

Claudin18.2 Testing in Gastric & GEJ Adenocarcinoma

A Diagnostic Imperative in the Era of Targeted Therapy



Youngsoo Park M.D., Ph.D.

Department of Pathology, Asan Medical Center,
University of Ulsan College of Medicine, Seoul, Korea

Pre-lecture Survey



Pre-lecture Survey



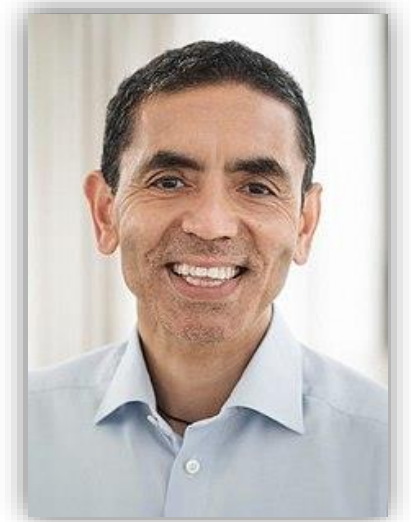
Claudin 18.2

a novel molecular biomarker for GC and PDAC

Human Cancer Biology

Claudin-18 Splice Variant 2 Is a Pan-Cancer Target Suitable for Therapeutic Antibody Development

Ugur Sahin,^{1,2} Michael Koslowski,² Karl Dhaene,³ Dirk Usener,¹ Gunda Brandenburg,¹ Gerhard Seitz,⁴ Christoph Huber,² and Özlem Türeci¹

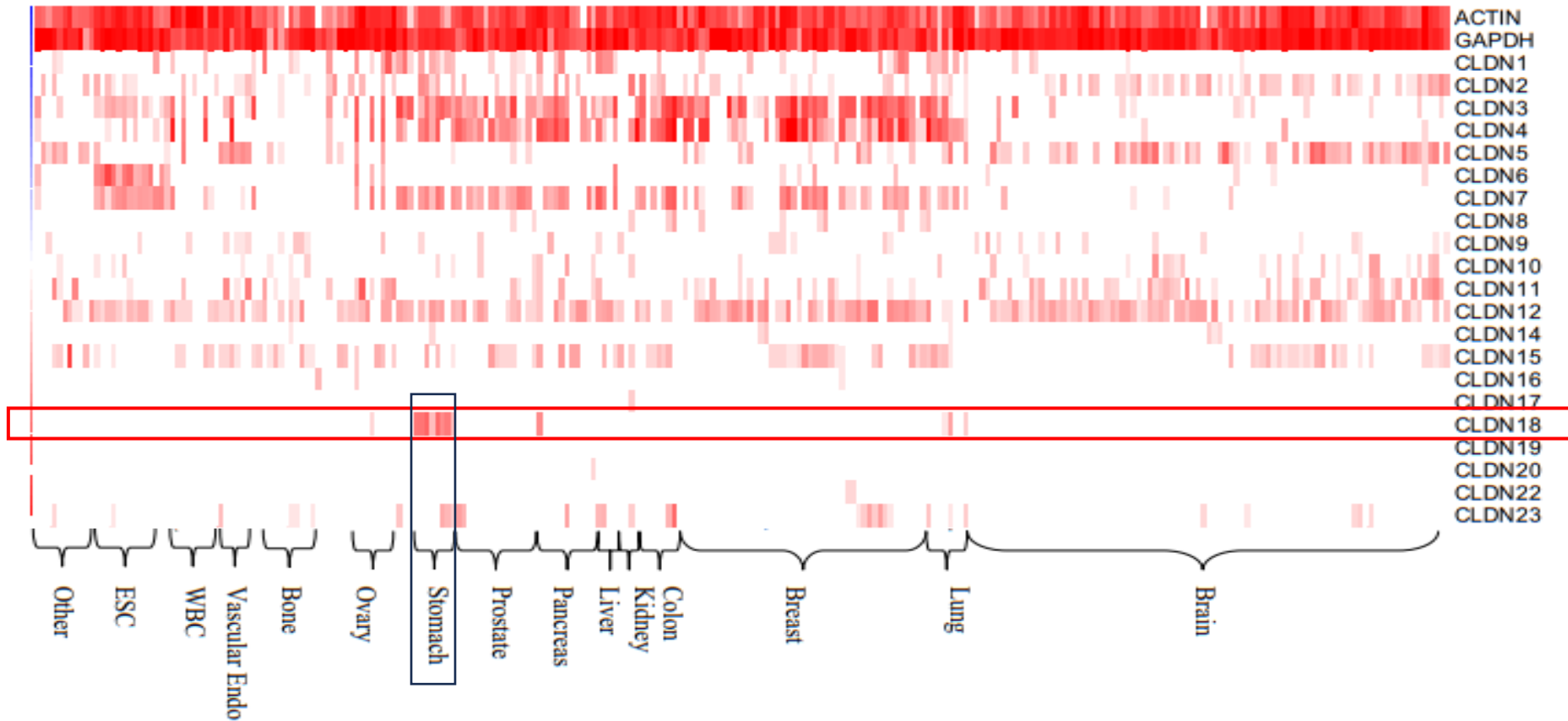


founder and CEO of [BioNTech](#)

Ugur Sahin et al. *Clin Cancer Res* 2008 Dec 1;14(23):7624-34

김현기. 대한병리학회 소화기병리연구회 2024 Dec.

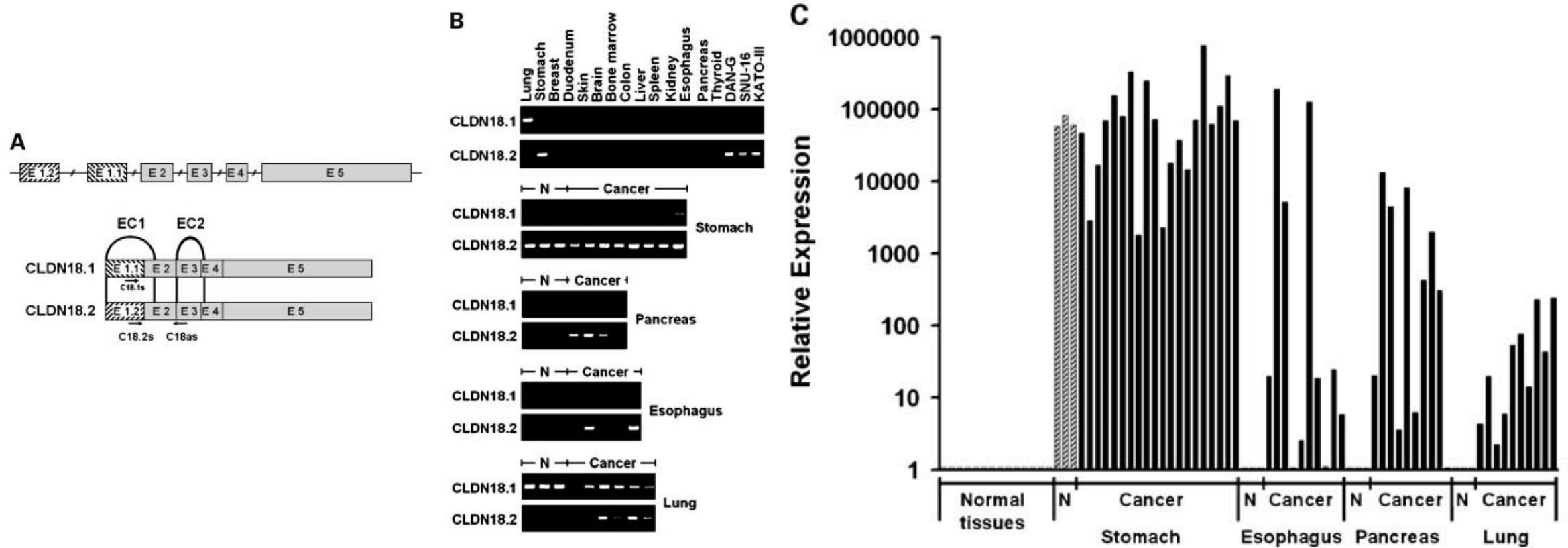
The key component of the tight junctions along with occludin, 23 genes in human (CLDN1 - 23)



Claudin

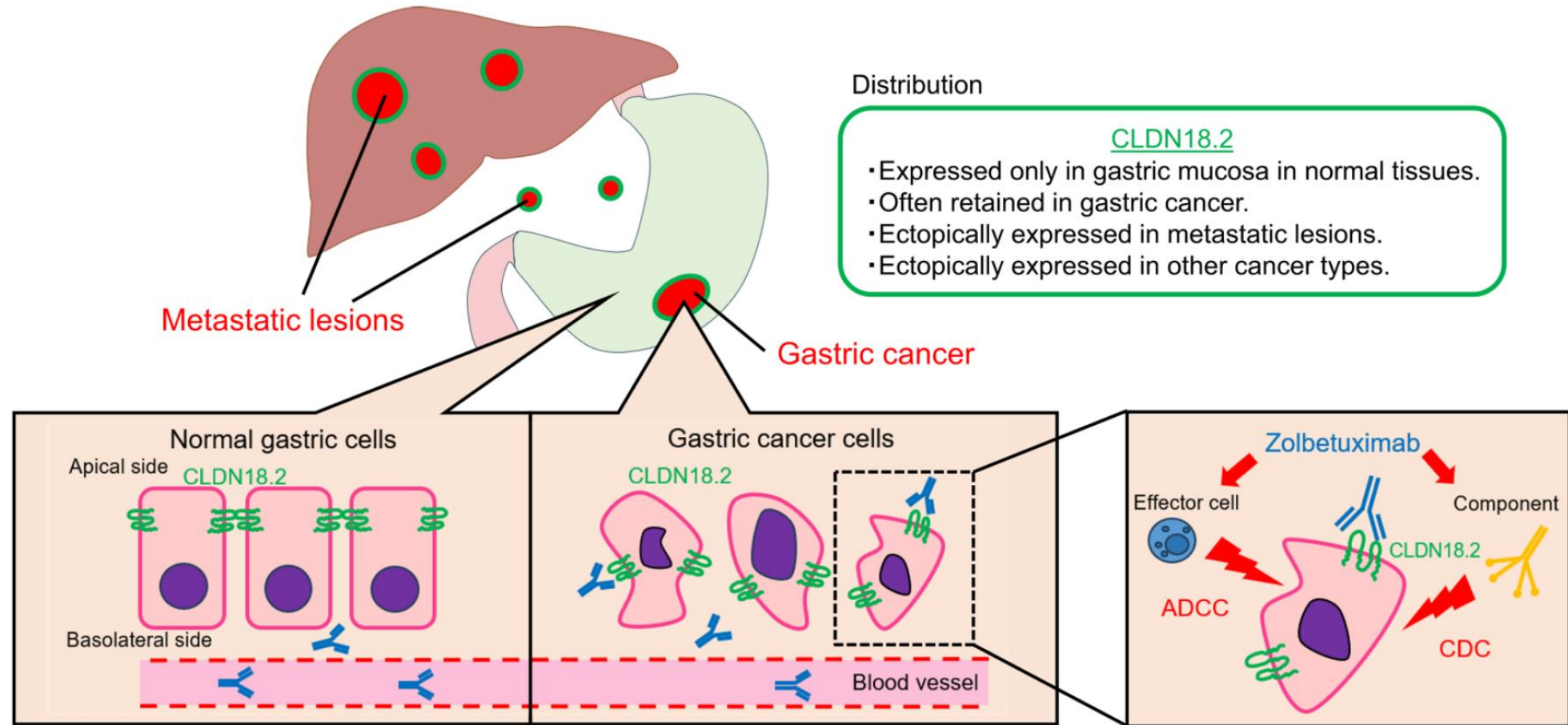
- Different claudins are expressed in different tissues
Ex. Claudin-1 (colon cancer), Claudin-18 (gastric cancer), Claudin-10 (hepatocellular carcinoma)
- Claudin 18.2
 - Isoform 2 of claudin 18 (splice variant)
 - Exclusively expressed in normal gastric mucosa
 - Retained after malignant transformationCf. Aberrant expression in pancreatic, esophageal, ovarian, and lung adenocarcinomas
- Claudin 18.1
 - Expressed in lung alveolar epithelial cells serving lung barrier function

Claudin 18.2 expression in normal & cancer



Ugur Sahin et al. *Clin Cancer Res* 2008 Dec 1;14(23):7624-34

김현기. 대한병리학회 소화기병리연구회 2024 Dec.



CLDN18.2 in normal gastric cells

- Presents on the apical side of tight junctions, and not exposed to the cell surface.
- Maintains the barrier function of gastric mucosa.
- Prevents the leakage of H⁺ in gastric acid through paracellular pathways [38].

CLDN18.2 in gastric cancer

- Exposed to cell surface of whole membrane due to perturbations in cell polarity upon malignant transformation [16, 35].
- CLDN18 gene fusion with mainly ARHGAP in around 2% of patients [50, 52].

Zolbetuximab

- A first-in-class chimeric immunoglobulin G1 monoclonal antibody highly specific for CLDN18.2.
- Stimulates immune effectors to induce ADCC and CDC.

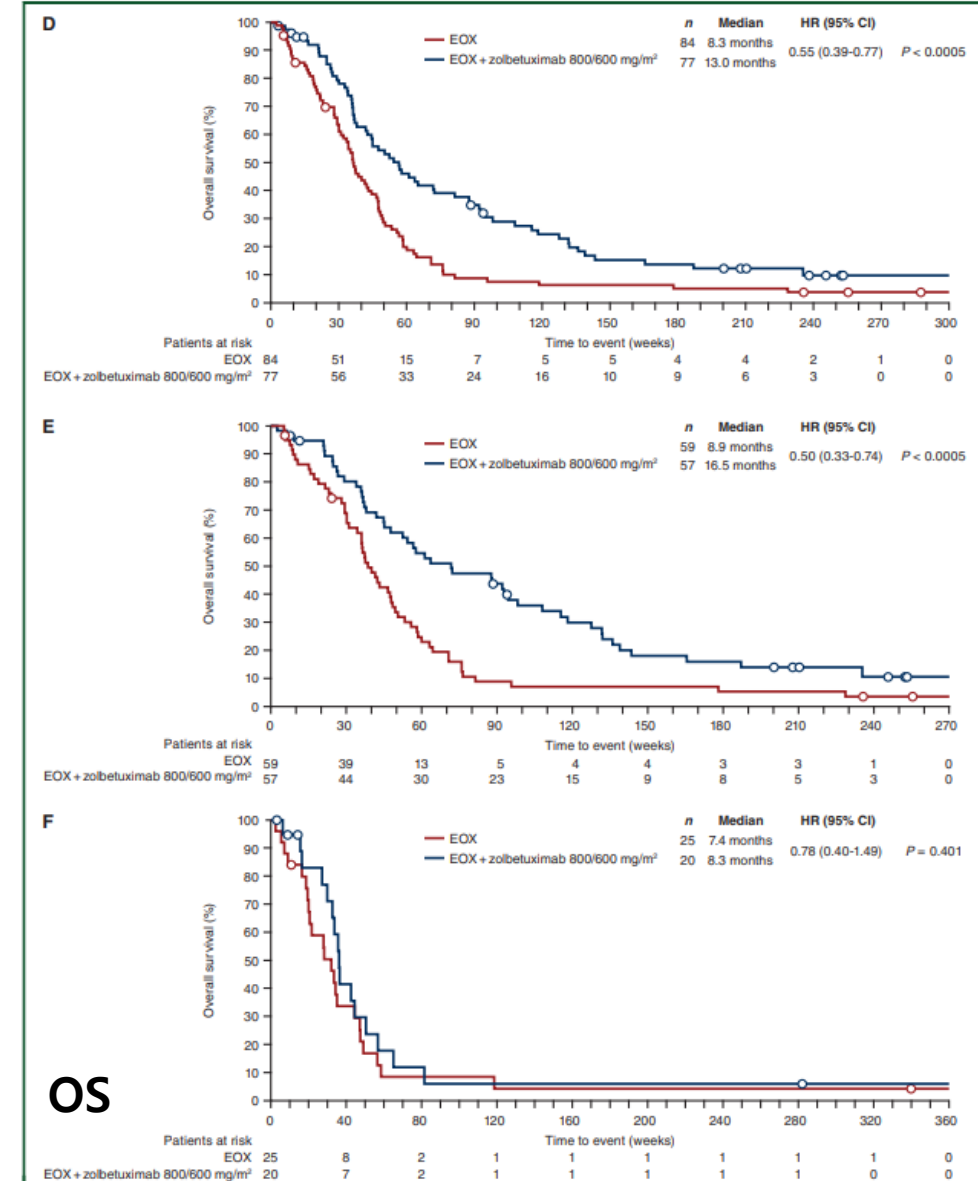
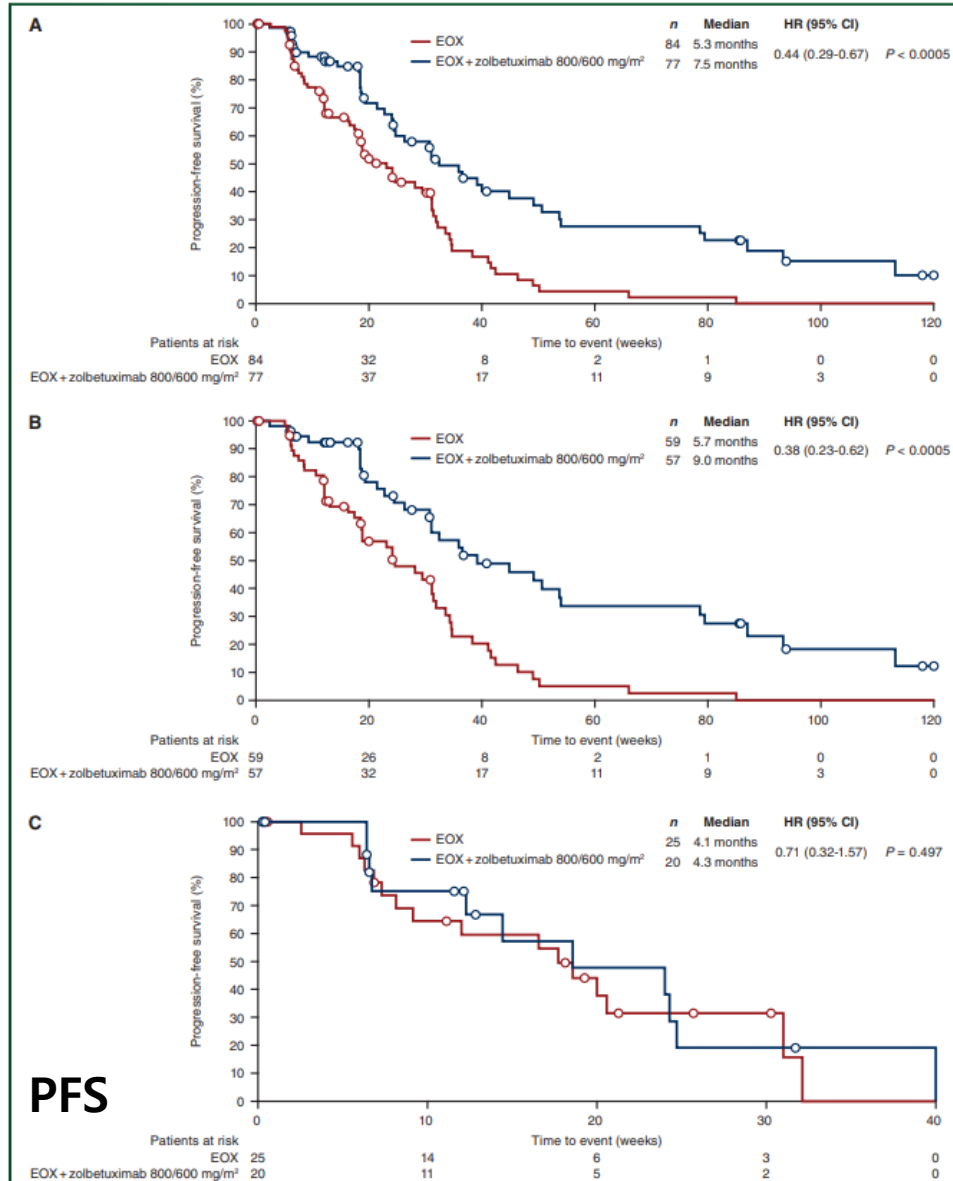
FAST trial. Ugur Sahin et al. *Ann Oncol* 2021 May;32(5):609-619.

A randomised phase II study of zolbetuximab plus EOX vs. EOX for 1st-line treatment of CLDN18.2-positive (>40%) AGC

Overall

>70%

40-70%





SPOTLIGHT and GLOW studies

Randomized phase 3 studies of Zolbetuximab + Chemo in Previously Untreated, CLDN18.2+, HER2-, LA Unresectable or mG/GEJ Adenocarcinoma

ADJUSTMENT OF CLDN18.2 POSITIVITY CUTOFF

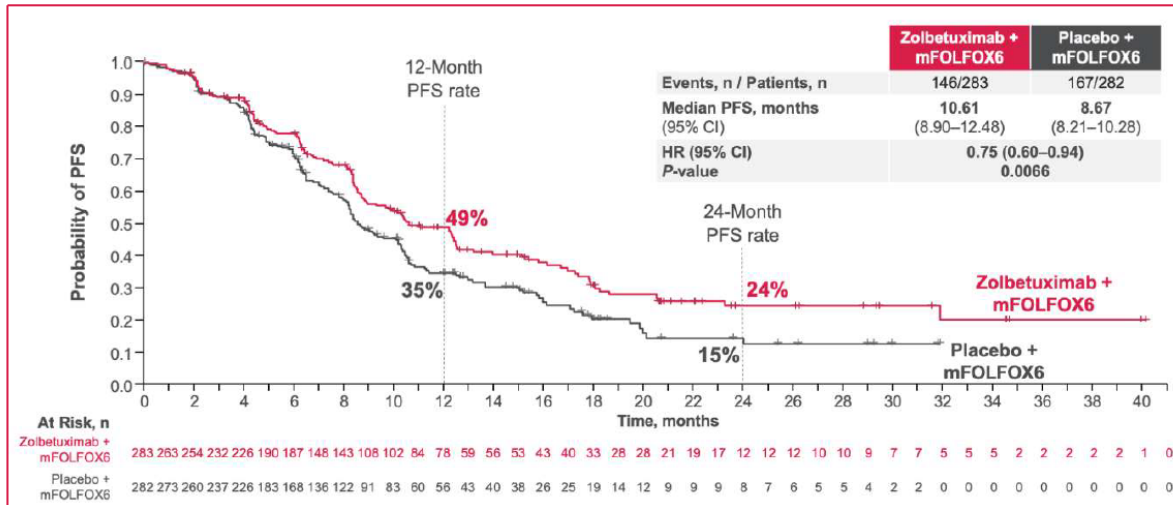
	FAST (Ph II study)	SPOTLIGHT/GLOW (Ph III studies)
CLDN18.2 positivity cutoff	$\geq 70\%$ (2+ or 3+ intensity)	$\geq 75\%$ (2+ or 3+ intensity)
IHC assay	Manual CLAUDETECT™ assay	VENTANA CLDN18[43-14A] RxDx Assay (Roche Diagnostic Solutions; Tucson, AZ, USA)

- This automatic CLDN18.2 IHC assay in SPOTLIGHT was changed from the manual CLAUDETECT™ assay used in FAST, which led to the **adjustment of CLDN18.2 positivity cutoff** from greater than or equal to 70% in FAST to greater than or equal to 75% in SPOTLIGHT **to identify a similar patient population.**

RESULTS: PFS

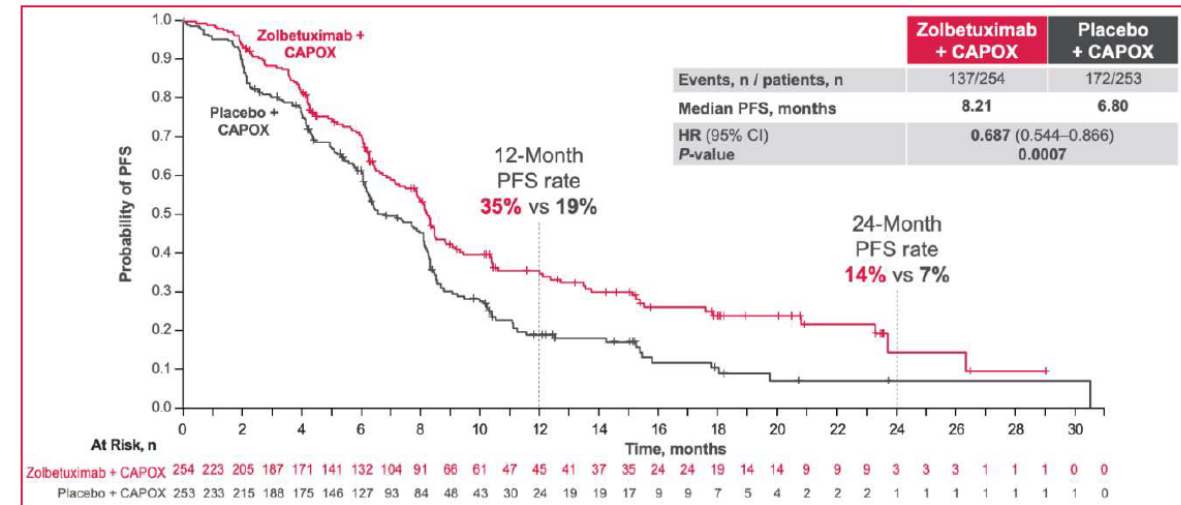
In both studies, PFS was significantly longer for patients treated with zolbetuximab + chemo vs. placebo + chemo.^{1,2}

SPOTLIGHT¹



Median follow-up: 12.94 months (zolbetuximab + mFOLFOX6) versus 12.65 months (placebo + mFOLFOX6).

GLOW²



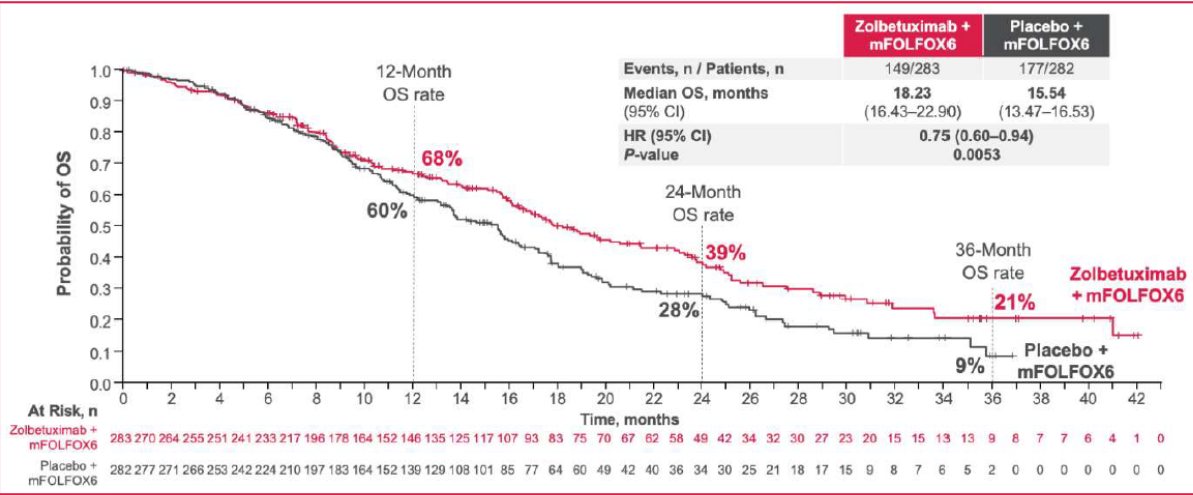
Median follow up = 12.62 months (zolbetuximab + CAPOX) versus 12.09 months (placebo + CAPOX).

The updated PFS analysis for SPOTLIGHT/GLOW continued to show a significant benefit for patients treated with zolbetuximab + chemo.^{3,4}

RESULTS: OS

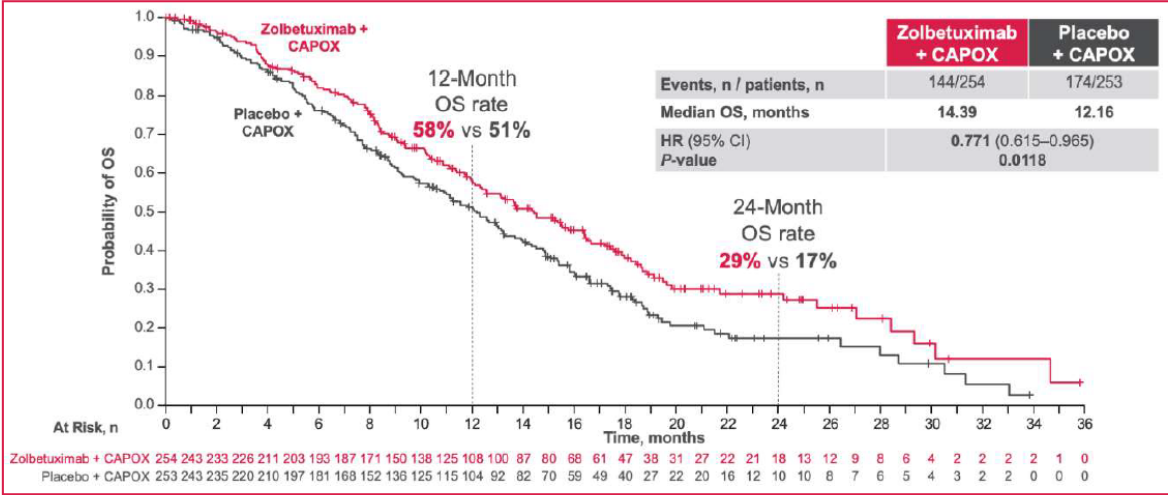
In both studies, OS was significantly longer for patients treated with zolbetuximab + chemo vs. placebo + chemo.^{1,2}

SPOTLIGHT¹



Median follow-up = 22.14 months (zolbetuximab + mFOLFOX6) versus 20.93 months (placebo + mFOLFOX6).

GLOW²



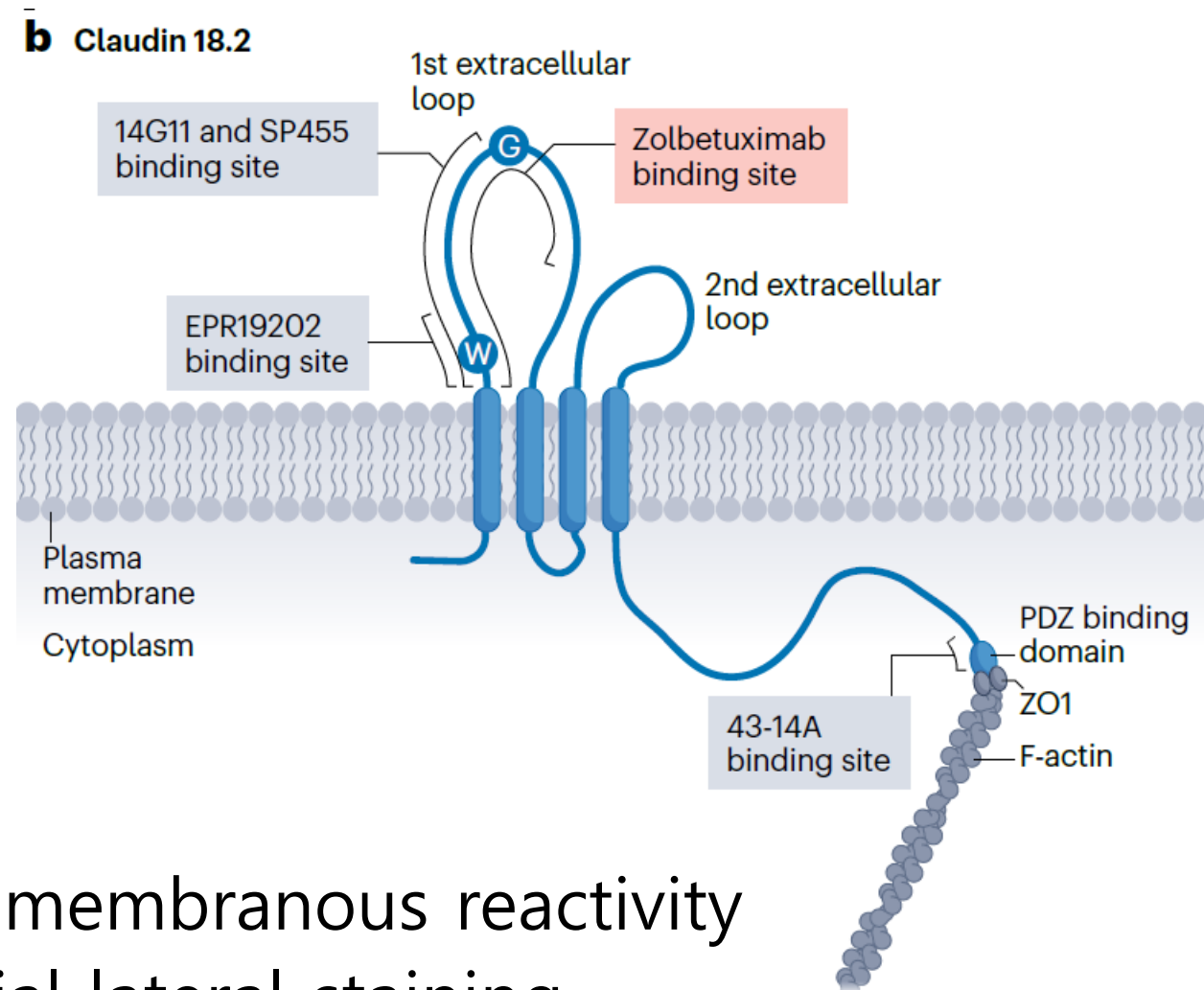
Median follow up = 17.71 months (zolbetuximab + CAPOX) vs 18.43 months (placebo + CAPOX).

The updated OS analysis for SPOTLIGHT/GLOW continued to show a significant benefit for patients treated with zolbetuximab + chemo.^{3,4}

CAPOX capecitabine + oxaliplatin regimen; CI confidence interval; HR hazard ratio; mFOLFOX6 modified folinic acid + 5-fluorouracil + oxaliplatin regimen; OS Overall Survival
1. Shitara K et al. *Lancet*. 2023; 401: 1655-1668. 2. Shah MA et al. *Nat Med*. 2023; 29: 2133–2141. 3. Ajani JA et al. ESMO 2023; Abstract LBA82. 4. Lordick F et al. ESMO 2023; Abstract LBA81.

