VENTANA CLDN18 (43-14A) RxDx Assay

REF 741-6067

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IVD Σ 50

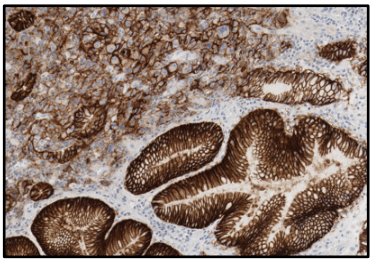

Figure 1. VENTANA CLDN18 (43-14A) RxDx Assay staining in gastric adenocarcinoma tissue.

INTENDED 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 IHC/ISH instrument.

The assay is indicated as a companion diagnostic to 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 cut-off 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.

SUMMARY AND EXPLANATION

VENTANA CLDN18 (43-14A) RxDx Assay is an immunohistochemistry (IHC) assay that utilizes a mouse monoclonal antibody (clone 43-14A) to detect the transmembrane CLDN18 proteins.

CLDN18 belongs to the claudin protein superfamily.[1] Claudins are tetramembrane proteins with two extracellular loops and the intracellular N- and C-termini regions in the cytoplasm.[1,2] Functionally, claudins are integral apical cell membrane proteins that form the tight junctions, a component of cell-cell adhesion.[1,2] In general, members of the claudin family play an essential role in maintaining a permeability barrier, regulating cell migration, and conferring polarity in epithelial cells.[1]

CLDN18 is expressed as two protein isoforms: CLDN18.1 and CLDN18.2.[1,2] Both isoforms are 261 amino acids in length; CLDN18.1 differs from CLDN18.2 in the N-terminal amino acids.[3] The primary antibody used in the VENTANA CLDN18 (43-14A) RxDx Assay targets the conserved C-terminus region of the CLDN18 protein and detects both CLDN18.1 and CLDN18.2.

CLDN18.1 is predominantly expressed in normal and neoplastic lung tissue.[1,2] CLDN18.2 is only expressed in differentiated epithelial cells of the gastric mucosa and not in other healthy tissues under normal physiological conditions.[1,2,4] Under malignant transformation, CLDN18.2 is frequently retained in gastric cancer and its metastases, and may be expressed in other neoplastic tissues (e.g., pancreas, lung, ovary).[1,2,4] Expression of CLDN18.2 in various solid tumors (e.g., gastric, pancreatic) has been reported to be associated with loss of cell-cell adhesion, epithelial-mesenchymal transition, and tumor progression and metastasis.[1,2]

Clinical Significance in Gastric Adenocarcinoma including GEJ

Gastric cancer (GC) including GEJ is one of the leading causes of cancer deaths worldwide and amongst the malignancies with the highest unmet medical needs.[5-8] Most cancers of the stomach (about 90% to 95%) are adenocarcinomas. CLDN18.2 expression is frequently noted in various solid tumors including GC/GEJ.[1,2,4]

PRINCIPLE OF THE PROCEDURE

VENTANA CLDN18 (43-14A) RxDx Assay is a mouse monoclonal primary antibody which binds to CLDN18 protein in FFPE tissue sections. The specific antibody can be located by using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the OptiView DAB IHC Detection Kit method sheet for further information.

MATERIAL PROVIDED

VENTANA CLDN18 (43-14A) RxDx Assay contains sufficient reagent for 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.

VENTANA CLDN18 (43-14A) RxDx Assay is a mouse monoclonal antibody IgG2b produced as cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Human gastric tissue with intestinal metaplasia for use as control tissue
2. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
3. Microscope slides, positively charged
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. General purpose laboratory equipment
14. BenchMark IHC/ISH Instrument
15. Permanent mounting medium
16. Cover glass
17. Automated coverslipper
18. Light Microscope

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin for 24 hours with a range from 6 to 48 hours fixation time.[9]

Sections should be cut at approximately 4 µm thick with a range from 3 µm to 5 µm and should be mounted onto positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

Ask your Roche representative for a copy of “Recommended Slide Storage and Handling” for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.10,11
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. For further information on the use of this device, refer to the Interpretation Guide, BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
9. Consult local and/or state authorities with regard to the recommended method of disposal.
10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet is available for professional user on request.
11. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must verify results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 741-6067.

