SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic IHC test for detection of Ki-67 antigen in formalin-fixed, paraffin-embedded (FFPE) human tissue sections
Device Trade Name:	Ki-67 IHC MIB-1 pharmDx (Dako Omnis)
Device Procode:	QQT
Applicant's Name and Address:	Agilent Technologies, Inc. 5301 Stevens Creek Blvd Santa Clara, CA 95051
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P210026
Date of FDA Notice of Approval:	10/12/21

II. INDICATIONS FOR USE

For In Vitro Diagnostic Use.

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) is a qualitative immunohistochemical (IHC) assay using monoclonal mouse anti-Ki-67, Clone MIB-1, intended for use in the detection of Ki-67 protein in formalin-fixed, paraffin-embedded (FFPE) breast carcinoma tissue using the EnVision FLEX visualization system on Dako Omnis.

Ki-67 protein expression in breast carcinoma is determined by using the Ki-67 pharmDx Score, which is the overall percentage of viable tumor cells in the invasive cancer component showing Ki-67 nuclear staining. The specimen should be considered to have Ki-67 expression if Ki-67 pharmDx Score is $\geq 20\%$.

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) is indicated as an aid in identifying patients with early breast cancer at high risk of disease recurrence for whom adjuvant treatment with Verzenio® (abemaciclib) in combination with endocrine therapy is being considered.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Ki-67 IHC MIB-1 pharmDx (Dako Omnis) labeling.

V. DEVICE DESCRIPTION

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) (GE020) is designed to be run on the Dako Omnis automated staining system (Dako Omnis) with Dako Omnis Software (OIS 2.1.1)and the Dako Link Omnis Workstation and Server software (WSS 2.2.1). Ki-67 IHC MIB-1 pharmDx (Dako Omnis) for use on the Dako Omnis platform will be marketed as a modular IHC assay system. Ki-67 IHC MIB-1 pharmDx (Dako Omnis) consists of the monoclonal mouse anti-Ki-67 antibody (Clone MIB-1) and Negative Control Reagent (NCR) and ancillary reagents that are individually labelled but are recognized together when the assay is run on Dako Omnis. The reagents running on Dako Omnis are individually packed with bar code outside the reagent container recognized by the instrument and supplied by Agilent. The Dako Omnis is designed to process slides on a continuous basis and can run different staining protocols for individual slides at the same time to optimize capacity utilization and patient case management. All reagents (primary and accessory) are required for proper use of the assay. An overview of the supplied kit components is shown in the Table 1 below.

Table 1: Ki-67 IHC MIB-1 pharmDx (Dako Omnis): Overview of Primary Components

Reagent	Description	Qty x Vol
Monoclonal Mouse Anti-Human Ki-67, Clone MIB-1	Monoclonal mouse anti-Human Ki-67 (Clone MIB-1) antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.	1 x 12mL
Negative Control Reagent (NCR)	Monoclonal mouse control IgG1 antibody (isotype matched to MIB-1) in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide	1 x 12mL

Ki-67 IHC MIB-1 pharmDx (Dako Omnis): Ancillary Reagents (sold separately)

- EnVision FLEX, Target Retrieval Solution Low pH (50x) (Dako Omnis
- EnVision FLEX, High pH (Dako Omnis)
    - EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis)
    - EnVision FLEX / HRP (Dako Omnis)
    - EnVision FLEX Substrate Buffer (Dako Omnis)
    - EnVision FLEX DAB+ Chromogen (Dako Omnis)
- Wash Buffer (20x) (Dako Omnis)

- Sulfuric Acid
- Counterstain: Dako Hematoxylin
- Clearify Clearing agent

Deparaffinization, rehydration, target retrieval, staining and counterstaining procedures are automatically performed by the Dako Omnis instrument. Coverslipping can be manual or automated. All required reagents to run the Ki-67 IHC MIB-1 pharmDx (Dako Omnis) assay were part of the device validation activities.

Specimen Preparation

Specimens must be handled to preserve the tissue for IHC staining. Standard histological methods of tissue processing should be used for all specimens. FFPE tissue specimens should be cut into sections of 4-5 µm. After sectioning, tissues should be mounted on Dako FLEX IHC Microscope Slides or SuperFrost Plus microscope slides, and then placed in a 58 ± 2°C calibrated oven for 1 hour. To preserve antigenicity, tissue sections mounted on slides should be stained within 2 months of sectioning when held in the dark at 2-8°C (preferred), or at room temperature up to 25°C. Slide storage and handling conditions should not exceed 25°C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls and Calibrators

Run controls are included in each staining run to establish the validity of the test results and is provided by the end-user laboratory. The recommended positive tissue control is tonsil tissue. Positive and negative run controls should be fresh biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). The positive control tissue should include weak staining for Ki-67 to detect subtle changes in assay sensitivity. Negative control tissue is required to detect unintended antibody cross reactivity to tissue and is expected to be negative for Ki-67 expression.

Additional information about the use of controls is available in the product labeling.

Principles of Procedure

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) contains optimized reagents and the protocol required to complete an IHC staining procedure of FFPE specimens using the Dako Omnis. Following incubation with the primary monoclonal antibody to Ki-67 (MIB-1) or the NCR, specimens are incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added diaminobenzidine (DAB) chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen may then be counterstained and coverslipped. Results are interpreted by a pathologist using a bright field microscope. Consult the Dako Omnis User Guide(s) for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

Staining protocol

Ki-67 IHC pharmDx (GE020) has been developed for Dako Omnis. Deparaffinization, target retrieval, staining and counterstaining is performed on Dako Omnis. When processing slides

for staining with Ki-67 IHC MIB-1 pharmDx (Dako Omnis), the Dako Omnis automated platform executes the following protocol:

