conducted to evaluate reproducibility of the assay to determine HER2 status including HER2-ultralow status of breast cancer tissue. The study included 28 de-identified, archival, FFPE breast cancer tissue specimens stained on 3 BenchMark ULTRA instruments on each of 5 non-consecutive days over 20 days at 3 external laboratories. For the purpose of calculating agreement rates, HER2 scores of IHC >0 <1+, IHC 1+, and IHC 2+ were considered positive, and HER2 scores of IHC 0+ or IHC 3+ were considered negative. The specimens represented the range of staining of the PATHWAY anti-HER2 (4B5), with equal distribution between positive and negative cases, and included several cases selected to exhibit staining near the lower diagnostic threshold for the assay.

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 IHC status.

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	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
2	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
3	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
4	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
5	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
6	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
7	0	30	100% (30/30)	0	0	0	0	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
8	>0<1+	30	3% (1/30)	93% (28/30)	3% (1/30)	0	0	90% (9/10)	100% (10/10)	100% (10/10)	97% (29/30)
9	>0<1+	30	10% (3/30)	87% (26/30)	3% (1/30)	0	0	90% (9/10)	100% (10/10)	80% (8/10)	90% (27/30)
10	>0<1+	30	0	63% (19/30)	37% (11/30)	0	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
11	>0<1+	30	0	87% (26/30)	13% (4/30)	0	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
12	>0<1+	30	0	57% (17/30)	30% (9/30)	13% (4/30)	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
13	>0<1+	30	0	53% (16/30)	47% (14/30)	0	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
14	1+	30	0	13% (4/30)	87% (26/30)	0	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
15	1+	30	0	0	83% (25/30)	17% (5/30)	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
16	1+	30	0	0	67% (20/30)	33% (10/30)	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
17	1+	30	0	0	83% (25/30)	17% (5/30)	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
18	2+	30	0	0	0	100% (30/30)	0	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
19	2+	30	0	0	0	100% (30/30)	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
20	2+	30	0	0	0	93% (28/30)	7% (2/30)	80% (8/10)	100% (10/10)	100% (10/10)	93% (28/30)
21	2+	30	0	0	0	97% (29/30)	3% (1/30)	90% (9/10)	100% (10/10)	100% (10/10)	97% (29/30)
22	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
23	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
24	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
25	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
26	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
27	3+	30	3% (1/30)	0	0	0	97% (29/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
28	3+	30	0	0	0	0	100% (30/30)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)

Twenty one out of 28 cases had 30 results with the same type of staining ("No staining" or "Faint, partial staining <=10%", "Faint, partial staining >10%" 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: between-reader, between-day, between-site and total. Results are summarized in below tables.

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

Case	Case category	HER 2 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
3	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
4	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
5	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
6	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0
7	No staining	0	30	0.0	0-0	0.0	0.0	0.0	0.0

Case	Case category	HER 2 Bin	N of reads	Median %TC	Range %TC (Min-Max)	SD			
						Between-reader	Between-day	Between-site	Total
8	Faint incomplete ≤10%	>0<1+	30	1.0	0 - 20	2.4	0.7	0.0	3.9
9	Faint incomplete ≤10%	>0<1+	30	2.5	0 - 15	3.1	1.1	0.0	3.8
10	Faint incomplete ≤10%	>0<1+	30	4.0	1 - 75	4.2	12.1	10.2	19.8
11	Faint incomplete ≤10%	>0<1+	30	5.0	1 - 25	5.0	1.9	0.0	6.2
13	Faint incomplete ≤10%	>0<1+	30	9.5	1 - 60	13.4	6.6	11.2	19.6
14	Faint incomplete >10%	1+	30	15.0	1 - 80	0.0	13.9	18.2	27.1
18	Weak to moderate complete	2+	30	45.0	20 - 95	6.5	8.9	14.4	22.4
19	Weak to moderate complete	2+	30	62.5	40 - 90	7.6	0.0	3.8	13.3
22	Intense complete	3+	30	95.0	70 - 100	2.7	4.3	5.7	8.2
23	Intense complete	3+	30	96.5	70 - 100	3.1	2.8	4.6	7.1
24	Intense complete	3+	30	96.5	90 - 100	1.7	0.0	2.6	3.9
25	Intense complete	3+	30	98.0	65 - 100	5.3	3.5	10.6	12.8
26	Intense complete	3+	30	98.0	70 - 100	3.6	2.9	6.9	8.8
28	Intense complete	3+	30	99.0	80 - 100	4.5	1.1	0.0	5.3