CLDN18.2 IHC methods & interpretation

- Claudin 18.2 specific
 - Clone **EPR19202, Abcam**
 - Clone SP455
- Pan Claudin 18
 - Clone **43-14A, Ventana**
 - **SPOTLIGHT, GLOW** trials
 - Clone 34H14L15, Invitrogen
 - polyclonal, Thermo Fisher
- **$\geq 75\%$** in tumor cells
- **Moderate (2+) to strong (3+)** membranous reactivity
- Complete, basolateral, or partial lateral staining



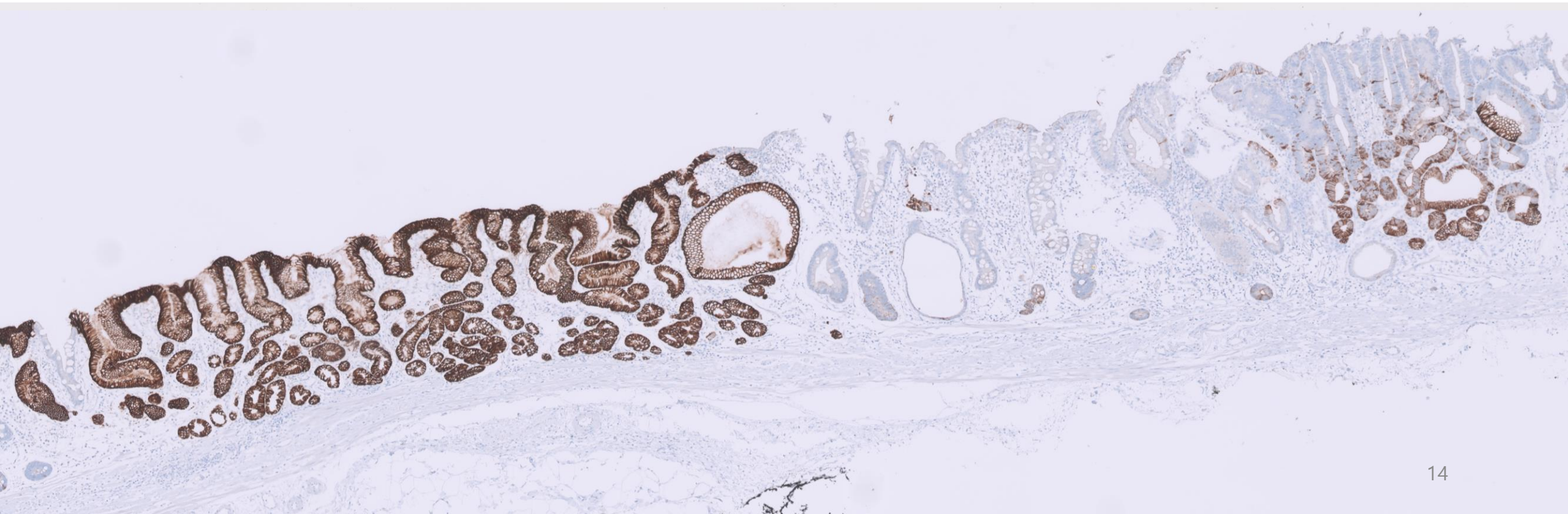
Positive & negative controls

Positive control

– Normal gastric mucosa

Negative control

– Intestinal metaplasia

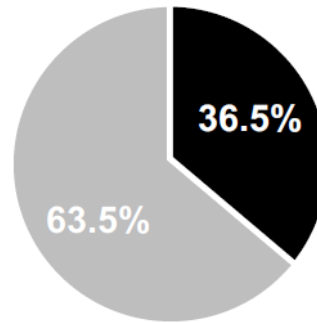


Global CLDN18.2-positivity in GC/GEJ cancer

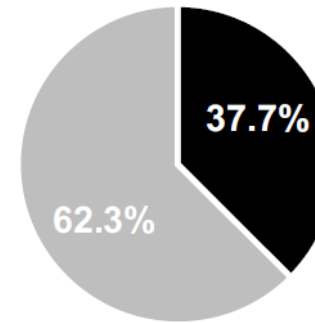
- **38.4%**
(1,730/4,507)

- SPOTLIGHT
- GLOW

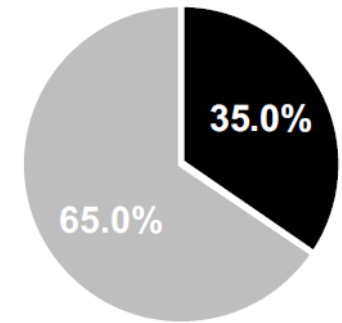
Asia Pacific
Excluding Mainland China
n = 1314



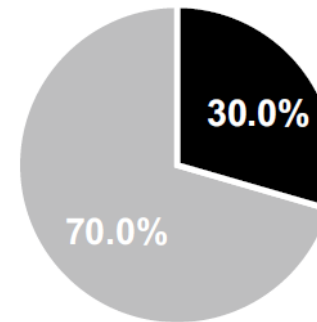
North America
n = 485



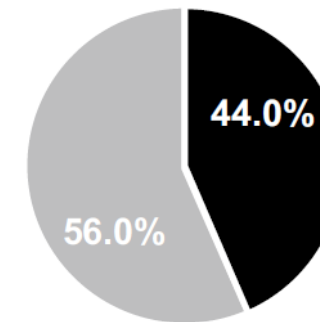
Mainland China
n = 844



South America
n = 340

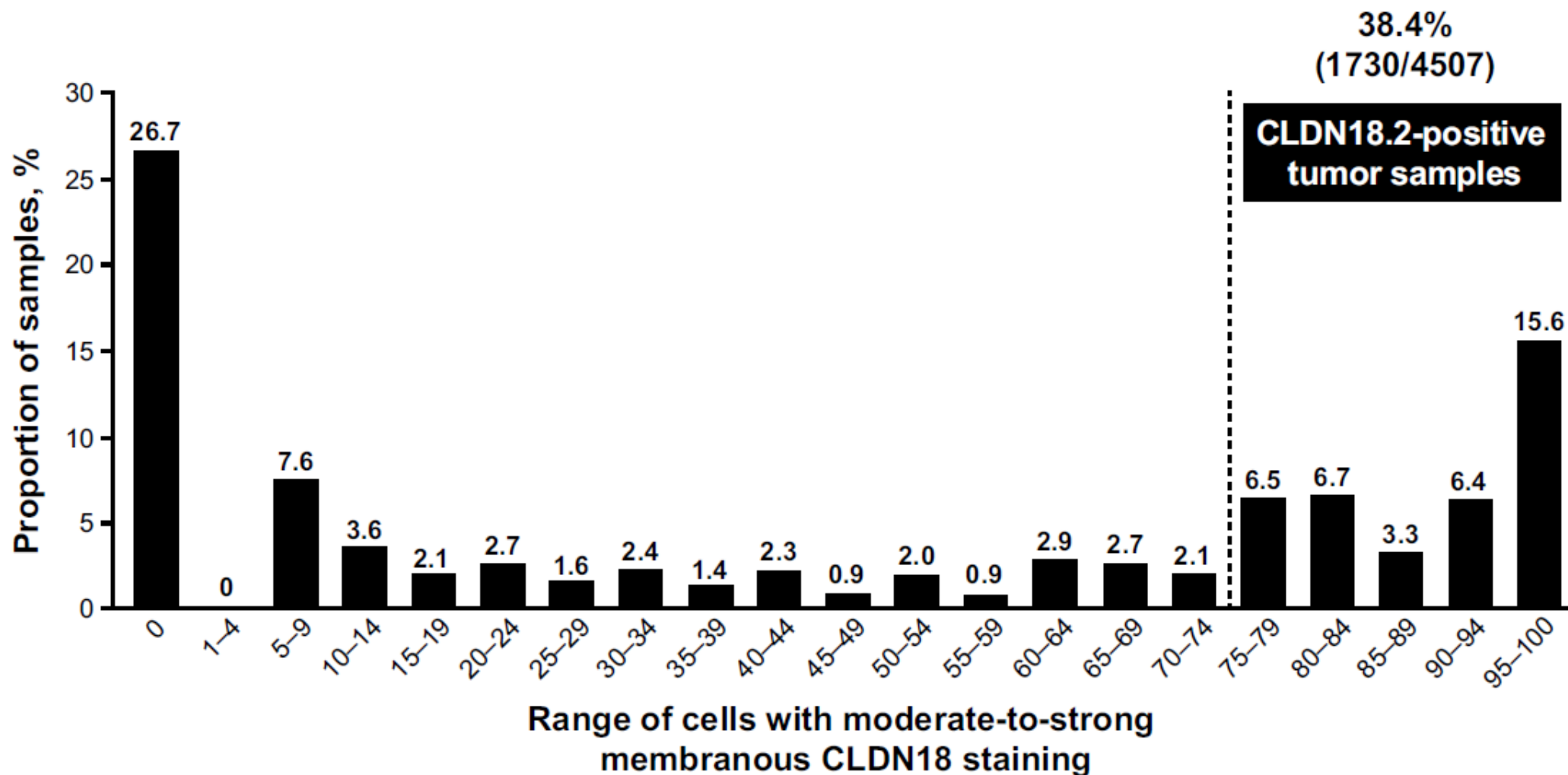


Europe/Middle East
n = 1524



■ CLDN18.2-positive ■ CLDN18.2-negative

Distribution of CLDN18.2 expression (Glow & Spotlight)

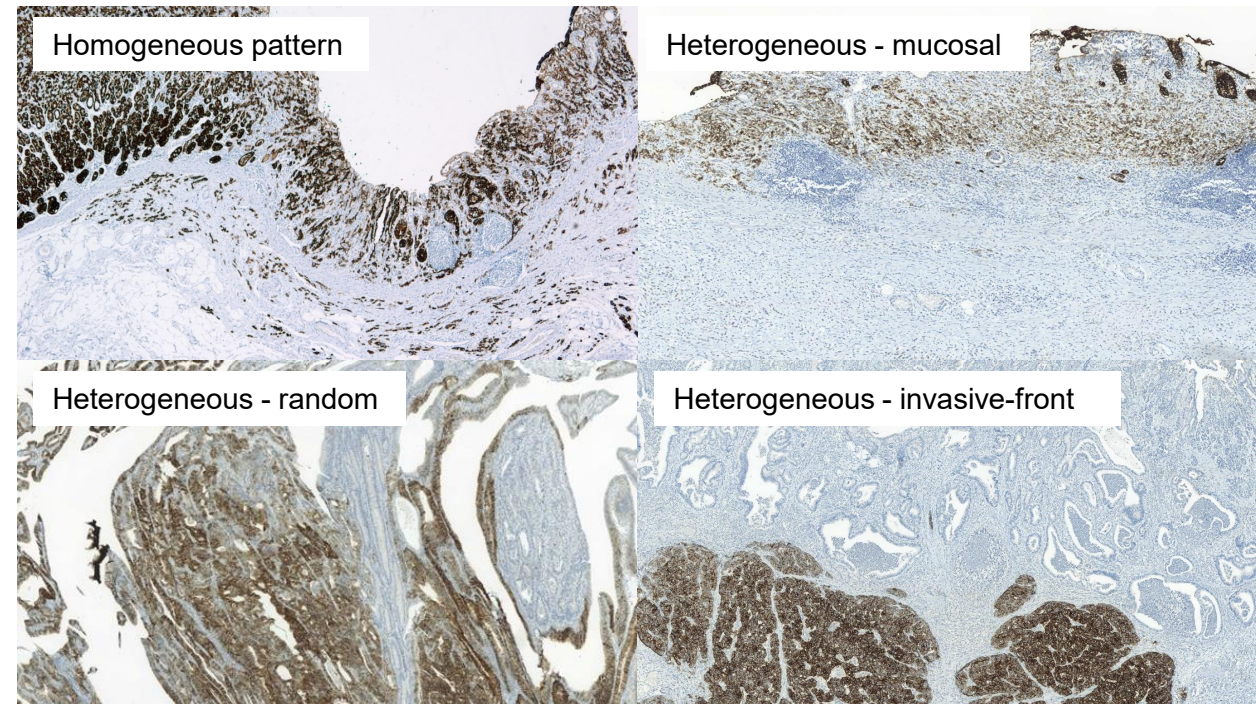


CLDN18.2 in **resectable GCs**

- **299** patients, AMC
 - Stage I (90), II (96) & III (113)
 - Curative surgical resection (2018 Mar ~ 2019 Feb)
- **43-14A** IHC with 75% cut-off
- CLDN18.2 positive GC
 - **46.5%**
 - Younger age
 - Lower invasion depth
 - Less lymphovascular invasion
 - **Borrmann type 4**
 - **Diffuse type**
 - **HER2 negative, EBV positive**
 - No prognostic significance

CLDN18.2 heterogeneity

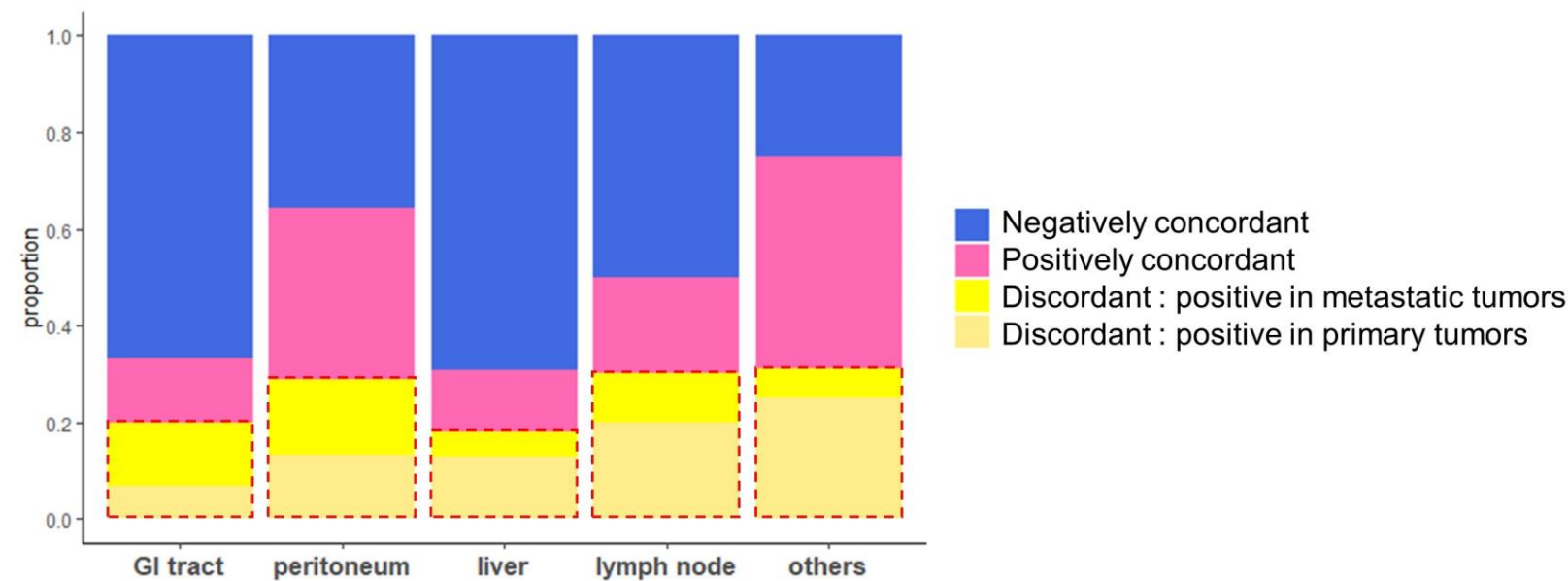
- Within claudin 18.2 positivity ($\geq 75\%$, i.e. **75 ~ 90% positive**)
 - **31%** (43/139 out of 299)
 - **Random type most common** (n=40)
- More in CLDN negative cases (i.e. $< 75\%$)
- Previous reports: 40 ~ 50%



CLDN18.2 in **stage IV GCs**

- **166** patients, AMC
 - 39 gastrectomy cases
 - 135 patients with primary – metastatic site pairs
- **43-14A** IHC with 75% cut-off
- CLDN18.2 positive GC
 - **47.0%**
 - **Peritoneal meta** > hepatic & distant LN meta
 - Intratumoral heterogeneity in **38.0%** of surgical specimens
 - Primary – metastatic discrepancy in **25.2%**
 - No prognostic significance

Figure 4.



	GI tract (n=15)	peritoneum (n=45)	liver (n=39)	lymph node (n=20)	Others (n=16)
Negatively concordant	10 (66.7)	16 (35.6)	27 (69.2)	10 (50.0)	4 (25.0)
Positively concordant	2 (13.3)	16 (35.6)	5 (12.8)	4 (20.0)	7 (43.8)
Discordant: positive in metastatic tumors	2 (13.3)	7 (15.6)	2 (5.1)	2 (10.0)	1 (6.2)
Discordant: positive in primary tumors	1 (6.7)	6 (13.3)	5 (12.8)	4 (20.0)	4 (25.0)

CLDN18.2 & other biomarkers

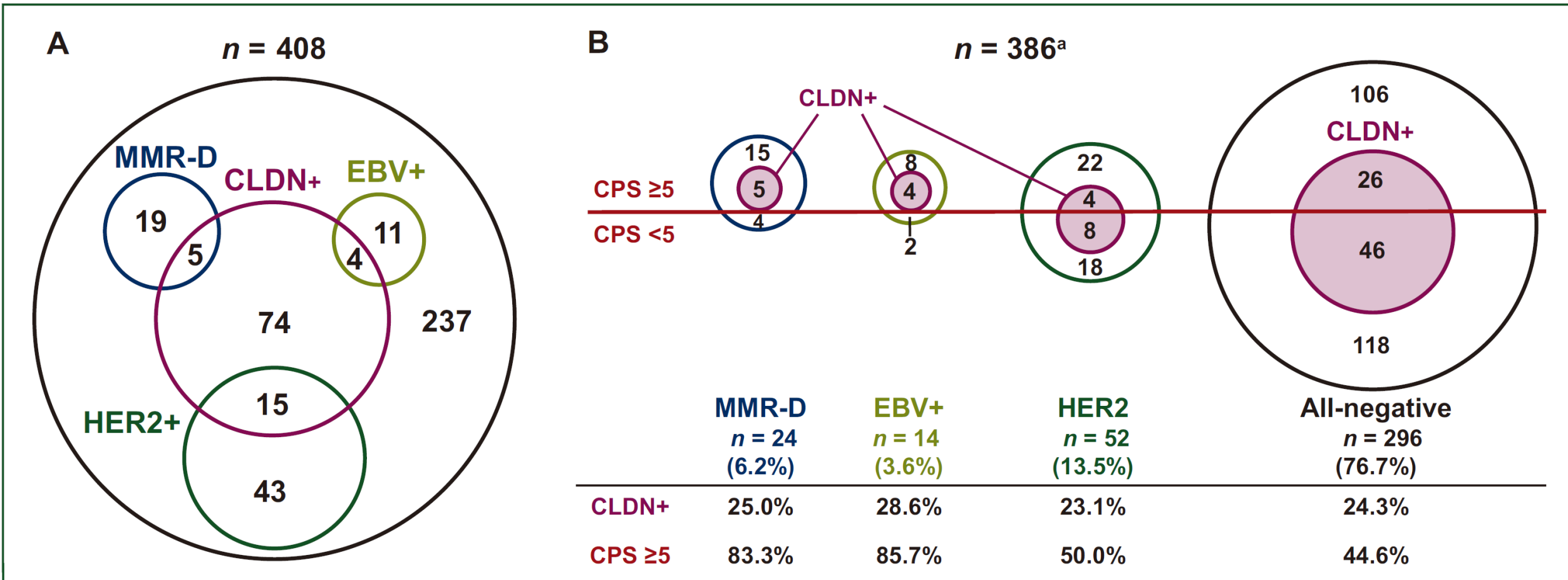
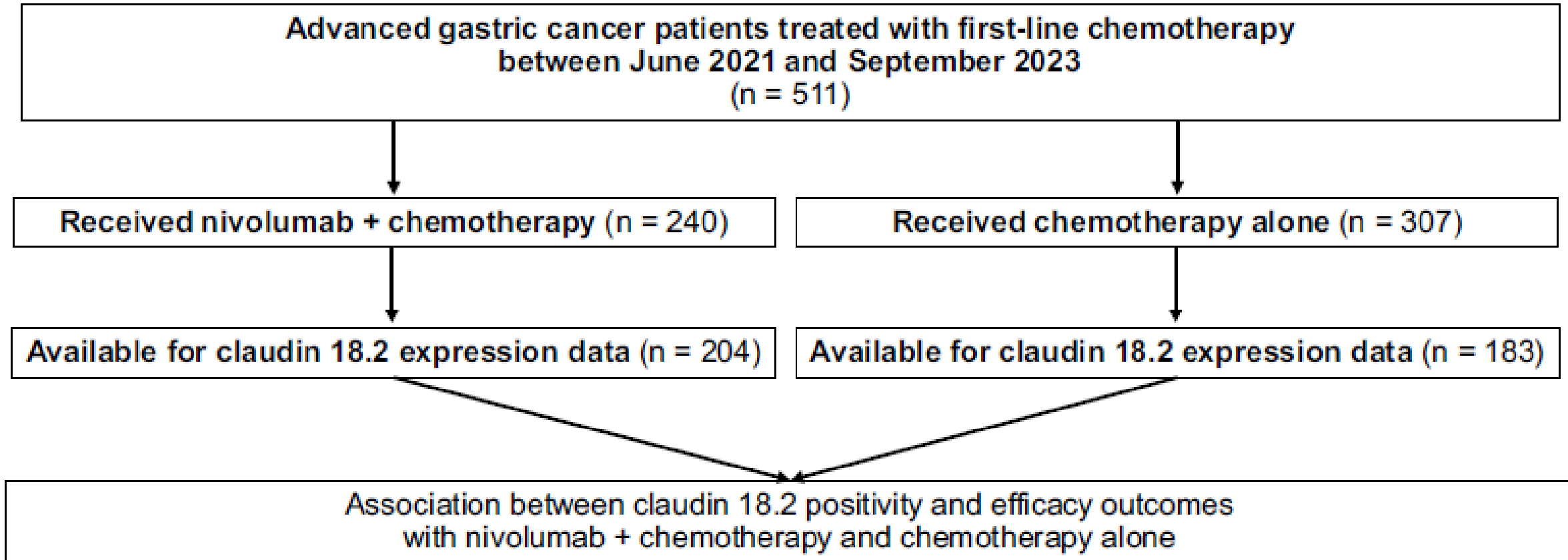


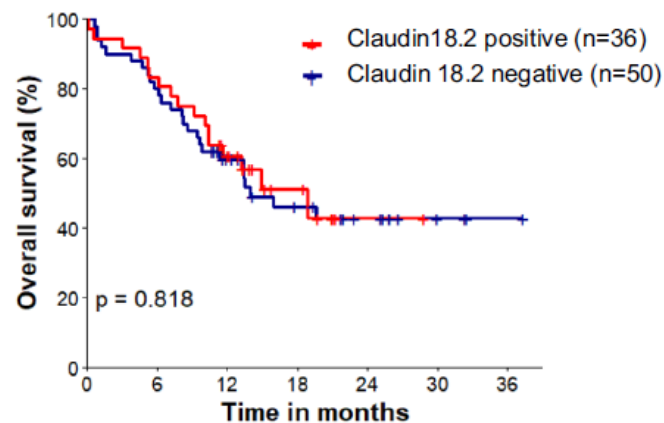
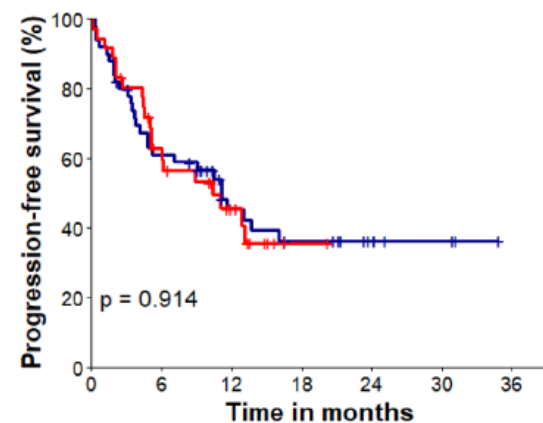
Figure 2. Relationship between CLDN and other biomarkers (A) and PD-L1 CPS (B). All-negative: negative for neither MMR-D, EBV nor HER2. CLDN, claudin; CPS, combined positive score; EBV, Epstein–Barr virus; HER2, human epidermal growth factor receptor 2; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient.

^aPatients with available CPS results.

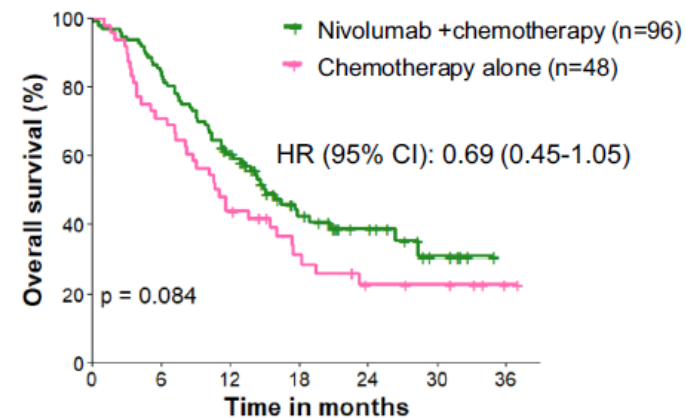
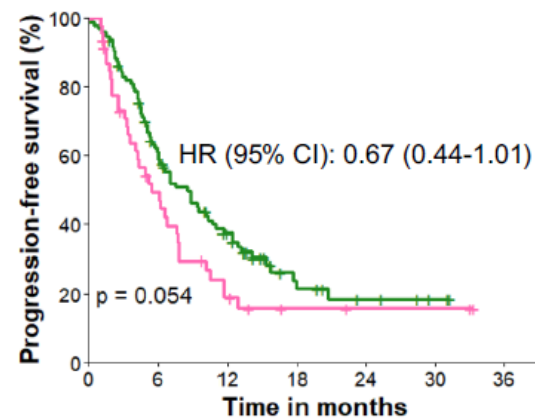
CLDN18.2 & Nivolumab treatment



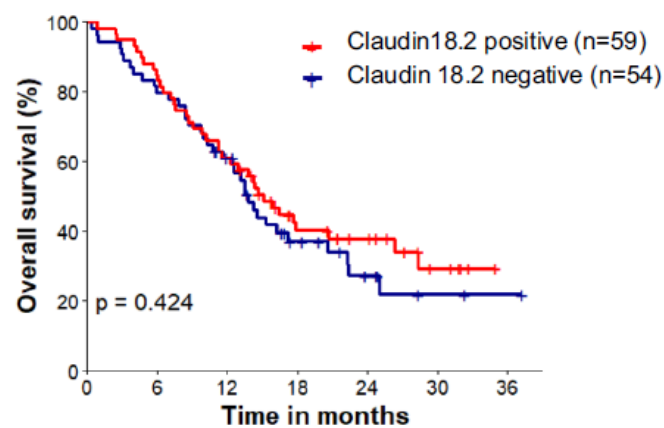
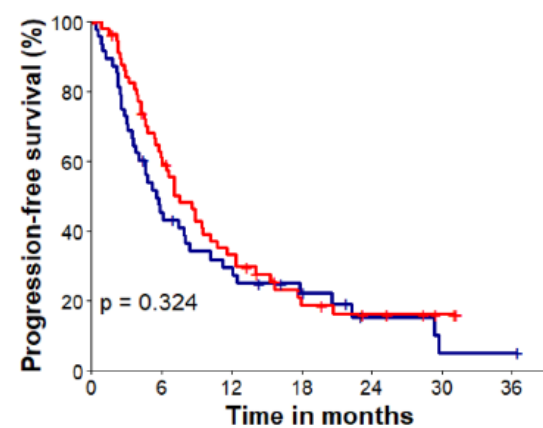
PD-L1 CPS ≥ 5 (treated with nivolumab plus chemotherapy)



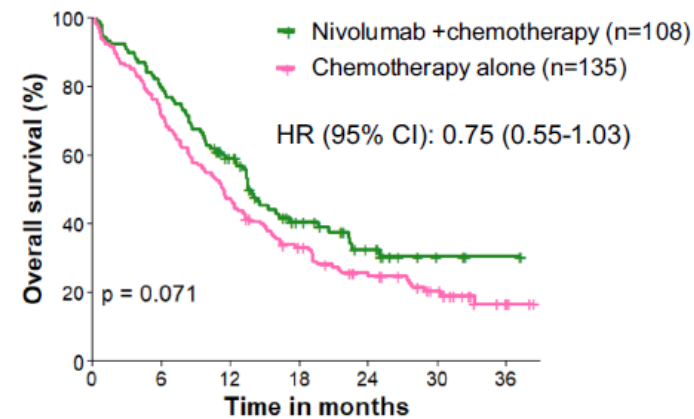
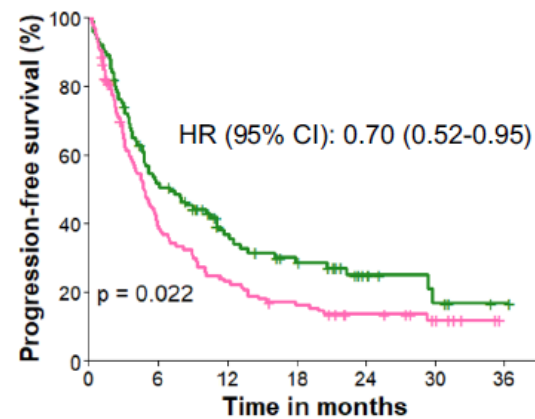
Claudin 18.2 positive



PD-L1 CPS < 5 (treated with nivolumab plus chemotherapy)



Claudin 18.2 negative

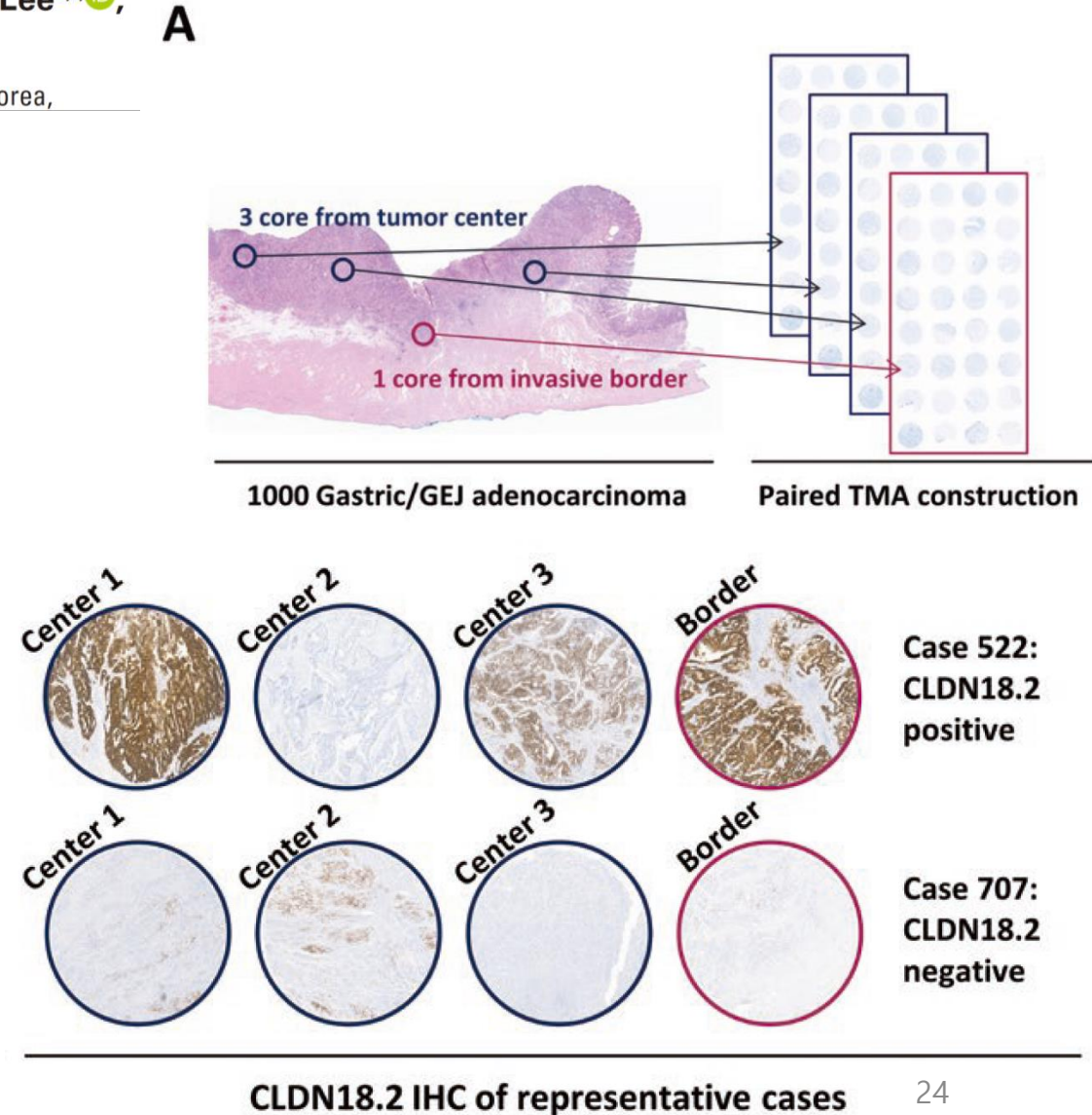


Clinicopathologic and molecular characterization of stages II-IV gastric cancer with Claudin 18.2 expression

Yoonjin Kwak^{1,‡}, Tae-Yong Kim^{2,‡}, Soo Kyung Nam³, Hye Jung Hwang¹, Daeyoung Han⁴,
Hyeon Jeong Oh⁵, Seong-Ho Kong⁶, Do Joong Park^{6,7}, Do-Youn Oh^{2,3,7}, Hyuk-Joon Lee^{6,7} ,
Seock-Ah Im^{2,3,7}, Han-Kwang Yang^{6,7} , Hye Seung Lee^{*,1,3,7} 

¹Department of Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea,

- **1000** gastrectomy cases, SNU
- Stage II-IV
- TMAs, 4 cores from each cases
- CLDN18 (43-13A), PD-L1 (22C3), HER2, FGFR2, EBV
- **34.4%** positive
 - Upper & middle third
 - **EBV, PD-L1 positive**
 - **HER2 negative**
 - No prognostic significance

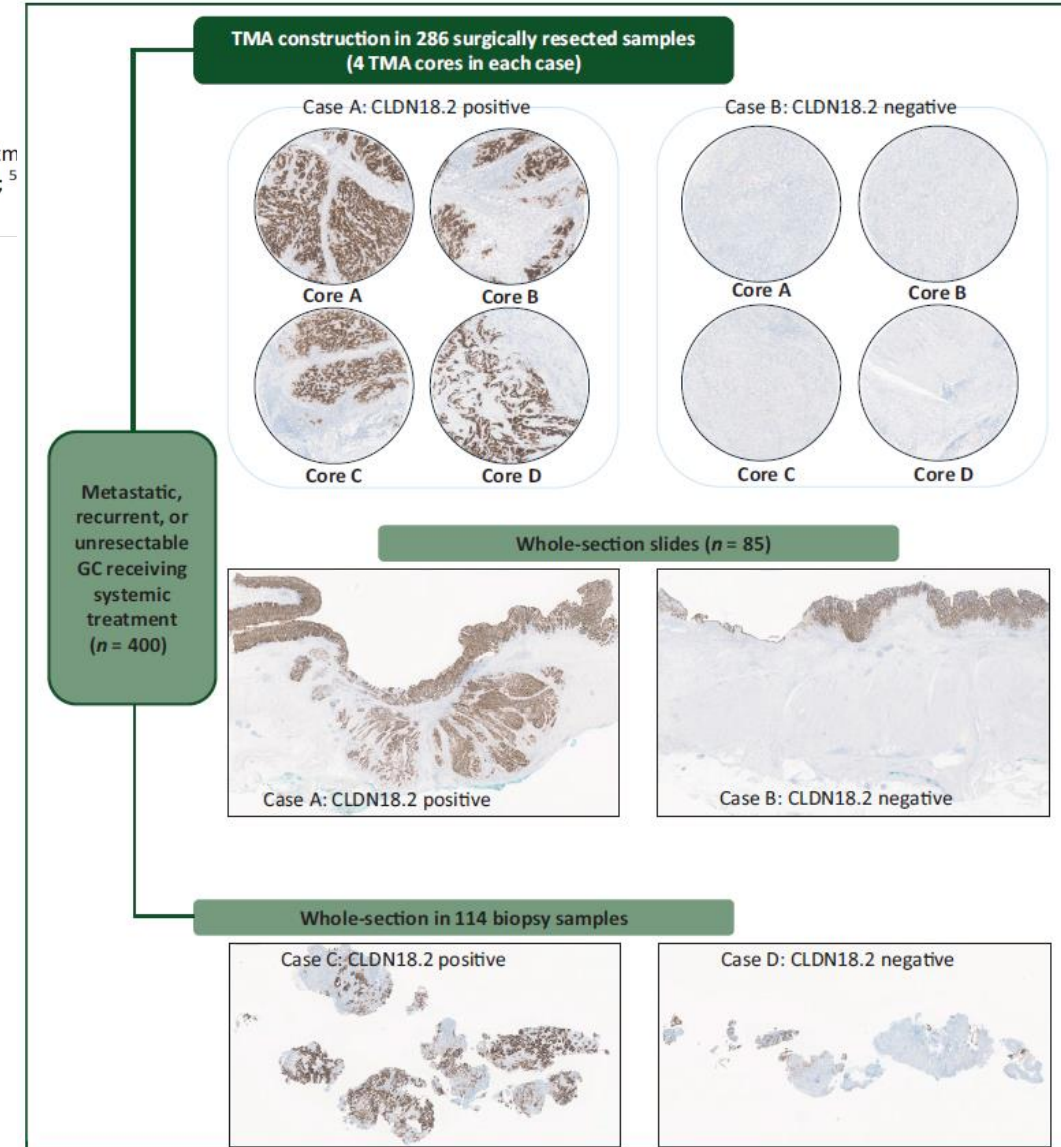


Clinicopathological analysis of claudin 18.2 focusing on intratumoral heterogeneity and survival in patients with metastatic or unresectable gastric cancer

T.-Y. Kim^{1†}, Y. Kwak^{2†}, S. K. Nam³, D. Han⁴, D.-Y. Oh^{1,3,5}, S.-A. Im^{1,3,5} & H. S. Lee^{2,3,5*}

Departments of ¹Internal Medicine; ²Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul; ³Department Interdisciplinary Program in Cancer Biology, Seoul National University College of Medicine, Seoul; ⁴Medical Affairs, Astellas Pharma Korea Inc., Seoul; ⁵Institute, Seoul National University College of Medicine, Seoul, Korea

- **400** gastrectomy cases, SNU
- Unresectable, metastatic, recurrent
- 286 TMAs & 114 bx whole-section
- CLDN18 (43-13A), PD-L1, HER2, FGFR2, EBV, MSI, CD3, CD4, FoxP3
- **31.3%** positive
 - Upper & middle third
 - **EBV, PD-L1 positive**
 - **HER2 negative**
 - No prognostic significance

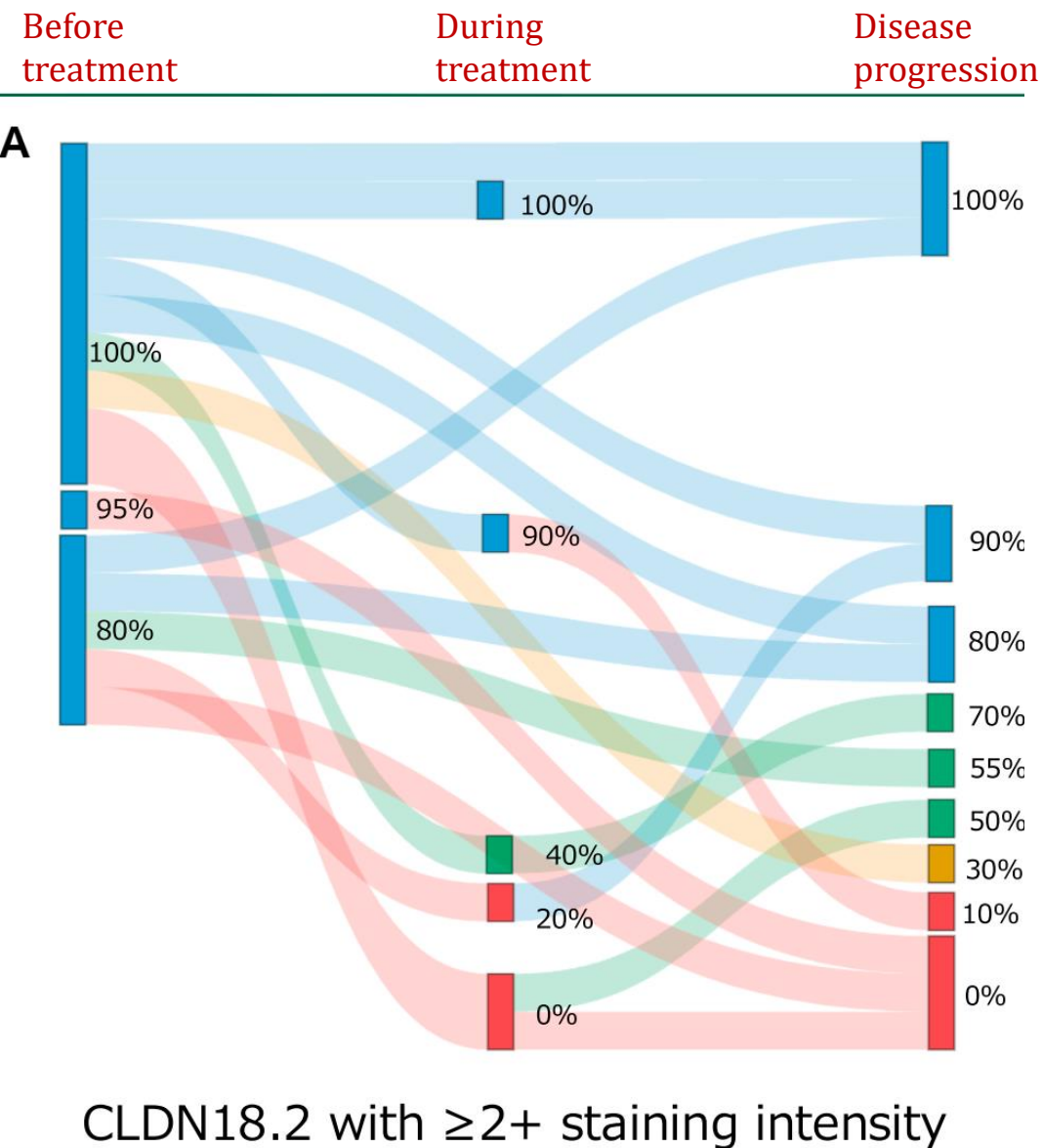


Temporal dynamics of CLDN18.2 expression following zolbetuximab treatment in advanced gastric cancer

K. Yamamoto¹, I. Nakayama^{1*}, N. Sakamoto^{2,3*}, Y. Matsubara¹, Y. Miyashita¹, A. Kobayashi¹, U. Okazaki¹, D. Okemoto¹, K. Seguchi¹, T. Hosokai¹, T. Ogura¹, S. Mishima¹, D. Kotani¹, A. Kawazoe¹, T. Hashimoto^{1,4}, Y. Kuboki^{1,5}, H. Bando¹, T. Kojima¹, T. Yoshino¹, T. Kuwata² & K. Shitara^{1,6}

Departments of ¹Gastroenterology and Gastrointestinal Oncology; ²Pathology and Clinical Laboratories, National Cancer Center Hospital East, Kashiwa, C; ³Division of Pathology, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, Kashiwa, Chiba; ⁴Translational Research Support S; National Cancer Center Hospital East, Kashiwa, Chiba; ⁵Department of Experimental Therapeutics, National Cancer Center Hospital East, Kashiwa, Chiba; ⁶Department of Precision Medicine, Kindai University Faculty of Medicine, Osaka-Sayama, Osaka, Japan

