Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples for Patient Identification in Two Phase 3 Trials of Zolbetuximab

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BACKGROUND

- Gastric cancer is the fifth most common cancer worldwide with about 769,000 deaths and >1 million new cases estimated in 2020; most gastric cancers are adenocarcinomas[1,2]
- Current treatment options for locally advanced (LA) unresectable or metastatic gastric or gastroesophageal junction (mG/GEJ) adenocarcinoma include chemotherapy, targeted therapies, and immunotherapy, but the efficacy is limited[2–4]
- There is an unmet need to develop additional targeted therapies to treat patients whose tumors are human epidermal growth factor receptor 2 (HER2)-negative[3,4]
- Claudin-18 isoform 2 (CLDN18.2) is expressed in normal gastric mucosa cells and retained in G/GEJ adenocarcinoma cells[3–8]
  - CLDN18 belongs to the family of claudin tight junction proteins which play an essential role in regulating permeability, cell migration, and polarity in epithelial cells[6,7]
  - Claudin-18 isoform 1 (CLDN18.1) is predominantly expressed only in normal and neoplastic lung cells[5,6,8]
  - CLDN18.2 is the dominant CLDN18 isoform in G/GEJ adenocarcinoma[3,6,8]
- Zolbetuximab, a monoclonal immunoglobulin G1 antibody that targets CLDN18.2, induces antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity in G/GEJ adenocarcinoma cells expressing CLDN18.2[9]
- The VENTANA CLDN18 (43-14A) RxDx Assay is an investigational immunohistochemistry (IHC) assay that utilizes a mouse monoclonal antibody and was designed for high sensitivity and specificity in the detection of CLDN18
- The investigational VENTANA CLDN18 (43-14A) RxDx Assay was used as an aid to identify patients with previously untreated, LA unresectable or mG/GEJ adenocarcinoma whose tumors are CLDN18.2-positive and HER2-negative in the global, phase 3 SPOTLIGHT (NCT03504397) and GLOW (NCT03653507) studies[3,4]
- We present data showing the robustness of analytical and clinical performance of the investigational VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma

METHODS

Investigational VENTANA CLDN18 (43-14A) RxDx Assay

- IHC was performed on slides prepared from formalin-fixed, paraffin-embedded (FFPE) tumor samples on the BenchMark ULTRA automated staining instrument (Roche) using the staining protocol in Table 1
  - The assay required 1 slide each for staining with (1) CLDN18 antibody, (2) negative reagent control, and (3) hematoxylin and eosin (H&E)
  - Each batch of case slides was also stained with a system level control slide of gastric tissue with intestinal metaplasia with CLDN18-positive and CLDN18-negative elements (Table 2)

Table 1. Staining procedure for the investigational VENTANA CLDN18 (43-14A) RxDx Assay

Procedure Parameter	Selection
Deparaffinization	Paraffin, Depara (72°C)
Cell conditioning solution 1	ULTRA CC1, 64 min (100°C)
Pre-primary peroxidase inhibitor	Used
CLDN18 (43-14A) or NRC	16 min (36°C)
OptiView HQ universal linker	8 min
OptiView HRP multimer	8 min
Counterstain: hematoxylin II	8 min
Post-counterstain: bluing reagent	4 min (selected)

CLDN18, claudin-18; NRC, negative reagent control; HRP, horseradish peroxidase.

Table 2. Criteria for evaluation of system level control (gastric tissue with intestinal metaplasia)

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

CLDN18, claudin-18.

- A staining result was considered valid if the H&E, system level control, negative reagent control, and CLDN18 slides were all acceptable—including case-tissue morphology and background staining (Table 3)

Table 3. Acceptability of background staining and case tissue morphology

Interpretation	Morphology	Background
Acceptable	Cellular elements of interest are visualized allowing interpretation of the stain	Nonspecific staining that is not obtrusive to interpretation of specific staining
Not acceptable	Cellular elements of interest are not visualized compromising interpretation of the stain	Nonspecific staining that is obtrusive to interpretation of specific staining

- Samples with valid CLDN18 IHC results were stratified as either positive or negative according to the scoring algorithm in Table 4

Table 4. Scoring algorithm for CLDN18 staining in G/GEJ adenocarcinoma

IHC Interpretation	Staining Description
Positive	≥75% of tumor cells demonstrating moderate-to-strong membrane CLDN18 staining
Negative	<75% of tumor cells demonstrating moderate-to-strong membrane CLDN18 staining

CLDN18, claudin-18.

