SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: In vitro diagnostic immunohistochemistry (IHC) test for detection of claudin 18 (CLDN18) protein in formalin-fixed, paraffin-embedded (FFPE) human tissue sections

Device Trade Name: VENTANA CLDN18 (43-14A) RxDx Assay

Device Procode: QZJ

Applicant's Name and Address: Ventana Medical Systems, Inc.
(Roche Tissue Diagnostics)
1910 E Innovation Park Drive
Tucson, AZ 85755

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P230018

Date of FDA Notice of Approval: October18 2024

II. INDICATIONS FOR USE

VENTANA CLDN18 (43-14A) RxDx Assay is a qualitative immunohistochemical assay using mouse monoclonal anti-claudin 18, clone 43-14A, intended for laboratory use in the assessment of claudin 18 (CLDN18) protein in formalin-fixed, paraffin-embedded (FFPE) gastric adenocarcinoma including gastroesophageal junction (GEJ) tissue specimens by light microscopy. This assay is used with OptiView DAB IHC Detection Kit for staining on a BenchMark ULTRA instrument.

The assay is indicated as an aid in identifying patients with gastric or GEJ adenocarcinoma who may be eligible for treatment with VYLOY (zolbetuximab) in accordance with the approved therapeutic product labeling. The clinical cutoff for the therapeutic product is ≥ 75% viable tumor cells (% TC) demonstrating moderate to strong membrane CLDN18 staining above background.

Test results of the VENTANA CLDN18 (43-14A) RxDx Assay should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VENTANA CLDN18 (43-14A) RxDx Assay device labeling (Package Insert/Method Sheet).

V. DEVICE DESCRIPTION

VENTANA CLDN18 (43-14A) RxDx Assay contains optimized reagents required to complete an immunohistochemical staining procedure for formalin-fixed paraffin embedded (FFPE) tissue specimens on the BenchMark ULTRA automated staining instrument visualized using the OptiView DAB IHC Detection Kit. VENTANA CLDN18 (43-14A) RxDx Assay mouse monoclonal antibody IgG2b is produced as cell culture supernatant. The antibody and detection reagents are provided as ready-to-use dispensers and each dispenser contains sufficient reagent for 50 tests. The VENTANA CLDN18 (43-14A) RxDx Assay components and their description is provided in Table 1 below. Materials required but not provided are listed in Table 2.

Table 1: VENTANA CLDN18 (43-14A) RxDx Assay Components

Component	Packaged Form	Description
VENTANA CLDN18 (43-14A) Mouse Monoclonal Primary Antibody	1 Flo-Lok Dispenser: 50 tests	One 5 mL dispenser of VENTANA CLDN18 (43-14A) RxDx Assay contains approximately 15 µg of mouse monoclonal antibody. The antibody is diluted in Tris buffered saline, EDTA, Brij-35 and 0.05% sodium azide, a preservative. There is a trace amount of bovine serum albumin, carrier protein. Specific antibody concentration is approximately 3 µg/mL.
OptiView DAB IHC Detection Kit	Set of 6 Flo-Lok dispensers, packaged in a kit: 250 tests	OptiView Peroxidase Inhibitor contains 3.0% hydrogen peroxide solution.
		OptiView HQ Universal Linker contains a cocktail of HQ-labeled (HQ is a proprietary hapten covalently attached to the goat antibodies) antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (<50 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		OptiView HRP Multimer contains a mouse monoclonal anti-HQ-labeled HRP tertiary antibody (<40 µg/mL) in a buffer containing protein with ProClin 300, a preservative
		OptiView H2O2 contains 0.04% hydrogen peroxide in a phosphate buffer solution

Component	Packaged Form	Description
		OptiView DAB contains 0.2% 3, 3′-diaminobenzidine tetrahydrochloride (DAB) in a proprietary stabilizer solution with a proprietary preservative.
		OptiView Copper contains copper sulfate (5.0 g/L) in an acetate buffer with a proprietary preservative
BenchMark ULTRA automated staining instrument and VSS software	Instrument installed with the VSS host system software; Version 12.3 to 12.5.4	A personal computer (PC) that runs on Microsoft Windows controls and monitors the BenchMark ULTRA instrument via the host operating software.
Negative Control (Monoclonal)	1 Flo-Lok dispenser: 250 test kit	A mouse monoclonal antibody intended for laboratory use as a control for nonspecific binding of the primary antibody in sections of FFPE tissue. One 25 mL dispenser contains approximately 25 µg (1 µg/mL) of mouse monoclonal antibody. The antibody is diluted in in phosphate buffered saline containing carrier protein and ProClin 300, a preservative.

Table 2: Ancillary Reagents Required for VENTANA CLDN18 (43-14A) RxDx Assay

Reagent	Description
Hematoxylin II counterstain	Modified Mayer's hematoxylin used for staining cellular nuclei on slides containing cells from frozen tissue, FFPE tissue, or cytologic preparations
Bluing Reagent	Aqueous solution of buffered lithium carbonate used for bluing hematoxylin-stained sections on glass slides
Reaction Buffer 10x	Tris based buffer solution (pH 7.6 ± 0.2) used to rinse slides between staining steps and provide a stable aqueous environment for reactions carried out on the BenchMark instrument
EZ Prep 10X	A detergent-containing reagent that removes paraffin from the tissue specimen during the IHC staining to prepare the tissue for antigen retrieval and subsequent reactions
ULTRA Liquid CoverSlip (LCS) predilute	Pre-diluted coverslip solution used as a barrier between aqueous reagents and air
ULTRA CC1 Cell Conditioning Solution	Pre-diluted solution used as a pretreatment step in the processing of tissue samples on the BenchMark ULTRA instrument

A. Device Instrumentation and Software

The VENTANA CLDN18 (43-14A) RxDx Assay is performed on the BenchMark ULTRA automated staining instrument using Ventana System Software (VSS)

versions 12.3 to 12.5.4. The VENTANA CLDN18 (43-14A) RxDx Assay staining protocol is assay specific. To ensure that all system reagents are used together, the software has been designed to recognize and group reagents required for staining per the VENTANA CLDN18 (43-14A) RxDx Assay staining protocol.

B. Specimen Preparation

Routinely processed FFPE tissues are suitable for use with the VENTANA CLDN18 (43-14A) RxDx Assay. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for 24 hours with a range from 6 to 48 hours fixation time. Use of alcohol-formalin-acetic acid (AFA), 95% alcohol, Prefer and Zinc Formalin fixatives demonstrated limitations either due to signal intensity for resection and biopsy samples or based on sectioning issues at all fixation times tested (1 to 72 hours), and therefore, are not recommended for use with this assay.

Tissue sections should be cut at approximately 4µm thickness with a range from 3µm to 5µm and should be mounted on positively charged glass slides. Roche recommends that the slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. See the VENTANA CLDN18 (43-14A) RxDx Assay device labeling (Package Insert) for additional details.

C. Quality Control Procedures

Run controls are included in each staining run to establish the validity of the test results. The following controls must be run with the VENTANA CLDN18 (43-14A) RxDx Assay.

1. Tissue Control – Human Gastric Tissue with Intestinal Metaplasia

For VENTANA CLDN18 (43-14A) RxDx Assay, a system level control (SLC) that is stained in the same manner as the patient specimens should be run for each set of test conditions to monitor the proper functioning of the reagents and instrument within the staining run. SLC tissue should be fixed and processed in the same manner as the patient specimens. Tissue specimens with autolysis, degeneration, or improper fixation should not be used as SLC.

Human gastric tissue with intestinal metaplasia, containing both CLDN18-positive and CLDN18-negative staining elements, can be used as positive tissue control and SLC for VENTANA CLDN18 (43-14A) RxDx Assay. If the SLC fail to demonstrate appropriate positive or negative staining, results of the test specimen should be considered invalid.

The interpretation of the CLDN18 staining in gastric tissue with intestinal metaplasia (SLC) is provided in Table 3 below.

Table 3: Scoring Criteria for Evaluation of Gastric Tissue with Intestinal Metaplasia (SLC)

Staining Elements	Acceptable	Unacceptable
Positive	Presence of strong membranous CLDN18 staining in normal gastric epithelial cells AND Presence of weak to moderate membranous CLDN18 staining of epithelial cells in the areas of metaplasia	Absence of any strong membranous CLDN18 staining in normal gastric epithelial cells OR Absence of weak to moderate membranous CLDN18 staining of epithelial cells in the areas of metaplasia
Negative	Absence of CLDN18 staining in lamina propria, lymphocytes, smooth muscle, blood vessels, and peripheral nerve	Excessive non-specific background staining of lamina propria, lymphocytes, smooth muscle, blood vessels, and peripheral nerve obscuring the evaluation of CLDN18 stained cells

2. Negative Reagent Control

A matched negative reagent control slide should be run for every specimen to aid in the interpretation of results. Negative Reagent (Monoclonal), a negative reagent control antibody, is specifically matched for this VENTANA CLDN18 (43-14A) RxDx Assay and is used in place of the primary antibody to evaluate nonspecific staining in the patient tissue that may result from a reaction with the detection chemistry and not the anti-claudin 18 (43-14A) primary antibody.