Table 1. Recommended staining protocol for VENTANA CLDN18 (43-14A) RxDx Assay with OptiView DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Staining Procedure	GX CLDN18 (43-14A) RxDx	XT CLDN18 (43-14A) RxDx	ULTRA CLDN18 (43-14A) RxDx
Baking	Not selected		
Antibody (Primary)	16 minutes, 37 °C	32 minutes, 37 °C	16 minutes, 36 °C
Negative Control	16 minutes, 37 °C	32 minutes, 37 °C	16 minutes, 36 °C
Post Counterstain	Bluing, 4 minutes		

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA CLDN18 (43-14A) RxDx Assay, a second slide should be stained with Negative Control (Monoclonal).

POSITIVE AND NEGATIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

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. For appropriate staining and evaluation of the human gastric tissue with intestinal metaplasia, refer to Table 2.

Table 2. VENTANA CLDN18 (43-14A) RxDx Assay 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

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by the VENTANA CLDN18 (43-14A) RxDx Assay. The cellular staining pattern for VENTANA CLDN18 (43-14A) RxDx Assay is membranous staining on gastric adenocarcinoma tissue including the gastroesophageal junction. Cytoplasmic staining can also be seen, but is not included in the scoring algorithm. A qualified pathologist experienced in immunohistochemical procedures must evaluate system-level controls and qualify the stained product before interpreting results.

Patient tissue must be evaluated according to the VENTANA CLDN18 (43-14A) RxDx Assay scoring algorithm, which is provided in Table 3. Refer to the VENTANA CLDN18 (43-14A) RxDx Assay Interpretation Guide 1016391EN for the indication tissue of gastric adenocarcinoma tissue including the gastroesophageal junction. Representative images are provided in the interpretation guide.

Table 3. VENTANA CLDN18 (43-14A) RxDx Assay scoring algorithm for gastric adenocarcinoma including the gastroesophageal junction.

IHC Interpretation	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

SPECIFIC LIMITATIONS

1. VENTANA CLDN18 (43-14A) RxDx Assay has been developed for BenchMark IHC/ISH instruments with the OptiView DAB IHC Detection Kit and is not approved with any other detection or instruments.
2. The primary antibody used in the VENTANA CLDN18 (43-14A) RxDx Assay targets the conserved C-terminus region of the CLDN18 protein and detects both CLDN18.1 and CLDN18.2. CLDN18.1 is either not detected or minimally expressed in gastric or GEJ adenocarcinoma tissues.[2,13,14]
3. A patient specimen slide should be stained with Negative Control (Monoclonal) 760-2014. Other negative control reagents are not suitable for this assay.
4. This assay has not been validated for use with cytology samples or decalcified bone specimens
5. VENTANA CLDN18 (43-14A) RxDx Assay may produce IHC staining in normal tonsil,[12] pneumocytes in normal lung tissue,[15,16] normal gastric tissue,[17,18] and paneth cells of normal small intestine tissue.[19]
6. It is not recommended to use normal tonsil as the negative control tissue for VENTANA CLDN18 (43-14A) RxDx Assay.
7. Cut slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slide stability within their own environment when storing beyond 45 days, when desired.
8. Use of alcohol-formalin-acetic acid (AFA), 95% ethanol, PREFER fixatives and Zinc Formalin are not recommended for use with this assay due to observed limitation with either signal intensity or based on issue of sectioning.
9. This assay might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity was evaluated by characterizing commercially acquired tissue samples. A cohort of 318 unique gastric or GEJ adenocarcinoma biopsy and resections tissue cases demonstrated 23.3% (74/318) positive IHC status with VENTANA CLDN18 (43-14A) RxDx Assay.

Analytical specificity was determined by staining normal and neoplastic tissues with VENTANA CLDN18 (43-14A) RxDx Assay. Staining results are listed in Table 4 and Table 5.

Table 4. Specificity of VENTANA CLDN18 (43-14A) RxDx Assay was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# 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[12]

[b] CLDN18 staining was present: membrane staining in pneumocytes[15,16]

[c] CLDN18 staining was present: membrane staining in the gastric epithelium[17,18]

[d] CLDN18 staining was present: membrane and cytoplasmic staining in paneth cells[19]

Table 5. Specificity of VENTANA CLDN18 (43-14A) RxDx Assay was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# 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

Pathology	# positive / total cases
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
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[20]

Precision

Precision of Slide Evaluation was determined by testing multiple pathologists who were trained with the assay scoring algorithm for their within-reader and between-reader reproducibility, using 100 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative on a BenchMark ULTRA instrument. Results are listed in Table 6 and Table 7.