Dewax
a. Clearify Clearing Reagent: 25 °C, 10 seconds incubation top, 1 min incubation bottom, 1 cycle b. DI water: 5 seconds incubation, 1 cycle
Target retrieval
a. EnV FLEX TRS, Low pH: 97 °C, 30 min incubation b. Cooling fluid DI water
Staining
a. Wash buffer: 2:40 min, 2 cycles b. Primary antibody: 20 min incubation (Ki-67 Primary Antibody or NCR) c. Wash buffer: 2:00 min, 10 cycles d. Endogenous enzyme block: 3 min incubation e. Wash buffer: 2:00, 10 cycles f. Labelled polymer: 20 min incubation g. Wash buffer: 2:00 min, 10 cycles h. Wash buffer: 2:00 min, 10 cycles i. Wash DI water: 31 seconds, 1 cycle j. Wash buffer: 2:00 min, 10 cycles k. Substrate chromogen: 5 min (DAB) l. Wash buffer: 2:00 min, 10 cycles m. Wash DI water: 31 seconds, 1 cycle n. Wash buffer: 2:00 min, 10 cycles
Counterstain
a. Hematoxylin: 3:00 min b. Wash DI water: 2:00 min, 10 cycles c. Wash buffer: 2:00 min, 10 cycles

Evaluation of Stained Slides, Interpretation of Ki-67 Staining

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) and hematoxylin & eosin (H&E) staining should be performed on serial sections from the same paraffin block of the specimen. After the histological diagnosis of invasive breast cancer is done based on the review of the H&E stained slide(s), the Ki-67 stained slide(s) are reviewed. Assessment of Ki-67 expression in breast carcinoma samples includes:

- Ensure that the specimen has been properly fixed and prepared for IHC analysis. Only well-preserved and well-stained areas of the specimen should be used to make the determination of the percentage of positive tumor cells.
- Only the invasive cancer component should be scored. Carcinoma in situ should not be scored.
- All viable tumor cells in the invasive cancer component on the entire slide must be evaluated and included in the Ki-67 scoring assessment.

- The specimen contains a minimum of 200 viable invasive tumor cells to determine the percentage of positive cells.

The Ki-67 pharmDx Score, which determines the Ki-67 expression of the breast cancer specimen, is the percentage of viable tumor cells in the invasive cancer component showing any nuclear staining. The specimen should be considered Ki-67 positive if Ki-67 pharmDx Score ≥ 20%, i.e. ≥ 20% of tumor cells from the invasive cancer component show positive Ki-67 staining.

The Ki-67 IHC MIB-1 pharmDx (Dako Omnis) Interpretation Manual for breast carcinoma is available to users to assist in the interpretation of assay results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is no other FDA-cleared or approved alternative immunohistochemistry assay available for detection of Ki-67 in formalin-fixed, paraffin-embedded (FFPE) breast carcinoma tissue in identifying patients with early breast cancer at high risk of recurrence for whom abemaciclib treatment is being considered.

VII. MARKETING HISTORY

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect Ki-67 test results and subsequently improper patient management decisions.

IX. SUMMARY OF NONCLINICAL STUDIES

The nonclinical studies conducted to support the safety and effectiveness of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) in breast cancer patients included sensitivity, specificity, robustness, precision, stability, and external reproducibility. Where applicable, the cutoff ≥ 20% (Ki-67 pharmDx Score ≥ 20%) was evaluated.

A. Laboratory Studies

1. Analytical Sensitivity

Analytical sensitivity of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was tested on 148 unique specimens of FFPE breast carcinoma specimens [100 resection tissues and 48 core needle biopsies (CNB)] using a manufactured production lot. Assessment of Ki-67 expression demonstrated staining across a range of 0-75% positive tumor cells and 0-3+

staining intensity. Seventy-two percent of the specimens were negative based on the cutoff (< 20% tumor cells positive) and 28% were positive when the cutoff was ≥ 20% (≥ 20% tumor cells positive).

2. Analytical Specificity

a. Western Blot

The specificity of the Ki-67 antibody was evaluated by Western Blot analysis. Cell lysates from three cancer cell lines MCF-7, SKBR3 and IM-9 were used in this study in which the Ki-67 protein was detected with the MIB-1 primary antibody and a goat anti-mouse immunoglobulin secondary antibody. These cell lines are known to be positive for Ki-67 and with known relative RNA expression levels for Ki-67. Results showed detection of protein bands at the expected sizes of 345 kDa and 395 kDa in the Ki-67 expressing IM-9 cell line that produced two high molecular weight bands, the low expressing MCF-7 cell line had a faint band of the higher molecular weight and the non-expressing SKBR3 cell line produced no bands.

b. Peptide inhibition studies

The purpose of this study was to evaluate the specificity of Ki-67 antibody clone MIB-1 for the target antigen, Ki-67, using peptide inhibition. Two different tests, immunohistochemistry (IHC) and western immunoblot, were conducted. It was expected that binding of the Ki-67 antibody to a peptide containing the Ki-67 MIB-1 antibody epitope would reduce the signal detected by the IHC antibody and western immunoblot, either completely or in a concentration-dependent manner.

The Ki-67 antibody (Clone MIB-1) staining showed a decrease in immunohistochemistry signal correlated to an increased concentration of peptide inhibitor expressing the Ki-67 epitope. The decrease in signal was observed in high, medium and low expressing tissues and was reflected in the percentage positivity of tumor staining and in the average staining intensity. No signal decrease was detected for Ki-67 antibody with the inclusion of a 100-fold excess of an irrelevant peptide having no sequence homology to the Ki-67 epitope region.

On the western immunoblot, binding of Ki-67 antibody (Clone MIB-1) to the Ki-67 protein could be completely abrogated by the addition of a peptide containing the region reported to contain the epitope of this antibody. When Ki-67 antibody was preincubated with 5 times excess by weight of the peptide, partial blocking of the binding was achieved, and when the peptide was added at 15 times excess by weight, no binding was detected.

c. Immunoreactivity normal human tissues (Tour of Body)

Immunoreacitivity of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was evaluated by staining a panel of 31 FFPE normal human tissues. Specificity testing was conducted to demonstrate that Ki-67 IHC MIB-1 pharmDx (Dako Omnis) will detect the target substance in the appropriate tissue elements and cellular compartment. The staining and localization for Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was evaluated by a

pathologist on normal and neoplastic tissues. The staining patterns and localization for Ki-67 IHC from one lot of the antibody was tested on 31 normal tissues.