D. Principles of Operation

The VENTANA CLDN18 (43-14A) RxDx Assay is fully automated for use on the BenchMark ULTRA automated slide stainer from deparaffinization through counterstaining. Patient FFPE tissue specimens are cut to approximately 4µm thickness and mounted on positively charged glass slides. These slides are loaded into the Benchmark ULTRA instrument. This system first removes the paraffin wax from the tissue (deparaffinization), and then subjects the tissue to heated antigen retrieval (cell conditioning). Endogenous peroxidases that could potentially react with the horseradish peroxidase conjugates (HRP) are blocked with OptiView Inhibitor (3% H_2O_2). After the endogenous peroxidase block, the VENTANA CLDN18 (43-14A) Mouse Monoclonal Primary Antibody is dispensed during the antibody incubation step and allowed to bind to its antigen. The slides are then incubated with the reagents in the OptiView DAB IHC Detection Kit, which is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit primary antibodies and which produces a visible dark brown precipitate (3,3'-Diaminobenzidine) via a horseradish peroxidase (HRP) enzymatic reaction at the antigen site. Slides are then counterstained using

Hematoxylin II and Bluing Reagent to create brown/blue contrast to aid the pathologist when reviewing the slides using bright field microscopy.

The VENTANA CLDN18 (43-14A) RxDx Assay staining protocol is shown in Table 4 below.

Table 4: VENTANA CLDN18 (43-14A) RxDx Assay Staining Protocol on BenchMark Ultra Instrument

Procedure Type	Protocol Parameter
Staining Procedure	U CLDN18 (43-14A) RxDx Assay PMA
Baking*	Not selected
Deparaffinization	4 minutes (default), 72°C
Cell Conditioning (Antigen Unmasking)	ULTRA CC1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	4 minutes, 36°C
Antibody (Primary)*	16 minutes, 36ºC
Negative Control*	16 minutes, 36ºC
OptiView HQ Linker	8 minutes (default), 36ºC
OptiView HRP Multimer	8 minutes (default), 36ºC
Counterstain	Hematoxylin II, 8 minutes, 36ºC
Post Counterstain*	Bluing, 4 minutes 36ºC

*** Selectable by Customer**

E. Staining Interpretation/ Expected Results

In gastric adenocarcinoma including the GEJ, neoplastic cells labeled with the VENTANA CLDN18 (43-14A) RxDx Assay are evaluated for percent tumor cell staining and intensity of the 3,3'-Diaminobenzidine (DAB) signal. The VENTANA CLDN18 (43-14A) RxDx Assay exhibits a membranous staining pattern with a varying range of intensities (weak, moderate, and strong) in non-neoplastic gastric tissue, intestinal metaplasia, and in gastric adenocarcinoma including the GEJ. Cytoplasmic staining may also be present, but it is not scored.

1. Hematoxylin & Eosin (H&E) Slide:

The pathologist will determine whether the H&E slide contains sufficient tumor tissue (defined as presence of ≥ 50 viable tumor cells) consistent with gastric adenocarcinoma including GEJ cases to allow interpretation of the case-matched IHC slides. If the H&E is not acceptable, the case-matched NRC and CLDN18 patient case slides will not be evaluated.

2. System-level Control Slide – Tissue Control Slide(s):

One system-level control (SLC) slide containing gastric tissue with intestinal metaplasia should be included in each BenchMark ULTRA staining run to confirm the validity of the staining run and should be evaluated by a qualified pathologist. Gastric tissue with intestinal metaplasia contains both positive-staining and negative-staining elements when stained with VENTANA

CLDN18 (43-14A) RxDx Assay. The SLC slide stained with CLDN18 (43-14A) antibody is expected to exhibit strong membranous staining in normal gastric epithelial cells and weak to moderate membranous staining of epithelial cells in the areas of metaplasia. Lamina propria, lymphocytes, smooth muscle, blood vessels and peripheral nerves should not exhibit CLDN18 (43-14A) staining and is used for assessment of background staining. The pathologist determines whether the SLC slide is acceptable or unacceptable based on criteria presented in Table 3 above.

3. **Negative Reagent Control (NRC) Slide:**

   The NRC slide is evaluated based on the level of non-specific staining (background) and the degree of background staining. If the NRC slide is considered not acceptable, the CLDN18 slide will not be evaluated, and the assay should be repeated, which can be done either on the same tissue or on another patient tissue, as applicable.

4. **Patient Tissue Slide:**

   CLDN18 IHC status will only be assigned if the H&E slide, SLC slide, NRC slide, and VENTANA CLDN18 (43-14A) antibody case slide (including background, morphology, and overall staining) are all acceptable. Patient specimens should have ≥ 50 viable tumor cells identified on the H&E in order to determine CLDN18 IHC status. Gastric and GEJ tissues stained with the VENTANA CLDN18 (43-14A) RxDx Assay are scored for percent tumor cell staining, which is defined as the proportion of viable tumor cells exhibiting membrane staining of moderate or stronger intensity out of the total number of viable tumor cells. Membrane staining pattern may be apical, circumferential (partial or complete), basolateral/ lateral or microluminal. Gastric adenocarcinoma including GEJ tissue cases are considered positive if ≥ 75% of viable tumor cells (TC) demonstrate moderate to strong membrane CLDN18 staining.

   The scoring algorithm for the VENTANA CLDN18 (43-14) RxDx Assay is provided in Table 5 below along with the staining intensities description. For detailed information on the Assay scoring and algorithm, refer to the device Package Insert and Interpretation Guide.

Table 5: VENTANA CLDN18 (43-14A) RxDx Assay Scoring Algorithm for Gastric Adenocarcinoma Including Gastroesophageal Junction (GEJ)

CLDN18 Status	Staining Description
Positive	≥ 75% viable tumor cells demonstrating moderate to strong membrane CLDN18 staining
Negative	< 75% viable tumor cells demonstrating moderate to strong membrane CLDN18 staining

The CLDN18 staining pattern with a varying range of intensities (weak, moderate, and strong) as described below:

- Weak intensity is staining with a light brown hue, and the membrane staining may appear to be partial or circumferential.
- Moderate intensity is staining with a chocolate brown hue, and the membrane staining may appear to be partial or circumferential.
- Strong intensity is staining with a dark brown to black hue, saturated DAB, and the membrane staining is thickened and may be partial or circumferential.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently no alternative FDA-cleared or approved assay available for detection of CLDN18 in FFPE gastric adenocarcinoma, including gastroesophageal junction (GEJ) tissue as an aid in identifying patients who may be eligible for treatment with VYLOY (zolbetuximab).

VII. MARKETING HISTORY

The VENTANA CLDN18 (43-14A) RxDx Assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

VENTANA CLDN18 (43-14A) RxDx Assay 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 test results may lead to improper patient management decisions.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Non-clinical studies were performed using the VENTANA CLDN18 (43-14A) RxDx Assay to establish analytical performance of the device in gastric adenocarcinoma including GEJ. These studies were performed using a BenchMark ULTRA instrument using the VSS software version 12.3 to 12.5.4. These studies were conducted to characterize the VENTANA CLDN18 (43-14A) RxDx Assay, demonstrate the impact of pre-analytical variables on assay performance, evaluate assay precision and robustness, and establish assay stability. The study results detailed below establish sensitivity, specificity, precision, robustness, stability, external reproducibility, and other performance characteristics of the device.

1. Analytical Sensitivity

The analytical sensitivity of the VENTANA CLDN18 (43-14A) RxDx Assay was assessed based on the prevalence of CLDN18 staining on a commercial cohort of 318 unique gastric or GEJ adenocarcinoma biopsy and resection tissue cases. The slides were read by one pathologist and were scored using the scoring algorithm as listed in Table 5 above. Positive CLDN18 status (≥75% was observed in 23.3% (74/318) of cases, whereas the negative CLDN18 status (<75%) was reported in 76.7% (244/318) of cases. Borderline cases, defined as 75% ±10%% TC, accounted for 14.2% (45/318) of the cases.

