HKIAP SCIENTIFIC CONGRESS 2026

Dr. Brendan Pang

From Biomarker to Routine Practice: Practical Considerations for Implementing CLDN18.2 Testing in Gastric Cancer

6–8 November 2026 | Postgraduate Education Center, Prince of Wales Hospital, Hong Kong

Disclaimer: This educational symposium is sponsored by Astellas. The content is intended for healthcare professionals only and complies with local regulations governing scientific exchange and communication of approved medicines. The opinions expressed are those of the speakers and may not necessarily reflect those of Astellas. Please refer to local prescribing information for prescription.

MA-MM-24845 Nov 2026

Disclosures

Consultancy	AstraZeneca, Merck Serono, Merck Sharp & Dohme, Novartis, Amgen, Pfizer, Roche, Astellas, Daiichi Sankyo
Honoraria (speaker)	Merck Sharp & Dohme, Novartis, Roche, Ventana, Amgen, Agilent, Astellas, Daiichi Sankyo, AstraZeneca

Vyloy:
Addressing a Critical Unmet Need in HER2-Negative Gastric Cancer



Drug Advisory Committee (DAC) – May 2026 Outcome:

Zolbetuximab (VYLOY) Granted SFI Approval



Indicated for the **first-line** treatment of adult patients with **locally advanced unresectable or metastatic** HER2-negative **gastric or gastro-oesophageal junction (GEJ) adenocarcinoma** whose tumours are **Claudin (CLDN) 18.2 positive**

Source: https://www.ha.org.hk/hadf/en/News/Dac-Meeting-Outcome-May-2026.html#gsc.tab=0

Guidelines for metastatic G/GEJ adenocarcinoma recommend biomarker testing to help support clinical decision-making for patients

NCCN National Comprehensive Cancer Network®

Assessment of Positivity of **Claudin 18 Isoform 2** in Gastric cancer
- For patients with untreated inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the stomach for whom zolbetuximab therapy is being considered.

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Metastatic Disease > First – line HER2-negative
- **CLDN18.2** test has been recommended with an ESCAT score of I (ready for routine use)

JGCA Japanese Gastric Cancer Association

CLDN18.2 is a useful novel biomarker for gastric cancer, and its expression needs to be confirmed before treatment.

대한위암학회 The Korean Gastric Cancer Association

Palliative first-line systemic therapy with zolbetuximab combined with capecitabine or fluorouracil plus oxaliplatin (CAPOX or FOLFOX) is recommended in patients with HER2-negative and **claudin 18.2-positive** and locally advanced unresectable or metastatic gastric cancer. [Grade of Recommendation: Strong for]

1. NCCN Clinical Practice Guidelines in Oncology: Gastric Cancer 2025.V3
2. Ann Oncol 2022;33(10):1005-1020 [ESMO Gastric Cancer Living Guideline, v1.4 September 2024]
3. Biomarker Guidelines for Unresectable Advanced and Recurrent Gastric Cancer Version 1.1 (Japan Gastric Cancer Society 2024)
4. Korean Practice Guidelines for Gastric Cancer 2024: An Evidence-based, Multidisciplinary Approach J Gastric Cancer. 2025 Jan;25(1):5-114 https://doi.org/10.5230/jgc.2025.25.e11pISSN 2093-582X·eISSN 2093-5641

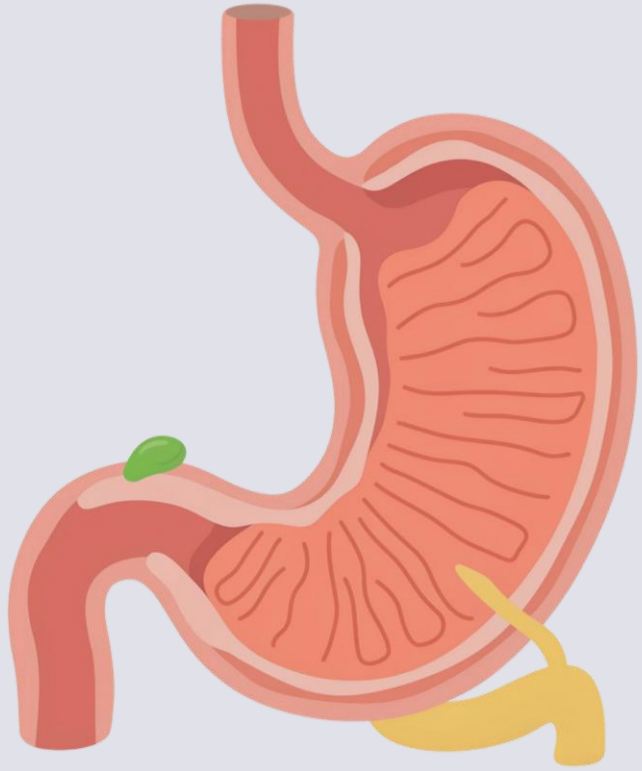
Management of HER2-Negative Locally Advanced & Metastatic Gastric/GEJ Cancers

A Modified Delphi Consensus from the **Hong Kong Society of Digestive Oncology**

Dr. Ka-On Lam

President

Hong Kong Society of Digestive Oncology



Statement 3. Upfront Biomarker Testing (100% agreement)

Recommendation

Upfront biomarker testing, including HER2, CLDN18.2, PD-L1, and MSI/MMR, is recommended in all patients with locally advanced unresectable or metastatic gastric or GEJ adenocarcinoma.

HER2

Determines eligibility for HER2-targeted therapy; negative status triggers further biomarker workup

CLDN18.2

Drives zolbetuximab eligibility; IHC using VENTANA assay, ≥75% moderate-to-strong staining[1,2]

PD-L1

Guides ICI addition to chemotherapy; CPS/TAP ≥ 10/10% associated with greatest survival benefit[3]

MSI / MMR

MSI-high/MMR-deficient identifies patients who respond exceptionally well to ICI-based therapy[4-6]

A reflex testing pathway at initial pathology is recommended to minimise time-to-treatment. NGS for less frequent actionable alterations (*NTRK*, *BRAF*, *FGFR2b*, *RET*) may also be considered.

CLDN18.2, claudin 18.2; CPS, combined positive score; GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1; TAP, tumour area positivity.

1. Shitara K, et al. Lancet. 2023;401(10389):1655-68. 2. Shah MA, et al. Nat Med. 2023;29(8):2133-41. 3. U.S. Food and Drug Administration. Available from: https://www.fda.gov/media/184453/download. Accessed on 24 Aug 2026. 4. Shitara K, et al. Nature. 2022;603(7903):942-8. 5. Chao J, et al. JAMA Oncol. 2021;7(6):895-902. 6. Maio M, et al. Ann Oncol. 2022;33(9):929-38.

ESTABLISHED IHC BIOMARKERS IN ADVANCED AND METASTATIC G/GEJ ADENOCARCINOMA

HER2

A biomarker associated with activation of downstream signalling that leads to uncontrolled **cell cycle progression, motility, adhesion, differentiation** and **apoptosis**[1]

MSI/dMMR

A biomarker identifying **deficiency in the DNA MMR system** resulting in an increased rate of somatic mutations within the cells, which can also be identified through **mutations in microsatellite sequences**[2,3]

PD-L1

A biomarker that can bind to the immune checkpoint receptor PD-1, which allows **tumours to escape immune surveillance** by inhibiting cytotoxic T cell responses[4]

CLDN18.2

A splice variant of CLDN18, CLDN18.2 is a component of **tight junctions** and has a critical role in **cell-to-cell epithelial adhesion** and regulating selective **barrier properties**[5–8]

Recently established

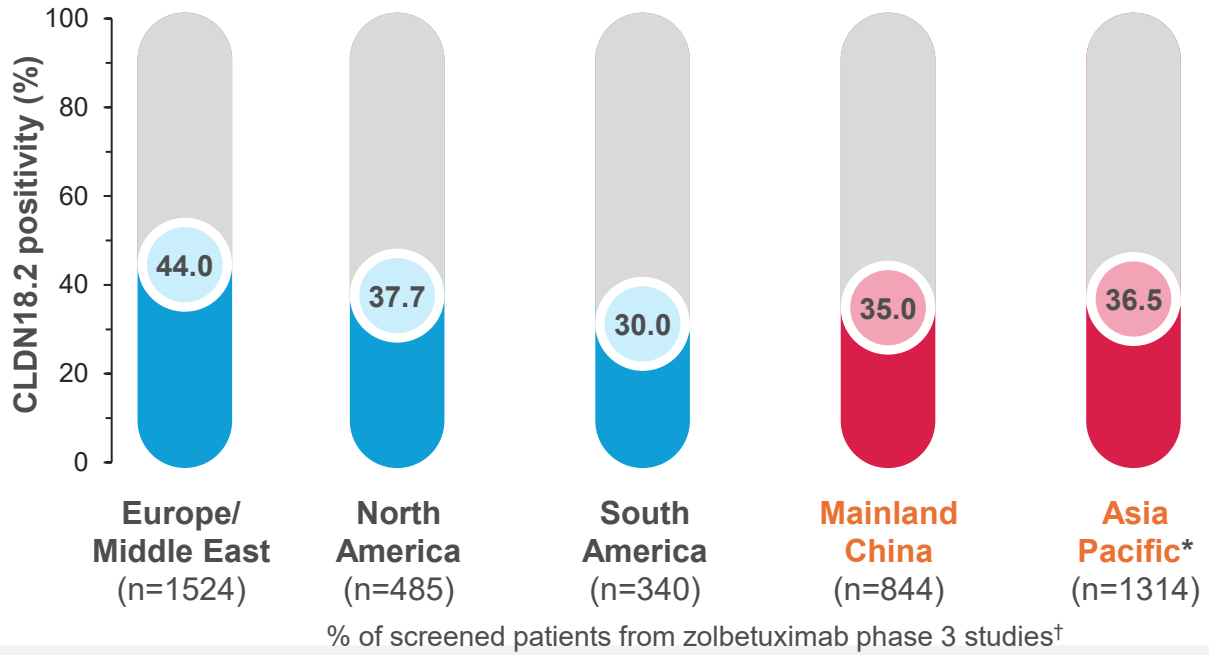
CLDN18, claudin 18; CLDN18.2, claudin 18.2; dMMR, mismatch repair deficient; G/GEJ, gastric/gastro-oesophageal junction; HER2, human epidermal growth factor receptor 2; MMR, mismatch repair; MSI, microsatellite instability; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

1. Van Cutsem E, et al. *Gastric Cancer* 2015;18(3):476–484; 2. Matsuoka T and Yashiro M. *World J Gastroenterol* 2018;24(26):2818–2832; 3. Baudrin LG, et al. *Front Oncol* 2018;8:621; 4. Kawazoe A, et al. *Gastric Cancer* 2019;22(1):69–76; 5. Sahin U, et al. *Clin Cancer Res* 2008;14(23):7624–7634; 6. Dottermusch M, et al. *Virchows Arch* 2019;475(5):563–571; 7. Coati I, et al. *Br J Cancer* 2019;121(3):257–263; 8. Li J, et al. *Aging (Albany NY)* 2020;12(14):14271–14284.

Prevalence of CLDN18.2 in unresectable or metastatic G/GEJ adenoca[1]

CLDN18.2 positivity is defined as ≥75% of tumour cells demonstrating moderate to strong membranous CLDN18 staining

Regional prevalence of CLDN18.2 positivity for all patients screened in SPOTLIGHT and GLOW



% of screened patients from zolbetuximab phase 3 studies†

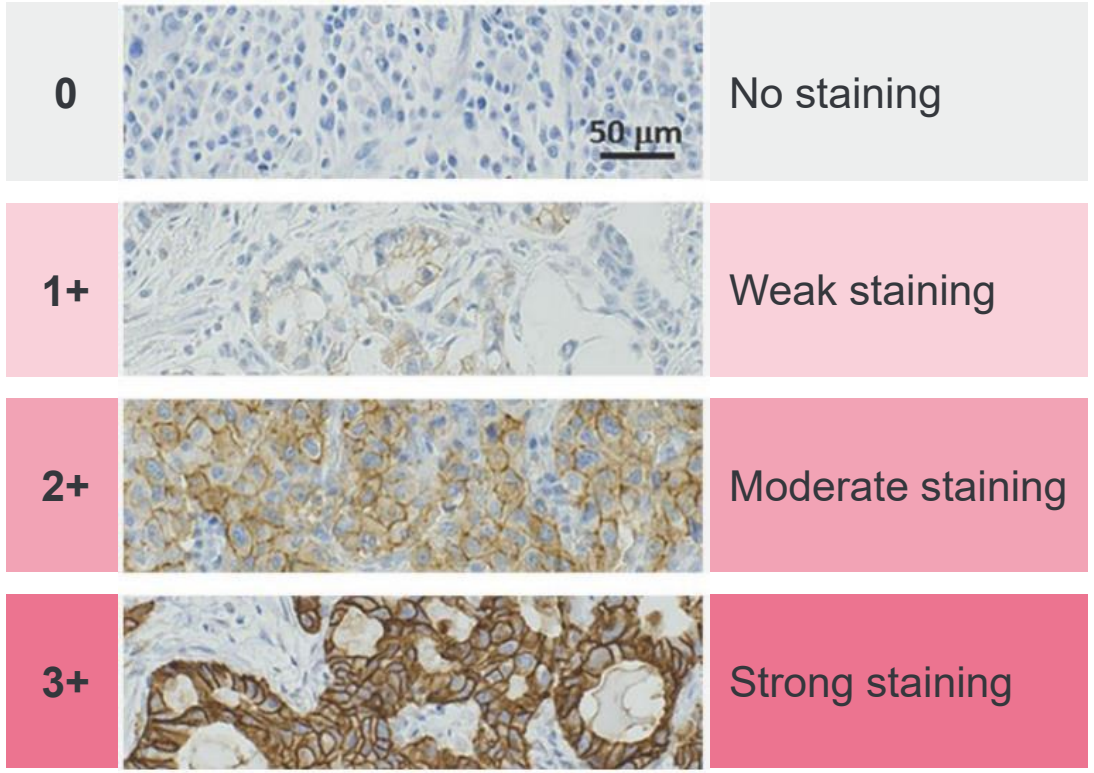
- Across the SPOTLIGHT and GLOW studies, regional prevalence of CLDN18.2 positivity ranged from 30−44%
- Prevalence of CLDN18.2 positivity was **consistent across SPOTLIGHT and GLOW** regardless of region[2]

Data from Shitara K, el al. *Gastric Cancer*. Volume 27, pages 1058–1068, (2024).

*Excluding Mainland China; †Includes both HER2-positive and HER2-negative patients.
CLDN18, claudin 18; CLDN18.2, claudin 18.2; HER2, human epidermal growth factor receptor 2; LA, locally advanced; mG/GEJ metastatic gastric/gastro-oesophageal junction
1. Shitara K, et al. *Gastric Cancer*. Volume 27, pages 1058–1068, (2024). Published online July 2, 2024; https://doi.org/10.1007/s10120-024-01518-1. 2. Shitara K, Xu RH, Moran D, et al. Poster presentation at ASCO 2023. Poster 4035.

DETECTING CLDN18.2 IN G/GEJ ADENOCARCINOMA

IHC scoring for CLDN18.2 in G/GEJ adenocarcinoma (membrane staining of tumour cells)[1]



CLDN18.2 positivity is defined by **≥75% of tumour cells** demonstrating **moderate (2+)** to **strong (3+)** membranous CLDN18 staining[2]

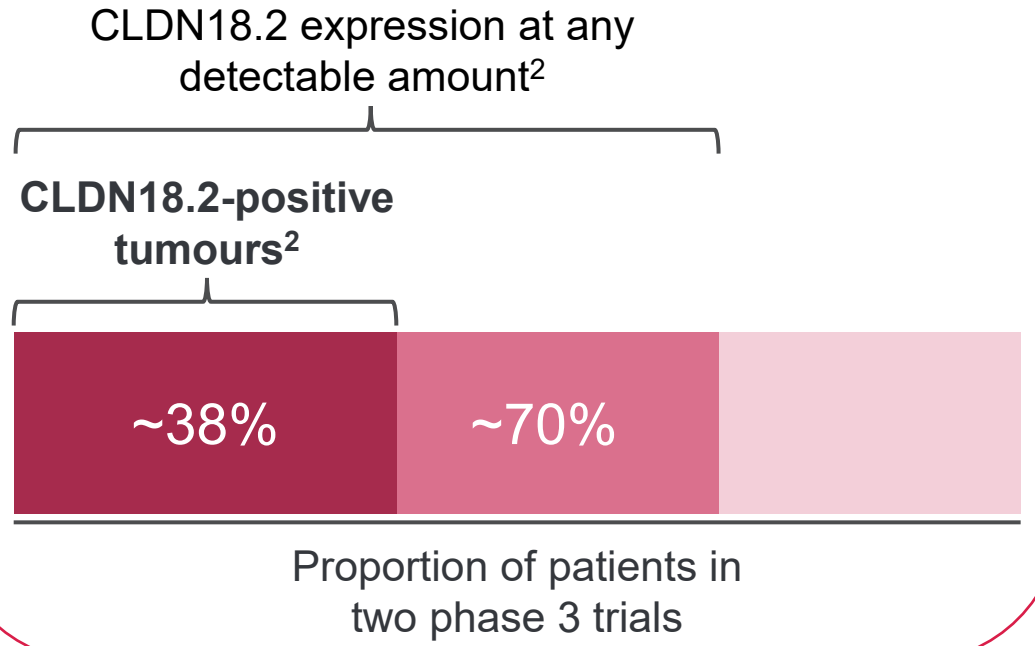


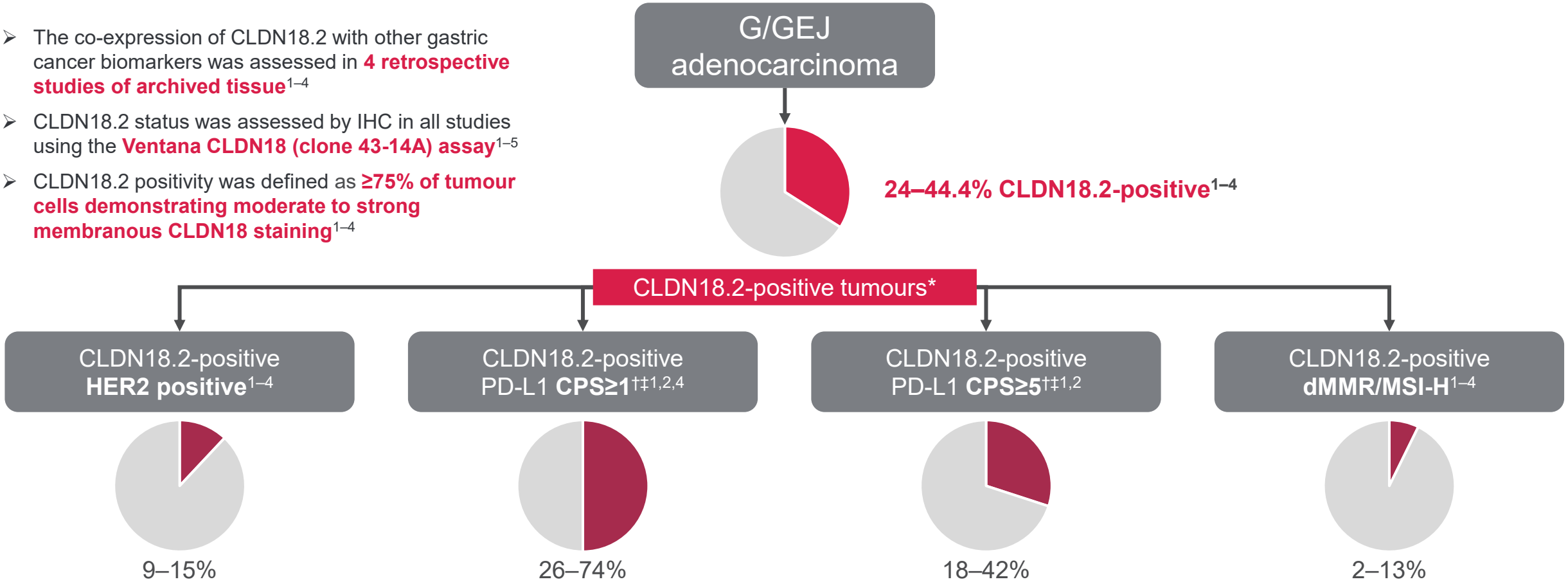
Figure adapted from Sahin U, et al. *Ann Oncol* 2021. Supplementary appendix.[1]

CLDN18, claudin 18; CLDN18.2, claudin 18.2; G/GEJ, gastric/gastro-oesophageal junction; IHC, immunohistochemistry.