Analytical Performance Studies

- G/GEJ adenocarcinoma tissue cases, representing a range of CLDN18 expression levels, were obtained from commercial sources and tested across the scenarios in Table 5

Table 5. Analytical performance studies of the investigational VENTANA CLDN18 (43-14A) RxDx Assay

Study	Description
Between-day[a,b]	24 Tissue cases were stained across 3 nonconsecutive days
Within-run, between-instrument, and between-lot[a–c]	24 Tissue cases were stained with 3 lots of VENTANA CLDN18 (43-14A) RxDx Assay antibody, 3 lots of OptiView IHC Detection Kit, and 3 BenchMark ULTRA instruments
Between-reader and within-reader precision[a,b]	100 Tissue cases were independently evaluated by 3 trained pathologists; each pathologist read the same set of samples twice, with a minimum of 2 weeks wash-out period between assessments
Interlaboratory reproducibility[a,b]	28 Tissue cases were stained in 3 external clinical pathology laboratories within 5 nonconsecutive staining days per site, and were evaluated by 2 pathologists at each site

[a]For studies besides within-run, the result from each case was compared to its respective case-level modal staining result and deemed concordant or discordant.
[b]Results were aggregated across cases for agreement rates.
[c]For within-run, duplicate samples from each case and condition were compared and deemed concordant or discordant.
CLDN18, claudin-18.

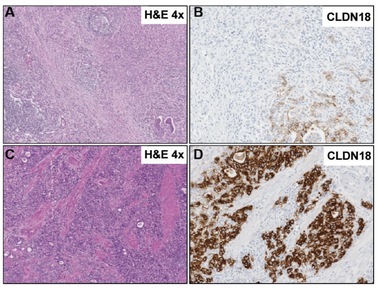
Clinical Performance Studies

- The investigational VENTANA CLDN18 (43-14A) RxDx Assay was used to evaluate CLDN18.2 status by IHC on the BenchMark ULTRA instrument to assess patient eligibility for enrollment in SPOTLIGHT and GLOW[3,4]
  - Ideally, slides were prepared with 4 µM thick sections from FFPE samples fixed in 10% neutral buffered formalin for 6–48 h (routinely processed FFPE samples were suitable); slides were stained within 6 months of cutting from the block
  - IHC was performed at Q[2] laboratories (Beijing, China; Edinburgh, UK; Singapore; Valencia, USA)
- Tumor samples from screened patients who completed CLDN18 testing per the diagnostic protocol and met eligibility criteria per the clinical protocol were evaluated for staining acceptability rates
  - Overall staining acceptability rates were calculated at the subject-level as the percentage of patients with a valid CLDN18 result

RESULTS

- Representative G/GEJ adenocarcinoma tumor samples with positive and negative IHC status are shown in Figure 1

Figure 1. Tumor-cell staining detected by the investigational VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma



A–B. H&E (A) and CLDN18 IHC (B) staining of tumor sample with CLDN18-negative IHC status demonstrating moderate-to-strong membrane staining in 20% of tumor cells.
C–D. H&E (C) and CLDN18 IHC (D) staining of tumor sample with CLDN18-positive IHC status demonstrating moderate-to-strong membrane staining in 95% of tumor cells.
CLDN18, claudin-18; H&E, hematoxylin and eosin; G/GEJ, gastric or gastroesophageal junction; IHC, immunohistochemistry.