2. Analytical Specificity

The antibody used in the VENTANA CLDN18 (43-14A) RxDx Assay is Mouse monoclonal anti-claudin 18 antibody (Clone 43-14A). The CLDN18 (43-14A) antibody clone detects the transmembrane CLDN18 proteins and was generated using the immunogen specifically targeting CLDN18 C-terminal amino acids, which is a conserved region for CLDN18 protein that is common to both the CLDN18.1 and CLDN18.2 protein isoforms. The analytical specificity tests demonstrated that VENTANA CLDN18 (43-14A) RxDx Assay detects both CLDN18.1 and CLDN18.2 protein isoforms. To evaluate the impact this would have on the ability of the VENTANA CLDN18 (43-14A) RxDx Assay to identify CLDN18.2-positive patients in the intended use population, a bioinformatics analysis of The Cancer Genome Atlas (TCGA) database was performed. The analysis showed that CLDN18.1 mRNA was generally expressed near or below baseline expression level in gastric adenocarcinoma. Among the gastric adenocarcinoma samples with CLDN18.1 expression detected above baseline, CLDN18.2 was expressed at a higher level in 99% of those samples, with a median CLDN18.2 mRNA expression level 93-fold higher than CLDN18.1.

The analytical specificity of the VENTANA CLDN18 (43-14A) RxDx Assay was evaluated in three (3) studies: Western blot, Peptide Inhibition, and Immunoreactivity in Human Tissues.

a. Western Blot

A Western blot (WB) study analyzed the specificity of the VENTANA CLDN18 (43-14A) antibody binding to endogenous protein in whole cell lysates prepared from cell lines with a range of CLDN18 expression levels. The range of CLDN18 expression in the cell lines was confirmed by both staining intensity when stained with VENTANA CLDN18 (43-14A) RxDx Assay and assessment of mRNA expression levels. CLDN18 (43-14A) reacted with ~22kD bands in the cell lysates prepared from the cells that express high and moderate levels of CLDN18 (NUGC-4 and SNU-620, respectively). No bands were observed in the lysates from the CLDN18-negative cell line (HH).

Additional WB analyses were performed to ensure that the VENTANA CLDN18 (43-14A) antibody is specific for CLDN18 and does not cross-react with the other CLDN proteins. The VENTANA CLDN18 (43-14A) antibody showed no reactivity for CLDN2, 4, 5, 7, 9, 10, 11, 12, and 15 in the WB assays. The antibody also showed slight reactivity for CLDN1, 3, and 20. Additional Western blot studies using different protein concentrations with densitometry measurements, as well as staining studies paired with mRNA analysis, showed that the VENTANA CLDN18 (43-14A) antibody is far less reactive to CLDN1, 3, and 20, compared to its reactivity to CLDN18.

Additionally, HEK293 cells transfected with expression vectors for CLDN18.1 and CLDN18.2 were tested with VENTANA CLDN18 (43-14A) RxDx Assay. CLDN18 staining was observed in both cell lines, demonstrating that the VENTANA CLDN18 (43-14A) RxDx Assay detects both CLDN18.1 and CLDN18.2 protein. Thus, the VENTANA CLDN18 (43-14A) antibody cannot distinguish between CLDN18.1 and CLDN18.2.

b. Peptide Inhibition

Peptide inhibition studies were performed to confirm that the VENTANA CLDN18 (43-14A) antibody binds its epitope within the CLDN18 protein. The study confirmed that the VENTANA CLDN18 (43-14A) antibody recognizes its corresponding peptide immunogen within the C-terminal region of the CLDN18 protein.

c. Immunoreactivity in Human Tissues

This study assessed the analytical specificity of VENTANA CLDN18 (43-14A) RxDx Assay in normal (Tour of Body) and neoplastic tissue (Tour of Tumor) samples. One lot each of VENTANA CLDN18 (43-14A) RxDx Assay, SLC, and Negative Control (Monoclonal) were used to stain slides containing multi-tissue arrays (TMA) of normal and neoplastic tissue. Slides were evaluated for CLDN18 reactivity, acceptable background staining and stain intensity, and potential cross reactivity.

In the Tour of Body (TOB) study, 105 cores, representing 35 normal tissue types, were analyzed. All evaluable test samples showed acceptable CLDN18 background staining (100% . CLDN18 (43-14A) staining was observed on three (3) normal stomach cores, two (2) normal small intestine cores, one (1) normal lung core, and two (2) normal tonsil resections. The results are summarized in Table 6.

Table 6: Specificity of VENTANA CLDN18 (43-14A) RxDx Assay in Normal FFPE Tissues.

Tissue	Number of positive/total cases	Tissue	Number of positive/total cases
Cerebrum	0/3	Myeloid (bone marrow)	0/3
Cerebellum	0/3	Lung [b]	1/3
Adrenal gland	0/3	Heart	0/3
Ovary	0/3	Pharynx	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach [c]	3/3
Pituitary gland	0/3	Small intestine [d]	2/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Appendix	0/3
Breast	0/3	Liver	0/3
Spleen	0/3	Salivary gland	0/3
Tonsil [a]	2/3	Kidney	0/3
Lymph node	0/3	Bladder	0/3
Endometrium (Uterus)	0/3	Prostate	0/3
Skeletal muscle	0/3	Cervix	0/3
Soft tissue	0/3	Skin	0/3
Peripheral nerve	0/3	Mesothelium	0/3
Thymus	0/3		

[a] CLDN18 staining was present: cytoplasmic and membrane staining may be seen in a subpopulation of antigen presenting cells in the reticulated crypt epithelium[1]

[b] CLDN18 staining was present: membrane staining in pneumocytes[2,3]

[c] CLDN18 staining was present: membrane staining in the gastric epithelium[4,5]

[d] CLDN18 staining was present: membrane and cytoplasmic staining in paneth cells[6]

In the Tour of Tumor (TOT) study, 64 neoplastic tissue cores, representing 61 distinct tumor types were evaluated. CLDN18 (43-14A) staining was observed in the liver cholangiocarcinoma core and the cerebellar ependymoma core. The data is summarized in Table 7.

Table 7: Specificity of VENTANA CLDN18 (43-14A) RxDx Assay in Neoplastic FFPE Tissues

Pathology	Number of positive/total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebellum) [a]	1/1
Oligodendroglioma (Cerebellum)	0/1
Adenoma (Adrenal gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Serous adenocarcinoma (Ovary)	0/1
Teratoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Pheochromocytoma (Adrenal gland)	0/1
Embryonal carcinoma (Testis)	0/1
Seminoma (Testis)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Invasive lobular carcinoma (Breast)	0/1
B-cell lymphoma, NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Adenocarcinoma (Esophagus)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Gastrointestinal)	0/2
Gastrointestinal stromal tumor (GIST) (Gastrointestinal)	0/2
Adenocarcinoma (Colon)	0/1
Adenosquamous carcinoma (Colon)	0/1
Carcinoid tumor (Appendix)	0/1
Hepatocellular carcinoma (Liver)	0/1
Cholangiocarcinoma (Liver) [b]	1/1
Clear cell carcinoma (Kidney)	0/1
Papillary adenoma (Kidney)	0/1
Urothelial carcinoma (Bladder)	0/1
Squamous cell carcinoma (Bladder)	0/1
Adenocarcinoma (Prostate)	0/2
Clear cell carcinoma (Uterus)	0/1

Pathology	Number of positive/total cases
Endometrial carcinoma (Uterus)	0/1
Leiomyoma (Uterus)	0/1
Leiomyosarcoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/1
Endocervical adenocarcinoma (Cervix)	0/1
Alveolar rhabdomyosarcoma (Striated muscle)	0/1
Melanoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Basal cell carcinoma (Skin)	0/1
Follicular carcinoma (Thyroid)	0/1
Schwannoma (Nerve)	0/1
Neurofibrosarcoma (Nerve)	0/1
Mesothelioma (Mesothelium)	0/1
Pleural solitary fibrous tumor (Mesothelium)	0/1
Follicular lymphoma (Lymph node)	0/1
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Warthin tumor (Salivary gland)	0/1
Pleomorphic adenoma (Salivary gland)	0/1
Squamous cell carcinoma (Head and neck)	0/1
Adenocarcinoma (Head and neck)	0/1
Multiple myeloma (Bone)	0/1
Liposarcoma (Soft tissue)	0/1
Angiosarcoma (Soft tissue)	0/1
Myxoma (Heart)	0/1

a CLDN18 staining was present as cytoplasmic staining in rare tumor cells
b CLDN18 staining was present as membrane and rare cytoplasmic staining in scattered tumor cells[7]

3. Precision

Precision of the VENTANA CLDN18 (43-14A) RxDx Assay on BenchMark ULTRA was evaluated in three studies: Reader (pathologist) Precision, Intermediate Precision and Repeatability, and Inter-Laboratory (External Reproducibility).

a. Reader Precision

In the Reader Precision study for VENTANA CLDN18 (43-14A) RxDx Assay, within-reader and between-reader components of precision for gastric

adenocarcinoma including GEJ tissue reads were evaluated. The study included 100 unique gastric adenocarcinoma including GEJ tissue cases (50 CLDN18-positive and 50 CLDN18-negative) that were stained with VENTANA CLDN18 (43-14A) RxDx Assay. For the Between-Reader study, three (3) trained pathologists (readers) evaluated the same set of slides, and for the Within-Reader study, three (3) trained pathologists evaluated the same slides twice with a minimum of 2 weeks washout period between assessments. Specimens were blinded and randomized prior to evaluation. The agreement rates for these studies are summarized in Table 8 below.