1. Sahin U, et al. *Ann Oncol* 2021;32(5):609–619. Supplementary appendix; 2. Shitara K, et al. *Gastric Cancer* 2024;27(5):1058–1068.

NO CLEAR CORRELATION FOR CLDN18.2 CO-EXPRESSION WITH OTHER BIOMARKERS IN G/GEJ ADENOCARCINOMA[1–4]

- The co-expression of CLDN18.2 with other gastric cancer biomarkers was assessed in **4 retrospective studies of archived tissue**[1–4]
- CLDN18.2 status was assessed by IHC in all studies using the **Ventana CLDN18 (clone 43-14A) assay**[1–5]
- CLDN18.2 positivity was defined as **≥75% of tumour cells demonstrating moderate to strong membranous CLDN18 staining**[1–4]

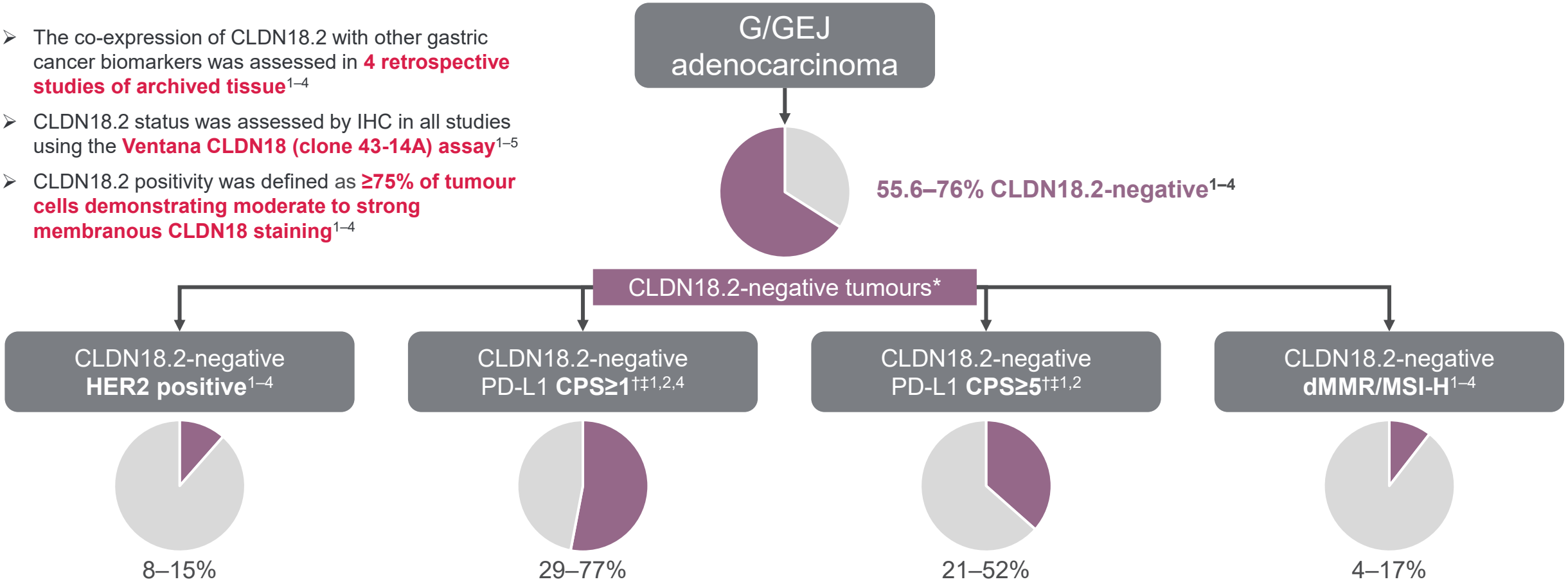


*Pellino A, et al. n=117, Kubota Y, et al. n=98, Sewastjanow M, et al. and Waters R, et al. n=135. The Sewastjanow M, et al. and Waters R, et al. studies used the same archive samples;[1–4] †PD-L1 testing antibodies: Pellino A, et al. and Waters R, et al. used the 22C3 (Dako) antibody. Kubota Y, et al. used the SP142 (Ventana) or SP263 (Ventana) antibody;[1,2,4] ‡In Kubota Y, et al., PD-L1 was evaluated in 93 CLDN18.2-positive and 293 CLDN18.2-negative specimens, and authors noted that relatively old archival specimens used may have affected the positivity rate.[2] In Waters R, et al, PD-L1 was evaluated in 53 CLDN18.2-positive and 61 CLDN18.2-negative tumours.[4] CLDN18, claudin 18; CLDN18.2, claudin 18.2; CPS, combined positive score; dMMR, mismatch repair deficient; G/GEJ, gastric/gastro-oesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; PD-L1, programmed death-ligand 1.

1. Pellino A, et al. *J Pers Med* 2021;11(11):1095; 2. Kubota Y, et al. *ESMO Open* 2023;8(1):100762; 3. Sewastjanow M, et al. Poster presentation at AACR 2023. Poster 2141; 4. Waters R, et al. *JCO Precis Oncol* 2024;8:e2300543; 5. Roche. VENTANA CLDN18 (43-14A) Assay. https://elabdoc-prod.roche.com/eLD/api/downloads/ff8f8fc9-3536-ea11-fc90-005056a71a5d?countryIsoCode=us. Accessed August 2025.

NO CLEAR CORRELATION FOR CLDN18.2 CO-EXPRESSION WITH OTHER BIOMARKERS IN G/GEJ ADENOCARCINOMA[1–4]

- The co-expression of CLDN18.2 with other gastric cancer biomarkers was assessed in **4 retrospective studies of archived tissue**[1–4]
- CLDN18.2 status was assessed by IHC in all studies using the **Ventana CLDN18 (clone 43-14A) assay**[1–5]
- CLDN18.2 positivity was defined as **≥75% of tumour cells demonstrating moderate to strong membranous CLDN18 staining**[1–4]



*Pellino A, et al. n=223, Kubota Y, et al. n=310, Sewastjanow M, et al. and Waters R, et al. n=169. The Sewastjanow M, et al. and Waters R, et al. studies used the same archive samples;[1–4] †PD-L1 testing antibodies: Pellino A, et al. and Waters R, et al. used the 22C3 (Dako) antibody. Kubota Y, et al. used the SP142 (Ventana) or SP263 (Ventana) antibody;[1,2,4] ‡In Kubota Y, et al., PD-L1 was evaluated in 93 CLDN18.2-positive and 293 CLDN18.2-negative specimens, and authors noted that relatively old archival specimens used may have affected the positivity rate.[2] In Waters R, et al, PD-L1 was evaluated in 53 CLDN18.2-positive and 61 CLDN18.2-negative tumours.[4] CLDN18, claudin 18; CLDN18.2, claudin 18.2; CPS, combined positive score; dMMR, mismatch repair deficient; G/GEJ, gastric/gastro-oesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; PD-L1, programmed death-ligand 1.

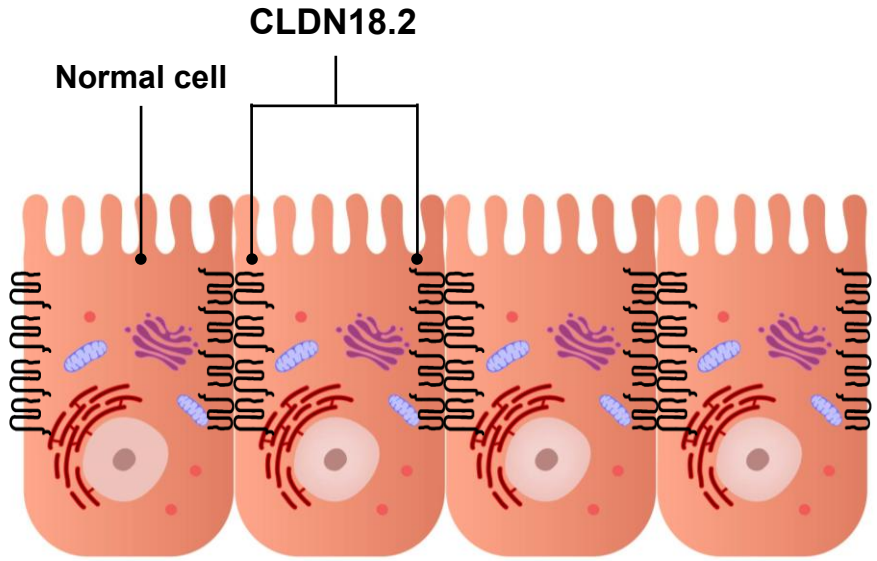
1. Pellino A, et al. *J Pers Med* 2021;11(11):1095; 2. Kubota Y, et al. *ESMO Open* 2023;8(1):100762; 3. Sewastjanow M, et al. Poster presentation at AACR 2023. Poster 2141; 4. Waters R, et al. *JCO Precis Oncol* 2024;8:e2300543; 5. Roche. VENTANA CLDN18 (43-14A) Assay. https://elabdoc-prod.roche.com/eLD/api/downloads/ff8f8fc9-3536-ea11-fc90-005056a71a5d?countryIsoCode=us. Accessed August 2025.

CLDN18.2 IS CONFINED TO TIGHT JUNCTIONS IN HEALTHY TISSUE[1]

Claudin proteins

- Family of at least **27** transmembrane proteins[2,3]
- Composed of **4 transmembrane** helix domains and **2 extracellular** loops[1,2]
- Major structural components of **tight junctions**,[2] playing a critical role in **maintenance of cell polarity** and in selective paracellular permeability[4–6]
- Expressed throughout the body and display **tissue-specific expression** patterns[1,7]

Gastric epithelium



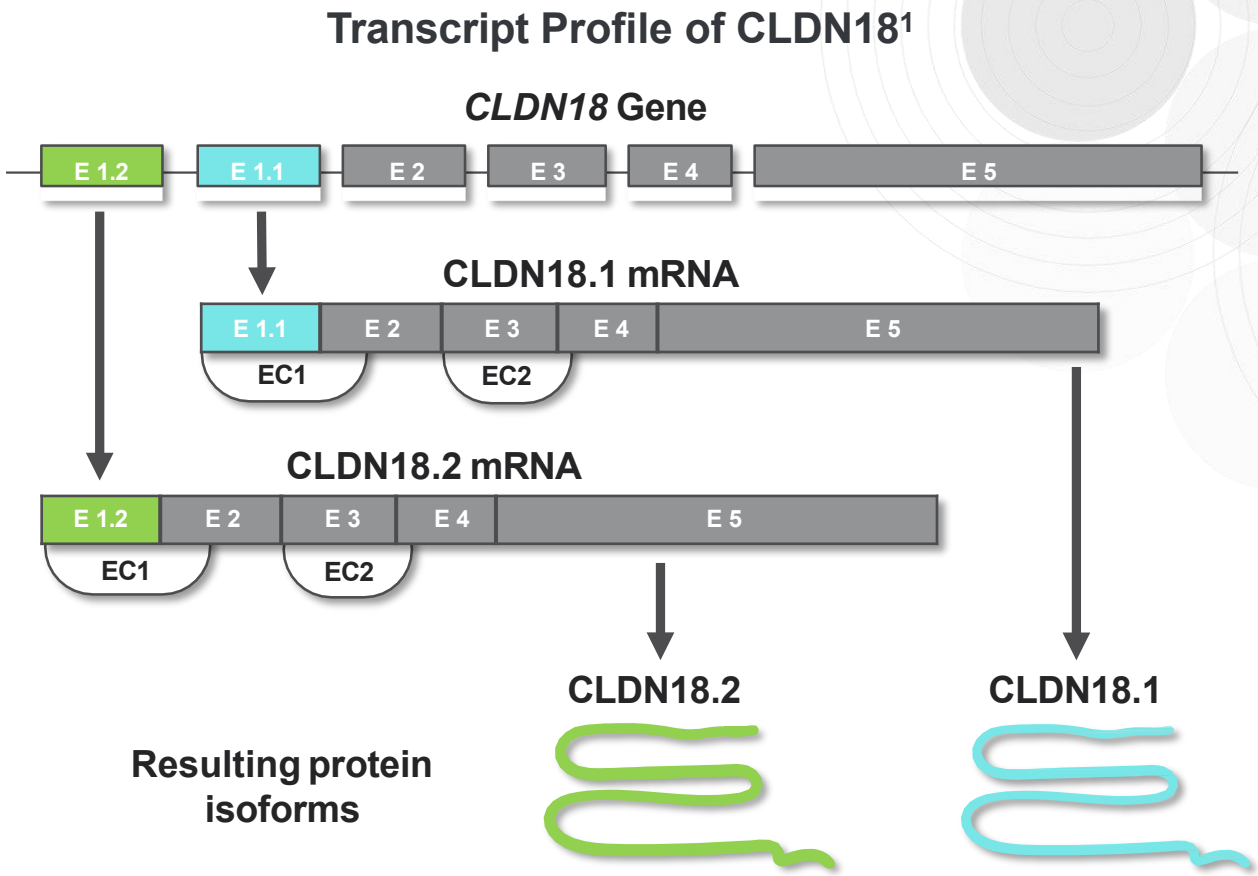
Alternative splicing of the *CLDN18* gene results in 2 protein isoforms: CLDN18.1, which is found in lung tissue, and **CLDN18.2, which is highly conserved in gastric tissue**[1]

CLDN18.1, claudin 18.1; CLDN18.2, claudin 18.2.

1. Sahin U, et al. *Clin Cancer Res* 2008;14(23):7624–7634; 2. Singh P, et al. *Journal of Hematology & Oncology* 2017;10:105; 3. Mineta K, et al. *FEBS Lett* 2011;585(4):606–612; 4. Otani T, et al. *Trends Cell Biol* 2020;30(12):1014; 5. Tsukita S, et al. *Trends Biochem Sci* 2019;44(2):141–152; 6. Hu YJ, et al. *Mol Biol Rep* 2013;40:6123–6142; 7. Hewitt KJ, et al. *BMC Cancer* 2006;6:186.

TWO ISOFORMS OF CLDN18 EXIST: CLDN18.1 AND CLDN18.2

- **The *CLDN18* gene** is located on **chromosome 3q22** and encodes for the homonymous protein (CLDN18)
- The *CLDN18* gene has **2 alternative first exons (E 1.2 and E 1.1),** leading to splice variants encoding **2 protein isoforms (CLDN18.1 and CLDN18.2)**[1]
- **CLDN18.1 and CLDN18.2 share a high degree of homology**[2]
  - Differ by 8 of the 51 amino acids in the first extracellular domain, with identical sequences of the second extracellular loops[1]



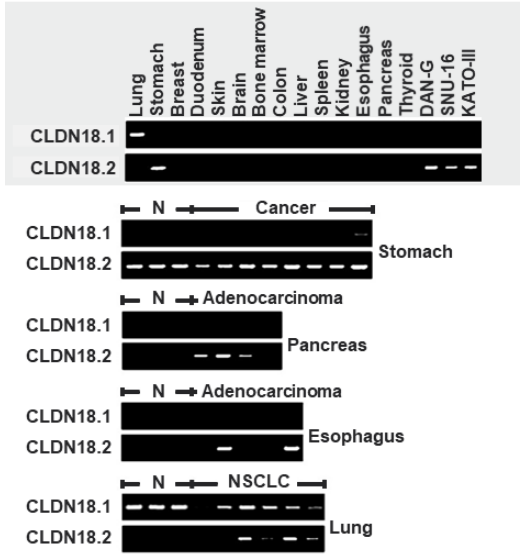
CLDN18 claudin 18; **CLDN18.1,** claudin 18 isoform 1; **CLDN18.2** claudin 18 isoform 2; **E** exon; **EC** extracellular loop; **mRNA** messenger RNA.

1. Sahin U, et al. *Clin Can Res.* 2008;14(23):7624–7634. 2. Saito T, et al. *ESMO World Congress on Gastrointestinal Cancer.* 2021.

CLDN18.1 and CLDN18.2 Expression Patterns

- Claudins are present throughout the body, but two specific splicing isoforms of CLDN18 are localised to SPECIFIC tissue types[1,2]

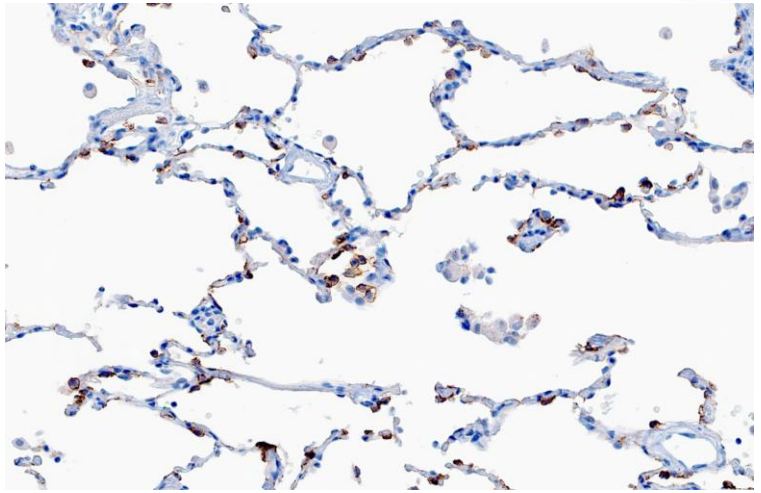
Comparative analysis of CLDN18 isoform expression[1]



CLDN18 claudin 18; CLDN18.1 claudin 18 isoform 1; CLDN18.2 claudin 18 isoform 2; DAN-G pancreatic cancer cell line; KATO-III gastric cancer cell line; N normal tissue; NSCLC non-small cell lung cancer; RT-PCR reverse transcription-polymerase chain reaction; SNU-16 gastric cancer cell line.
Normal tissue, primary tumour specimens, and tumour cell lines by endpoint RT-PCR.

CLDN18.1

- **Found in healthy lung tissue**[3]

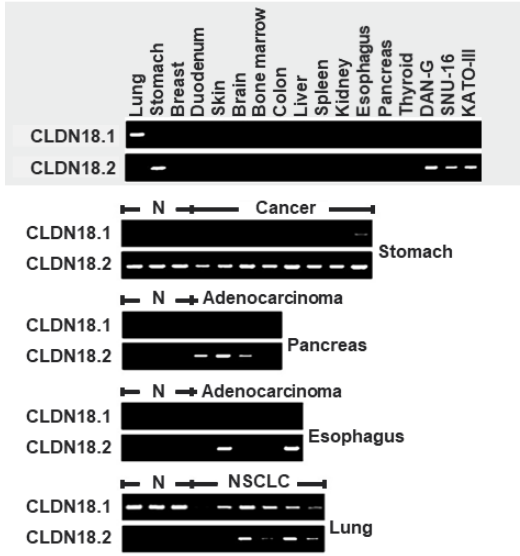


1. Sahin U, Koslowski M, Dhaene K, et al. Clin Cancer Res 2008;14(23):7624–7634;
2. Niimi T, Nagashima K, Ward JM, et al. Mol Cell Biol 2001;21(21):7380–73902.
3. PathologyOutlines.com

CLDN18.1 and CLDN18.2 Expression Patterns

- Claudins are present throughout the body, but two specific splicing isoforms of CLDN18 are localised to SPECIFIC tissue types[1,2]

Comparative analysis of CLDN18 isoform expression[1]



CLDN18 claudin 18; CLDN18.1 claudin 18 isoform 1; CLDN18.2 claudin 18 isoform 2; DAN-G pancreatic cancer cell line; KATO-III gastric cancer cell line; N normal tissue; NSCLC non-small cell lung cancer; RT-PCR reverse transcription-polymerase chain reaction; SNU-16 gastric cancer cell line.
Normal tissue, primary tumour specimens, and tumour cell lines by endpoint RT-PCR.