Results: All specimens that displayed positive staining showed nuclear stain localization and zero nonspecific staining with the exception of peripheral nerve, which showed a small amount of patchy cytoplasmic staining.

Nuclear staining was observed in a subset of tissues. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for Ki-67 IHC expression in normal tissues.

Table 2 summarizes Immunoreacitivity of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) (Dako Omnis) in normal tissues.

Table 2: Summary of Ki-67 Antibody Staining of 31 Normal Tissues

Tissue Type (# tested)	Positive Cell Staining: Tissue Elements	Non-specific Staining
Adrenal (3)	2/3 Scattered adrenal cortex cells	0/3
Bone marrow (3)	2/3 Marrow cells	0/3
Breast (3)	2/3 subset ductal epithelial cells 2/3 rare myoepithelial cells	0/3
Cerebellum (3)	0/3	0/3
Cerebrum (3)	1/3 Rare oligodendrocytes	0/3
Cervix (3)	2/3 Rare epithelial cells 1/3 parabasal squamous cells	0/3
Colon (3)	3/3 Crypt epithelial cells	0/3
Esophagus (3)	3/3 Parabasal squamous cells	0/3
Kidney (3)	3/3 Rare tubule cells	0/3
Liver (3)	3/3 Rare hepatocytes	0/3
Lung (3)	3/3 Rare type 1 alveolar cells 1/3 Pulmonary macrophages	0/3
Mesothelial cells (3)	1/3 Rare mesothelial cells	0/3
Muscle, cardiac (3)	1/3 Few cardiac myocytes	0/3
Muscle, skeletal (3)	0/3	0/3
Nerve, peripheral (3)	0/3	0/3
Ovary (3)	2/3 Follicle cyst lining cells 1/3 Rare ovarian stromal cells	0/3
Pancreas (3)	3/3 Rare acinar cells	0/3
Parathyroid (3)	3/3 Rare endocrine cells/Chief cells	0/3
Pituitary (3)	1/3 Lymphocytes 2/3 Pituicytes	0/3
Prostate (3)	3/3 Rare epithelial cells and stromal cells	0/2
Salivary gland (3)	1/3 Rare acinar epithelial cells	0/3
Skin (3)	3/3 Suprabasal squamous cells	0/3
Small intestine (3)	2/3 Crypt epithelium	0/3

Tissue Type (# tested)	Positive Cell Staining: Tissue Elements	Non-specific Staining
Spleen (3)	3/3 Few red pulp cells 1/3 White pulp cells	0/3
Stomach (3)	3/3 Gastric epithelial cells	0/3
Testis (3)	3/3 Spermatogonia	0/3
Thymus (3)	3/3 Thymic cortex	0/3
Thyroid (3)	0/3	2/3 Scattered interspersed inflammatory cells
Tonsil (3)	3/3 Squamous parabasal cells 3/3 Germinal centers 3/3 interfollicular cells	0/3
Urinary bladder (3)	3/3 Urothelial cells	2/3 Interspersed inflammatory cells
Uterus (3)	3/3 Endometrial epithelium and stroma	0/3

The numbers in each cell of the Table above indicate the number of tissues showing staining out of the total number of tissues of that type tested.

d. Immunoreactivity neoplastic tissues (Tour of Tumor)

The Tour of Tumor specificity study was conducted using a commercial tissue microarray (TMA). The TMA contained 80 unique tissues in total, including two normal tissues as control sections, seven benign tumors, and 71 malignant tumors. Three sections of the TMA were stained using one of three different reagent lots each, thus 3 replicates of each sample were used.

Among the 71 malignant tumors reported, the following cancer types included more than one unique sample in the TMA:

- Bladder transitional cell carcinoma (2)
- Breast carcinoma, lobular (2)
- Breast carcinoma, NOS* (4)
- Lung adenocarcinoma (2)
- Melanoma (3)
- Renal cell carcinoma, NOS* (2)
- Thymic carcinoid Tumor (2)

* not otherwise specified

Table 3 summarizes monoclonal mouse anti-Ki-67 immunoreactivity on a panel of 71 neoplastic tissues. Nuclear staining was observed in the majority of tumor types evaluated. All tissues were formalin-fixed and paraffin-embedded and stained with Ki-67 IHC MIB-1 pharmDx (Dako Omnis). There were no unexpected results observed in the tumor specimens tested.

Table 3: Summary of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) neoplastic tissue reactivity (N=71)

Tumor Type by System		Tumor Type by System	

	Number of Positive Cases with Any Staining (out of 3 replicates)		Number of Positive Cases with Any Staining (out of 3 replicates)
Gastrointestinal tract		**Lung and mediastinum**	
Colon mucinous adenocarcinoma	1/3	Lung bronch**iol**oalveolar carcinoma	3/3
Gastric adenocarcinoma	2/3	Lung squamous carcinoma	3/3
Gastric adenocarcinoma metastasis	2/3	Lung adenocarcinoma #1	1/3
Colon adenocarcinoma in liver	0/3	Lung adenocarcinoma #2	3/3
Gastrointestinal stromal tumor (GIST)	2/3	Lung carcinoma, NOS	3/3
Pancreatic adenocarcinoma	2/3	Fibrous tumor of pleura	0/3
Hepatoma (hepatocellular carcinoma)	3/3	Thymoma	3/3
Cholangiocarcinoma	3/3		
		Female reproductive tract	
Head and neck		Papillary serous carcinoma	3/3
Squamous carcinoma of the ear	3/3	Endometrial carcinoma	0/3
		Ovarian mucinous adenocarcinoma	0/3
Urinary tract		Well differentiated serous carcinoma	0/3
Kidney transitional cell carcinoma	3/3	Metastatic ovarian carcinoma, NOS	3/3
Bladder transitional cell carcinoma #1	3/3	Endometrial stromal sarcoma	3/3
Bladder transitional cell carcinoma #2	3/3	Ovary granulosa cell tumor	3/3
Papillary renal cell carcinoma	1/3	Squamous carcinoma, cervix	3/3
Renal cell carcinoma, NOS #1	0/3	Ovarian Dysgerminoma	3/3
Renal cell carcinoma, NOS #2	1/3		