- **65** cases, NCC Japan
 - 1L Zolbetuximab
 - **29** disease progression, **15** bx
- At disease progression, **53.3%** converted to CLDN18.2 negative
- CLDN18.2 $\geq 2+$ retained
 - $\geq 75\%$ in 7/15 (**46.7%**)
 - $\geq 40\%$ in 10/15 (**66.7%**)
 - $\geq 25\%$ in 11/15 (**73.3%**)
- Prior CTx (non-target) studies
 - CLDN18.2 reduction in **40-60%**

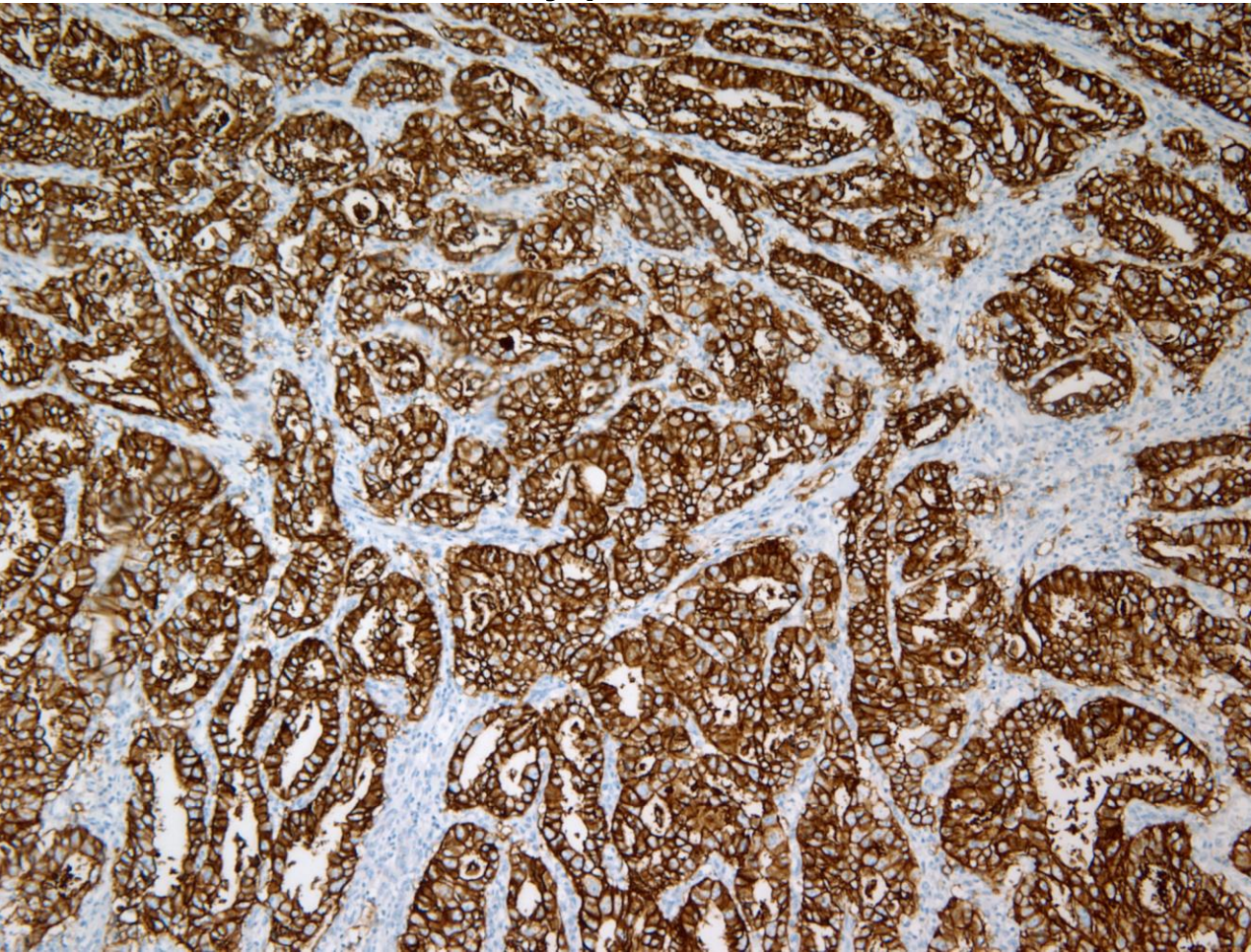


Pitfalls in interpretation

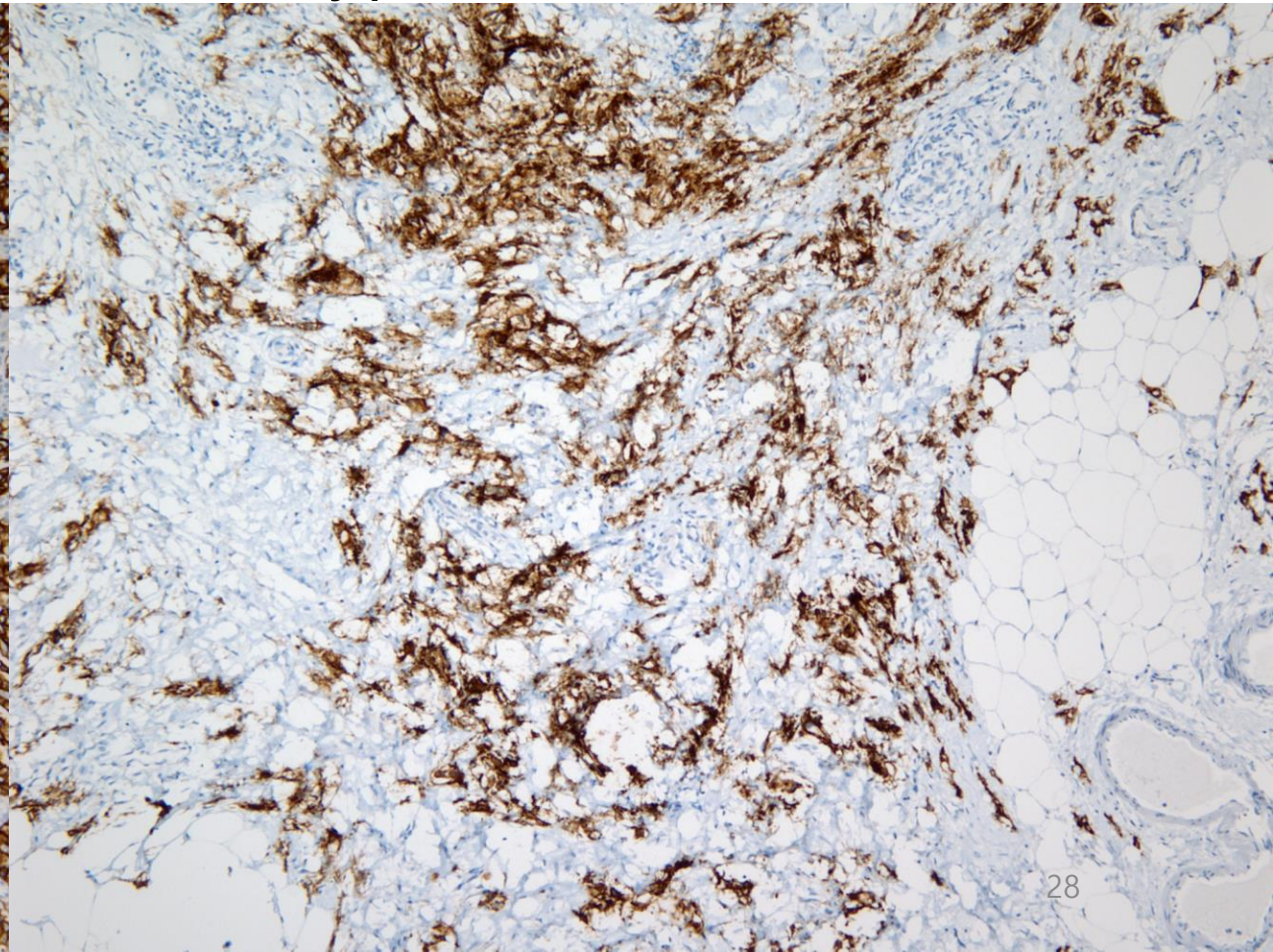
- Membranous vs cytoplasmic
 - Strong membranous staining tends to overstain cytoplasm
 - Especially in **diffuse type** cancers
- Intensity decision **1+** vs **2+**
 - Use Her2 IHC intensity criteria
 - **1+** if perceptible at **x200** or higher magnification
 - **2+** if perceptible at **x100** or lower magnification
- Estimating positive cell proportion
 - Especially in **diffuse type** cancers
 - Estimation of total cancer cells is difficult with counterstaining

Membranous vs cytoplasmic

Intestinal type

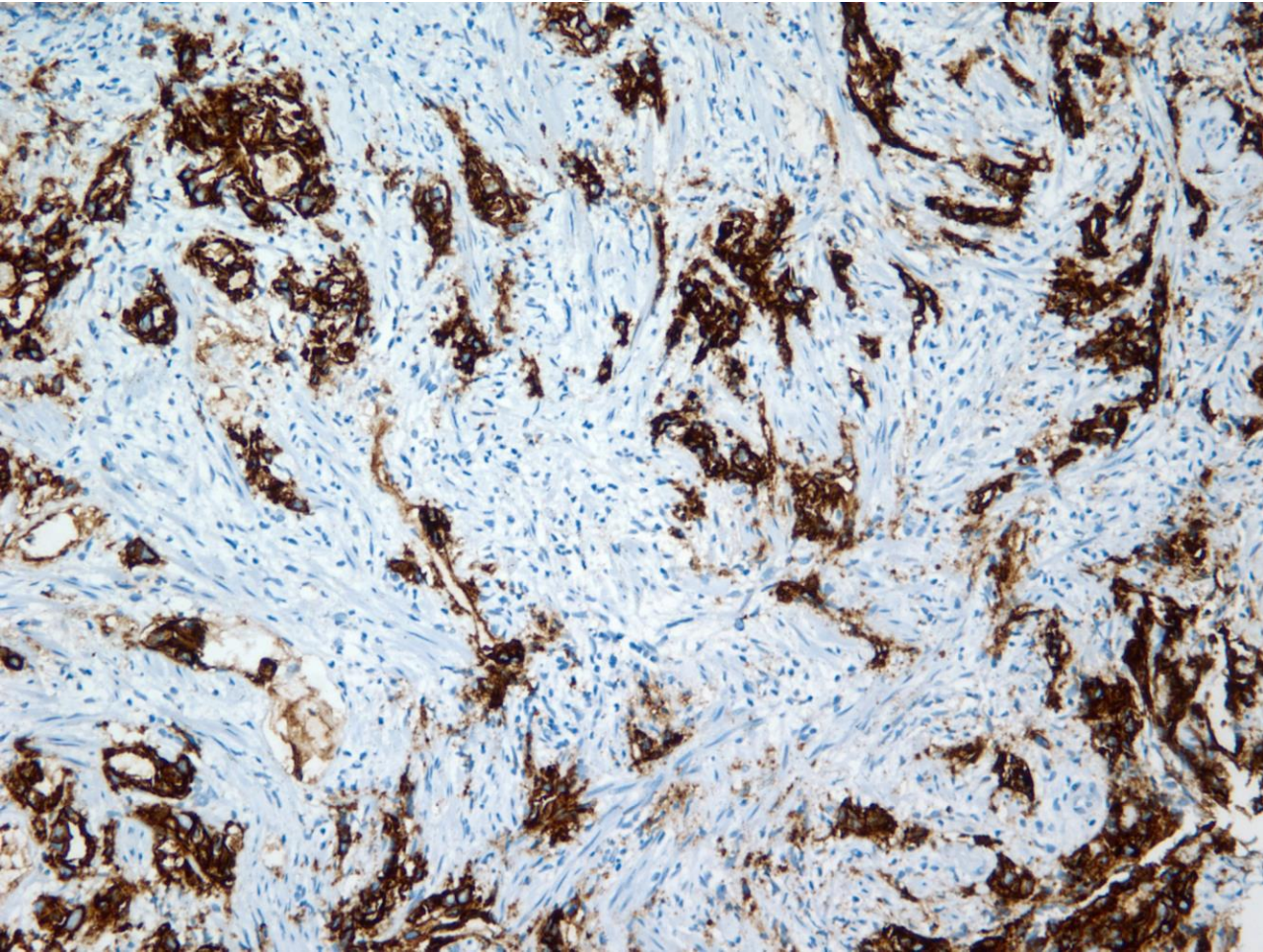


Diffuse type (frozen FFPE)

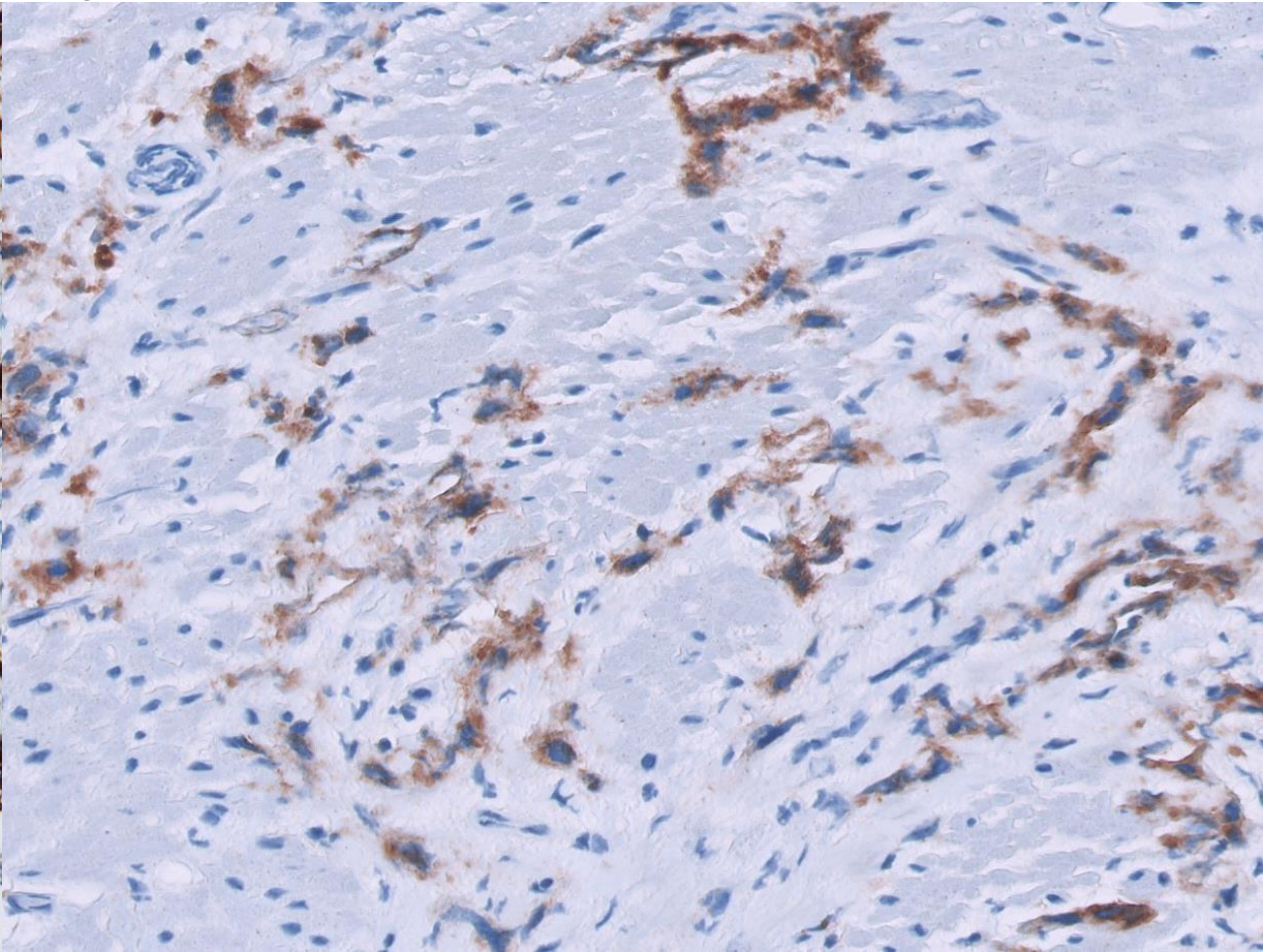


Membranous vs cytoplasmic

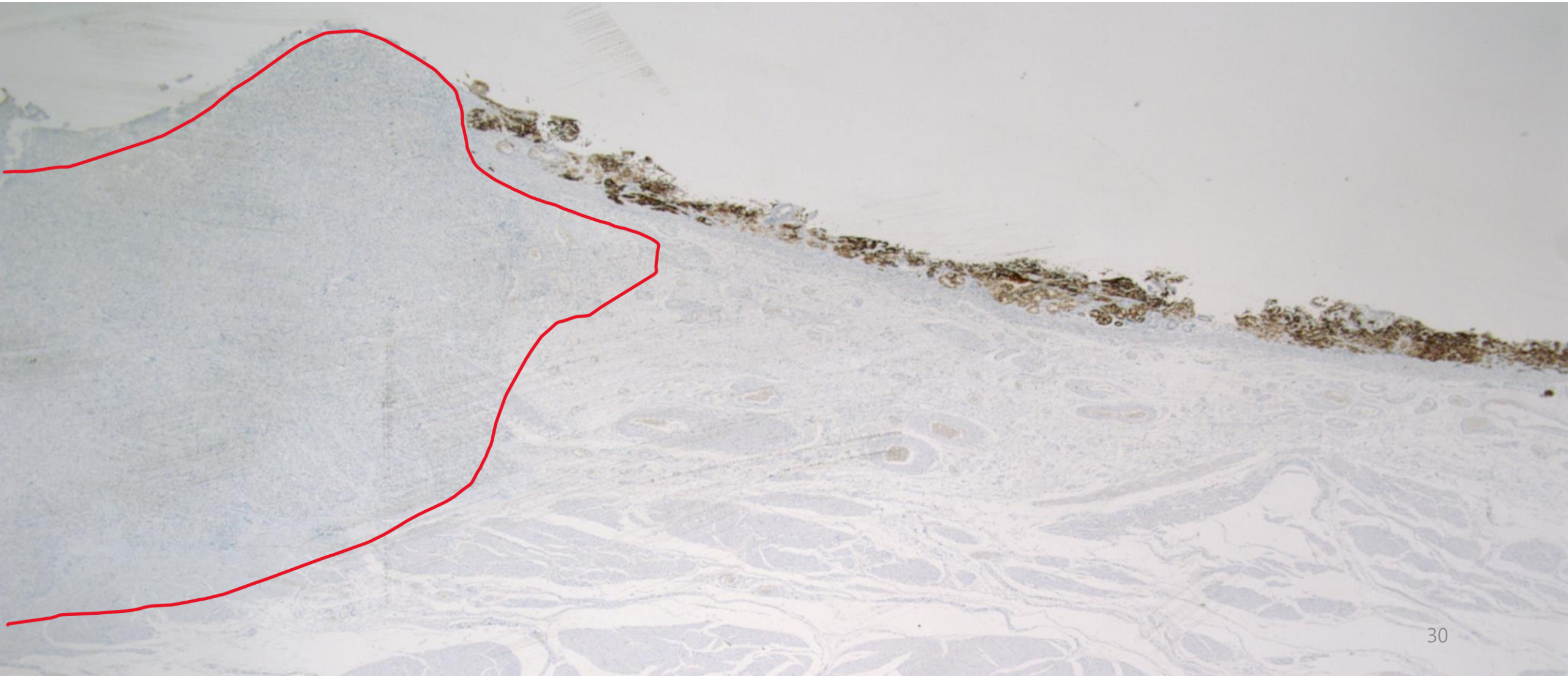
Overstaining (x200)



Cytoplasmic (x400)

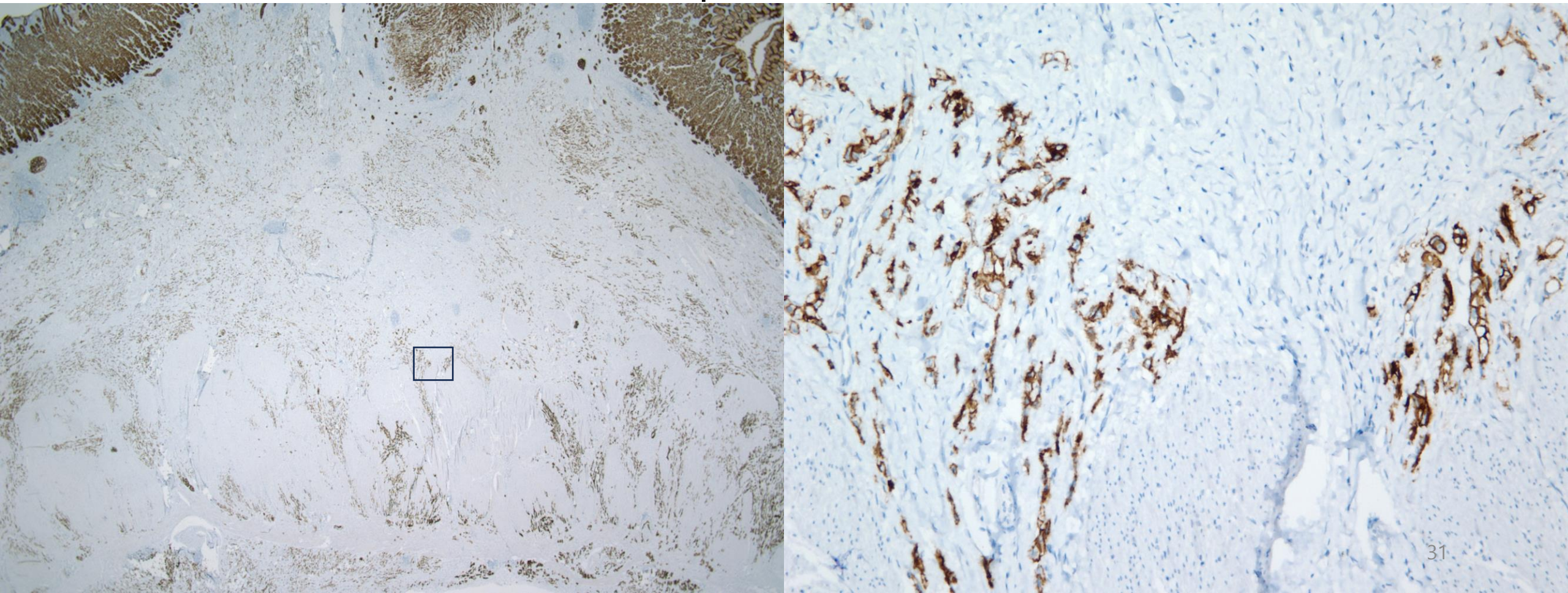


Estimating positive cell proportion



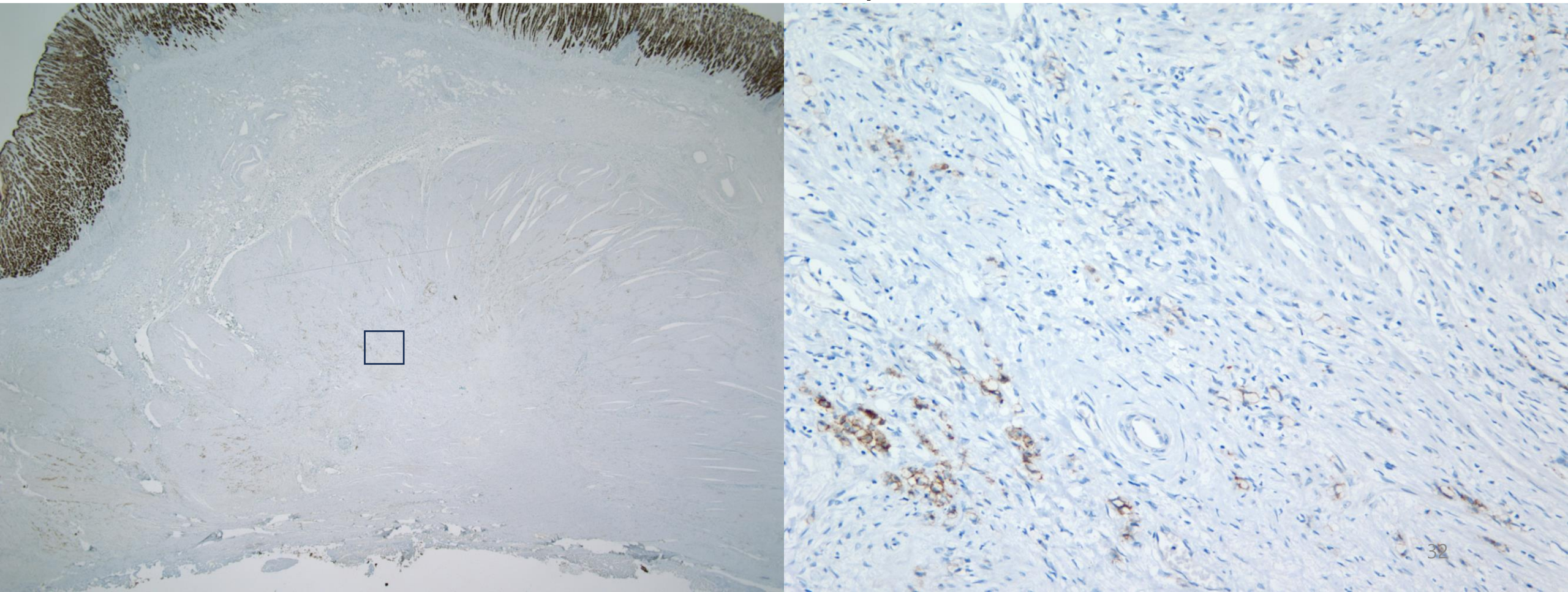
Estimating positive cell proportion

- Almost all tumor cells are positive

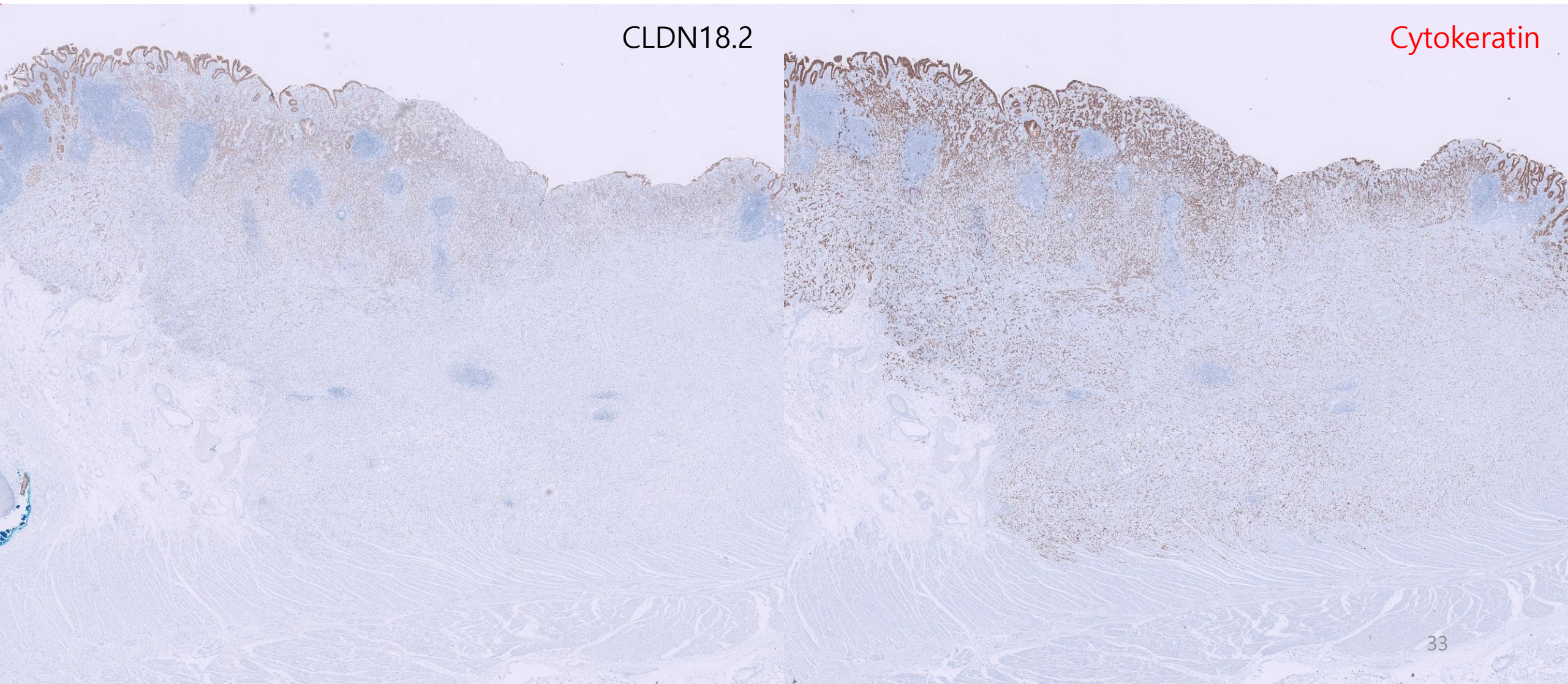


Estimating positive cell proportion

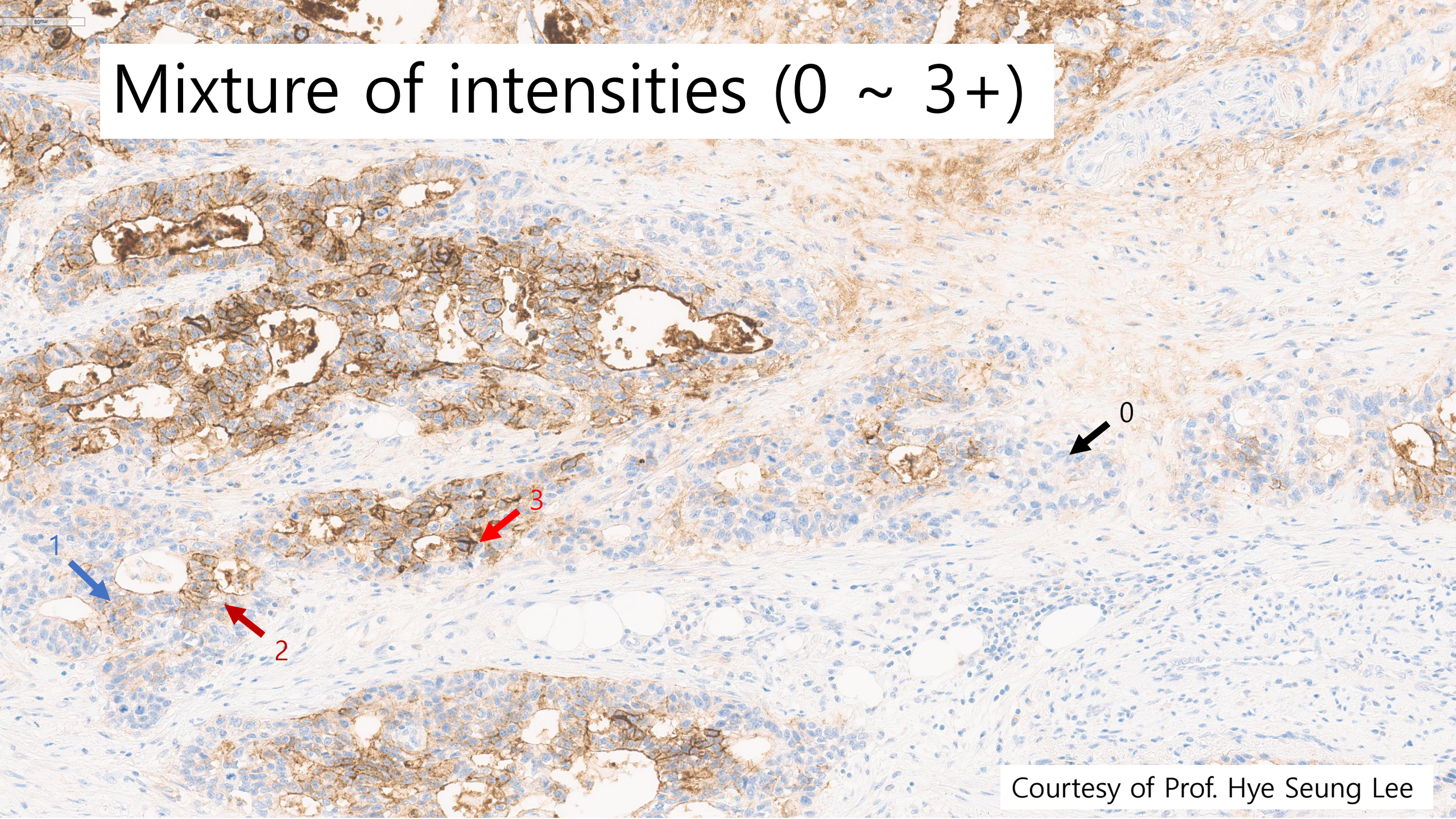
- About 50% of the tumor cells are positive



Estimating positive cell proportion

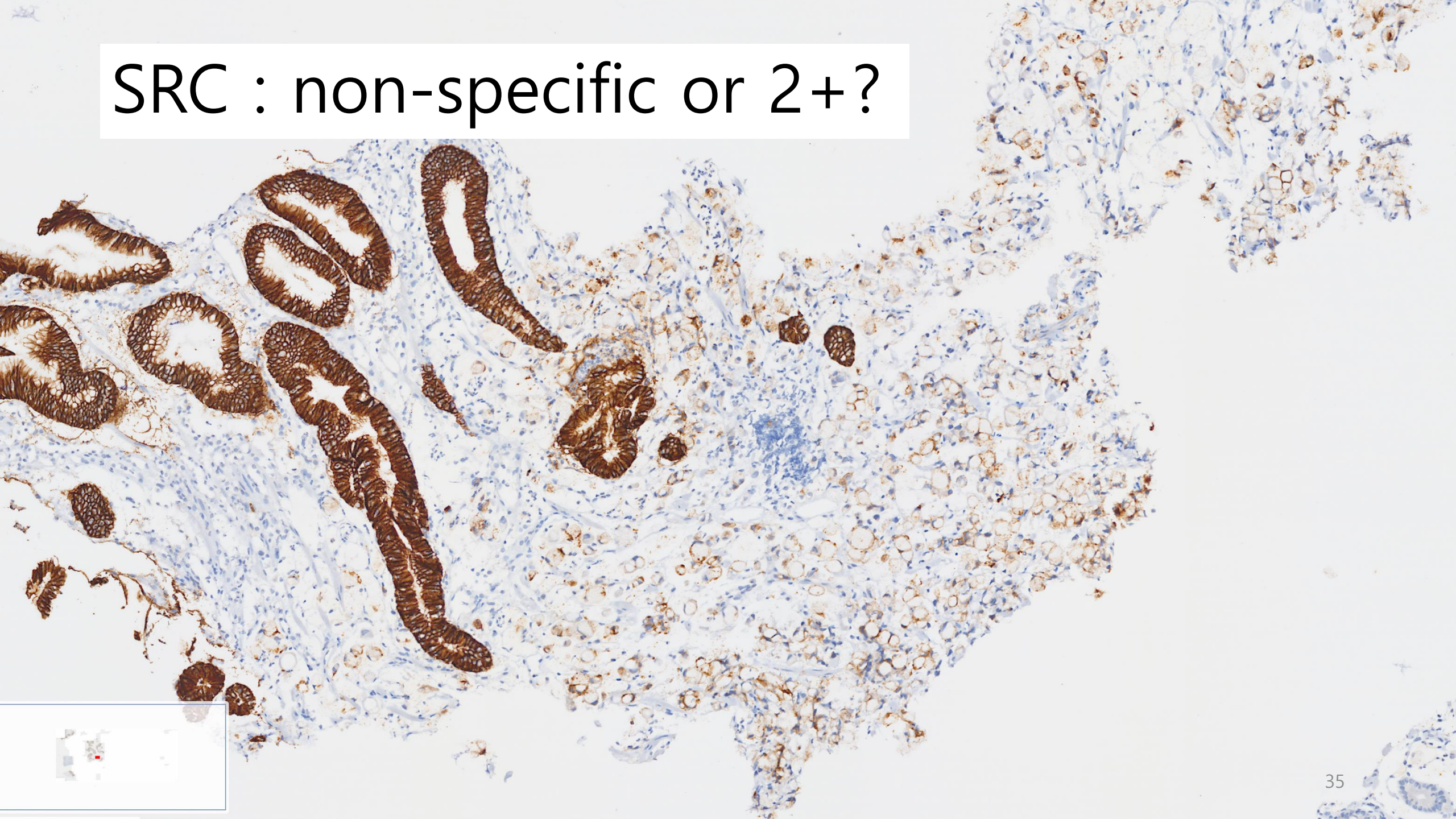


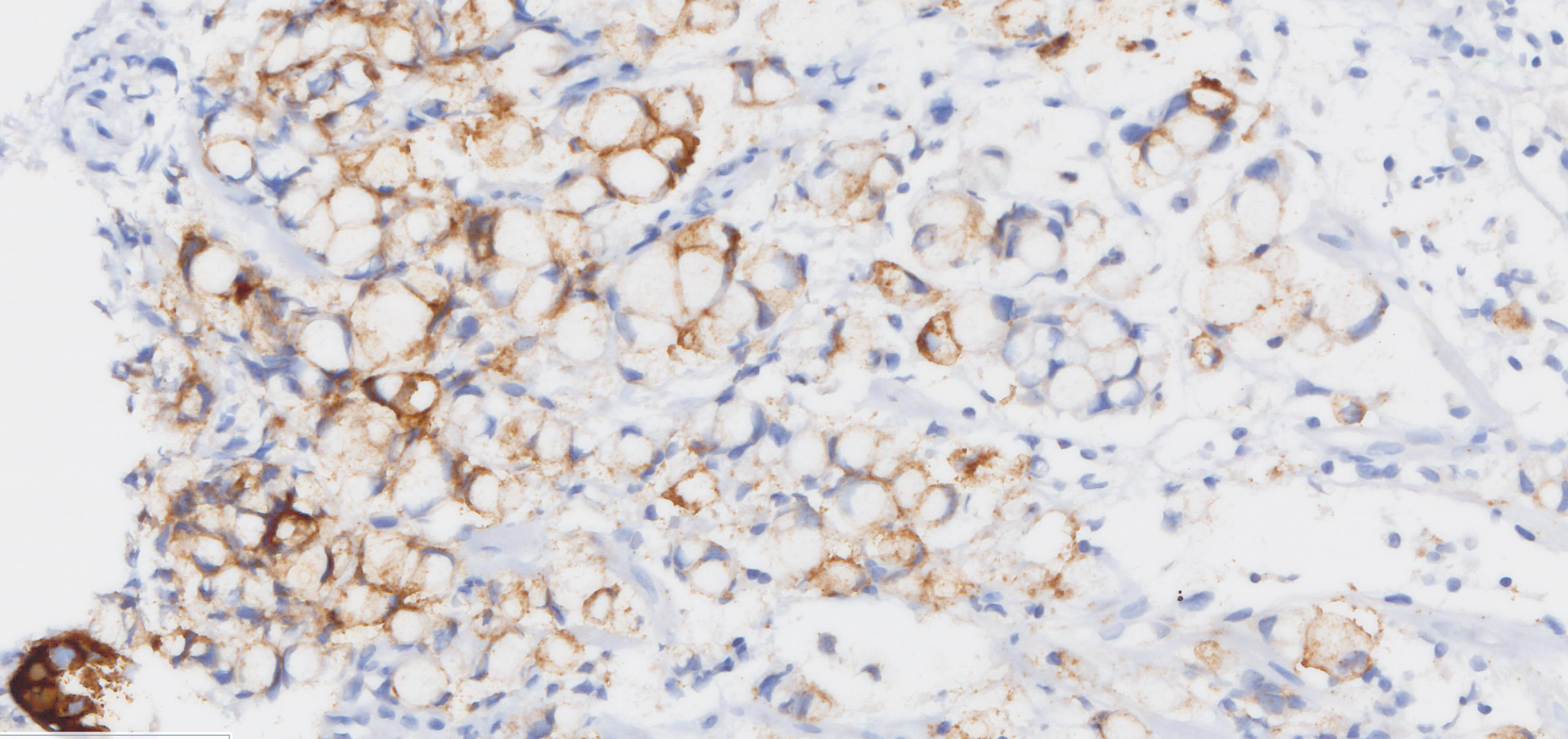
Mixture of intensities (0 ~ 3+)



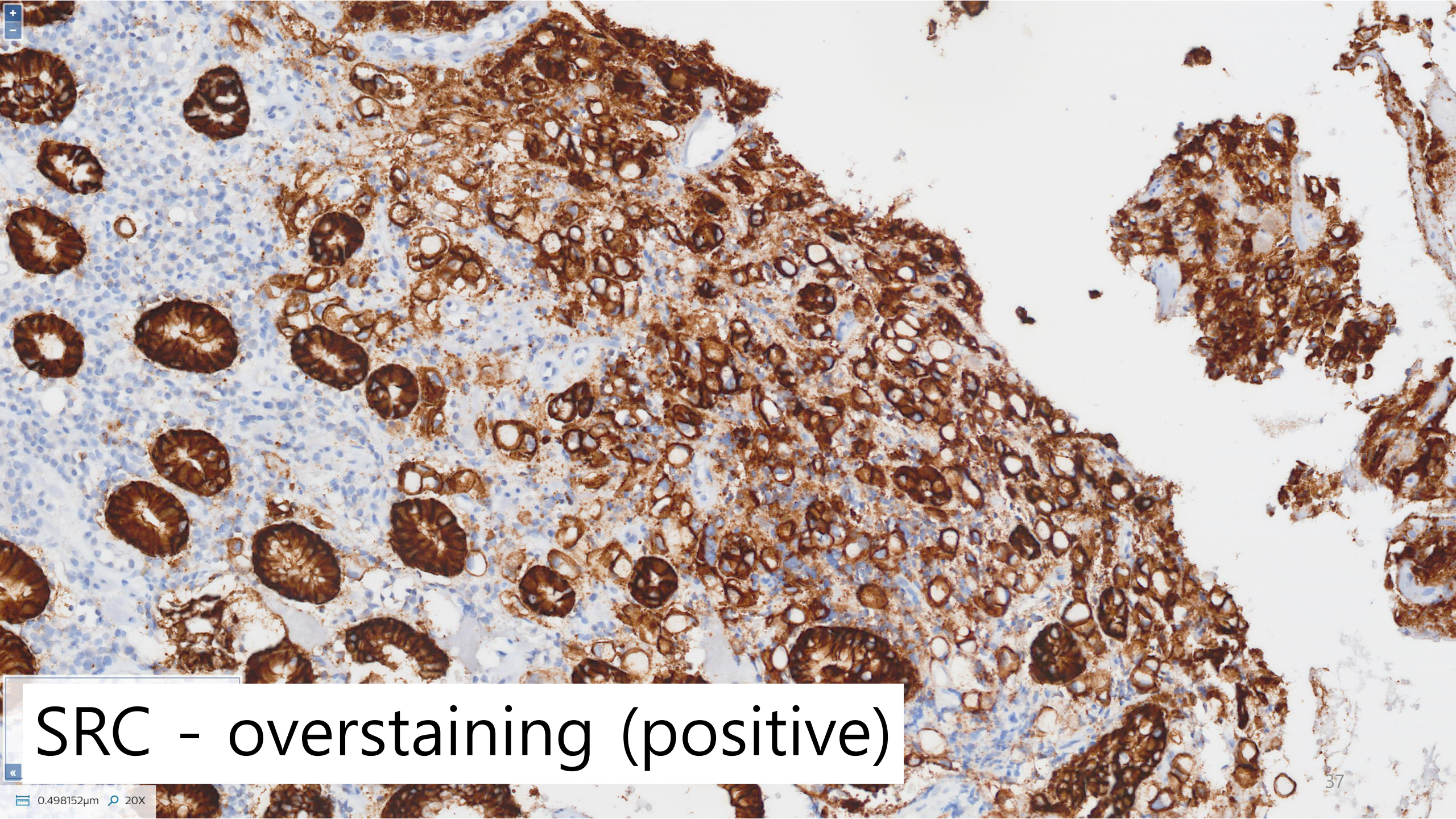
Courtesy of Prof. Hye Seung Lee

SRC : non-specific or 2+?