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

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

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	413/420	98.3	(96.7, 99.8)
	NPA	420/420	100.0	(99.1, 100.0)
	OPA	833/840	99.2	(98.3, 99.9)
Within-Site	PPA	413/420	98.3	(96.8, 99.1)
	NPA	420/420	100.0	(99.1, 100.0)
	OPA	833/840	99.2	(97.7, 99.4)
Within-Reader	PPA	413/420	98.3	(96.7, 99.8)
	NPA	420/420	100.0	(99.1, 100.0)
	OPA	833/840	99.2	(98.3, 99.9)

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

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

Note: For the purposes of study analysis, HER2 scores 0 absent membrane staining and 3+ were grouped together as negative cases because they were ineligible for the clinical trial investigating HER2-low and HER2-ultralow breast cancer. HER2 scores of 0 with membrane staining, 1+ and 2+ were grouped together as positive cases as they were eligible or potentially eligible for the clinical trial.

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

Table 19. Inter-Laboratory Reproducibility Pairwise Agreement Rates for PATHWAY anti-HER2 (4B5) antibody

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	APA	8124/8260	98.4	(96.7, 99.8)
	ANA	8404/8540	98.4	(96.9, 99.8)
	OPA	8264/8400	98.4	(96.8, 99.8)
Between-Reader	APA	406/413	98.3	(96.6, 99.8)
	ANA	420/427	98.4	(96.8, 99.8)

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
	OPA	413/420	98.3	(96.7, 99.8)
Between-Day	APA	1628/1652	98.5	(97.2, 99.8)
	ANA	1684/1708	98.6	(97.4, 99.8)
	OPA	1656/1680	98.6	(97.3, 99.8)

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

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

Note: For the purposes of study analysis, HER2 scores 0 absent membrane staining and 3+ were grouped together as negative cases because they were ineligible for the clinical trial investigating HER2-low and HER2-ultralow breast cancer. HER2 scores of 0 with membrane staining, 1+ and 2+ were grouped together as positive cases as they were eligible or potentially eligible for the clinical trial.

6. Stability Studies

a. Real-time and Ship Stress Stability

A supplemental study was performed to assess the stability (shelf-life and in-use) and shipping category of VENTANA/PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody and evaluating HER2 (4B5) staining performance on breast carcinoma cases that included the HER2-ultralow (IHC 0 with membrane staining) scoring category. Three breast carcinoma FFPE specimens in the category of HER2-ultralow score were analyzed in addition to six breast carcinoma FFPE specimens spanning the HER2 (4B5) range of staining from HER2 score 0 to 3+. Three production lots of PATHWAY anti-HER2 (4B5) antibody were evaluated under following conditions:

- Intended Storage (2-8ºC)
- Hot Ship Stress Category F (37°C±2ºC 192 hours)
- Cold Ship Stress (Freeze/Thaw Cycle) (-25°C±5ºC 192 hours)

Based on the study results, the current product dating of 18 months and current shipping conditions is applicable to HER2-ultralow (IHC 0 with membrane staining) scoring category as well.

b. Cut Slide Stability

A supplemental study was performed to confirm that cut slide stability in HER2-ultralow breast cancer dating of 45 days was maintained. Based on the study results, the cut slide stability is 45 days when slides are stored at 5ºC ± 3 ºC (15% Relative Humidity ± 10%).

Refer to the Summary of Safety and Effectiveness Data P990081/S047 (Section IX.A.5.a) for more information.

B. Animal Studies

Not applicable.

C. Additional Studies

1. Primary vs. Metastatic Tumor

This study characterized the staining performance of PATHWAY anti-HER2 (4B5) antibody on the primary tumor versus patient-matched metastatic tumor sample.

Of the 32 matched pairs of breast cancer tissue resections and metastatic tumors, patient cases included in analysis, 81.3% of cases (26/32) demonstrated concordant HER2 status between the primary tumor block and the patient-matched metastatic tumor block. Six paired cases were discordant changing HER2-ultralow expression status negative to positive or positive to negative. These data indicate that HER2-ultralow expression level can be different in the patient's primary and metastatic tumors stained with HER2 (4B5) Assay.