Table 6. Within-reader precision across three readers.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Average Positive Agreement	296/300	98.7% (97.3%, 99.7%)
Average Negative Agreement	296/300	98.7% (97.3%, 99.7%)
Overall Percent Agreement	296/300	98.7% (97.3%, 99.7%)

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

Table 7. Between-reader precision across three readers.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Average Positive Agreement	296/300	98.7% (96.6%, 100.0%)
Average Negative Agreement	296/300	98.7% (96.6%, 100.0%)
Overall Percent Agreement	296/300	98.7% (96.7%, 100.0%)

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

Precision Study of Antibody Lots

VENTANA CLDN18 (43-14A) RxDx Assay antibody lot-to-lot reproducibility was tested with 24 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative, using three antibody lots on a BenchMark ULTRA instrument. Results are listed in Table 8.

Table 8. Agreement rate between-antibody lots.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Negative Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Overall Percent Agreement	144/144	100.0% (97.4%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of OptiView Detection System Lots

Three lots of OptiView DAB IHC Detection Kit lot-to-lot reproducibility was tested with 24 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative, using VENTANA CLDN18 (43-14A) RxDx Assay antibody on a BenchMark ULTRA instrument. Results are listed in Table 9.

Table 9. Agreement rate between-detection kit lots.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Negative Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Overall Percent Agreement	144/144	100.0% (97.4%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of BenchMark ULTRA Instruments

Between BenchMark ULTRA instrument reproducibility was tested with 24 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative on three BenchMark ULTRA instruments. Results are listed in Table 10.

Table 10. Agreement rate between BenchMark ULTRA instruments.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Negative Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Overall Percent Agreement	144/144	100.0% (97.4%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of Between Staining Days

Between-day reproducibility was tested with 24 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative on a BenchMark ULTRA instrument. Results are listed in Table 11.

Table 11. Agreement rate between-days.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Negative Percent Agreement	72/72	100.0% (94.9%, 100.0%)
Overall Percent Agreement	144/144	100.0% (97.4%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of Within Staining Run

Within-run reproducibility evaluated the duplicate slides from the precision studies of 24 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative. Results are listed in Table 12.

Table 12. Agreement rate within-runs.

Agreement Rate	n/N (Sample Pairs)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	108/108	100.0% (96.6%, 100.0%)
Negative Percent Agreement	108/108	100.0% (96.6%, 100.0%)
Overall Percent Agreement	216/216	100.0% (98.3%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Study of BenchMark GX, XT and ULTRA Instruments

Staining on BenchMark GX and XT instruments were tested using BenchMark ULTRA instrument as the reference. This study tested with 44 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative. Results are listed in Table 13.

Table 13. Agreement rate comparing to BenchMark ULTRA instruments.

Agreement Rate	n/N (GX Sample)	GX Percentage (95% Confidence Interval) a	n/N (XT Sample)	XT Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	21/21	100.0% (84.5%, 100.0%)	20/20	100.0% (83.9%, 100.0%)
Negative Percent Agreement	23/23	100.0% (85.7%, 100.0%)	23/23	100.0% (85.7%, 100.0%)
Overall Percent Agreement	44/44	100.0% (92.0%, 100.0%)	43/43	100.0% (91.8%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of BenchMark GX Instruments

Between BenchMark GX instrument reproducibility was tested with 14 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative on three BenchMark GX instruments. Results are listed in Table 14.

Table 14. Agreement rate between BenchMark GX instruments.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	42/42	100.0% (91.6%, 100.0%)
Negative Percent Agreement	42/42	100.0% (91.6%, 100.0%)
Overall Percent Agreement	84/84	100.0% (95.6%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

Precision Study of BenchMark XT Instruments

Between BenchMark XT instrument reproducibility was tested with 14 gastric adenocarcinoma including GEJ tissue cases that encompassed CLDN18 IHC staining status range of positive and negative on three BenchMark XT instruments. Results are listed in Table 15.