Table 8: Between-Reader and Within-Reader Precision for VENATNA CLDN18 (43-14A) RxDx Assay

Precision	Agreement			
	Type	n/N	%	95% CI[a]
Between-Reader	APA	296/300	98.7	96.6, 100.0
	ANA	296/300	98.7	96.6, 100.0
	OPA	296/300	98.7	96.7, 100.0
Within-Reader	APA	296/300	98.7	97.3, 99.7
	ANA	296/300	98.7	97.3, 99.7
	OPA	296/300	98.7	97.3, 99.7

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

[a] 2-sided 95% confidence interval calculated using the percentile bootstrap method.

b. Intermediate Precision and Repeatability

In this study, one pathologist evaluated 24 gastric adenocarcinoma including GEJ tissue cases (12 CLDN18-positive and 12 CLDN18-negative). There were 2 replicates per condition, and all slides were randomized prior to being evaluated. The following design factors included:

- 3 antibody lots of VENTANA CLDN18 (43-14A) RxDx Assay (between-antibody lots)
- 3 BenchMark ULTRA instruments (between-instruments)
- 3 lots of OptiView DAB IHC Detection Kits (between-detection kit lots)
- Across 3 non-consecutive days (between-staining days)
- Across all intermediate precision conditions (within-staining run)

Results are summarized in Table 9 below.

Table 9: Intermediate Precision for VENTANA CLDN18 (43-14A) RxDx

Repeatability/ Precision	Agreement		
	Type	n/N	% (95% CI)[a]
Between-Antibody Lots	PPA	72/72	100.0 (94.9, 100.0)
	NPA	72/72	100.0 (94.9, 100.0)

Repeatability/ Precision	Agreement		
	Type	n/N	% (95% CI)[a]
	OPA	144/144	100.0 (97.4, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	72/72	100.0 (94.9, 100.0)
	NPA	72/72	100.0 (94.9, 100.0)
	OPA	144/144	100.0 (97.4, 100.0)
Between-Detection Kits	PPA	72/72	100.0 (94.9, 100.0)
	NPA	72/72	100.0 (94.9, 100.0)
	OPA	144/144	100.0 (97.4, 100.0)
Between-Day	PPA	72/72	100.0 (94.9, 100.0)
	NPA	72/72	100.0 (94.9, 100.0)
	OPA	144/144	100.0 (97.4, 100.0)
Within-Run	PPA	108/108	100.0 (96.6, 100.0)
	NPA	108/108	100.0 (96.6, 100.0)
	OPA	216/216	100.0 (98.3, 100.0)

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA)

[a] 2-sided 95% CIs calculated using the Wilson score method

c. Inter-Laboratory (External Reproducibility) Study

An inter-laboratory reproducibility study was conducted to evaluate reproducibility of the VENTANA CLDN18 (43-14A) RxDx Assay in determining CLDN18 IHC status in gastric adenocarcinoma including GEJ cancer cases. The study included 28 gastric adenocarcinoma including GEJ tissue specimens (14 CLDN18-positive and 14 CLDN18-negative) stained and scored at three external laboratories (Sites A, B, C). Each site stained five sets of specimens from the 28 cases on five non-consecutive days over a period of at least 20 days. Two qualified pathologists at each site independently evaluated stained slides. Results from sites A, B, and C are listed in Tables 11 and 12. Performance of the two readers at one site was significantly different from each other, as well as from the other 4 readers from the other 2 sites.

Table 10 shows the per-site results of the between-reader, between-site, and within-site inter-laboratory reproducibility study at Sites A, B, and C.

Table 10. Between-Reader, Between-Site, and Within-Site Inter-Laboratory Reproducibility for Sites A, B, and C

Per-Site Inter-Laboratory Reproducibility at Sites A, B, and C			
Between-Reader Agreement			
	Type	**n/N**	**% (95% CI) [a]**
Site A	APA	86/129	66.7 (51.1, 78.0)
	ANA	106/149	71.1 (60.3, 79.2)
	OPA	96/139	69.1 (57.9, 78.5)
Site B	APA	128/140	91.4 (83.1, 97.2)
	ANA	126/138	91.3 (84.6, 97.0)
	OPA	127/139	91.4 (84.1, 97.1)
Site C	APA	130/143	90.9 (86.6, 96.7)
	ANA	124/137	90.5 (87.3, 96.1)
	OPA	127/140	90.7 (87.1, 96.4)
Between-Site Agreement			
	Type	**n/N**	**% (95% CI) [a]**
Site A -vs- B	APA	2230/2691	82.9 (75.4, 89.0)
	ANA	2408/2869	83.9 (77.2, 89.2)
	OPA	2319/2780	83.4 (76.6, 89.0)
Site A -vs- C	APA	2266/2720	83.3 (76.2, 89.1)
	ANA	2406/2860	84.1 (77.3, 89.2)
	OPA	2336/2790	83.7 (76.9, 89.2)
Site B -vs- C	APA	2632/2830	93.0 (88.3, 97.0)
	ANA	2552/2750	92.8 (89.1, 96.5)
	OPA	2592/2790	92.9 (88.9, 96.8)
Within-Site Agreement			
	Type	**n/N**	**%**
Site A	PPA	118/150	78.7
	NPA	118/129	95.7
	OPA	236/279	96.4
Site B	PPA	133/139	91.5
	NPA	132/140	94.3
	OPA	265/279	94.3
Site C	PPA	135/140	84.6
	NPA	132/140	95
	OPA	267/280	95.4

Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).
Positive Percent Agreement (PPA), Negative Positive Agreement (NPA)
[a] 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2000 bootstrap samples.

Due to the poor agreement at the original 3 sites, the slides that were stained at sites A, B, and C were distributed to three additional external sites (X, Y, Z). Each site received five sets of stained slides from the 28 cases that were previously stained on five non-consecutive days over a period of 20 days. Two qualified pathologists at each site independently evaluated the stained slides. Results from sites X, Y, and Z are also listed in Tables 11 and 12.

Table 11: Inter-Laboratory Reproducibility for VENTANA CLDN18 (43-14A) RxDx Assay for Sites A, B, C and Sites X, Y, and Z

Inter-laboratory Reproducibility	Type	Agreement			
		Sites A, B, C		Sites X, Y, Z	
		n/N	% (95% CI) [b]	n/N	% (95% CI) [b]
Overall [a]	PPA	380/419	90.7 (85.5, 95.9)	408/420	97.1 (94.4. 99.5)
	NPA	386/419	92.1 (86.5, 97.8)	390/420	92.9 (86.7, 97.4)
	OPA	766/838	91.4 (88.2, 94.2)	798/840	95.0 (91.7, 97.7)
Within-site	PPA	386/429	90.0 (84.7, 95.2)	433/450	96.2 (93.8, 98.4)
	NPA	382/409	93.4 (88.9, 99.4)	385/390	98.7 (97.7, 99.7)
	OPA	768/838	91.6 (88.7, 94.3)	818/840	97.4 (96.0, 98.7)
Within-reader	PPA	399/409	97.6 (96.1, 98.9)	431/440	98.0 (96.5, 99.1)
	NPA	415/429	96.7 (94.6, 98.6)	393/400	98.3 (96.9, 99.5)
	OPA	814/838	97.1 (95.9, 98.2)	824/840	98.1 (97.0, 99.2)

Positive Percent Agreement (PPA), Negative Positive Agreement (NPA), Overall Percent Agreement (OPA)

a Agreement of study results with the case-level modal CLDN18 IHC status

b 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2000 bootstrap samples.

