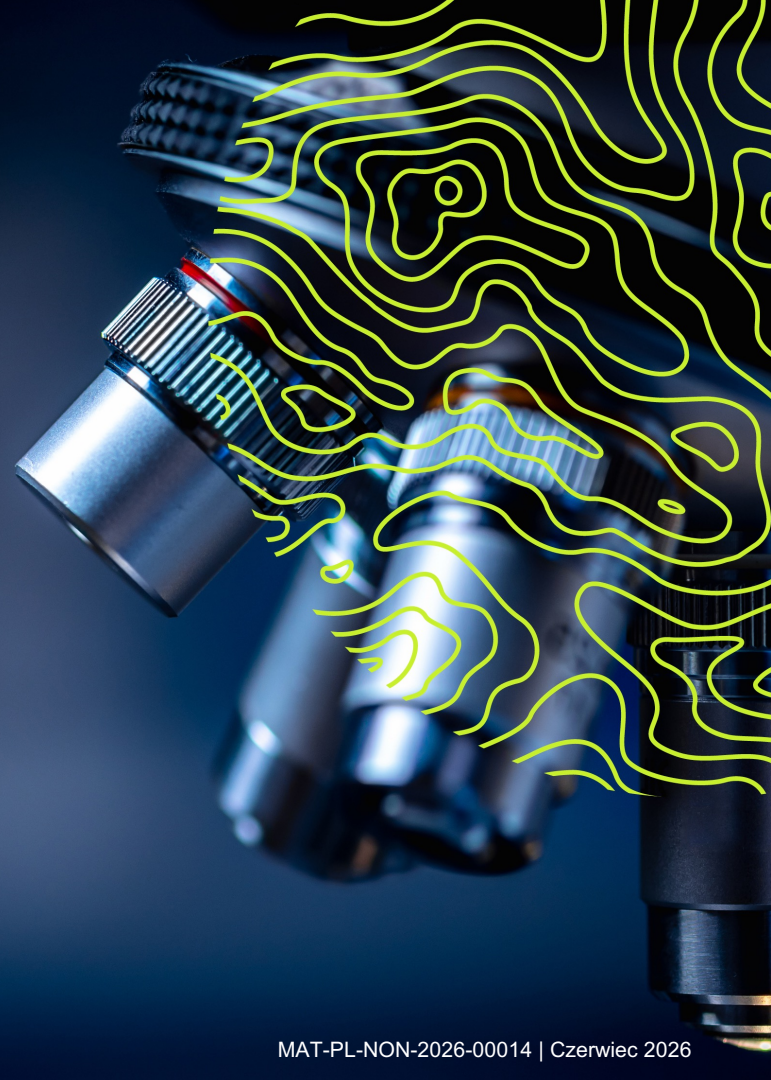
CLDN18.2 Biomarker in Gastric Cancer



astellas

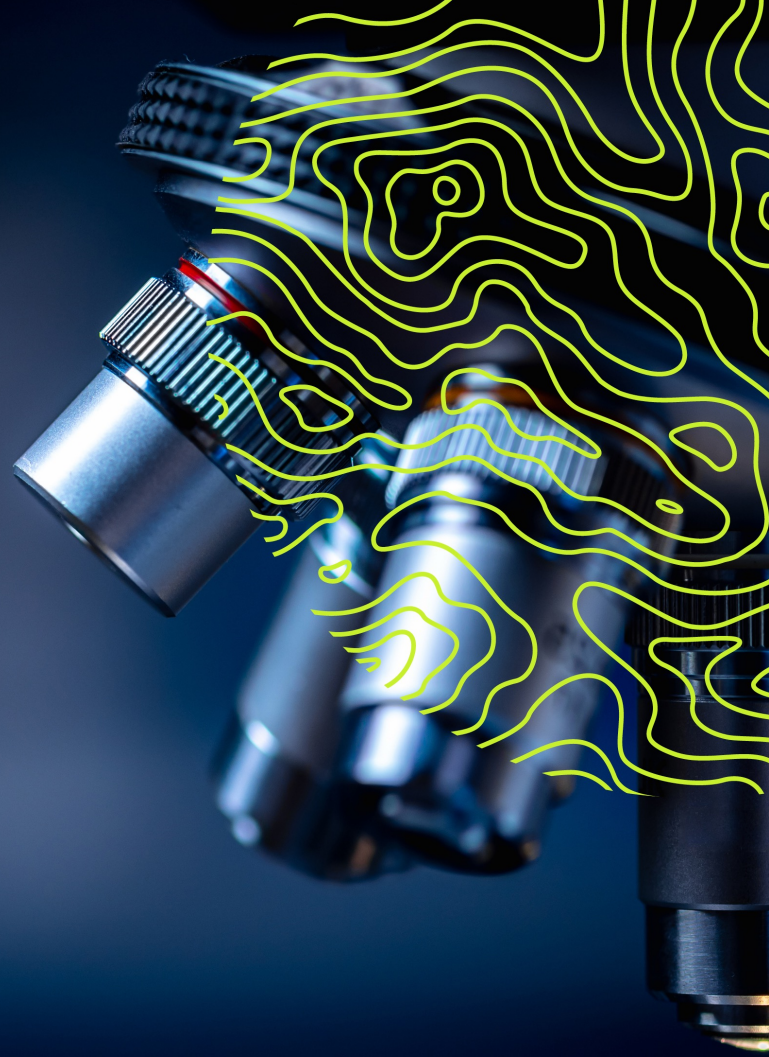
MAT-PL-NON-2026-00014 | Czerwiec 2026

Agenda

1	Biomedical Background	Slide 3
2	Anti-CLDN18 Assays & Antibodies	Slide 17
3	IHC Staining Evaluation	Slide 23
4	Quality Control	Slide 31
5	Clinical Pathology Features	Slide 39
6	FAQs	Slide 42
7	Training on Sample Cases	Slide 54

CLDN18, claudin 18; **FAQs,** frequently asked questions; **IHC,** immunohistochemistry.

Biomedical background



astellas

The unmet need in G/GEJ cancers

Worldwide (1/2)

In 2020, over **1 million new cases of GC** were diagnosed worldwide, making it the **5th most diagnosed cancer**[1]



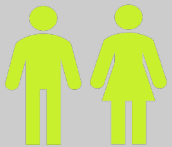
In 2020, **~769,000 people** (equating to one in every 13 deaths globally) **died** worldwide due to GC, making it the **4th most deadly cancer.**[1] In Europe, **>95,000** people **died** of GC, making it Europe's **6th most deadly cancer**[3]



In 2020, **~136,000 new cases of GC** were diagnosed in Europe, making it Europe's **9th most prevalent cancer**[2,3]



Males are nearly **twice** as likely to be diagnosed with GC than females.[4] The age-specific incidence of GC rises steadily from **~45–49 years of age**, with the highest rate of incidence observed in the **85–89 years age group**[5]



GC, gastric cancer; **G/GEJ,** gastric/gastroesophageal junction.

[1]Sung H, et al. Global cancer statistics 2020. CA *Cancer J Clin*. 2021;
[2]Morgan E, et al. *E Clinical Medicine*. 2022;
[3]WHO. Global Cancer Observatory, International Agency for Research on Cancer 2022; https://gco.iarc.fr/today/explore. Accessed November 2024;
[4]National Cancer Institute. Surveillance, Epidemiology, and End Results Program. Cancer stat facts: stomach cancer. https://seer.cancer.gov/statfacts/html/stomach.html. Accessed November 2024; [5]Cancer Research UK. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/stomach-cancer/incidence. Accessed November 2024.

The unmet need in G/GEJ cancer

Worldwide (2/2)

The overall 5-year survival rate of GC (all stages)

26%
Europe[1]



20–30%
Global[2]



The overall 5-year survival rate in advanced GC (stages III/IV)

5–10%
Global[2]

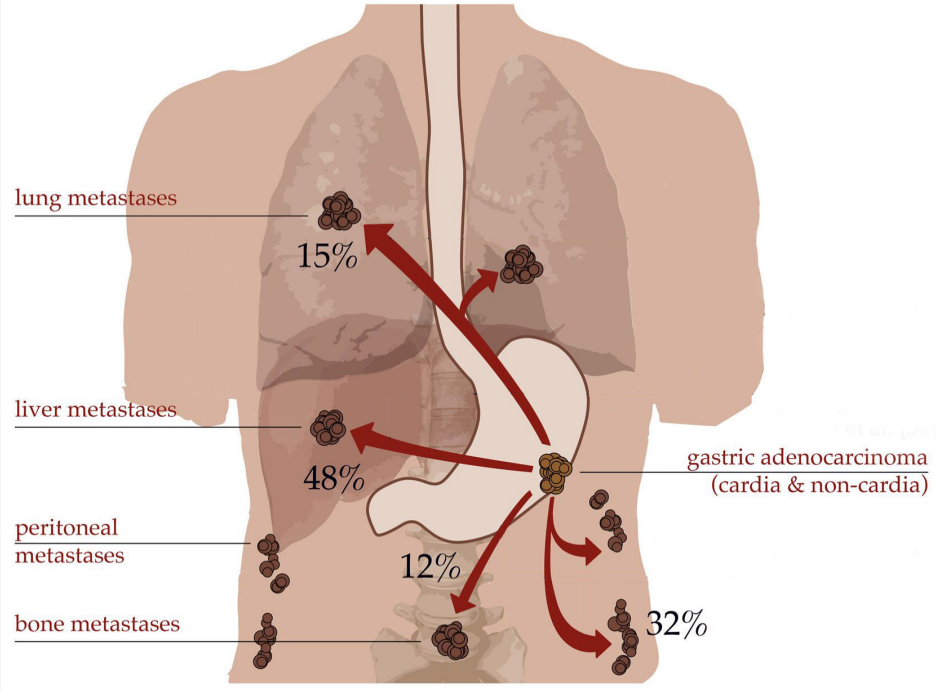


GC, gastric cancer; **G/GEJ,** gastric/gastroesophageal junction.

[1]Rawla P, Barsouk A. *Gastroenterol Rev.* 2019;14(1):26–38;
[2]Casamayor M, et al. *Ecancermedicalscience.* 2018;12:883.

G/GEJ cancer

Metastatic sites[1, 2] and stage at diagnosis



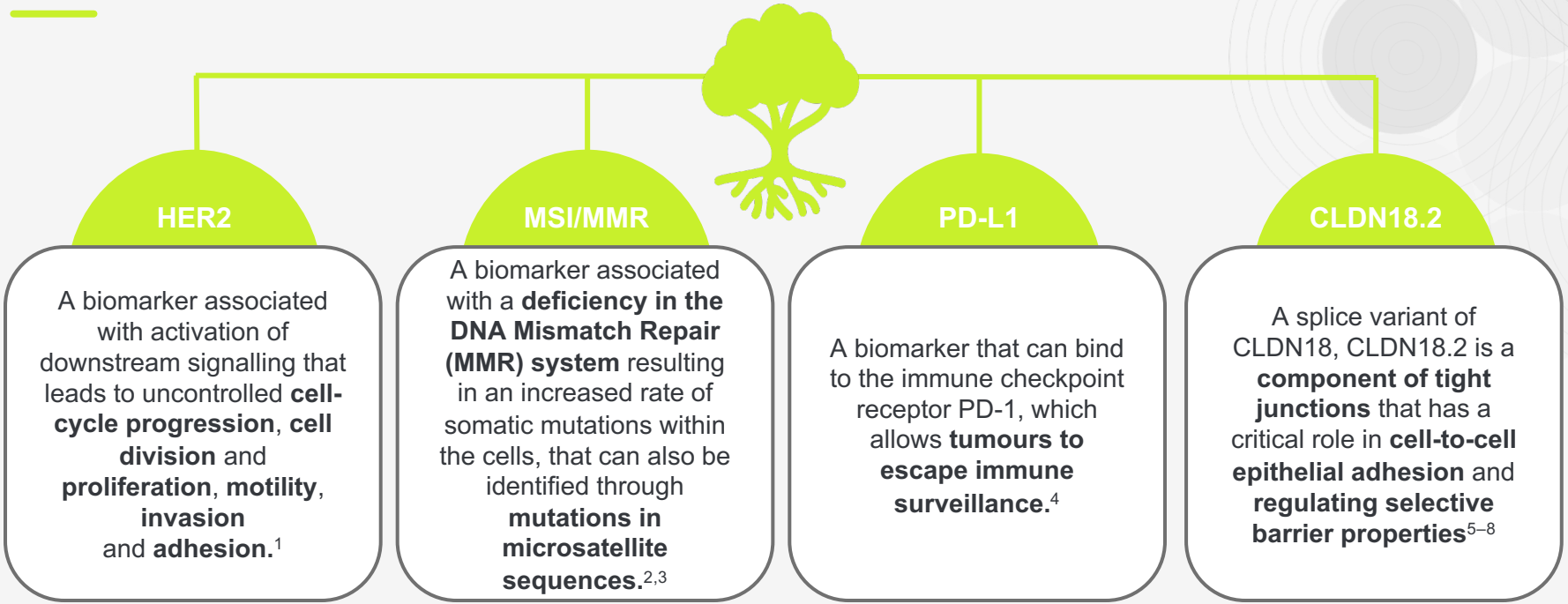
The majority of patients are diagnosed at advanced stages (in Western countries).[3] In 2024 in the US, nearly 62% of gastric cancer cases were detected after the cancer had already spread regionally/distantly.[4]

G/GEJ, gastric/gastroesophageal junction.

[1]Mod acc. Jung J-O, et al. *Int. J. Mol. Sci*. 2020; (frequencies from Swedish registry, [2]Riihimäki M, et al. *Oncotarget*. 2016;7(32):52307-52316; [3]Siebenhüner, AR, et al. *Oncol Res Treat*. 2021;44(9):485-494.; [4]National Cancer Institute. Cancer Stat Facts. Stomach Cancer. https://seer.cancer.gov/statfacts/html/stomach.html. Accessed November 2024.

Established biomarkers in G/GEJ cancer

Biological significance



G/GEJ: gastric/gastroesophageal junction; **HER2:** human epidermal growth factor receptor 2; **MMR:** mismatch repair deficiency; **MSI:** microsatellite instability; **PD-1:** programmed cell death protein 1; **PD-L1:** programmed death-ligand 1

[1]Van Cutsem E, Bang YJ, Feng-Yi F, et al. *Gastric Cancer*;18(3):476–484, 2015; [2]Matsuoka T and Yashiro M. *World J Gastroenterol*;24(26):2818–2832, 2018; [3]Baudrin LG, Deleuze JF, How-Kit A. *Front Oncol*;8:621, 2018; [4]Kawazoe A, Shitara K, Kuboki Y, et al. *Gastric Cancer*;22(1):69–76,2019; [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.

Established biomarkers in G/GEJ cancer

Prevalence in G/GEJ cancer, biomarker testing methodology & patient response to targeted therapy



HER2 (IHC & ISH)

~22 % of G/GEJ cancer cases are HER2+.[1] Detection is typically performed by IHC and ISH. Positive HER2 tumours are associated with a higher likelihood of response to HER2-targeted treatments.[1]

MSI/MMR (IHC/PCR/NGS)

4% of patients with advanced GC exhibit MSI-H/MMR; associated with higher response rates and improved survival when treated with ICI.[2-4]

PD-L1 (IHC → CPS)

Several positivity thresholds for PD-L1 expression are reported across various studies. When a threshold of CPS ≥ 1 is applied, the prevalence is shown to be ~75% (CPS ≥ 5, < 10; 12.7% and CPS ≥ 10; 16.4%).[5] A higher CPS (≥5 or ≥ 10) is associated with an improved response to ICI treatment.[6]

CLDN18.2 (IHC)

~70% of advanced G/GEJ cancers express CLDN18.2 (at any detectable amount); ~38% of patients have high expression of CLDN18.2.[7-9]

CPS, combined positive score; **CLDN18.2,** CLDN18 isoform 2; **G/GEJ,** gastric/gastroesophageal junction.**GC,** gastric cancer; **G/GEJ,** gastric/gastroesophageal junction; **HER2,** human epidermal growth factor receptor 2; **ICI,** immune checkpoint inhibitor; IHC, immunohistochemistry; **ISH,** in situ hybridization; **MMR,** mismatch repair deficiency; **MSI (-H),** microsatellite instability (-high); **NGS,** next-generation sequencing; **PCR,** polymerase chain reaction; **PD-L1,** programmed death-ligand 1.

[1]Van Cutsem E, *et al. Gastric Cancer,*18(3):476-84, 2015; [2]Cancer Genome Atlas Research Network. *Nature,* 513, 202–209, 2014; [3]Pietrantonio F, *et al. ESMO Open*, 6, 100036, 2021; [4]Fuchs CS, *et al. JAMA Oncol*;4(5):e180013, 2018; [5]Schoemig-Markiefka *et al. Gastric Cancer*. 2021;24(5):1115–1122. [6]Fuchs CS, *et al. Gastric Cancer* 25(1):197-206, 2022; [7]Shitara K, et al. *Lancet.* 6736, 1655–1668, 2023; [8]Xu RH, et al. Abstract 405736: Presented at ASCO Annual Meeting, March 22, 2023; [9]Pellino A, et al. *J Pers Med*. 11, 1095, 2021;

Emerging biomarkers in G/GEJ cancer

Biological significance



FGFR2b

A splice variant of FGFR2, FGFR2b is a **transmembrane signalling protein** that intermediates diverse cellular behaviours and cellular processes, such as **mitogenesis**, **differentiation**, **cell proliferation**, **angiogenesis** and **invasion**[1,2]

FGFR2b, fibroblast growth factor receptor 2b; **G/GEJ,** gastric/gastroesophageal junction.

[1]Matsuoka T and Yashiro M. *World J Gastroenterol.* 2018;24(26):2818–2832, 2018.
[2]Ahn S, Lee J, Hong M, et al. *Modern Pathology*; 2016;29:1095–1103.