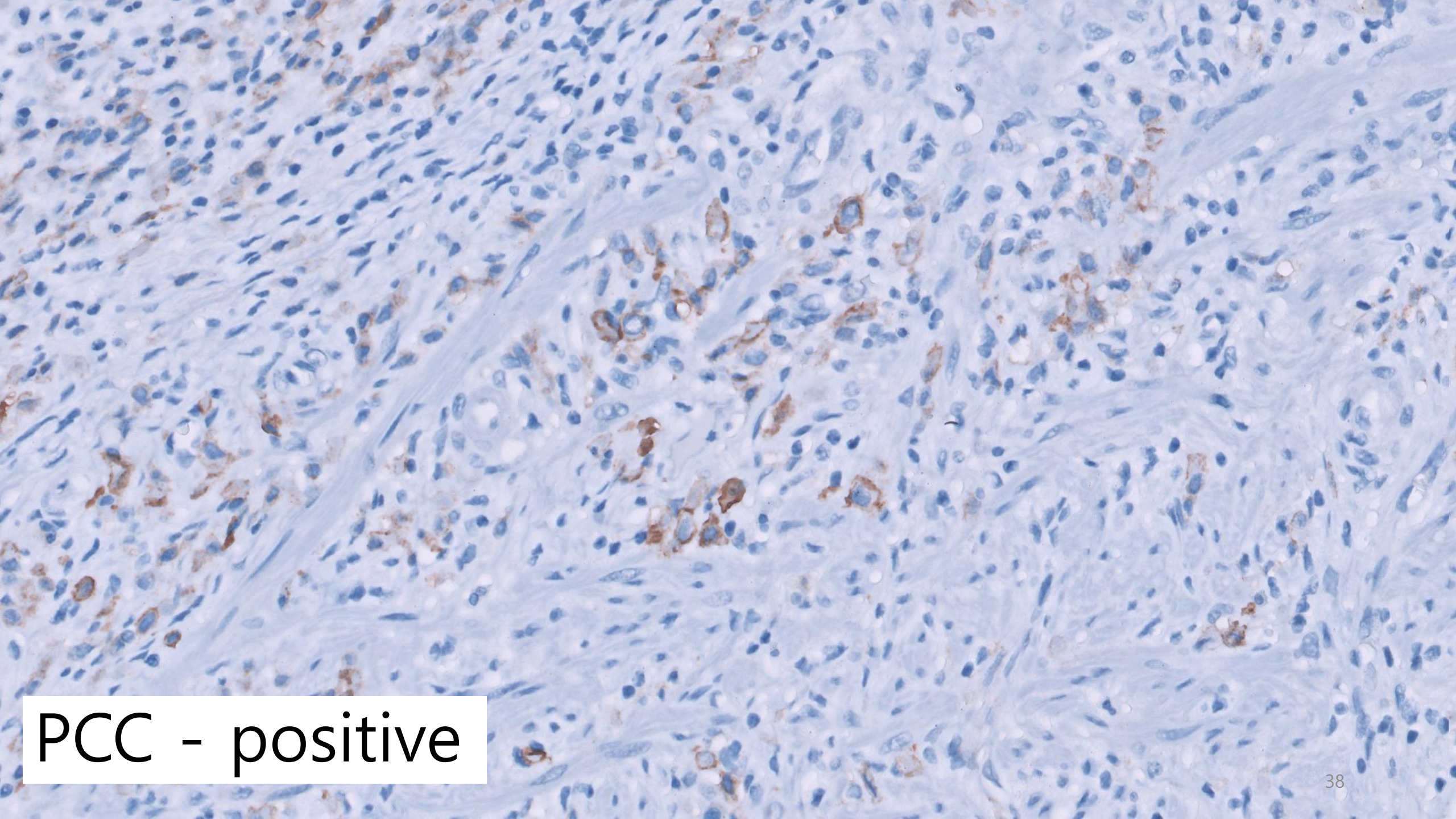




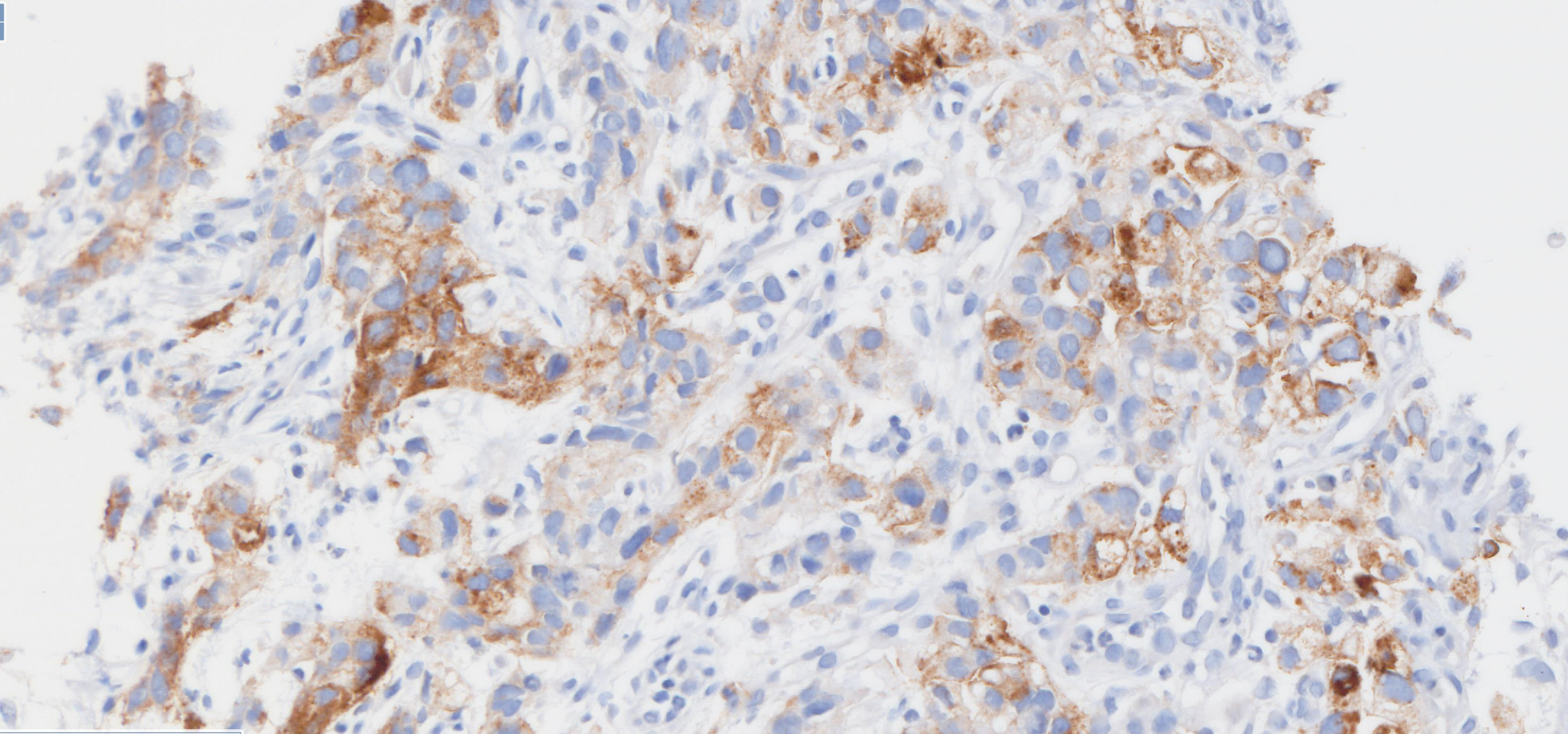
SRC : non-specific or 2+?



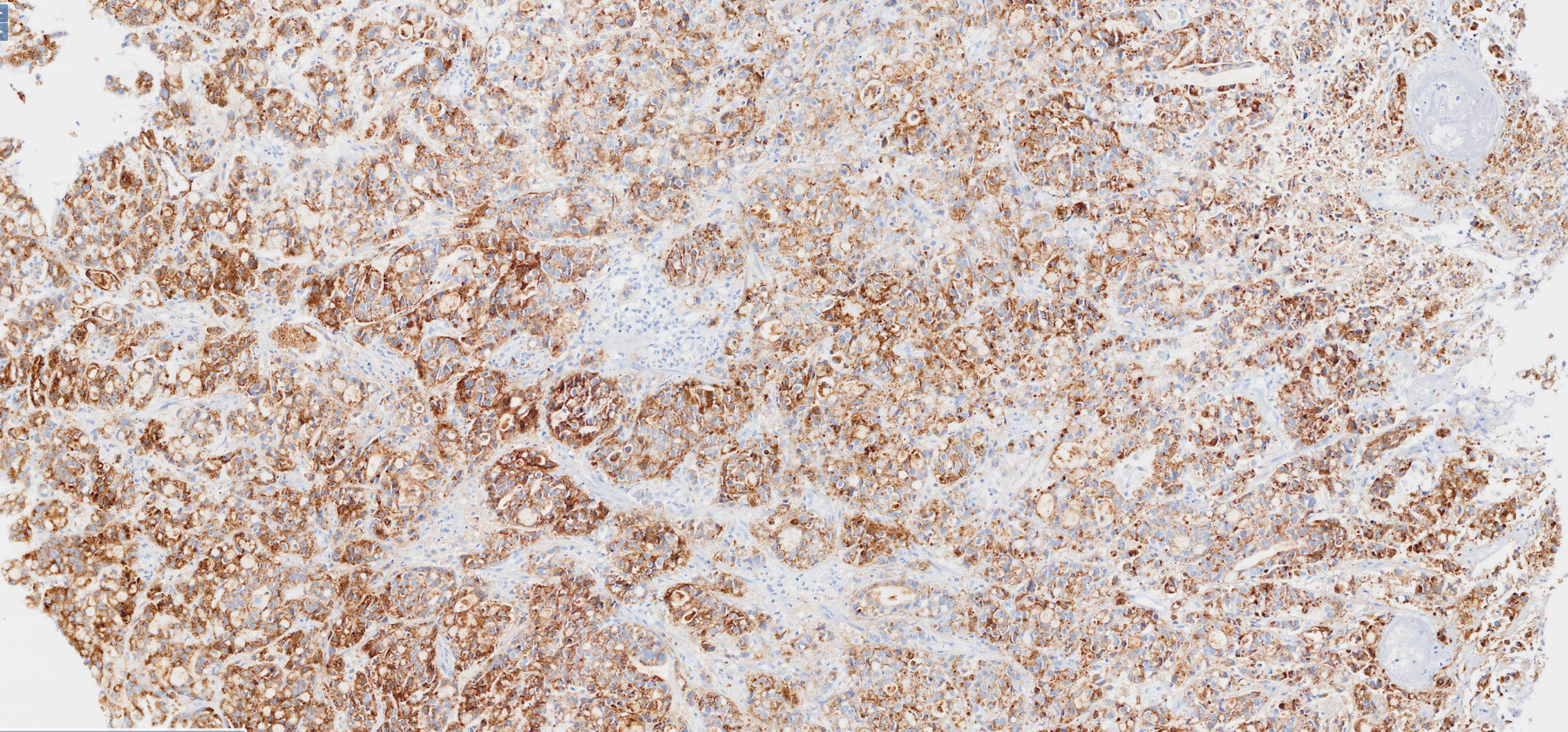
SRC - overstaining (positive)



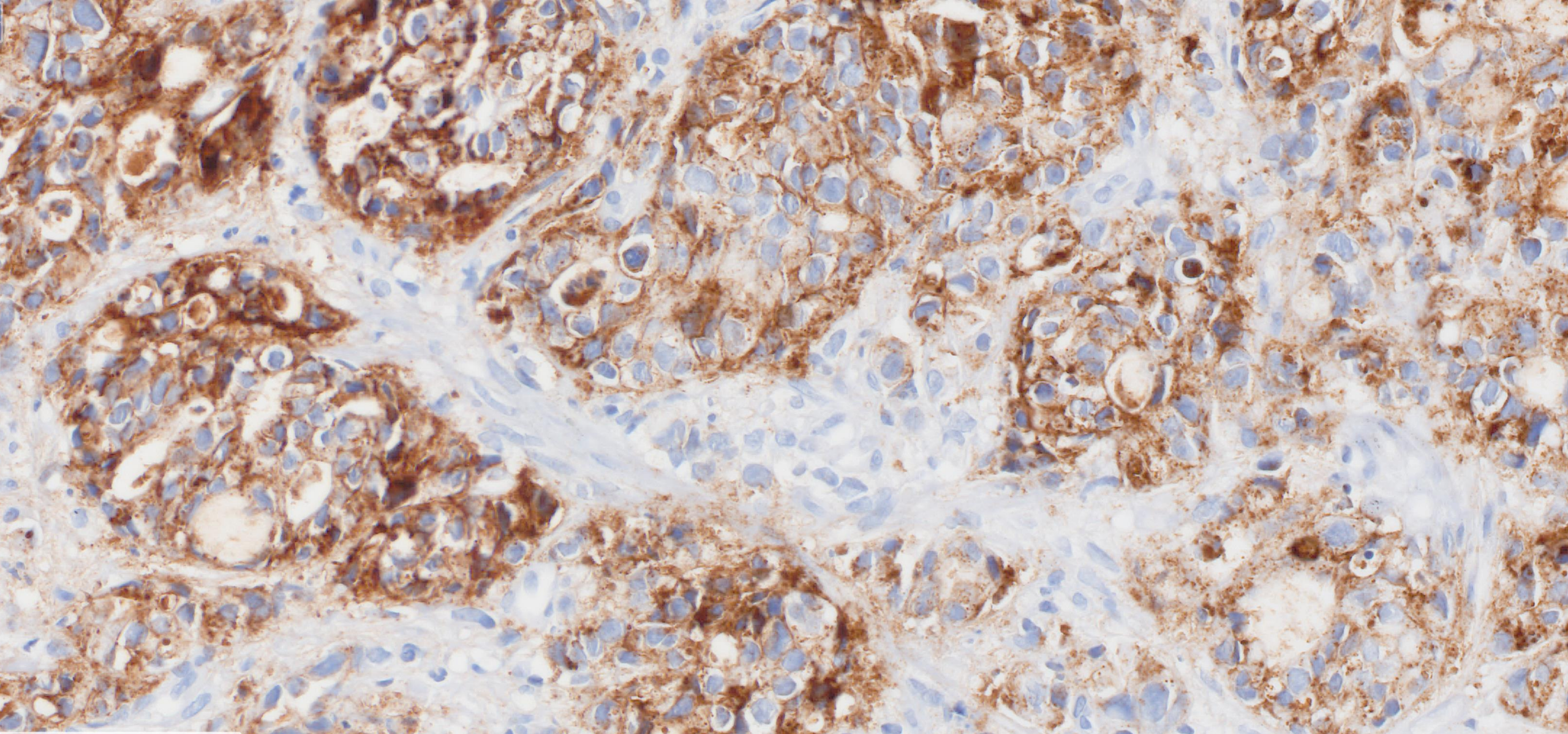
PCC - positive



Cytoplasmic dot pattern

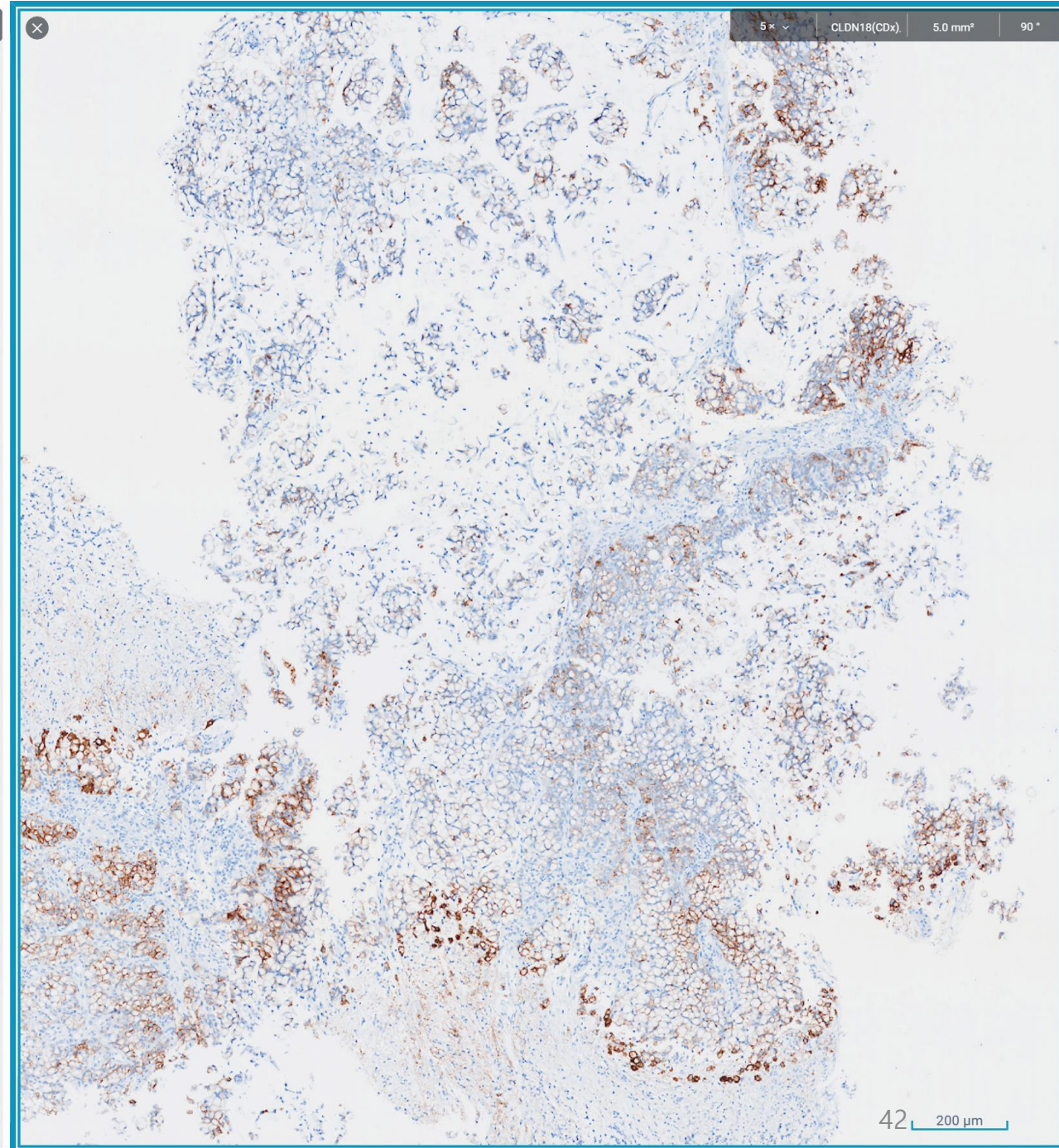
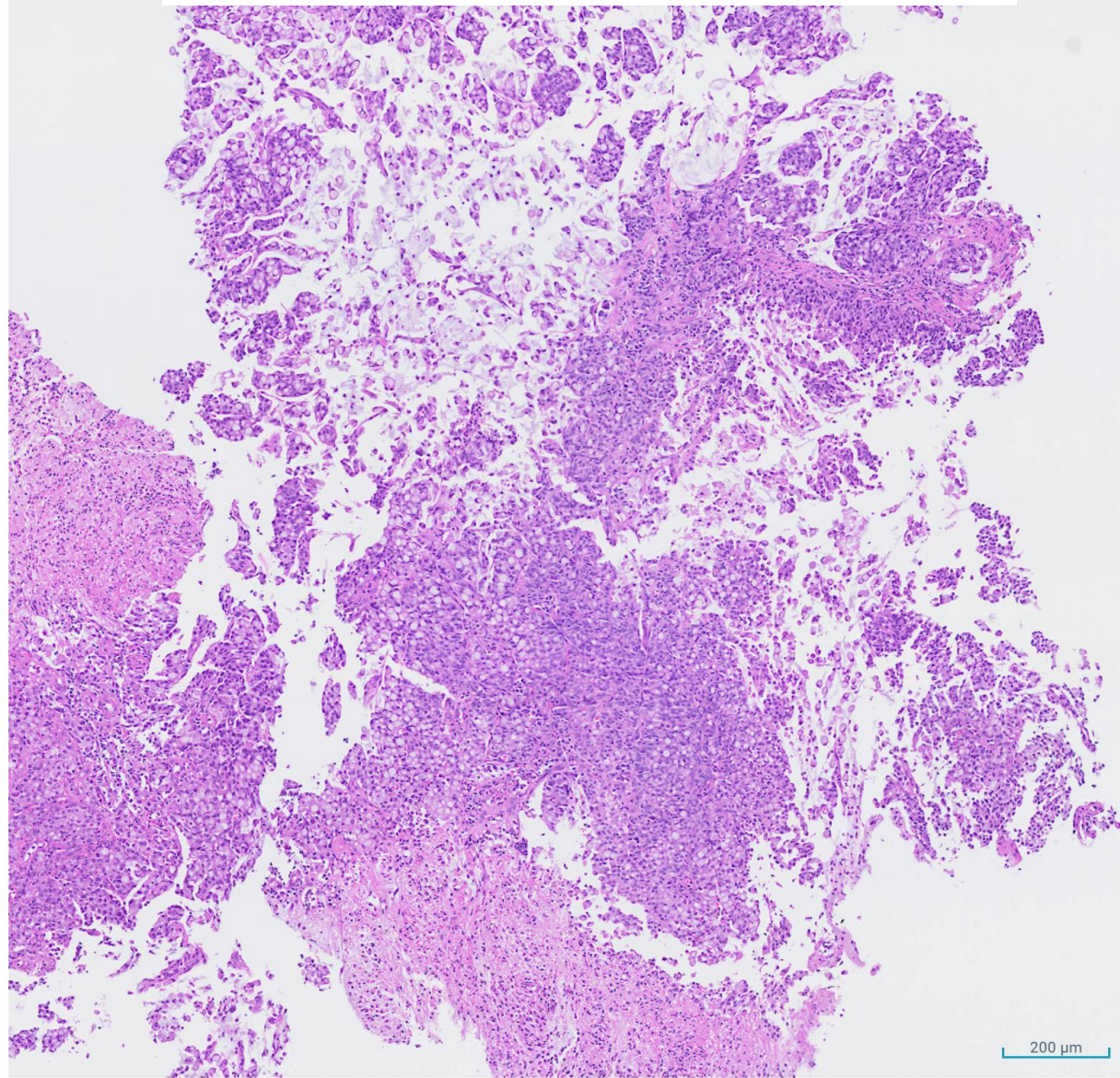


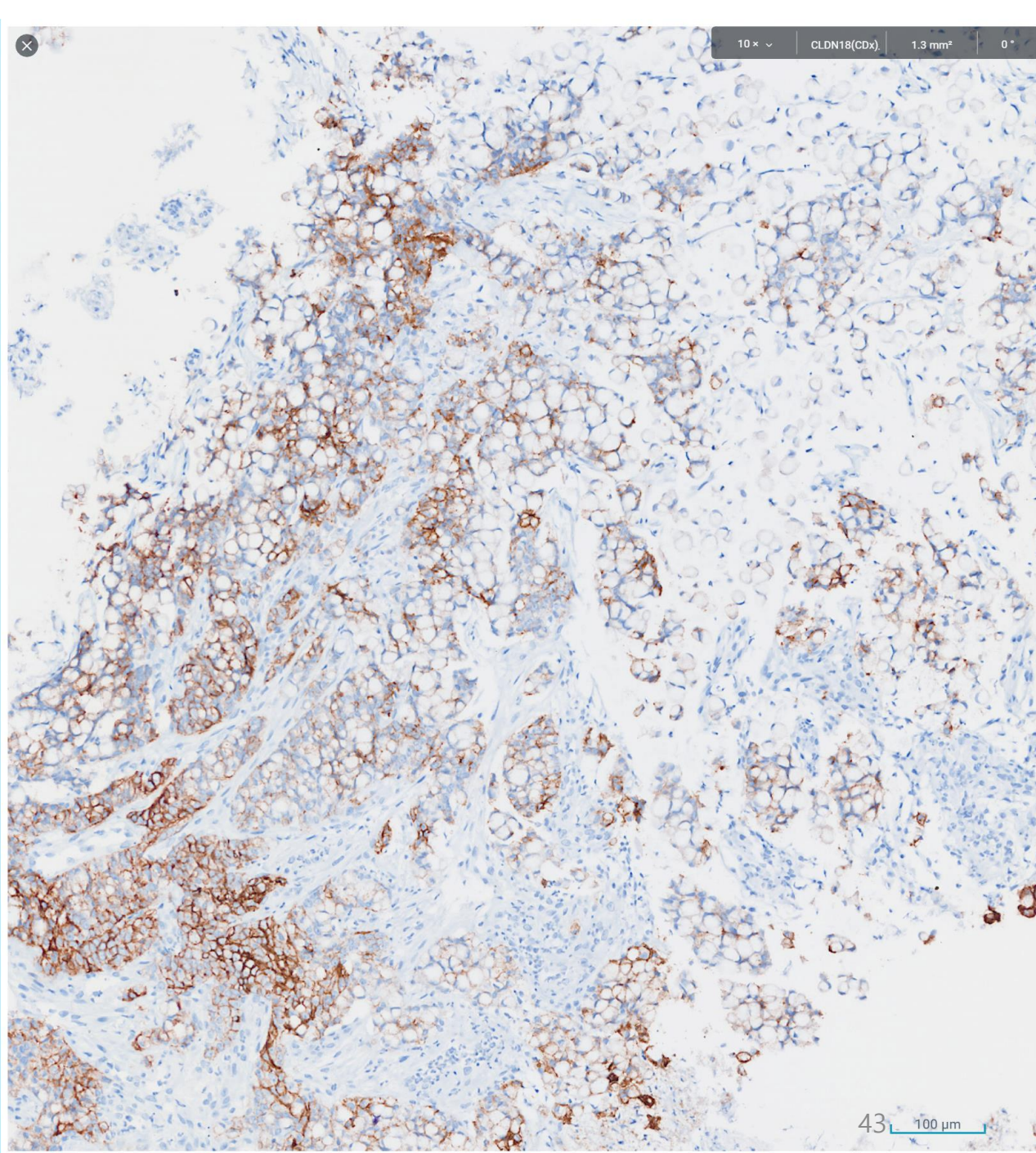
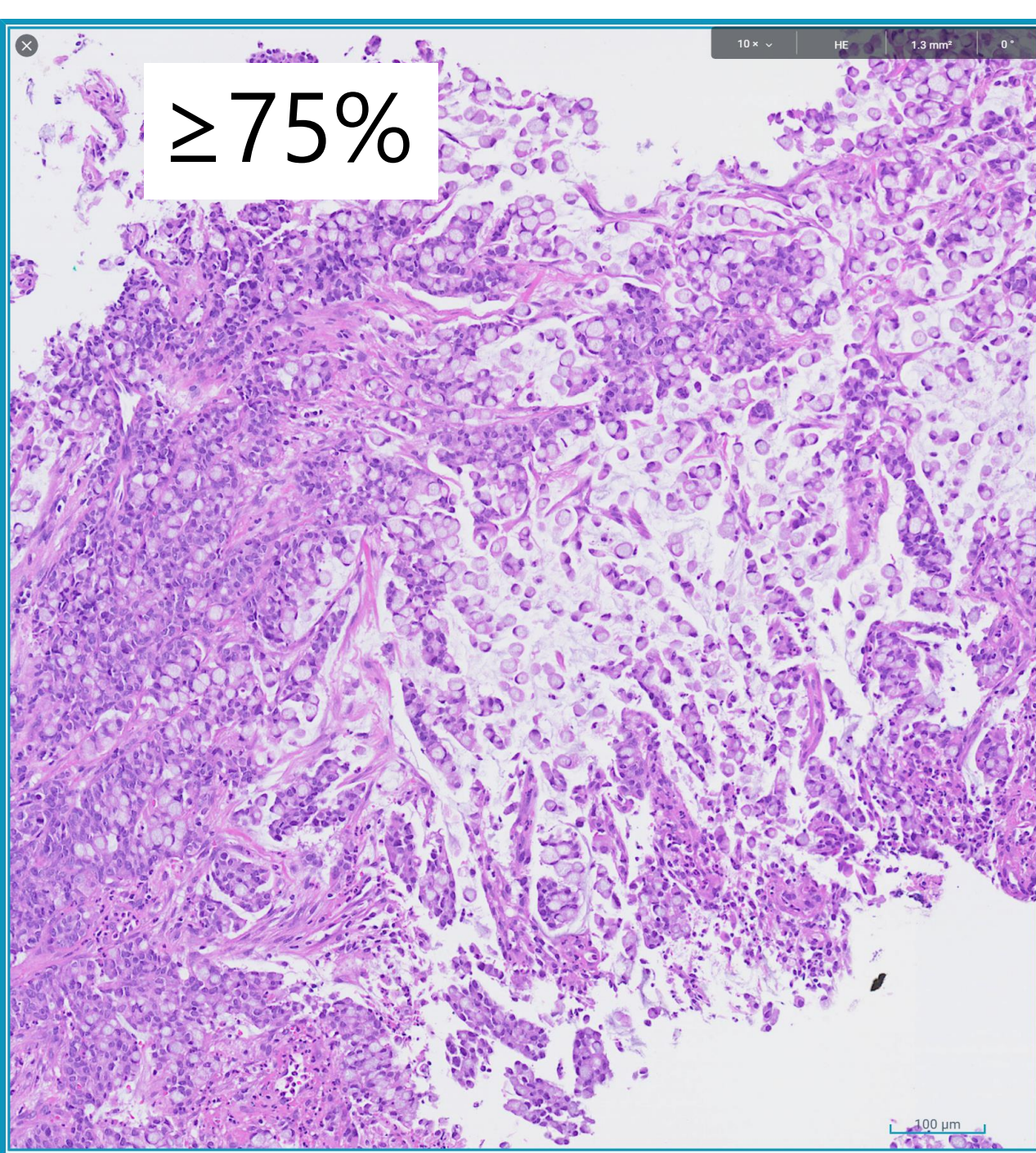
Strong cytoplasmic dot pattern

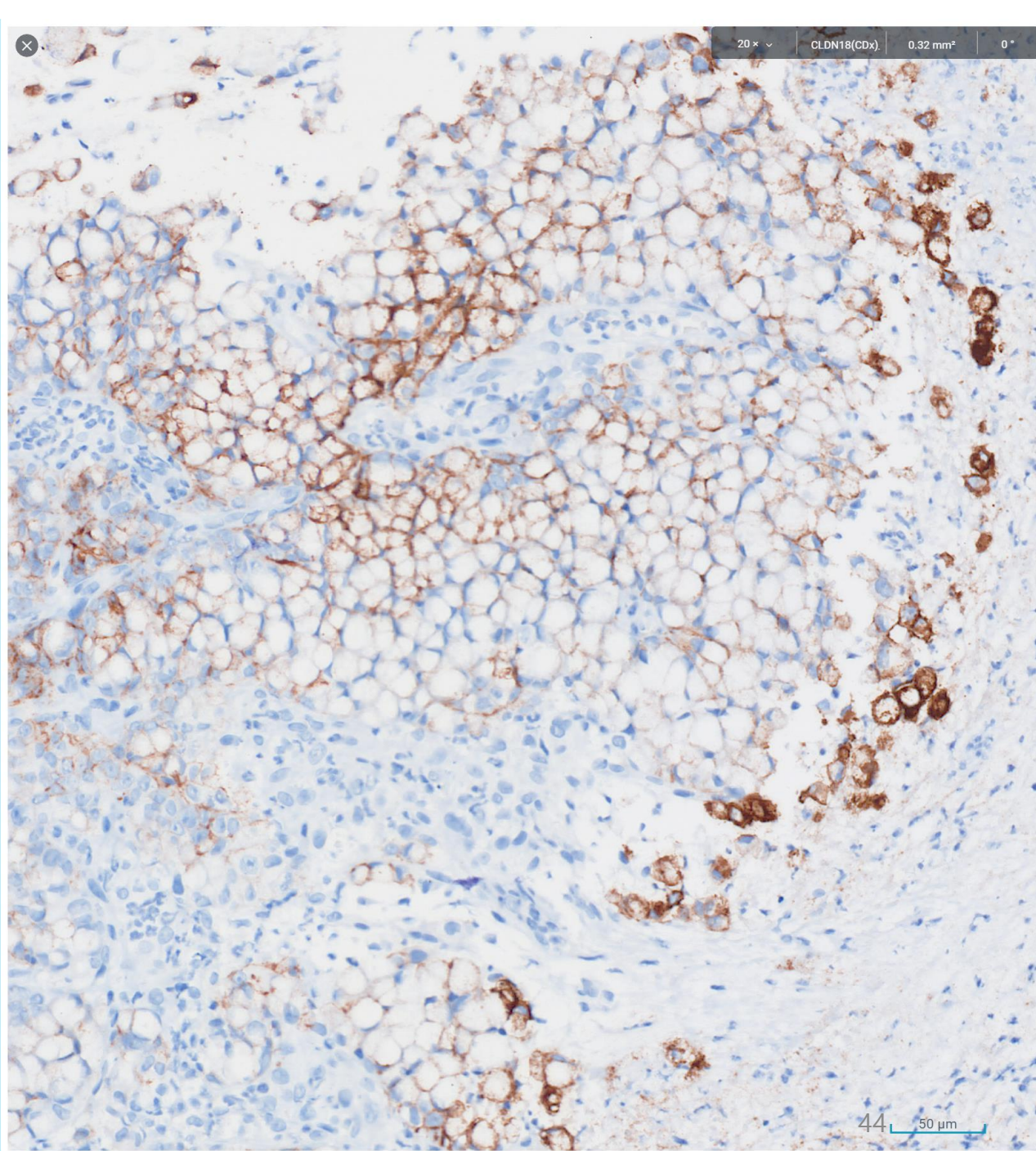
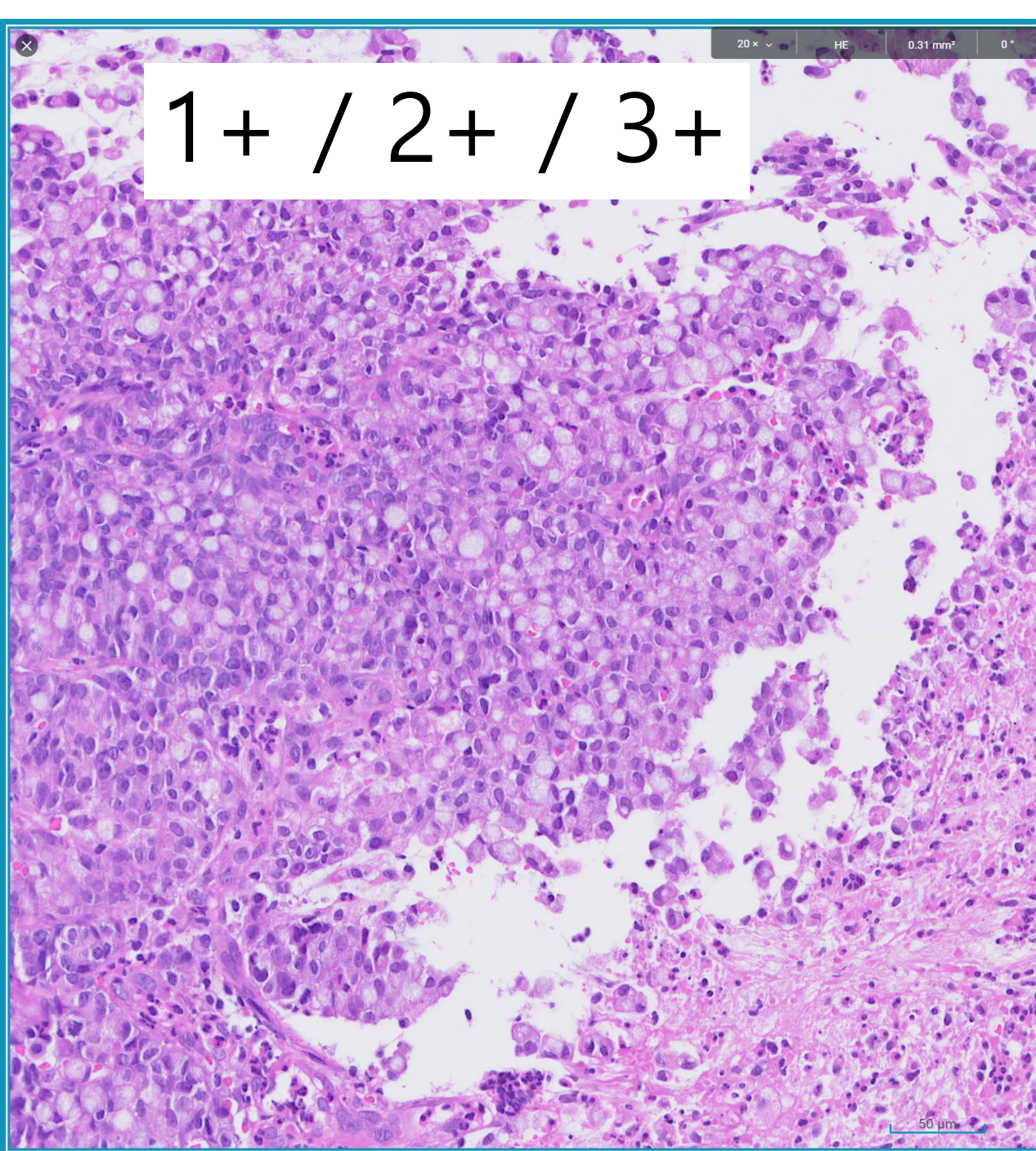