2. Tissue Heterogeneity

a. Case Heterogeneity

This study characterized case heterogeneity by evaluating staining performance of VENTANA/PATHWAY anti-HER2/neu (4B5) (hereafter, HER2 (4B5)) on multiple blocks from the same patient case. Case heterogeneity compares biomarker status between multiple blocks from one patient case.

All 20 FFPE breast cancer resection cases (10 cases, 2 blocks per case) demonstrated 100% concordant HER2 status. The results from this study demonstrate that heterogeneity among tumor blocks from the same patient case may exist in a low proportion across breast cancer tissues stained with the PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody, and therefore the tumor block from the same patient did not significantly impact the HER2 status.

b. Block Heterogeneity

This study characterized within-block heterogeneity by evaluating staining outcome from multiple samples from the same tissue block. Block heterogeneity compares biomarker status between multiple slides sectioned from one block.

Of the 15 cases enrolled, 14 cases demonstrated concordant HER2 status within the FFPE breast cancer tissue blocks. In the 1 case that displayed heterogeneity, the change of HER2 clinical interpretation status between tissue sections was due to reduction in viable

tumor expressing HER2, as the block was sectioned to exhaustion. The results from this study demonstrate that heterogeneity between samples from the same tissue blocks may exist in a low proportion across breast cancer tissues stained with the PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody, and therefore the sample from the block did not significantly impact the HER2 status.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of PATHWAY anti-HER2 (4B5) antibody assay as a companion diagnostic (CDx) device to aid in identify patients with breast cancer who are likely to benefit from ENHERTU® (fam-trastuzumab deruxtecan-nkxi, T-DXd) treatment was evaluated in the Phase 3 clinical trial DESTINY-Breast06 (D9670C00001).

A. Study Design

The DESTINY-Breast06 (DB06) study [A Phase 3, Randomized, Multi-center, Open-label Study of Trastuzumab Deruxtecan (T-DXd) Versus Investigator's Choice Chemotherapy in HER2-Low, Hormone Receptor Positive Breast Cancer Patients whose Disease has Progressed on Endocrine Therapy in the Metastatic Setting] enrolled adult patients with advanced (unresectable) or metastatic hormone receptor positive breast cancer who have received at least one endocrine therapy (ET) in the metastatic setting. Patients were required to have HER2 low (HER2 IHC 1+ or HER2 IHC 2+/ISH-negative or HER2 ultralow (IHC 0 with membrane staining, i.e., membrane staining in >0 and <10% of tumor cells staining) tumor IHC scores.

A total of 713 patients were enrolled as HER2-low (IHC 1+ or 2+/ISH-) and 153 patients were enrolled as HER2-ultralow (IHC >0<1+, IHC 0 with membrane staining). Patients were randomized 1:1 to receive either ENHERTU® (T-DXd) or investigator's choice single agent chemotherapy (capecitabine, paclitaxel or nab-paclitaxel) treatment arms. Patients were stratified according to prior CDK4/6 inhibitor (CDK4/6i) use (yes vs no), HER2 IHC expression (IHC 2+/ISH- vs IHC 1+ vs IHC > 0 < 1+) and prior taxane use in the non-metastatic setting (yes vs no). The PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody was used centrally to assess HER2 protein expression by immunohistochemistry (IHC) and to identify patients with low levels of HER2 expression (HER2-low and HER2-ultralow) breast cancer who may benefit from treatment with ENHERTU®.

1. Clinical Inclusion and Exclusion Criteria

Key Inclusion Criteria

Subjects were required to fulfill the criteria in the DB06 clinical protocol, including but not limited to the following:

1. Male or female patients ≥ 18 years of age.
2. Pathologically documented breast cancer that:
   a. Is advanced or metastatic.

b. Has a history of HER2-low or negative expression, defined as IHC 2+/ISH- or IHC 1+ (ISH- or untested) or IHC 0 (ISH- or untested) with a validated assay.
c. Has HER2-low expression or HER2 IHC >0 <1+ expression as determined by the central laboratory result from a metastatic setting.
d. Was never previously reported as HER2-positive (IHC 3+ or ISH+) as per ASCO-CAP guidelines.
e. Is documented as HR+ (either ER and/or PgR positive [ER or PgR ≥1%]) per ASCO-CAP guidelines (Allison et al 2020) in the metastatic setting. If a patient has had multiple ER/PgR results after metastatic disease, the most recent test result will be used to confirm eligibility.