Table 12: External Reproducibility Pairwise Comparison Results for VENTANA CLDN18 (43-14A) RxDx Assay for Sites A, B, C and Sites X, Y, and Z

Inter-laboratory Reproducibility	Type	Agreement			
		Sites A, B, C		Sites X, Y, Z	
		n/N	% (95% CI) [a]	n/N	% (95% CI) [a]
Between-site	APA	7128/8241	86.5 (80.9, 91.2)	8014/8760	91.5 (85.9, 96.3)
	ANA	7366/8479	86.9 (81.7, 91.0)	7294/8040	90.7% (84.4, 95.6)
	OPA	7247/8360	86.7 (81.4, 91.0)	7654/8400	91.1% (85.3, 96.0)
Between-reader	APA	344/412	83.5 (76.8, 89.2)	416/438	95.0% (92.3, 97.6)
	ANA	356/424	84.0 (78.5, 88.7)	380/402	94.5% (91.6, 97.2)
	OPA	350/418	83.7 (77.9, 88.9)	398/420	94.8% (91.9, 97.4)
Between-day	APA	1562/1648	94.8 (92.3, 96.8)	1698/1752	96.9% (94.9, 98.7)
	ANA	1610/1696	94.9 (92.8, 96.7)	1554/1608	96.6% (94.7, 98.4)
	OPA	1586/1672	94.9 (92.7, 96.8)	1626/1680	96.8% (94.9, 98.6)

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

a 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2000 bootstrap samples.

4. Robustness

a. Tissue Thickness

Tissue thickness was evaluated with 5 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative and 3 total gastric tissue with intestinal metaplasia tissue (SLC) cases, using VENTANA CLDN18 (43-14A) RxDx Assay antibody on a BenchMark ULTRA instrument. Duplicate sections at 2, 3, 4, 5, 6, and 7 µm were tested for each case. A 4 µm thick sample was used as a reference for each case. Significant variability in the CLDN18 staining intensity was observed in at least one (1) SLC sample at the 2, 6, and 7 µm thicknesses. The recommended section thickness for gastric adenocarcinoma including GEJ indication and gastric tissue with intestinal metaplasia (SLC) is 3µm to 5µm for use with the VENTANA CLDN18 (43-14A) RxDx Assay.

b. Protocol Limitations and Failure Mode

The purpose of this study was to identify protocol conditions (protocol parameters and simulated dispense errors) that might lead to a potential false positive, false negative, or unacceptable result. Results are used to r provide as troubleshooting guidance for the assay in the device labeling (package insert).

The test conditions included:

- One lot of VENTANA CLDN18 (43-14A) RxDx Assay
- One lot of OptiView DAB IHC Detection Kit
- One BenchMark ULTRA instrument system
- One Negative Reagent Control per case per test condition
- One Pathologist (Reader)
- 5 Gastric including GEJ adenocarcinoma samples
- 3 Gastric with intestinal metaplasia tissue (SLC) samples

Protocol limitation parameters included the following conditions:

- Baking conditions:
  - Offline
  - Online (2 conditions with different times and temperatures)
  - Double (offline and online)
- CC1 (Cell Conditioning 1) incubation times:
  - Low
  - High
- Antibody incubation times:

    - Low
    - High
- Counterstain (Bluing, Hematoxylin) incubation times:
    - High
- Stacking (CC1, antibody, Bluing, and Hematoxylin II) incubation times:
    - Low
    - High
- Turnaround Time

Failure Mode analysis included the following conditions:

- Antibody over and partial-dispense
    - 4x dispense
    - 1/8 of dispense
    - 1/16 of dispense
- HQ Block (Linker) over and partial-dispense
    - 4x dispense
    - 1/8 of dispense
- HRP Block (Multimer) over and partial-dispense
    - 4x dispense
    - 1/8 of dispense
- Chromogen Block (H2O2/DAB) over and partial-dispense
    - 4x dispense
    - 1/8 of dispense

The CLDN18 (43-14A) staining performance on gastric adenocarcinoma including GEJ and gastric tissue with intestinal metaplasia (SLC) was not affected by any of the baking test condition ranges and incubation time test condition ranges. The Failure Modes testing resulted in failures from partial dispense of the Chromogen Block (H2O2/DAB), and the internal control (SLC) was able to accurately identify the failures. The test results identified which staining procedures should be locked in the final staining procedure for the VENTANA CLDN18 (43-14A) RxDx Assay. Thus, the final staining protocol specified in Table 4 above should be followed. Refer to the Labeling (Package Insert) for additional instructions.

5. Stability Studies

a. Cut Slide Stability

This study evaluated the stability of the CLDN18 antigen in FFPE tissue that had been sectioned onto positively charged glass microscope slides and stored for an extended duration of time at 5±3°C and 30±5°C. Cut slide stability was evaluated on 7 gastric adenocarcinoma including GEJ cases and 3 gastric tissue with intestinal metaplasia (SLC) cases. Slides sectioned and stained at the Day 0 time point served as the baseline comparator for the

remainder of the time points tested. Tissue was sectioned from each of the seven cases and separated into two different storage conditions for the duration of the study. One set of slides was stored at the refrigerated temperature condition (5±3°C) and one at the incubator temperature condition (30±5°C). Slides were stained at each pre-defined designated time and staining results for each time point were compared to the Day 0 baseline slides.

The study results support cut slide stability for up to 6 months; however, to align the cut slide stability dating for CDx assays, the cut slide stability for VENTANA CLDN18 (43-14A) RxDx Assay will be assigned 45 days for both gastric adenocarcinoma tissue including GEJ and gastric tissue with intestinal metaplasia.

b. Real-Time Stability Study

The objective of this study was to assess the shelf-life and in-use stability of the VENTANA CLDN18 (43-14A) RxDx Assay. Stability was assessed by evaluating CLDN18 (43-14A) antibody staining performance on formalin fixed paraffin embedded (FFPE) human gastric adenocarcinoma tissue including GEJ at specified intervals. Three (3) production lots of VENTANA CLDN18 (43-14A) RxDx Assay were placed at the intended storage condition (2-8°C) for the duration of testing.

Based on the study results, the stability dating for the VENTANA CLDN18 (43-14A) RxDx Assay is 24 months when stored at 2-8°C.

c. Ship Stress Stability Study

The objective of this study was to assess the shipping category of the VENTANA CLDN18 (43-14A) RxDx Assay. Stability was assessed by evaluating CLDN18 (43-14A) antibody staining performance on formalin fixed paraffin embedded (FFPE) human gastric adenocarcinoma tissue including GEJ at specified intervals. Three (3) production lots of VENTANA CLDN18 (43-14A) RxDx Assay were subjected to different stressed conditions and then placed at the intended storage condition (2-8°C).

The conditions tested were as follows:

- Hot Ship Stress Cat. A (33°C±3ºC 192 hours)
- Hot Ship Stress Cat. B (18°C±3ºC 192 hours)
- Hot Ship Stress Cat. F (37°C±2ºC 150 hours)
- Hot Ship Stress Cat. F (37°C±2ºC 192 hours)
- Cold Ship Stress Freeze/Thaw (-20°C±5ºC 192 hours)
- Cold Ship Stress Freeze/Thaw cycles (-25°C±5ºC 192 hours)

Based on the study results, the product was assigned a Shipping Categories B > 0 °C and ≤ +15 °C). The results may also support a Shipping Category F (> -20 °C and ≤ +35 °C).

B. Animal Studies

None

C. Additional Studies

1. Tissue Heterogeneity

a. Within-Block Heterogeneity

This study evaluated the staining outcome of multiple samples from the same tissue block by comparing the CLDN18 status between multiple slides sectioned from one block. Five gastric adenocarcinoma tissue including GEJ blocks were tested, including 3 positive cases and 2 negative cases. For each block, approximately every 10th slide was stained with VENTANA CLDN18 (43-14A) RxDx Assay, resulting in a total of 11 slides from each block. Four of the five (80%) gastric adenocarcinoma tissue including GEJ blocks maintained CLDN18 status throughout the block. The fifth block was a borderline positive case that resulted in a CLDN18 status change. The overall percent agreement (OPA) for CLDN18 status across all stained slides from all blocks was 94.5%. The results of this study demonstrate that some heterogeneity may be observed in the CLDN18 expression within each gastric adenocarcinoma including GEJ tissue block from the same case when stained with the VENTANA CLDN18 (43-14A) RxDx Assay.

b. Within-Case Heterogeneity

This study evaluated the staining outcome of multiple blocks from the same patient case by comparing the CLDN18 status between multiple blocks from one patient case. Twenty-eight (28) blocks that encompassed 9 total gastric adenocarcinoma including GEJ tissue patient cases were tested with the VENTANA CLDN18 (43-14A) RxDx Assay. Six of the nine gastric adenocarcinoma tissue including GEJ patient cases (66.7%) maintained CLDN18 IHC status across the multiple blocks. Across all blocks, the OPA for case heterogeneity was 89.3% (25/28). Case-level heterogeneity was observed in 3 of the 28 cases (10.7%). These results demonstrate that variation may be observed in the CLDN18 expression within of different gastric adenocarcinoma including GEJ tissue blocks from the same patient case when stained with the VENTANA CLDN18 (43-14A) RxDx Assay.

c. Matched Primary vs Metastatic Study

This study evaluated the concordance of CLDN18 status between FFPE matched primary and metastatic gastric adenocarcinoma including GEJ tissues when stained with the VENTANA CLDN18 (43-14A) RxDx Assay on the BenchMark ULTRA instrument. There were 40 matched sets evaluated, 35 sample sets were evaluable. Of the 35 evaluable matched sets, 29 pairs were concordant (82.9%), and 6 pairs were discordant (17.1%). These results demonstrate that variation may be observed in the CLDN18 expression depending on the location of tissue collection from the patient.