Male reproductive tract		**Endocrine system (including neuroendocrine tumors)**	
Testicular yolk sac tumor	3/3	Islet cell tumor of Pancreas	2/3
Testicular embryonal carcinoma	3/3	Pancreatic glucagonoma	3/3
Prostate adenocarcinoma (metastatic)	3/3	Thymic carcinoid Tumor #1	3/3
Prostate adenocarcinoma	1/3	Thymic carcinoid Tumor #2	3/3
		Pheochromocytoma	3/3
Breast		Paraganglioma	0/3
Breast carcinoma, lobular #1	0/3	Thyroid medullary carcinoma	3/3
Breast carcinoma, lobular #2	1/3	Thyroid papillary carcinoma	1/3
Breast carcinoma, NOS #1	0/3	Thyroid follicular adenoma	3/3
Breast carcinoma, NOS #2	3/3		
Breast carcinoma, NOS #3	3/3	**Soft tissue and bone**	
Breast carcinoma, NOS #4	1/3	Pleomorphic rhabdomyosarcoma	3/3
Breast carcinoma, metastasis in lymph node	3/3	Spindle cell rhabdomyosarcoma	0/3
		Ewing sarcoma	0/3
Hematopoietic system		Primitive neuroendocrine tumor in scrotum	0/3
Splenic lymphoma	3/3	Leiomyosarcoma	3/3
Lymphoma of cecum	3/3	Round cell liposarcoma	3/3
		Synovial sarcoma	0/3
Skin		Malignant fibrous Histocytoma	3/3
Melanoma #1	3/3	Extraskeletal. myxoid chondrosarcoma	0/3
Melanoma #2	3/3		
Melanoma #3	3/3		
Merkel cell tumor	3/3		
Basal cell carcinoma, skin	0/3		
Nervous system			
Glial tumor, NOS	3/3		
Meningioma	3/3		
Ganglioneuroma	0/3		
Schwanomma	3/3		

NOS = not otherwise specified

The numbers in each cell of the Table above indicate the number of tissues showing staining out of the total number of tissues of that type tested.

3. Precision

The precision of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was evaluated at Agilent. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method. Pre-specified acceptance criteria required that the lower bound of the two-sided 95% confidence interval (CI) computed on % agreement must meet or exceed 85% for all precision studies. Table 4 below shows the precision study results as evaluated at one internal site.

Table 4: Precision of Ki-67 IHC MIB-1 pharmDx (Dako Omnis)

Precision Study	Study Design	% Agreement (95% CI)
Between-day	Each 32 breast carcinoma specimens (16 Ki-67-negative and 16 Ki-67-positive, including specimens around the cutoff*) representing a range of Ki-67 expression were tested on a single Dako Omnis instrument over 5 non-consecutive days using a unique set of reagents from a single lot per test day**.	NPA 98.3% (96.2%; 100.0%) PPA 99.2% (97.5%; 100.0%) OA 98.8% (97.5%; 99.8%)
Between-instrument	Each of 32 breast carcinoma specimens (16 Ki-67-negative and 16 Ki-67-positive, including specimens around the cutoff*) representing a range of Ki-67 expression were tested on each of three Dako Omnis instruments using one lot of assay reagents on one day.	NPA 98.8% (96.7%; 100.0%) PPA 97.5% (94.6%, 100.0%) OA 98.1% (96.5%; 99.6%)
Between-lot	Each of 40 breast carcinoma specimens (20 negative and 20 positive, including specimens around the cutoff*) representing a range of Ki-67 expression were tested using three unique lots of all Ki-67 pharmDx reagents (including buffer, Target Retrieval Solution, GV800 visualization system, and sulfuric acid), on a single instrument.	NPA 98.9% (97.2%; 100.0%) PPA 97.2% (92.8%; 100.0%) OA 98.1% (95.8%; 99.7%)
Between-lot (Ancillary reagents)	Each of 32 breast carcinoma specimens (17 negative and 15 positive, including specimens around the cutoff*) representing a range of Ki-67 expression	NPA 95.1% (88.2-100.0%) PPA 100.0% (95.9-100.0%) OA 97.4% (93.8-100.0%)

Precision Study	Study Design	% Agreement (95% CI)
	were tested using three unique lots of accessory reagents.	
Repeatability Within-instrument/ Within-rack/ Within-day	Each of 32 breast carcinoma specimens (16 Ki-67 negative and 16 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression were tested within one rack on three different Dako Omnis instrument using reagents from one kit lot. Pairwise comparisons were only performed on slides that were stained within the same rack.	NPA 98.8% (96.7%; 100.0%) PPA 97.5% (94.6%; 100.0%) OA 98.1% (96.2%; 99.6%)
Between-observer	One set of 60 stained specimens (32 Ki-67 negative and 28 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was evaluated three times by three different trained and certified pathologists. Between-observer analysis was performed between observers on a total of 537 comparisons.	NPA 98.9% (97.2%; 100.0%) PPA 95.2% (91.7%; 98.0%) OA 97.2% (95.4%; 98.7%)
Within-observer	One set of 60 stained specimens (32 Ki-67 negative and 28 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was evaluated three times by the same trained and certified Pathologist. Within-observer analysis was performed on a total of 537 comparisons.	NPA 99.3% (98.3%; 100.0%) PPA 96.8% (94.4%; 98.8%) OA 98.1% (96.8%; 99.3%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement

*Around the cut-off is defined as specimens with a Ki-67 score of 10-30% which includes approximately 20% of the study samples.

** One replicate per specimen was stained with NCR.

4. External Reproducibility

The reproducibility of the Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was evaluated at three external sites (testing laboratories). NPA, PPA, and OA were computed with two-sided 95% confidence intervals using the bootstrap method for the ≥ 20% cutoff.