3. ECOG performance status of 0 or 1
4. Radiologic or objective evidence of disease progression on or after the last systemic therapy prior to starting study treatment
5. Must have had either:
   a. Disease progression on endocrine therapy + CDK4/6 inhibitor within 6 months of starting first line treatment for metastatic disease and considered appropriate for chemotherapy as the next treatment by the investigator, OR
   b. Disease progression on at least 2 previous lines of ET with or without a targeted therapy (such as CDK4/6, mTOR or PI3-K inhibitors) administered for the treatment of metastatic disease.

Key Exclusion Criteria

Subjects were excluded if they met any of the exclusion criteria in the DB06 clinical protocol, including but not limited to the following:

1. Uncontrolled intercurrent illness, including but not limited to, ongoing or active infection, uncontrolled or significant cardiovascular disease, serious chronic gastrointestinal conditions associated with diarrhea, or psychiatric illness/social situations that would limit compliance with study requirement, substantially increase risk of incurring AEs or compromise the ability of the patient to give written informed consent.
2. Uncontrolled or significant cardiovascular disease.
3. Subjects with uncontrolled or significant cardiovascular disease, prior non-infectious interstitial lung disease (ILD) that required steroids or current/ suspected ILD, lung-specific intercurrent clinically significant illnesses, spinal cord compression, or clinically active central nervous system (CNS) metastases.
4. Previous treatment with anti-HER2 therapy.

2. Follow-up Schedule

For each patient there was a 40-day (+7 days) follow-up visit after the last study treatment administration, followed by long-term/survival follow-up visits every 3

months (±14 days) until death, withdrawal of consent, or study closure, whichever occurs first.

3. **Clinical Endpoints**

Primary Efficacy and Key Secondary Endpoints of the DESTINY-Breast06 study

The primary efficacy endpoint was Progression-Free Survival (PFS) by a blinded independent central review (BICR) according to Response Evaluation Criteria in (RECIST) 1.1 in the hormone receptor (HR) +, HER2-low population (IHC 1+ or IHC 2+/ISH-).

The key secondary endpoints were Overall Survival (OS) in the HR+, HER2-low population, PFS by BICR according to RECIST 1.1 in the intent-to-treat (ITT) population [HER2-ultralow (IHC 0 with membrane staining) and HER2-low (IHC 1+ or IHC 2+/ISH-)], and OS in the ITT population.

Other secondary endpoints included Objective Response Rate (ORR) and Duration of Response (DoR) by BICR and Investigator assessment according to RECIST 1.1 in the HR+, HER2-low population, PFS by Investigator assessment according to RECIST 1.1 in the HR+, HER2-low population and ORR and DoR by BICR and Investigator assessment according to RECIST 1.1 in the ITT population (HER2 IHC >0 <1+ and HER2-low).

B. Accountability of PMA Cohort

A total of 2311 patients were screened for DESTINY-Breast06 study screening. Out of the screened population, 1952 were tested with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay. Out of these 1952 patients tested, only 866 patients were enrolled (in Treatment Arms A and B), as seen in Tables 20 and 21 below. Out of the remaining 1086 patients, 937 were not enrolled due to failed study protocol inclusion and/or exclusion criteria, and 149 were not enrolled as randomization had completed/closed, patient exceeded 28 day screening window, or patient died during screening.

Also, an accountability of the PMA cohort for total number of patients screened, number tested with the PATHWAY anti-HER2 (4B5) antibody assay, and the final number of patients enrolled into the DESTINY-Breast06 study is provided in table below.