2. Impact of Tissue Specimen Preparation and Treatment Studies

a. Ischemia Study (Time to Fixation)

The objective of this study was to evaluate the effects of ischemic time on CLDN18 antigenicity as detected by staining with VENTANA CLDN18 (43-14A) RxDx Assay. This study examined the effects of delay to fixation (ischemia) for HEK293p318 xenograft samples at 0 hours, 0.5 hours, 1 hour, 2 hours, 6 hours, and 24 hours post excision. The sample fixed immediately in 10% neutral buffered formalin (NBF) for 24 hours was the reference condition. The study demonstrated concordant CLDN18 staining results for all samples tested (0 - 24 hours); however, the recommendation is to immediately fix samples.

b. Fixation Study

The objective of this study was to evaluate the effects of fixative type and fixation time on CLDN18 antigenicity as detected by staining with VENTANA CLDN18 (43-14A) RxDx Assay. Six HEK293p318 xenograft tissues were fixed for 1, 6, 12, 24, 48 and 72 hours in 6 fixatives: 10% NBF, Zinc formalin, 95% alcohol, alcohol-formalin-acetic acid (AFA), alcohol formalin, and PREFER for a total of 86 samples. The data were then compared to the reference standard of 10% NBF for 24 hours.

Based on the study results, the recommended fixation conditions are 10% NBF between 6 and 48 hours. Other acceptable fixation methods and types are the 2+2 fixation method for 10% NBF and Alcoholic Formalin between 6 and 48 hours. Tissues fixed in AFA, Prefer, 95% Ethanol, and Zinc Formalin were unacceptable when stained with VENTANA CLDN18 (43-14A) RxDx Assay and are therefore not recommended fixatives.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of VENTANA CLDN18 (43-14A) RxDx Assay as a companion diagnostic (CDx) device to identify patients with gastric or gastroesophageal

adenocarcinoma who may be eligible for treatment with VYLOY (zolbetuximab) was evaluated in two (2) pivotal Phase 3 clinical trials: SPOTLIGHT study (8951-CL-0301) and GLOW study (8951-CL-0302). The detailed overview of both studies is provided below.

A. Study Design

Both SPOTLIGHT study (8951-CL-0301, NCT03504397) and GLOW study (8951-CL-0302, NCT03653507) were phase 3, global, multicenter, double-blind, randomized trial to evaluate the efficacy and safety of VYLOY (zolbetuximab) in combination with chemotherapy as first-line treatment in patients with HER2-negative locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma whose tumors are CLDN18.2 positive. The chemotherapy backbone in SPOTLIGHT was mFOLFOX6 (leucovorin [folinic acid], fluorouracil [5-FU], and oxaliplatin) and in GLOW was CAPOX (capecitabine and oxaliplatin).

For both studies, CLDN18.2 positivity was defined as ≥75% of viable tumor cells demonstrating moderate to strong membranous CLDN18 staining. CLDN18.2 positivity was determined by immunohistochemistry (IHC) on gastric or GEJ tumor tissue specimens from all patients with the VENTANA CLDN18 (43-14A) RxDx Assay performed in a central laboratory.

In SPOTLIGHT, a total of 565 patients were randomized 1:1 to receive VYLOY in combination with mFOLFOX6 (n=283) or placebo in combination with mFOLFOX6 (n=282).

In GLOW, a total of 507 patients were randomized 1:1 to receive VYLOY in combination with CAPOX (n=254) or placebo in combination with CAPOX (n=253).

1. Clinical Inclusion and Exclusion Criteria

a. Key Trial Inclusion Criteria

Enrollment in the SPOTLIGHT and GLOW studies was limited to patients who met the following inclusion criteria:

i. Participants were considered adults (e.g., ≥ 18 years of age in the United States) according to local regulation at the time of signing the informed consent.
ii. Participant's tumor expressed CLDN 18.2 in ≥ 75% of tumor cells demonstrating moderate to strong membranous staining as determined by central IHC testing.
iii. Participant had a known HER2-negative tumor as determined by local or central testing on a gastric or GEJ tumor specimen.
iv. Participants must have had:
- A histologically confirmed diagnosis of gastric or GEJ adenocarcinoma.

- Radiologically confirmed locally advanced unresectable or metastatic disease.
- Radiologically evaluable disease (measurable and/or non-measurable disease according to RECIST 1.1) per local assessment.

b. Key Trial Exclusion Criteria

Patients were not permitted to enroll in the SPOTLIGHT and GLOW studies if they met any of the following exclusion criteria:

i. Prior systemic chemotherapy for locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma.
ii. Radiotherapy for locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma ≤ 14 days prior to randomization and had not recovered from any related toxicity.

2. **Follow-up Schedule**

For both the SPOTLIGHT and GLOW studies, treatment continued until disease progression, or a subsequent anticancer treatment was initiated. Tumor assessments were performed every 9 weeks up to and including Week 54, then every 12 weeks thereafter.

3. **Clinical Endpoints**

For both SPOTLIGHT and GLOW:

a. The primary efficacy endpoint was Progression-free survival (PFS), defined as the time from the date of randomization until the date of radiological progressive disease (per RECIST 1.1 by IRC) or death from any cause, whichever is earliest.
b. The key secondary efficacy endpoint included Overall Survival (OS), defined as the time from the date of randomization until the date of death from any cause.

B. Accountability of PMA Cohort

1. SPOTLIGHT Study - Accountability of PMA Cohort

A total of 2735 patients were screened (i.e., patients who signed the informed consent form for SPOTLIGHT study), and 565 patients were randomized into the SPOTLIGHT study. Among the 2735 screened patients, 276 did not have a sample accessioned for central CLDN18 testing, and 26 did not meet the specimen inclusion/exclusion criteria for diagnostic (Dx) testing (e.g., insufficient tumor for evaluation). Tissue specimens from the remaining 2433 patients were tested with VENTANA CLDN18 (43-14A) RxDx Assay. Out of the 2433 patients, 1868 patients did not meet the inclusion/exclusion criteria of SPOTLIGHT study. The remaining 565 patients were randomized to the study and comprised the efficacy population. The patient accountability is summarized in Table 13.

Table 13: Accountability of Patients in SPOTLIGHT Study (8951-CL-0301)

Patient Disposition for SPOTLIGHT Study	N
Total number of patients screened	**2735**
-Samples not accessioned for central testing	276
-Samples did not meet specimen criteria for Dx testing	26
Number Tested with the VENTANA CLDN18 (43-14A) RxDx Assay	**2433**
-Study 8951-CL-0301 Inclusion/Exclusion Criterion Not Met	1868
Final number of patients in the trial	**565**

2. GLOW Study - Accountability of PMA Cohort

A total of 2333 patients were screened (i.e., patients who signed the informed consent form for GLOW study), and 507 patients were randomized into the GLOW study. Among the 2333 screened patients, 168 did not have a sample accessioned for central CLDN18 testing, and 29 did not meet the specimen inclusion/exclusion criteria for diagnostic (Dx) testing (e.g., insufficient tumor for evaluation). Tissue specimens from the remaining 2136 patients were tested with VENTANA CLDN18 (43-14A) RxDx Assay. Out of the 2136 patients, 1629 patients did not meet the inclusion/exclusion criteria of GLOW study. The remaining 507 patients were randomized to the study and comprised the efficacy population. The patient accountability is summarized in Table 14.

Table 14: Accountability of Patients in GLOW Study (8951-CL-0302)

Patient Disposition for GLOW Study	N
Total number of patients screened	**2333**
-Samples not accessioned for central testing	168
-Samples did not meet specimen criteria for Dx testing	29
Number Tested with the VENTANA CLDN18 (43-14A) RxDx Assay	**2136**
-Study 8951-CL-0302 Inclusion/Exclusion Criterion Not Met	1629
Final number of patients in the trial	**507**

C. Study Population Demographics and Baseline Parameters

1. SPOTLIGHT Study - Population Demographics and Baseline Parameters

Table 15 below summarizes the patient demographic and specimen characteristic information for patients tested with VENTANA CLDN18 (43-14A) RxDx Assay in the SPOTLIGHT study.