CLDN18.1

- Found in healthy lung tissue

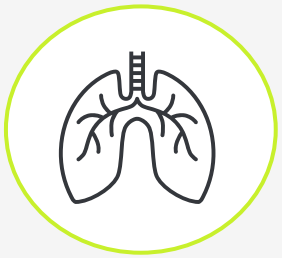
CLDN18.2

- Found in **healthy gastric epithelial cells**
- Its expression is **maintained in malignant gastric tissue**.
- Overexpressed in **malignant gastric, pancreatic and oesophageal tissue**[1,3]

1. Sahin U, Koslowski M, Dhaene K, et al. Clin Cancer Res 2008;14(23):7624–7634;
2. Niimi T, Nagashima K, Ward JM, et al. Mol Cell Biol 2001;21(21):7380–73902.
3. Saito T, et al. ESMO World Congress on Gastrointestinal Cancer. 2021.

Detection of CLDN18.2 in GC/GEJ

- CLDN18 antibodies can identify but not distinguish CLDN18 isoforms - CLDN18.1 and CLDN18.2.
- When evaluating G/GEJ tumour tissue, staining can be attributed to the presence of CLDN18.2 due to localisation in certain tissue types:
    - CLDN18.1 is primarily expressed in adenocarcinoma lung tissue and its expression is negligible or absent in G/GEJ cancers
    - CLDN18.2 is normally present in gastric epithelium and is often retained in malignant gastric tissue[1]



CLDN18.1



CLDN18.2

CLDN18, claudin 18; CLDN18.2, claudin 18.2; IVD, *in vitro* diagnostic; IVD, *in vitro* diagnostic

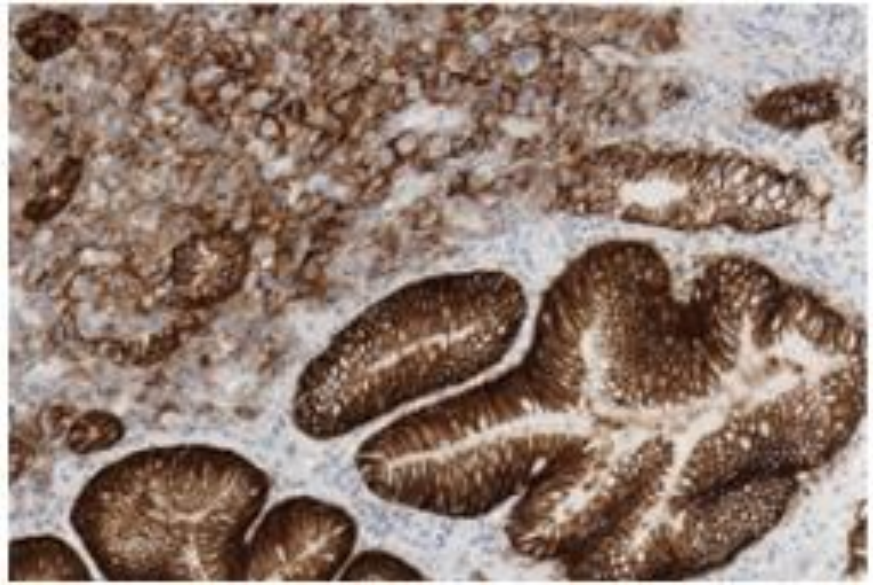
1. Saito T, Matsuda T, Moran D. ESMO World Congress on Gastrointestinal Cancer 2021; Poster 286

VENTANA CLDN18 (43-14A) RxDx Assay

The tetramembrane claudin proteins comprise a major component of tight junctions, a component of cell-cell adhesion.[1,2] 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 has two isoforms and does not exhibit broad tissue expression. CLDN18.1 is predominantly expressed in normal and neoplastic lung tissue.[1,2] CLDN18.2 is the predominant isoform expressed in gastric and pancreatic cancers.[3]

The VENTANA CLDN18 (43-14A) RxDx Assay is a mouse monoclonal antibody that exhibits a membranous staining pattern.

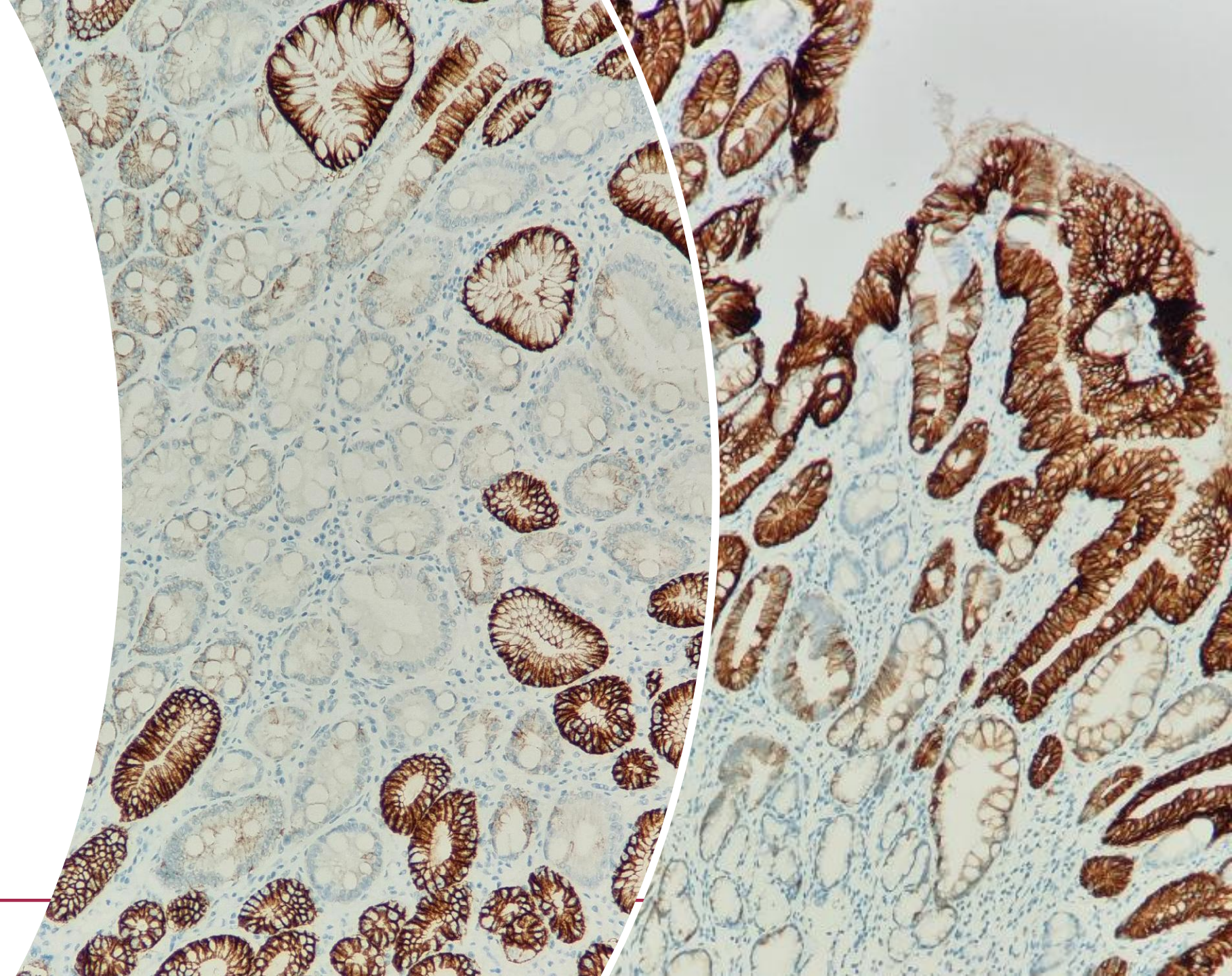


Gastric adenocarcinoma tissue stained with the VENTANA CLDN18 (43-14A) Assay

1. Hashimoto I, Oshima T, Claudins and gastric cancer: An overview. Cancers (Basel), 2022;14(2):290
2. Singh P, Toom S, Huang Y. Anti-Claudin 18.2 antibody as new targeted therapy for advanced gastric cancer J Hematol Oncol. 2017;10(1):105
3. T. Saito, T. Matsuda, D. Moran, P-114 Claudin 18 isoform expression in gastric adenocarcinoma and pancreatic adenocarcinoma, Annals of Oncology. Volume 32, Supplement 3, 2021, Page 5138, ISSN 0923-7534. doi.org/10.1016/j.annonc.2021.05.169

astellas

STAINING INTENSITIES OF NORMAL STOMACH VS INTESTINAL METAPLASIA



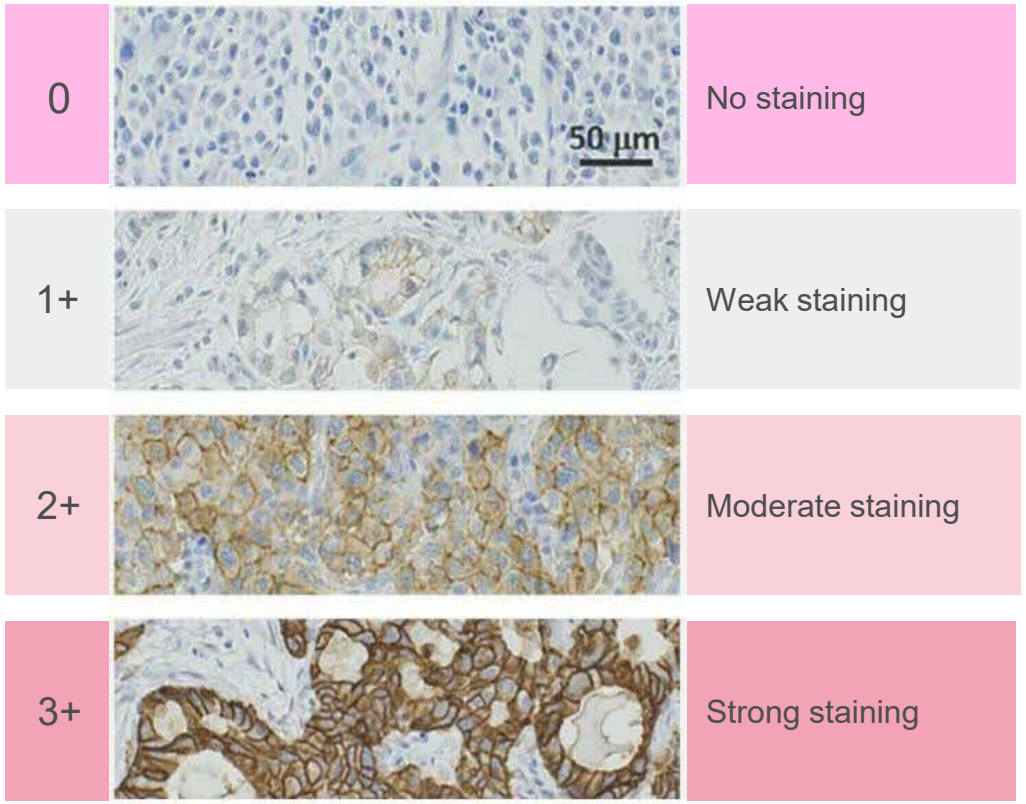
CLDN18 IHC EVALUATION

- For the evaluation of CLDN18.2 expression, both the intensity of tumour membranous staining and the percentage of cancer cells with complete, basolateral or lateral membrane staining are assessed[1]
- Pathologists must consider the entire tumour area to obtain an accurate evaluation of CLDN18.2 expression[1,2]

General rules for CLDN18 IHC evaluation[1,3–5]

Cells included nominator	Stained viable invasive tumour cells
Subcellular localisation	Cell membrane
Staining intensity	Moderate (2+) to strong (3+)
Staining pattern	Apical, circumferential (partial & complete), basolateral/lateral and micro-luminal
Scoring denominator	Total number of viable invasive tumour cell: ≥50

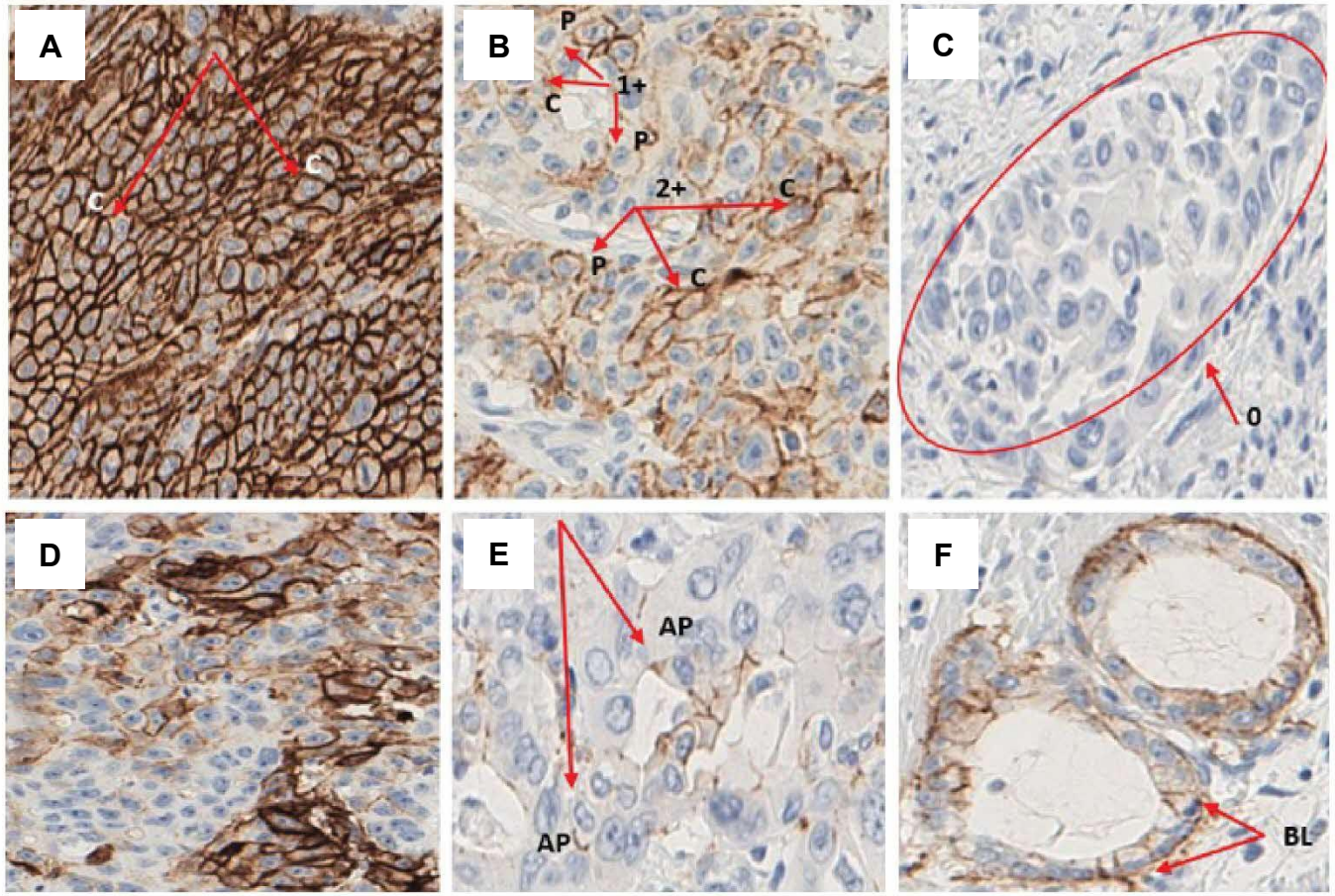
CLDN18 IHC Evaluation in G/GEJ cancer (membrane staining of tumour cells)[4]



CLDN18.2, claudin 18.2; G/GEJ, gastric/gastro-oesophageal; IHC, immunohistochemistry.

1. Pellino A, Brignola S, Riello E, et al. *J Pers Med* 2021;11(11):1095; 2. Angerilli V, Ghelardi F, Nappo F, et al. *Pathol Res Pract* 2024;254:155145; 3. Rohde C, Yamaguchi R, Mukhina S, et al. *Jpn J Clin Oncol* 2019;49(9):870–876; 4. Sahin U, Türeci Ö, Manikhas G, et al. *Ann Oncol* 2021;32(5):609–619. Supplementary appendix; 5. Jasani B, Taniere P, Schildhaus H-U, et al. Lab Invest 2024;104(1):100284.