Analytical Performance Studies

- Reproducibility and precision rates for CLDN18 staining in G/GEJ adenocarcinoma samples using the investigational VENTANA CLDN18 (43-14A) RxDx Assay are shown in Tables 6–8

Table 6. Repeatability of the investigational VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma

Study	Agreement Rate	% (n/N)	95% CI
Between-antibody-lot	PPA, NPA	100.0 (72/72)	94.9, 100.0
	OPA	100.0 (144/144)	97.4, 100.0
Between-detection-kit-lot	PPA, NPA	100.0 (72/72)	94.9, 100.0
	OPA	100.0 (144/144)	97.4, 100.0
Between-instruments	PPA, NPA	100.0 (72/72)	94.9, 100.0
	OPA	100.0 (144/144)	97.4, 100.0
Between-day	PPA, NPA	100.0 (72/72)	94.9, 100.0
	OPA	100.0 (144/144)	97.4, 100.0
Within-run	PPA, NPA	100.0 (108/108)	96.6, 100.0
	OPA	100.0 (216/216)	98.3, 100.0

CI, confidence interval; CLDN18, claudin-18; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement.

Table 7. Between-reader and within-reader precision of the investigational VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma

Agreement Rate	Between-Reader Precision		Within-Reader Precision	
	% (n/N)	95% CI	% (n/N)	95% CI
APA	98.7 (296/300)	96.6, 99.7	98.7 (296/300)	97.3, 99.7
ANA	98.7 (296/300)	96.6, 99.7	98.7 (296/300)	97.3, 99.7
OPA	98.7 (296/300)	96.6, 99.7	98.7 (296/300)	97.3, 99.7

ANA, average negative agreement; APA, average positive agreement; CI confidence interval; CLDN18, claudin-18; OPA, overall percent agreement.

Table 8. Interlaboratory reproducibility of the investigational VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma

Agreement Rate	Overall Inter-site Agreement		Overall Inter-reader Agreement	
	% (n/N[a])	95% CI	% (n/N[a])	95% CI
APA	91.5 (8014/8760)	85.9, 96.3	95.0 (416/438)	92.3, 97.6
ANA	90.7 (7294/8040)	84.4, 95.6	94.5 (380/402)	91.6, 97.2
OPA	91.1 (7654/8400)	85.3, 96.0	94.8 (398/420)	91.9, 97.4

[a]Counts indicate the number of pairwise comparisons and do not represent the number of unique cases.
ANA, average negative agreement; APA, average positive agreement; CI confidence interval; CLDN18, claudin-18; OPA, overall percent agreement.

Clinical Performance Studies

- Staining acceptability rates for the investigational VENTANA CLDN18 (43-14A) RxDx Assay in a clinical use setting from the SPOTLIGHT and GLOW studies are shown in Table 9

Table 9. Staining acceptability rates of the investigational VENTANA CLDN18 (43-14A) RxDx Assay in SPOTLIGHT and GLOW

Patient Characteristics	SPOTLIGHT				GLOW			
	Initial[a]		Final[b]		Initial[a]		Final[b]	
	n/N	Rate (%), 95% CI[c]	n/N	Rate (%), 95% CI[c]	n/N	Rate (%), 95% CI[c]	n/N	Rate (%), 95% CI[c]
Overall staining	1916/2020	94.9 (93.8, 95.7)	1994/2020	98.7 (98.1, 99.1)	1672/1763	94.8 (93.7, 95.8)	1737/1763	98.5 (97.8, 99.0)
Background	1950/1968	99.1 (98.6, 99.4)	2001/2005	99.8 (99.5, 99.9)	1698/1714	99.1 (98.5, 99.4)	1741/1744	99.8 (99.5, 99.9)
Morphology	1946/1968	98.9 (98.3, 99.3)	1996/2005	99.6 (99.1, 99.8)	1686/1714	98.4 (97.6, 98.9)	1740/1745	99.7 (99.3, 99.9)

[a]The initial staining attempt is the attempt with the earliest staining date in the first sample accessioned.
[b]The final staining attempt is the attempt associated with the staining result used to determine patient CLDN18.2 status in SPOTLIGHT and GLOW.
[c]Two-sided 95% confidence interval calculated using the score method.
CI, confidence interval; CLDN18, claudin-18; CLDN18.2, claudin-18 isoform 2.