Emerging biomarkers in G/GEJ cancer

Prevalence in G/GEJ cancers and biomarker testing methodology



FGFR2b

FGFR2b is overexpressed in ~30% of advanced G/GEJ cancers[1]

FGFR2b, fibroblast growth factor receptor 2b; **G/GEJ,** gastric/gastroesophageal

[1]Wainberg Z.A, et al. *Lancet Oncol*. 23, 1430–1440, 2022;
;

Detection of G/GEJ cancer biomarkers

Established and emerging biomarkers are detected using validated tests

	Biomarker	IHC	ISH	PCR	NGS*
Established	HER2†[1,2]	✓	✓		(✓)
	MSI/MMR[1]	✓ MMR		✓ MSI	(✓) MSI
	PD-L1‡[1,3]	✓			
	CLDN18.2[4]	✓			
Emerging	FGFR2b§[5]	✓			✓

✓ : Recommeded method of testing

In-situ tests (validated in clinical trials, CTA; identifying heterogeneity)
*Assay to be validated prior use, according to specific indication. †Includes FISH, SISH, CISH and DDISH[2]; ‡Varying diagnostic IHC assays[3]; §IHC detects FGFR2b overexpression and NGS detects FGFR2b gene amplification in ctDNA.[5]

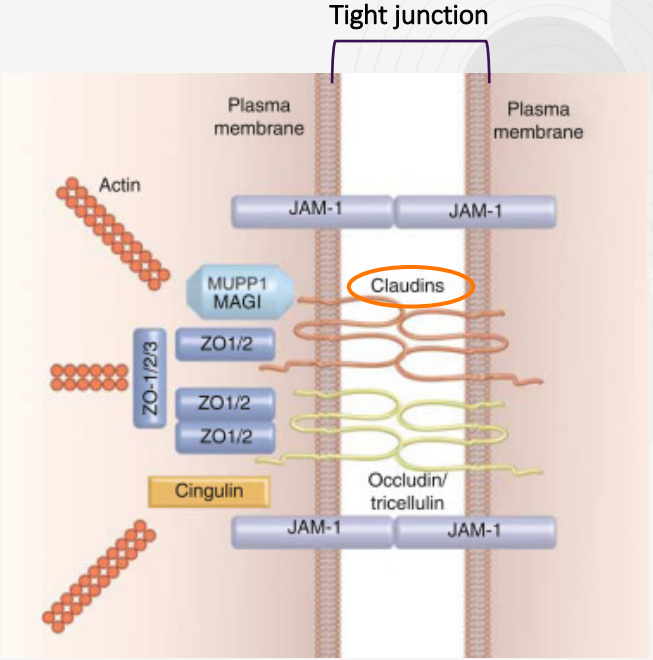
CISH, chromogenic ISH; **CLDN,** claudin; **CTA,** clinical trial assay; **ctDNA,** circulating tumour DNA; **DDISH,** dual-colour dual-hapten ISH; **FGFR2b,** fibroblast growth factor receptor 2b; **FISH,** fluorescent ISH; **GC,** gastric cancer; **G/GEJ,** gastric/gastroesophageal junction; **HER2,** human epidermal growth factor receptor 2; **IHC,** immunohistochemistry; **ISH,** in situ hybridisation; **MMR,** mismatch repair; **MSI,** microsatellite instability; **NGS,** next-generation sequencing; **PCR,** polymerase chain reaction; **PD-L1,** programmed death-ligand 1; **SISH**, silver ISH.

[1]National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology for Gastric Cancer V1.2024. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1434. Accessed November 2024; [2]Abrahao-Machado LF and Scapulatempo-Neto C. *World J Gastroenterol.* 2016;22(19):4619–4625; [3]Meng X, *et al. Cancer Treat Rev. 2015;*41(10):868–876; [4]Sahin U, *et al. Ann Oncol.* 2021;32(5):609–619; [5]Catenacci DVT, *et al. Future Oncol.* 2019;15(18):2073–2082.

Claudin proteins

Biology

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



Basic structural components of tight junctions[8]

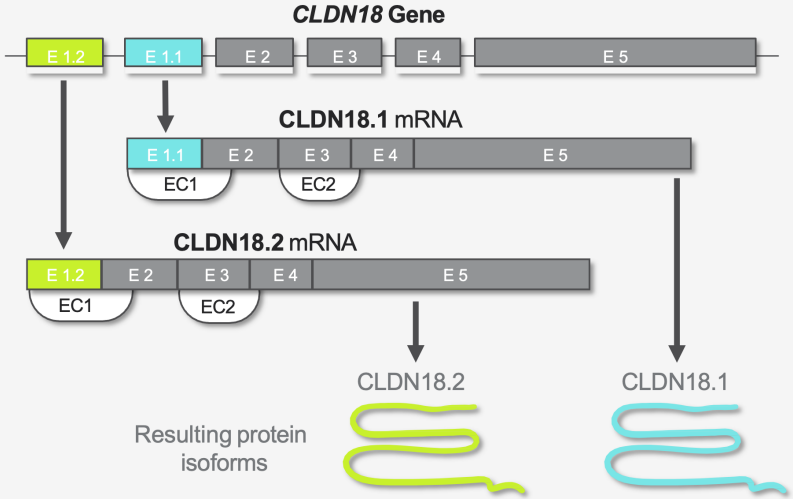
JAM, junctional adhesion molecules; **MAPI,** membrane-associated guanylate kinase inverted; **MUPP1,** multi- PDZ domain protein 1**; ZO,** zonula occludens.

[1]Singh AB, *et al. Semin Cell Dev Biol.*;42(1096-3634(Electronic)):58-65, 2015; [2]Mineta K, *et al. FEBS Lett.*;585(4):606-12, 2011; [3]Sahin U, et al. *et al. Clin Cancer Res.*;14(23):7624-7634, 2008; [4]Otani T, *et al. Trends Cell Biol.*;30(12):1014, 2020. [5]Tsukita S, *et al. Trends Biochem Sci.*;44(2):141-152, 2019; [6]Hu YJ, *et al. Mol Biol Rep.* 2013;40:6123-42; [7]Hewitt KJ, *et al. BMC Cancer.* 6, 186, 2006; [8]Image adapted from Niessen CM. *J Invest Dermatol.* 2007;127(11):2525-32.

Two isoforms of CLDN18 exist

CLDN18.1 & CLDN18.2

Transcript Profile of CLDN18[1]



- 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]
    - The isoforms differ by 8 of the 51 amino acids in the first extracellular domain, with identical sequences of the second extracellular loops[1]

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

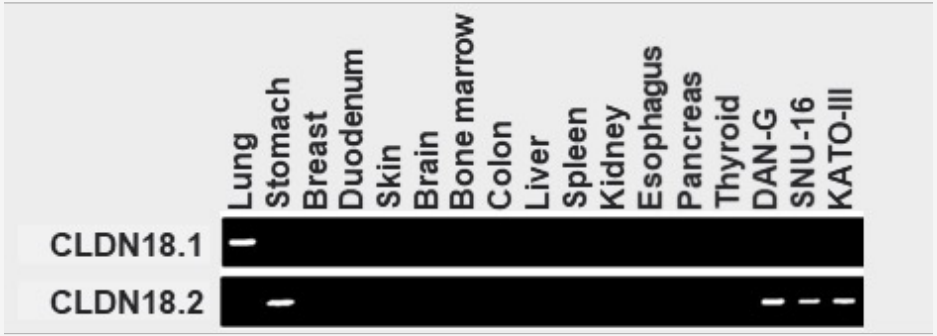
[1]Sahin U, *et al. Clin Can Res.* 2008;14(23):7624–7634.
[2]. Saito T, et al. Presented at ESMO World Congress on Gastrointestinal Cancer 2021, 30 June–3 July. Online. Poster #P-286.

CLDN18.1 & CLDN18.2

Expression in normal tissue

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

Comparative analysis of CLDN18 isoform expression in normal tissue and cell lines (RT-PCR) [1]





CLDN18.1 is predominantly found in healthy lung tissue



CLDN18.2 is predominantly found in healthy gastric epithelial cells

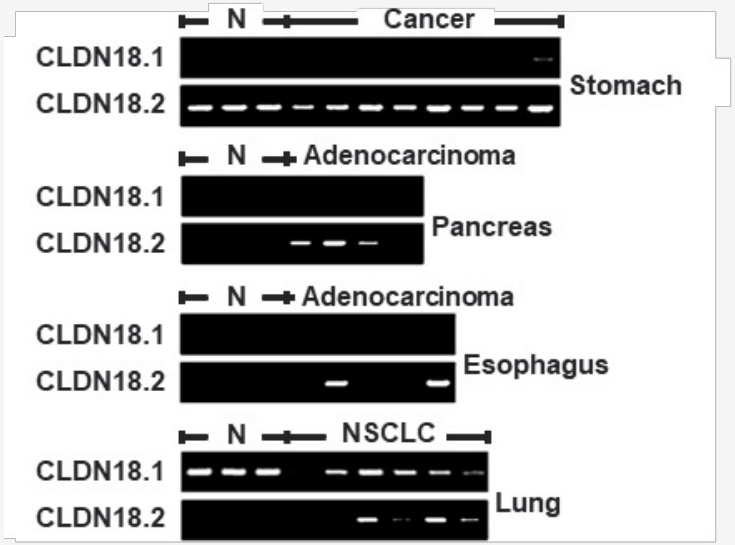
CLDN18.1, claudin18 isoform 1; **CLDN18.2,** claudin18 isoform 2;
DAN-G, pancreatic cancer cell line; **KATO-III,** gastric cancer cell line;
RT-PCR, reverse transcription-polymerase chain reaction; **SNU-16,** gastric cancer cell line.

[1]Sahin U, et al. *Clin Cancer Res*; 2008;14(23):7624–7634;
[2]Niimi T, et al. *Mol Cell Biol*. 2001;21(21):7380–7390.

CLDN18.2

Expression in normal tissue and cancer tissue

Comparative analysis of CLDN18 isoform expression in normal and cancer tissue (RT-PCR)[1]



- Among cancer tissues, CLDN18.2 is most prevalent in malignant G/GEJ and pancreatic tissue[1,2]
- CLDN18.1 expression is negligible or absent in G/GEJ cancers[1,2]
- Ectopic CLDN18.2 expression may also be observed in various other cancer types:
  - Biliary tract tumours, non-small cell lung cancer, and ovarian mucinous adenocarcinoma[1,3]

CLDN, claudin; **G/GEJ,** gastric and gastroesophageal junction; **N,** normal tissue; **NSCLC,** non-small cell lung cancer; **RT-PCR,** reverse transcription-polymerase chain reaction.

[1]Sahin, U, *et al*. *Clin. Cancer Res*. 2008;14:7624-34;
[2]Saito T, et al. Presented at ESMO World Congress on Gastrointestinal Cancer 2021, 30 June–3 July. Online. Poster #P-286;
[3]Shinozaki A, *et al. Virchows Arch.* 2011;459:73–80.

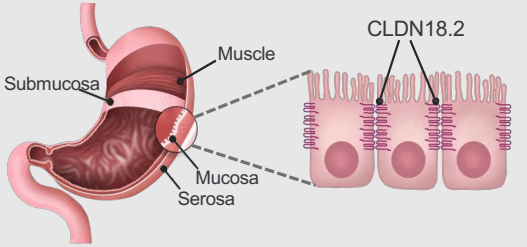
CLDN18.2

During tumourigenesis

CLDN18.2 confined in healthy tissue

Stomach

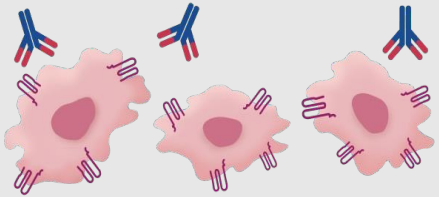
Gastric epithelium



- 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

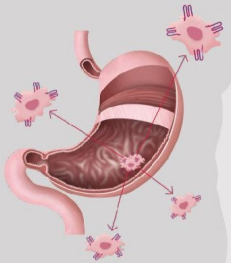
Gastric epithelium



- 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 more exposed and thus accessible to antibodies[1,6,7]

Maintained in metastatic progression

Metastatic sites

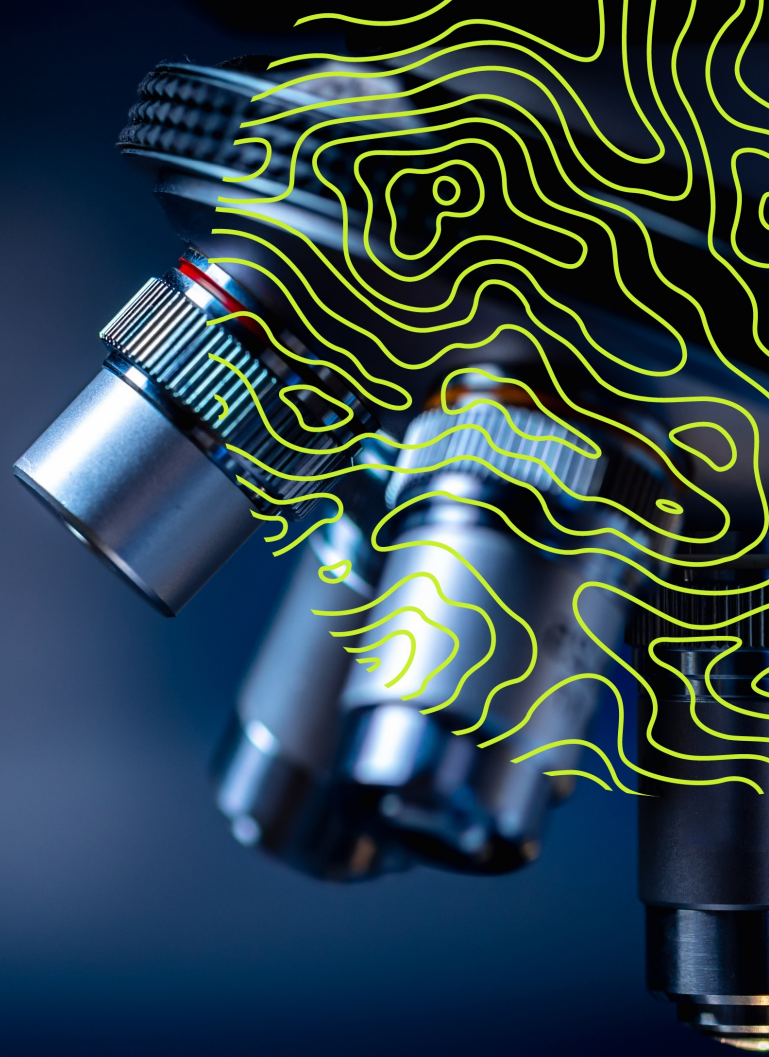


- CLDN18.2 may also be expressed in lymph node metastases of gastric adenocarcinoma as well as other distant metastatic sites[1,8-10]

CLDN, claudin..

[1]Sahin U, *et al. Clin Can Res.* 2008;14(23):7624-34; [2]Sahin U, *et al. Eur J Cancer.* 2018; [3]Turner JR, *et al. Semin Cell Dev Biol.* 2014; 36:204-12; [4]Tsukita S, *et al.Trends Biochem Sci.* 2019; 44(2):141-152; [5]Hu YJ, *et al. Mol Biol Rep.* 2013; 40(11):6123-42; [6]Mitnacht-Kraus R, *et al. Presented at ESMO 2017, 8–12 September. Madrid, Spain.* Poster #378P; [7]Shah M, *et al. Presented at ASCO 2020, 29–31 May. Online.* TPS4648; [8]Pellino A, *et al. J Pers Med.* 2021; 26;11(11):1095; [9]Coati I, *et al. Br J Cancer.*2019;121:257–263;[10]Rohde C, *et al. Jpn J Clin Oncol.* 2019; 49(9):870-876.

Anti-CLDN18 assays & antibodies



astellas

CLDN18.1 and CLDN18.2

Identification with CLDN18 antibody

- Claudins are present throughout the body, but the two alternative splicing isoforms of the *CLDN18* gene are localised to certain tissue types[1,2]
- CLDN18 antibodies can identify both CLDN18 isoforms — CLDN18.1 and CLDN18.2; however, when evaluating G/GEJ tumour tissue, staining can be attributed to the presence of CLDN18.2 due to localisation in certain tissue types[3]



CLDN18.2 is normally present in gastric epithelium and is often retained in malignant gastric tissue[1,3]



CLDN18.1 is primarily expressed in adenocarcinoma lung tissue and its expression is negligible or absent in G/GEJ cancers[1,3]

CLDN18.1, claudin 18 isoform 1**; CLDN18.2,** claudin 18 isoform 2; **G/GEJ,** gastric/gastroesophageal junction.

[1]Sahin U, et al. *Clin Cancer Res.*14(23):7624–7634, 2008;
[2]Niimi T, et al. *Mol Cell Biol.*21(21):7380–7390, 2001;
[3]Saito T, et al. Presented at ESMO World Congress on Gastrointestinal Cancer 2021, 30 June–3 July. Online. Poster #P-286.

Assays & antibodies

Test for CLDN18.2 expression in G/GEJ cancers by IHC

- Several assays, antibodies, and platforms are available for the assessment of CLDN18.2 expression. The choice of assays and antibodies includes but is not limited to*:

Name	Company	Clone/catalog #	Clonality	Host	Field of Use
VENTANA® CLDN18 (43-14A) RxDx Assay[1]	Roche Tissue Diagnostics	43-14A (08285918001)	Monoclonal	Mouse	IVD (CDx assay)
VENTANA® CLDN18 (43-14A) Assay[2]	Roche Tissue Diagnostics	43-14A (08504148001)	Monoclonal	Mouse	IVD (analytical assay)
Anti-Claudin18 antibody[3]	abcam	43-14A (ab314690)	Monoclonal	Mouse	Research Use only
PathPlus™ Monoclonal Mouse anti-Human CLDN18 / Claudin 18 Antibody[4]	LSBio	LS-B16145	Monoclonal	Mouse	Research Use only
Claudin-18 antibody[5]	Novus Biologicals	NBP2-32002	Polyclonal	Rabbit	Research Use Only

*Please note the list of antibodies/assays and platforms is not exhaustive. With the exception of VENTANA CLDN18 (43-14a) RxDx Assay, the tests mentioned above are not currently approved companion diagnostics. Please use the appropriate test to guide clinical decision-making.
Based on findings from a global ring study, caution may be needed with the Novus antibody as some of the parameters did not reach the study's 85% acceptance criteria.[6]

CDx, companion diagnostic; CLDN18, claudin 18; **G/GEJ**: gastric/gastrooesophageal junction; **IHC,** immunohistochemistry; **IVD,** *in vitro* diagnostic; **RUO,** research use only

[1]Ventana CLDN18 (43-14A) RxDx assay [package insert]. Mannheim, Germany: Roche Diagnostics GmbH; [2]Ventana CLDN18 (43-14A) assay [package insert]. Mannheim, Germany: Roche Diagnostics GmbH; [3]Recombinant Anti-Claudin18 antibody Overview Sheet, available at https://www.abcam.com/en-gb/products/primary-antibodies/claudin18-antibody-43-14a-ab314690. Accessed November 2024; [4]PathPlusTM Claudin18 Antibody Specifications, available at https://www.lsbio.com/pathplus-antibodies/pathplus-cldn18-antibody-claudin-18-antibody-ihc-ls-b16145/789855. Accessed November 2024; [5]Claudin-18 Antibody Product details, available at https://www.novusbio.com/products/claudin-18-antibody_nbp2-32002. Accessed November 2024; [6]Jasani B, et al. Lab Invest. 2024;104(1):100284.