EXAMPLES OF CLDN18 IHC STAINING
VENTANA (43-14A) ANTIBODY ON THE VENTANA BENCHMARK ULTRA PLATFORM

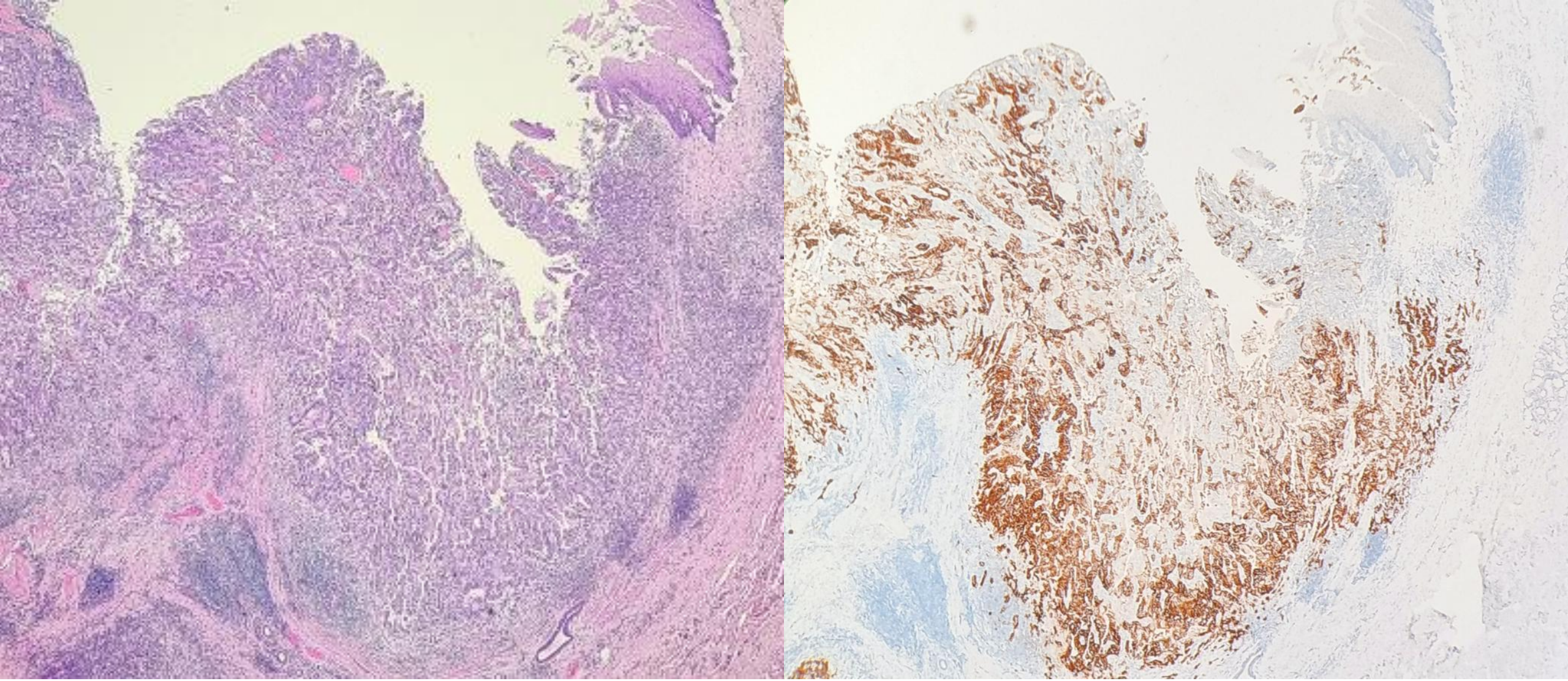


Various IHC staining intensities and membranous staining patterns using the VENTANA (43-14A) antibody on the VENTANA BenchMark Ultra platform:

A. Strong (3+) complete circumferential staining
B. Areas of partial (P) and complete (C) membranous staining with 2+ (moderate) or 1+ (weak) intensity
C. Negative (0) staining
D. Heterogeneous staining with a mix of staining intensities and staining patterns
E. Apical (AP) staining cells
F. Basal lateral (BL) staining cells

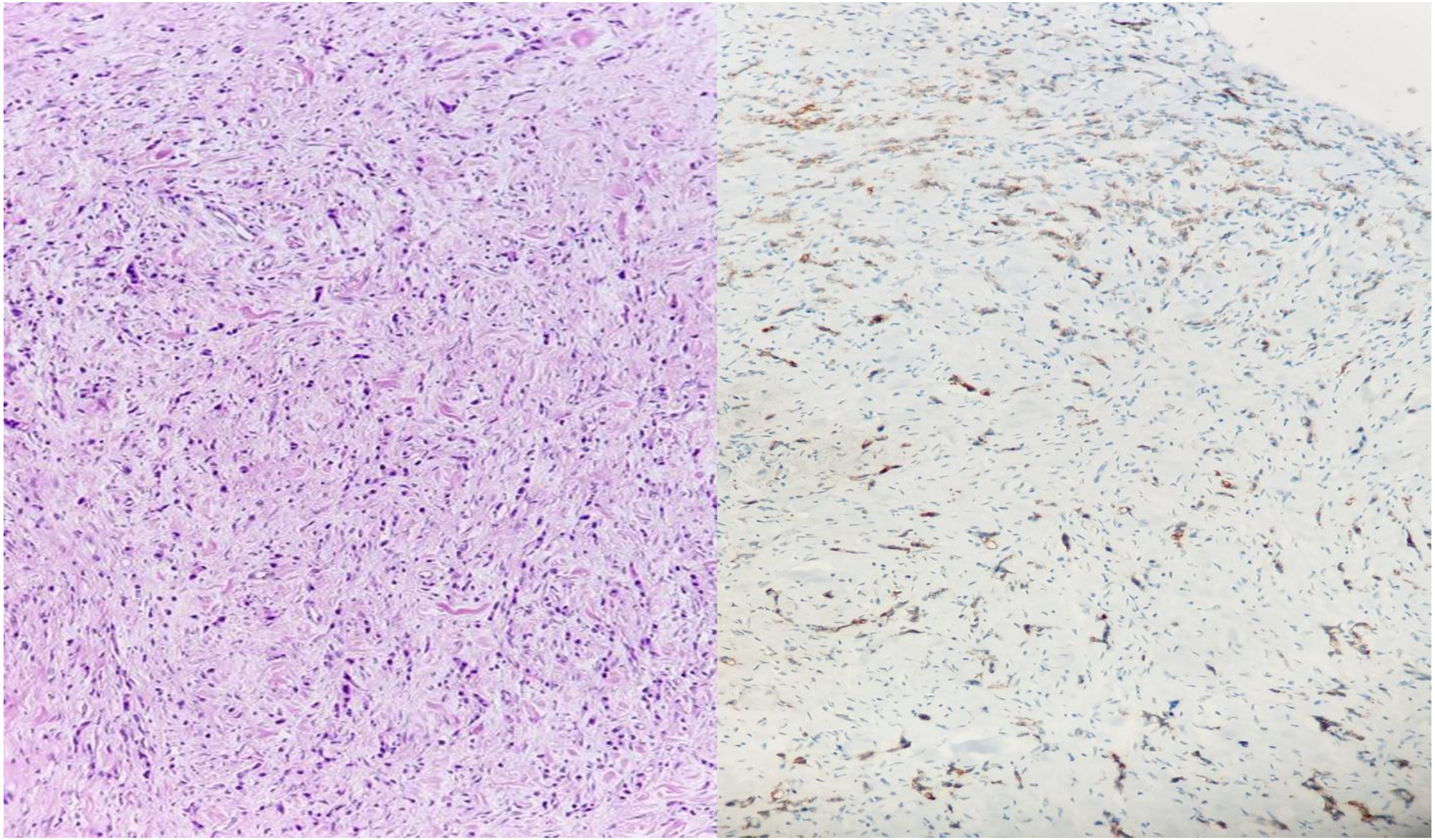
CLDN18, claudin 18; IHC immunohistochemistry.

Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284.

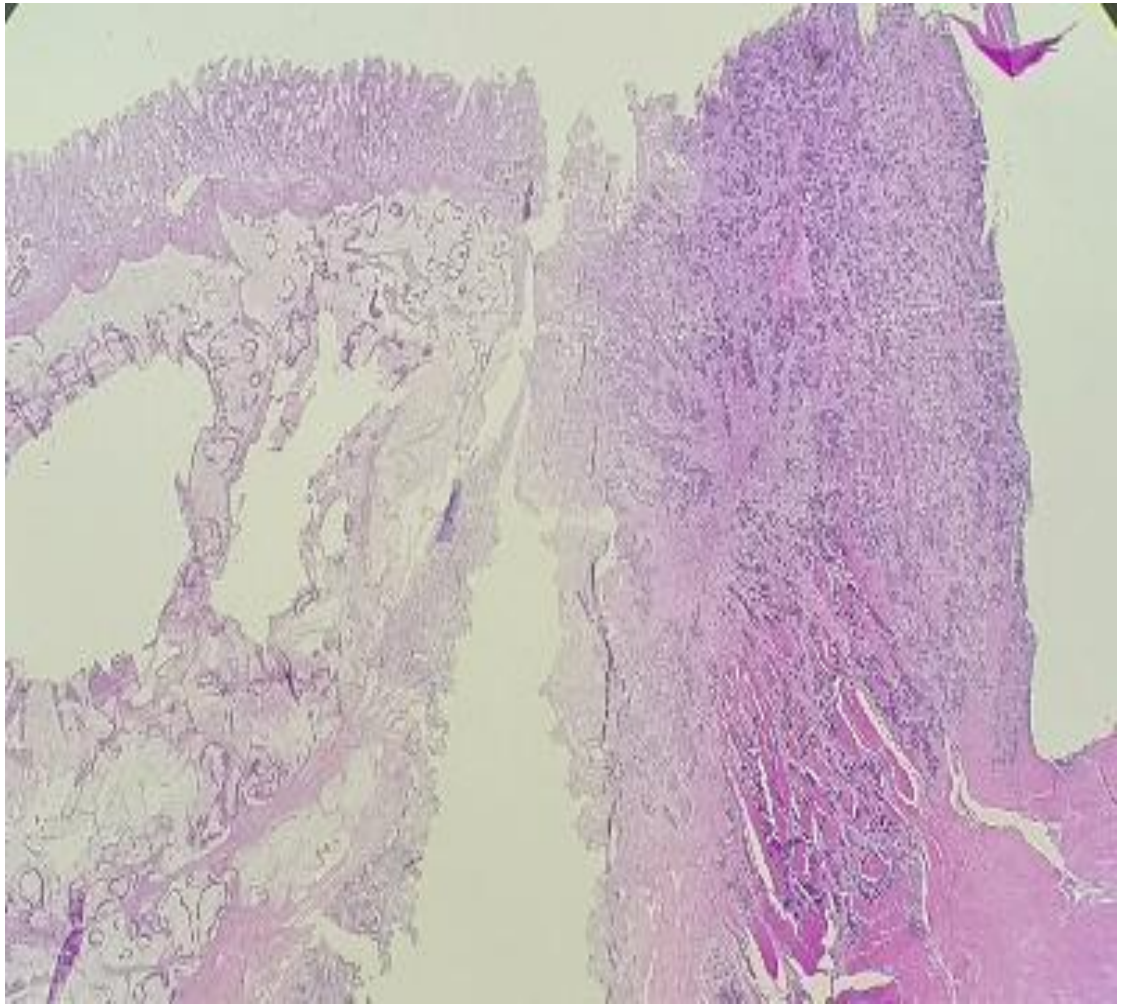


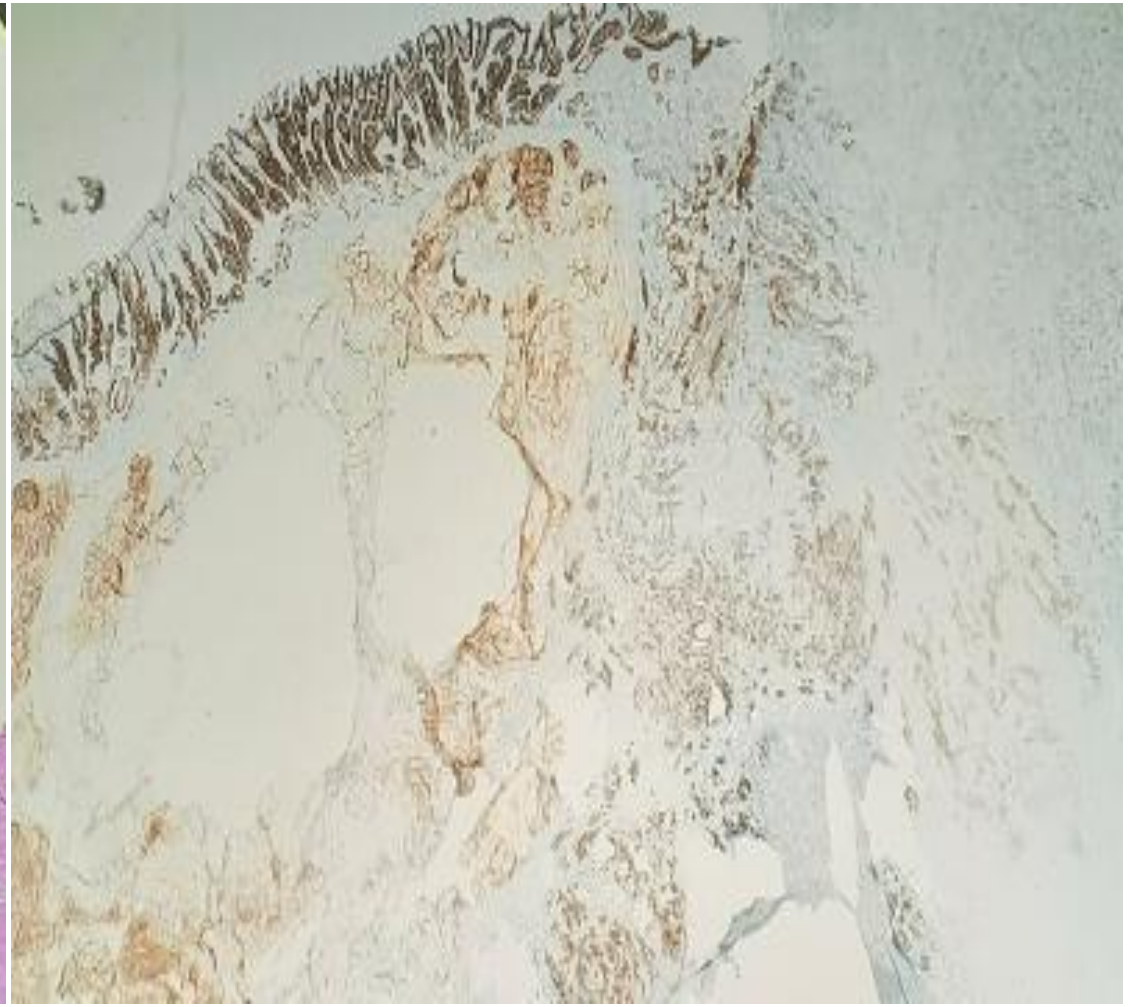
Positive: 80% at least 2/3+

astellas



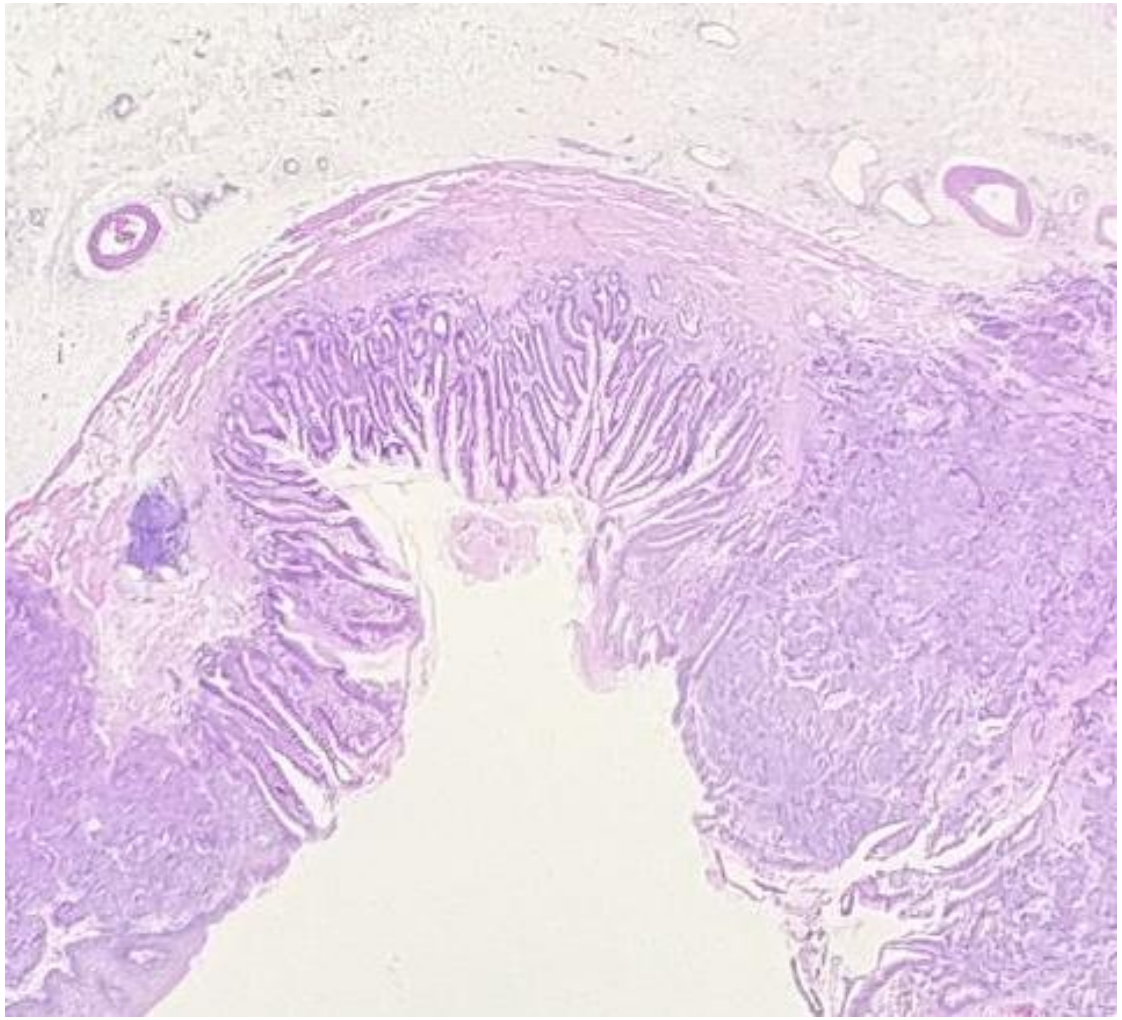
Diffuse gastric cancer: Positive 2/3+ staining

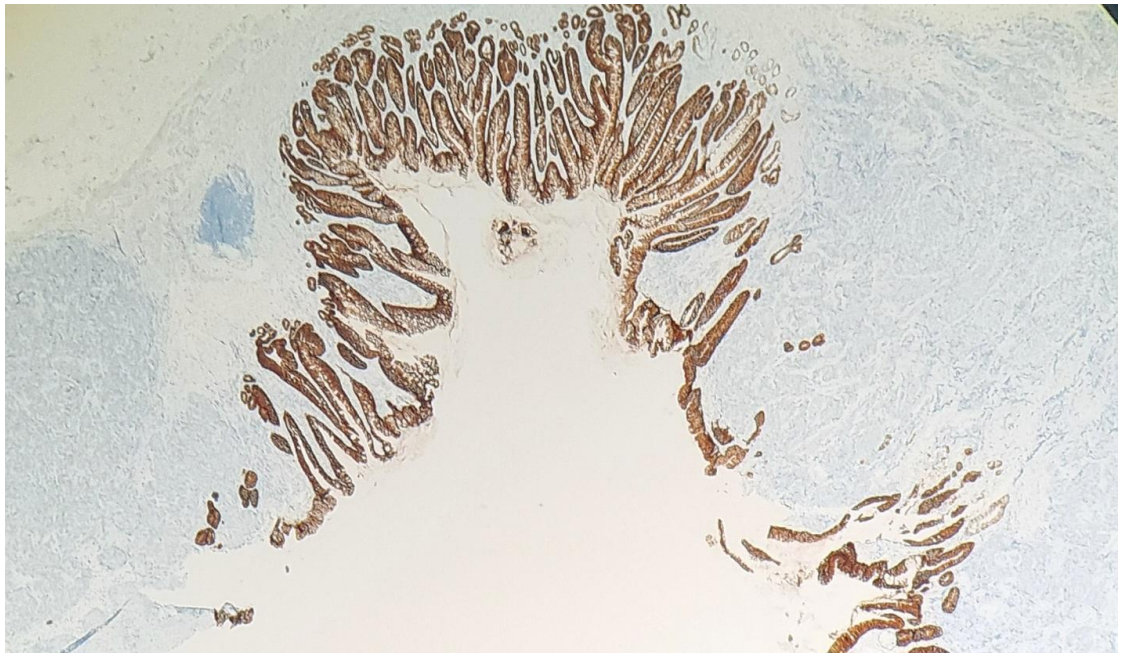




Borderline Negative: 60% at least 2/3+

astellas



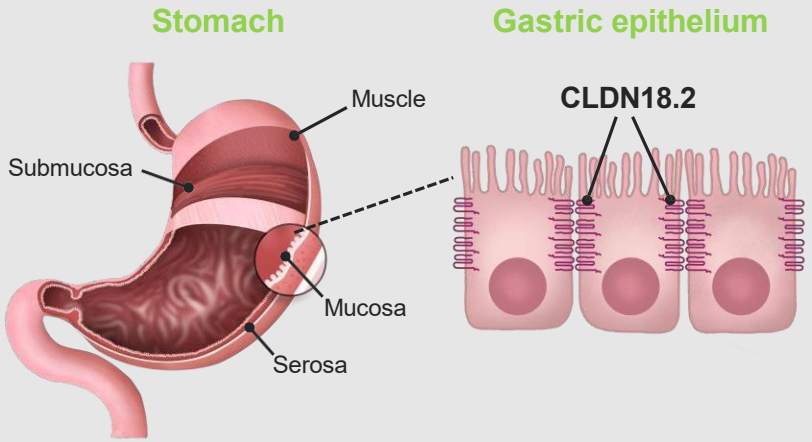


Negative: 0% at least 2/3+

astellas

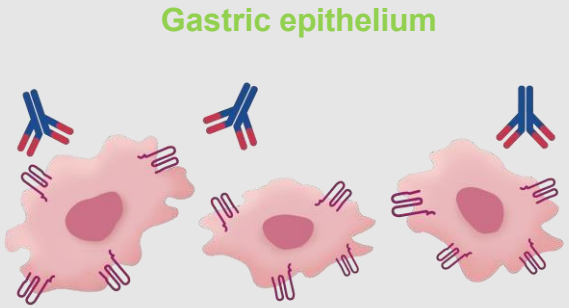
CLDN18.2 during Tumourigenesis

CLDN18.2 confined in healthy tissue



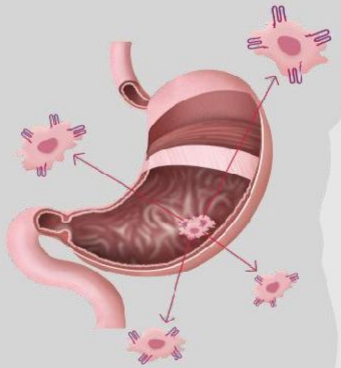
- In normal gastric mucosa, CLDN18.2 is typically buried within **tight junctions**.[1,2]
- As a component **of tight junctions**, claudins are involved in the regulation of **permeability**, **barrier** function, and **polarity** of epithelial layers.[3-5]

CLDN18.2 retained and exposed during malignant transformation



- CLDN18.2 is often retained during malignant transformation.[1]
- During this stage, the perturbations in the gastric mucosa cell polarity and structural loss may result in CLDN18.2 becoming **even more exposed** and thus accessible to antibodies.[1,6,7]

Maintained in metastatic progression



- CLDN18.2 may also be expressed in lymph node **metastases** of gastric adenocarcinoma as well as other distant metastatic sites.[1,8-10]

CLDN18.2 claudin 18 isoform 2

1. Sahin U, et al. *Clin Can Res.* 2008;14(23):7624–7634. **2.** Sahin U, et al. *Eur J Cancer.* 2018;100:17-26. **3.** Turner JR, Buschmann MM, Romero-Calvo I, Sailer A, Shen L. *Semin Cell Dev Biol.* 2014 Dec;36:204-12 **4.** Tsukita S, Tanaka H, Tamura A. *Trends Biochem Sci.* 2019 Feb;44(2):141-152. **5**. Hu YJ, Wang YD, Tan FQ, Yang WX. *Mol Biol Rep.* 2013 **6.** Mitnacht-Kraus R, et al. *ESMO* 2017. Poster #378P. **7.** Shah M, et al. *ASCO* 2020. TPS4648. **8**. Pellino A, et al. *J Pers Med.* 2021;11(11):1095. **9**. Coati I, et al. *Br J Cancer.* 2019;121(3):257-263. **10.** Rohde C, et al. *Jpn J Clin Oncol.* 2019; 49(9):870-876.