The study was divided into Part A (between- and within-laboratory endpoints) and Part B (between and intra-observer endpoints).

- **Part A** assessed between- and within-laboratory reproducibility. It was conducted at 3 CAP/CLIA certified clinical laboratories in the United States. At each laboratory, 30

breast carcinoma specimens were stained five times on non-consecutive days, over a minimum of 20 days.

- **Part B** assessed between- and within-observer reproducibility. Sixty (60) breast carcinoma specimens were stained at Dako and read three times by each of three external pathologists, with a washout period of 14 to 30 days between reads.

All slides were relabeled between rounds/reads to blind the study pathologists from specimen identity from previous evaluations. The slide sets were read by study pathologists trained and certified on the scoring algorithm.

Table 5 below shows between-site, within-site/ between-day, between -observer, and within-observer reproducibility results for Ki-67 IHC MIB-1 pharmDx (Dako Omnis) met the acceptance criteria of NPA/PPA/OA ≥ 85% at the lower bound of a 95% confidence interval (LBCI) for the ≥ 20% cut-off.

Table 5: Reproducibility of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) tested at three external sites

Reproducibility Study	Study Design	% Agreement (95% CI)
Between-site	Each of 30 breast carcinoma specimens (15 Ki-67 negative and 15 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was tested on 5 non-consecutive days. Inter-site analysis was performed between three sites on a total of 450 comparisons.	NPA 94.7% (88.4%; 100.0%) PPA 100% (98.3%; 100.0%) OA 97.3% (94.2%; 100.0%)
Within-site	Each of 30 breast carcinoma specimens (15 Ki-67 negative and 15 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was tested on 5 non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 450 comparisons.	NPA 100% (98.2%; 100.0%) PPA 98.8% (96.9%; 100.0%) OA 99.3% (98.2%; 100.0%)
Between-observer	One set of 60 stained specimens (29 Ki-67 negative and 31 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was rotated across three sites and evaluated three times by the same pathologist at each site. Inter-observer analysis was performed between three sites on a total of 540 comparisons.	NPA 98.9% (97.7%; 100.0%) PPA 97.8% (95.3%; 99.6%) OA 98.3% (96.9%; 99.4%)

Reproducibility Study	Study Design	% Agreement (95% CI)
Within-observer	One set of 60 stained specimens (29 Ki-67 negative and 31 Ki-67 positive, including specimens around the cutoff*) representing a range of Ki-67 expression was rotated across three sites and evaluated three times by the same pathologist at each site. Intra-observer analysis was performed for three sites on a total of 540 comparisons.	NPA 98.5% (97.0%; 99.6%) PPA 98.6% (97.1%; 99.6%) OA 98.5% (97.4%; 99.4%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement

*Around the cut-off is defined as specimens with a Ki-67 score of 10-30% which includes approximately 20% of the study samples.

5. Robustness

Robustness of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) was tested using breast carcinoma specimens in each study. Agreement estimates were calculated for Ki-67 pharmDx Score ≥ 20% cutoff. The following parameters were evaluated in robustness testing:

a. Tissue thickness: 3µm, 4 µm-standard, 5 µm and 6 µm; 32 specimens (8 CNB, 24 resections) were included in the study.

b. Slide type tolerance: Superfrost™ Plus and Dako FLEX IHC Microscope Slides-standard. Sixty specimens (16 CNB, 44 resections) were included in the study. Six sections were stained for each condition: three slides stained with Ki-67 primary antibody and three slides with NCR. Specimen sections were mounted on Dako FLEX IHC slides (Dako K8020) and Superfrost Plus Microscope Slides.

c. Target Retrieval Solution pH values: pH 5.9, pH 6.1-standard, pH 6.3 and pH 6.5; 32 specimens (8 CNB, 24 resections) were included in the study.

d. Overnight and Over-weekend delayed staining: Within working hours of day 1-standard, Overnight and Over-weekend: Thirty specimens (all resection) were included in the study. Four sections were stained for each condition: three slides stained with Ki-67 primary antibody and one slide with NCR. Three conditions were tested, standard, overnight and over-weekend.

Overall, the robustness studies demonstrate that the use of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) on breast carcinoma specimens produces consistent results under the conditions tested when scored at the ≥ 20 % cutoff.

- TRS pH from 5.9 to 6.5
- Tissue section thickness from 3µm to 6µm
- Overnight/Over-weekend delayed staining
- Slide Type of both Superfrost Plus and Dako FLEX IHC

6. Stability Studies

a. Real time Stability

The purpose of real-time stability study was to confirm the shelf life of Ki-67 IHC pharmDx (Dako Omnis). Three-unique kit lots of the device were tested on 2 replicates of 4 breast cancer specimens with the following Ki-67 expression levels: One (1) Ki-67 negative breast cancer specimen (<10%); One (1) Ki-67 positive breast cancer specimen (>30%) and Two (2) breast cancer specimens near the Ki-67 20% cut-off (10-30%. One slide from each of these specimens was also used for staining with the NCR. One benign tonsil specimen was also used. The test lots were stored at 2-8°C before and after transport (where applicable) and after each in use/ on-board cycle or test point. Testing was performed at specified intervals and compared to testing at time point zero which served as the reference. The total shelf-life at 2-8°C was calculated using the shortest passing result for all three test lots. The assigned product shelf-life at 2-8°C is the total shelf-life minus two (2) months margin of safety. Based on a real-time stability study with three lots of Ki-67 IHC MIB-1 pharmDx (Dako Omnis). the stability dating is as follows:

Total Shelf Life: 12 months at 2-8 °C

b. Transport Simulation: Transport simulation subjects 2-8°C product class stability test sample(s) to worst case scenario conditions for stock shipments and customer shipments under ambient conditions. A passing result confirms that product may be shipped without controlled temperatures or specialized packaging for up to two (2) stock shipments and one (1) customer shipment. Vials from one lot of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) underwent transport simulation at temperatures between -20 ºC to 37 ºC. Each reagent lot was tested on 4 intended use specimens for each time point:

- 1 Negative Breast Carcinoma (<10%)
- 2 Near-cutoff (NCO) Breast Carcinomas (10-30%)
- 1 Positive Breast Carcinoma (>30%)

Each of the 4 specimens mentioned above were tested with the primary antibody (in duplicate/2 slides/2 sections) and negative control reagent (NCR) (1 slide/1 section). Note that different specimens (blocks) were used for testing the different reagent lots, and at T0 additional backup blocks were qualified for each category. During transport simulation, reagent bottle caps were closed. Based on results to date, Ki-67 IHC MIB-1 pharmDx (Dako Omnis) have passed transport simulation.

c. In-Use/On-Board Stability Testing: On-board stability was performed on specific test sample vials (in the 25 °C group) of one lot of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) during the intervals of T0-T1 and T1-T2. Each reagent lot was tested on one tonsil specimen for each time point. The tonsil specimen was tested with the primary antibody (in triplicate/3 slides/3 sections) and NCR (1 slide/1 section). On-board simulation was performed by placing the reagents at 18˚C (on-board an Omnis instrument) for various hours and back to 25 °C. The caps were open at 18 ºC and closed

at 25 °C. Based on results to date, the on-board stability for Ki-67 IHC MIB-1 pharmDx (Dako Omnis) is as follows:

- 27 cycles from 2-8°C to 18°C for at least 5 hours and back to 2-8°C
- 375 hours at 18°C

d. Cut Section Stability

The stability of the Ki-67 antigen in breast carcinoma cut sections was evaluated after storage at ambient (25°C) and 2-8°C. Seven breast carcinoma tissue blocks fixed in 10% NBF (neutral buffered formalin) were selected to represent a range of Ki-67 expression. The blocks were cut through and mounted on slides at T0 (testing time point zero). Slide-mounted sections from each block were stained and scored at T0 and at each subsequent study timepoint. The total percent positive and average intensity scores at each timepoint were compared to the scores assigned at the same time point to a T0 section from the same specimen (if stained slide stability was confirmed for the time point) or to the scores given at T0 (otherwise). Data from these comparisons were analyzed to determine cut section stability. The cut section stability is as follows:

- Cut Section Stability for specimens stored at 25 °C is 4 months.
- Cut Section Stability for Specimens stored at 2-8 °C is 5 months.

It is recommended that tissue sections mounted on slides are stained within 2 months of sectioning when stored in the dark at 2-8 °C (preferred) or at room temperature up to 25 °C to preserve antigenicity.

B. <u>Animal Studies</u>

None

C. <u>Additional Studies</u>

- **Pre-Analytical Variables**

This study assessed the effect of pre-analytical variables on the Ki-67 antigen when stained with Ki-67 IHC MIB-1 pharmDx (Dako Omnis) assay. Samples prepared using different fixative types, fixation times and ischemia times were tested. Evaluation of pre-analytical variables was performed for informational purposes only and was not subjected to acceptance criteria. Three different tonsil specimens were used for these studies (3 tonsil specimens X 15 conditions), except for specimens that have an ischemic time of 30 minutes for which 7-10 different tonsils were prepared.

The study investigated the following:

- Fixation times between 6-72 hours
- Ischemia times between 30 min and 72 hours
- Fixatives
  - 10% Neutral-buffered Formalin (NBF)
  - AFA (Acetic Formalin Alcohol)
  - Bouin's
  - UBF (10% unbuffered Formalin)

- 10% NBF followed by 4-5 days in 70% EtOH

An ischemia time of 1 hour or less and fixation time for 6-72 hours in 10% neutral buffered formalin (NBF) is recommended.

- **Within-Block Heterogeneity**

This study assessed intra-block heterogeneity in breast carcinoma specimens stained with Ki-67 IHC MIB-1 pharmDx (Dako Omnis) between sections across a span of 200µm. For the purposes of this study a block is defined as a single tumor specimen from which sequential tissue sections can be taken. Thirty-six specimens were included in this study. Cut tissue sections (1st, 11th, 28th and 52th cut section were stained with Ki-67 IHC MIB-1 pharmDx (Dako Omnis). Section number 29 was stained with NCR. Slides were blinded and randomized before being read. Stained slides were read by a trained and certified observer. The overall agreement was found to be 96.5%.

- **Within-Case Heterogeneity**

This study assessed within-case (sister block) heterogeneity between 25 unique breast carcinoma specimens (consisting of 50 total tissue blocks) stained with Ki-67 IHC MIB-1 pharmDx (Dako Omnis). For the purposes of this study, sister blocks are defined as blocks that were embedded separately but contain tissue from the same case (i.e., they have the same sample ID and are from the same patient). The overall agreement was found to be 92% (23/25 cases).

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The clinical performance of Ki-67 pharmDx was based on a Phase 3 clinical study I3Y-MC-JPCF (monarchE) for patients with node positive, early stage, hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) breast cancer who completed definitive locoregional therapy (with or without neoadjuvant or adjuvant chemotherapy) and are at high risk for disease recurrence.

A. Study Design

monarchE is a multicenter, randomized, open-label, Phase 3 study to compare the efficacy of abemaciclib plus standard adjuvant endocrine therapy (ET) to ET alone in patients with node positive, invasive, resected, hormone receptor positive (HR+), human epidermal receptor 2 negative (HER2-) early breast cancer (EBC) who completed definitive locoregional therapy, with or without neoadjuvant or adjuvant chemotherapy, and whose cancer was at high risk of disease recurrence. Abemaciclib is a kinase inhibitor for oral administration. Ki-67 is a marker of cell proliferation.

Patients with available untreated breast tissue samples were retrospectively tested using a central immunohistochemistry clinical trial assay to establish if the Ki-67 score was ≥20% or <20%. Ki-67 IHC MIB-1 pharmDx (Dako Omnis) Score ≥ 20% was evaluated as the

diagnostic cutoff. The data described below are from patients with Ki-67 positive tumors (>20%).