Strong cytoplasmic dot pattern

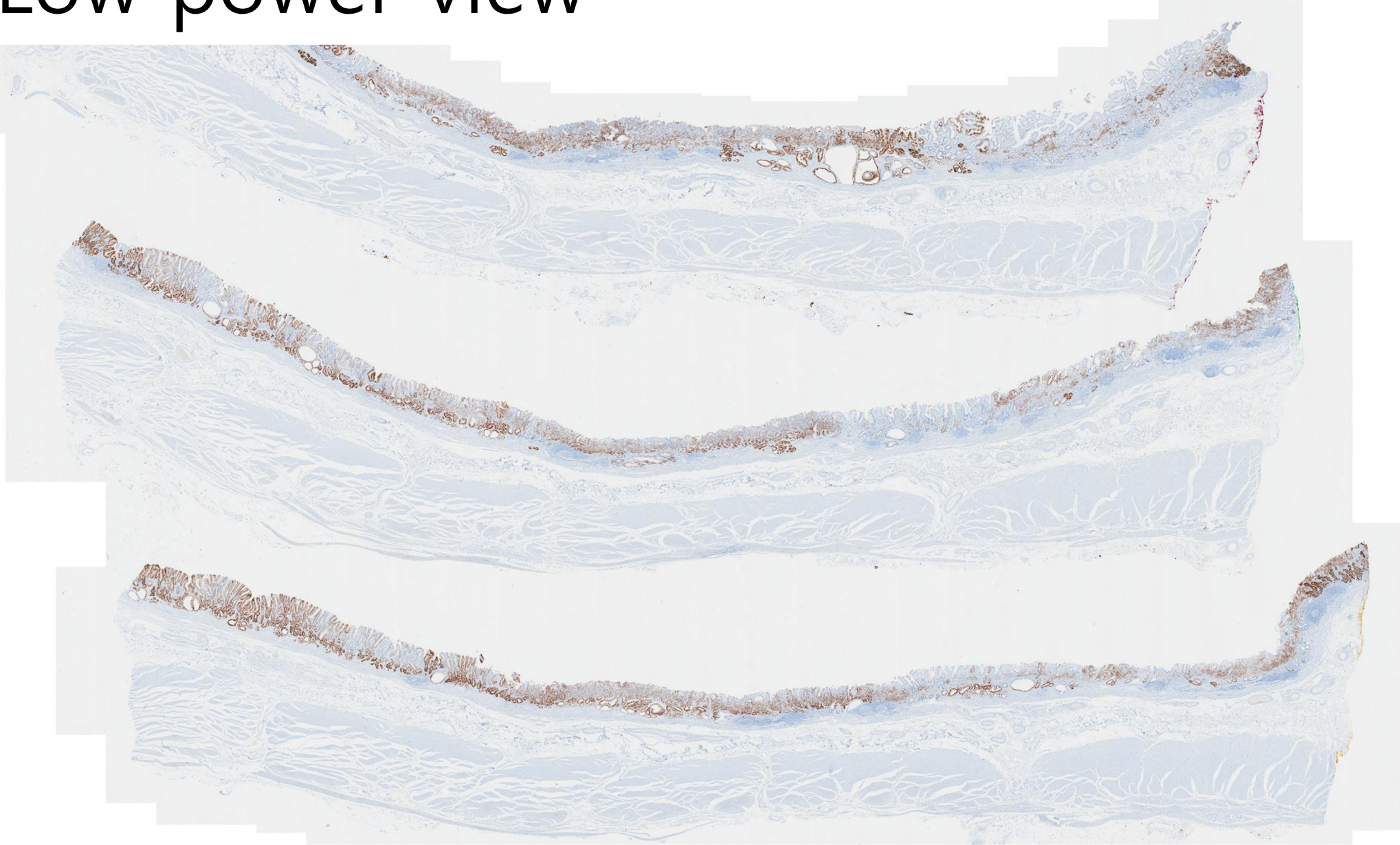
$\geq 75\%$ or $< 75\%$?