CLDN18.2 during Tumourigenesis and a Focus on Distant Metastases

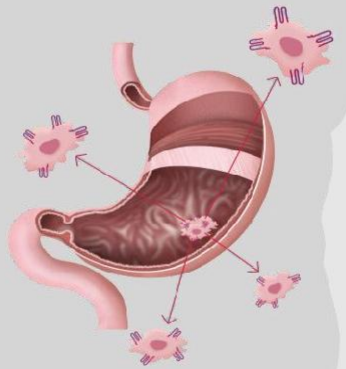
CLDN18.2 expression concordance between primary and metastatic sites:

-The **concordance rate** between **primary tumors** and **peritoneal metastases** was **75.0%** in a recent investigation, with higher positivity rates in primary tumors than in peritoneal metastases (28.6% [24/84] vs. 20.2% [17/84], respectively).

-The **concordance** across **different metastatic sites** (peritoneum, liver, lung, bone, and distant LND) was **74.8%** (101/135) in another recent study, with peritoneal metastases showing the highest CLDN18.2 positivity (44.3%) and liver metastases the lowest (17.9%).

The high rate of concordance among primary and different metastatic sites is a very significant foundation for supporting anti-CLDN18 therapies in metastatic setting.

Maintained in metastatic progression



- CLDN18.2 may also be expressed in lymph node **metastases** of gastric adenocarcinoma as well as other distant metastatic sites.[1,8-10]

CLDN18.2 claudin 18 isoform 2

1. Sahin U, et al. *Clin Can Res.* 2008;14(23):7624–7634. **2.** Sahin U, et al. *Eur J Cancer.* 2018;100:17-26. **3.** Turner JR, Buschmann MM, Romero-Calvo I, Sailer A, Shen L. *Semin Cell Dev Biol.* 2014 Dec;36:204-12 **4.** Tsukita S, Tanaka H, Tamura A. *Trends Biochem Sci.* 2019 Feb;44(2):141-152. **5**. Hu YJ, Wang YD, Tan FQ, Yang WX. *Mol Biol Rep.* 2013 **6.** Mitnacht-Kraus R, et al. *ESMO* 2017. Poster #378P. **7.** Shah M, et al. *ASCO* 2020. TPS4648. **8**. Pellino A, et al. *J Pers Med.* 2021;11(11):1095. **9**. Coati I, et al. *Br J Cancer*. 2019;121(3):257-263. **10.** Rohde C, et al. *Jpn J Clin Oncol*. 2019; 49(9):870-876. **11.** Choi E, et al. *Sci Rep*. 2024;14:17648. **12.** Ogawa H, et al. *Gastric Cancer*. 2024;27:802–810. **13.** Lee HS. *J Gastric Cancer*. 2025 Jan;25(1):192-209.

Other evidence regarding CLDN18.2 expression in Primary GC and Metastases

Lymph node metastases: A study of 523 primary G/GEJ adenocarcinomas and 135 pair-matched, synchronous nodal metastases demonstrated

86.7% concordance	between matched samples of primary and **lymph node metastases.**[17]

Peritoneal metastases: Another recent studies suggest similar high concordance of CLDN18.2 expression between primary tumours and peritoneal metastases (PMs)[23]

86.5% concordance	between matched samples of primary and **peritoneal metastases.**[23]

These results further support anti-CLDN18 therapies in metastatic setting

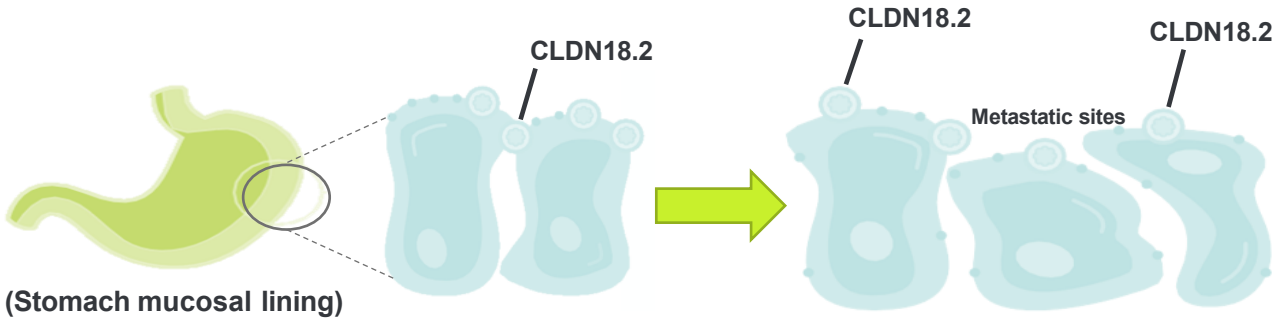
astellas

17. -> Shah; Nat Med; 2023; 2133-2141 (v1.0) - Glow demonstrated (p.4). Shitara K et al;2023;1-13 (v1.0) - p4/col2/Results/para1/ln1-4 (p.4). Between June 21, 2018, and April 1, 2022, 2735 patients were screened (figure 1) figure 1). Of the 2403 patients assessable for CLDN18.2 sta. Shitara K et al;2023;1-13 (v1.0) - p3/col1/Methods/para2/ln1-11 (p.3). Eligible patients were aged 18 years or older with CLDN18.2-positive (defined as ≥75% of tumour cells showing moderate-to-strong membranous Shitara K et al;2023;1-13 (v1.0) - p3/col1/Methods/para1/ln1-3 (p.3) SPOTLIGHT is a global, randomised, placebo-controlled, double-blind, phase 3 trial that enrolled patients from 215 centres in 20 countries (

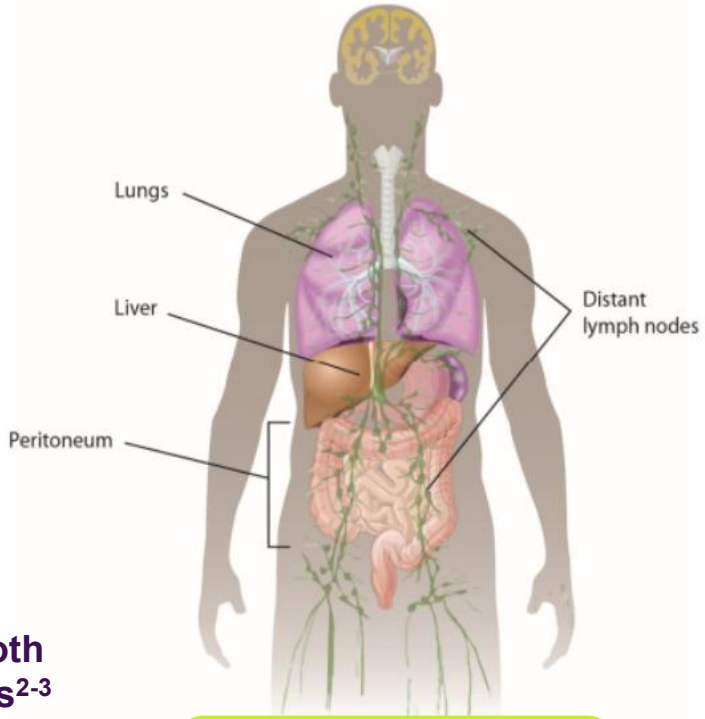
23. Angerilli V et al. Heterogeneity of predictive biomarker expression in gastric and esophago-gastric junction carcinoma with peritoneal dissemination. Gastric Cancer. 2025 Jul;28(4):569-578.]

CLDN18.2 expression

Primary GC & Metastatic sites



CLDN18.2 expression in both primary and metastatic sites[2-3]



Most common sites of metastasis for G/GEJ cancer[4]

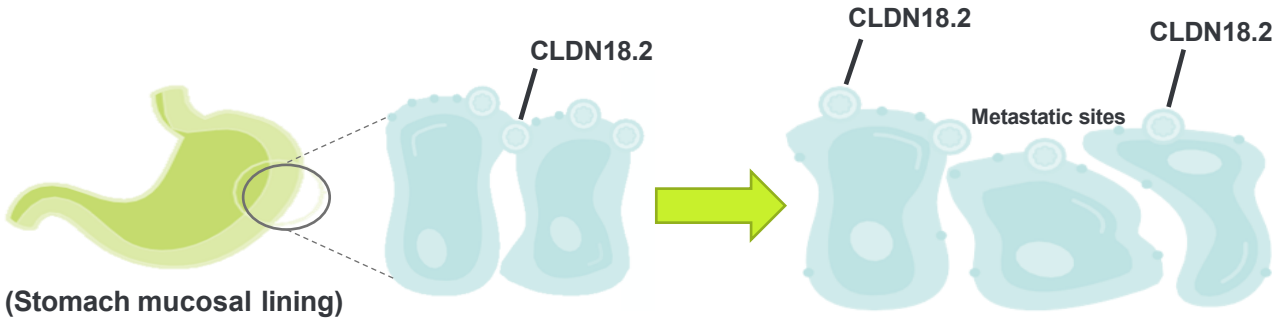
Image adapted from: Verstegen MHP, *et al*. *World J Gastroenterol.* 2020.

Data supports CLDN18.2 expression is maintained in metastatic progression, suggesting accurate CLDN18.2 assessment may be performed from either primary tumour and/or sites of metastases.[1]

[1]Pellino A, *et al*. *J Pers Med*. 11, 1095, 2021;
[2]Rohde C, *et al*..*J Clin Oncol*. 49(9):870-876, 2019;
[3]Sahin U, *et al*. *Clin Cancer Res*.14(23):7624-34, 2008;
[4]Verstegen MHP,*et al*. *World J Gastroenterol*. 26(39):6037-46, 2020.

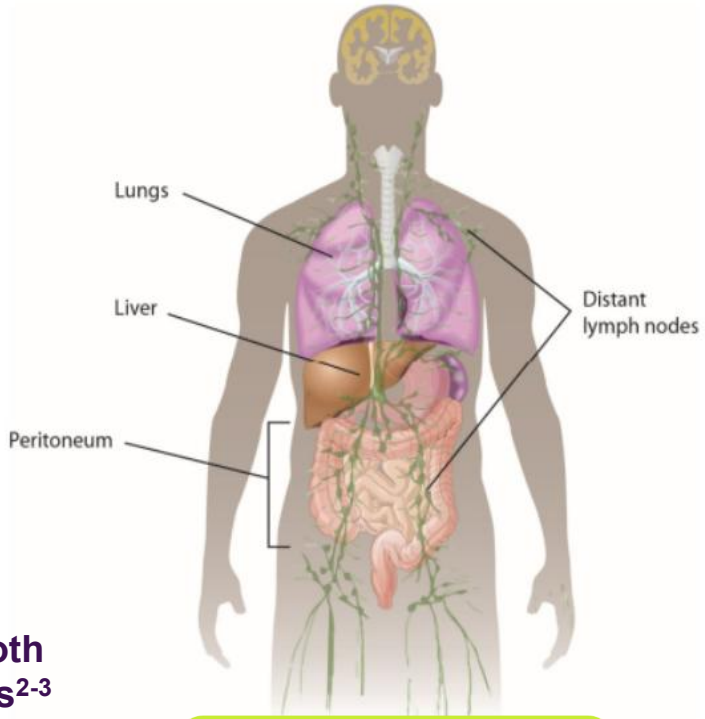
CLDN18.2 expression

Primary GC & Metastatic sites



CLDN18.2 expression in both primary and metastatic sites[2-3]

METASTATIC SITES: A RELIABLE MATERIAL FOR TESTING THE EXPRESSION OF CLDN18



Most common sites of metastasis for G/GEJ cancer[4]

Image adapted from: Verstegen MHP, *et al*. *World J Gastroenterol.* 2020.

Data supports CLDN18.2 expression is maintained in metastatic progression, suggesting accurate CLDN18.2 assessment may be performed from either primary tumour and/or sites of metastases.[1]

astellas

[1]Pellino A, *et al*. *J Pers Med*. 11, 1095, 2021;
[2]Rohde C, *et al*..*J Clin Oncol*. 49(9):870-876, 2019;
[3]Sahin U, *et al*. *Clin Cancer Res.*14(23):7624-34, 2008;
[4]Verstegen MHP,*et al*. *World J Gastroenterol.* 26(39):6037-46, 2020.

Anti-CLDN18 Assays & Antibodies

Aim - Discuss various assays, IHC scoring results & ring studies

Antibody options for evaluating CDLN18.2 expression via IHC

A number of assays and antibodies are available to assess CLDN18.2 expression. The list below is not exhaustive. An IHC ab/assay with high performance should be used when guiding clinical decision-making.

Name	Company	Clone Name	Clonality & Host	Use
VENTANA® CLDN18 (43-14A) RxDx Assay	Roche Diagnostics	43-14A	Mouse, monoclonal	IVD
Recombinant Anti-Claudin18 antibody (43-14A)	Abcam	43-14A	Mouse, monoclonal	RUO
AmoyDx CLDN18 (43-14A)	Amoy Diagnostics	43-14A	Mouse, monoclonal	RUO
LBP CLDN18 (43-14A)	LBP Diagnostics	43-14A	Mouse, monoclonal	RUO
PathPlus™ CLDN18/Claudin18 Antibody	LSBio, an Absolute Biotech Company	LS-B16145	Mouse, monoclonal	RUO

VENTANA® CLDN18 (43-14A) Assay
IVD

- Roche Diagnostics developed an assay for *CLDN18.2* (mouse MoAb, clone 43-14A)
- The Ventana **assay detects both *CLDN18* isoforms in FFPE tissues**, however CLDN18.2 is the dominant isoform in gastric tissue → CLDN18.1 expression is negligible.[1]
- VENTANA CLDN18 (43-14A) Assay is intended for the immunohistochemical detection of the Claudin 18 (CLDN18) protein in formalin-fixed, paraffin embedded (FFPE) neoplastic tissues stained with BenchMark IHC/ISH instruments[1].
- The regulatory status is CE-IVD.
- Antibody has been optimised for specific incubation times on the BenchMark IHC/ISH instrument in combination with the OptiView DAB IHC Detection Kit, but the user must validate results obtained with this reagent.



BenchMark ULTRA Automated IHC/ISH slide staining system

[1]VENTANA CLDN18 (43-14A) Assay, Package Insert Sheet, CE-IVD 1017589EN Rev C, 2023-09-28.

astellas

Global Concordance Study: Overview[1]

Proficiency evaluations have identified variability in antibody and platform performance

LABORATORY INVESTIGATION

journal homepage: https://laboratoryinvestigation.org/

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY Creating a Better Pathologist

Research Article

Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer

Bharat Jasani[a], Philippe Taniere[b], Hans-Ulrich Schildhaus[a], Kevin Blighe[c], Suzanne Parry[d], Dawn Wilkinson[d], Neil Atkey[e], Scott Clare-Antony[e], Clare McCabe[e], Christine Quinn[e,*], CLDN Study Group[f], Andrew Dodson[d]

Global Concordance Study

In this study, which assessed the **reproducibility** and **comparability** of three CLDN18 antibodies—VENTANA (43-14A), LSBio (LS-B16145), and Novus (NBP2-32002)—and IHC staining platforms across a cohort of 27 global laboratories, **sensitivity**, **specificity**, **accuracy**, and **precision** were analyzed.

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

astellas

Global Concordance Study: Overview[1]

Proficiency evaluations have identified variability in antibody and platform performance

Global Concordance Study

In the global concordance study, which assessed the reproducibility and comparability of three CLDN18 antibodies—VENTANA (43-14A), LSBio (LS-B16145), and Novus (NBP2-32002)—and IHC staining platforms across a cohort of 27 global laboratories, sensitivity, specificity, accuracy, and precision were analyzed.