Table 15. Agreement rate between BenchMark XT instruments.

Agreement Rate	n/N (Sample)	Percentage (95% Confidence Interval)[a]
Positive Percent Agreement	42/42	100.0% (91.6%, 100.0%)
Negative Percent Agreement	42/42	100.0% (91.6%, 100.0%)
Overall Percent Agreement	84/84	100.0% (95.6%, 100.0%)

[a] 2-sided 95% confidence interval calculated using the Wilson Score method.

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 cases, using VENTANA CLDN18 (43-14A) RxDx Assay antibody on a BenchMark ULTRA instrument. The recommended section thickness range is 3-5 µm for the VENTANA CLDN18 (43-14A) RxDx Assay.

Inter-Laboratory Reproducibility

An inter-laboratory reproducibility study for VENTANA CLDN18 (43-14A) RxDx Assay was conducted to demonstrate reproducibility of the assay in determining CLDN18 IHC status in gastric adenocarcinoma including GEJ cancer cases that were stained and scored at three external sites (laboratories) A, B, C. This study analyzed 28 gastric adenocarcinoma including GEJ tissue specimens encompassed of 14 CLDN18 positive and 14 CLDN18 negative cases. 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 to assign a CLDN18 IHC status at the 75% cut-off. Results from sites A, B, and C are listed in Table 16.

Slides 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 to assign a CLDN18 IHC status at the 75% cut-off. Results from sites X, Y, and Z are also listed in Table 16.

Table 16. Inter-laboratory reproducibility.

Reproducibility Tested	Agreement Rate	Sites A, B, C		Sites X, Y, Z	
		n/N (Sample)	Percentage (95% CI)[b]	n/N (Sample)	Percentage (95% CI)[b]
Overall agreement[a] (across sites, days and readers)	Positive Percent Agreement	380/419	90.7% (85.5%, 95.9%)	408/420	97.1% (94.4%. 99.5%)
	Negative Percent Agreement	386/419	92.1% (86.5%, 97.8%)	390/420	92.9% (86.7%, 97.4%)
	Overall Percent Agreement	766/838	91.4% (88.2%, 94.2%)	798/840	95.0% (91.7%, 97.7%)
Inter-site agreement (average of site-to-site pairwise comparisons)	Average Positive Agreement	7128/8241	86.5% (80.9%, 91.2%)	8014/8760	91.5% (85.9%, 96.3%)
	Average Negative Agreement	7366/8479	86.9% (81.7%, 91.0%)	7294/8040	90.7% (84.4%, 95.6%)
	Overall Percent Agreement	7247/8360	86.7% (81.4%, 91.0%)	7654/8400	91.1% (85.3%, 96.0%)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	Average Positive Agreement	344/412	83.5% (76.8%, 89.2%)	416/438	95.0% (92.3%, 97.6%)
	Average Negative Agreement	356/424	84.0 % (78.5%, 88.7%)	380/402	94.5% (91.6%, 97.2%)
	Overall Percent Agreement	350/418	83.7% (77.9%, 88.9%)	398/420	94.8% (91.9%, 97.4%)

[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.

CLINICAL PERFORMANCE

Clinical Outcome Studies - SPOTLIGHT and GLOW

The clinical performance of VENTANA CLDN18 (43-14A) RxDx Assay was evaluated in two phase 3, double-blind, randomized, multicenter studies that enrolled 1072 patients whose tumors were CLDN18.2 positive, HER2-negative, with locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma [SPOTLIGHT and GLOW]. CLDN18.2 positivity (defined as ≥75% of tumor cells demonstrating moderate to strong membranous CLDN18 staining) was determined by immunohistochemistry on gastric or GEJ tumor tissue specimens from all patients with the VENTANA CLDN18 (43-14A) RxDx Assay performed in a central laboratory.

Patients were randomized 1:1 to receive zolbetuximab in combination with chemotherapy (n=283 in SPOTLIGHT, n=254 in GLOW) or placebo in combination with chemotherapy (n=282 in SPOTLIGHT, n=253 in GLOW). Zolbetuximab was administered intravenously at a loading dose of 800 mg/m2 (Day 1 of cycle 1) followed by maintenance doses of 600 mg/m2 every 3 weeks in combination with either mFOLFOX6 (oxaliplatin, folinic acid and fluorouracil), or CAPOX (oxaliplatin and capecitabine).