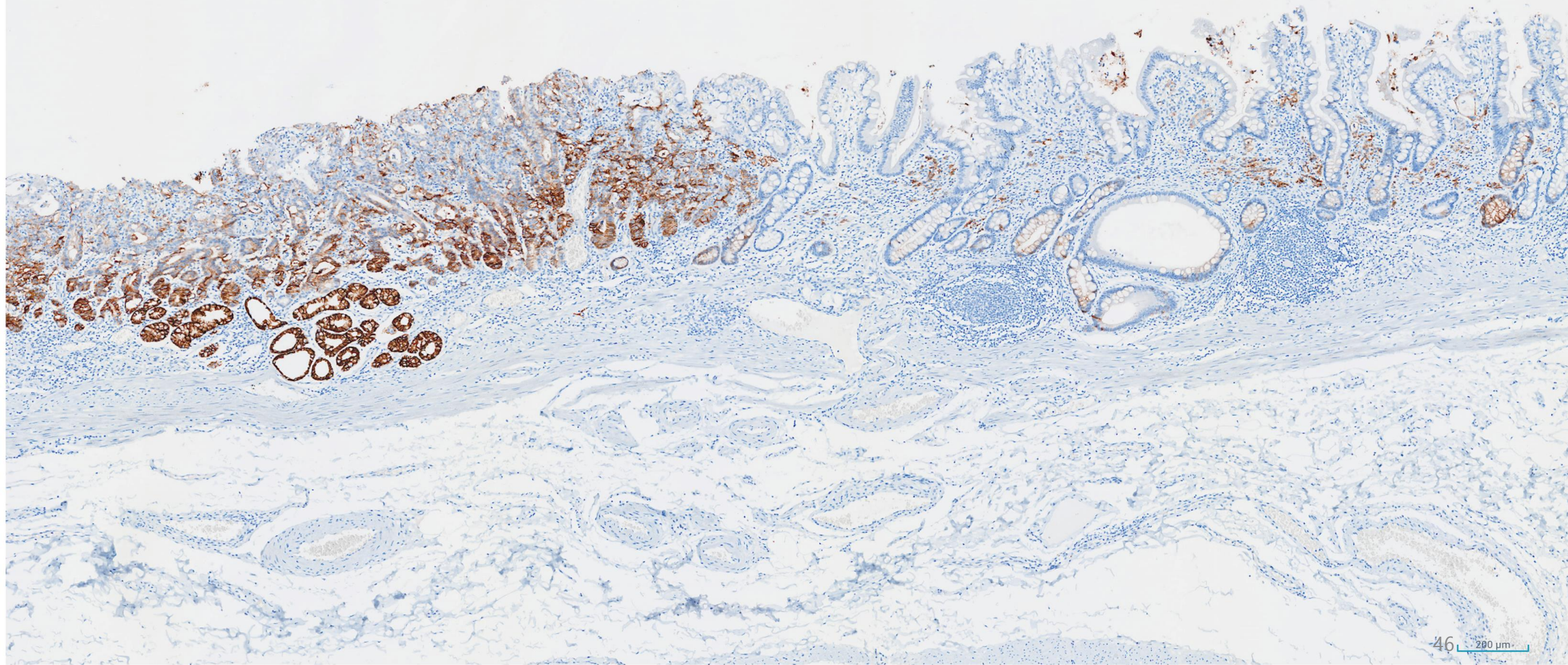




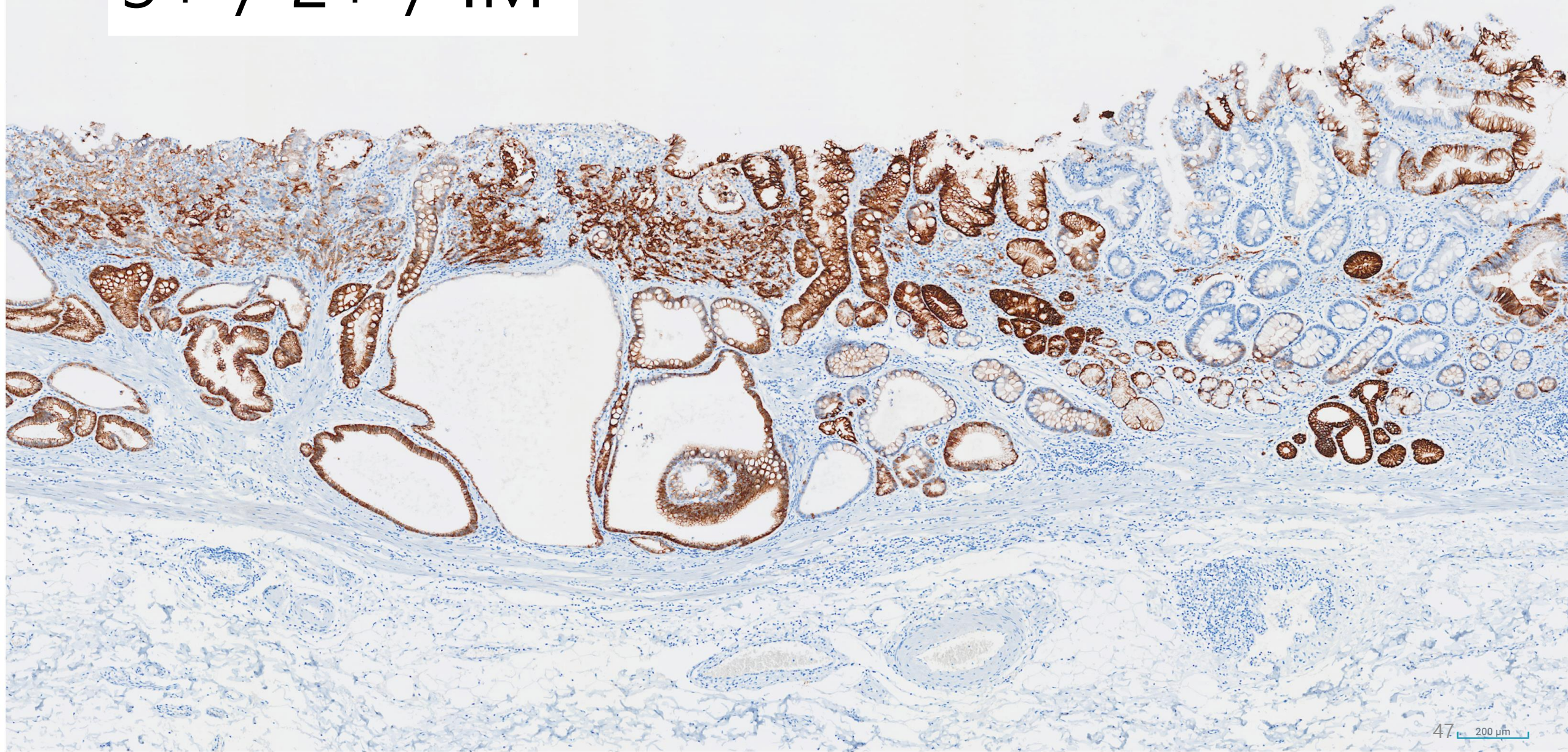
Low power view



3+ / 2+ / IM

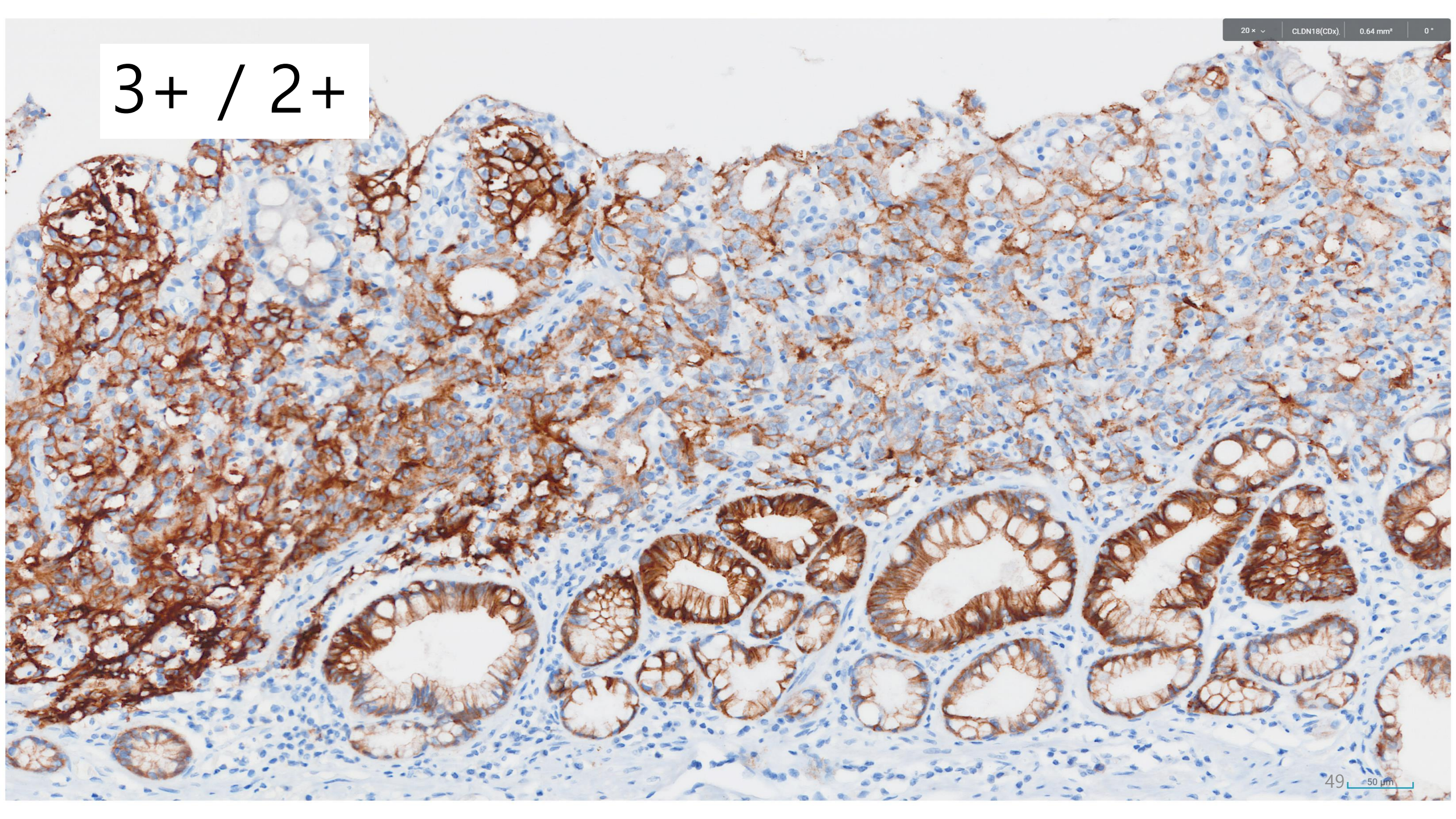


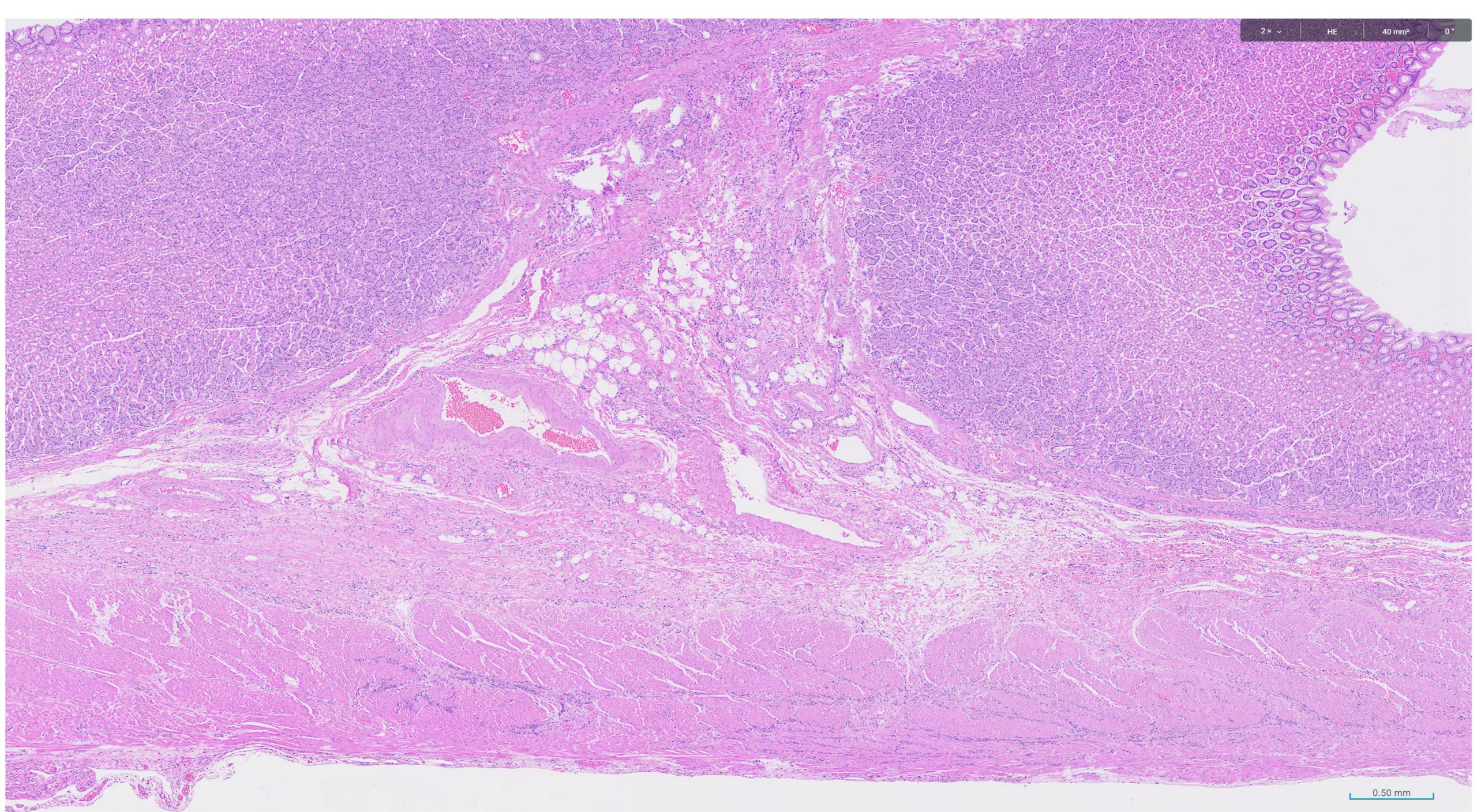
3+ / 2+ / IM

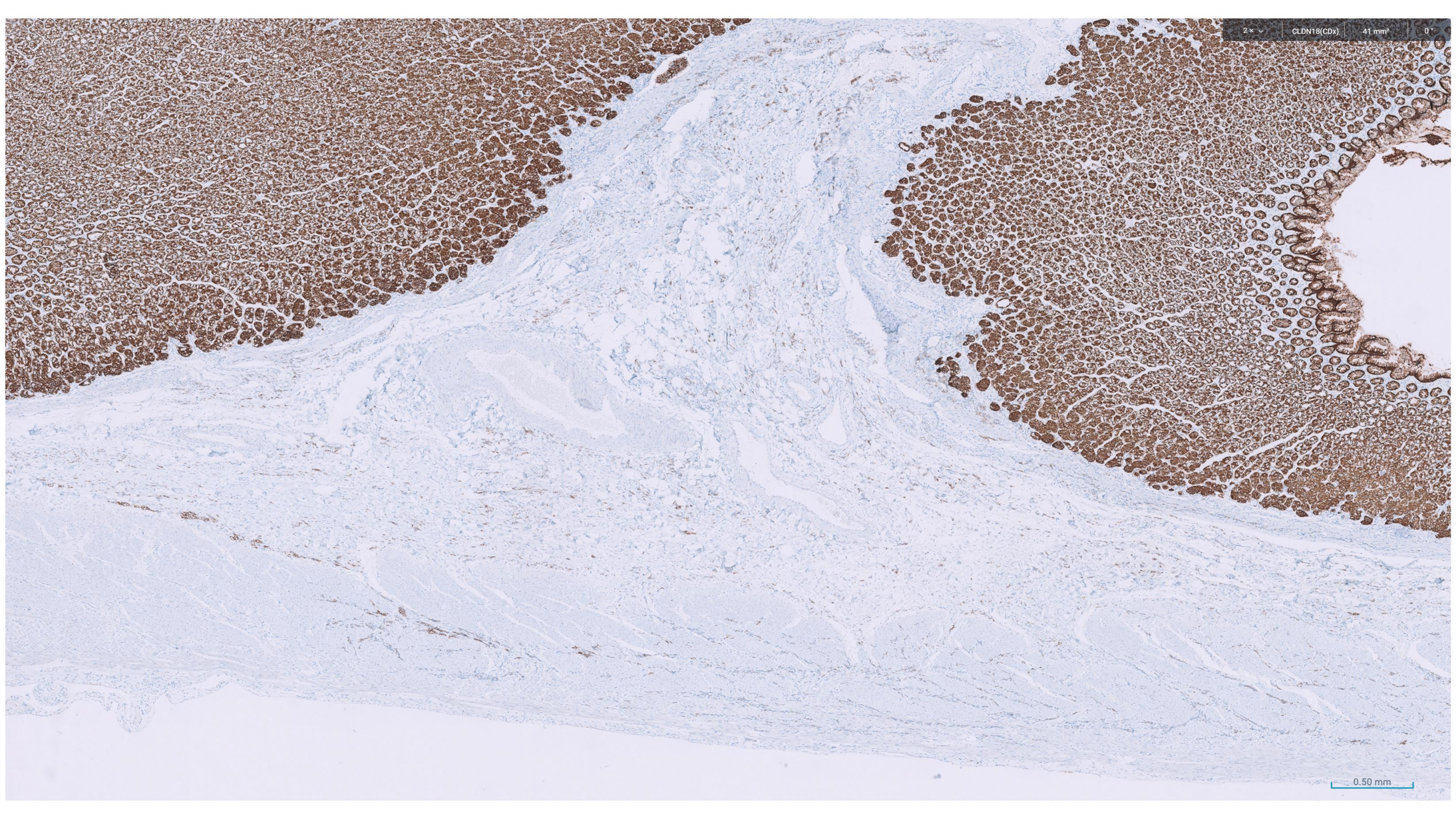


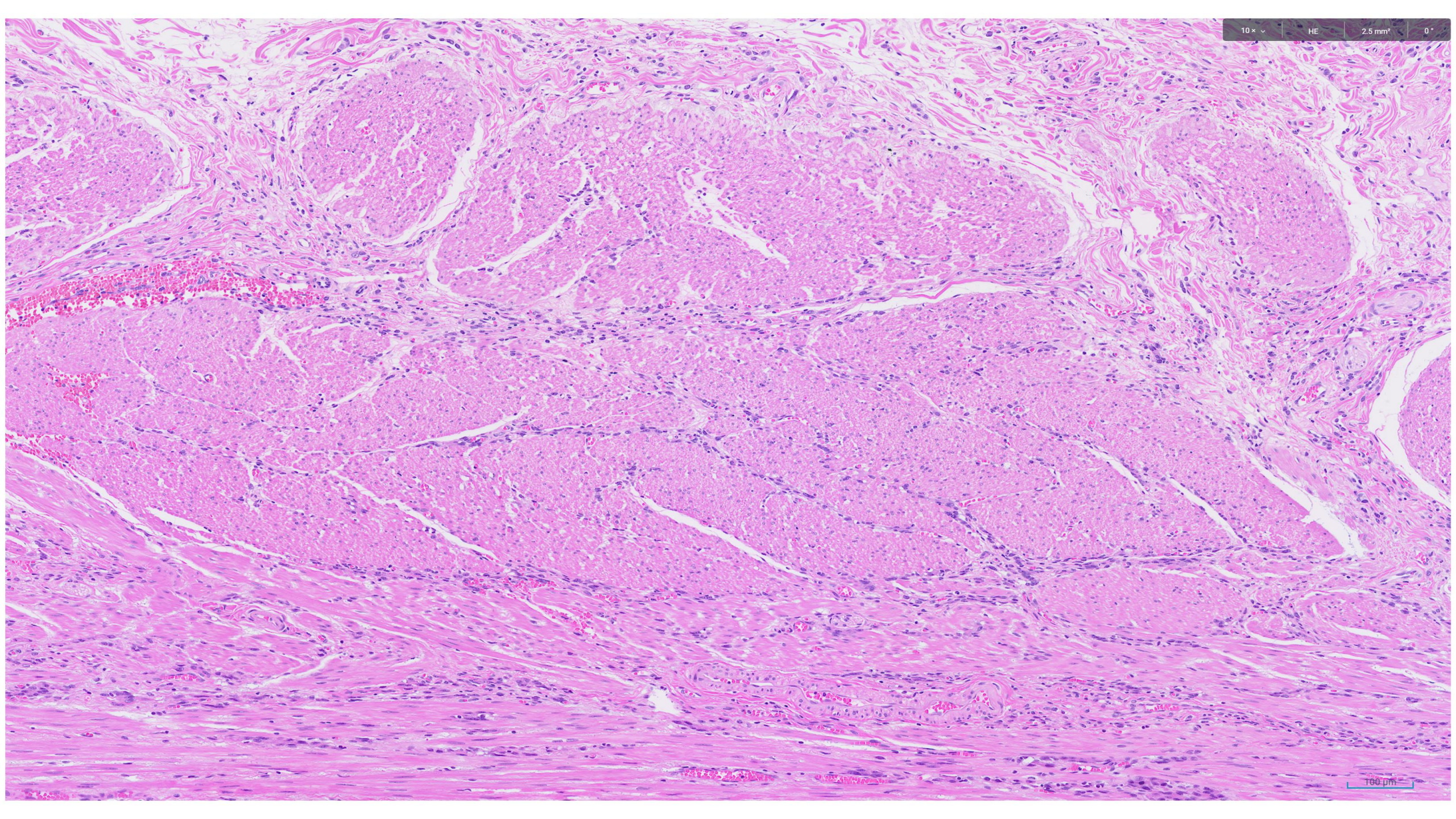
1+ / 2+

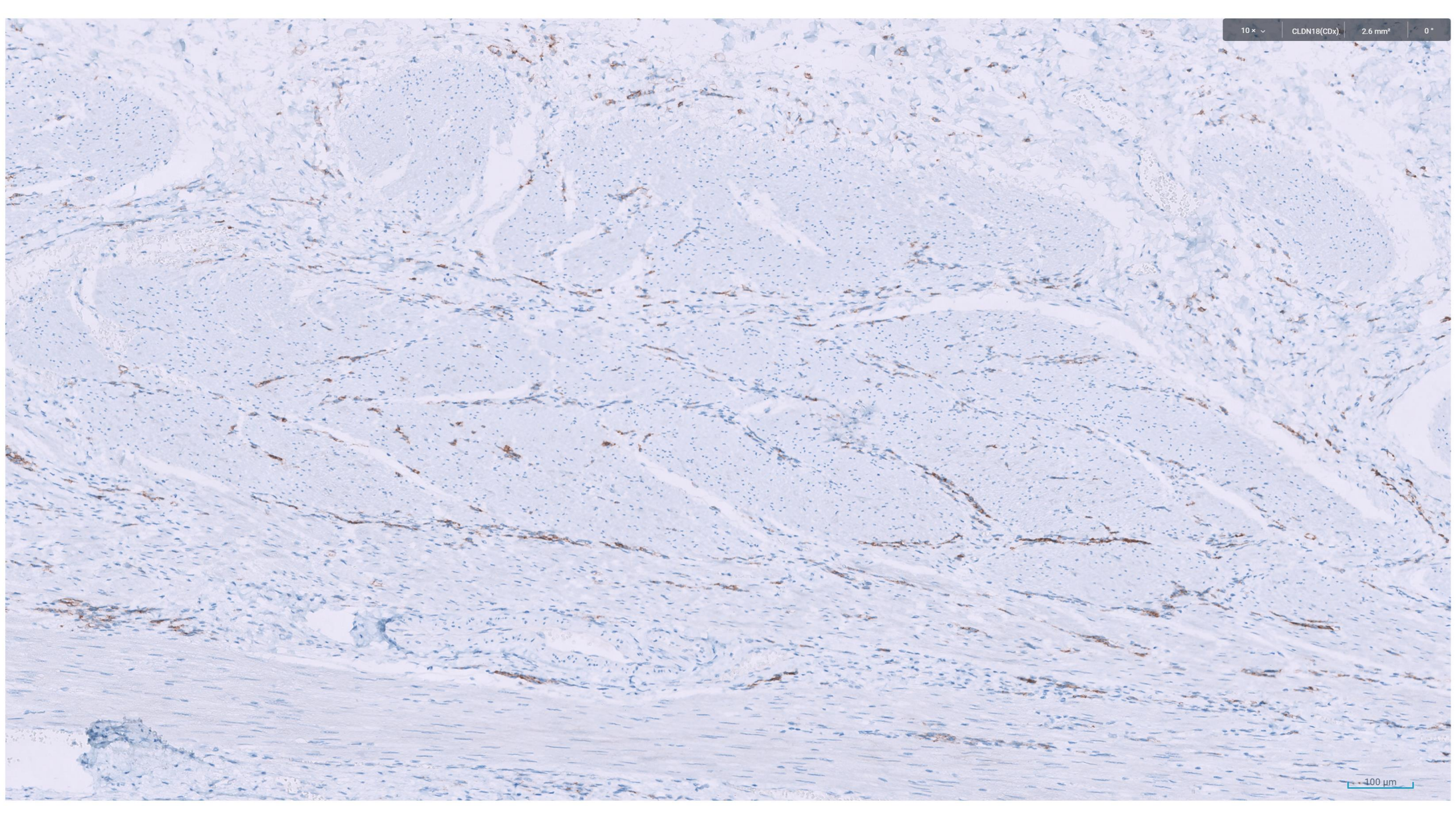
3+ / 2+

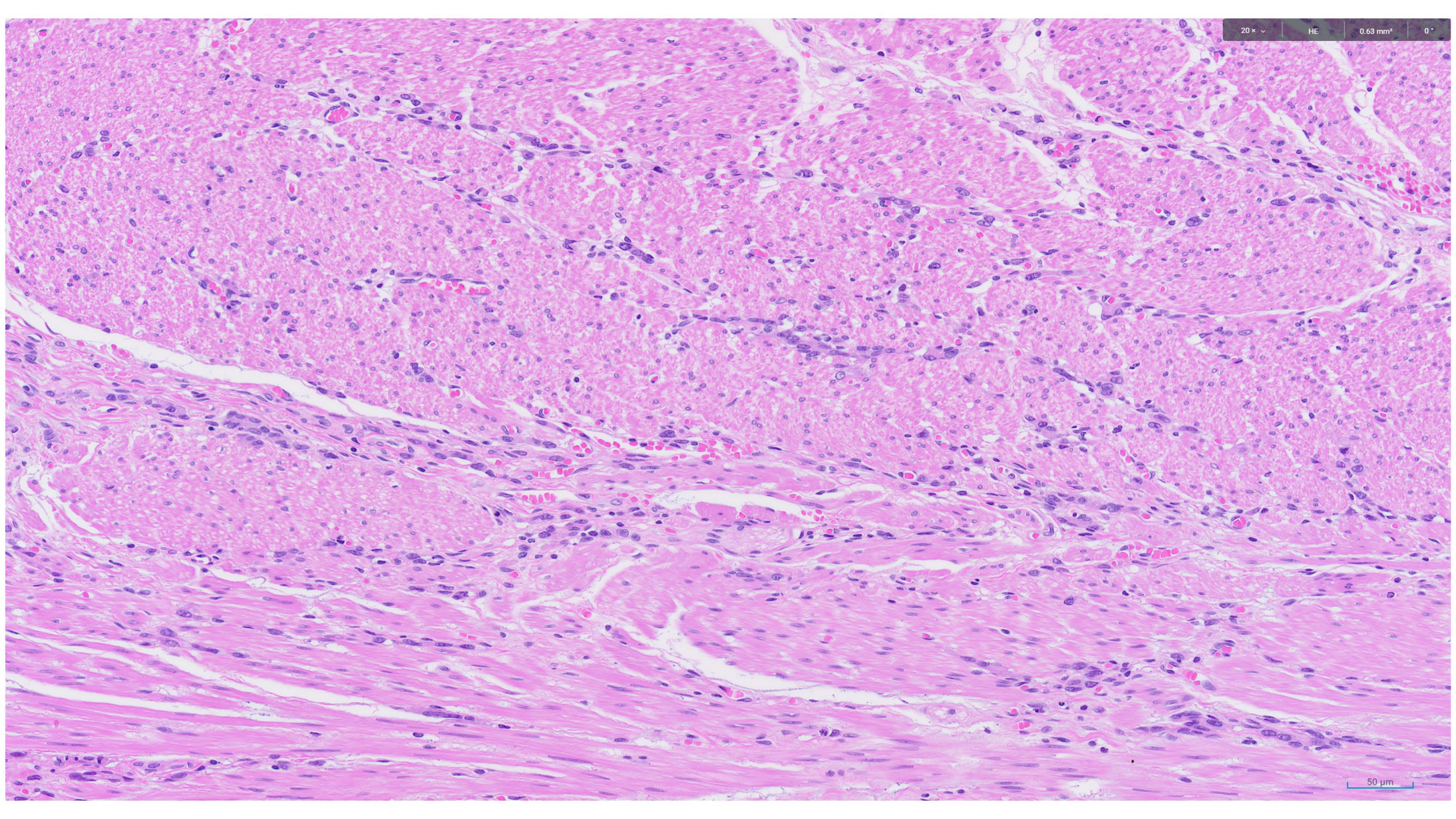


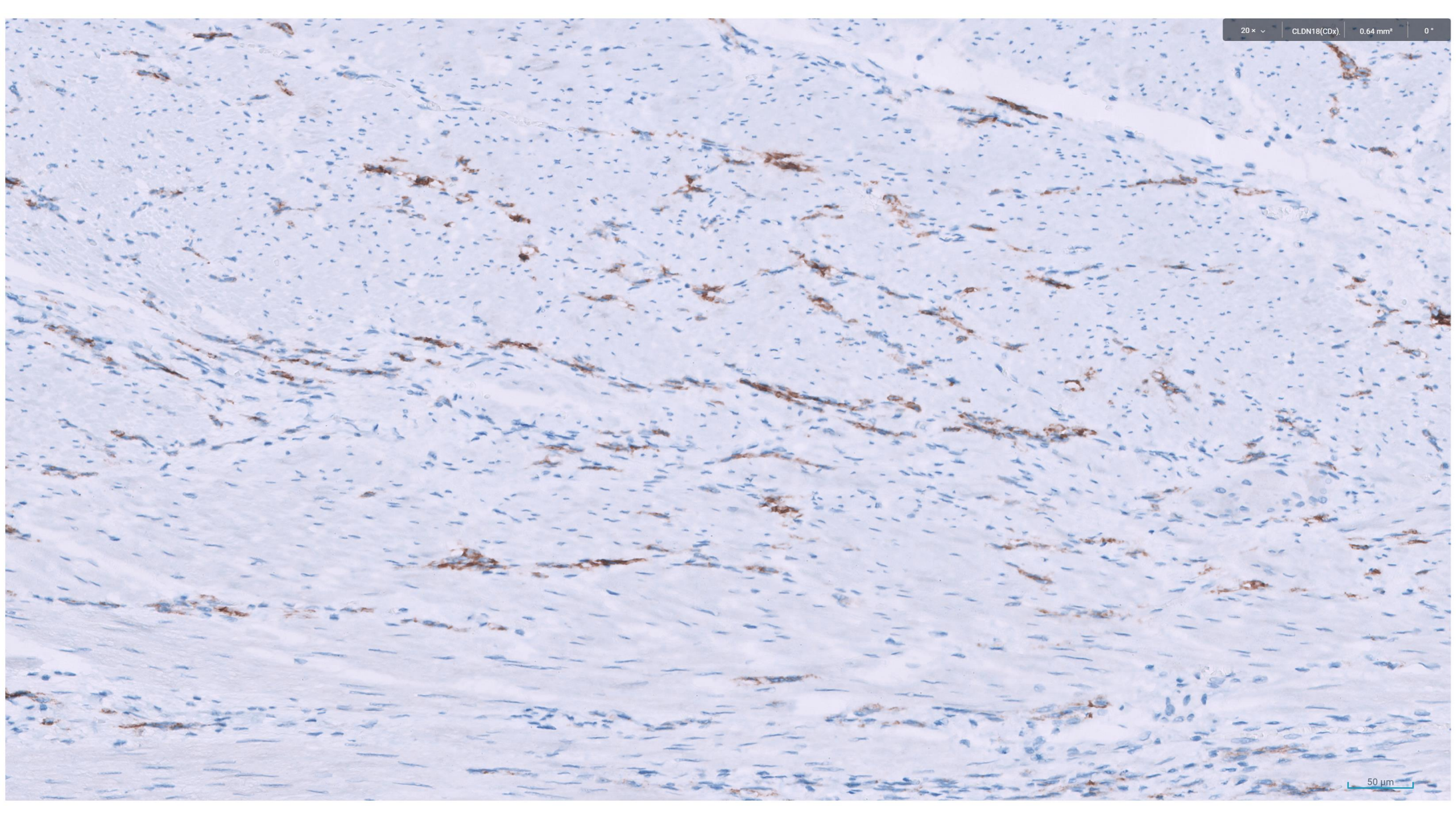


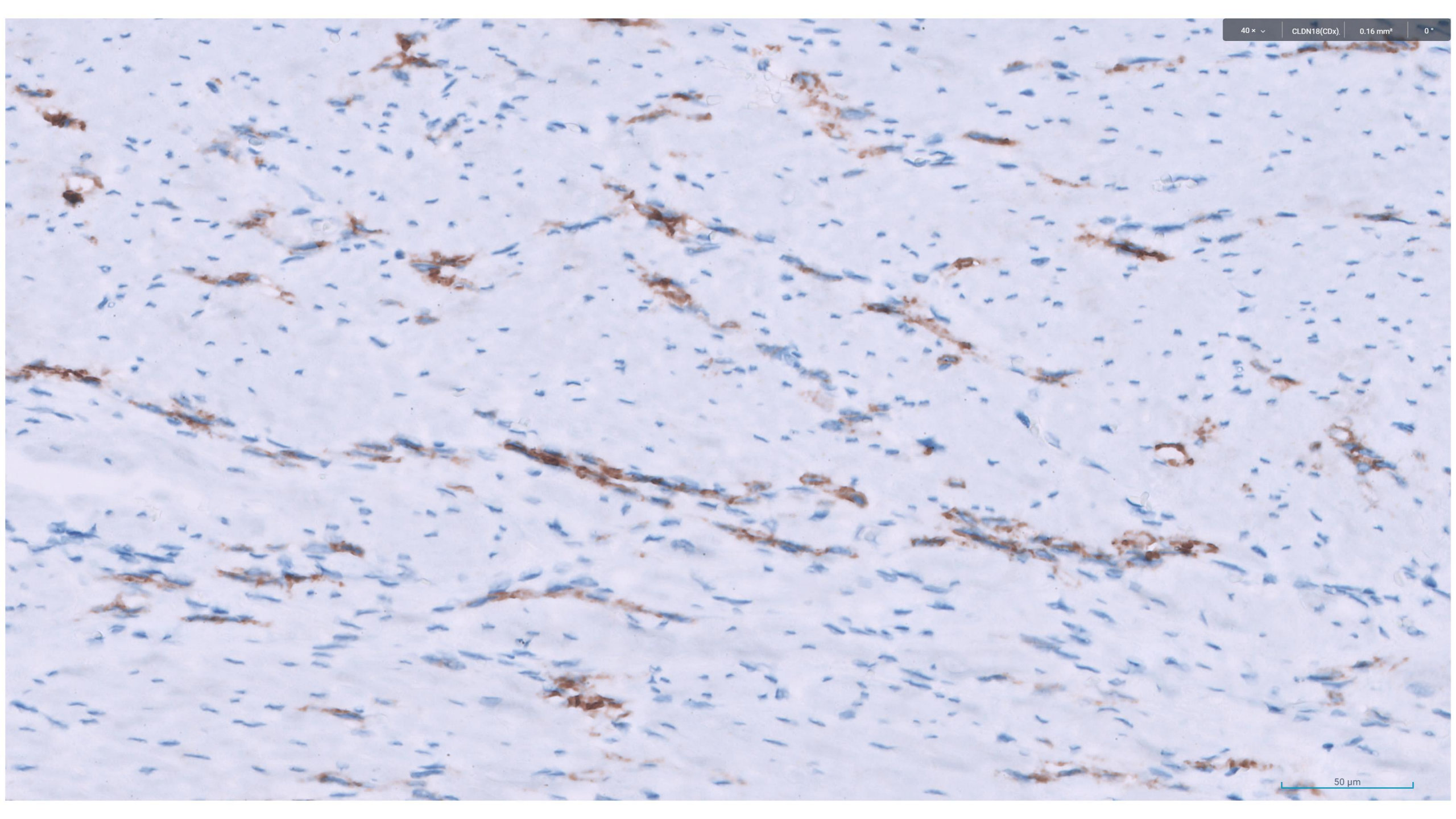


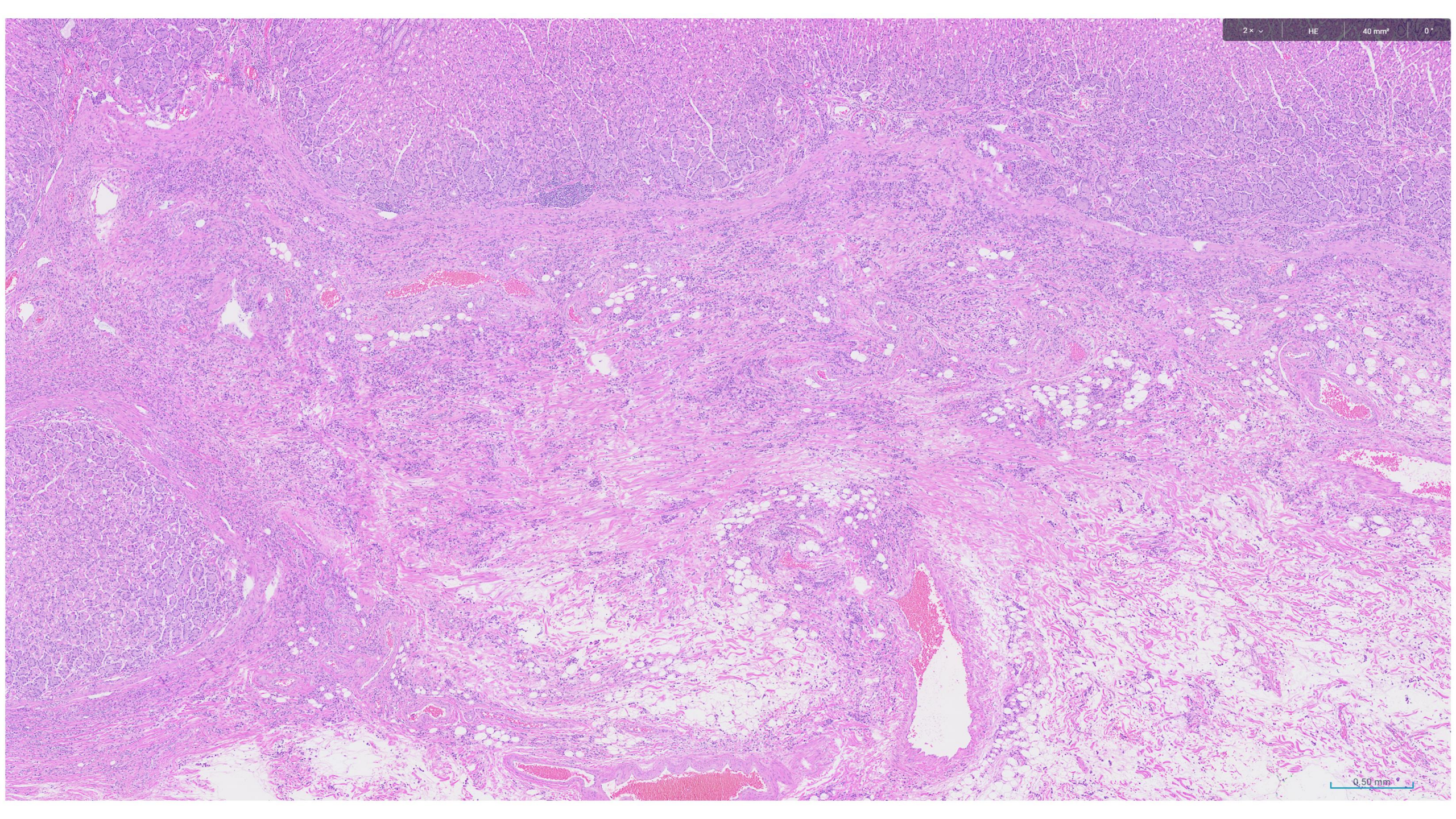


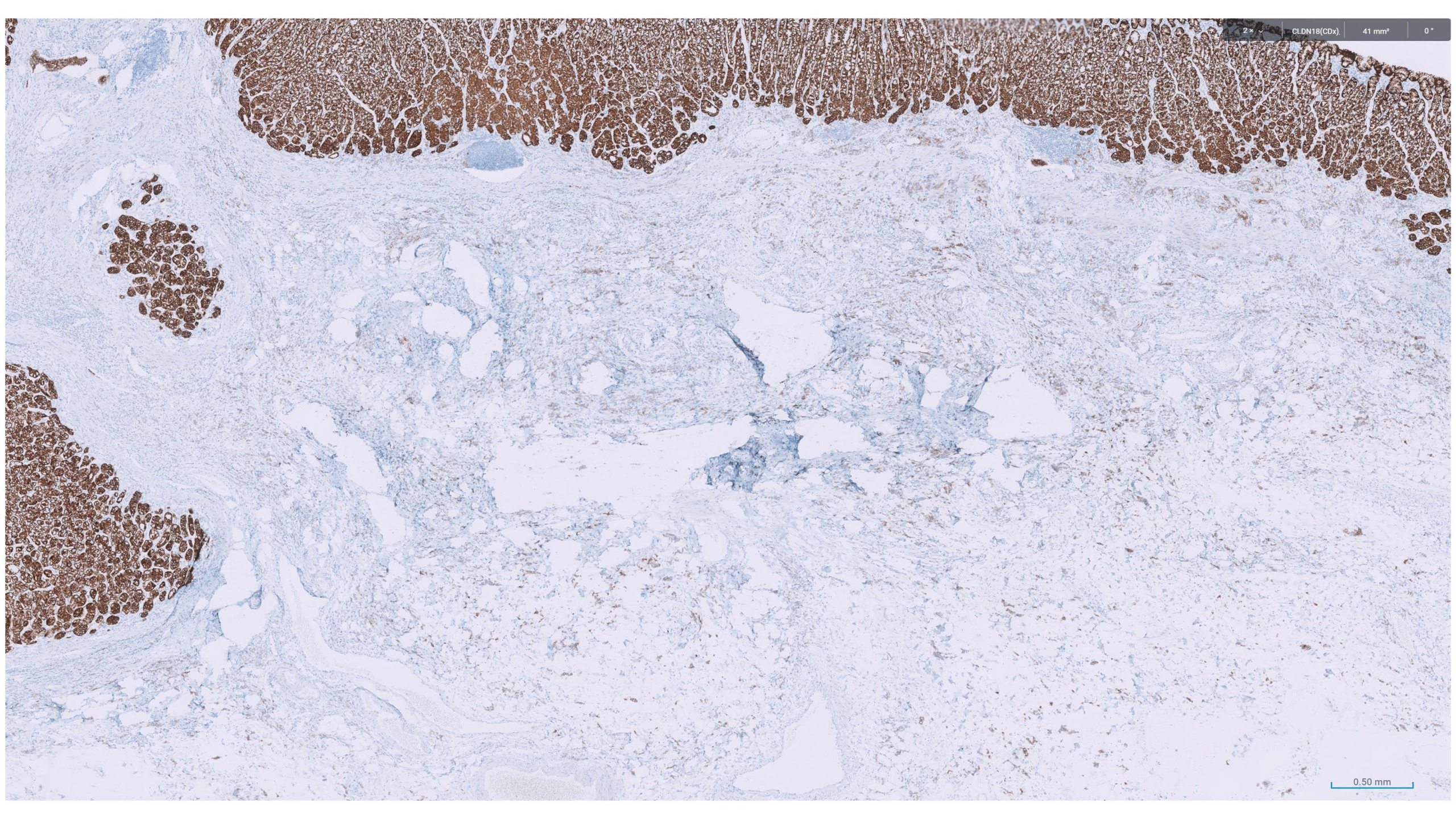


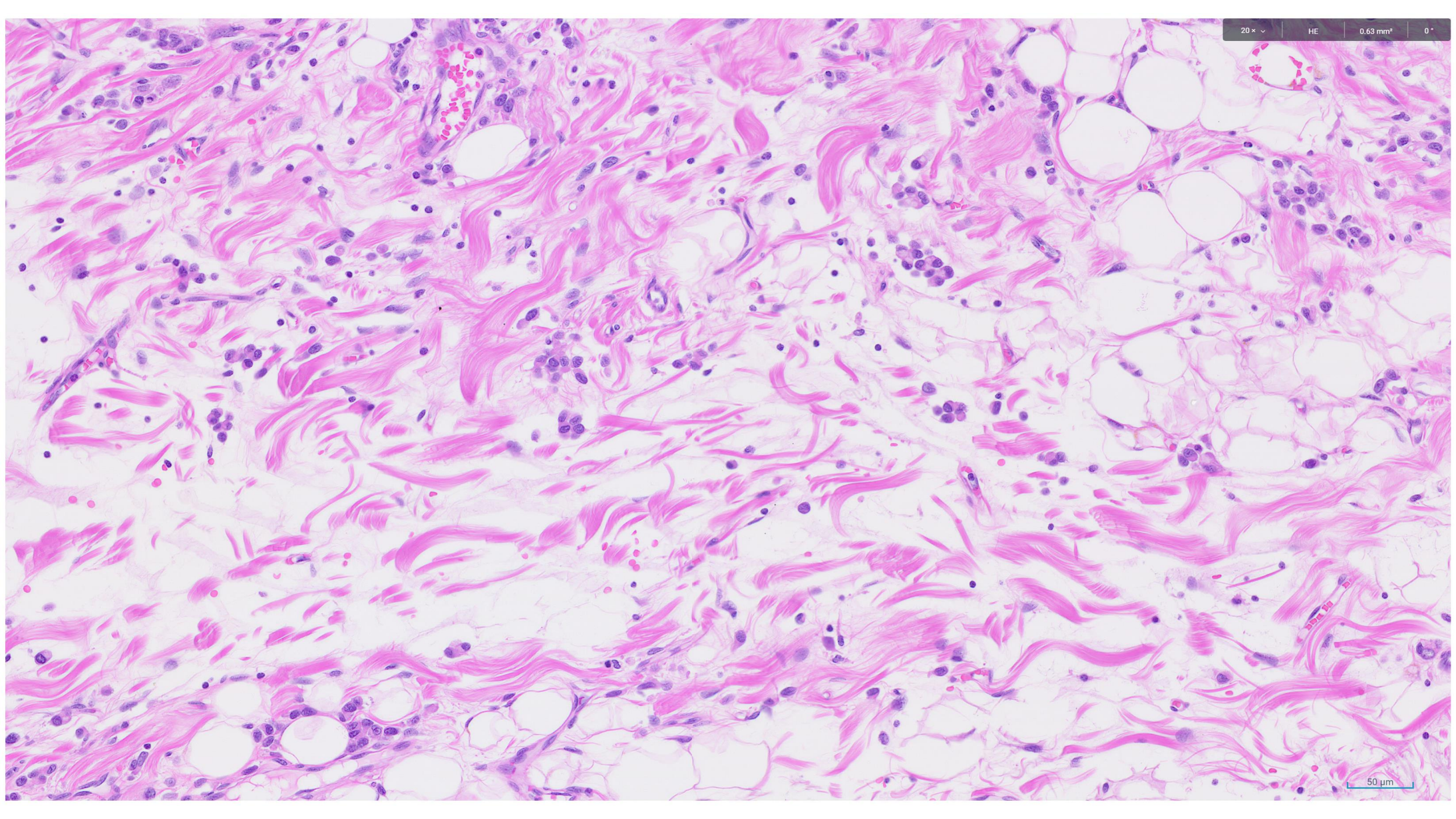


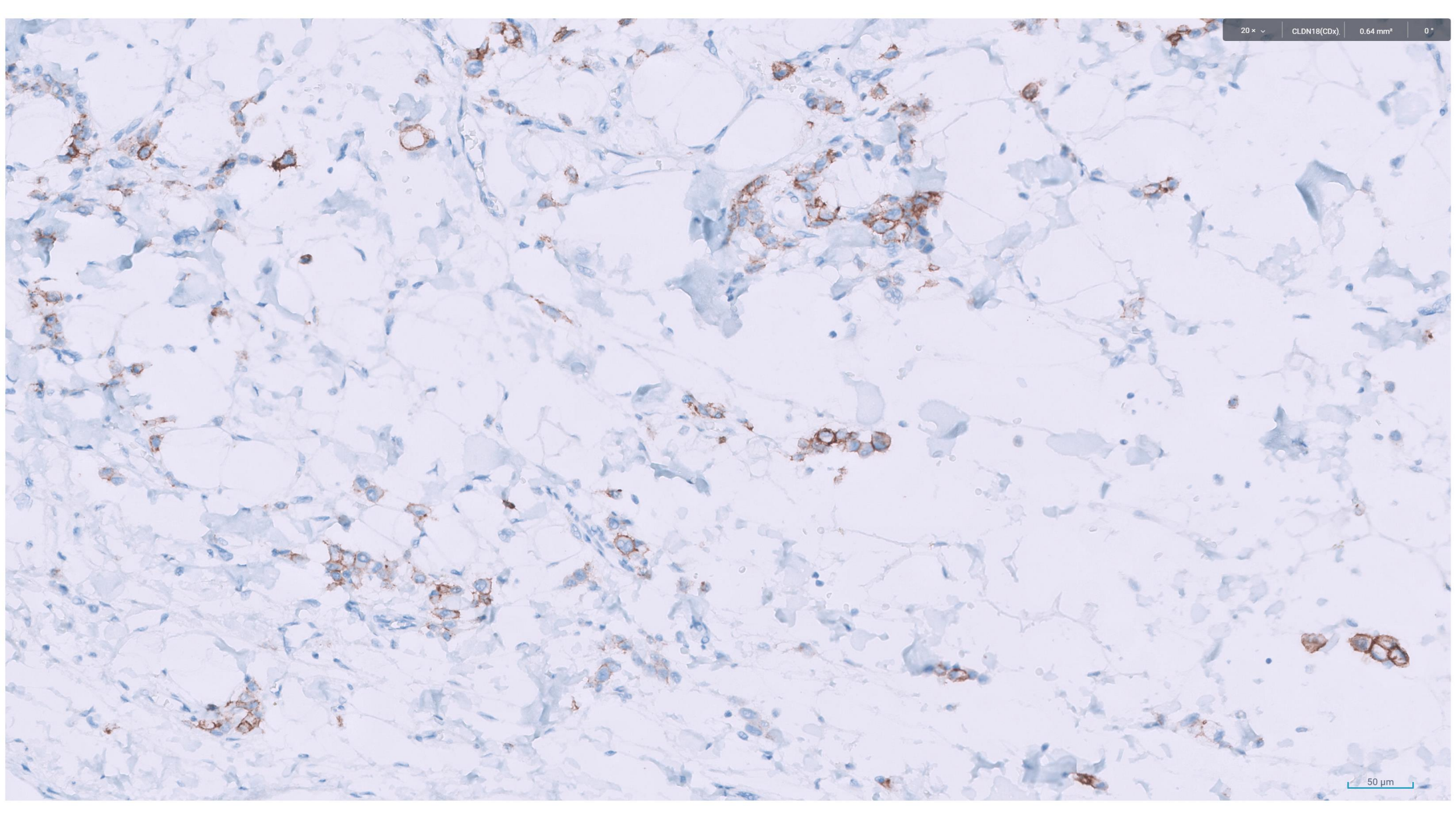












Interpreting stains

Studies have evaluated both membranous staining and percentage of tumor cells stained for CLDN18.2 expression.^{1,2}

Based on 2 studies: one study comparing claudin 18.2 expression in primary tumors and lymph node metastases in Japanese patients with gastric adenocarcinoma and one study investigating the immunohistochemical (IHC) profile of claudin 18 in a series of gastric and GEJ adenocarcinomas.^{1,2}

<https://www.claudin182.com>

CLDN18.2 testing

- Biomarker for **Zolbetuximab** (SPOTLIGHT & GLOW trials)
- IHC using clone **43-14A**
- **≥75%** with moderate to strong intensity
- **Heterogenous** expression like other biomarkers
- Relationship with other biomarkers
 - Her2- & EBV+
 - PD-L1 ?

Summary

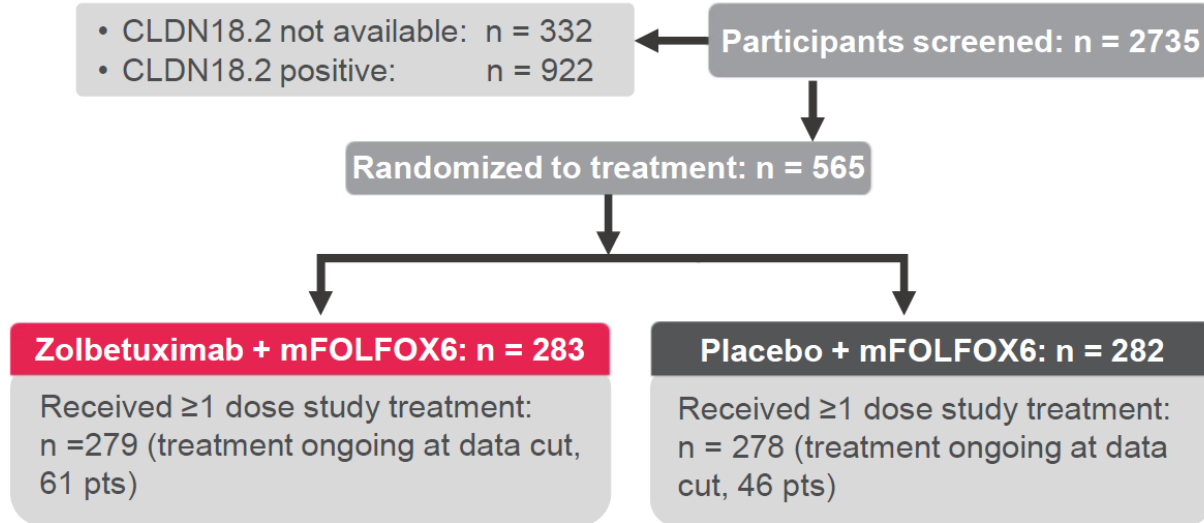
- **Multiple** biomarkers to test in mGC
 - HER2 PD-L1 MSI (PCR or IHC)
 - EBV CLDN18.2 FGFR2b
 - Others (cMET, TRK etc.)
- **Limited** tissue sample in biopsies
 - Should prioritize testing
 - Initial HER2 test (IHC → ISH)
 - Simultaneous staining is favored over stepwise staining (tissue loss)
- **Various** testing methods
 - IHC, ISH, PCR, NGS
- **Heterogeneity** is the issue
 - Multiple biopsy pieces (6-8) or *re-biopsy (primary or metastatic site)*
- Treatment of choice in **multiple biomarker positive** cases

Post lecture survey



PATIENT DISPOSITION

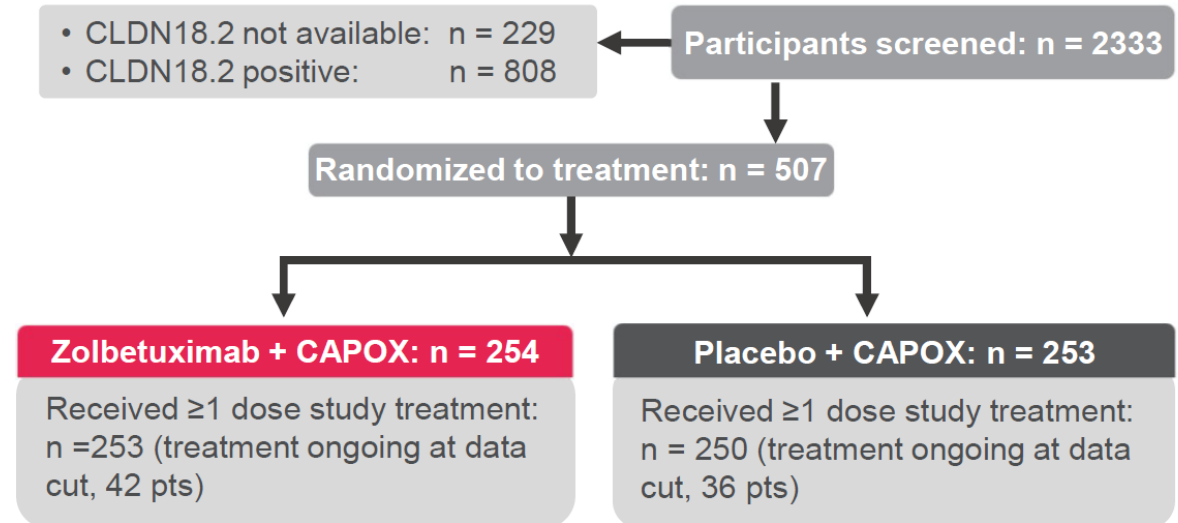
SPOTLIGHT¹



Of all screened patients assessable for CLDN18.2 status, **38.4% met the cut-off for CLDN18.2 positivity***. **PD-L1 CPS of ≥5** was observed in 41/311 (**13%**) of the assessed patients.

Main reason for treatment discontinuation in both arms was disease progression - 133/218 (61%) in the zolbetuximab arm and 177/232 (76%) in the placebo arm.

GLOW²



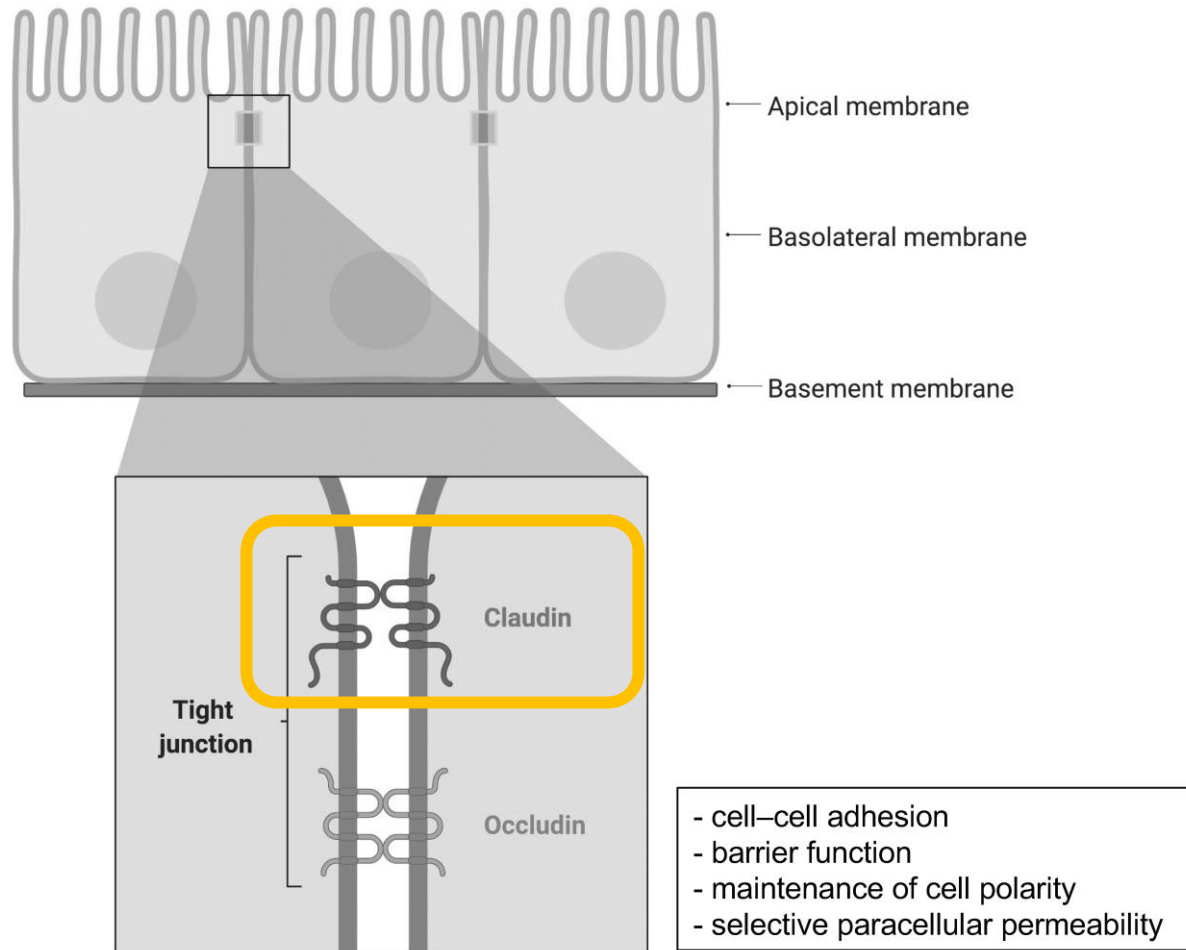
Of all screened patients assessable for CLDN18.2 status, **38.4% met the cut-off for CLDN18.2 positivity***. **PD-L1 CPS of ≥5** was observed in 63/288 (**22%**) of the assessed patients.

Main reason for treatment discontinuation in both arms was disease progression - 100/212 (47%) in the zolbetuximab arm and 143/213 (67%) in the placebo arm.

* Moderate-to-strong staining in ≥ 75% of tumor cells.

CAPOX capecitabine + oxaliplatin regimen; CLDN18.2 claudin-18 isoform 2; mFOLFOX6 modified folinic acid + 5-fluorouracil + oxaliplatin regimen; PD-L1 CPS programmed death-ligand combined positive score

1. Shitara K et al. *Lancet*. 2023; 401: 1655-1668. 2. Shah MA et al. *Nat Med*. 2023; 29: 2133-2141.



b Claudin 18.2

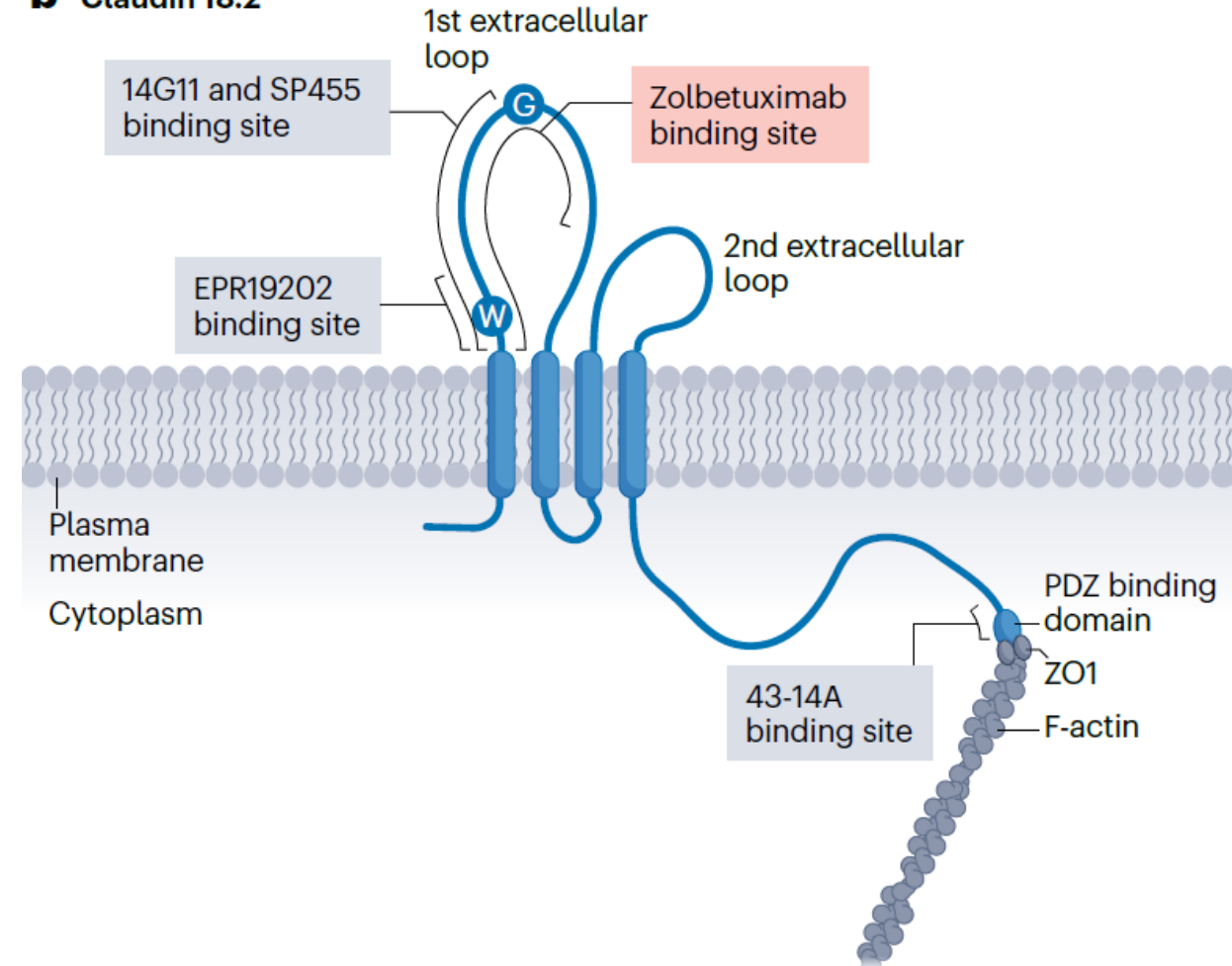
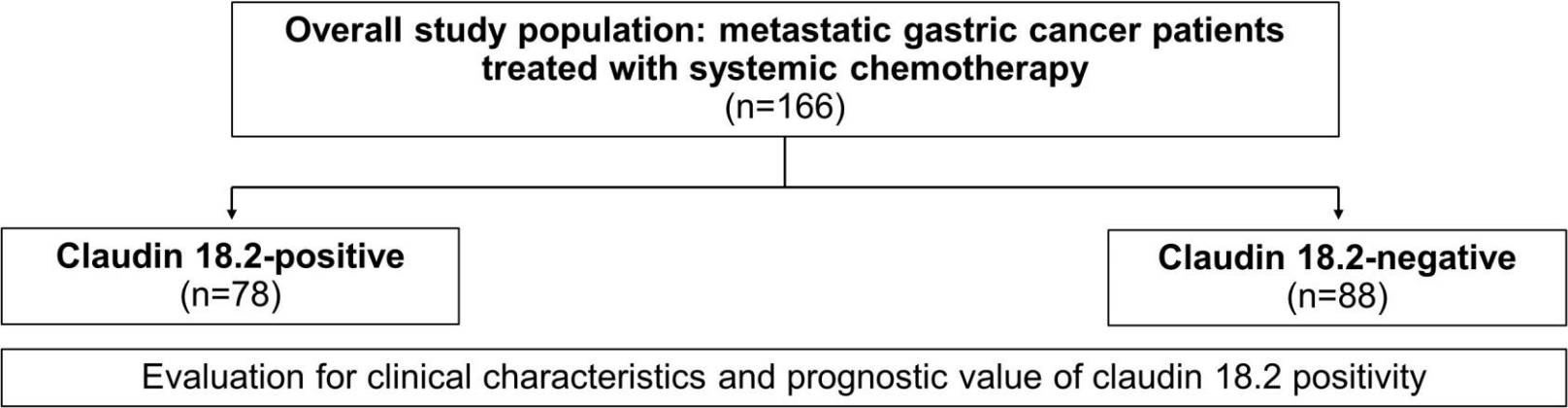
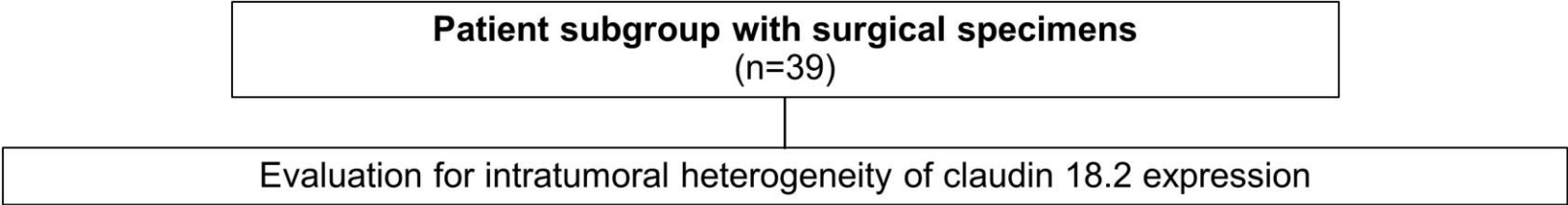


Figure 1.