Antibodies investigated in the study

Supplier	Product name	Type	Catalogue number
Roche Tissue Diagnostics	VENTANA CLDN18 (43-14A) Assay	Anti-human mouse monoclonal	08504148001
LSBio	PathPlus™ CLDN18 Antibody for IHC LS-B16145	Anti-human mouse monoclonal	LS-B16145-100
Novus Biologicals	CLDN18 Antibody	Anti-human rabbit polyclonal	NBP2-32002

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

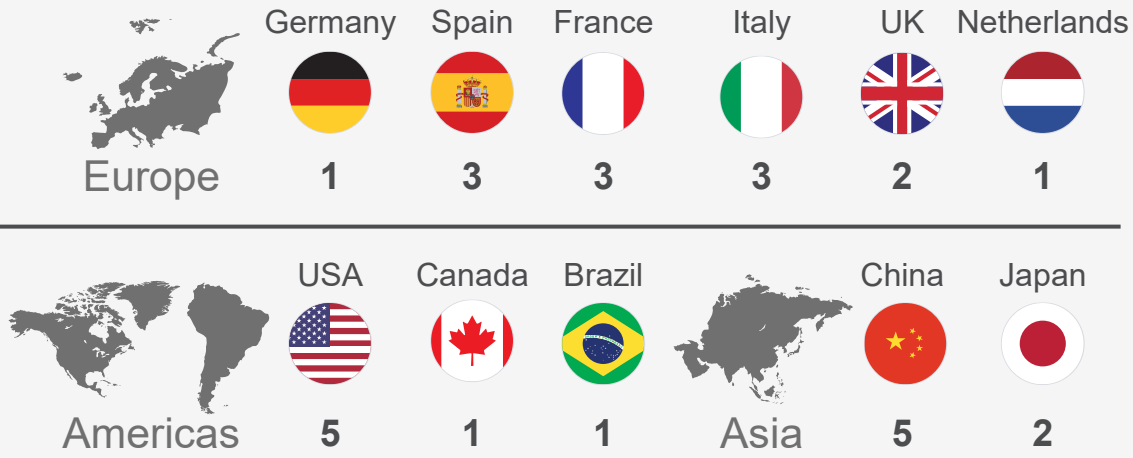
astellas

Global Concordance Study: Method[1]

Study locations

- Up to **5 sets of a TMA comprising 15 FFPE GC samples per array** (represented in triplicate by 1.5 mm cores) were distributed to **27 laboratories in 11 countries**

Number of laboratories



Number of laboratories using each of the platforms and antibodies

Antibodies and platforms		Number of laboratories
Antibodies	Ventana (Roche)	26
	LSBio	25
	Novus	27
Platforms	Ventana BenchMark	27
	Dako AutoStainer	10
	Leica BOND	13

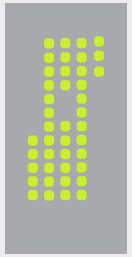
- Laboratories received the **Ventana CLDN18 IVD (43-14A) Assay*** and **CLDN18 antibodies and protocols for the LSBio and Novus antibodies** that had previously been optimised by UK NEQAS

*This assay is an antibody reagent product that is classified as Class I in the USA and General IVD (CE-IVD Analytic) under IVDD in the EU. It is not a CDx assay.
CDx, companion diagnostic; CE, conformité européenne; CLDN18, claudin 18; FFPE, formalin-fixed paraffin-embedded; GC, gastric cancer; IVD, *in vitro* diagnostic; IVDD, *in vitro* diagnostic directive; NEQAS, National External Quality Assessment Services; TMA, tissue microarray.

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

Global Concordance Study: Trial Design[1]

TMA containing 15 gastric cancer samples and controls

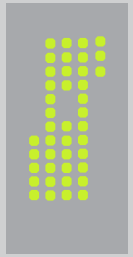


TMAs provided to 27 laboratories

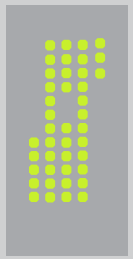
Each laboratory processed a maximum of five TMAs*

Ventana BenchMark IHC platform

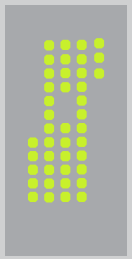
43-14A



LSBio

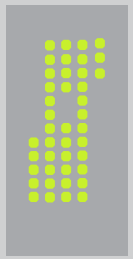


Novus

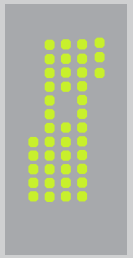


Leica Bond or Dako Autostainer IHC platform

LSBio



Novus



Laboratory scoring results

Slides sent to UK NEQAS for digitization

Central pathological review

Statistical analysis

Laboratories were provided with staining protocols, an instructional video, and reference guide for understanding of CLDN18 IHC staining characteristics

Expected reference results were obtained for each sample stained by a central laboratory using the VENTANA CLDN18 (43-14A) Assay

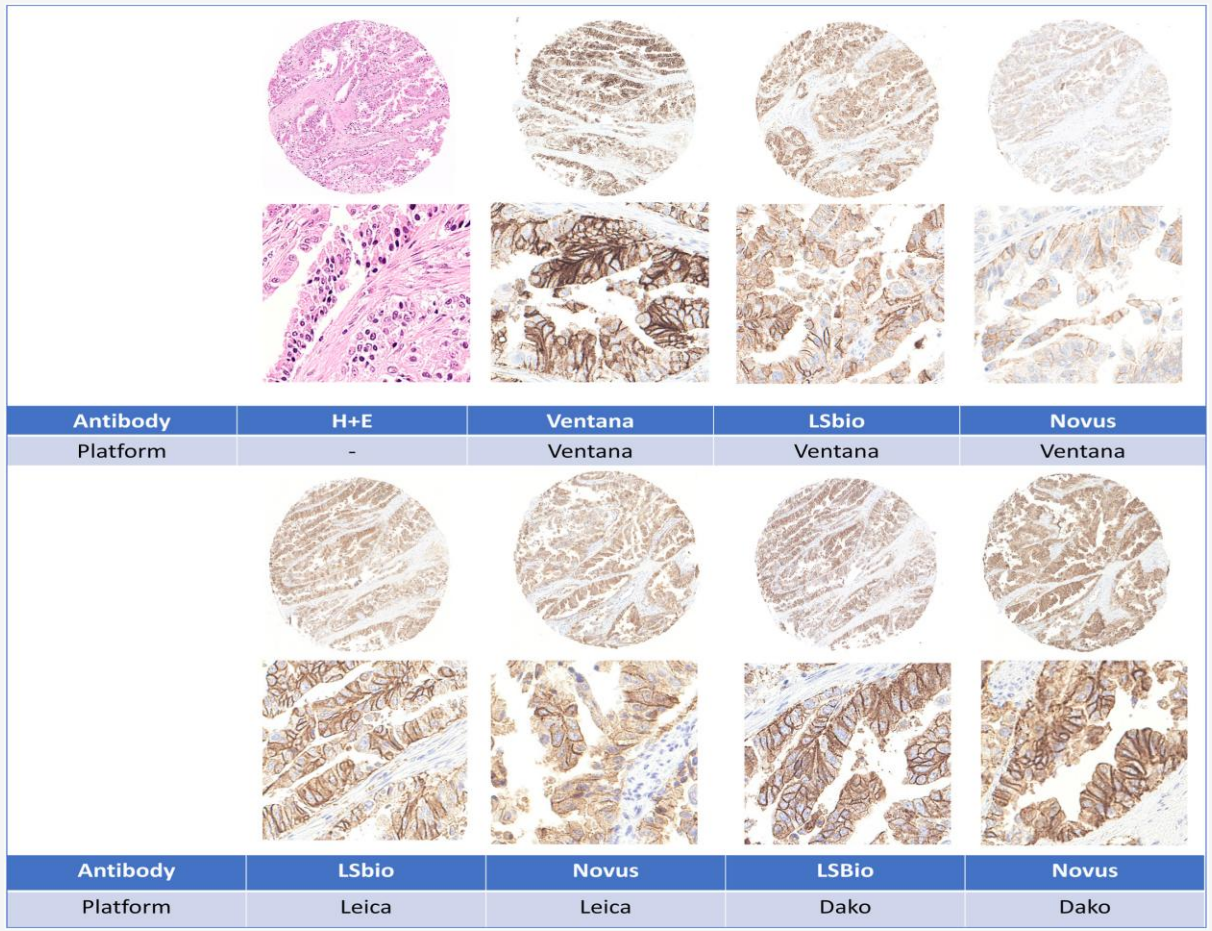
CLDN18.2 positivity was defined as an IHC score of ≥75% of tumor cells demonstrating moderate to strong (≥2+) membranous CLDN18 staining intensity, as used in two clinical trials

*Each laboratory was required to test a maximum of five TMAs using the antibody/assay combinations.

CLDN18.2 claudin 18 isoform 2; GC gastric cancer; IHC immunohistochemistry; NEQAS National External Quality Assessment Scheme; TMA tissue microarray.

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

Global Concordance Study: Results[1]



Antibody	H+E	Ventana	LSbio	Novus
Platform	-	Ventana	Ventana	Ventana

Antibody	LSbio	Novus	LSBio	Novus
Platform	Leica	Leica	Dako	Dako

Images taken from serial sections of the same TMA core stained with H&E and 7 different CLDN18 antibody and platform combinations.

The Ventana assay shows the darkest (brown/black) positive staining, with clear and sharp membranous staining.

Both the LSBio and Novus show weaker staining on the Ventana platform and more defined membranous staining on the Leica and Dako platforms.

CLDN, claudin; H&E, hematoxylin and eosin; TMA, tissue microarray.

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

Global ring study

Discussion

- Ring studies facilitate early biomarker test adoption and emphasize quality testing to produce accurate, reliable results.
- The benefits of conducting a ring study are multifactorial: educates relevant stakeholders on both technical & analytical requirements; provides real-life, hands-on experience to prime laboratories for early integration of new biomarker tests; offers data on available testing options beyond the approved assay.
- Although variation usually occurs between antibodies, the sensitivity and specificity of the Ventana CLDN18 IVD Assay (43-14A) and LSBio antibody met the pre-defined acceptance criteria of the study (≥85%). Caution may be needed with the Novus antibody, as some of the parameters did not reach the 85% acceptance criteria.
- Results highlight that there is a high level of similarity across the Dako and Ventana platforms, suggesting that IHC testing for CLDN18.2 does not have to be limited to one platform.
- An interpretation and scoring manual was used to ensure consistency in CLDN18 IHC staining evaluation.

CLDN: claudin; **IHC:** immunohistochemistry

[1]Jasani, B. *et al*. Global Ring Study …..Claudin 18 Antibodies for Evaluation in Gastric Cancer. *Laboratory Investigation*. 2024.

astellas

Global Concordance Study: Conclusions[1]

1. **Analytical performance (accuracy, sensitivity, and specificity) of the VENTANA CLDN18 (43-14A) IVD Assay was >95% and reproducible across the 27 laboratories vs consensus reference results**

2. The **VENTANA CLDN18 (43-14A) IVD (CE-IVD) Assay and LSBio antibody** met the acceptance criteria (85%) for **all performance metrics after result correction** for discrepant cases by central pathology review, while the Novus antibody assay did not meet the acceptance criteria for some of the performance metrics, even after result correction.

3. **The VENTANA CLDN18 (43-14A) IVD (CE-IVD) assay showed the most overall consistency in performance**. **Note:** The concordance of results after **central pathology review** of CLDN18.2 staining evaluation in GC was found to increase, i.e., when the interpretation discrepancies were removed. E.G., the Ventana antibody on the Ventana platform showed 84% sensitivity which increased to 94% sensitivity after central pathology review.

4. Although the results from this study demonstrate a generally **high-quality performance** among pathologists, they **emphasise the significance of pathologist training** in attaining optimal scoring outcomes



Lab Invest 104 (2024) 100284

LABORATORY INVESTIGATION

journal homepage: https://laboratoryinvestigation.org/

Research Article

Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer

Bharat Jasani[a], Philippe Taniere[b], Hans-Ulrich Schildhaus[a], Kevin Blighe[c], Suzanne Parry[d], Dawn Wilkinson[d], Neil Atkey[a], Scott Clare-Antony[a], Clare McCabe[e], Christine Quinn[e,*], CLDN Study Group[f], Andrew Dodson[d]

[a] Discovery Life Sciences (DLS) Biomarker Services GmbH, Kassel, Germany; [b] University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK; [c] Clinical Bioinformatics Research Ltd, London, UK; [d] UK National External Quality Assessment Scheme for Immunocytochemistry & In-Situ Hybridisation, London, UK; [e] Diaceutics PLC, Belfast, UK; [f] See the details of CLDN Study Group members in the acknowledgments

ARTICLE INFO

Article history:
Received 28 July 2023
Revised 23 October 2023
Accepted 6 November 2023
Available online 8 November 2023

Keywords:
Claudin-18.2
CLDN18.2
zolbetuximab
gastroesophageal junction adenocarcinoma
gastric adenocarcinoma

ABSTRACT

Claudin 18.2 (CLDN18.2), the dominant isoform of CLDN18 in gastric tissues, is a highly specific tight junction protein of the gastric mucosa with variably retained expressions in gastric and gastro-esophageal junction cancers. Additionally, CLDN18.2-targeted treatment with zolbetuximab, in combination with chemotherapy, has recently been assessed in 2 phase-III studies of patients with HER2-negative, locally advanced, unresectable, or metastatic gastric or gastroesophageal junction adenocarcinoma. These trials used the investigational VENTANA CLDN18 (43-14A) RxDx immunohistochemistry (IHC) assay on the Ventana BenchMark platform to identify patients eligible for CLDN18.2-targeted treatment. We report the findings of a global ring study evaluating the analytical comparability of concordance of the results of 3 CLDN18 antibodies (Ventana, LSBio, and Novus) stained on 3 IHC-staining platforms (Ventana, Dako, and Leica). A tissue microarray (TMA), comprising 15 gastric cancer cases, was stained by 27 laboratories across 11 countries. Each laboratory stained the TMAs using at least 2 of the 3 evaluated CLDN18 antibodies. Stained TMAs were assessed and scored using an agreed IHC-scoring algorithm, and the results were collated for statistical analysis. The data confirmed a high level of concordance for the VENTANA CLDN18 (43-14A; Ventana platform only) and LSBio antibodies on both the Dako and Leica platforms, with accuracy, precision, sensitivity, and specificity rates all reaching a minimum acceptable >85% threshold and good-to-excellent levels of concordance as measured by Cohen's kappa coefficient. The Novus antibody showed the highest level of variability against the reference central laboratory results for the same antibody/platform combinations. It also failed to meet the threshold for accuracy and sensitivity when used on either the Dako or Leica platform. These results demonstrated the reliability of IHC testing for CLDN18 expression in gastric tumor samples when using commercially available platforms with an appropriate methodology and primary antibody selection.

© 2023 THE AUTHORS. Published by Elsevier Inc. on behalf of the United States & Canadian Academy of Pathology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

* Corresponding author.
E-mail address: christine.quinn@diaceutics.com (C. Quinn).

0023-6837/© 2023 THE AUTHORS. Published by Elsevier Inc. on behalf of the United States & Canadian Academy of Pathology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
https://doi.org/10.1016/j.labinv.2023.100284

Global Concordance Study

https://www.sciencedirect.com/science/article/pii/S0023683723002271?via%3Dihub

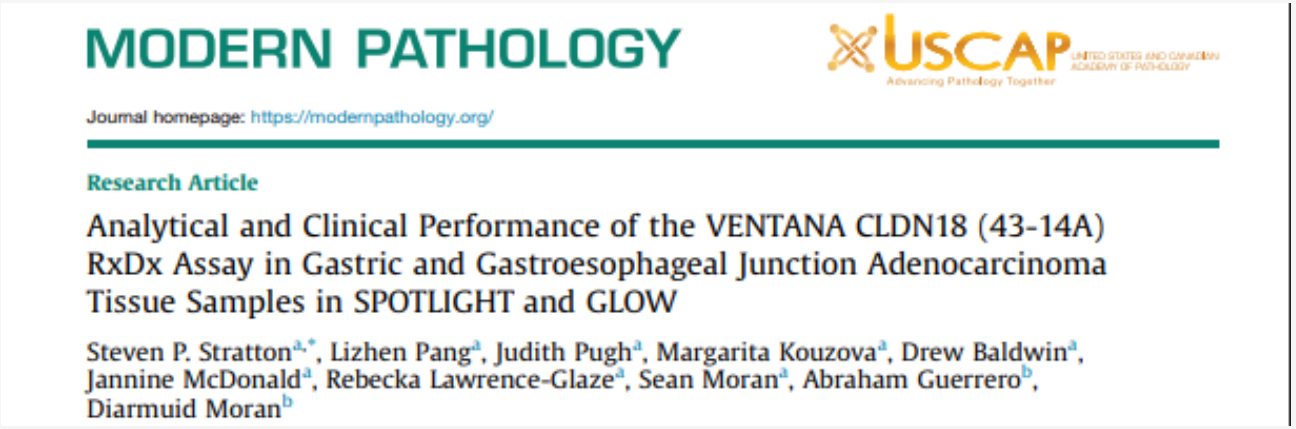
CLDN18.2, claudin 18.2; IHC, immunohistochemistry.

1. Jasani B, Taniere P, Schildhaus H-U, et al. *Lab Invest* 2024;104(1):100284

Analytical Performance Study: Overview[1]

Ventana CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma

- The VENTANA CLDN18 (43-14A) RxDx Assay is an investigational immunohistochemistry (IHC) test that utilizes a mouse monoclonal antibody and was designed for high sensitivity and specificity in the detection of CLDN18.
- The VENTANA CLDN18 (43-14A) RxDx Assay was used to identify patients with previously untreated, locally advanced unresectable or mG/GEJ adenocarcinoma whose tumours are CLDN18.2-positive and HER2-negative in 2 phase 3 clinical trials[1,2].
- The aim of the study was to study the robustness of analytical and clinical performance of the VENTANA CLDN18 (43-14A) RxDx Assay in mG/GEJ adenocarcinoma.



MODERN PATHOLOGY

USCAP

Journal homepage: https://modernpathology.org/

Research Article

Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW

Steven P. Stratton[a,*], Lizhen Pang[a], Judith Pugh[a], Margarita Kouzova[a], Drew Baldwin[a], Jannine McDonald[a], Rebecka Lawrence-Glaze[a], Sean Moran[a], Abraham Guerrero[b], Diarmuid Moran[b]

astellas

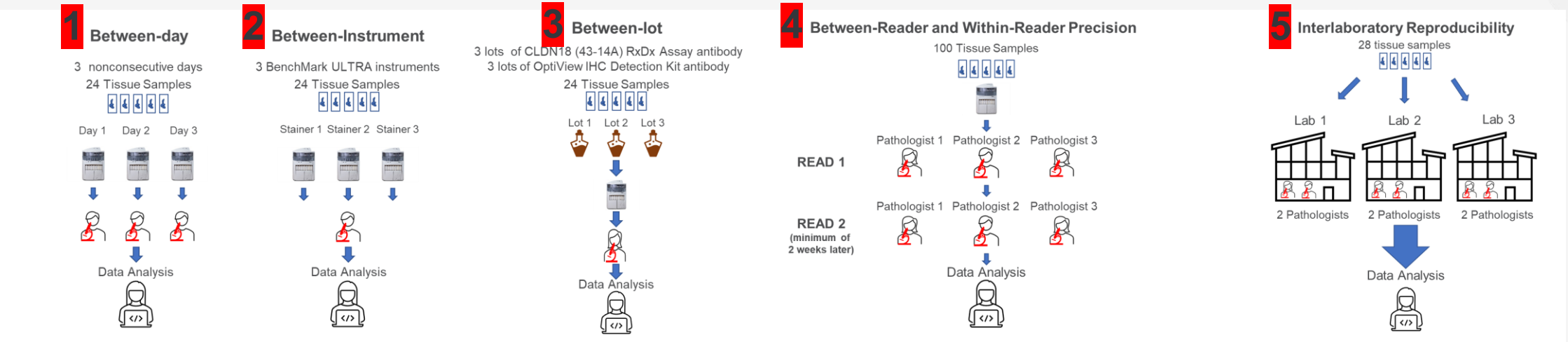
CLDN18 claudin 18; **CLDN18.2** claudin 18 isoform 2; **IHC** immunohistochemistry; **HER2** human epidermal growth factor receptor; **mG/GEJ** metastatic gastric or gastroesophageal junction.