VENTANA® CLDN18 (43-14A) assay

IVD

- Roche Diagnostics developed an assay for CLDN18.2 (mouse MAb, 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[1,2]
- VENTANA CLDN18 (43-14A) assay is intended for the immunohistochemical detection of the CLDN18 protein in FFPE neoplastic tissues stained with BenchMark IHC/ISH instruments[1]
- The regulatory status is CE-IVD
- The 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 the performance of this IVD in their institution



BenchMark ULTRA Automated IHC/ISH slide staining system

CE-IVD, Conformité Européenne - *in vitro* diagnostic; **CLDN18,** claudin 18; **CLDN18.**1, claudin 18 isoform 1; **CLDN18.2,** claudin 18 isoform 2; **DAB,** 3,3'-diaminobenzidine tetrahydrochloride; **FFPE,** formalin-fixed, paraffin embedded; **Mab,** monoclonal antibody; **IHC,** immunohistochemistry; **ISH,** in situ hybridization.

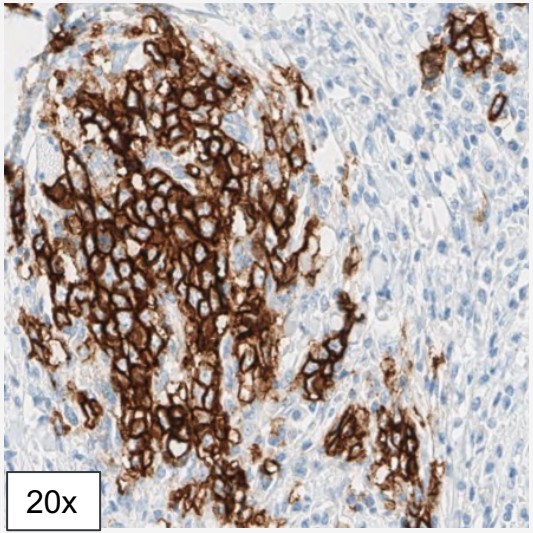
[1]VENTANA CLDN18 (43-14A) Assay, Package Insert Sheet, CE-IVD 1017589EN Rev C, 2023-09-28;

[2]Saito T, et al. Presented at ESMO World Congress on Gastrointestinal Cancer 2021, 30 June–3 July. Online. Poster #P-286.

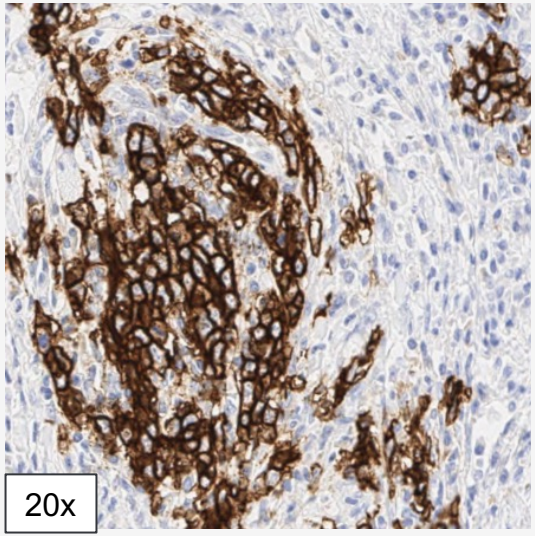
CLDN18 (43-14A) antibody

Comparability across different platforms

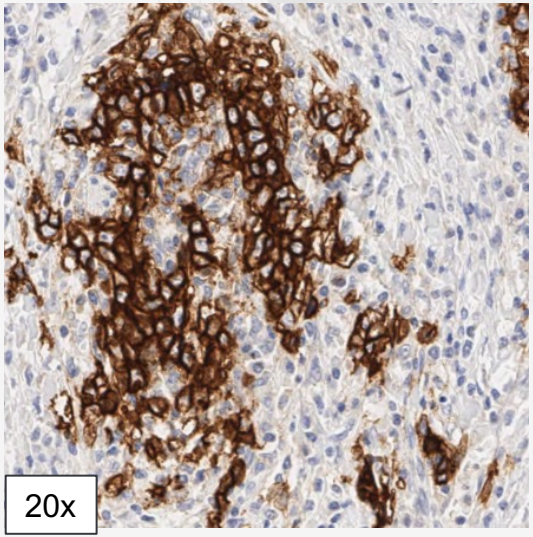
VENTANA



Dako



Leica



Recombinant anti-Claudin18 antibody [43-14A] (ab314690)

CLDN18, claudin 18.

https://www.abcam.com/products/primary-antibodies/claudin18-antibody-43-14a-ab314690.html
Astellas. Data on file. Document REF-50697. 2024.

Specimen preparation

VENTANA (43-14A) Assay[1]

- Tissue in 10% neutral buffered formalin, with 6 to 48 hours fixation time
- Cut at approximately 4 µm thick (tissue sections can range from 3 to 6 µm*) and mount on positively charged glass slides
- Before staining, the cut slides should be dried completely either at room temperature (air-dried) or offline baking (baked in oven) at 60°C for 60 minutes*
- Slides should be stained promptly, as antigenicity of cut tissue sections may diminish; studies support a minimum of 45 days of antigen stability on unstained slides
- It is recommended that positive and negative controls are run simultaneously with unknown specimens

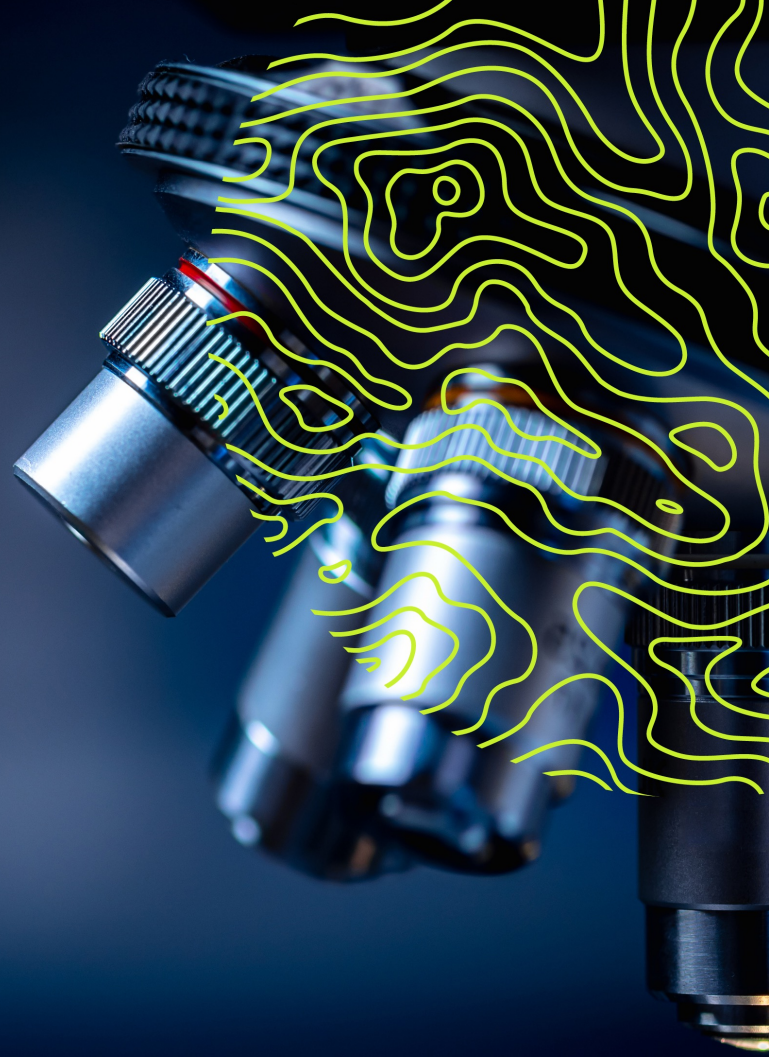
CLDN18, claudin 18; **CAP,** Collage of American Pathologists..

*CLDN18-specific guidance here aligns with CAP guidelines[2]

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

[2]Compton CC, et al. *Arch Pathol Lab Med*. 2019;143(11):1346-63.

CLDN18 staining evaluation in GC



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Interpretation of CLDN18.2 expression

Intensity & percentage of membranous staining

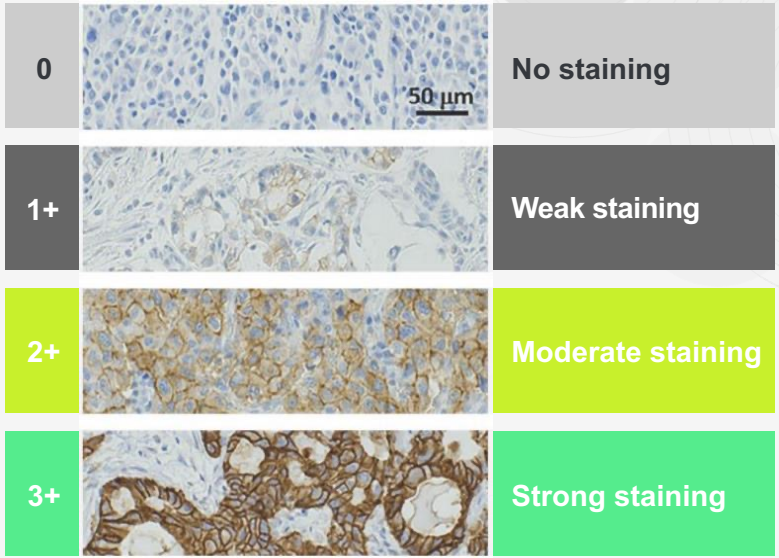
For the evaluation of CLDN18.2 expression, both the ***intensity*** of membranous staining and the ***percentage*** of tumour cells with partial and/or complete, apical, basolateral, lateral and micro-luminal staining are assessed[1]

- The entire tumour section should be considered for the scoring[1]

General Rules for CLDN18 Staining Evaluation[1–3]

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

IHC scoring for CLDN18 in G/GEJ cancer (membranous staining of tumour cells)[3]

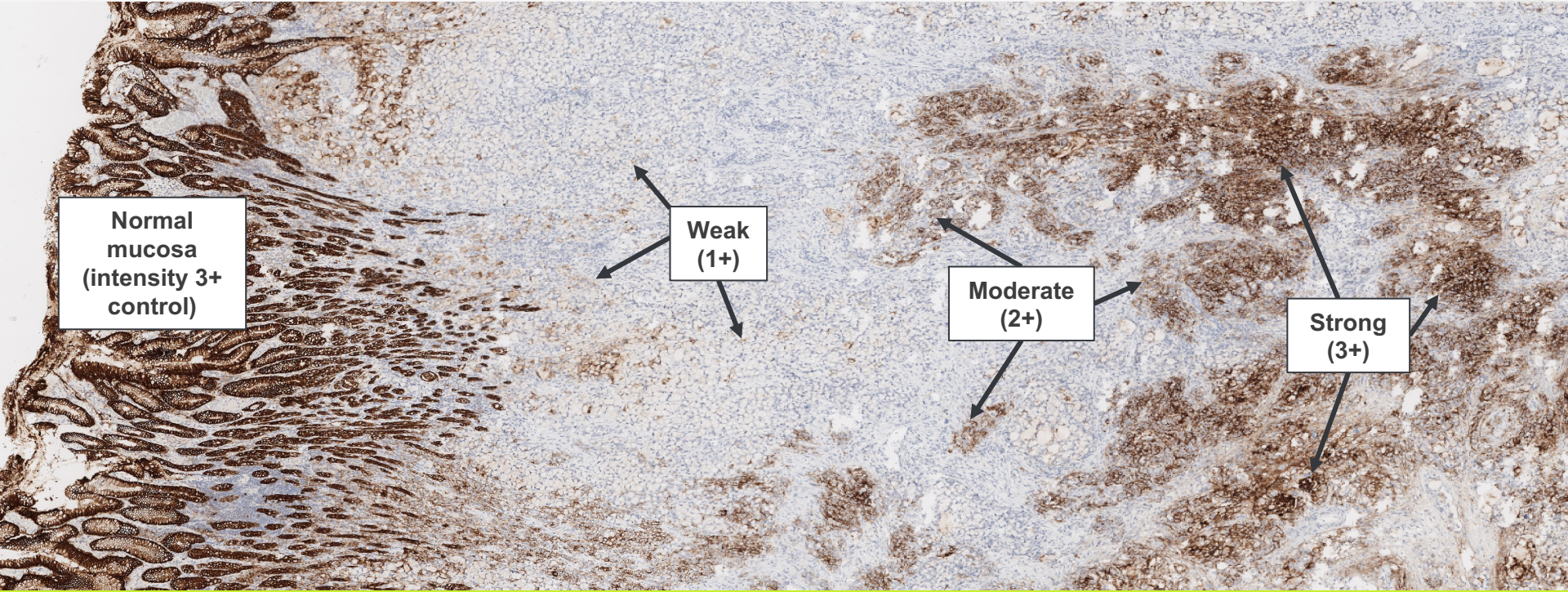


CLDN18.2, claudin 18 isoform 2; **G/GEJ:** gastric and gastroesophageal junction; **IHC**, immunohistochemistry.

[1]Pellino, A. *et al. J. Pers. Med*. 2021;
[2]Rohde C, *et al. Jpn J Clin Oncol* 2019;
[3]Sahin U et al. Ann Oncol. 2021;32(5):609-619(Suppl).

General rules—CLDN18 staining evaluation

Scoring: percentage of invasive tumour cells with moderate-to-strong membranous staining

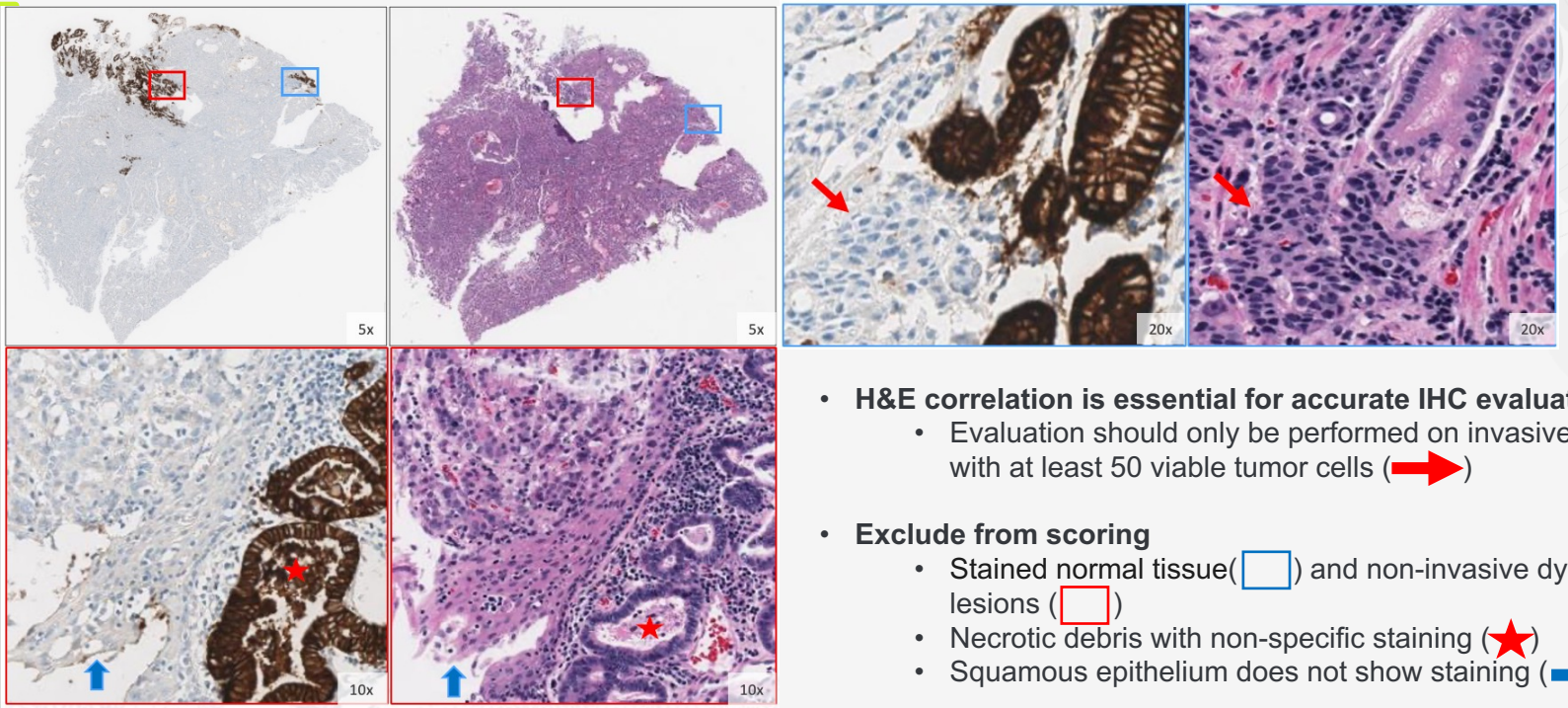


CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Stepwise CLDN18 staining evaluation

STEP 1: Identify invasive carcinoma by H&E review



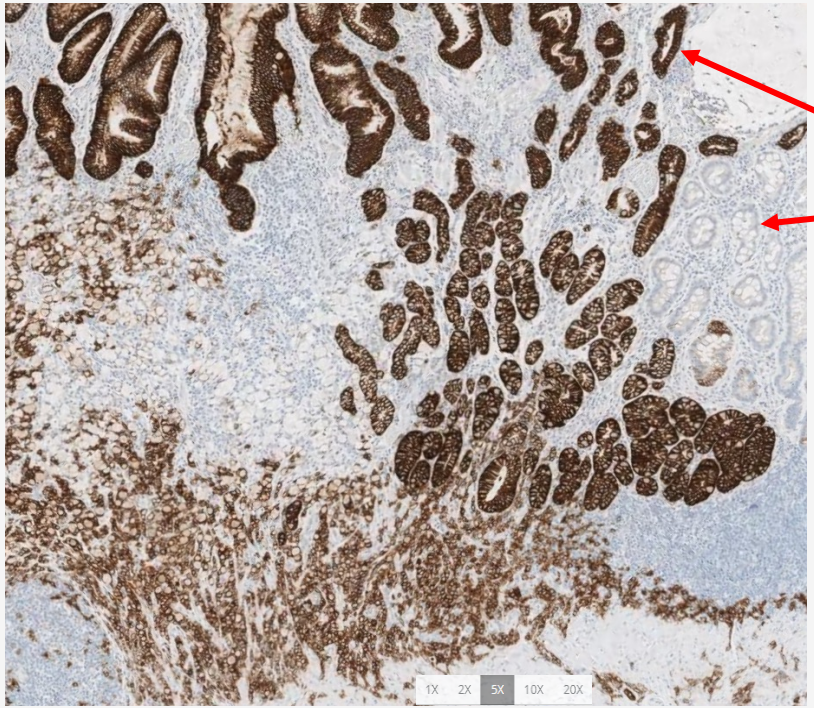
- **H&E correlation is essential for accurate IHC evaluation**
  - Evaluation should only be performed on invasive tumor with at least 50 viable tumor cells (➡)
- **Exclude from scoring**
  - Stained normal tissue(▭) and non-invasive dysplastic lesions (▭)
  - Necrotic debris with non-specific staining (★)
  - Squamous epithelium does not show staining (➡)

CLDN18, claudin 18, **H&E,** Hematoxylin and eosin; IHC, immunohistochemistry.