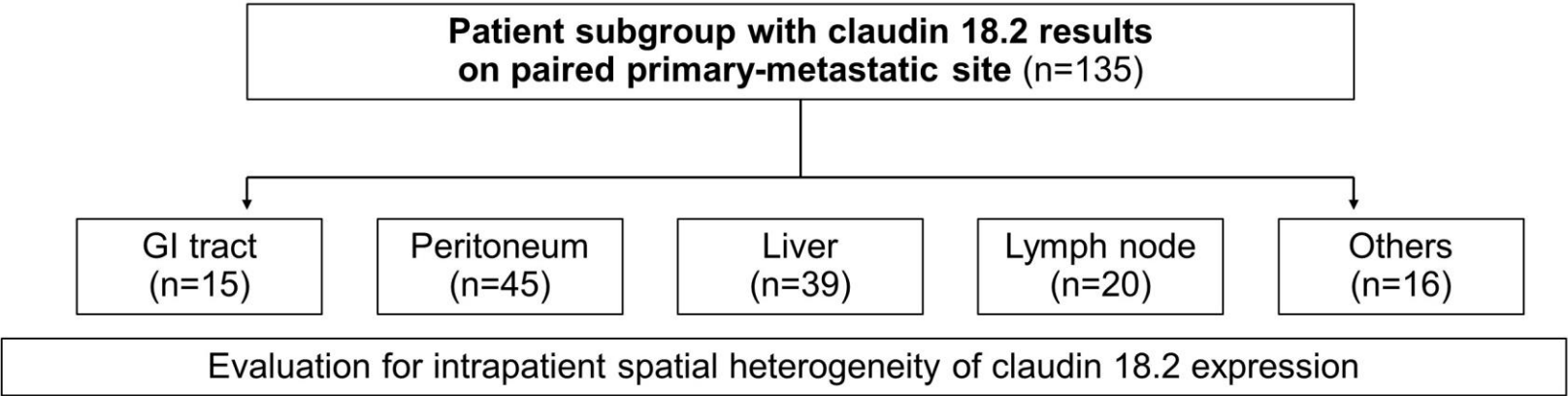
A



B



C



Heterogeneity

- Homogeneous
 - $\geq 90\%$ of the tumor area (moderate or strong intensity): **43.6%** (17/39)
 - 0% (complete negative): **17.9%** (7/39)
- **Heterogeneous**
 - **38.5%** (15/39)
 - **random** pattern: 17.9% (n = 7)
 - superficial pattern: 12.8% (n = 5)
 - invasive-front pattern: 7.7% (n = 3)

Assay & antibodies

Test for CLDN18.2 expression in GC/GEJ cancers

- 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*:

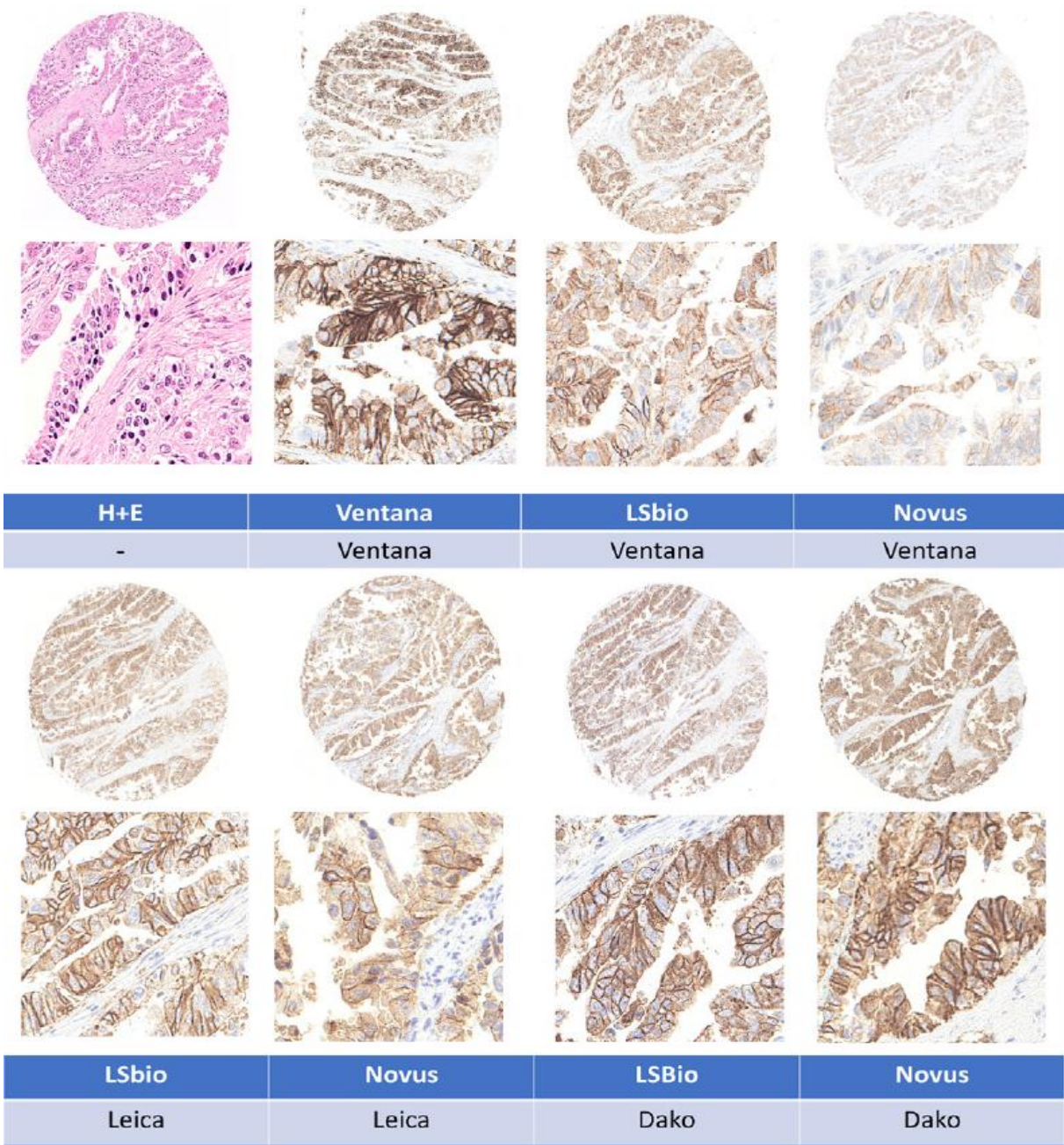
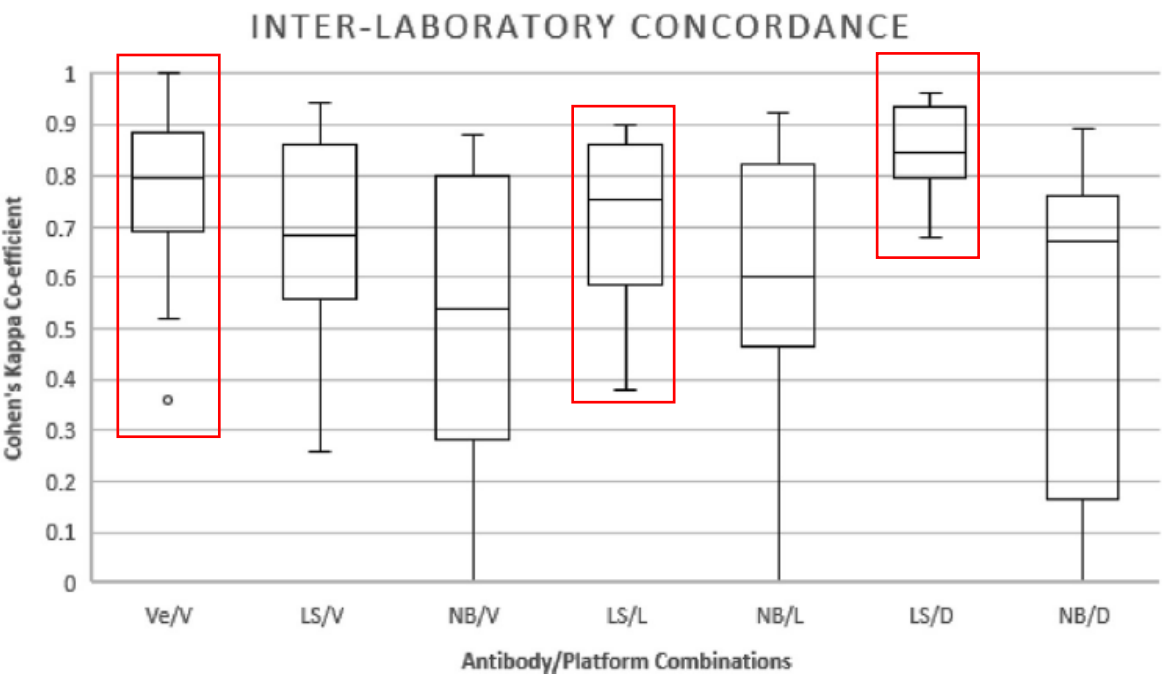
Name	Company	Clone Name	Clonality	Host	Field of Use
VENTANA CLDN18 (43-14A) ASSAY	Roche Tissue Diagnostics	43-14A	Monoclonal	Mouse	IVD
Recombinant Anti-Claudin18 antibody	Abcam	43-14A	Monoclonal	Mouse	RUO
PathPlus™ Claudin 18 Antibody	LSBio	LS-B16145	Monoclonal	Mouse	RUO
CLDN18 antibody	Novus Biologicals	NBP2-32002	Polyclonal	Rabbit	RUO

- 43-14A, LS-B16145, NBP2-32002: pan-CLDN18
- SP455 and EPR19202(Abcam): CLDN18.2-specific

Analytical concordance study (Global ring study)

Bharat Jasani et al. *Lab Invest* 2024 Jan;104(1):100284

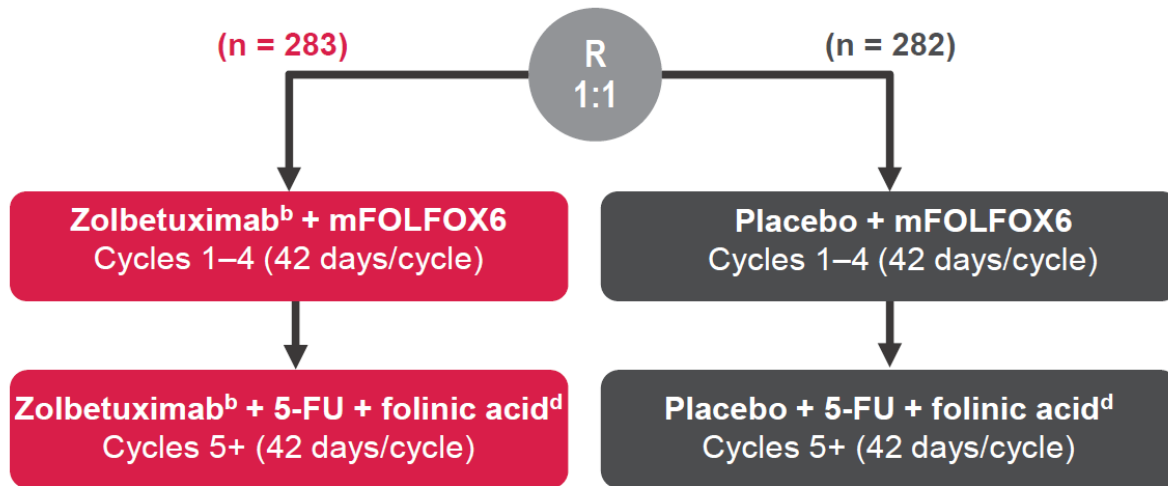
- 3 antibodies / 3 platforms
- 27 Labs
- 15 GCs / TMA



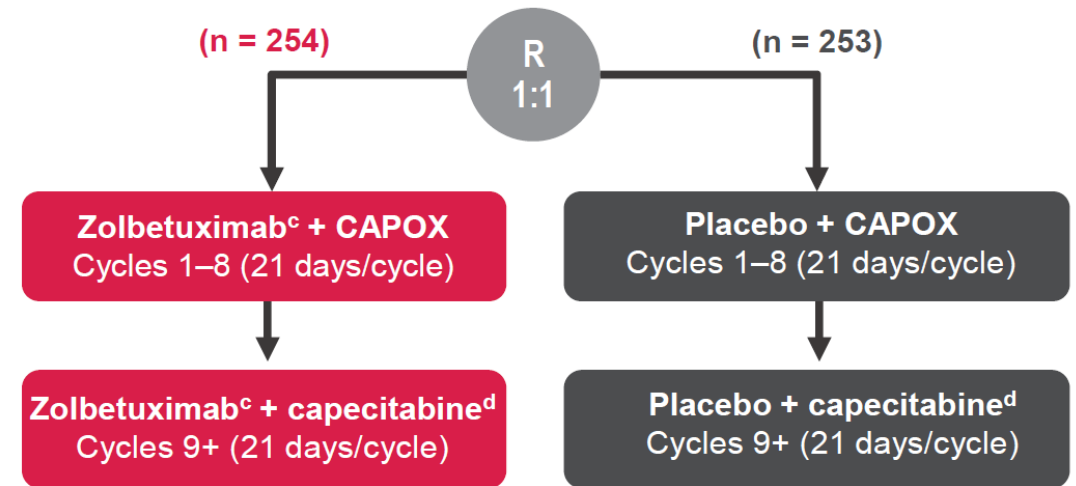
STUDY DESIGNS

SPOTLIGHT and **GLOW** are both global, randomized, double-blinded, placebo-controlled, **phase 3 studies**. Both studies investigated the use of **Zolbetuximab + chemo** (mFOLFOX6 for SPOTLIGHT and CAPOX for GLOW) in patients with **previously untreated, CLDN18.2 positive^a, HER2-, LA unresectable or mG/GEJ adenocarcinoma**. The **primary endpoint** in both studies was **PFS**.^{1,2}

SPOTLIGHT¹



GLOW²



^aModerate-to-strong staining in $\geq 75\%$ of tumor cells; ^bQ3W, 800 mg/m² at cycle 1 day 1 followed by 600 mg/m² on cycle 1 day 22 and days 1 and 22 of subsequent cycles; ^cQ3W, 800 mg/m² at cycle 1 day 1 followed by 600 mg/m² on day 1 of subsequent cycles; ^dChemo could be continued at the investigator's discretion

5-FU 5-fluorouracil; CAPOX capecitabine + oxaliplatin regimen; CLDN18.2 claudin-18 isoform 2; HER2 human epidermal growth factor receptor; LA locally advanced; mFOLFOX6 modified folinic acid + 5-fluorouracil + oxaliplatin regimen; mG/GEJ metastatic gastric or gastroesophageal junction; PFS progression-free survival; R Randomized; Q3W every 3 weeks;

1. Shitara K et al. *Lancet*. 2023; 401: 1655-1668. 2. Shah MA et al. *Nat Med*. 2023; 29: 2133–2141.

CLDN18.2 in **resectable GCs**

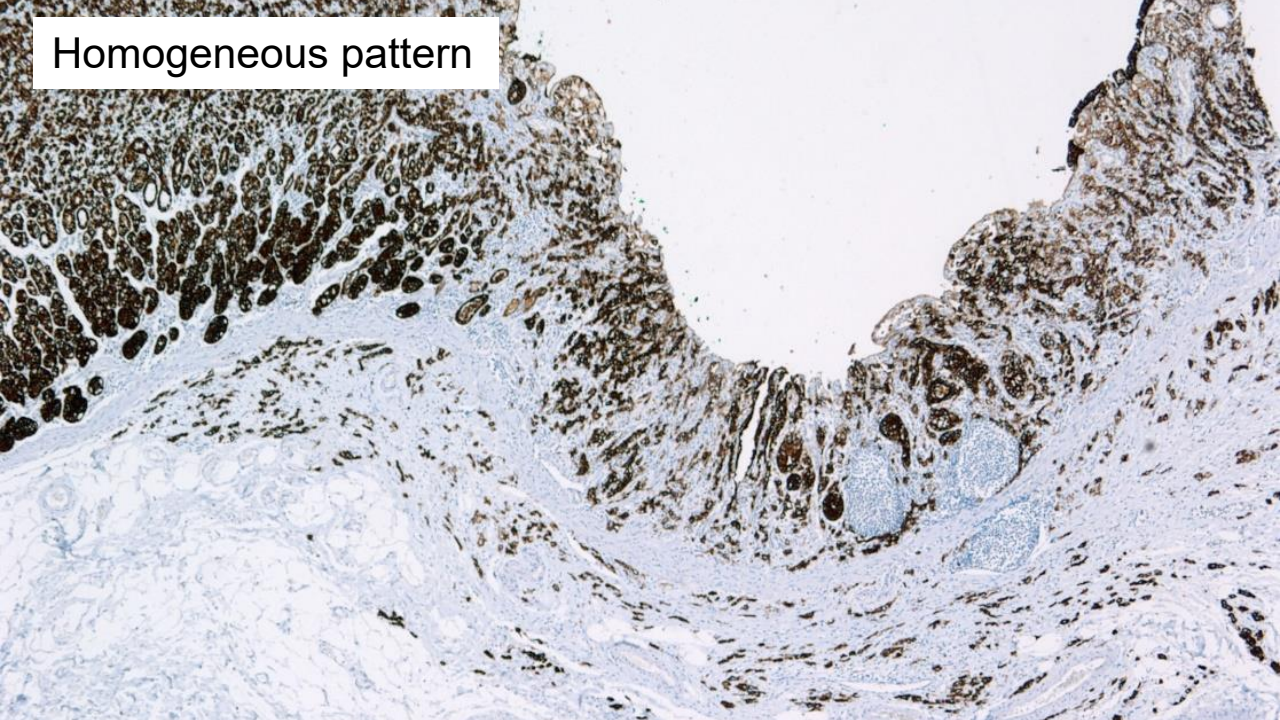
- **299** patients
 - Stage I (90), II (96) & III (113)
 - Curative surgical resection (2018 Mar ~ 2019 Feb)
- **43-14A** IHC with 75% cut-off
- CLDN18.2 positive GC
 - **46.5%**
 - Younger age
 - Lower invasion depth
 - Less lymphovascular invasion
 - **Borrmann type 4**
 - **Diffuse type**
 - **HER2 negative, EBV positive**
 - No prognostic significance

Clinicopathologic characteristics according to claudin 18.2 positivity

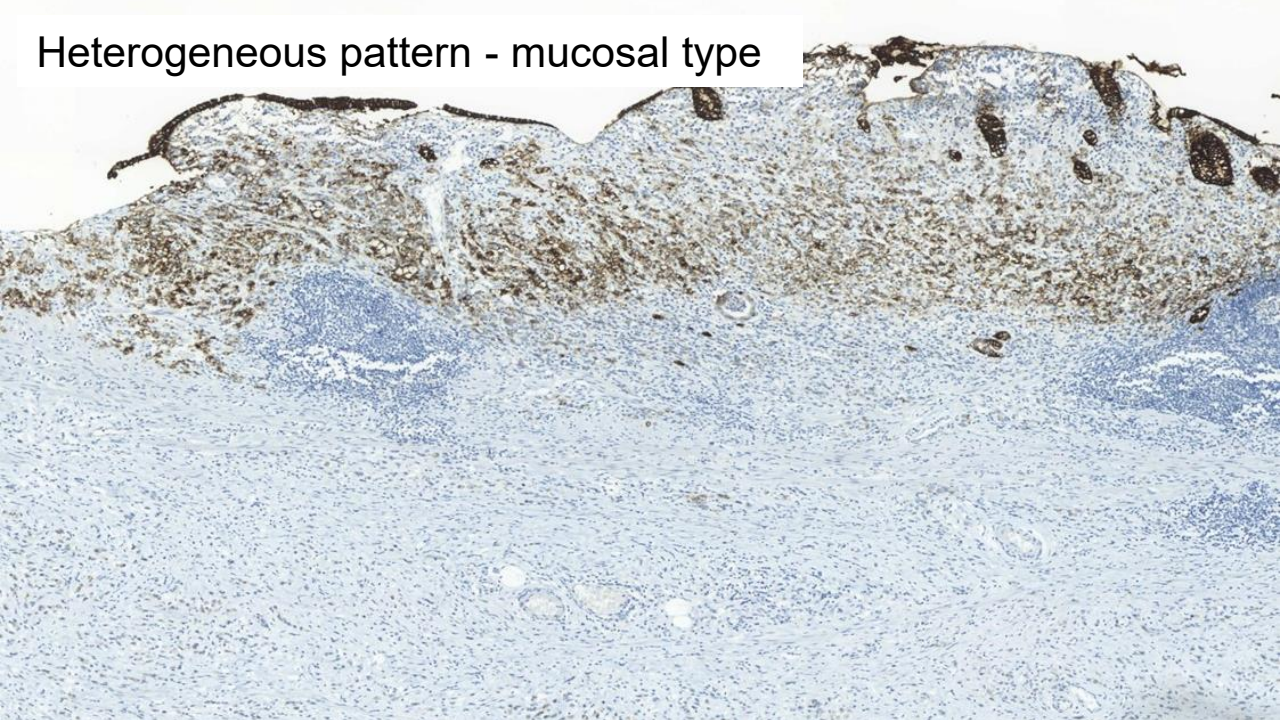
Characteristics	Overall study population (n = 299)	Claudin 18.2 negative (n = 160)	Claudin 18.2 positive (n = 139)	p-value
Median age (years)	63 (27–95)	66 (33–95)	61 (27–83)	<0.001
Male sex	205 (68.6)	118 (73.8)	87 (62.6)	0.051
Gross type				0.014
EGC	70 (23.4)	28 (17.5)	42 (30.2)	
AGC	229 (76.6)	132 (82.5)	97 (69.8)	
AGC subtype	(n = 299)	(n = 132)	(n = 97)	0.008
Borrmann type 1	9 (3.9)	8 (6.1)	1 (1.0)	
Borrmann type 2	49 (21.4)	36 (27.3)	13 (13.4)	
Borrmann type 3	108 (47.2)	57 (43.2)	51 (52.6)	
Borrmann type 4	34 (14.8)	14 (10.6)	20 (20.6)	
EGC-like or unclassifiable	29 (12.7)	17 (12.9)	12 (12.4)	
Location				0.537
Lower/middle	237 (79.3)	129 (80.6)	108 (77.7)	
Upper	58 (19.4)	30 (18.8)	28 (20.1)	
Entire	4 (1.3)	1 (0.6)	3 (2.2)	74

Characteristics	Overall study population (n = 299)	Claudin 18.2 negative (n = 160)	Claudin 18.2 positive (n = 139)	p-value
WHO classification				0.089
WD/MD/papillary	110 (36.8)	68 (42.5)	42 (30.2)	
PD/PD with SRC/SRCa	166 (55.5)	81 (50.6)	85 (61.2)	
Others	23 (7.7)	11 (6.9)	12 (8.6)	
Lauren classification				0.011
Intestinal type	113 (37.8)	72 (45.0)	41 (29.5)	
Diffuse type	120 (40.1)	53 (33.1)	67 (48.2)	
Mixed/indeterminate type	66 (22.1)	35 (21.9)	31 (22.3)	
Invasion depth				0.035
Mucosa/submucosa	70 (23.4)	28 (17.5)	42 (30.2)	
Proper muscle	62 (20.7)	40 (25.0)	22 (15.8)	
Subserosa	73 (24.4)	42 (26.2)	31 (22.3)	
Serosa/adjacent organ	94 (31.4)	50 (31.2)	44 (31.7)	
Lymph node metastasis	183 (61.4)	106 (66.7)	77 (55.4)	0.061
Lymphovascular invasion	198 (66.4)	120 (75.5)	78 (56.1)	<0.001
Perineural invasion	116 (38.8)	64 (40.0)	52 (37.4)	0.734
Overall pathologic stage (AJCC 7 th)				0.379
I	90 (30.1)	44 (27.5)	46 (33.1)	
II	96 (32.1)	50 (31.2)	46 (33.1)	
III	113 (37.8)	66 (41.2)	47 (33.8)	75

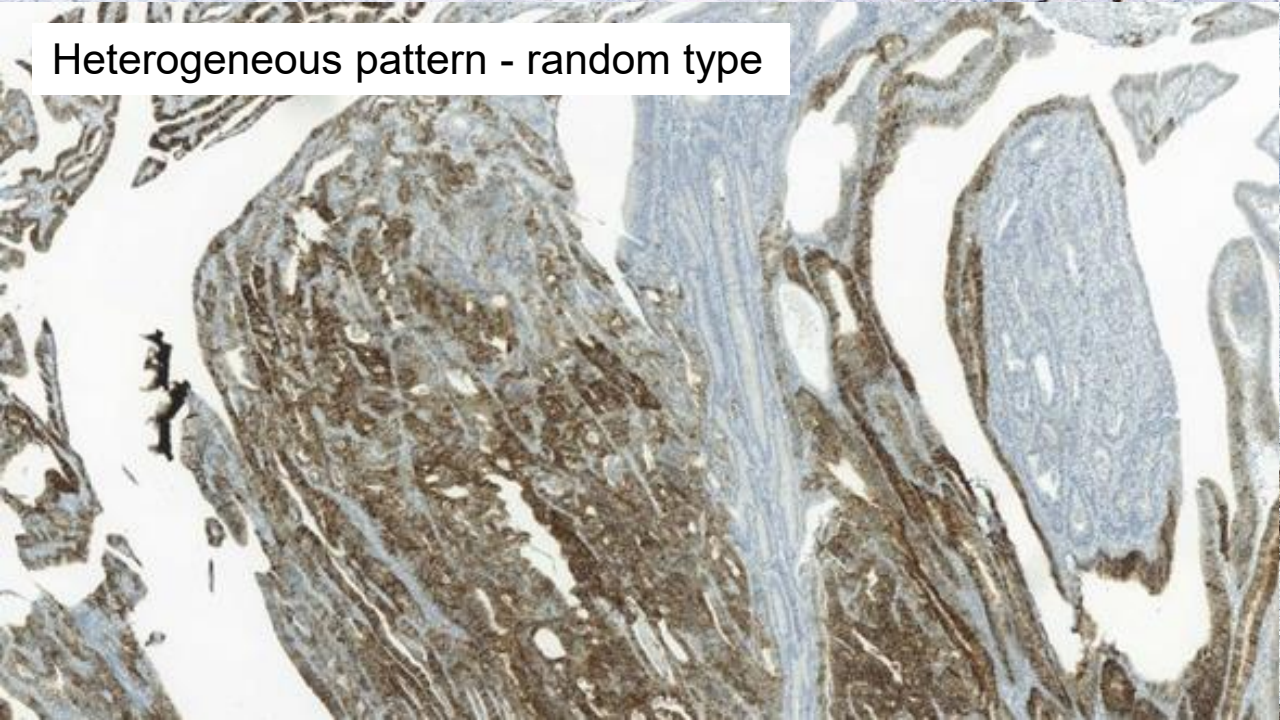
Homogeneous pattern



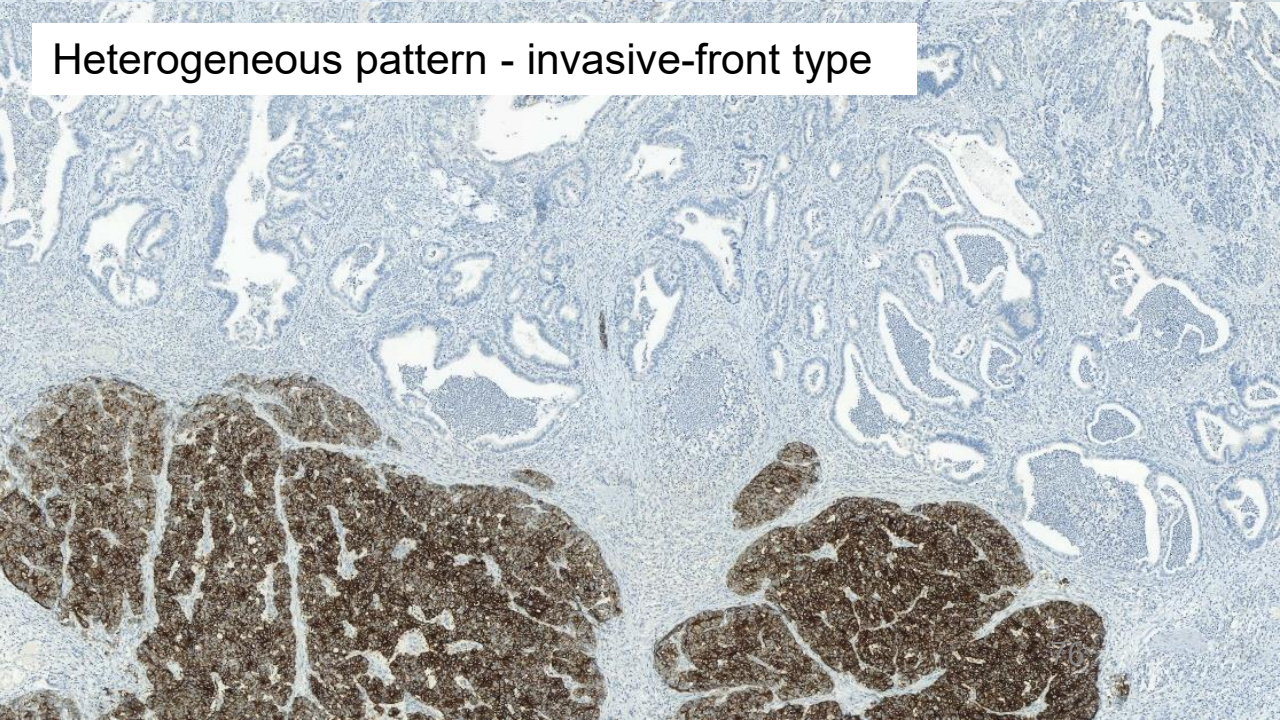
Heterogeneous pattern - mucosal type



Heterogeneous pattern - random type



Heterogeneous pattern - invasive-front type



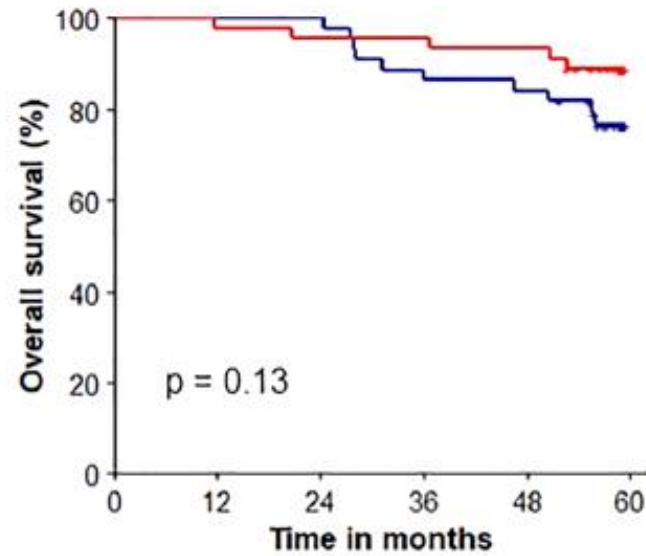
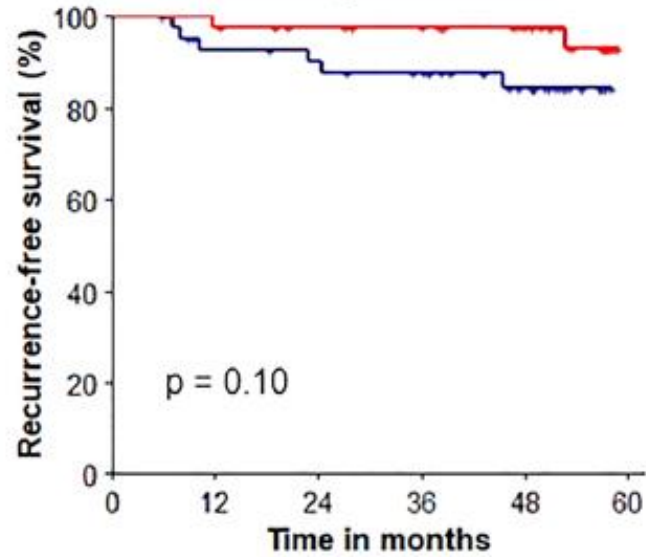
Heterogeneity

- Homogeneous
 - $\geq 90\%$ of the tumor area (moderate or strong intensity)
 - 69% (96/139 out of 299)
- **Heterogeneous**
 - within claudin 18.2 positivity ($\geq 75\%$), i.e. 75 ~ 90% positive
 - 31% (43/139 out of 299)
 - Random type most common (n=40)
 - previous reports: 40 ~ 50%
 - All CLDN negative cases (i.e. $< 75\%$)

— Claudin 18.2 positive
— Claudin 18.2 negative

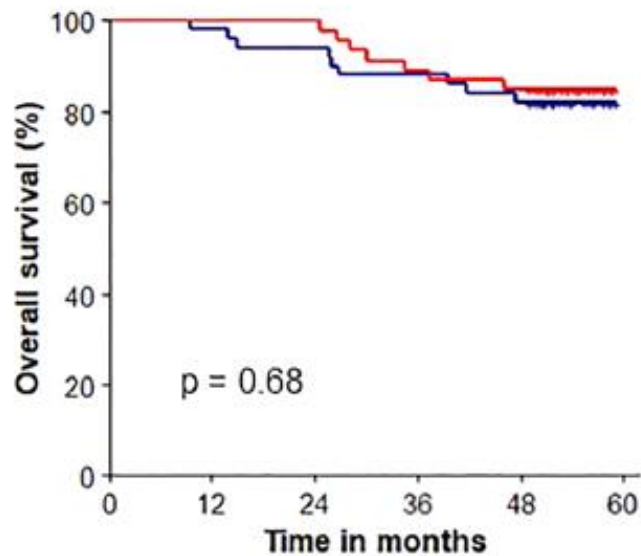
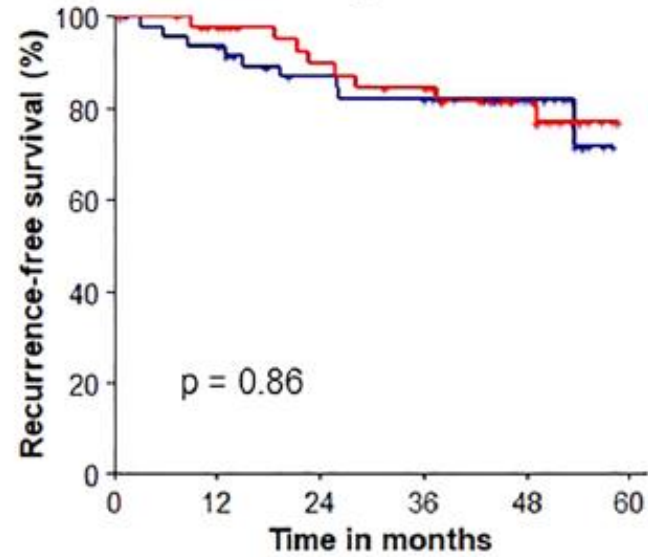
A

Stage I



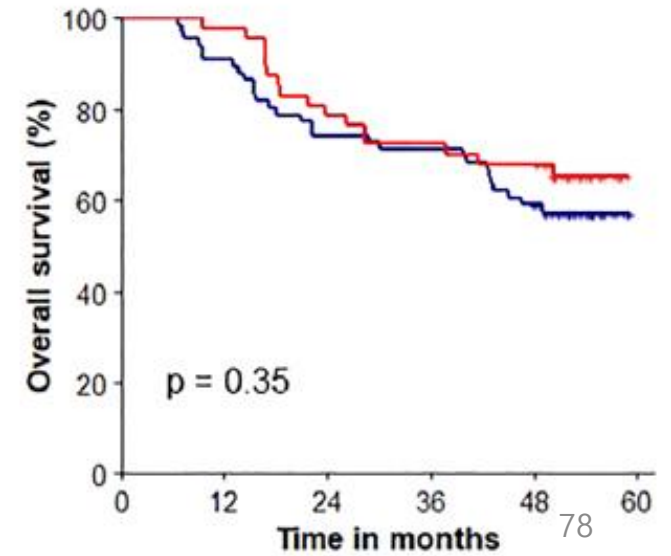
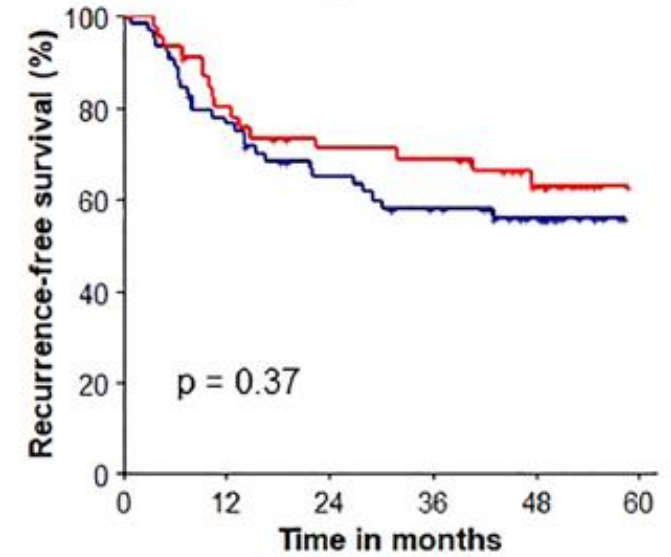
B

Stage II



C

Stage III



CLDN18.2 & other biomarkers (stage ≤ 3)

Claudin 18.2 positivity according to HER2

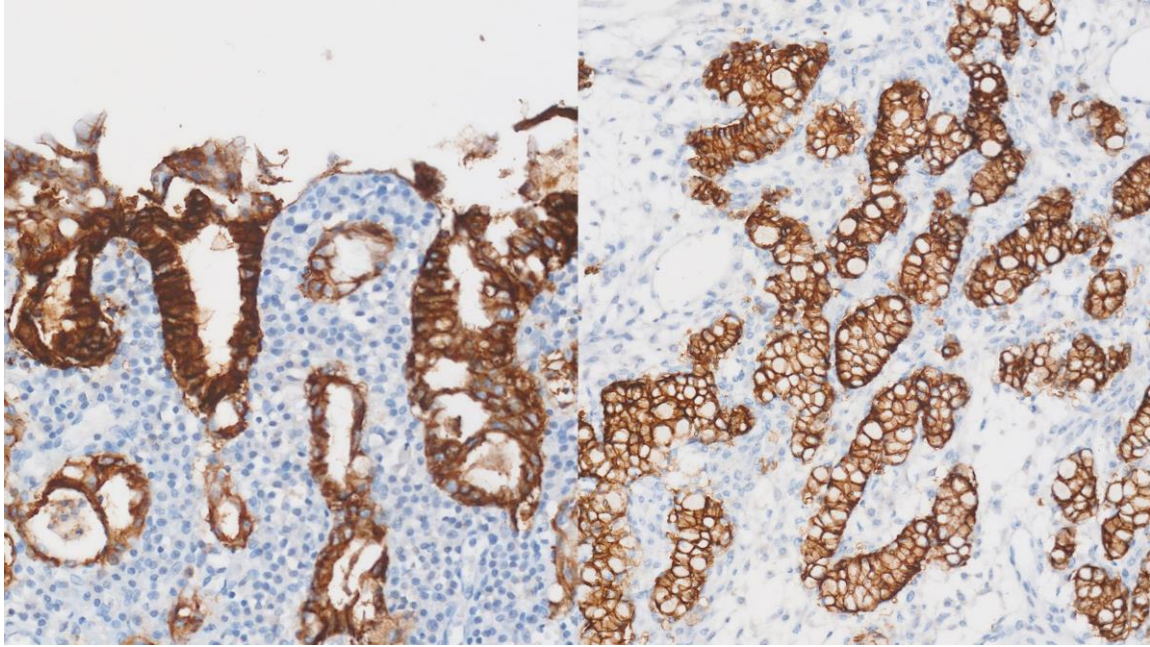
	HER2 positive (n = 19)	HER2 negative (n = 271)	p-value
Claudin 18.2 negative	16 (84.2)	139 (51.3)	0.011
Claudin 18.2 positive	3 (15.8)	132 (48.7)	