1. S Stratton, et al. Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW, Modern Pathology, Volume 38, Issue 12, 2025

Analytical Performance Study: Overview[1]

Ventana CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma

- The VENTANA CLDN18 (43-14A) RxDx Assay is an investigational immunohistochemistry (IHC) test that utilizes a mouse monoclonal antibody and was designed for high sensitivity and specificity in the detection of CLDN18.
- The VENTANA CLDN18 (43-14A) RxDx Assay was used to identify patients with previously untreated, locally advanced unresectable or mG/GEJ adenocarcinoma whose tumours are CLDN18.2-positive and HER2-negative in 2 phase 3 clinical trials[1,2].
- The aim of the study was to study the robustness of analytical and clinical performance of the VENTANA CLDN18 (43-14A) RxDx Assay in mG/GEJ adenocarcinoma.
- The analytical performance studies included **5 different steps of analysis**, as follows:



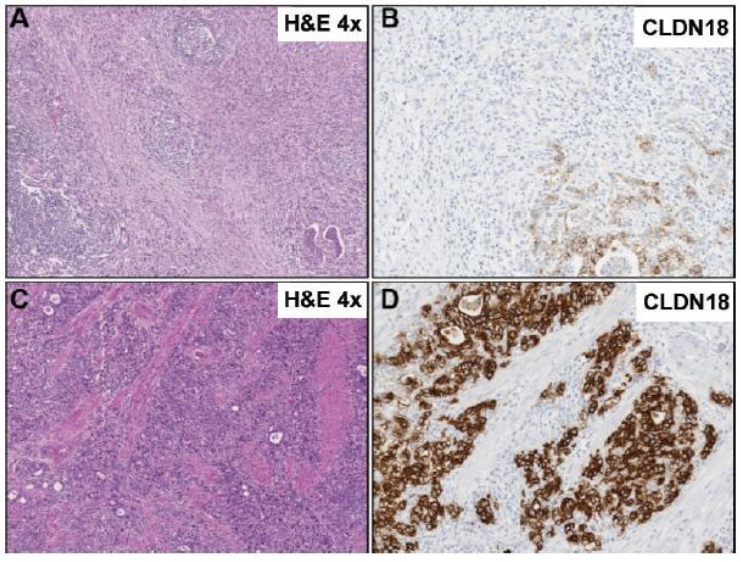
CLDN18 claudin 18; **CLDN18.2** claudin 18 isoform 2; **IHC** immunohistochemistry; **HER2** human epidermal growth factor receptor; **mG/GEJ** metastatic gastric or gastroesophageal junction.

1. S Stratton, et al. Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW, Modern Pathology, Volume 38, Issue 12, 2025

Analytical Performance Study: Results[1]

- **High repeatability** of the VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma was observed for the test in verification studies
  - Between **day**
  - Between **instrument**
  - Between **lot**
- **High precision** was observed for **between-reader** and **within-reader** precision of the VENTANA CLDN18 (43-14A) RxDx Assay in G/GEJ adenocarcinoma
- **High reproducibility** was observed for the assay in the **interlaboratory reproducibility** study showing the assay performs consistently across laboratories.

Tumour-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 tumour sample with CLDN18-negative IHC status demonstrating moderate-to-strong membranous staining in 20% of tumour cells.
C–D. H&E (C) and CLDN18 IHC (D) staining of tumour sample with CLDN18-positive IHC status demonstrating moderate-to-strong membranous staining in 95% of tumour cells.

CLDN18, claudin-18; H&E, haematoxylin and eosin; G/GEJ, gastric or gastroesophageal junction; IHC, immunohistochemistry.

CLDN18 claudin 18; **CLDN18.2** claudin 18 isoform 2; **IHC** immunohistochemistry; **HER2** human epidermal growth factor receptor; **mG/GEJ** metastatic gastric or gastroesophageal junction.
Reader = Pathologist

1. S Stratton, et al. Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW, Modern Pathology, Volume 38, Issue 12, 2025

Analytical Performance Study: Conclusions[1]

1. The investigational VENTANA CLDN18 (43-14A) RxDx Assay demonstrated robust analytical and clinical performance in G/GEJ adenocarcinoma tissue.

2. Analytical performance studies demonstrated reproducibility across reagent lots, instruments, testing days and sites, and pathologists' readings.

3. Performance in two phase 3 clinical trials demonstrated high overall staining acceptability rates.



Mod Pathol 38 (2025) 100844

MODERN PATHOLOGY

USCAP

Journal homepage: https://modernpathology.org/

Research Article

Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW

Steven P. Stratton[a,*], Lizhen Pang[a], Judith Pugh[a], Margarita Kouzova[a], Drew Baldwin[a], Jannine McDonald[a], Rebecka Lawrence-Glaze[a], Sean Moran[a], Abraham Guerrero[b], Diarmuid Moran[b]

[a] Roche Diagnostics Solutions, Tucson, Arizona; [b] Astellas Pharma Global Development Inc, Northbrook, Illinois

ARTICLE INFO

Article history:
Received 21 February 2025
Revised 16 June 2025
Accepted 2 July 2025
Available online 16 July 2025

Keywords:
companion diagnostic
Claudin 18
Claudin 18 isoform 2
gastric adenocarcinoma
gastroesophageal junction adenocarcinoma
zolbetuximab

ABSTRACT

New therapies are needed to treat patients with human epidermal growth factor receptor 2 (HER2)-negative, locally advanced (LA) unresectable or metastatic gastric or gastroesophageal junction (mG/GEJ) adenocarcinoma. Zolbetuximab is a monoclonal antibody that targets the tight junction protein claudin 18 isoform 2 (CLDN18.2) in gastric or gastroesophageal junction (G/GEJ) adenocarcinoma cells. We describe the analytical and clinical performance of the VENTANA CLDN18 (43-14A) RxDx Assay (Roche Diagnostics Solutions) as a companion diagnostic immunohistochemistry assay for treatment with zolbetuximab. Analytical performance was assessed in commercially available G/GEJ adenocarcinoma samples. Clinical performance was assessed in the context of 2 phase 3 trials (SPOTLIGHT, NCT03504397; GLOW, NCT03653507) of first-line zolbetuximab plus chemotherapy in patients with HER2-negative, LA unresectable or mG/GEJ adenocarcinoma, whose tumors were CLDN18.2 positive (≥75% of viable tumor cells demonstrating moderate-to-strong membrane CLDN18 staining). Staining acceptability rates were assessed in patients whose tumors were tested for CLDN18.2 status using the VENTANA CLDN18 (43-14A) RxDx Assay and who met enrollment eligibility criteria not related to CLDN18.2 status. Progression-free survival and overall survival were evaluated in the enrolled patients. Analytical performance: positive and negative percent agreement compared with immunohistochemistry reference scoring was 100% for between-antibody, between-detection kit, between-instrument, between-day, and within-run precision studies. Interlaboratory reproducibility exceeded 90% average positive and negative agreement between sites and 94% average positive and negative agreement between readers across 3 sites. Clinical performance: among 3783 patient tumor samples, staining acceptability rates exceeded 94%. Patients identified for eligibility using the VENTANA CLDN18 (43-14A) RxDx Assay demonstrated statistically significant improvement in progression-free survival and overall survival with zolbetuximab plus chemotherapy in both phase 3 trials. The VENTANA CLDN18 (43-14A) RxDx Assay demonstrated robust, reproducible analytical performance and clinical utility as a companion diagnostic for first-line zolbetuximab plus chemotherapy in patients with HER2-negative, LA unresectable or mG/GEJ adenocarcinoma, whose tumors were CLDN18.2 positive.

© 2025 THE AUTHORS. Published by Elsevier Inc. on behalf of the United States & Canadian Academy of Pathology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* Corresponding author.
E-mail address: steven.stratton@roche.com (S.P. Stratton).

0893-3952/© 2025 THE AUTHORS. Published by Elsevier Inc. on behalf of the United States & Canadian Academy of Pathology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
https://doi.org/10.1016/j.modpat.2025.100844

Analytical Performance Study

https://www.sciencedirect.com/science/article/pii/S0893395225001413?via%3Dihub

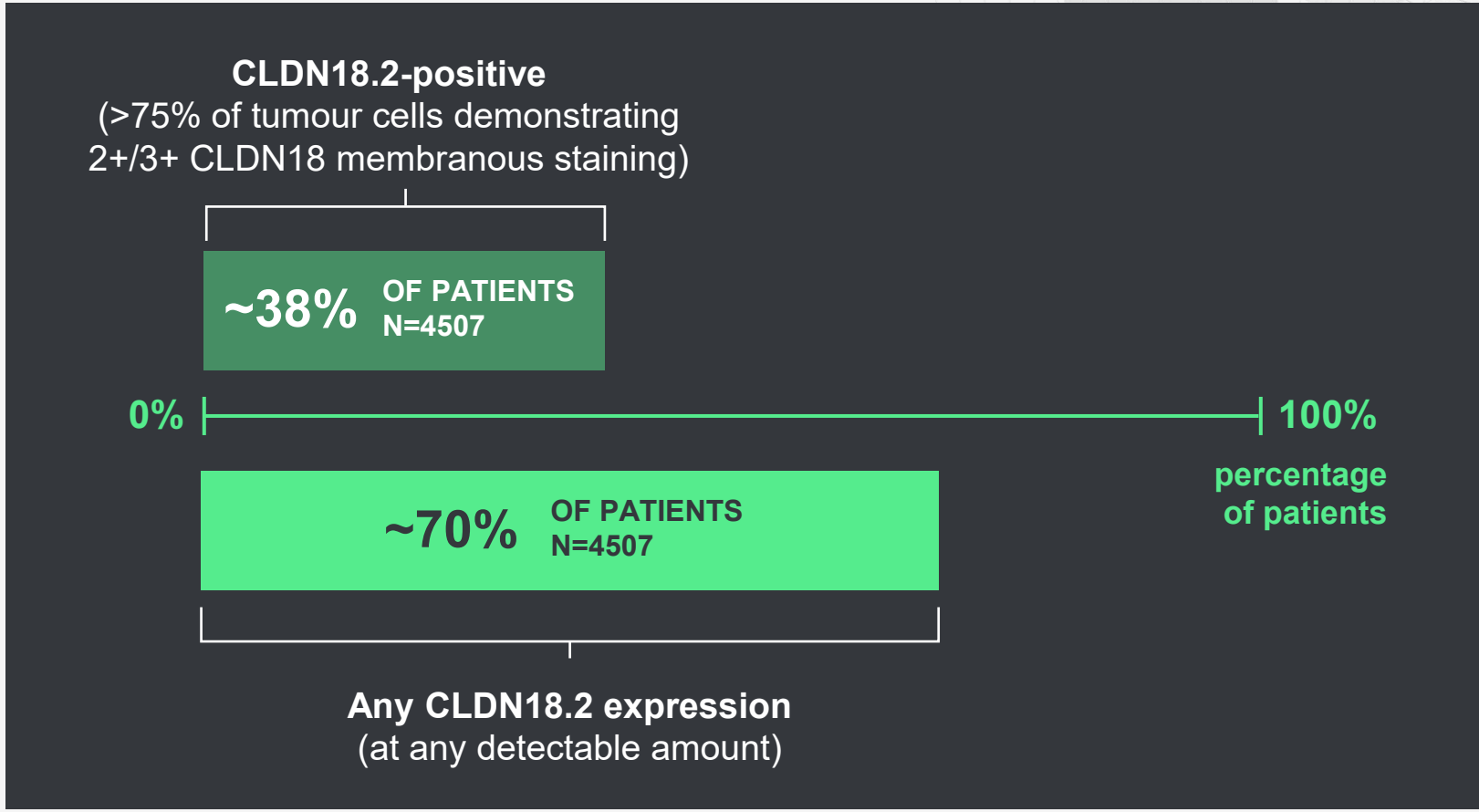
astellas

1. S Stratton, et al. Analytical and Clinical Performance of the VENTANA CLDN18 (43-14A) RxDx Assay in Gastric and Gastroesophageal Junction Adenocarcinoma Tissue Samples in SPOTLIGHT and GLOW, Modern Pathology, Volume 38, Issue 12, 2025

CLDN18.2 is a highly prevalent biomarker in advanced G/GEJ adenocarcinoma[1-3]

According two recent global studies which included 4507 patients with locally advanced unresectable or metastatic G/GEJ adenocarcinoma, ~38% of cases demonstrated ≥75% of tumour cells with moderate-to-strong (2/3+) membranous CLDN18 staining.[2,3]

- Among advanced G/GEJ biomarkers, CLDN18.2 is highly prevalent[2,3]
- CLDN18.2 expression can be readily detected using IHC testing[2,3]



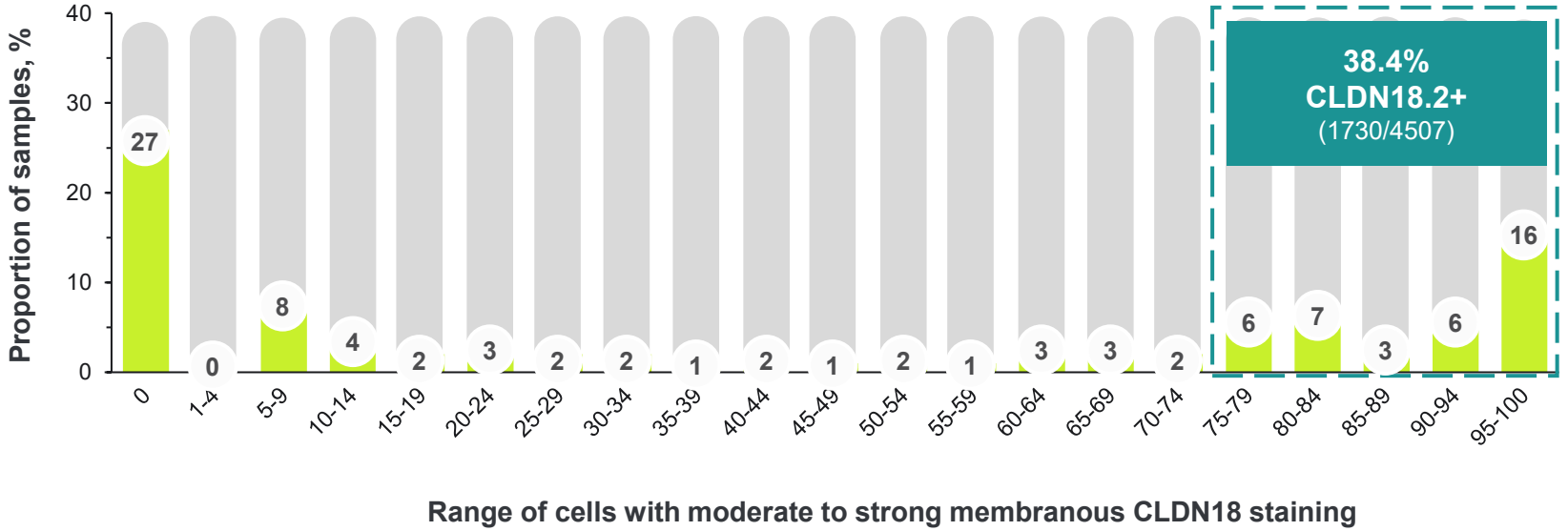
1. Shitara K, Lordick F, Bang YJ, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. Lancet 2023;401(10389):1655-68.
2. Xu RH, Shitara K, Ajani JA, et al. Zolbetuximab + CAPOX in 1L Claudin-18.2+ (CLDN18.2+)/HER2– locally advanced (LA) or metastatic gastric or gastroesophageal junction (mG/GEJ) adenocarcinoma: primary phase 3 results from GLOW. Presented at: March American Society of Clinical Oncology Plenary Series; March 22, 2023.

New slide

Global Prevalence of CLDN18.2 Across two Phase 3 Clinical Trials Was Assessed

Distribution of CLDN18 Expression (n=4507)[1,*,†]

Screened patients in two phase 3 clinical trials



38.4% of screened patients across two global phase 3 clinical trials had CLDN18.2+ tumors[1]

CLDN18.2 positivity defined as ≥75% of tumor cells demonstrating moderate to strong membranous CLDN18 staining

astellas

CLDN18, claudin 18; CLDN18.2, claudin 18 isoform 2; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; LA, locally advanced.
*CLDN18 staining by IHC using the investigational VENTANA CLDN18 (43-14A) RxDx Assay (Ventana Medical Systems, Inc. [Roche Tissue Diagnostics]) on the BenchMark ULTRA instrument. All IHC testing was performed by central laboratory testing (Q2 Solutions). †Includes both HER2+ and HER− patients.

1. Shitara K, et al. *Gastric Cancer*. 2024;27:1058-1068.

CLDN18.2 in G/GEJ cancer

Prevalence in global studies

- Overall CLDN18.2 prevalence in two global phase 3 clinical studies: 38.4% (1730 among 4507 patients).[1,2,3]
- Of 3705 patients with HER2-negative disease with valid CLDN18 IHC results, 1568 (42.3%) had CLDN18.2-positive tumours
- **Conclusion from two global clinical trials is that CLDN18.2 positivitiy prevalence is similar across regions (ranging from 30% to 44%).**

*CLDN18.2 positivity was defined as an IHC score of ≥75% of tumour cells demonstrating moderate-to-strong (≥2+) membranous CLDN18 staining intensity.

Prevalence of CLDN18.2 in Global Studies[1,2,3]

CLDN18.2 status	% of patients (n=4507)
Negative (0/1+)	26.5%
<50%(≥2+)	24.9%
50-74%(≥2+)	10.6%
≥75%(≥2+)	38.4%

Prevalence of CLDN18.2-positivity* by region[1,2,3]

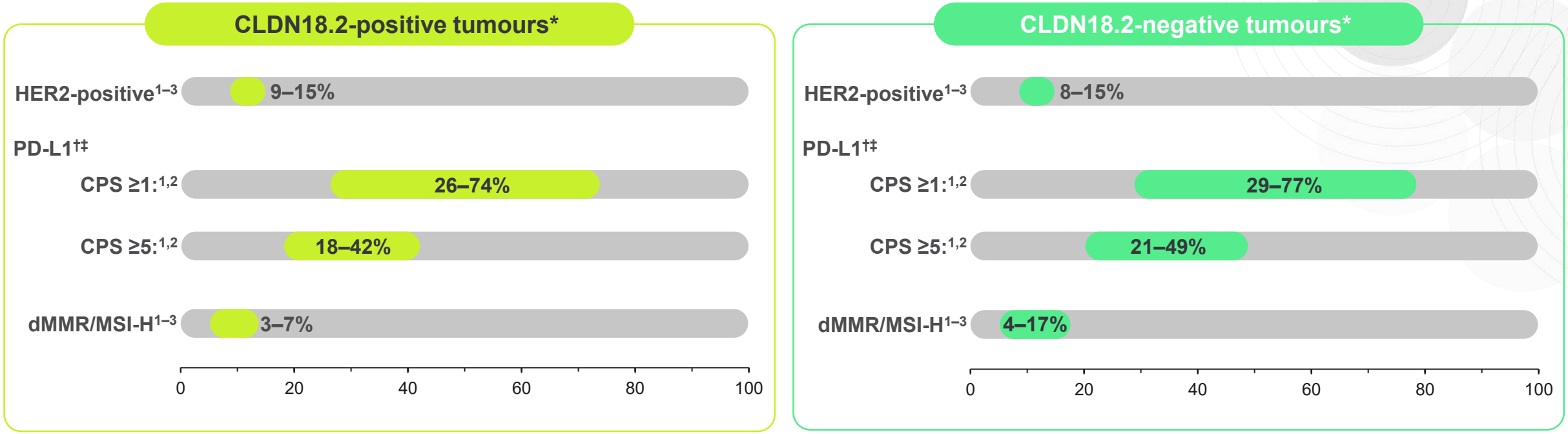
Region	% of patients
Asia Pacific (excluding China)	36.5% (479/1314)
China	35.0% (295/844)
Europe/ Middle East	44% (671/1524)
North America	37.7% (183/485)
South America	30% (102/340)

astellas

[1]Shitara, K, *et al*. Poster 4035: Presented at the 2023 ASCO Annual Meeting; June 2–6, 2023;
[2]Shah, MA, *et al*. *Nat Med*. 29:2133-2141, 2023;
[3]Shitara, K, *et al*. *Lancet*. 401(10389):1655-1668, 2023.