- SPOTLIGHT and GLOW demonstrated clinically meaningful and statistically significant improvements in progression-free survival (PFS) and overall survival (OS) in patients randomly assigned to receive zolbetuximab plus chemotherapy versus placebo plus chemotherapy (Table 10)[3,4]

Table 10. Efficacy of zolbetuximab plus chemotherapy in SPOTLIGHT and GLOW[3,4]

	SPOTLIGHT		GLOW	
	Zolbetuximab plus mFOLFOX6 (n = 283)	Placebo plus mFOLFOX6 (n = 282)	Zolbetuximab plus CAPOX (n = 254)	Placebo plus CAPOX (n = 253)
PFS				
Events, n (%)	146 (51.6)	167 (59.2)	137 (53.9)	172 (68.0)
Median, months (95% CI)	10.61 (8.90, 12.48)	8.67 (8.21, 10.28)	8.21 (7.46, 8.84)	6.80 (6.14, 8.08)
HR (95% CI); *P*-value	0.751 (0.598, 0.942); 0.0066		0.687 (0.544, 0.866); 0.0007	
OS				
Events, n (%)	149 (52.7)	177 (62.8)	144 (56.7)	174 (68.8)
Median, months (95% CI)	18.23 (16.43, 22.90)	15.54 (13.47, 16.53)	14.39 (12.29, 16.49)	12.16 (10.28, 13.67)
HR (95% CI); *P*-value	0.750 (0.601, 0.936); 0.0053		0.771 (0.615, 0.965); 0.0118	

CAPOX, capecitabine and oxaliplatin regimen; CI, confidence interval; HR, hazard ratio; mFOLFOX6, modified folinic acid, fluorouracil, and oxaliplatin regimen; PFS, progression-free survival; OS, overall survival.

CONCLUSIONS

- The VENTANA CLDN18 (43-14A) RxDx Assay demonstrated robust analytical and clinical performance in G/GEJ adenocarcinoma tissue
- Analytical performance studies demonstrated reproducibility versus variation in reagent lot, instrument, day, site, and reader
- Performance in phase 3 SPOTLIGHT and GLOW studies demonstrated high overall staining acceptability rates
- The clinically significant improvements in PFS and OS in patients whose tumors are CLDN18.2-positive in SPOTLIGHT and GLOW, identified by the investigational VENTANA CLDN18 (43-14A) RxDx Assay, demonstrate the potential clinical utility of this assay as a companion diagnostic for reliably identifying patients who may benefit from first-line treatment with zolbetuximab plus chemotherapy

References

1. Sung H et al. *CA Cancer J Clin*. 2021;71:209-249.
2. Lordick F et al. *Ann Oncol*. 2022;33:1005-1020.
3. Shitara K et al. *Lancet*. 2023;401:1655-1668.
4. Shah MA et al. *Nat Med*. 2023;29:2133–2141.
5. Niimi T et al. *Mol Cell Bio*. 2001;21:7380–7390.
6. Hashimoto I and Oshima T. *Cancers (Basel)*. 2022;14:290.
7. Singh P et al. *J Hematol Oncol*. 2017;10:105.
8. Li J et al. *Aging*. 2020;12:14271–14284.
9. Mitnacht-Kraus R et al. *Ann Oncol*. 2018;28:v126.

Acknowledgements

The authors thank the patients who participated in the studies and their families. Thank you to Julie Buckmeier for her contributions to this study. BenchMark and VENTANA are trademarks of Roche.

Funding

This study was funded by Astellas Pharma Inc. Medical writing support was provided by Ann Ferguson, PhD of Oxford PharmaGenesis Inc., Newtown, PA, USA, and funded by Astellas Pharma Inc.

Conflicts of interest

SS, **LP**, **JP**, **MK**, **DB**, and **JM** are employees of Roche.
AG and **DM** are full-time employees of Astellas Pharma US, Inc.

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