Baseline characteristics were generally similar between studies, except for the proportion of Asian versus non-Asian patients in each study.

In the SPOTLIGHT study, the median age was 61 years (range: 20 to 86); 62% were male; 53% were Caucasian, 38% were Asian; 31% were from Asia and 69% were not from Asia. Patients had a baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0 (43%) or 1 (57%). Patients had a mean body surface area of 1.7 m^2 (range: 1.1 to 2.5). The median time from diagnosis was 56 days (range: 2 to 5366); 36% of tumor types were diffuse, 24% were intestinal; 76% had gastric adenocarcinoma, 24% had GEJ adenocarcinoma; 16% had locally advanced disease and 84% had metastatic disease.

In the GLOW study, the median age was 60 years (range: 21 to 83); 62% were male; 37% were Caucasian, 63% were Asian; 62% were from Asia and 38% were not from Asia. Patients had a baseline ECOG performance status of 0 (43%) or 1 (57%). Patients had a mean body surface area of 1.7 m^2 (range: 1.1 to 2.3). The median time from diagnosis was 44 days (range: 2 to 6010); 37% of tumor types were diffuse, 15% were intestinal; 84% had gastric adenocarcinoma, 16% had GEJ adenocarcinoma; 12% had locally advanced disease and 88% had metastatic disease.

The primary efficacy outcome was progression free survival (PFS) as assessed per RECIST v1.1 by independent review committee (IRC). The key secondary efficacy outcome was overall survival (OS). Other secondary efficacy outcomes were objective response rate (ORR) and duration of response (DOR) as assessed per RECIST v1.1 by IRC.

In the primary analysis (final PFS and interim OS), the SPOTLIGHT study demonstrated a statistically significant benefit in PFS (as assessed by IRC) and OS for patients who received zolbetuximab in combination with mFOLFOX6 compared with patients who received placebo in combination with mFOLFOX6 treatment. The PFS HR was 0.751 (95% CI: 0.598, 0.942; 1-sided p = 0.0066) and the OS HR was 0.750 (95% CI: 0.601, 0.936; 1-sided p = 0.0053). The updated PFS and final OS for SPOTLIGHT are presented in Table 17.

Table 17. Efficacy Results in SPOTLIGHT

Endpoint	Zolbetuximab with mFOLFOX6 N = 283	Placebo with mFOLFOX6 N = 282
Progression-Free Survival (PFS)		
Number (%) of patients with events	159 (56.2)	187 (66.3)
Median PFS[a] (95% CI)	11.0 (9.7, 12.5)	8.9 (8.2, 10.4)
HR[b,c] (95% CI)	0.734 (0.591, 0.910)	
Overall Survival (OS)		
Number (%) of patients with events	197 (69.6)	217 (77.0)
Median OS[a] (95% CI)	18.2 (16.1, 20.6)	15.6 (13.7, 16.9)
HR[b,c] (95% CI)	0.784 (0.644, 0.954)	
Objective Response Rate (ORR), Duration of Response (DOR)		
ORR[d] (%) (95% CI)	48.1 (42.1, 54.1)	47.5 (41.6, 53.5)
Median DOR in months[d] (95% CI)	9.0 (7.5, 10.4)	8.1 (6.5, 11.4)

Clinical cutoff date: 08-Sep-2023; median follow-up time of zolbetuximab in combination with mFOLFOX6 arm was 18.0 months.

CI = confidence interval, HR = hazard ratio

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

[b] Stratification factors were region, number of metastatic sites, prior gastrectomy from interactive response technology and study ID (SPOTLIGHT/GLOW).

[c] Based on Cox proportional hazards model with treatment, region, number of organs with metastatic sites, prior gastrectomy as the explanatory variables and study ID (SPOTLIGHT/GLOW).

[d] Based on IRC assessment and unconfirmed responses.