Table 15: SPOTLIGHT Patient and Sample Characteristics by Treatment Arm

Characteristic	Treatment Arm			Overall (N=2433)
	VYLOY plus mFOLFOX6 (N=283)	Placebo plus mFOLFox6 (N=282)	Not Enrolled (N=1868)	
Age (years)				
Mean (SD)	59.7 (11.75)	58.8 (12.96)	61.8 (12.05)	61.2 (12.17)
Median	62	60	63	63
Min, Max	27, 83	20, 86	20, 91	20, 91
Sex				
Female	107 (37.8%)	107 (37.9%)	569 (30.5%)	783 (32.2%)
Male	176 (62.2%)	175 (62.1%)	1299 (69.5%)	1650 (67.8%)
Ethnicity				
Hispanic or Latino	36 (12.7%)	37 (13.1%)	322 (17.2%)	395 (16.2%)
Not Hispanic or Latino	225 (79.5%)	213 (75.5%)	1392 (74.5%)	1830 (75.2%)
Missing	22 (7.8%)	32 (11.3%)	154 (8.2%)	208 (8.5%)
Race				
American Indian or Alaska Native	9 (3.2%)	8 (2.8%)	98 (5.2%)	115 (4.7%)
Asian	96 (33.9%)	97 (34.4%)	635 (34.0%)	828 (34.0%)
Black or African American	5 (1.8%)	2 (0.7%)	40 (2.1%)	47 (1.9%)
Native Hawaiian or Other Pacific Islander	0	0	1 (0.1%)	1 (0.0%)
White	140 (49.5%)	134 (47.5%)	851 (45.6%)	1125 (46.2%)
Other	11 (3.9%)	12 (4.3%)	87 (4.7%)	110 (4.5%)
Missing	22 (7.8%)	29 (10.3%)	156 (8.4%)	207 (8.5%)
ECOG Status				
0	125 (44.2%)	115 (40.8%)	0	240 (9.9%)
1	153 (54.1%)	163 (57.8%)	0	316 (13.0%)
2	1 (0.4%)	0	0	1 (0.0%)
Missing	4 (1.4%)	4 (1.4%)	1868 (100.0%)	1876 (77.1%)
Prior Gastrectomy				
Yes	82 (29.0%)	83 (29.4%)	0	165 (6.8%)
No	201 71.0%)	199 (70.6%)	0	400 (16.4%)
Missing	0	0	1868 (100.0%)	1868 (76.8%)
Medical Condition				
Gastric Adenocarcinoma	219 (77.4%)	210 (74.5%)	1267 (67.8%)	1696 (69.7%)
GEJ Adenocarcinoma	64 (22.6%)	72 (25.5%)	464 (24.8%)	600 (24.7%)
Missing	0	0	137 (7.3%)	137 (5.6%)

Characteristic	Treatment Arm			Overall (N=2433)
	VYLOY plus mFOLFOX6 (N=283)	Placebo plus mFOLFox6 (N=282)	Not Enrolled (N=1868)	
Sample Collection Method				
Resection	71 (25.1%)	67 (23.8%)	323 (17.3%)	461 (18.9%)
Biopsy	212 (74.9%)	215 (76.2%)	1544 (82.7%)	1971 (81.0%)
Unknown	0	0	1 (0.1%)	1 (0.0%)
Tumor Type				
Metastatic	239 (84.5%)	238 (84.4%)	1310 (70.1%)	1787 (73.4%)
Primary	44 (15.5%)	44 (15.6%)	351 (18.8%)	439 (18.0%)
Unknown	0	0	207 (11.1%)	207 (8.5%)

2. GLOW Study - Population Demographics and Baseline Parameters

Table 16 below summarizes the patient demographic and specimen characteristic information for patients tested with VENTANA CLDN18 (43-14A) RxDx Assay in the GLOW study.

Table 16: GLOW Patient and Sample Characteristics by Treatment Arm

Characteristic	Treatment Arm			Overall (N=2136)
	VYLOY plus CAPOX (N=254)	Placebo plus CAPOX (N=253)	Not Enrolled (N=1629)	
Age (years)				
Mean (SD)	58.6 (12.09)	56.7 (13.04)	59.8 (12.04)	59.3 (12.21)
Median	61	59	62	61
Min, Max	22, 82	21, 83	21, 90	21, 90
Sex				
Female	95 (37.4%)	97 (38.3%)	537 (33.0%)	729 (34.1%)
Male	159 (62.6%)	156 (61.7%)	1092 (67.0%)	1407 (65.9%)
Ethnicity				
Hispanic or Latino	10 (3.9%)	7 (2.8%)	72 (4.4%)	89 (4.2%)
Not Hispanic or Latino	242 (95.3%)	241 (95.3%)	1526 93.7%)	2009 (94.1%)
Missing	2 (0.8%)	5 (2.0%)	31 (1.9%)	38 (1.8%)
Race				
American Indian or Alaska Native	0	0	4 (0.2%)	4 (0.2%)
Asian	158 (62.2%)	158 (62.5%)	1035 (63.5%)	1351 (63.2%)
Black or African American	0	0	2 (0.1%)	2 (0.1%)
Native Hawaiian or Other Pacific Islander	0	0	2 (0.1%)	2 (0.1%)

Characteristic	Treatment Arm			Overall (N=2136)
	VYLOY plus CAPOX (N=254)	Placebo plus CAPOX (N=253)	Not Enrolled (N=1629)	
White	94 (37.0%)	90 (35.6%)	548 (33.6%)	732 (34.3%)
Other	0	0	7 (0.4%)	7 (0.3%)
Missing	2 (0.8%)	5 (2.0%)	31 1.9%)	38 (1.8%)
ECOG Status				
0	108 (42.5%)	108 (42.7%)	0	216 (10.1%)
1	145 (57.1%)	142 (56.1%)	0	287 (13.4%)
Missing	1 (0.4%)	3 (1.2%)	1629 (100.0%)	1633 (76.5%)
Prior Gastrectomy				
Yes	73 (28.7%)	66 (26.1%)	0	139 (6.5%)
No	181 71.3%)	187 (73.9%)	0	368 (17.2%)
Missing	0	0	1629 (100.0%)	1629 (76.3%)
Medical Condition				
Gastric Adenocarcinoma	219 (86.2%)	209 (82.6%)	1271 (78.0%)	1699 (79.5%)
GEJ Adenocarcinoma	35 (13.8%)	44 (17.4%)	240 (14.7%)	319 (14.9%)
Missing	0	0	118 (7.2%)	118 (5.5%)
Sample Collection Method				
Resection	51 (20.1%)	44 (17.4%)	338 (20.7%)	433 (20.3%)
Biopsy	203 (79.9%)	209 (82.6%)	1287 (79.0%)	1699 (79.5%)
Unknown	0	0	4 (0.2%)	4 (0.2%)
Tumor Type				
Metastatic	222 (87.4%)	222 (87.7%)	1273 (78.1%)	1717 (80.4%)
Primary	32 (12.6%)	31 (12.3%)	232 (14.2%)	295 (13.8%)
Unknown	0	0	124 (7.6%)	124 (5.8%)

D. Safety and Effectiveness Results

1. Safety Results

No adverse events associated with use of VENTANA CLDN18 (43-14A) RxDx Assay were reported during the SPOTLIGHT and GLOW clinical studies.

For the complete safety evaluation of VYLOY (zolbetuximab) and specific adverse events that occurred in the SPOTLIGHT and GLOW studies, please see the VYLOY (zolbetuximab) PI available at Drugs@FDA.

2. Effectiveness Results

a. SPOTLIGHT Study - Effectiveness Results

The major efficacy outcome measure for the SPOTLIGHT study was Progression Free Survival (PFS) as assessed per RECIST v1.1 by independent review committee (IRC). Additional efficacy outcome measures were Overall Survival (OS), Objective Response Rate (ORR), and Duration of Response (DOR). In patients who were positive for CLDN18 expression, VYLOY in combination with mFOLFOX6 demonstrated a statistically significant improvement in PFS and OS compared with placebo in combination with mFOLFOX6. Results are summarized in Table 17 below.