Astellas. Data on file. Document REF-50697. 2024.

Stepwise CLDN18 staining evaluation

STEP 2: Evaluate staining intensity and specificity



Intensity 3+ reference:

(1) **non neoplastic tissue**[1]

- normal mucosa usually 3+ (*“chocolate brown”)*
- intestinal metaplasia 1+/2+, if any

(2) use the **magnification rule**[2,3]

in case of missing internal staining control and for further subclassification: 3+/2+/1+

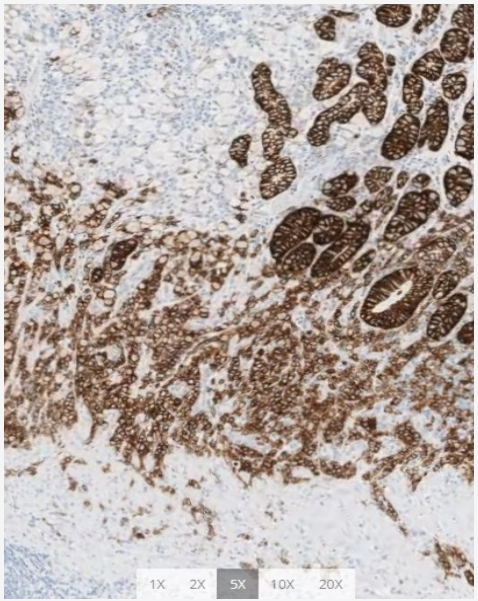
CLDN18, claudin 18.

[1]Fassan M, et al. *Mod Pathol.* 37(11):100589; 2024.
[2]Astellas. Data on file. Document REF-50697. 2024.
[3]Scheel AH, et al. *Diagn Pathol.* 13(1):19; 2018.

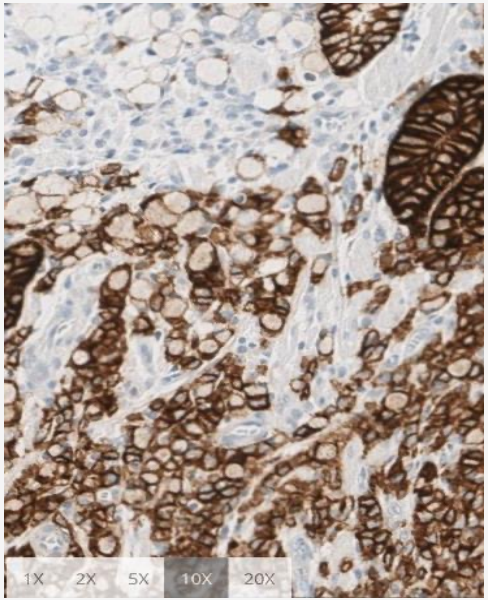
Stepwise CLDN18 staining evaluation

STEP 2: Evaluate staining intensity and specificity

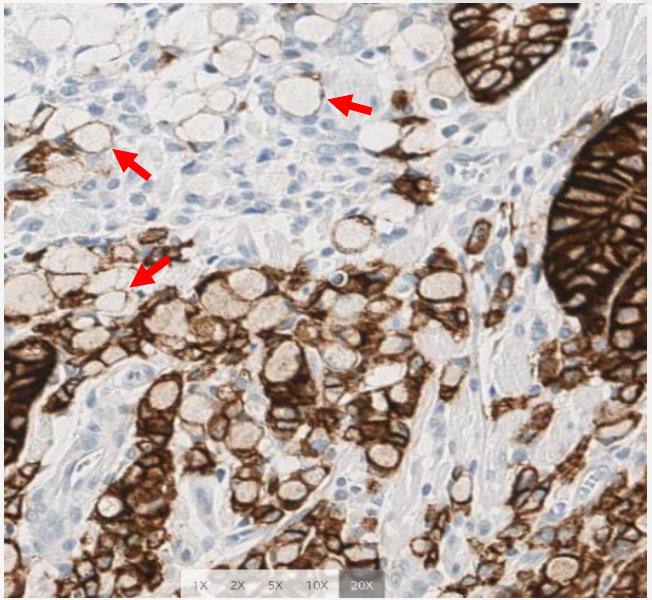
→ Control for specific membranous staining (vs cytoplasmic artifacts)



Most membranous staining visible at 5x (chicken wire) → **strong staining 3+**



Some membranous staining shows up at 10x
→ **moderate staining 2+**



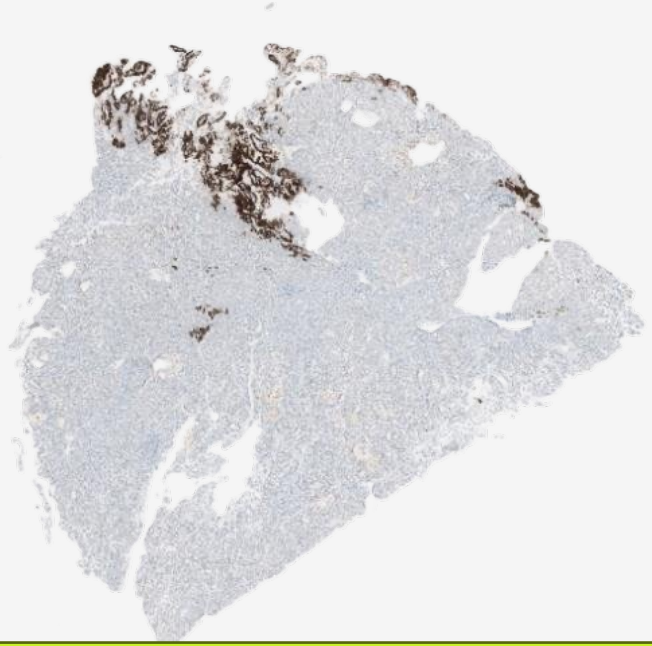
Barely visible membranous staining at 20x
(→) **weak staining 1+**

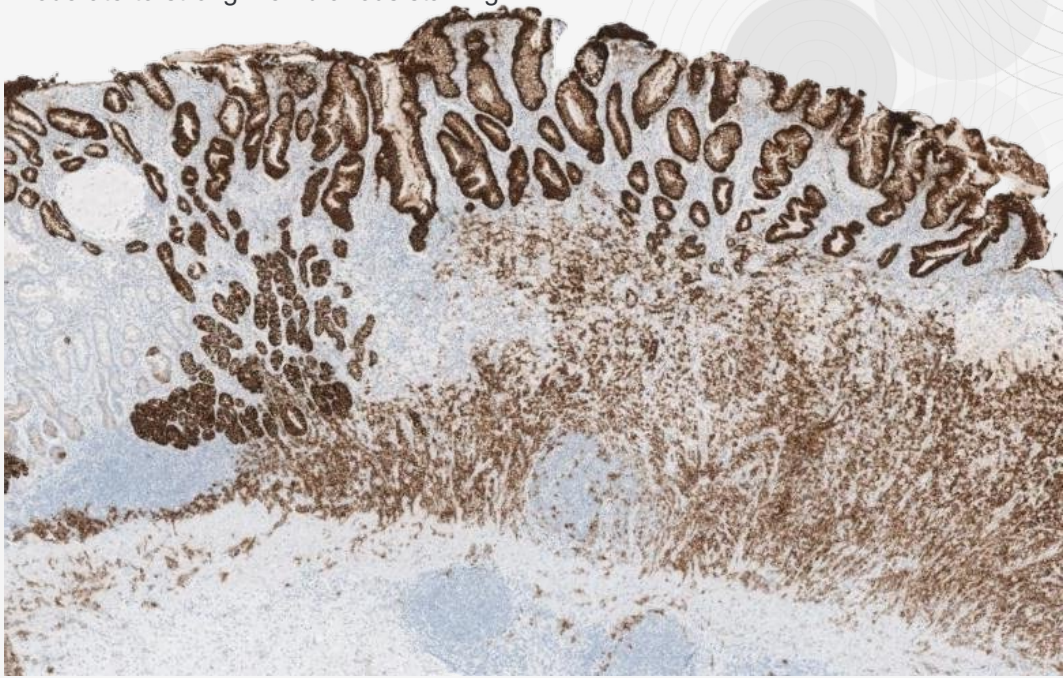
CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Stepwise CLDN18 staining evaluation

STEP 3: Estimate percentage of stained invasive tumour cells → moderate-to-strong membranous staining





CLDN18 score:	0%	98%

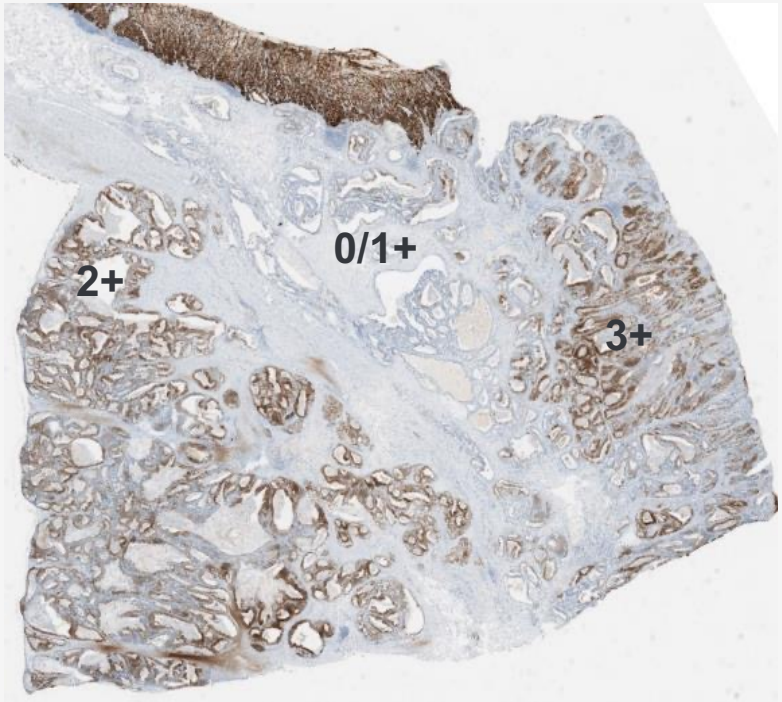
→ go back to low magnification and consider heterogeneity

CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Stepwise CLDN18 staining evaluation

Special topics of training

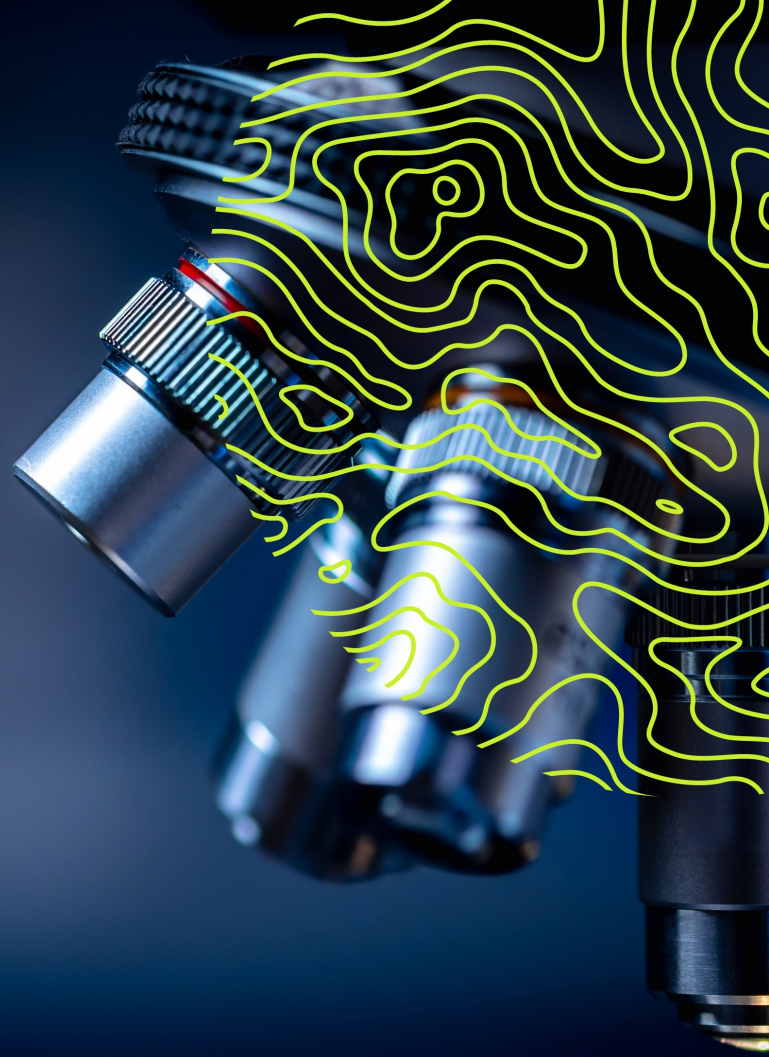


- **Assessment of denominator**: number of viable invasive tumour cells
  - Viewing what is not stained (tumour cell density: solid vs scirrhous growth)
  - Exclusion of dysplasia and metaplasia
  - Exclusion of non-viable tumour cells (associated with ulceration and necrosis)
- **Assessment of staining intensities**:
  - IHC 0 / 1+ / 2+ / 3+
  - Focus on IHC 2+/3+, which are relevant to scoring
- **Staining patterns:**
  - Inclusion criteria: membranous and circumferential ─ complete or partial; basal-/basolateral; apical/microluminal
  - Exclusion criteria: necrotic tumour and basal membrane-like; cytoplasmic, granular (if not membrane bound), and mucin staining

CLDN18, claudin 18; **IHC**, immunohistochemistry.

Astellas. Data on file. Document REF-50697. 2024.

Quality control of CLDN18 staining

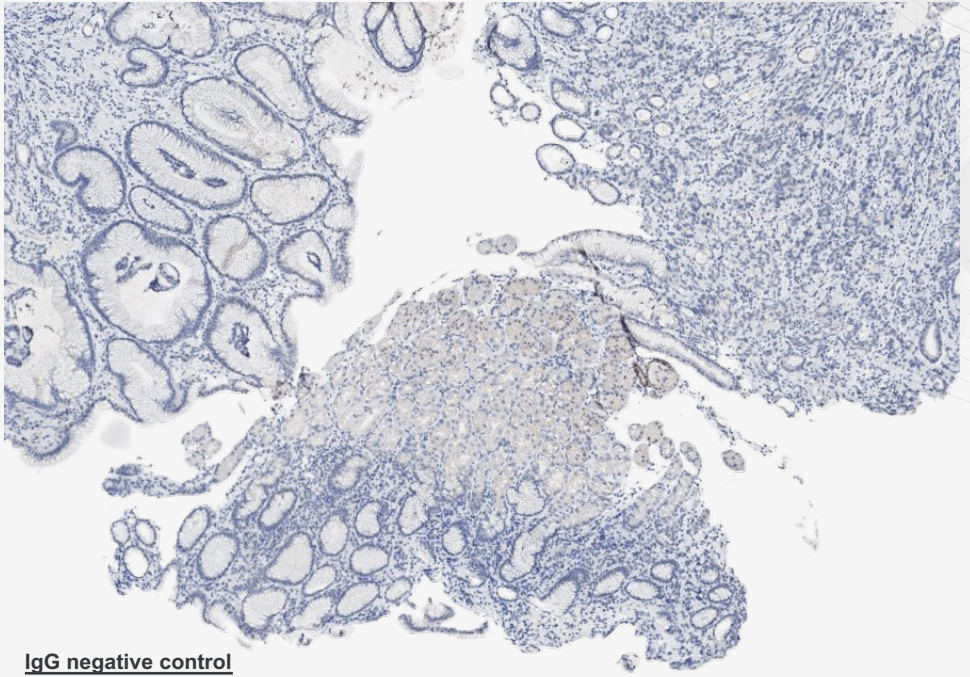


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Non-specific staining

Negative reagent IgG control

- Negative reagent control slide for each case
- Reject if specific membrane staining is present
- Blush staining in parietal cells is acceptable



IgG negative control

IgG, immunoglobulin G.

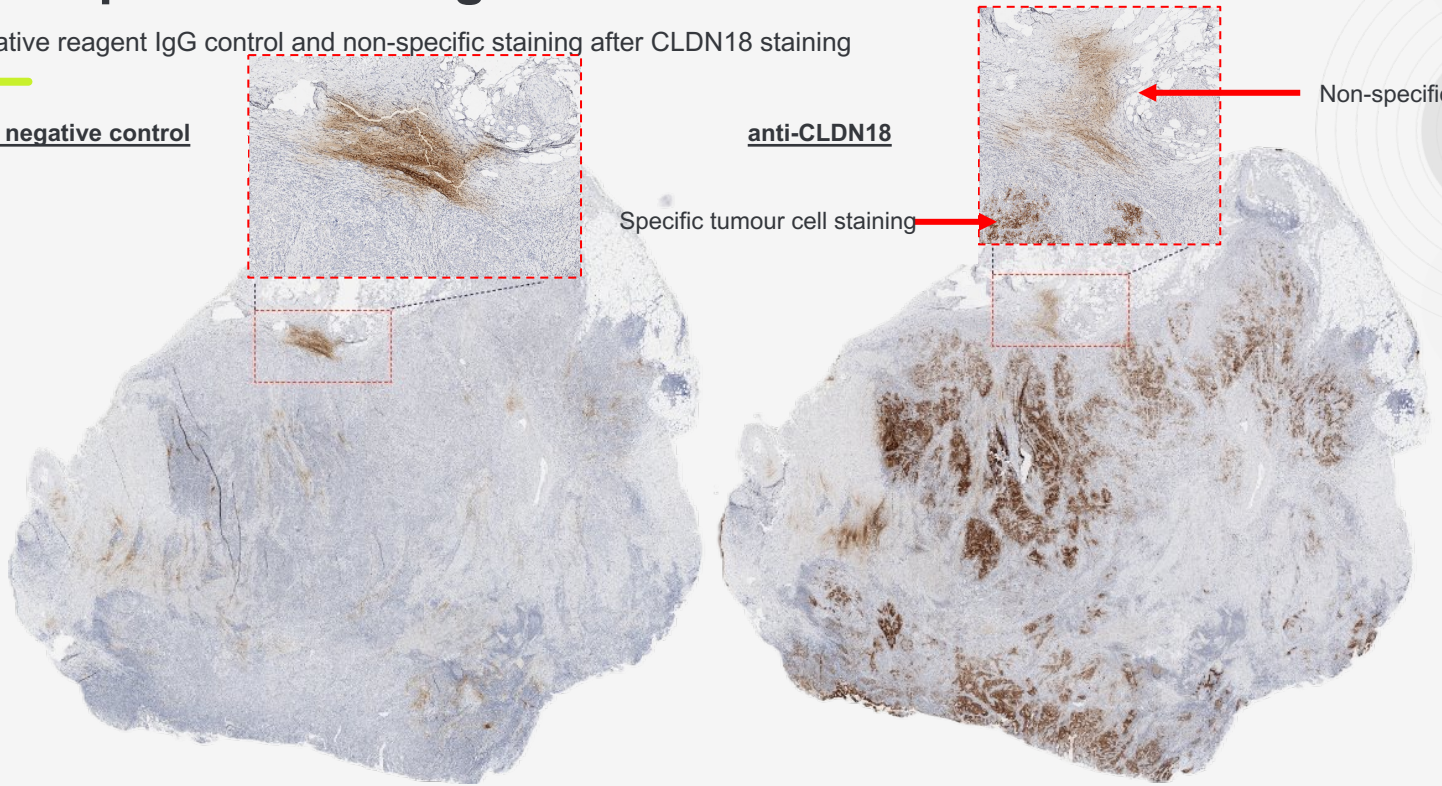
Astellas. Data on file. Document REF-50697. 2024.