Table 20. Accountability of the PMA Cohort for Study DESTINY-Breast06

Patients Disposition for study **DESTINY-Breast06**	N
Total number of patients screened	2311
No sample accessioned	312
Samples did not meet the study eligibility criteria	47
Number tested with the PATHWAY anti-HER2 (4B5) antibody assay	1952
Protocol Deviation for IHC testing	12
Not evaluable	60
IHC 0 (no membrane staining)	225
IHC 0 (with membrane staining)	400
IHC 1+ (HER2-Low)	828

IHC 3+ (HER2-positive)	4
IHC 2+ (reflex to ISH) – ISH tested samples	423
IHC 2+ / ISH Not evaluable	29
IHC 2+ /ISH negative (HER2 Non-amplified)	383
IHC 2+ /ISH positive (HER2 Amplified)	11
Final number of enrolled patients	866
IHC 0 (with membrane staining)	152
IHC 1+ (HER2-Low)	473
IHC 2+/ISH negative (HER2 Non-amplified)	234
Protocol Deviations	1
Randomization Error	6

C. Study Population Demographics and Baseline Characteristics

The demographics for patients in the IU population are presented by HER2 IHC score in table 22 below. The majority of patients were female (99.4%), the mean and the median age were approximately 57.7 and 57.0 years, respectively, with 46.4% <65 years old. Of the patients in the overall IU population with reported characteristics, the majority of patients were Not Hispanic or Latino (86.3%). The majority of patients were White (52.0%) or Asian (33.3%) and from Europe (47.9%) or Asia (33.2%). The patient demographic and clinical characteristics by treatment arm in the HER2-low and ITT populations were very similar including the distribution of IHC scores (which was a stratification factor) across the treatment arms as shown in table below.

Table 21. Study Population Demographics and Baseline Parameters - Patient Characteristic

	Treatment Arm			
Characteristic	Arm A (N=435)	Arm B (N=430)	Not Enrolled (N=1075)	Overall (N=1940)
Age (Years)				
n	435	430	415	1280
Mean (SD)	58.1 (11.46)	58.1 (10.91)	56.7 (11.25)	57.7 (11.22)
Median	58.0	57.0	57.0	57.0
Min, Max	28, 87	32, 83	31, 95	28, 95
Missing	0	0	660	660
Age Group				
<65	302 (69.4%)	298 (69.3%)	301 (28.0%)	901 (46.4%)
>=65	133 (30.6%)	132 (30.7%)	114 (10.6%)	379 (19.5%)
Missing	0	0	660 (61.4%)	660 (34.0%)
Sex				
Female	435 (100.0%)	429 (99.8%)	1064 (99.0%)	1928 (99.4%)

Characteristic	Treatment Arm			
	Arm A (N=435)	Arm B (N=430)	Not Enrolled (N=1075)	Overall (N=1940)
Male	0	1 (0.2%)	11 (1.0%)	12 (0.6%)
Ethnicity				
Hispanic or Latino	28 (6.4%)	32 (7.4%)	120 (11.2%)	180 (9.3%)
Not Hispanic or Latino	396 (91.0%)	387 (90.0%)	892 (83.0%)	1675 (86.3%)
Missing	11 (2.5%)	11 (2.6%)	63 (5.9%)	85 (4.4%)
Race				
American Indian or Alaskan Native	1 (0.2%)	0	2 (0.2%)	3 (0.2%)
Asian	154 (35.4%)	151 (35.1%)	341 (31.7%)	646 (33.3%)
Black or African American	4 (0.9%)	3 (0.7%)	22 (2.0%)	29 (1.5%)
White	230 (52.9%)	230 (53.5%)	548 (51.0%)	1008 (52.0%)
Other	7 (1.6%)	12 (2.8%)	12 (1.1%)	31 (1.6%)
Not Reported	39 (9.0%)	34 (7.9%)	129 (12.0%)	202 (10.4%)
Missing	0	0	21 (2.0%)	21 (1.1%)
Region				
Asia	149 (34.3%)	147 (34.2%)	349 (32.5%)	645 (33.2%)
Europe	227 (52.2%)	213 (49.5%)	490 (45.6%)	930 (47.9%)
North America	47 (10.8%)	47 (10.9%)	151 (14.0%)	245 (12.6%)
Rest of the World	12 (2.8%)	23 (5.3%)	85 (7.9%)	120 (6.2%)
Stratification Prior CDK4/6 Inhibitor Use [b]				
Yes	393 (90.3%)	389 (90.5%)	0	782 (40.3%)
No	42 (9.7%)	41 (9.5%)	0	83 (4.3%)
Missing	0	0	1075 (100.0%)	1075 (55.4%)
Stratification Prior Taxane Use in the Non-metastatic Setting [b]				
Yes	174 (40.0%)	172 (40.0%)	0	346 (17.8%)
No	261 (60.0%)	258 (60.0%)	0	519 (26.8%)
Missing	0	0	1075 (100.0%)	1075 (55.4%)
Stratification HER2 IHC Expression [b]				
IHC >0 <1+	77 (17.7%)	76 (17.7%)	0	153 (7.9%)
IHC 1+	239 (54.9%)	235 (54.7%)	0	474 (24.4%)
IHC 2+/ISH-	119 (27.4%)	119 (27.7%)	0	238 (12.3%)
Missing	0	0	1075 (100.0%)	1075 (55.4%)