Table 17: Efficacy Results in SPOTLIGHT Study

Endpoint	VYLOY with mFOLFOX6 N = 283	Placebo with mFOLFOX6 N = 282
Progression-Free Survival (PFS)		
Number (%) of patients with events	146 (51.6)	167 (59.2)
Median PFS [a] 95% CI)	10.6 (8.9, 12.5)	8.7 (8.2, 10.3)
HR [b,c] 95% CI)	0.751 (0.598, 0.942)	
P-value [b,d]	0.0066	
Overall Survival (OS)		
Number (%) of patients with events	149 (52.7)	177 (62.8)
Median OS [a] 95% CI)	18.2 (16.4, 22.9)	15.5 (13.5, 16.5)
HR [b,c] 95% CI)	0.750 (0.601, 0.936)	
P-value [b,d]	0.0053	
Objective Response Rate (CR + PR) [e]		
ORR (%) (95% CI) [f]	40.3 (34.5, 46.3)	39.7 (34.0, 45.7)
Complete response rate (%)	14 (4.9)	8 (2.8)
Partial response rate (%)	100 (35.3)	104 (36.9)
Duration of Response	N=114	N=112
Median in months (95% CI)	10.3 (8.3, 10.9)	10.5 (7.7, 13.3)

Endpoint	VYLOY with mFOLFOX6 N = 283	Placebo with mFOLFOX6 N = 282
Clinical cut-off date: 09-Sep-2022 CI = confidence interval, HR = hazard ratio [a] Months, based on Kaplan-Meier estimates. [b] Stratification factors were region, number of metastatic sites, and prior gastrectomy from IRT. [c] Based on a stratified Cox proportional hazards model. [d] Based on a 1-sided stratified log-rank test. [e] Based on confirmed response. [f] Based on binomial distribution (Clopper-Pearson).		

The SPOTLIGHT study showed a statistically significant improvement in PFS and OS compared with placebo. The median PFS was 10.6 months for patients who received VYLOY plus mFOLFOX6 compared with 8.7 months for patients who received placebo plus mFOLFOX6. The median OS was 18.2 months for patients who received VYLOY plus mFOLFOX6 compared with 15.5 months for patients who received placebo plus mFOLFOX6.

b. **GLOW Study - Effectiveness Results**

The major efficacy outcome measure for the GLOW study was PFS as assessed per RECIST v1.1 by IRC. Additional efficacy outcome measures were OS, ORR, and DOR. In patients who were positive for CLDN18 expression, VYLOY in combination with CAPOX demonstrated a statistically significant improvement in PFS and OS compared with placebo in combination with CAPOX. Results are summarized in the Table 18 below.

Table 18: Efficacy Results in GLOW Study

Endpoint	VYLOY with CAPOX N = 254	Placebo with CAPOX N = 253
Progression-Free Survival (PFS)		
Number (%) of patients with events	137 (53.9)	172 (68.0)
Median PFS [a] 95% CI)	8.2 (7.5, 8.8)	6.8 (6.1, 8.1)
HR [b,c] 95% CI)	0.687 (0.544, 0.866)	
P-value [b,d]	0.0007	
Overall Survival (OS)		
Number (%) of patients with events	144 (56.7)	174 (68.8)
Median OS [a] 95% CI)	14.4 (12.3, 16.5)	12.2 (10.3, 13.7)
HR [b,c] 95% CI)	0.771 (0.615, 0.965)	

Endpoint	VYLOY with CAPOX N = 254	Placebo with CAPOX N = 253
P-value [b,d]	0.0118	
Objective Response Rate (CR + PR) [e]		
ORR (%) (95% CI) [f]	32.3 (26.6, 38.4)	31.2 (25.6, 37.3)
Complete response rate (%)	6 (2.4)	2 (0.8)
Partial response rate (%)	76 (29.9)	77 (30.4)
Duration of Response	N=82	N=79
Median in months (95% CI)	8.3 (6.3, 11.4)	6.2 (6.0, 7.6)

Clinical cut-off date: 07-Oct-2022

CI = confidence interval, HR = hazard ratio

[a] Months, based on Kaplan-Meier estimates.

[b] Stratification factors were region, number of metastatic sites, and prior gastrectomy from IRT.

[c] Based on a stratified Cox proportional hazards model.

[d] Based on a 1-sided stratified log-rank test.

[e] Based on confirmed response.

[f] Based on binomial distribution (Clopper-Pearson).

The GLOW study demonstrated a statistically significant improvement in PFS and OS compared with placebo. The median PFS was 8.2 months for patients who received zolbetuximab plus CAPOX compared with 6.8 months for patients who received placebo plus CAPOX. The median OS was 14.4 months for patients who received zolbetuximab plus CAPOX compared with 12.2 months for patients who received placebo plus CAPOX.

3. **Subgroup Analyses**

Refer to VYLOY (zolbetuximab) package insert for information regarding subgroup analysis.

4. **Pediatric Extrapolation**

In this premarket application, existing clinical data from SPOTLIGHT and GLOW were leveraged to support reasonable assurance of safety and effectiveness of the proposed device in the pediatric sub-population age 18-22 years old. While in existing clinical data from SPOTLIGHT and GLOW no subjects between ages of 18-21 years old were tested in VYLOY (zolbetuximab) arm, there are no tangible clinical differences expected between a patient diagnosed at the age of 18-21 and a patient diagnosed at the age of 22 or older and data generated in population older than 21 is considered generalizable to subjects between ages 18 and 21 years old.

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. None of the clinical investigators from both SPOTLIGHT and GLOW 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 data from the analytical validation and the clinical outcome data from SPOTLIGHT and GLOW clinical studies support the reasonable assurance of safety and effectiveness of use of VENTANA CLDN18 (43-14A) RxDx Assay as a companion diagnostic device for treatment with VYLOY (zolbetuximab) in combination with chemotherapy in the target patient population with gastric or gastroesophageal (GEJ) adenocarcinoma. The efficacy analyses demonstrate a clinically significant treatment benefit in PFS and OS for patients with HER2-negative, locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma whose tumors express CLDN18.2 as determined by the VENTANA CLDN18 (43-14A) RxDx Assay (75% viable TC demonstrating moderate to strong membrane CLDN18 staining).

B. Safety Conclusions

The risks of the device are based on data collected in the non-clinical laboratory studies as well as the SPOTLIGHT and GLOW clinical studies conducted to support PMA approval as described above.

The VENTANA CLDN18 (43-14A) RxDx Assay is an in vitro diagnostic device which is used to test FFPE tumor specimens collected from patients with gastric and gastroesophageal junction (GEJ) adenocarcinoma. No adverse events associated with

the diagnostic testing procedure were reported during these studies. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely collected for diagnosis.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in the SPOTLIGHT and GLOW clinical studies (8951-CL-0301 and 8951-CL-0302, respectively) conducted to support the PMA approval as described above. The clinical performance of the VENTANA CLDN18 (43-14A) RxDx Assay was demonstrated in the clinical validation studies. Patients selected with VENTANA CLDN18 (43-14A) RxDx Assay in both clinical studies showed statistically significant and clinically meaningful PFS and OS benefits:

- The SPOTLIGHT study showed statistically significant and clinically meaningful PFS and OS benefit with a 25% reduction in the risk of disease progression or death compared with placebo.
- The GLOW study showed statistically significant and clinically meaningful PFS and OS benefit with a 31.3% and 22.9% reduction in the risk of disease progression or death, respectively, compared with placebo.

The magnitude of benefit was consistent between the two studies and the OS benefit in both studies was clinically meaningful. The data from these clinical studies support the use of VENTANA CLDN18 (43-14A) RxDx Assay as an aid to identify patients with gastric or GEJ adenocarcinoma who may be eligible for treatment with zolbetuximab in accordance with approved therapeutic labeling.

The main risk of the VENTANA CLDN18 (43-14A) RxDx assay is obtaining a false result. A false positive result could lead to the treatment with a reduced probability of benefit. This could unnecessarily expose the patient to toxicity of the drug. False negative result could deprive a patient of the potential benefit of VYLOY (zolbetuximab). The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with acceptable accuracy, mitigating the potential risk for false results.

In conclusion, given the available information above, the potential for clinical benefit outweighs the clinical risk for gastric or GEJ adenocarcinoma patients tested with the VENTANA CLDN18 (43-14A) RxDx to determine eligibility for VYLOY (zolbetuximab) therapy.

1. **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 SPOTLIGHT and GLOW studies support the use of the VENTANA CLDN18 (43-14A) RxDx Assay as a companion diagnostic to identify patients with gastric or gastroesophageal junction (GEJ) adenocarcinoma who may be eligible for treatment with VYLOY (zolbetuximab).

XV. CDRH DECISION

CDRH issued an approval order on October 18, 2024.

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

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVII. REFERENCES

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