CLDN: claudin; **G/GEJ:** gastric and gastro-oesophageal junction

No clear co-expression patterns for CLDN18.2 with other biomarkers in G/GEJ adenocarcinoma



- CLDN18.2 status was assessed by IHC in all studies using the Ventana CLDN18 (clone 43-14A) assay[4]
- CLDN18.2 positivity was defined as ≥75% of tumour cells demonstrating moderate-to-strong membranous CLDN18 staining[1–3]

*Pellino A, et al. n=117, Kubota Y et al. n =98, Sewastjanow M, et al. n = 135;[1–3] †PD-L1 testing antibodies: Pellino et al. used the 22C3 (Dako) antibody. Kubota et al. used the SP142 (Ventana) or SP263 (Ventana) antibody; ‡In Kubota et al. PD-L1 was evaluated in 93 CLDN18.2-positive and 293 CLDN18.2-negative and authors noted that relatively old archival specimens used may have affected the positivity rate.[2]
CLDN18, claudin 18; CLDN18.2, claudin 18.2; CPS, combined positive score; dMMR, mismatch repair deficient; G/GEJ, gastric/gastro-oesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; PD-L1, programmed death-ligand 1.

1. Pellino A, et al. *J Pers Med* 2021;11(11):1095; 2. Kubota Y, et al. *ESMO Open* 2023;8(1):100762; 3. Sewastjanow M, et al. Poster presentation at AACR 2023. Poster 2141;
4. Roche. https://elabdoc-prod.roche.com/eLD/api/downloads/ff8f8fc9-3536-ea11-fc90-005056a71a5d?countryIsoCode=us. Accessed November 2024.

CLDN18.2 prevalence

By tumour disease and sample characteristics

Disease Characteristics[1]

Prevalence of CLDN18.2-positivity was 39.9% (1341/3357) in patients with **gastric adenocarcinoma** and 37.5% (338/901) in patients with **GEJ adenocarcinoma**

There was a statistically significant association between CLDN18.2 status and tumour type (P = 0.0002); patients with **diffuse tumours** (48.3%; 553/1145) had the highest prevalence of CLDN18.2-positivity compared to patients with **intestinal** (38.8%; 308/794) and **mixed type tumours** (42.9%; 134/312)

Sample Characteristics[1]

Prevalence of CLDN18.2-positivity was similar regardless of collection method; 38.6% (1403/3632) for **biopsy** and 37.6% (327/870) for **resection.**

Prevalence of CLDN18.2-positivity was 39.4% (201/510) in samples collected from **metastatic sites** and 38.0% (1498/3939) in samples collected from **primary sites**. This suggests samples from primary or metastatic sites can be used to screen for CLDN18 expression by IHC

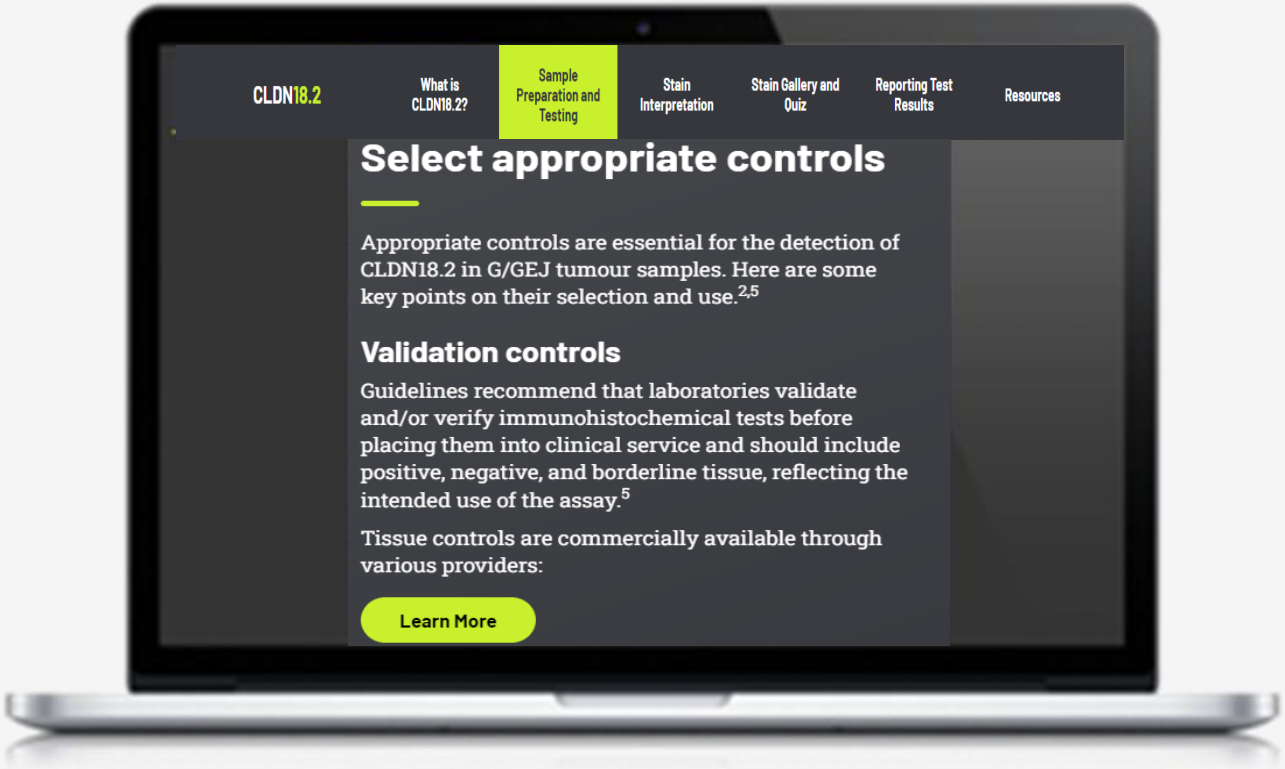
Overall CLDN18.2 prevalence in two global phase 3 clinical studies: 38.4% (1730/4507).[2,3]

- CLDN18.2 positivity was defined as an IHC score of ≥75% of tumour cells demonstrating moderate to strong (≥2+) membranous CLDN18 staining intensity.

1. Shitara K, Lordick F, Bang YJ, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. Lancet 2023;401(10389):1655-68.
2. Xu RH, Shitara K, Ajani JA, et al. Zolbetuximab + CAPOX in 1L Claudin-18.2+ (CLDN18.2+)/HER2– locally advanced (LA) or metastatic gastric or gastroesophageal junction (mG/GEJ) adenocarcinoma: primary phase 3 results from GLOW. Presented at: March American Society of Clinical Oncology Plenary Series; March 22, 2023.

astellas

For more info on: Validation



Go to CLDN182.com
"Sample Preparation & Testing"

astellas

38th European Congress of Pathology (ECP) · 12–16 September 2026

SG_11305_Dx_21102026

Evaluation of CLDN18.2 IHC concordance across multiple cut-offs and sample types in gastric carcinoma

Jing Li[1*], Miranda E Clements[2], Sophie E Willis[3], Tsu-Shuen Tsao[4], Mark D Robida[4], Deborah Berry[2], Philip Martin[2], Gemma N Jones[3], Marlon Rebelatto[2], Maria Broggi[5], John Longshore[6], Nicolas Giraldo[7]

1. Global Oncology Diagnostics, AstraZeneca, Cambridge, UK; 2. Translational Pathology, Oncology R&D, AstraZeneca, Gaithersburg, MD, USA; 3. Translational Pathology, Oncology R&D, AstraZeneca, Cambridge, UK; 4. Roche Tissue Diagnostics, Tucson, AZ, USA; 5. Translational Medicine, Oncology R&D, AstraZeneca, Gaithersburg, MD, USA; 6. Global Oncology Diagnostics, AstraZeneca, Durham, NC, USA; 7. Global Oncology Diagnostics, AstraZeneca, Barcelona, Spain.
*Presenting author: jing.li43@astrazeneca.com

Objective

- Assess concordance between the pan-CLDN18 43-14A and CLDN18.2-specific SP455 IHC assays across clinical cut-offs (≥25/50/75%) and intensities (moderate–strong 2+/3+; any 1+/2+/3+)
- Evaluate concordance between paired primary and metastatic gastric carcinoma samples

Conclusions

- Building on previously-established analytical concordance, 43-14A and SP455 agreed highly across all cut-offs (OPA 86.7–96.7%; Cohen's κ 0.72–0.93)
- Strong agreement at both clinically-relevant cut-offs — ≥75% moderate–strong (approved; OPA 93.3%) and ≥25% any intensity (of interest; OPA 92.2%)
- Paired primary–metastatic samples were strong trend towards concordance (76.2%) with no significant expression shift
- Provides an evidence-based reference for CLDN18.2 IHC testing in gastric carcinoma

Limitations

- Commercially-sourced samples; scoring by a single board-certified pathologist
- Modest paired cohort (n=21) limits power to detect subtle primary–metastatic differences
- Metastatic analysis used the EPR19202 research-use-only assay

Plain language summary



Why did we perform this research?

LDN18.2 expression in gastric cancer can be targeted therapeutically, but different therapies have been developed using different assays and cut-offs to define biomarker positivity We checked whether two widely-used IHC tests agree across those cut-offs, and whether results match between the original and metastatic (spread) tumour.



How did we perform this research?

We tested 90 stomach-cancer samples with two assays across several cut-offs, and compared 21 matched primary–metastatic pairs.



What did we find, and what are the implications?

The tests agreed well across all cut-offs, and matched pairs mostly agreed with no consistent difference. These exploratory findings help guide how CLDN18.2 testing could be implemented.

astellas

Introduction

- CLDN18.2 (claudin-18.2) is a tight-junction protein normally confined to differentiated gastric mucosa; on malignant transformation its epitopes become exposed on the cell surface, making it an attractive therapeutic target in gastric and gastro-oesophageal junction (G/GEJ) adenocarcinoma[1]. Approximately 38% of G/GEJ adenocarcinomas are CLDN18.2-positive by the ≥75% moderate–strong definition[2].
- A CLDN18.2-targeted antibody is approved (with chemotherapy) for CLDN18.2-positive, HER2-negative advanced G/GEJ adenocarcinoma; its companion diagnostic — the VENTANA CLDN18 (43-14A) IHC assay — defines positivity as ≥75% of tumour cells with moderate-to-strong (2+/3+) membranous staining[3,4]. A next generation of CLDN18.2-directed therapies — antibody–drug conjugates, bispecific antibodies and CAR-T — is in development, several using different (often lower or any-intensity) positivity thresholds[5,6,7]. The definition of "CLDN18.2-positive" therefore varies by asset and trial.
- Multiple assays are in use — the pan-CLDN18 43-14A assay and CLDN18.2-specific assays such as SP455 and EPR19202 — so concordance between assays and across thresholds is central to consistent patient selection. Concordance between primary and metastatic disease is also clinically important.
- Prior work established analytical concordance between 43-14A, SP455 and EPR19202 by H-score correlation (43-14A vs SP455 R=0.94; EPR19202 vs SP455 R=0.92; n=91; Clements et al., ASCO 2024)[8]; this study extends that work to concordance across clinical cut-offs, and to protein expression changes between paired primary and metastatic samples

Methods

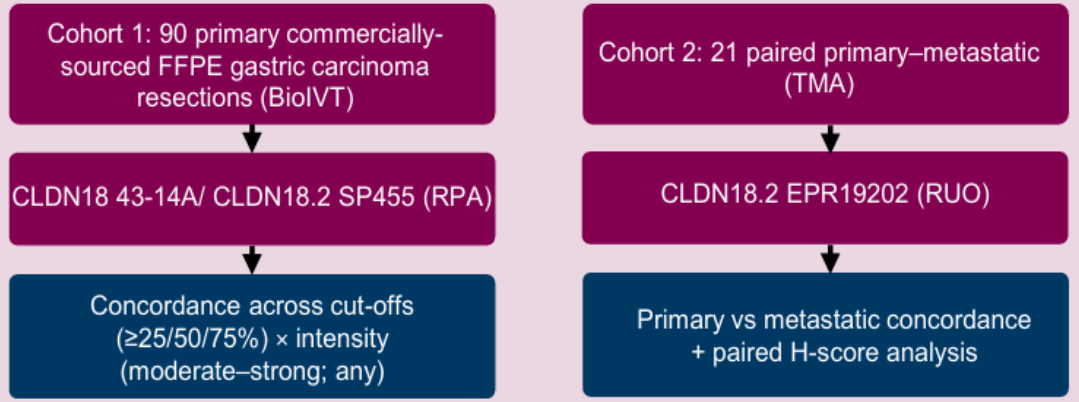
Samples & assays & scoring

- 90 commercially-sourced primary GC resections (BioIVT); FFPE
- Stained with CLDN18 43-14A (formula-locked) and CLDN18.2 SP455 (robust prototype), both Ventana
- 21 paired primary–metastatic samples (TMA) stained with CLDN18.2 EPR19202 (RUO)
- Tumour positivity (%) scored at each intensity (1+/2+/3+) by a board-certified pathologist

Concordance analysis

- 43-14A vs SP455 evaluated at moderate–strong (2+/3+) and any intensity (1+/2+/3+) across ≥25/50/75% cut-offs
- Agreement summarised as OPA, PPA and NPA (vs SP455) and Cohen's κ
- Cut-offs of clinical interest: ≥75% moderate–strong (approved CDx threshold) and ≥25% any intensity (of interest)
- Paired primary–metastatic H-scores compared by paired t-test and Wilcoxon signed-rank test

Figure 1. Study design



astellas

Results

Concordance of assays across clinical cut-offs (43-14A vs SP455, n=90)

- Across all cut-offs, overall agreement between 43-14A and SP455 exceeded 86% (OPA 86.7–96.7%); Cohen's κ 0.72–0.93
- Across all cut-offs, PPA (vs SP455) 85.0–98.0%; NPA 85.7–95.7%
- Agreement was generally higher using any-intensity thresholds (OPA 92.2%, 96.7%, 90.0% at ≥25/50/75%)
- At ≥75% moderate–strong: OPA 93.3%, κ 0.81
- At ≥25% any intensity: OPA 92.2%, κ 0.83 (**Table 1, Figure 2**).

Figure 2. 43-14A vs SP455 agreement by cut-off and intensity (n=90)

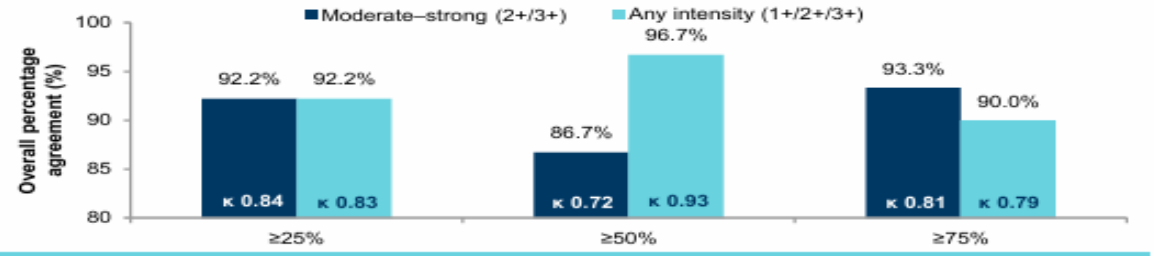


Table 1. 43-14A vs SP455 agreement metrics (n=90)

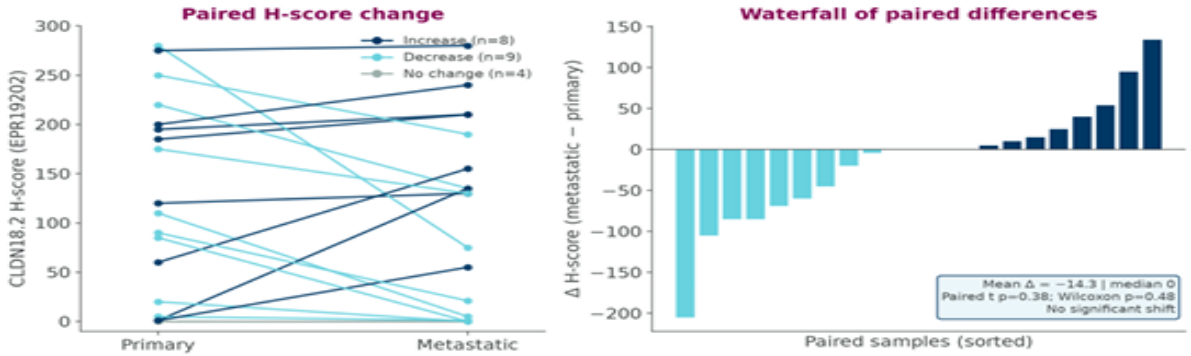
Intensity	Cut-off	TP	FP	FN	TN	OPA	PPA*	NPA*	κ	Clinical relevance
Moderate–strong	≥25%	47	6	1	36	92.2%	97.9%	85.7%	0.84	
Moderate–strong	≥50%	28	8	4	50	86.7%	87.5%	86.2%	0.72	
Moderate–strong	≥75%	17	3	3	67	**93.3%**	85.0%	95.7%	**0.81**	**Approved drug threshold**
Any intensity	≥25%	56	3	4	27	**92.2%**	93.3%	90.0%	**0.83**	**Cut-off of interest**
Any intensity	≥50%	50	2	1	37	96.7%	98.0%	94.9%	0.93	
Any intensity	≥75%	32	6	3	49	90.0%	91.4%	89.1%	0.79	

**vs SP455 (reference). Moderate–strong = 2+/3+; any intensity = 1+/2+/3+. Shaded rows: approved drug threshold (≥75% moderate–strong) and cut-off of interest (≥25% any intensity). OPA/PPA/NPA, overall/positive/negative percentage agreement; κ, Cohen's kappa.*

Concordance between paired primary and metastatic samples (n=21)

- Paired H-scores (EPR19202) showed no statistically significant shift between primary and metastatic samples (mean Δ −14.3; median 0; paired t-test p=0.38; Wilcoxon p=0.48) (**Figure 3**)
- 16/21 pairs (76.2%) were concordant at the ≥25% any-intensity cut-off of interest, and at the ≥75% moderate–strong approved threshold (**Table 2**)

Figure 3. Paired primary–metastatic H-score (EPR19202, n=21)



Left: paired change (navy = increase, light blue = decrease, grey = no change). Right: waterfall of Δ (met − primary). No significant overall shift.

Table 2. Primary vs metastatic concordance at clinical cut-offs (n=21)

≥25% any	Prim +	Prim −
Met +	**10**	2
Met −	3	**6**

Overall agreement: 76.2% (16/21)

≥75% mod–strong	Prim +	Prim −
Met +	**2**	2
Met −	3	**14**

Overall agreement: 76.2% (16/21)

Abbreviations

CDx, companion diagnostic; FFPE, formalin-fixed paraffin-embedded; G/GEJ, gastric / gastro-oesophageal junction; GC, gastric carcinoma; IHC, immunohistochemistry; OPA/PPA/NPA, overall/positive/negative percentage agreement; RUO, research use only; TMA, tumour microarray; κ, Cohen's kappa.

References

1. Shah MA, et al. GLOW. Nat Med 2023;29:2133–41. 2. Shitara K, et al. Gastric Cancer 2024;27:1058–68. 3. Shitara K, et al. SPOTLIGHT. Lancet 2023;401:1655–68. 4. FDA. Zolbetuximab + VENTANA CLDN18 (43-14A) RxDx (PMA P230018), 18 Oct 2024. 5. Yamamoto K, et al. Expert Opin Biol Ther 2026;26:65–80. 6. Ruan D-Y, et al. CMG901. Lancet Oncol 2025;26:227–38. 7. Qi C, et al. satri-cel. Lancet 2025;405:2049–60. 8. Clements ME, et al. Presented at ASCO 2024; abstr 4089

THANK YOU

FOR INTERNAL USE ONLY. NOT FOR DISTRIBUTION, DISPLAY OR PROMOTION.