Non-specific staining

Negative reagent IgG control and non-specific staining after CLDN18 staining

IgG negative control

anti-CLDN18



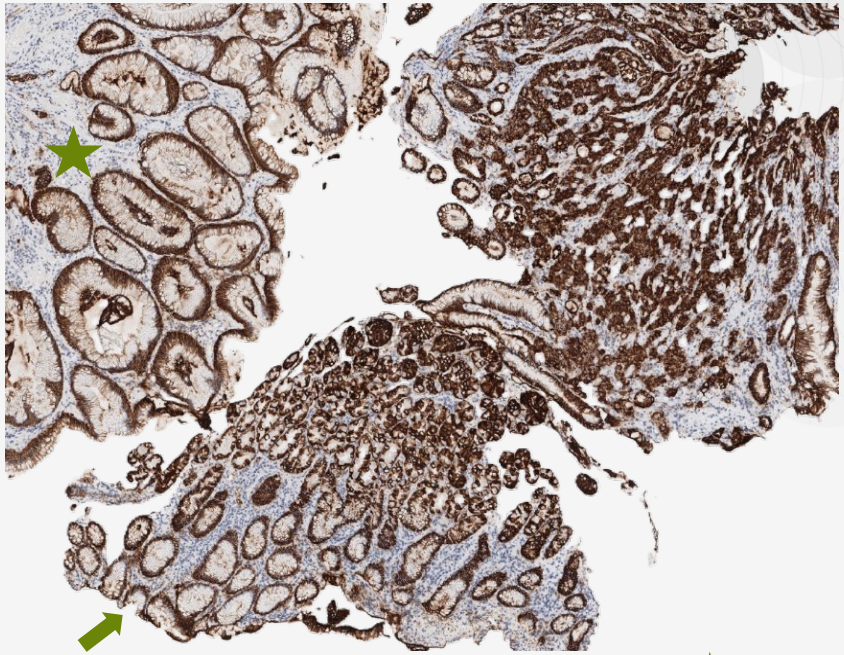
CLDN18, claudin 18; **IgG,** immunoglobulin G.

Astellas. Data on file. Document REF-50697. 2024.

Staining level control

Internal positive controls: normal mucosa - intestinal metaplasia

	Acceptable	Unacceptable
Positive CLDN18 staining characteristics	Strong membrane staining in normal gastric epithelium. AND Weak-to-moderate membrane staining in metaplastic areas of gastric epithelium.	No strong membrane staining in normal gastric epithelium. AND/OR Lack of weak-to-moderate membrane staining in metaplastic areas of gastric epithelium.



Tissue fragment with extensive intestinal metaplasia (★)

Tumour-bearing particle with superficially normal mucosa (➡)

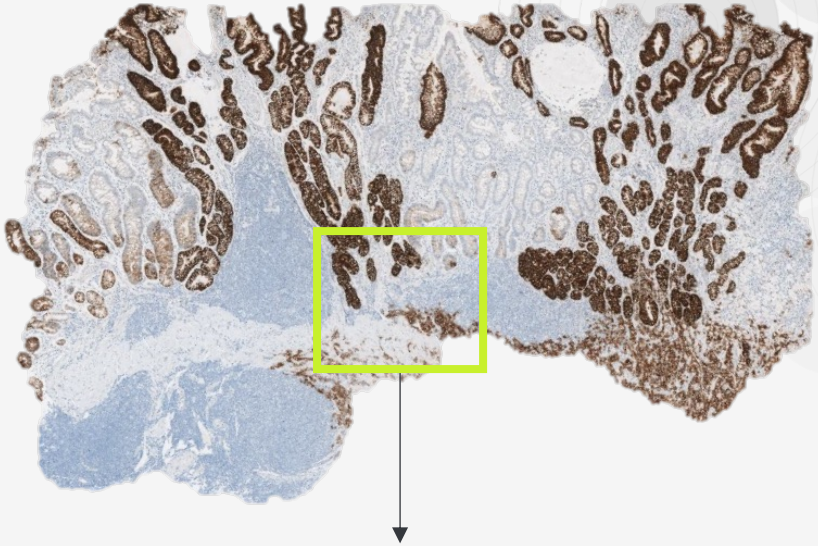
CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Staining level control

Internal negative controls

	Acceptable	Unacceptable
Negative CLDN18 staining characteristics	No CLDN18 staining in blood vessels, lamina propria, lymphocytes, peripheral nerve and smooth muscle.	High amount of non-specific background staining in blood vessels, lamina propria, lymphocytes, peripheral nerve and smooth muscle.



See next slide for 10x magnification

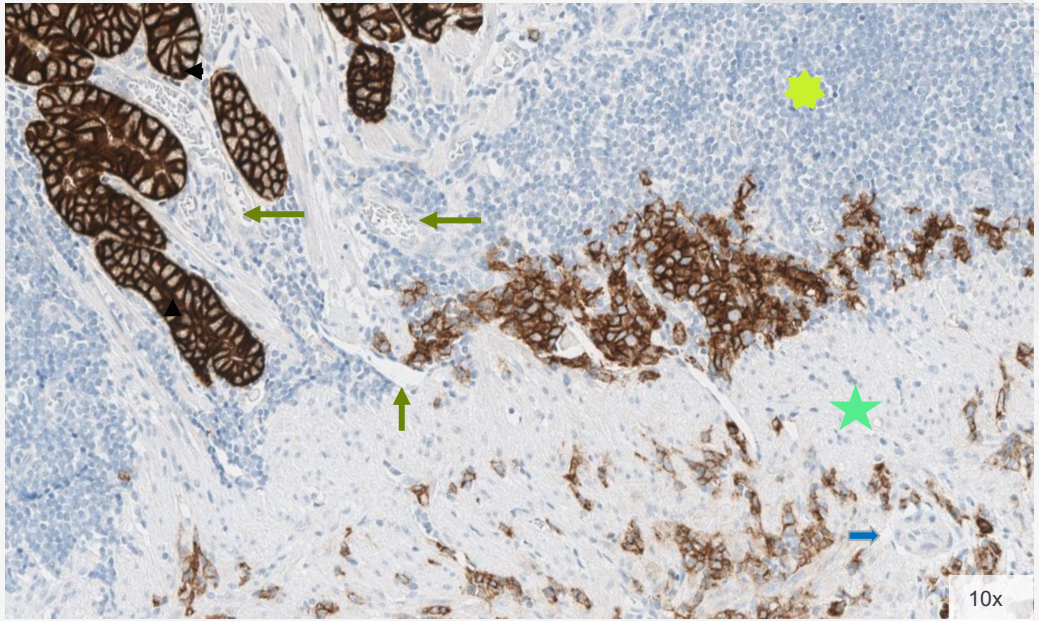
CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Staining level control

Internal negative controls

	Acceptable	Unacceptable
Negative CLDN18 staining characteristics	No CLDN18 staining in blood vessels, lamina propria, lymphocytes, peripheral nerve and smooth muscle	High amount of non-specific background staining in blood vessels, lamina propria, lymphocytes, peripheral nerve and smooth muscle



Lamina propria mucosae/smooth muscles

Lymphocytic aggregate

Blood and lymph vessels

Peripheral nerve

CLDN18, claudin 18.

Astellas. Data on file. Document REF-50697. 2024.

Controls

Summary[1,2]

Internal controls	**Positive tissue control (PTC):** normal gastric epithelial or dysplastic cells within test section **Negative tissue control (NTC):** e.g. connective tissue and immune cells within test section
External controls (on slide or separate slide)	**PTC**: normal gastric epithelium (strong membranous) or epithelial cells in the areas of metaplasia (weak to moderate membranous) **NTC**: any normal non-gastric tissue (e.g. lamina propria, lymphocytes, smooth muscle, blood vessels and peripheral nerve)
Negative reagent control (NRC)	**NRC-primAb**: replace primary antibody with “non-specific” Ig **NRC-detSys**: omit primary antibody
Validation controls (reference slides)	Unique control samples representing the dynamic staining range (from 0 to 100%) of CLDN18.2 expression, including borderline cases

CLDN18.2 validation tissue controls can be purchased through various options, including DLS.
Learn more here: www.dls.com/cldn182

Ab: antibody**, CLDN**: claudin; **CLDN18.2,** claudin 18 isoform 2; **DLS,** Discovery Life Sciences**; Ig, immunoglobulin**

[1]Torlakovic E.E. *et al*. International Ad Hoc Expert Panel. *Appl Immunohistochem Mol Morphol*. 2014; [2] Torlakovic, E.E. *et al*. International Ad Hoc Expert Committee. *Appl Immunohistochem Mol Morphol*. 2015.

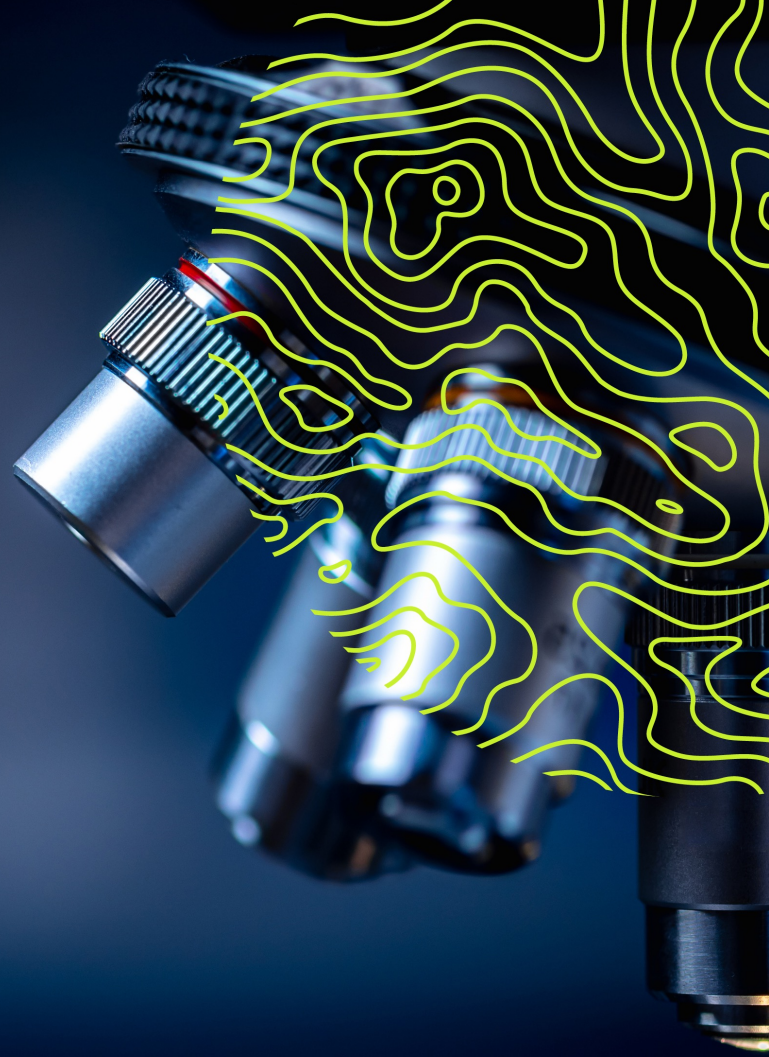
External quality assurance (EQA)

Selected international quality assurance programme links to learn more about how countries monitor the quality of tests and their reporting

- Australia https://www.australianpathology.com/
- CAP https://www.cap.org/
- European Society of Pathology https://www.esp-pathology.org/
- France https://www.afaqap.fr/
- Germany https://www.quip.eu/de_DE/
- NordiQC https://www.nordiqc.org/
- UK NEQAS https://ukneqas.org.uk/

CAP, College of American Pathologists; **NEQAS,** National External Quality Assessment Scheme; **NordiQC,** Nordic immunohistochemical Quality Control.

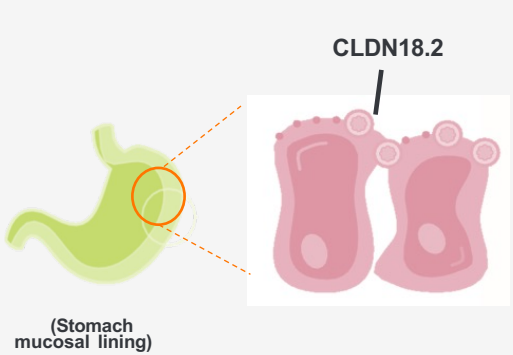
Clinical pathology features



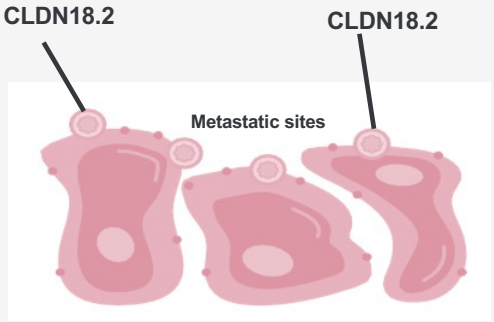
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CLDN18.2 expression

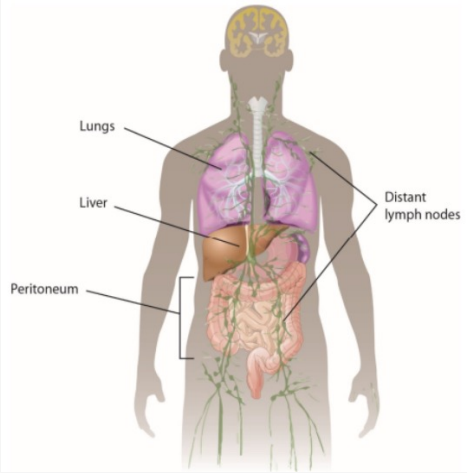
Primary GC and metastatic sites



***De novo* CLDN18.2 expression in GC[1-3]**



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



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]

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

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

CLDN18.2, claudin 18 isoform 2; G/GEJ, gastric and gastroesophageal; **GC,** gastric cancer.

[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 prevalence in LA unresectable or mG/GEJ adenocarcinoma

By tumour disease and sample characteristics

Overall CLDN18.2 prevalence in two global phase 3 clinical studies: 38.4% (1730/4507)[1,2]

Disease Characteristics[3]

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[3]

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

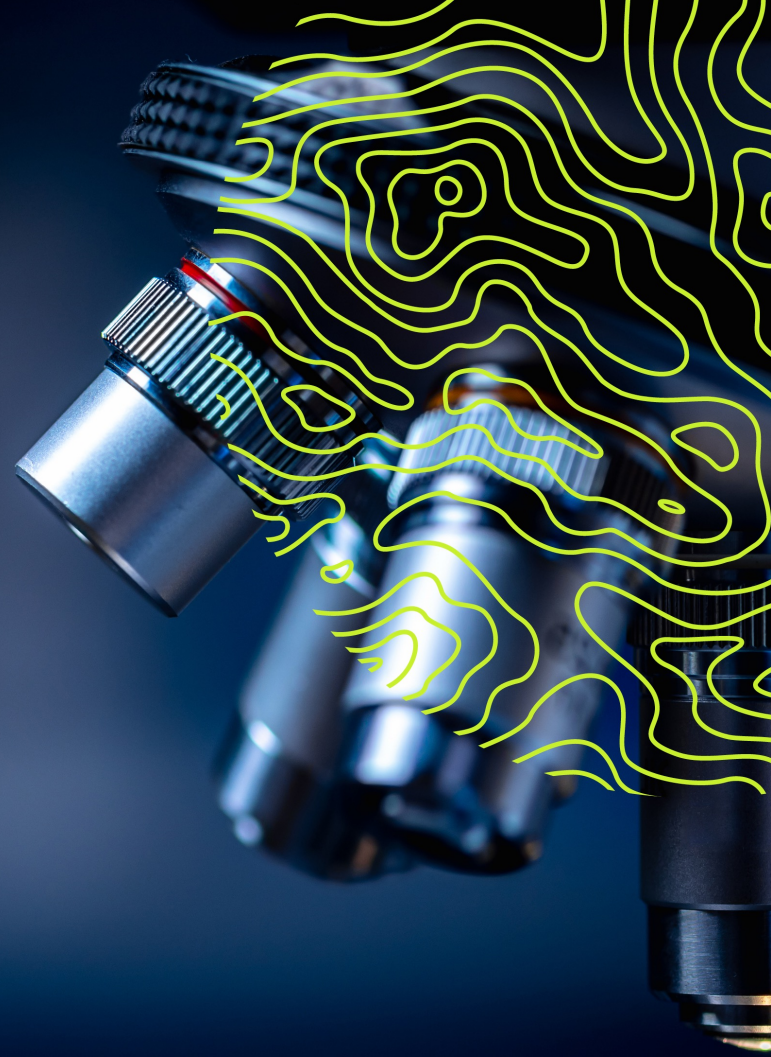
Prevalence of CLDN18.2-positivity was 39.4% (201/510) in samples collected from **metastatic sites** and 38.0% (1498/3939) in samples collected from **primary sites**. This suggests samples from primary or metastatic sites can be used to screen for CLDN18.2 expression by IHC

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

CLDN18.2: claudin 18 isoform 2; **G/GEJ**: gastric/gastroesophageal junction; **IHC:** immunohistochemistry; **LA**: locally advanced

[1]Shah MA, et al. *Nat Med*. 29:2133-2141, 2023;
[2]Shitara K, et al. *Lancet*. 401(10389):1655-1668, 2023;
[3]Shitara K., et al. *Gastric Cancer*. 27.5:1058-1068, 2024.