The primary objective of the study was to investigate the efficacy of abemaciclib plus adjuvant endocrine therapy (ET) versus adjuvant ET alone in patients with HR+, HER2- early breast cancer. A secondary objective was to investigate the efficacy of abemaciclib plus adjuvant ET versus adjuvant ET alone in patients with HR+, HER2- early breast cancer who had a pretreatment Ki-67 pharmDx Score ≥ 20% assessed by the central testing laboratory.

Key Inclusion Criteria

1. Female (regardless of menopausal status) or male ≥18 years of age (or of an acceptable age according to local regulations whichever is older).
2. The patient has confirmed HR+, HER2-negative (HER2-) early stage resected invasive breast cancer without evidence of distant metastases.
3. The patient must have undergone definitive surgery of the primary breast tumor(s).
4. The patient must have tumor tissue from breast (preferred) or lymph node for exploratory biomarker analysis available prior to randomization.
5. Patients must be node positive.
6. Pathological tumor involvement in ≥4 ipsilateral axillary lymph nodes.
   OR
   Pathological tumor involvement in 1 to 3 ipsilateral axillary lymph node(s) and meet at least one of the following criteria:
   - Grade 3 as defined by a combined score of at least 8 points per the modified Bloom-Richardson grading system (Elston et al. 1991) also known as the Nottingham scale or equivalent.
   - Pathological primary invasive tumor size ≥5 cm (for patients who received neoadjuvant therapy primary tumor size ≥5 cm on breast imaging is allowed).

Key Exclusion Criteria

1. Patients with occult breast cancer, metastatic disease, or node-negative breast cancer were excluded.
2. Patients who had received treatment with endocrine treatment for breast cancer prevention, raloxifene, and/or a CDK4/6 inhibitor, and those with a history of venous thromboembolic events.

1. Follow-up Schedule

Short-term follow-up monthly visits during the 2-year study treatment (or until discontinued). After treatment ended, patients are followed up to 10 years. No repeat biopsies planned for Ki-67 IHC MIB-1 pharmDx (Dako Omnis) testing.

2. Clinical Endpoints

The primary end point was invasive disease–free survival (IDFS). IDFS was defined as the time from randomization to the first occurrence of ipsilateral invasive breast tumor recurrence, regional invasive breast cancer recurrence, distant recurrence, contralateral invasive breast cancer, second primary non-

breast invasive cancer, or death attributable to any cause.

Accountability of the PMA cohort:

	Number of Study Subjects
Enrolled	5120
Primary tumor samples submitted	4186
Specimens with insufficient tissue	269
Not evaluable	126
Not applicable	143
Tissue sample not submitted	934
Tumor samples submitted and with results (N=3917)	
Total evaluable Ki-67 expression	3917
Ki-67 ≥20%	2003
Ki-67 <20%	1914
Site of tumor: (N=3917 evaluable samples)	
Primary site (breast)	3917
Metastatic site	0
Specimen type (N=3917 evaluable samples)	
Newly obtained	0
Archival	3917
Sample procedure (N=3917 evaluable samples)	
Biopsy	1530
Resection	2351
Unknown	36

Comparison of the demographics/clinical and sample characteristics between missing and evaluable clinical samples demonstrated that there is no impact on the CDx clinical effectiveness outcome. It was determined that the distribution of demographic/clinical covariates were similar between evaluable and missing CDx samples and there were no concerns.

B. Study Population Demographics of Cohort 1:

Among the 2003 patients with Ki-67 ≥20 in cohort 1, patient median age was 51 years (range: 24-88 years), 99% were women, 68% were White, and 25% were Asian. Forty-six percent of patients were premenopausal. Most patients received prior chemotherapy (37% neoadjuvant, 60% adjuvant) and prior radiotherapy (95%). Fifty-seven percent of the patients had 4 or more positive lymph nodes with 20% having ≥10 positive lymph nodes, 58% had Grade 3 tumor, and 19% had pathological tumor size ≥50 mm. Most patients were progesterone receptor positive (84%).

Key demographics for the Cohort 1, Ki-67 ≥20 are summarized in Table 7. Patients enrolled had a median age of 51 years old, and 12.6% were under the age of 40 years old. Overall, baseline

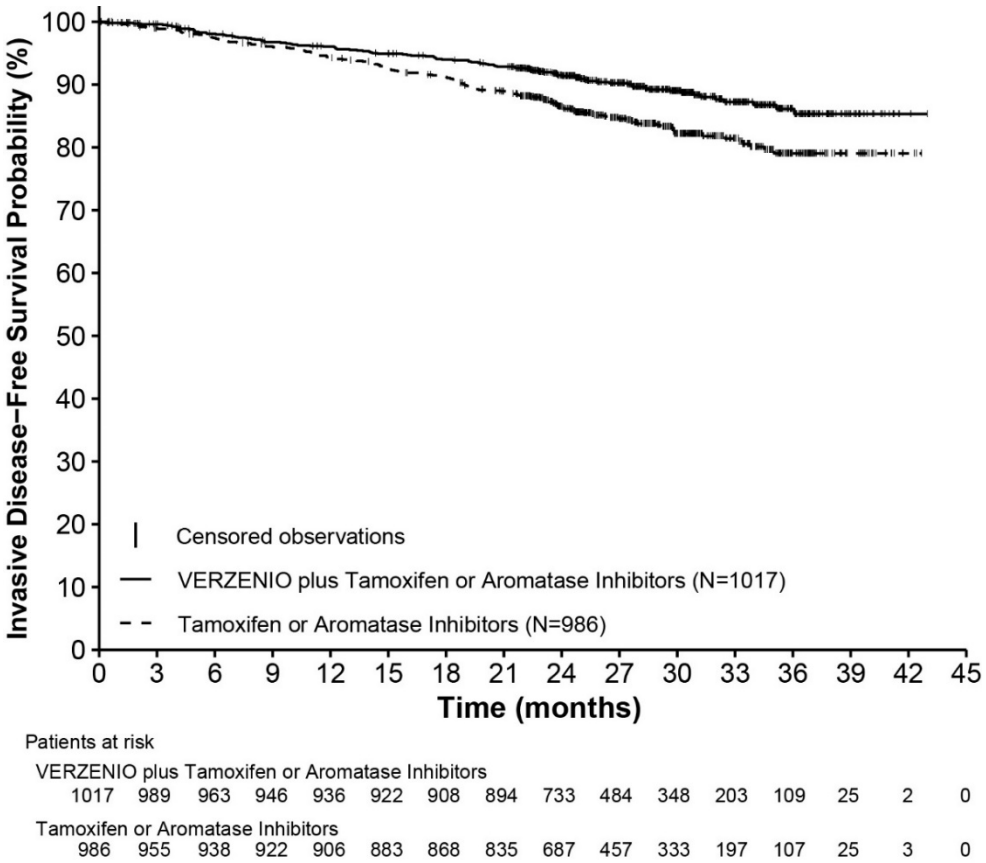
demographic characteristics were well-balanced between treatment arms. Efficacy results are summarized in Table 7 and Figure 1.