In the primary analysis (final PFS and interim OS), the GLOW study demonstrated a statistically significant benefit in PFS (as assessed by IRC) and OS for patients who received zolbetuximab in combination with CAPOX compared with patients who received placebo in combination with CAPOX treatment. The PFS HR was 0.687 (95% CI: 0.544, 0.866; 1-sided p = 0.0007) and the OS HR was 0.771 (95% CI: 0.615, 0.965; 1-sided p = 0.0118). The updated PFS and final OS analysis for GLOW are presented in Table 18.

Table 18. Efficacy Results in GLOW

Endpoint	Zolbetuximab with CAPOX N = 254	Placebo with CAPOX N = 253
Progression-Free Survival (PFS)		
Number (%) of patients with events	153 (60.2)	182 (71.9)
Median PFS[a] (95% CI)	8.2 (7.3, 8.8)	6.8 (6.1, 8.1)
HR[b,c] (95% CI)	0.689 (0.552, 0.860)	
Overall Survival (OS)		
Number (%) of patients with events	180 (70.9)	207 (81.8)
Median OS[a] (95% CI)	14.3 (12.1, 16.4)	12.2 (10.3, 13.7)
HR[b,c] (95% CI)	0.763 (0.622, 0.936)	
Objective Response Rate (ORR), Duration of Response (DOR)		
ORR[d] (%) (95% CI)	42.5 (36.4, 48.9)	39.1 (33.1, 45.4)
Median DOR in months[d] (95% CI)	6.3 (5.4, 8.3)	6.1 (4.4, 6.3)

Clinical cutoff date: 12-Jan-2024; median follow-up time of zolbetuximab in combination with CAPOX arm was 20.6 months.

CI = confidence interval, HR = hazard ratio

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

[b] Stratification factors were region, number of metastatic sites, prior gastrectomy from interactive response technology and study ID (SPOTLIGHT/GLOW).

[c] Based on Cox proportional hazards model with treatment, region, number of organs with metastatic sites, prior gastrectomy as the explanatory variables and study ID (SPOTLIGHT/GLOW).

[d] Based on IRC assessment and unconfirmed responses.

A combined efficacy analysis of SPOTLIGHT and GLOW of the final OS and updated PFS resulted in a median PFS (as assessed by IRC) of 9.2 months (95% CI: 8.4, 10.4) for zolbetuximab in combination with mFOLFOX6/CAPOX versus 8.2 months (95% CI: 7.6, 8.4) for placebo with mFOLFOX6/CAPOX (HR 0.712, 95% CI: 0.610, 0.831) and a median OS for zolbetuximab in combination with mFOLFOX6/CAPOX of 16.4 months (95% CI: 15.0, 17.9) versus 13.7 months (95% CI: 12.3, 15.3) for placebo with mFOLFOX6/CAPOX (HR 0.774, 95% CI: 0.672, 0.892).

Exploratory subgroup analyses of efficacy for SPOTLIGHT and GLOW showed a difference in PFS and OS for Caucasian versus Asian patients.

For SPOTLIGHT, in Caucasian patients this resulted in a PFS (as assessed by IRC) with a HR of 0.872 (95% CI: 0.653, 1.164) and an OS HR of 0.940 (95% CI: 0.718, 1.231) for zolbetuximab in combination with mFOLFOX6 versus placebo with mFOLFOX6. In Asian patients, this resulted in a PFS (as assessed by IRC) with a HR of 0.526 (95% CI: 0.354, 0.781) and an OS HR of 0.636 (95% CI: 0.450, 0.899) for zolbetuximab in combination with mFOLFOX6 versus placebo with mFOLFOX6. For GLOW, in Caucasian patients this resulted in a PFS (as assessed by IRC) with a HR of 0.891 (95% CI: 0.622, 1.276) and an OS HR of 0.805 (95% CI: 0.579, 1.120) for zolbetuximab in combination with CAPOX versus placebo with CAPOX. In Asian patients, this resulted in a PFS (as assessed by IRC) with a HR of 0.616 (95% CI: 0.467, 0.813) and an OS HR of 0.710 (95% CI: 0.549, 0.917) for zolbetuximab in combination with CAPOX versus placebo with CAPOX.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. Extended stay of the slides on the instrument after run completion may affect quality and intensity of the staining. Remove slides promptly at the end of the run and proceed to post-instrument processing.
8. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:
https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information):

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
A	Initial Release

INTELLECTUAL PROPERTY

For USA: Rx only

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