FAQs



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Global ring study overview

Reproducibility and comparability of CLDN18.2 testing in GC

- A ring trial provides concordance analysis of test results against consensus reference values with regard to scoring, assay methods and platforms used among participating laboratories[1,2]
- The aim of the trial was to assess reproducibility and comparability of three CLDN18 antibodies and IHC staining platforms for determination of CLDN18.2 positivity in GC tissue specimens within a cohort of 27 global laboratories[1]
- Sensitivity, specificity, accuracy and precision were the four-performance metrics used for data analysis in this study[1]

Antibodies investigated in the global ring study[1]

Supplier	Product name	Type	Catalogue number
Roche Tissue Diagnostics	VENTANA CLDN18 Assay (43-14A)[3]*	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

*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; **CLDN**: claudin; CLDN18.2: claudin 18 isoform 2; **GC**: gastric cancer; **IHC:** immunohistochemistry; **IVD:** *in vitro* diagnostic; **IVDD:** *in vitro* diagnostic directive

[1]Jasani B, et al. *Lab Invest*. 2024;104(1):100284.
[2]Johnson P & Cabuang L. *Rev Sci Tech*. 2021;40(1):189-203.
[3]VENTANA CLDN18 (43-14A) Assay, Package Insert Sheet, CE-IVD 1017589EN Rev C, 2023-09-28;

Global ring study

Design and methodology (1/2)

Study locations

- Up to **5 TMA sets 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



Europe	Germany	Spain	France	Italy	UK	Netherlands
	1	3	3	3	2	1



Americas	USA	Canada	Brazil	Asia	China	Japan
	5	1	1		5	2

Number of laboratories using each of the platforms and antibodies

Antibodies and platforms		Number of laboratories
Antibodies	VENTANA 43-14A	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.

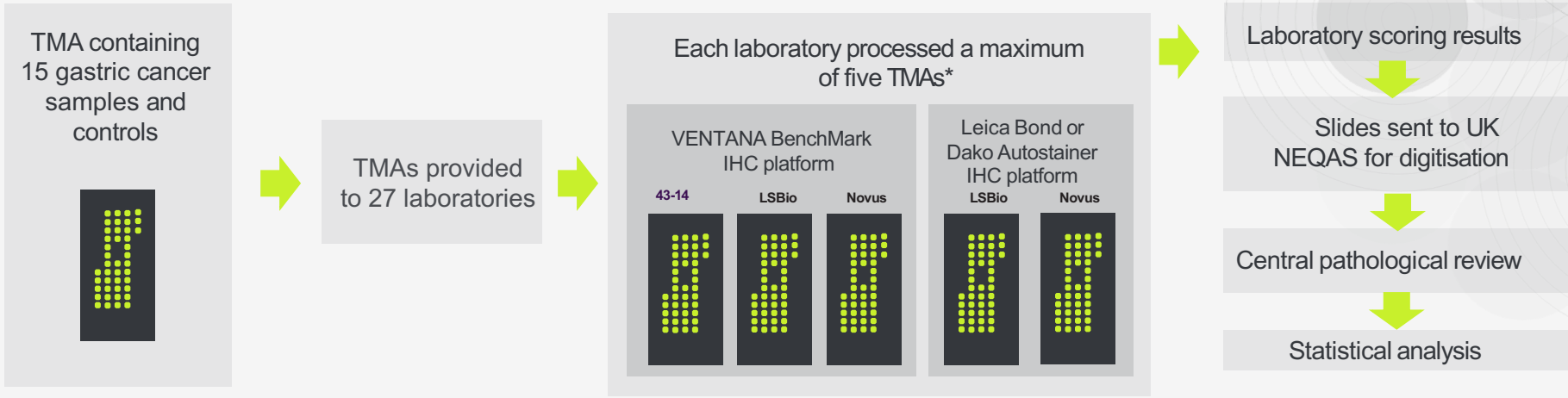
*This assay is an antibody reagent product that is classified as Class I in the USA and General IVD (CE-IVD Analytic) under IVDD in the EU. It is not a CDx assay.

CDx: companion diagnostic; **CE**: Conformité Européenne; **CLDN**: claudin; 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, et al. *Lab Invest*. 2024;104(1):100284

Global ring study

Design and methodology (2/2)



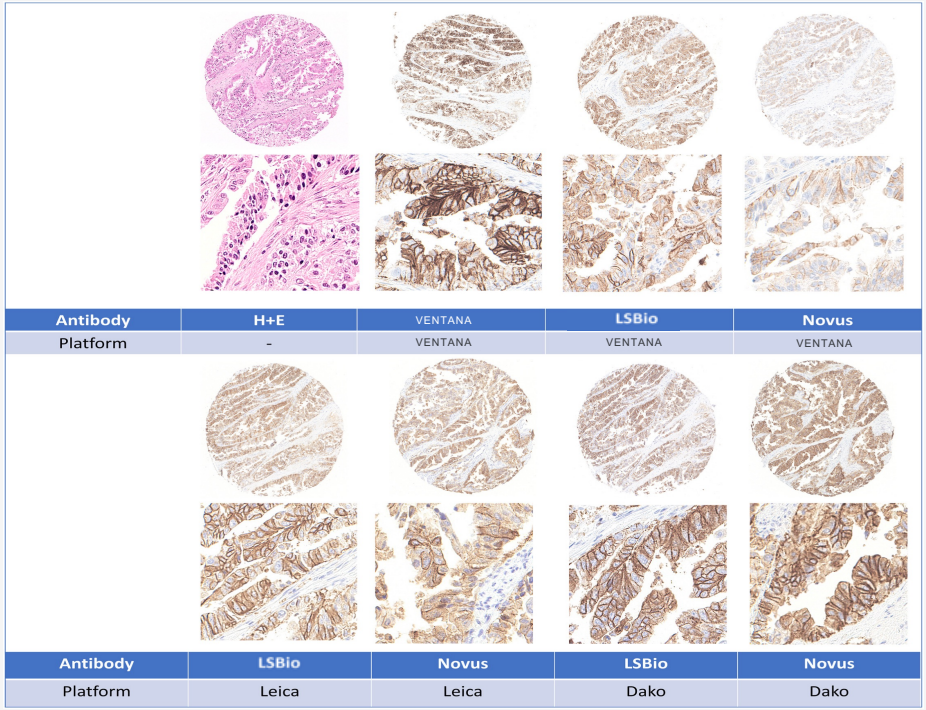
*Each laboratory was required to test a maximum of five TMAs using the antibody/assay combinations.

- Laboratories were provided with staining protocols, an instructional video and reference guide for understanding 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 tumour cells demonstrating moderate to strong (≥2+) membranous CLDN18 staining intensity, as used in two Phase 3 clinical trials

CLDN18.2: claudin 18 isoform 2; **IHC:** immunohistochemistry; **NEQAS:** National External Quality Assessment Scheme; **TMA:** tissue microarray

Global ring study

Results



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 seven different CLDN18 antibody and platform combinations. The VENTANA assay shows the darkest (brown/black) positive staining, with clear and sharp membranous staining. Both LSBio and Novus show weaker staining on the VENTANA platform and more defined membranous staining on the Leica and Dako platforms

CLDN18: claudin 18; **H&E:** haematoxylin and eosin; **TMA:** tissue microarray

[1]Jasani B, et al. *Lab Invest*. 2024;104(1):100284.

Global ring study

Discussion

- Ring studies facilitate early biomarker test adoption and emphasise quality testing to produce accurate, reliable results
- The benefits of conducting a ring study are multifactorial: it educates relevant stakeholders on both technical and analytical requirements; it provides real-life, hands-on experience to prime laboratories for early integration of new biomarker tests; and it 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 predefined 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

CLDN18.2: claudin 18 isoform 2; **IHC:** immunohistochemistry; **IVD**: *in vitro* diagnostic

[1]Jasani B, et al. *Lab Invest*. 2024;104(1):100284.

Global ring study

Conclusions

1. The **Global Ring Study** evaluated **CLDN18.2 expression by IHC** across multiple antibodies, and multiple staining platforms; reproducibility was assessed across a cohort of laboratories for the detection of CLDN18.2 in **GC.**

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. **Prior to central laboratory correction**, all three antibody assays failed to meet the acceptance criteria for **one or more performance metrics**, with the **Novus antibody assay demonstrating the least consistent performance metrics** on all platforms. 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 evaluation in GC was found to increase, i.e., when the interpretation discrepancies were removed. An example of this is at precentral pathology review: 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.**

CE: Conformité Européenne; **CLDN18.2:** claudin 18 isoform 2; **GC**: gastric cancer; **IHC:** immunohistochemistry; **IVD**: *in vitro* diagnostic

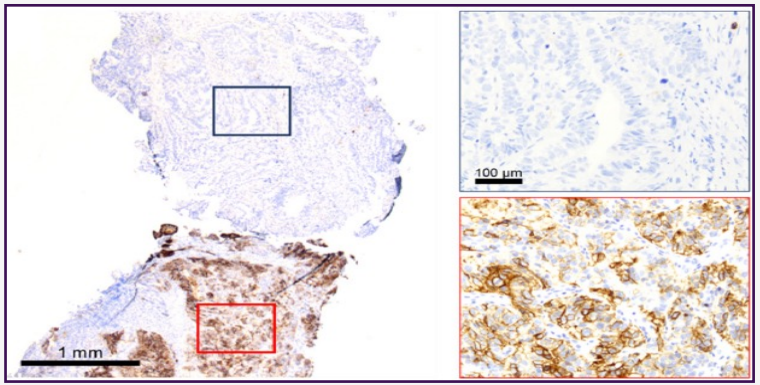
[1]Jasani B, et al. *Lab Invest.* 2024;104(1):100284.

CLDN18.2 in G/GEJ cancer

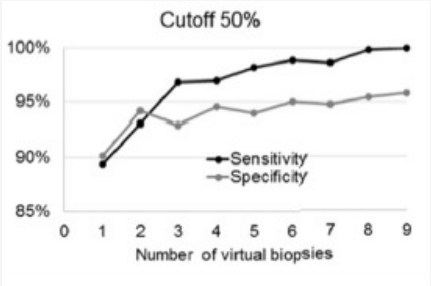
Intratumoural heterogeneity

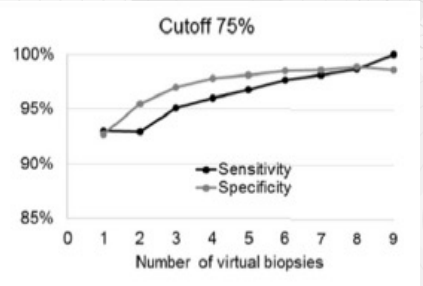
- In 2021, an Italian research team published data on intratumoural heterogeneity by conducting a virtual biopsy study on 93 surgically treated cases (77 GC and 16 GEC) from Padua University[1]
  - Digitally scanned images of stained GC cases used to simulate virtual endoscopic biopsies (five on each of the two digital slides for each tumour)
  - CLDN18 IHC evaluation by two blinded pathologists
  - Sensitivity increased from 2 to 9 biopsies (93 to 100%); after six biopsies, increase was minimal (~97.7 to 100%)
  - Specificity stable between 6-8 biopsies (98.5 to 98.9%)

REPRESENTATIVE EXAMPLE OF INTRATUMOUR CLDN18 HETEROGENEITY



SENSITIVITY AND SPECIFICITY OF CLDN18 TESTING ACCORDING TO THE NUMBER OF VIRTUAL BIOPSIES SCORED[1]





This study supports that (consistent with other established biomarkers in GC [e.g., HER2]) CLDN18.2 status is optimally assessed in multiple biopsies (approximately 6-8) obtained from the primary tumour.[1]

This may help to overcome intratumour heterogeneity for purposes of IHC evaluation.[1]

CLDN18.2: claudin 18 isoform 2; **GC**: gastric cancer; **GEC**: gastroesophageal cancer; **G/GEJ:** gastric/gastroesophageal junction cancer; **HER2**: human epidermal growth factor receptor 2; **IHC**: immunohistochemistry

[1]Pellino A, Brignola S, Riello E, *et al. J Pers Med*.;11,1095, 2021.

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)[1-3]
- Of 3705 patients with HER2-negative disease with valid CLDN18 IHC results, 1568 (42.3%) had CLDN18.2-positive tumours
- The conclusion from two global clinical trials is that CLDN18.2 positivity prevalence is similar across regions (ranging from 30% to 44%)

Prevalence of CLDN18.2 in global studies[1-3]

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%

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

CLDN18.2: claudin 18 isoform 2; **G/GEJ:** gastric and gastroesophageal junction

[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.

Biomarker-guided targeted therapies

In advanced G/GEJ cancers

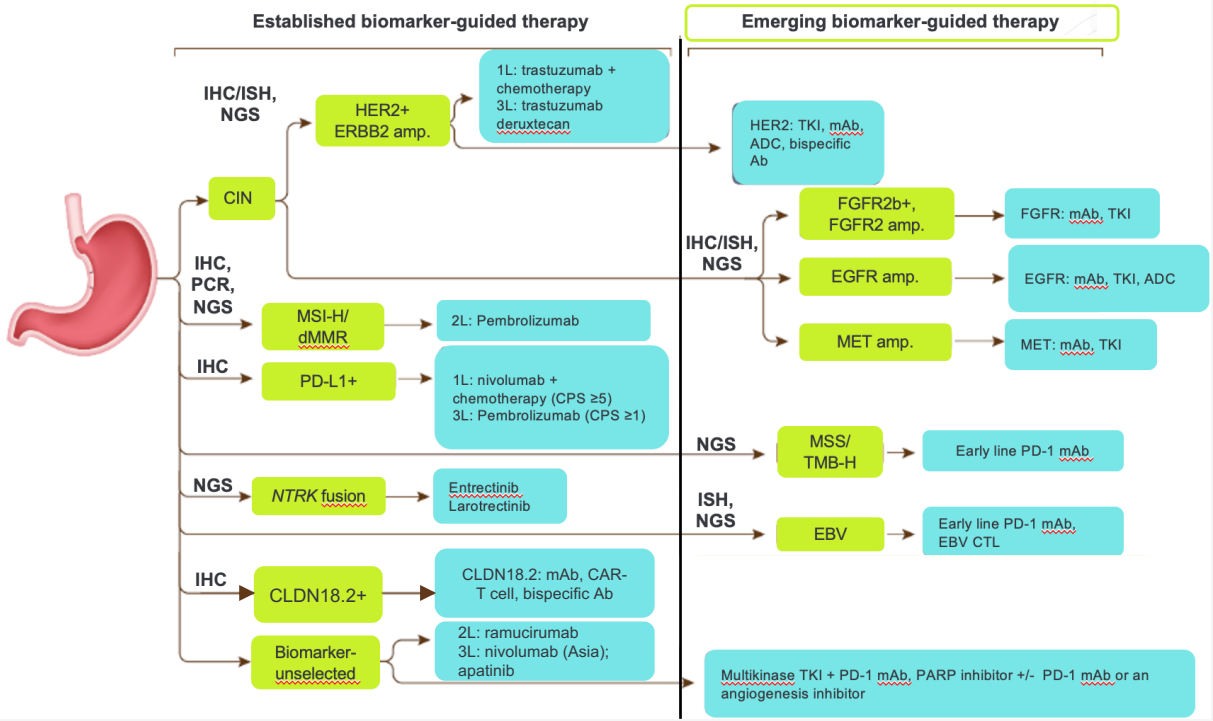


Image adapted from Nakamura Y, Kawazoe A, Lordick F, et al. *Nat Rev Clin Oncol*. 18(8):473-487, Aug 2021. Lordick F, et al. *Ann Oncol*. 2022;33(10):1005-1020 (modified) ESMO Gastric Cancer Living Guideline, v1.4 September 2024

1L/2L/3L: first-/second-/third-line; **ADC:** antibody-drug conjugate; **Amp:** amplification; **CAR:** chimeric antigen receptor; **CIN:** chromosome instability; **CLDN:** claudin; **CPS:** combined positive score; **CTL:** cytotoxic T- lymphocyte; **dMMR:** mismatch repair deficient; **EBV:** Epstein-Barr virus; **EGFR:** epidermal growth factor receptor; **ERBB2**: v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; **FGFR2(b)**: fibroblast growth factor receptor 2(b); **G/GEJ**: gastric/gastroesophageal junction; **HER2:** human epidermal growth factor receptor 2; **IHC:** immunohistochemistry; **ISH**: *in situ* hybridisation; **(m)Ab:** (monoclonal) antibody; **MET:** tyrosine-protein kinase Met; **MSI-H:** microsatellite instability-high; **MSS:** microsatellite stable; **NGS:** next-generation sequencing; **NTRK**: neurotrophic tyrosine receptor kinase; PARP**:** poly (ADP-ribose) polymerase; PCR: polymerase chain reaction; PD-1**:** programmed cell death protein 1; **PD-L1:** programmed death-ligand 1; **TKI:** tyrosine kinase inhibitor; **TMB-H:** tumour mutational burden-high.