Characteristic	Treatment Arm			
	Arm A (N=435)	Arm B (N=430)	Not Enrolled (N=1075)	Overall (N=1940)
Sample Collection Method				
Biopsy	374 (86.0%)	389 (90.5%)	938 (87.3%)	1701 (87.7%)
Excision/resection	61 (14.0%)	41 (9.5%)	137 (12.7%)	239 (12.3%)

Note: IHC = Immunohistochemistry; Arm A = T-DXd; Arm B = Chemotherapy

[a] All IHC ITD patients for whom the final patient-level VENTANA HER2 (4B5) assay staining attempt was performed according to the requirements of the Dx protocol.

[b] Patient stratification was based on prior CDK4/6 inhibitor use (yes vs no), HER2 IHC expression (IHC >0 <1+, IHC1+ or IHC 2+/ISH-), and prior taxane use in the non-metastatic setting (yes vs no). ISH testing was conducted with an on-market ISH assay.

D. Safety and Effectiveness Results

1. Safety Results

No Adverse Device Effects (ADEs) and no adverse events associated with use of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody were observed, supporting the safety of the device in its intended use environment.

For the complete safety evaluation of ENHERTU® and specific adverse events that occurred in the DESTINY-Breast06 study, please see the ENHERTU® PI available at Drugs@FDA.

2. Effectiveness Results

The clinical performance of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody was evaluated in Treatment Arm A in the HER2-low and HER2-ultralow (part of the ITT population) of the DESTINY-Breast06 study.

The primary efficacy outcome was PFS by BICR in the HER2 low IU Population. Key secondary endpoints in DESTINY-Breast06 study included the PFS by BICR according to RECIST 1.1 in the ITT population (HER2-ultralow and HER2-low), ENHERTU® demonstrated statistically significant and clinically meaningful improvement in PFS as assessed by BICR compared with chemotherapy in patients with HER2-low (IHC1+ and IHC2+/ISH-) and HER2-ultralow ((IHC 0 with membrane staining) hormone receptor positive breast cancer.

Efficacy results are summarized in Table 22.

Table 22 Efficacy Results in DESTINY-Breast06

Efficacy Parameter	HER2-low		Overall Population (HER2-low and HER2-ultralow)	
	ENHERTU (N=359)	Chemotherapy (N=354)	ENHERTU (N=436)	Chemotherapy (N=430)
Progression Free Survival (PFS) per BICR				

Number of events (%)	225 (62.7)	232 (65.5)	269 (61.7)	271 (63.0)
Median, months (95% CI)	13.2 (11.4, 15.2)	8.1 (7.0, 9.0)	13.2 (12.0, 15.2)	8.1 (7.0, 9.0)
Hazard ratio (95% CI)	0.62 (0.52, 0.75)[a]		0.64 (0.54, 0.76)[b]	
p-value	<0.0001[a]		<0.0001[b]	
Confirmed Objective Response Rate (ORR) per BICR[c]				
N	326	324	393	389
n (%)	202 (62.0)	114 (35.2)	246 (62.6)	134 (34.4)
95% CI	56.5, 67.3	30.0, 40.7	57.6, 67.4	29.7, 39.4
Complete Response n (%)	9 (2.8)	0	10 (2.5)	0
Partial Response n (%)	193 (59.2)	114 (35.2)	236 (60.1)	134 (34.4)
Duration of Response (DOR) per BICR[c]				
Median, months (95% CI)	14.1 (11.9, 15.9)	8.6 (6.7, 11.3)	14.3 (12.5, 15.9)	8.6 (6.9, 11.5)

CI = confidence interval

[a] Based on stratified analysis with stratification factors prior CDK4/6 inhibitor use (yes vs no) and HER2 IHC status of tumor samples (IHC 1+ vs IHC 2+/ISH-).