Table 7: Efficacy Results in monarchE in Patients with Ki-67 Score ≥20%

	VERZENIO Plus Tamoxifen or an Aromatase Inhibitor N=1017	Tamoxifen or an Aromatase Inhibitor N=986
Invasive Disease–Free Survival (IDFS)		
Number of patients with an event (n, %)	104 (10.2)	158 (16.0)
Hazard ratio (95% CI)	0.626 (0.488, 0.803)	
p-value	0.0042[a]	
IDFS at 36 months (%, 95% CI)	86.1 (82.8, 88.8)	79.0 (75.3, 82.3)

Abbreviation: CI = confidence interval.
[a]This p-value is from the pre-specified final IDFS analysis for cohort 1 patients with Ki-67 score ≥20%.

Figure 1: Kaplan-Meier Curves of Invasive Disease–Free Survival VERZENIO plus Tamoxifen or an Aromatase Inhibitor versus Tamoxifen or an Aromatase Inhibitor in Cohort 1 Patients with Ki-67 Score ≥20% (monarchE)



XI. Safety and Effectiveness Results

1. Safety Results

The observed safety profile of abemaciclib in monarchE was generally consistent with that previously reported for abemaciclib and ET in the advanced or metastatic breast cancer setting.

Safety of the device for patient management is related to safety and efficacy of the therapeutic. In general, risks of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) are associated with the possibility of inaccurate or false results. There was no device related serious adverse events (SAEs) or unanticipated adverse device effects (UADEs) reported in the monarchE clinical study.

2. Effectiveness Results

monarchE met the primary objective of the study at the second interim efficacy analysis (IA2), with abemaciclib plus ET achieving a statistically significant and clinically meaningful improvement in invasive disease free survival (IDFS) compared to ET alone in the Ki-67 pharmDx Score ≥ 20% population of Cohort1.

Invasive disease-free survival in patients in cohort 1 with Ki-67 pharmDx Score ≥ 20% was tested sequentially after IDFS in the Ki-67 Score ≥ 20% population and was demonstrated to be statistically significant, with a pre-specified 2-sided alpha level of 0.0426. The results shown in Table 8 indicate, abemaciclib plus ET demonstrated a statistically significant improvement in IDFS compared to ET alone in the Cohort 1-Ki67 Score ≥ 20%population (HR=0.643, 95% CI: 0.475, 0.872) along with a clinically meaningful improvement in 2-year IDFS rates for patients treated with abemaciclib plus ET.

3. Subgroup Analyses

There were no subgroup analyses performed in this clinical study.

4. Pediatric Extrapolation

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

XII. 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 multiple laboratory investigators. None of the laboratory 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.

XIII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

TBD based on FDA review.

XV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

In the monarchE clinical study, abemaciclib plus ET demonstrated a statistically significant and clinically meaningful improvement in IDFS compared to ET alone at the second interim efficacy analysis and a higher risk of recurrence among patients with high risk clinical and/or pathological factors whose tumors express Ki-67 at ≥ 20% Ki-67 pharmDx Score. There was a 35.7% reduction in the hazard of developing invasive disease in patients treated with abemaciclib plus ET (HR=0.643). Patients with high Ki-67 expression had an increased risk of developing IDFS events within 2 years, compared to patients with low Ki-67 expression (2-year IDFS rate = 86.1% vs 92.0% in the control arm, respectively). The data support the reasonable assurance of safety and effectiveness of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) when used in accordance with the indications for use in the adjuvant setting and product labeling. The study results support use of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) as an aid in identifying patients with early breast cancer at high risk of disease recurrence for whom adjuvant treatment with Verzenio® (abemaciclib) plus endocrine therapy is being considered.

B. Safety Conclusions

Safety of the device for patient management is related to safety and efficacy of the therapeutic. The observed safety profile of abemaciclib in monarchE was generally consistent with that previously reported for abemaciclib and ET in the advanced or metastatic breast cancer setting. In general, risks of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) are associated with failure of the device to perform as expected, or failure to correctly interpret test results. The process of testing FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for breast cancer diagnosis.

C. Benefit-Risk Determination

Additional factors to be considered in determining probable risks and benefits for Ki-67 IHC MIB-1 pharmDx (Dako Omnis) include the analytical performance of the device and the lack of availability of alternative standardized and validated tests. The primary risks associated with Ki-67 IHC MIB-1 pharmDx (Dako Omnis) are the possibility of inaccurate or false results. Thus, the probable benefits are based on results demonstrating that the test performs consistently and provides clinically relevant results for evaluating Ki-67 status in early breast cancer patients who are being considered for abemaciclib plus ET treatment.

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

In conclusion, given the available information above, the data support that for device indication (stated above) the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) when used in accordance with the indications for use and product labeling. The provided studies support use of Ki-67 IHC MIB-1 pharmDx (Dako Omnis) as an aid in identifying patients with early breast cancer at high risk of disease recurrence for whom adjuvant treatment with Verzenio® (abemaciclib) plus endocrine therapy is being considered.

XVI. CDRH DECISION

CDRH issued an approval order on 10/12/21. The final clinical conditions of approval cited in the approval order are described below.

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

XVII. 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.