Current Claudin18.2 targeted therapies under investigation in gastric cancer

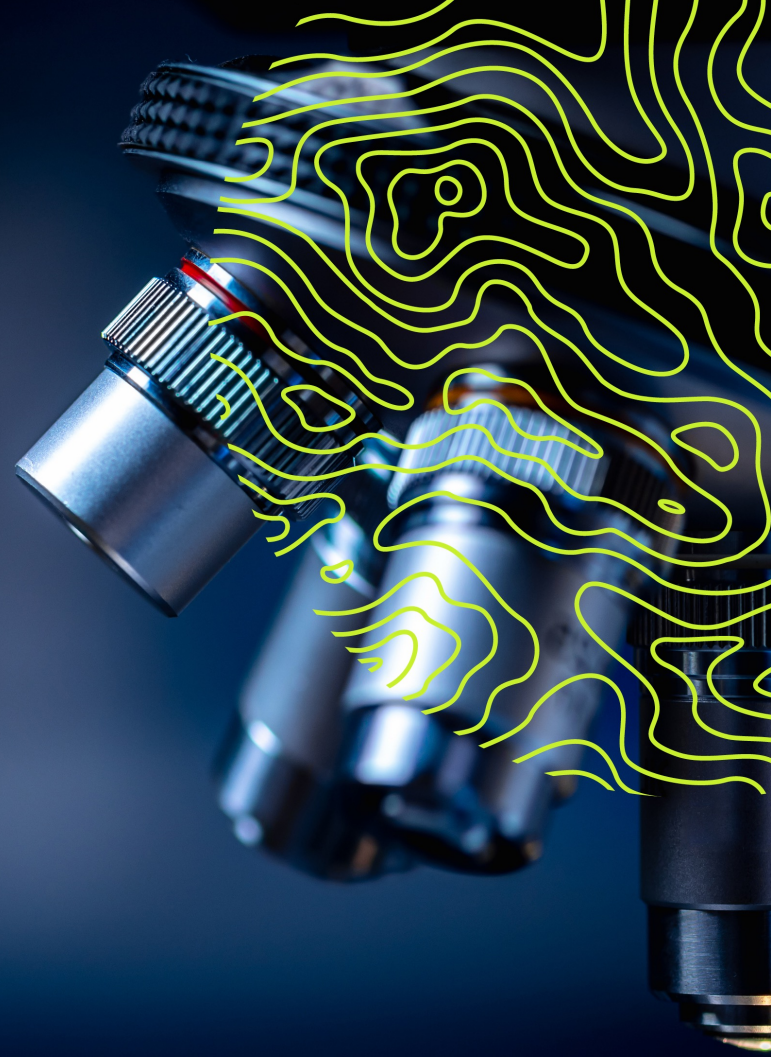
Therapy name (Company)	Class	Stage	NCT	Indication/Description	Definition of CLDN18.2 positivity
Zolbetuximab	mAb	Phase III	NCT03504397	1L+ CLDN18.2+ HER2- GC/GEJC	IHC 2+/3+ in TC ≥75%[1]
Osemitamab (Mabspace/Transcenta/BMS)	mAb	Phase III	NCT06093425	1L CLDN18.2+ GC/GEJC HER2 negative, known PD-L1 CPS status	IHC membrane staining ≥10% tumour cells with ≥1+ intensity per LDT assay[2]
ASKB589 (AskGene Pharma)	mAb	Phase III	NCT06206733	1L+ CLDN18.2+ GC/GEJC	Medium-high CLDN18.2 expression IHC 2+/3+ membrane staining in tumour cells ≥40%[3]
M108 (FutureGen Biopharma)	mAb	Phase III	NCT06177041	1L CLDN18.2+, HER2- GC/GEJC PD-L1 CPS < 5	IHC 1+/2+/3+ ≥10%*[4]
AZD0901/CMG901 (AstraZeneca)	ADC	Phase III	NCT06346392	2L+ CLDN18.2+ HER2- GC/GEJC	≥ 2+ intensity in ≥ 20% tumour cells*[5]
SHR-A1904 (Hengrui Medicine)	ADC	Phase I/III	NCT06350006	1L+ CLDN18.2+ HER2- GC/GEJC/PC	IHC 2+/3+ in tumour cells ≥ 50%*[6]
LM-302 (LaNova Medicines)	ADC	Phase I/II	NCT06351020	2L+ CLDN18.2+ HER2- GC/GEJC	Tumour cells ≥ 50%, IHC ≥ 2+[7]
CT041 (CARsgen)	CAR-T	Phase II	NCT04581473	3L+ CLDN18.2+ HER2- GC/GEJC	IHC 2+/3+ tumour cells ≥ 40% patients[8]
AZD5863 (AstraZeneca)	BsAb†	Phase I/II	NCT06005493	2L+ GC/GEJC/PC	Must show positive CLDN18.2 expression in tumour cells as determined by IHC[9]
IBI343 (Innovent)	ADC	Phase I	NCT05458219	2L+ CLDN18.2+ Locally Advanced Unresectable or Metastatic Solid Tumors	≥1% tumor cells with membranous staining (1+/2+/3+) by IHC[10]

The landscape is dynamic; this information is accurate as of August 2024.
*Definition based on Phase I/II trials.
1L/2L/3L: first-/second-/third-line; **ADC**: antibody-drug conjugate; BsAb: bispecific antibody; **CAR-T**: chimeric antigen receptor T cell; **CD3**: cluster of differentiation 3; **CHMP**: Committee for Medicinal Products for Human Use; **CLDN18.2**: claudin 18 isoform 2; **CPS**: combined positive score; **EMA**: European Medicines Agency; **GC**: gastric cancer; **GEJC**: gastroesophageal junction cancer; **GI**: gastrointestinal; **H/M**: high/medium; **HER2**: human epidermal growth factor receptor 2; **IHC**: immunohistochemistry; **LDT**: laboratory-developed test; **mAb**: monoclonal antibody; **PD-1**: programmed cell death protein 1; **PD-L1**: programmed death-ligand 1.

1. Shitara K, et al. *Lancet*. 401(10389):1655-1668, 2023; 2. Shen L, et al. ESMO World Congress on Gastrointestinal Cancer 2023. Poster #PD-7; 3. Peng Z, et al. *J Clin Oncol*. 2024;42:3_suppl:317; 4. Liu F, et al. *J Clin Oncol*. 2024;42:16_suppl:4142; 5. Xu G, et al. *Cell Rep Med*. 2024;5(9):101710; 6. NCT05277168. ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT05277168. Accessed October 2024; 7. Chunmei B, et al. *J Clin Oncol*. 2024 42:16_suppl, 3028-3028; 8. Qi C, et al. Presented at ASCO Annual Meeting 2022, 3–7 June. Chicago, USA. Abstract #4017; 9. NCT06005493. ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT06005493. Accessed October 2024; 10. Liu JJ, et al. *Annals of Oncology*. 2024;35:S160.

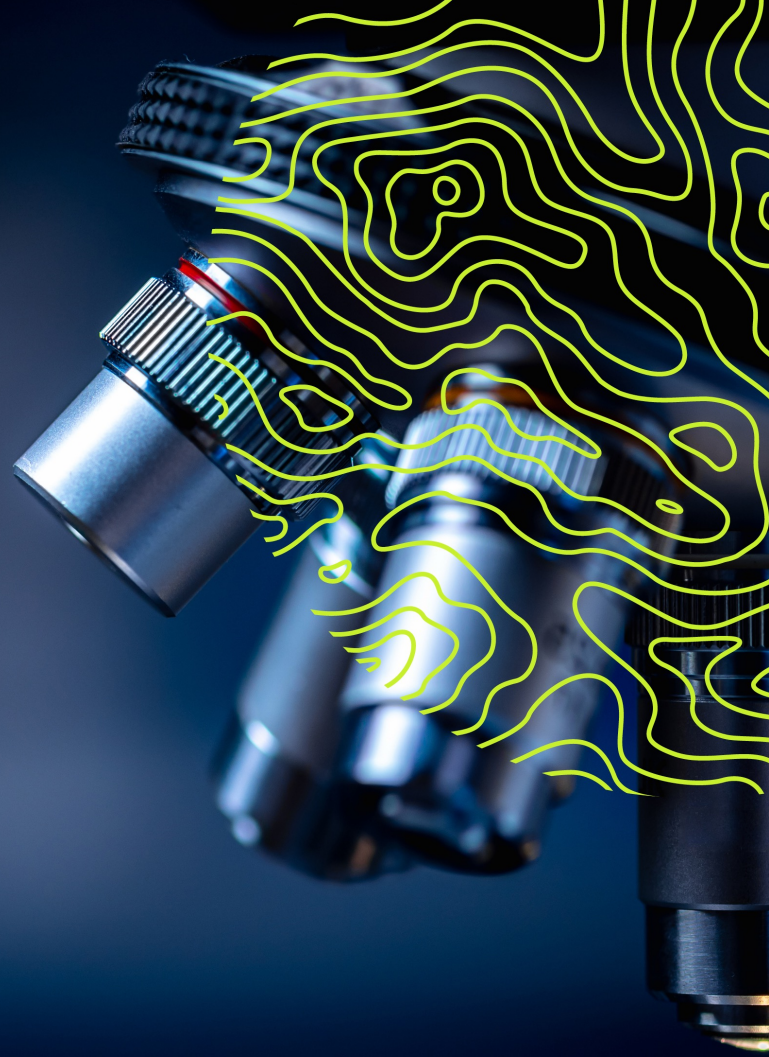
Thank You

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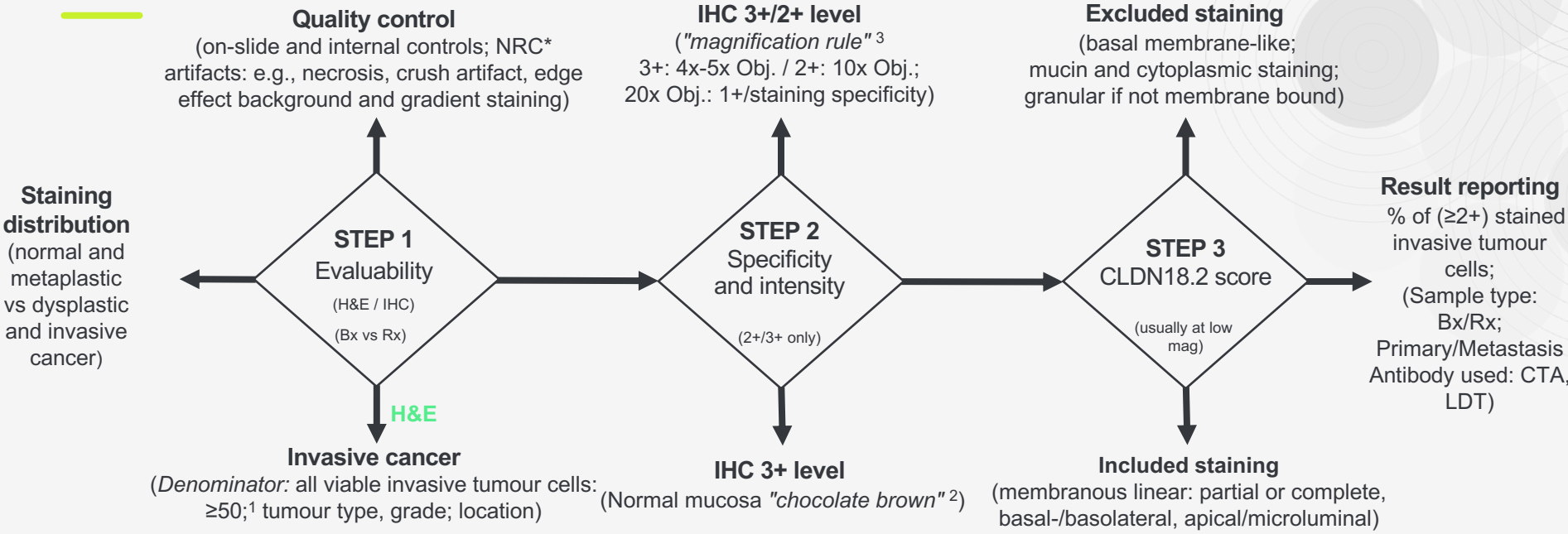
Training on sample cases



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CLDN18 staining evaluation

Scoring: stepwise approach

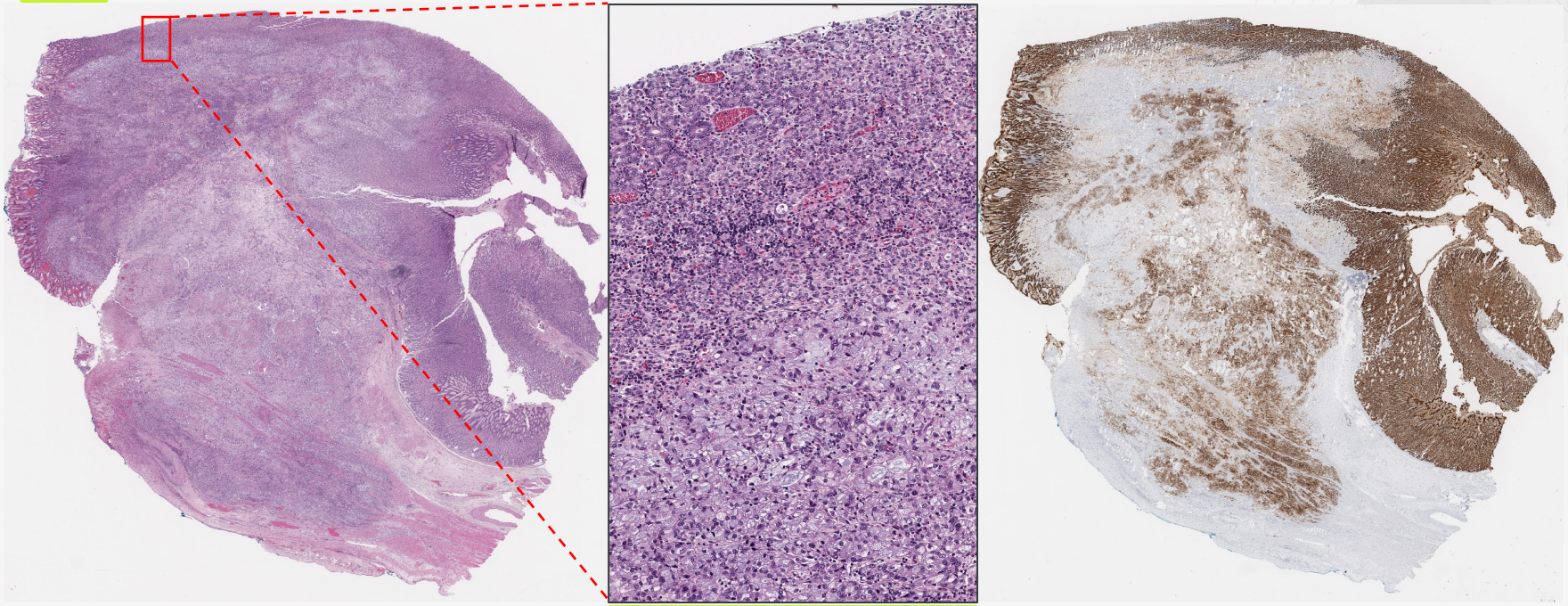


Bx: biopsies; **CTA**: clinical trial application; **H&E**: hemtoxylin and eosin;
IHC: immunohistochemistry; **LDT**: lab developed test; **NRC**: negative reagent control; **Obj**: microscope objective;
Rx: resection

[1]Ventana CLDN18 (43-14A) assay [package insert]. Mannheim, Germany: Roche Diagnostics GmbH[2]Schildhaus HU, Schultheis AM, Rüschoff J, *et al. Clin Cancer Res.* 21:907-15, 2015; [3]Scheel AH *et al*. Physical basis of the 'magnification rule' … gastric cancer. *Diagn Pathol*. 13(1):19. 2018.

H&E / CLDN18 staining evaluation (step 1)

Assessment of denominator: what is an **invasive** tumour and what is **not** stained (included)



Start at low power 2-5x objective

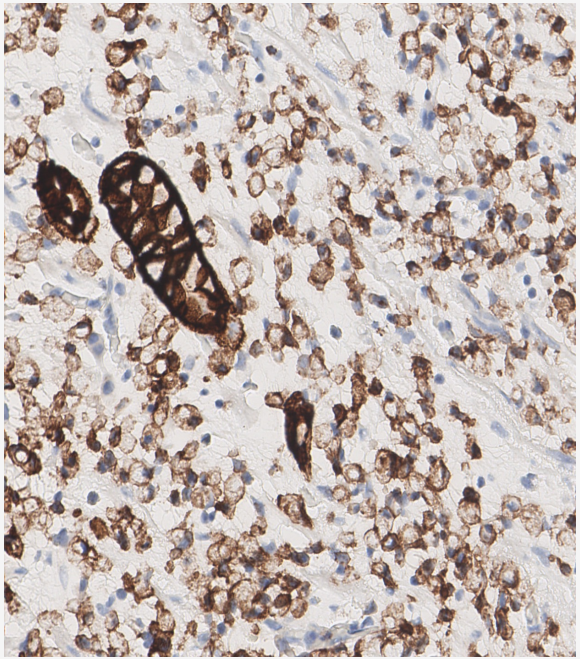
CLDN18: claudin 18; **H&E:** hematoxylin and eosin

Astellas. Data on file. Document REF-50697. 2024.

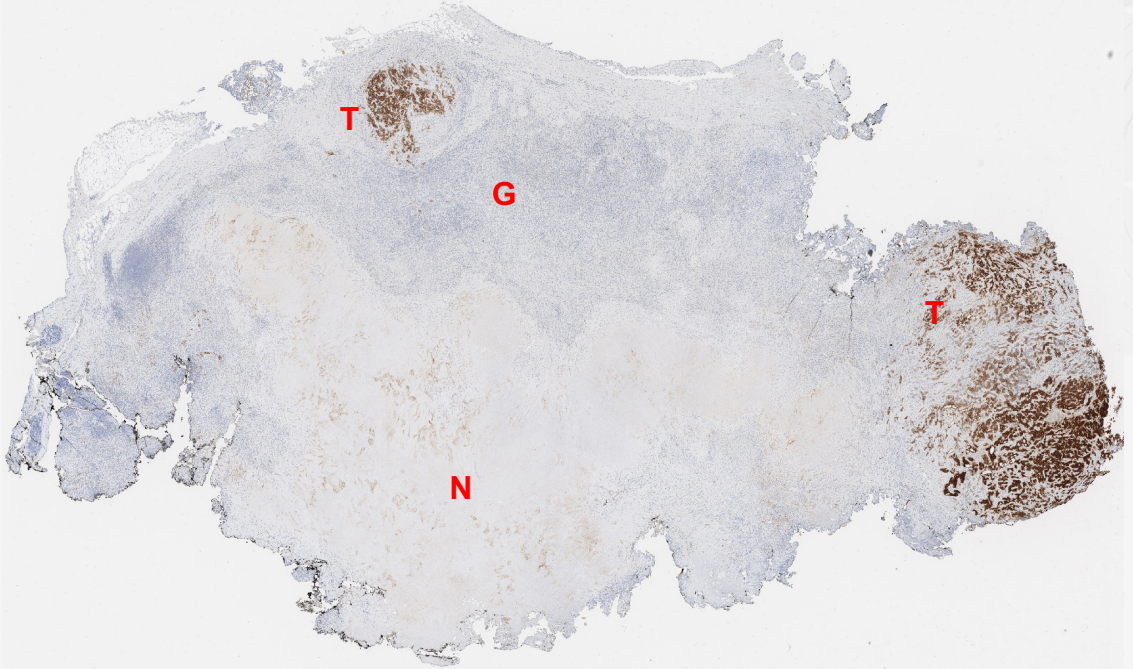
CLDN18 staining evaluation (step 1)

Assessment of denominator: non-invasive and non-viable tumour cells (excluded)

Metaplastic and dysplastic non-invasive cells



Necrosis (N) and granulation tissue (G) – evaluate invasive tumour only (T)

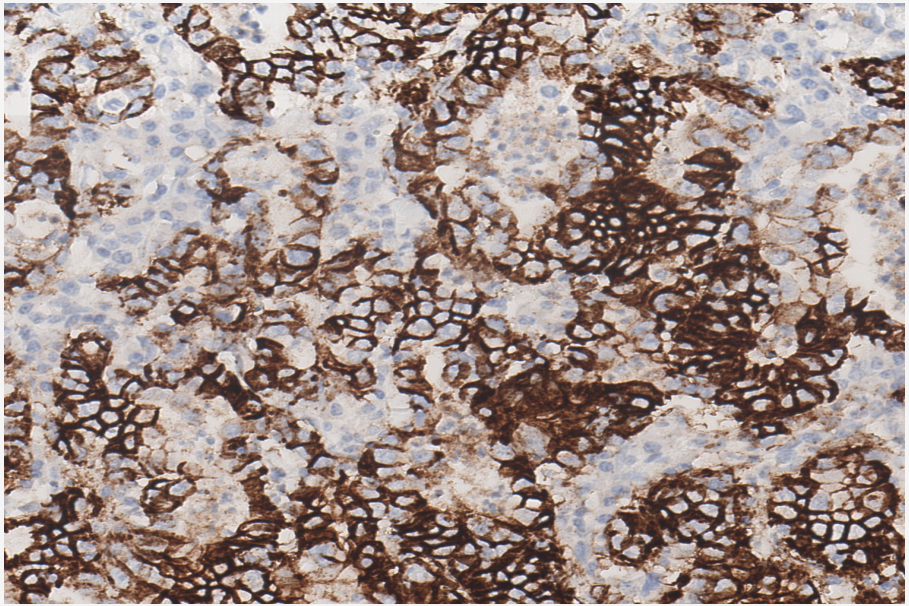


CLDN18: claudin 18

Astellas. Data on file. Document REF-50697. 2024.