[b] Based on unstratified analysis.

[c] Analysis was performed based on the patients with measurable disease assessed by BICR at baseline.

3. Relevant Subgroup Analyses

In the ITT population which included 152 patients (17.6%) whose tumors were HER2-ultralow (IHC 0 with membrane staining) per central laboratory categorization, the results demonstrated that the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay successfully selected HER2-ultralow (IHC 0 with membrane staining), hormone receptor-positive patients with unresectable or metastatic BC who were likely to benefit from ENHERTU® treatment in the DESTINY-Breast06 study. These results support extending the patient population eligible for ENHERTU® therapy to the HER2-ultralow(IHC 0 with membrane staining) subgroup.

4. Pediatric Extrapolation

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

Patients that were 18 years or older were eligible for enrollment into DESTINY-Breast06 study if they fulfilled other inclusion criteria. The youngest age enrolled in the study was 28 years. For the DESTINT-Breast06 study, data from adult patients 28 years or older is generalizable to the pediatric population (18-21 years) and can be relied on to establish safety and effectiveness within the 18-21 years age group.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included two (2) investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

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

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay as a CDx device for identifying breast cancer patients with HER2-ultralow expression who may be eligible for treatment with ENHERTU® is based on the clinical performance assessed in the DESTINY-Breast06 study. Overall, data show clinically meaningful efficacy for this patient population.

The performance of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary 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. The risks of the device are based on data collected in the clinical study, and no adverse events associated with the diagnostic testing procedure were reported during the DESTINY-Breast06 study. In the

context of an in vitro diagnostic test, there are no directly harmful events from testing FFPE breast cancer tissue sections. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely collected for diagnosis.

As PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody is intended to identify breast cancer patients for treatment with ENHERTU®, failure of the device to perform as expected may lead to incorrect or false results and breast cancer patients may not receive the proper treatment. Patients with false positive results may undergo treatment with ENHERTU® without much clinical benefit and may experience adverse reactions associated with ENHERTU® therapy. Patients with false negative results may not be considered for treatment with ENHERTU®, 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 DESTINY-Breast06 clinical study 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 overall benefit-risk assessment is positive and supports approval of ENHERTU® as monotherapy for the treatment of adult patients with unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) or HER2-ultralow (IHC 0 with membrane staining) breast cancer, as determined by the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody and, as needed reflex to ISH with an FDA-approved test, who have received at least one endocrine therapy in the metastatic setting.

As shown in Table 22, a clinically meaningful treatment effect of ENHERTU® was observed in the overall population investigated which included HER2-ultralow (IHC 0 with membrane staining) and HER2-low subgroups. The results in this population supported by subgroups analyses showed evidence of clinical benefit with a positive benefit-risk profile irrespective of HER2 IHC expression. ENHERTU® demonstrated a statistically significant and clinically meaningful improvement in PFS as assessed by BICR compared with chemotherapy in patients with HER2-low (IHC1+ and IHC2+/ISH) hormone receptor-positive BC as the primary endpoint. The key secondary endpoint for efficacy in the overall population [HER2-ultralow (IHC 0 with membrane staining) and HER2-low] also demonstrated a statistically significant and clinically meaningful improvement in PFS by BICR for patients treated with ENHERTU® compared with chemotherapy.

The main risk of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody is obtaining a false result. A false positive could lead to ENHERTU® treatment for a patient with a HER2-null (IHC 0 absent membrane staining) tumor, resulting in a reduced probability of benefit (since ENHERTU® has not been investigated in that subpopulation). 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-targetted 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 data from the DESTINY-Breast06 study support the use of the PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody as a companion diagnostic to identify patients with unresectable or metastatic breast cancer with HER2-ultralow (IHC 0 with membrane staining) expression who may be eligible for treatment with ENHERTU® in accordance with approved therapeutic labeling as the probable benefits outweigh the probable risks.

XV. CDRH DECISION

CDRH issued an approval order on January 27, 2025.

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

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.