CLDN18 staining evaluation (step 2)

Specific staining patterns: membranous complete – incomplete (included)



Usually needs higher power (10x/20x)

Circumferential

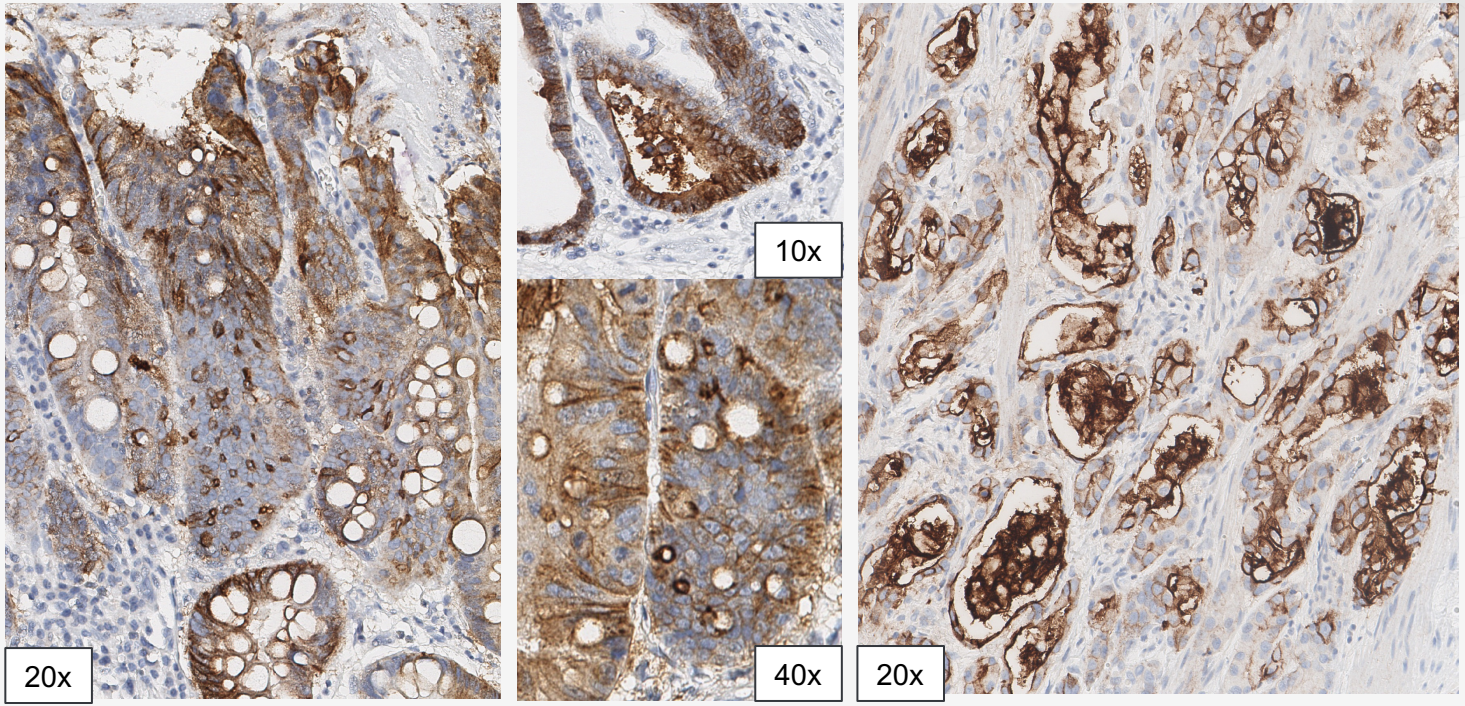
- Partial/complete
- Lateral-/basolateral

CLDN18: claudin 18

Astellas. Data on file. Document REF-50697. 2024.

CLDN18 staining evaluation (step 2)

Specific staining patterns: microluminal and apical (included)



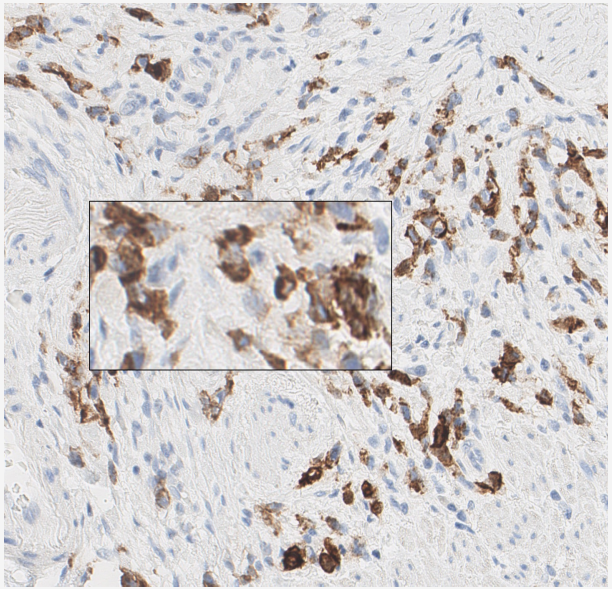
CLDN18: claudin 18

Astellas. Data on file. Document REF-50697. 2024.

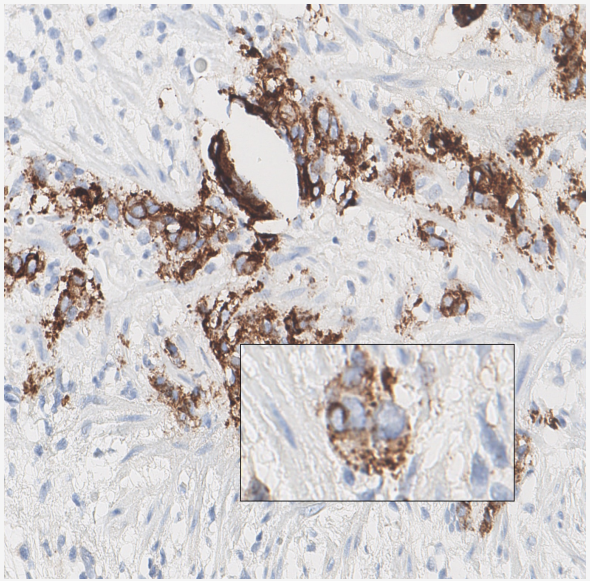
CLDN18 staining evaluation (step 2)

Non-specific staining patterns: cytoplasmic, granular and mucin staining (excluded)

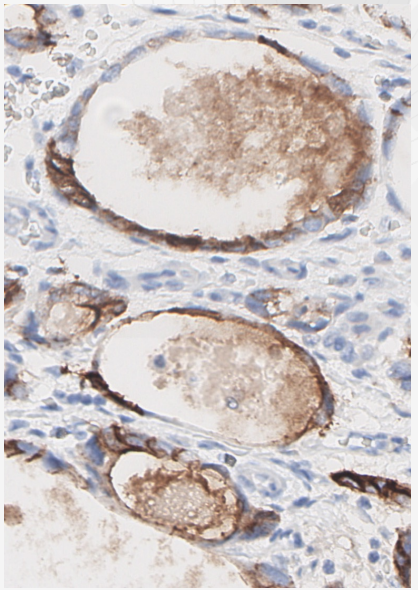
Cytoplasmic staining



Granular staining (if not membrane bound)



Mucin stains



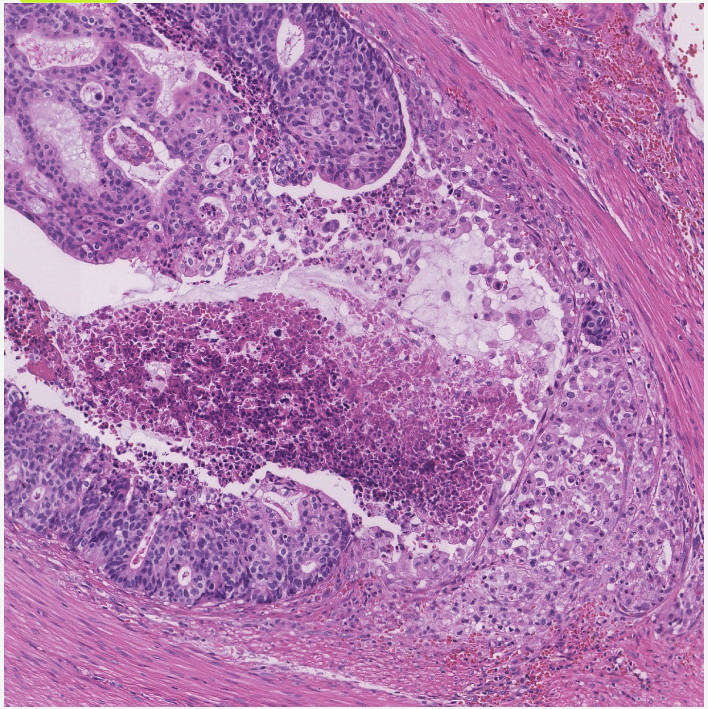
CLDN18: claudin 18

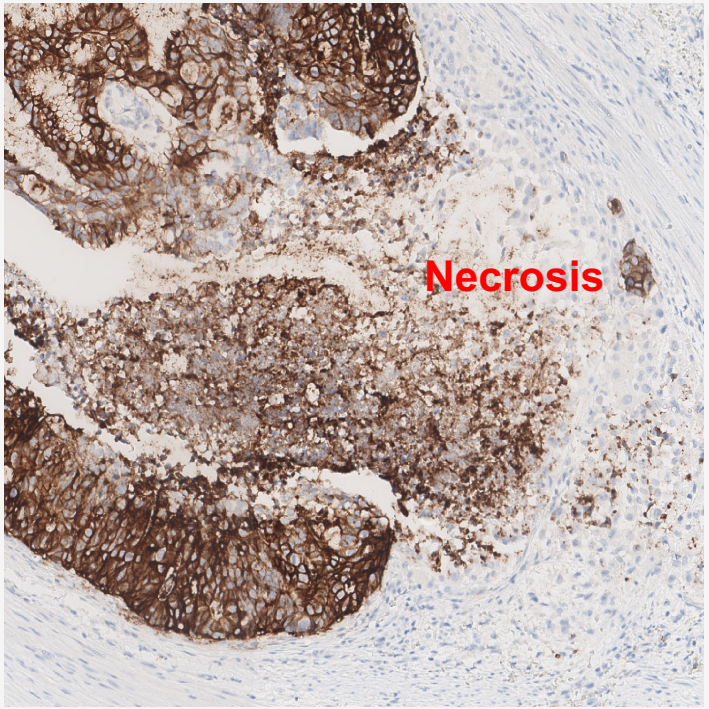
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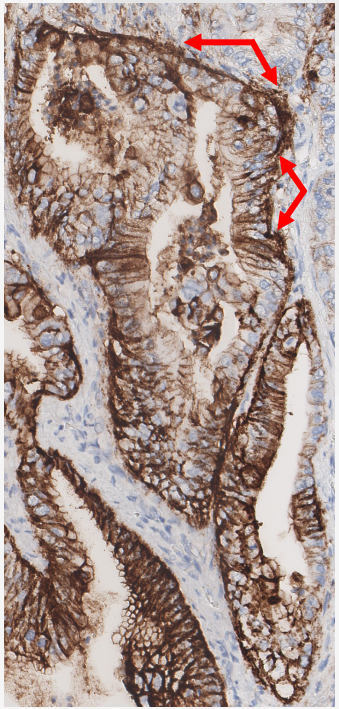
CLDN18 staining evaluation (step 2)

Non-specific staining patterns: necrotic tumour and basal membrane like (→ excluded)

Red arrows indicate basement membrane





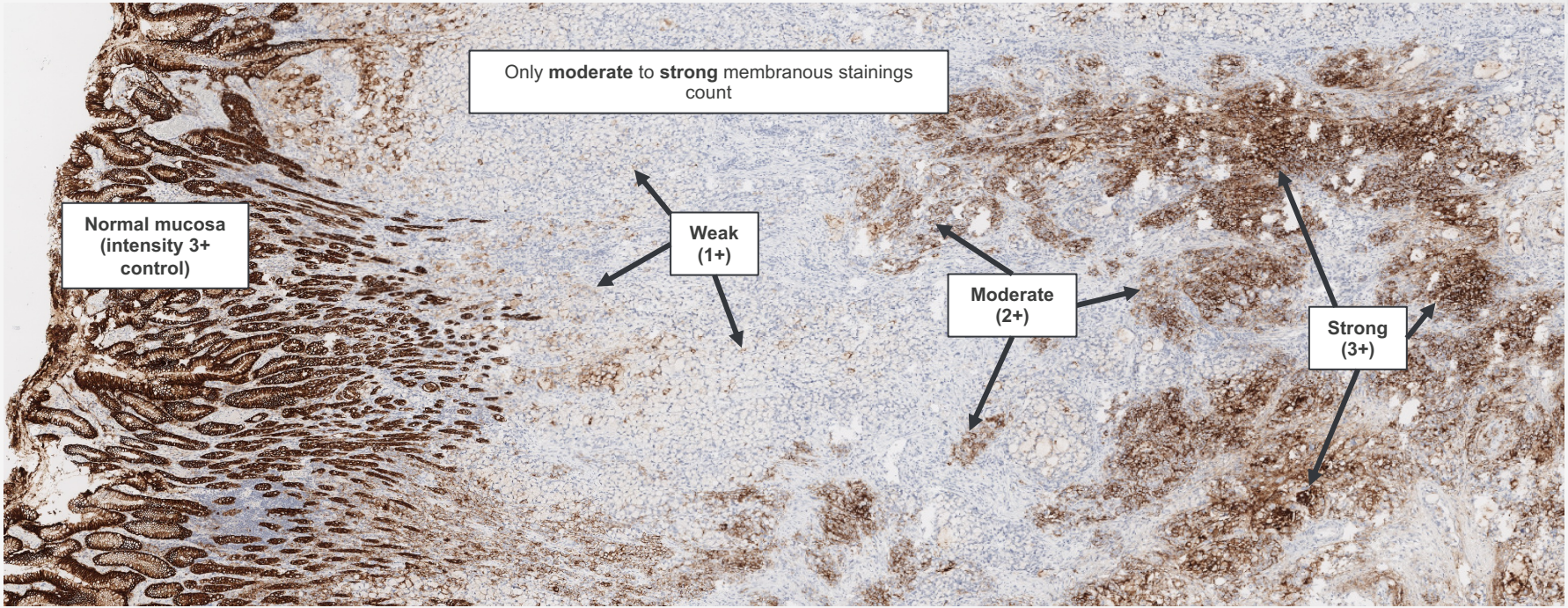


CLDN18: claudin 18

Astellas. Data on file. Document REF-50697. 2024.

CLDN18 staining evaluation (step 3)

Scoring: percentage of invasive tumour cells with moderate-to-strong membranous staining

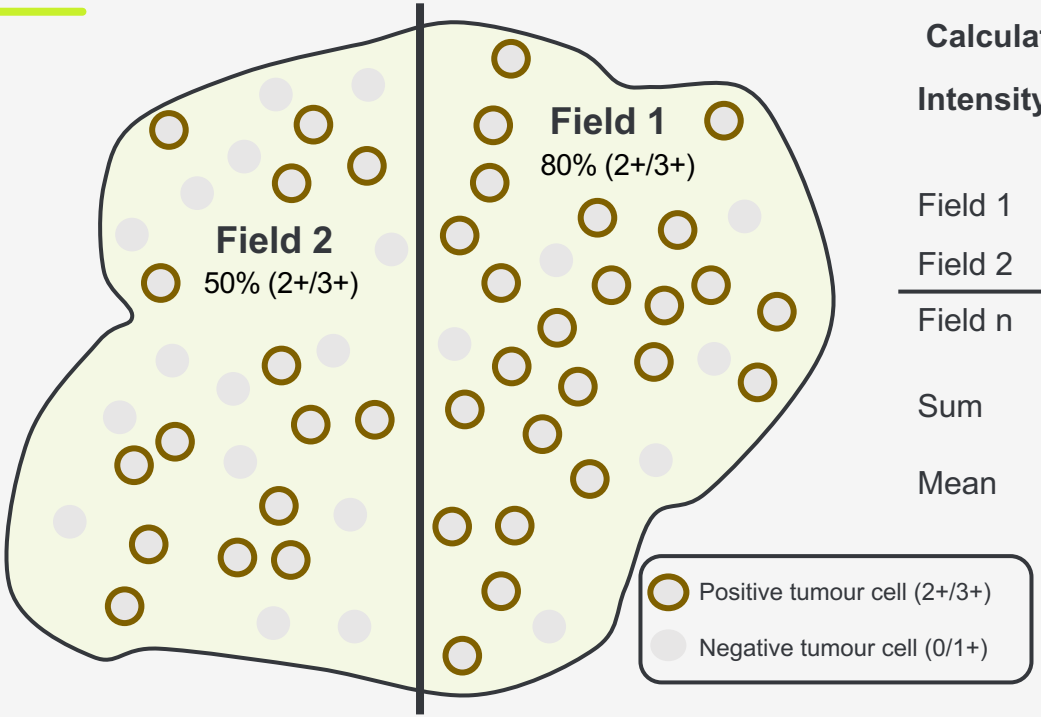


CLDN18: claudin 18

Astellas. Data on file. Document REF-50697. 2024.

CLDN18 staining evaluation (step 3)

Calculation of IHC results (covering whole tumour area)



Calculation of final CLDN18.2 Score:

Intensity	0	1+	2+	3+
Field 1	10	10	15	65
Field 2	20	30	10	40
Field n	..	..	..	..
Sum	30	40	25	105
Mean	15	20	12.5	52.5

Final CLDN18.2 Score: 65%

CLDN18.2: claudin 18 isoform 2; IHC: immunohistochemistry

CLDN18 staining evaluation

Different scoring approaches

Method	Approach	Application Area	Trade-offs
Structured Scoring (Cell number oriented raw-scoring)	• Counting of tumour cells (TCs) (2+/3+ positives and 0/1+ negatives) • Denominator (all invasive TCs) (~2500 TCs / 20x field)	• Training (beginners) • Difficult cases (borderline e.g., 1+ vs 2+)	• Exact positive score (% of ≥2+ tumour cells) • Time consuming
Categorical Scoring (“eyeballing“)	• Estimate % 2+/3+ positive TCs (usually at low power, 5x magnification) • Replace negative TCs (0/1+) by positive TCs (2+/3+)	• Daily routine work • Experienced pathologist	• Quick • Suited for obvious positive/negative cases
Reference Scoring	• Comparison with refercence pictures	• Interpretation manuals	• Only rough estimation possible

CLDN18: claudin 18; **TC:** tumour cell