Claudin 18.2 positivity according to EBV

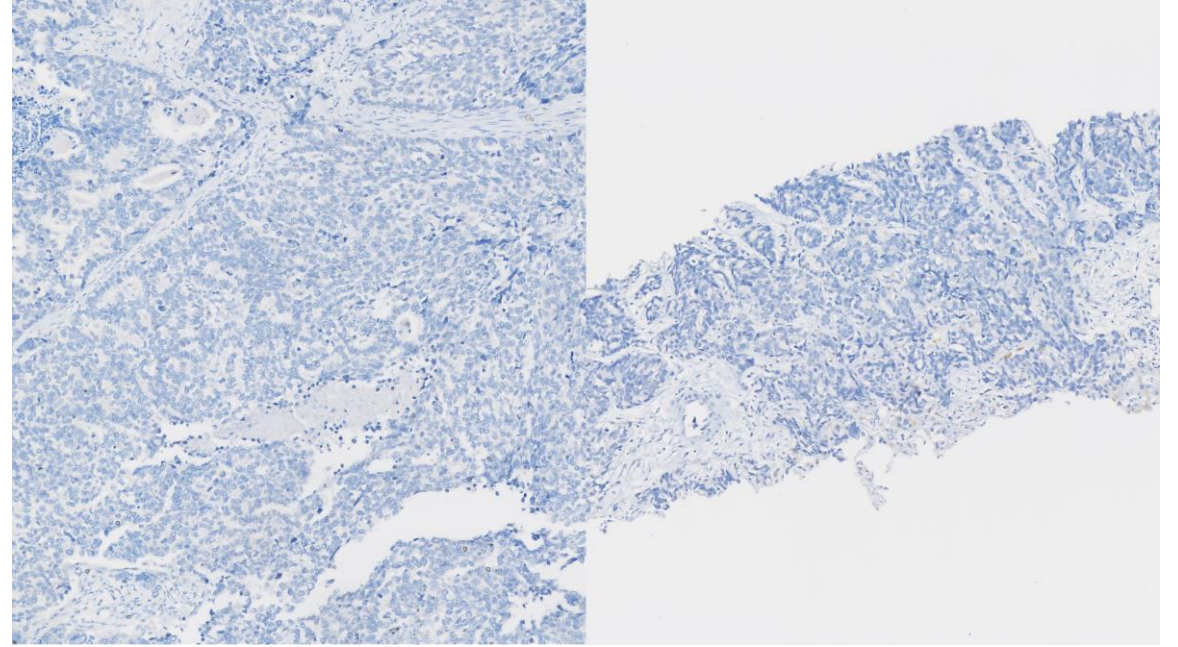
	EBV positive (n = 18)	EBV negative (n = 267)	p-value
Claudin 18.2 negative	5 (27.8)	146 (54.7)	0.049
Claudin 18.2 positive	13 (72.2)	121 (45.3)	

CLDN18.2 in **stage IV GCs**

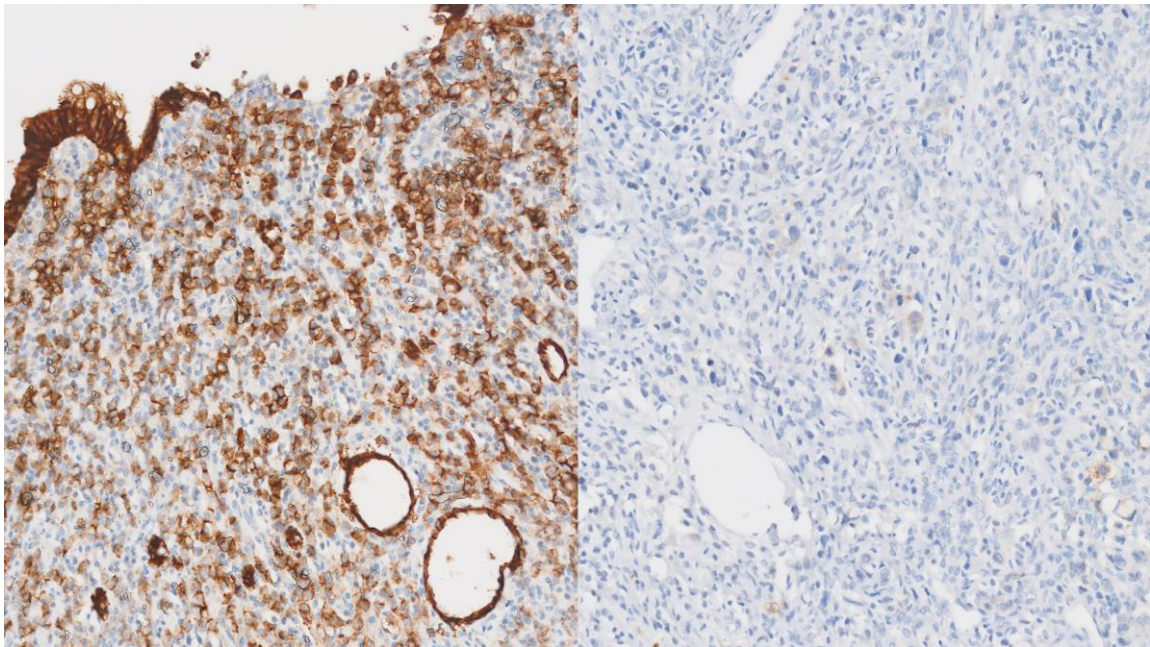
- **166** patients
 - 39 gastrectomy cases
 - 135 patients with primary – metastatic site pairs
- **43-14A** IHC with 75% cut-off
- CLDN18.2 positive GC
 - **47.0%**
 - **Peritoneal meta** > hepatic & distant LN meta
 - Intratumoral heterogeneity in **38.0%** of surgical specimens
 - Primary – metastatic discrepancy in **25.2%**
 - No prognostic significance



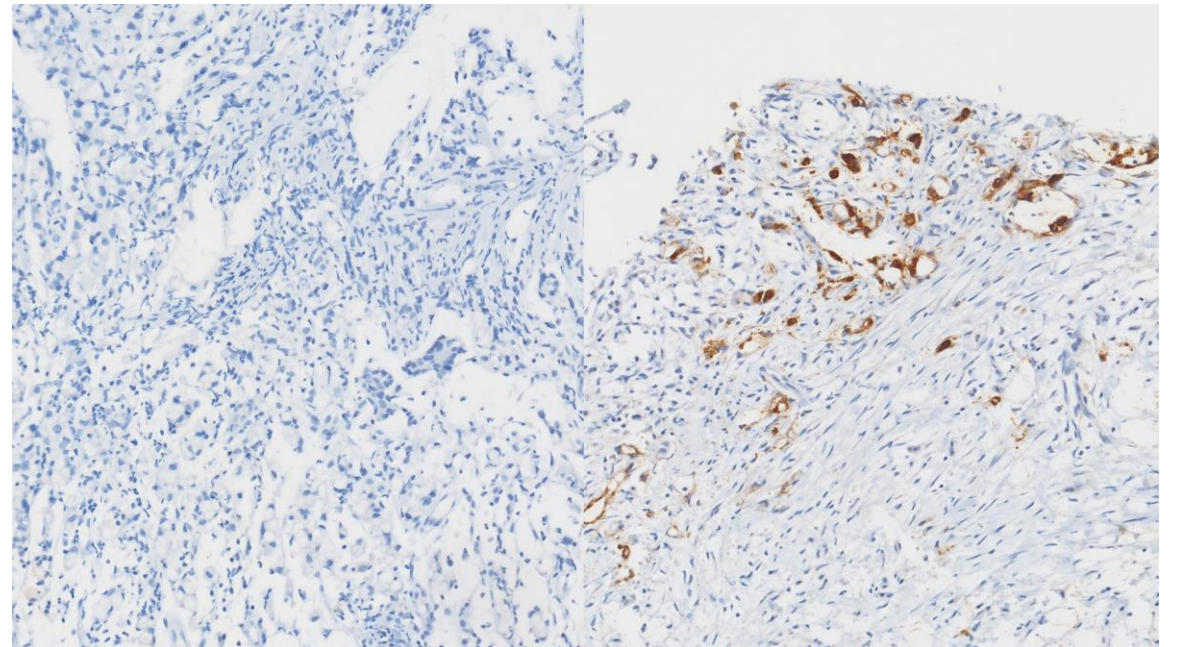
Positively concordant



Negatively concordant



Discordant: claudin 18.2 expression only in primary site



Discordant: claudin 18.2 expression only in metastatic site

Concordance of claudin 18.2 expression in primary and metastatic sites of paired cases (discrepancy in 25.2%)

		Claudin 18.2 at primary sites	
		Negative	Positive
Claudin 18.2 at the metastatic sites	Negative	67	14
	Positive	20	34

B

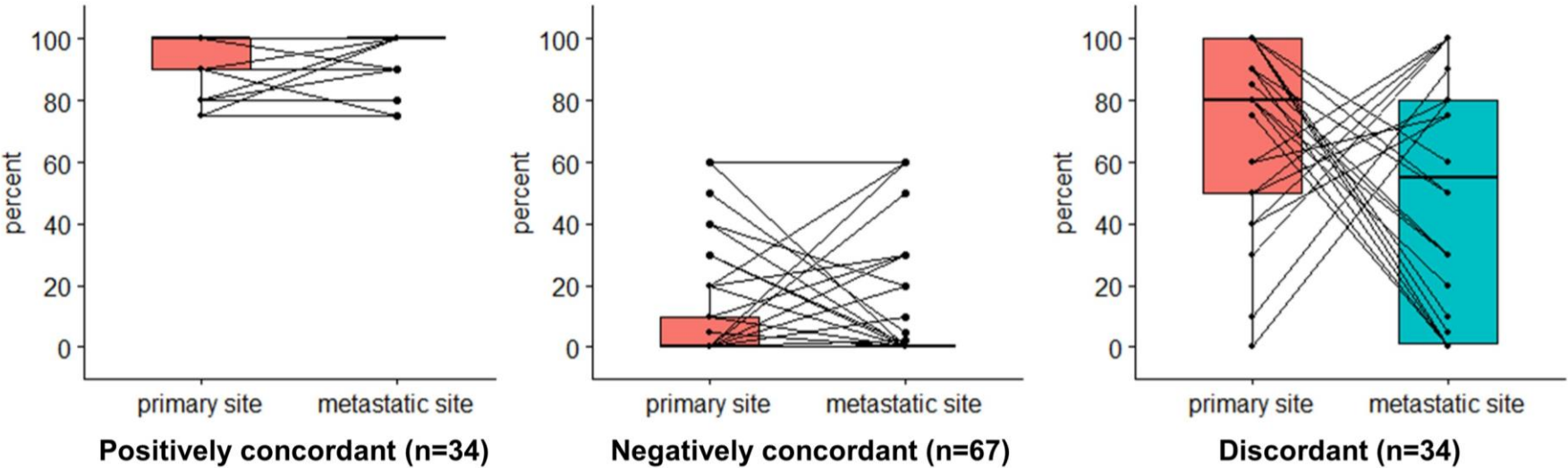
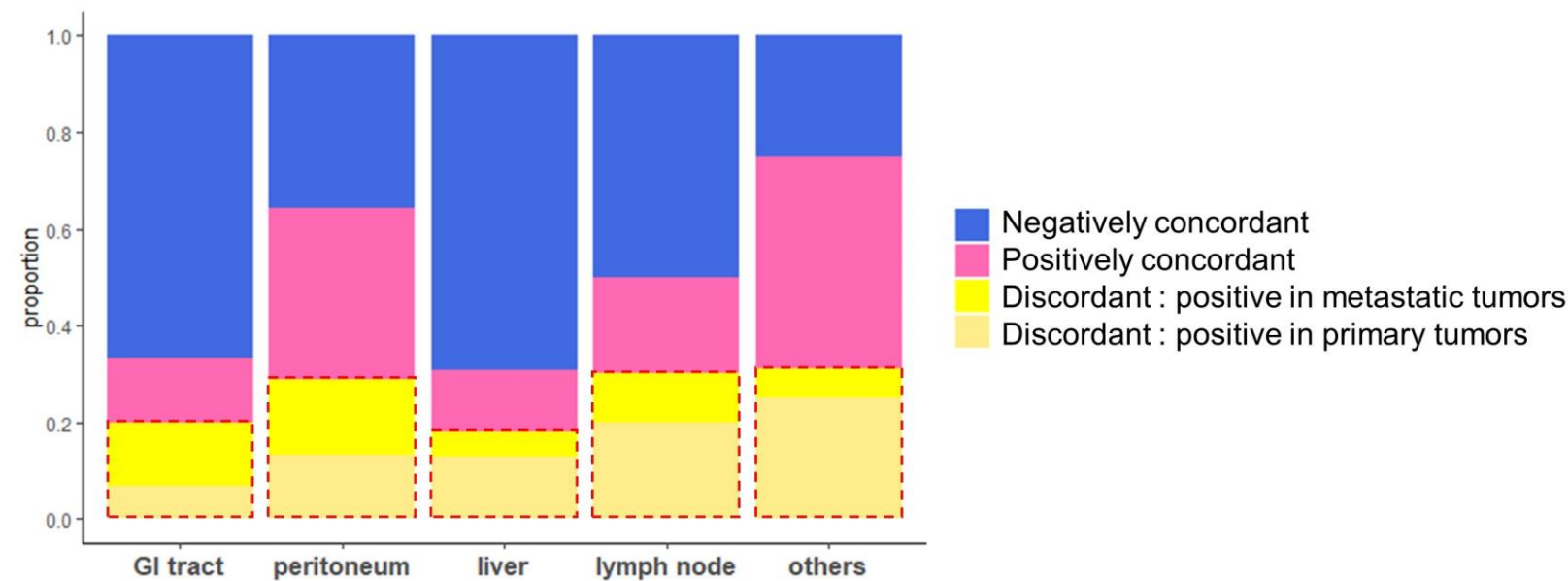


Figure 4.

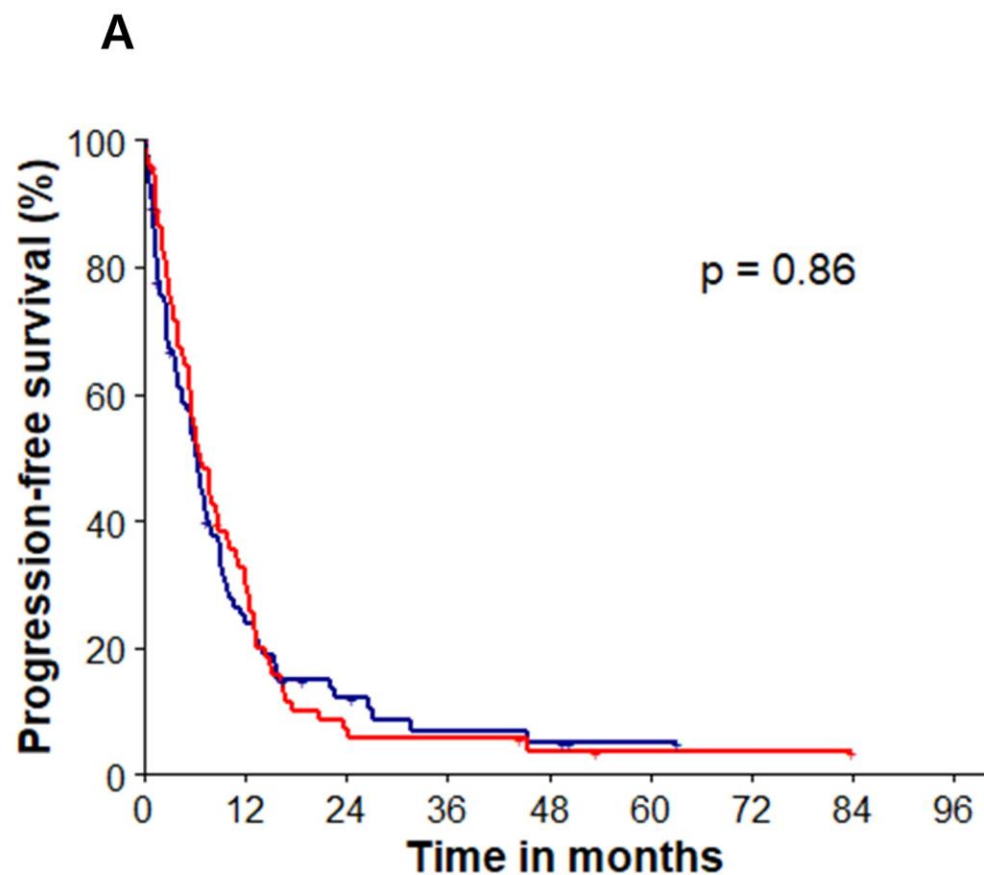


	GI tract (n=15)	peritoneum (n=45)	liver (n=39)	lymph node (n=20)	Others (n=16)
Negatively concordant	10 (66.7)	16 (35.6)	27 (69.2)	10 (50.0)	4 (25.0)
Positively concordant	2 (13.3)	16 (35.6)	5 (12.8)	4 (20.0)	7 (43.8)
Discordant: positive in metastatic tumors	2 (13.3)	7 (15.6)	2 (5.1)	2 (10.0)	1 (6.2)
Discordant: positive in primary tumors	1 (6.7)	6 (13.3)	5 (12.8)	4 (20.0)	4 (25.0)

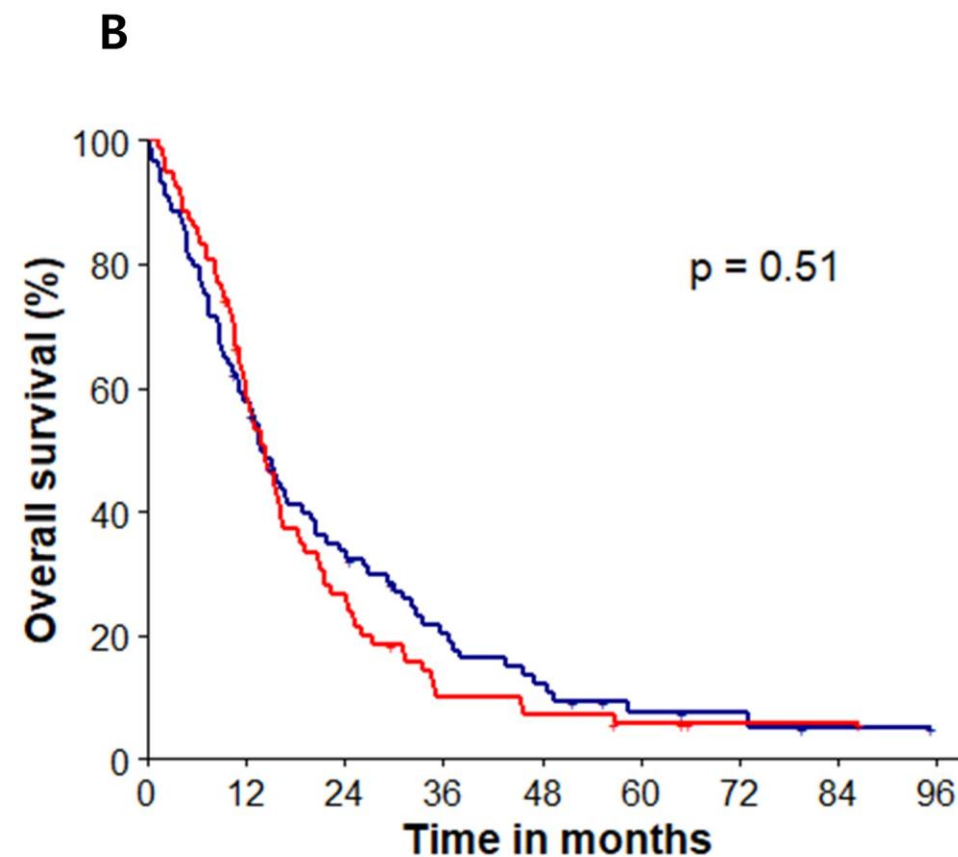
Figure 2.

— Claudin 18.2-negative (n=88)

— Claudin 18.2-positive (n=78)



Median PFS (95% CI):
Claudin 18.2 negative= 6.3 (4.2-7.8) months
Claudin 18.2 positive= 6.6 (5.3-9.0) months

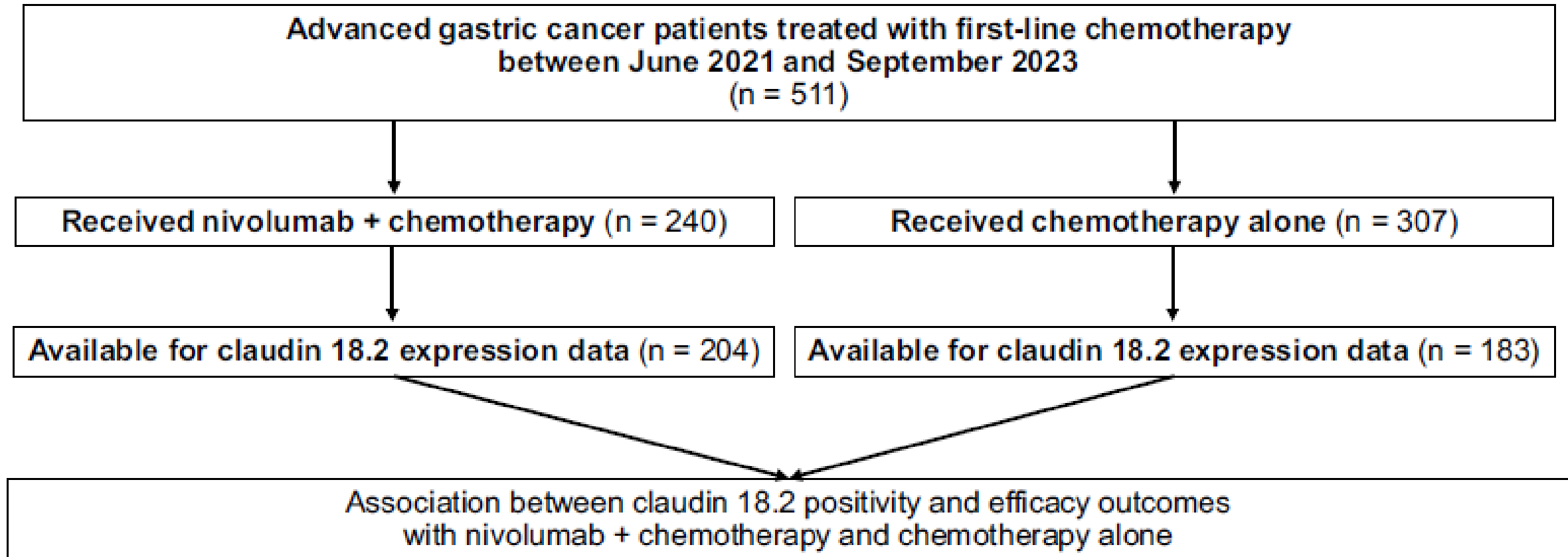


Median OS (95% CI):
Claudin 18.2 negative= 13.8 (11.2-18.9) months
Claudin 18.2 positive= 14.4 (11.7-16.2) months

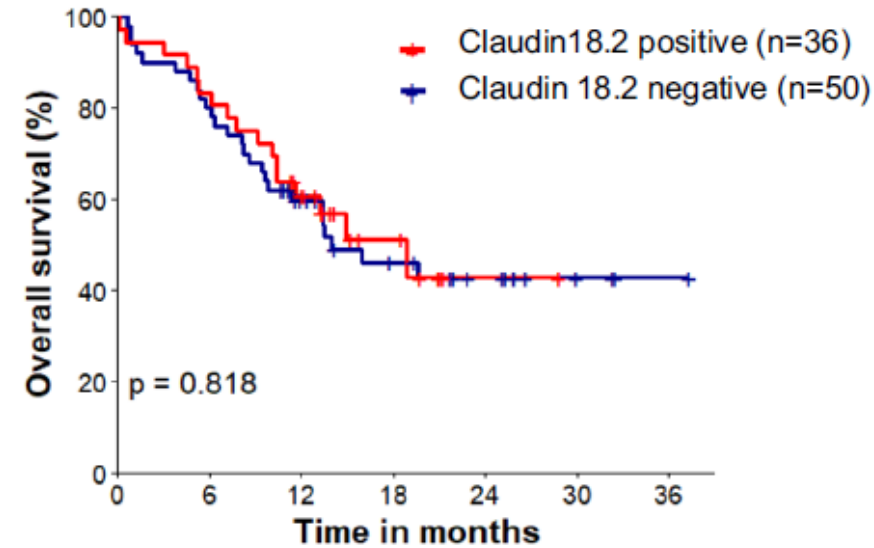
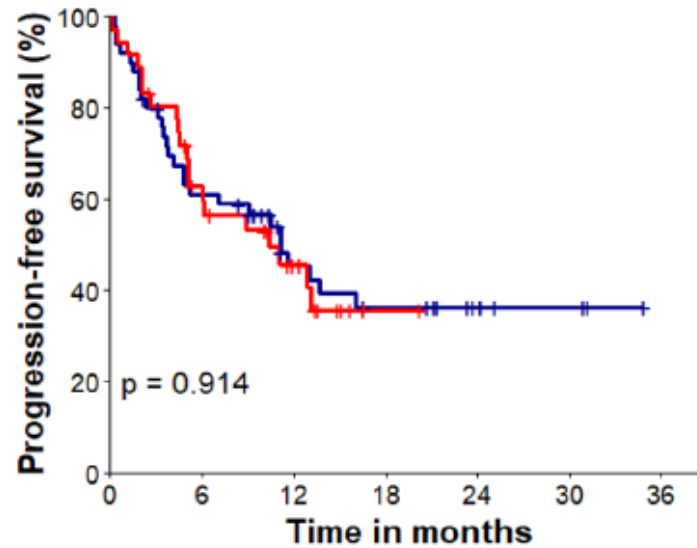
CLDN18.2 & other biomarkers (stage 4)

Variables	Claudin 18.2 negative (n = 88)	Claudin 18.2 positive (n = 78)	p-value
Histology			0.113
WD/MD	35 (39.8)	21 (26.9)	
PD	53 (60.2)	57 (73.1)	
EBV expression status	(n = 80)	(n = 71)	0.188
Negative	79 (98.8)	67 (94.4)	
Positive	1 (1.2)	4 (5.6)	
HER2 expression status			0.180
Negative	66 (75.0)	66 (84.6)	
Positive	22 (25.0)	12 (15.4)	
MSI status	(n = 60)	(n = 54)	0.999
MSS	59 (98.3)	53 (98.1)	
MSI high	1 (1.7)	1 (1.9)	
PD-L1 CPS	(n = 30)	(n = 26)	0.521
≥ 1	14 (46.7)	9 (34.6)	85

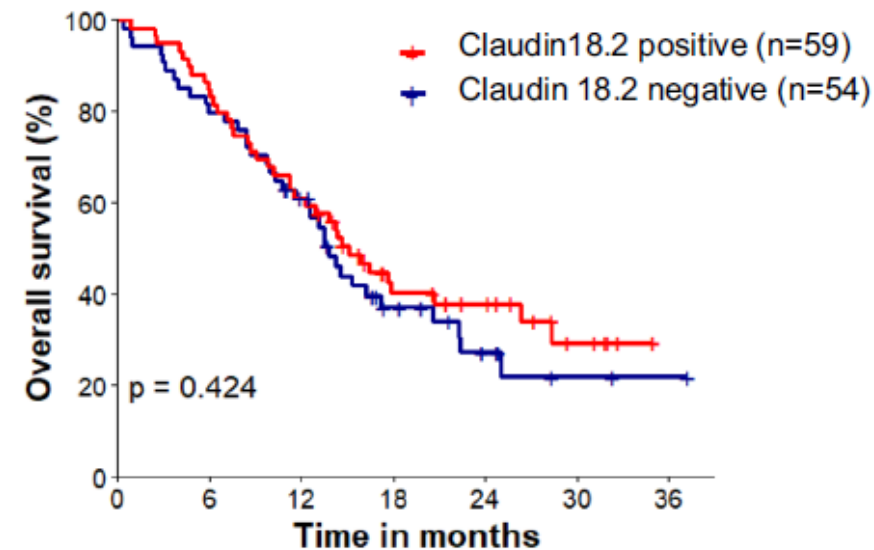
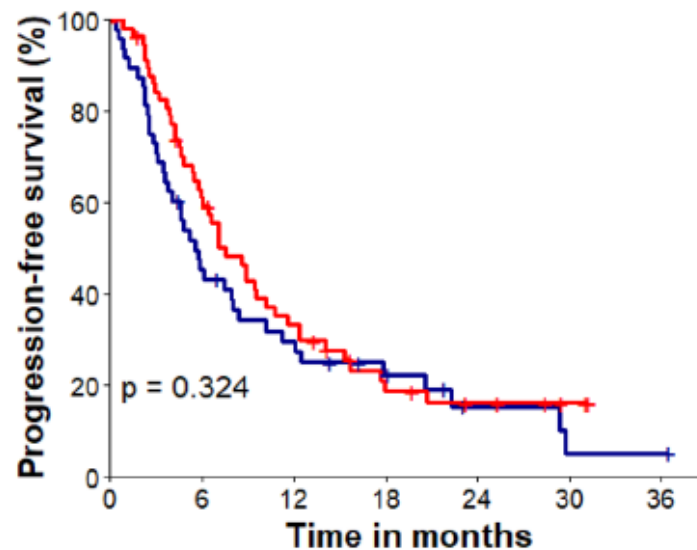
CLDN18.2 & Nivolumab treatment



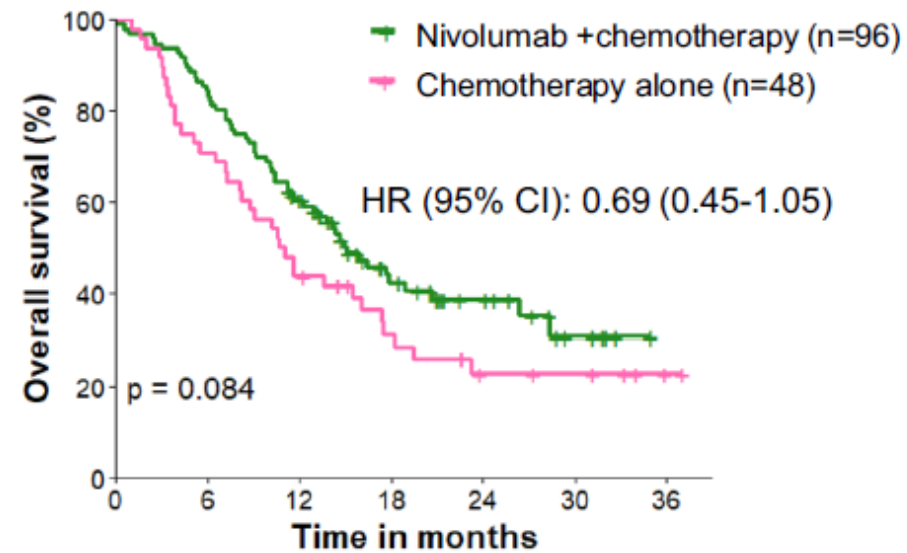
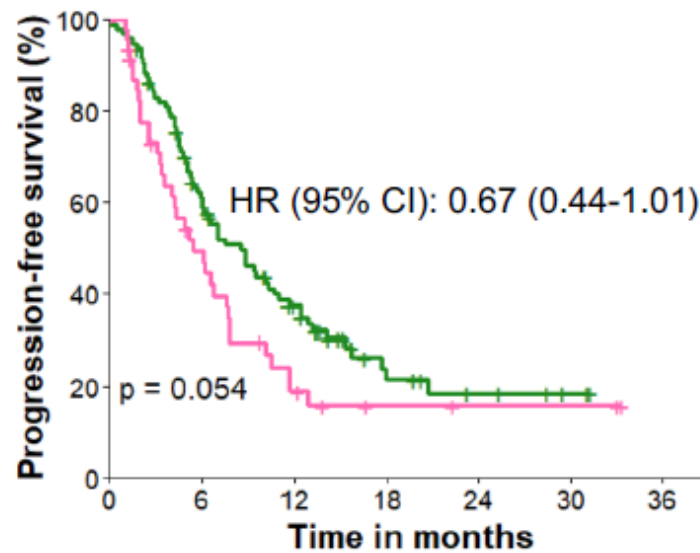
PD-L1 CPS ≥ 5 (treated with nivolumab plus chemotherapy)



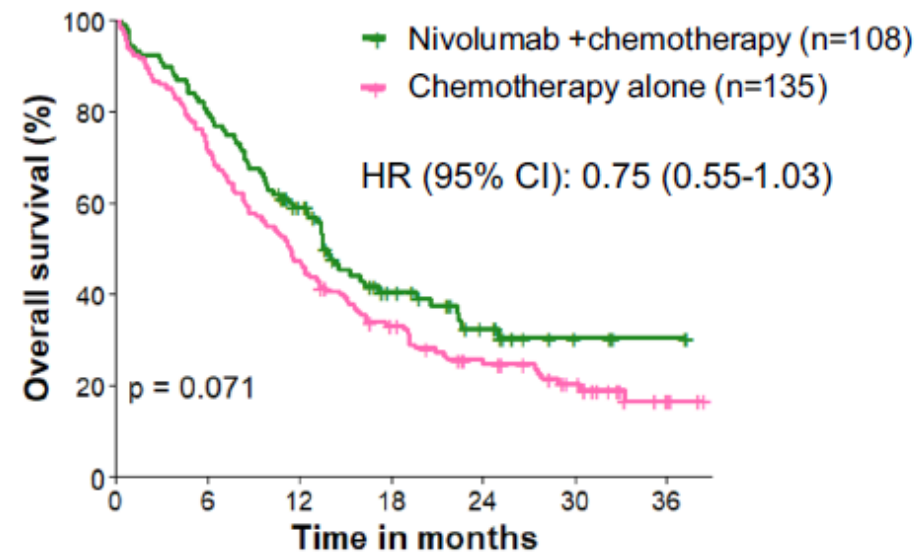
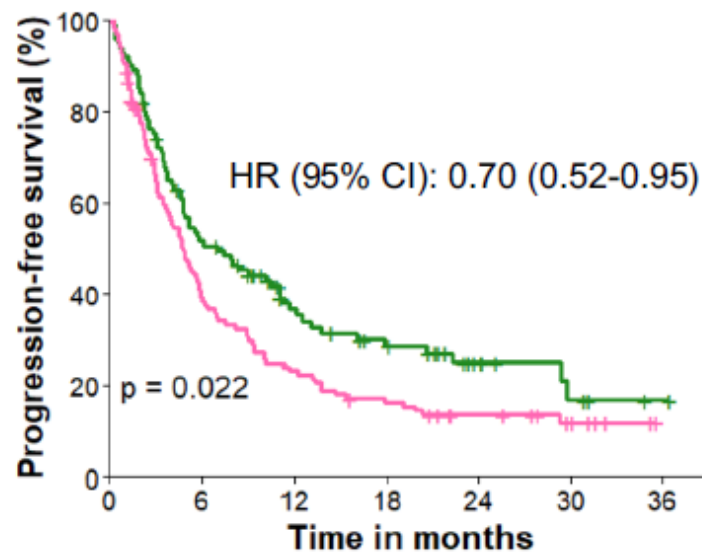
PD-L1 CPS < 5 (treated with nivolumab plus chemotherapy)



Claudin 18.2 positive



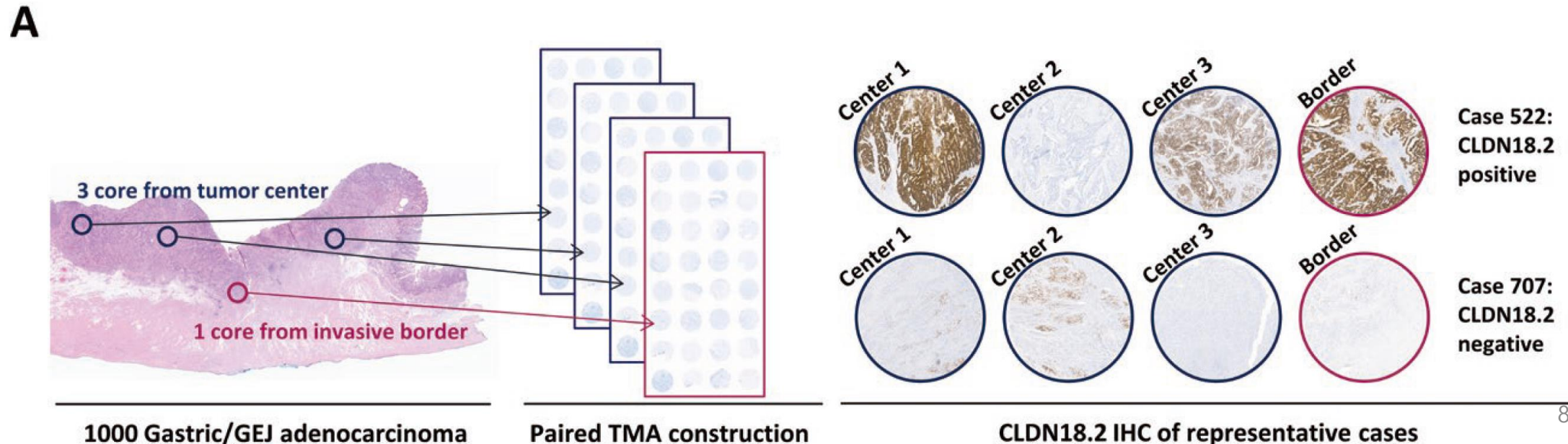
Claudin 18.2 negative



Clinicopathologic and molecular characterization of stages II-IV gastric cancer with Claudin 18.2 expression

Yoonjin Kwak^{1,†}, Tae-Yong Kim^{2,†}, Soo Kyung Nam³, Hye Jung Hwang¹, Daeyoung Han⁴, Hyeon Jeong Oh⁵, Seong-Ho Kong⁶, Do Joong Park^{6,7}, Do-Youn Oh^{2,3,7}, Hyuk-Joon Lee^{6,7} , Seock-Ah Im^{2,3,7}, Han-Kwang Yang^{6,7} , Hye Seung Lee^{*,1,3,7} 

¹Department of Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea,



Abstract

Background: Claudin 18.2 (CLDN18.2) is a promising target for targeted therapies in gastric cancer (GC). This study investigated the prevalence of CLDN18.2 expression in patients with stages II-IV GC or gastroesophageal junction (GEJ) adenocarcinoma and its correlation with clinicopathologic features and other crucial GC biomarkers.

Methods: We enrolled 1000 patients diagnosed with stages II-IV GC after surgical treatment. Immunohistochemistry for CLDN18 (43-14A clone), PD-L1 (22C3 pharmDx), HER2, and FGFR2 was performed. CLDN18.2 positivity was defined as moderate-to-strong (2+/3+) membranous staining in $\geq 75\%$ of tumor cells. CLDN18.2 expression was compared with biomarker expression, Epstein-Barr virus (EBV) association and microsatellite instability status, and clinicopathologic features.

Result: CLDN18.2 was positive in 34.4% of the patients. CLDN18.2 positivity was significantly higher in the middle and upper thirds than in the lower third gastric location ($P < .001$), but there was no correlation with age, sex, or stage ($P > .05$). CLDN18.2 positivity was rare (2.8%) in mucinous adenocarcinoma but frequent (90.9%) in a majority of gastric carcinomas with lymphoid stroma. CLDN18.2 positivity was higher in EBV-associated ($P < .001$) and PD-L1-positive (PD-L1 CPS ≥ 5) GC ($P = .014$) but lower in HER2 positive GC ($P = .005$). CLDN18.2 positivity was not significantly associated with overall survival and disease-free survival.

Conclusion: This study provides a comprehensive evaluation of CLDN18.2 status and its correlation with the clinicopathologic characteristics of patients with stages II-IV GC in Korea and with crucial biomarkers. It may be valuable for guiding future drug development, expanding treatment options, and ultimately improving patient outcomes in GC.

Key words: gastric cancer; claudin 18.2; immunohistochemistry; Epstein-Barr virus; microsatellite instability; PD-L1; HER2.

Table 3. Correlation between CLDN18.2 expression and other biomarkers.

		Total	CLDN18.2		P-value
			Negative	Positive	
		1,000	656 (65.6%)	344 (34.4%)	
PD-L1					.014
	Negative (CPS < 5)	633	433 (68.4%)	200 (31.6%)	
	Positive (CPS ≥ 5)	367	223 (60.8%)	144 (39.2%)	
PD-L1					.018
	CPS < 10	740	501 (67.7%)	239 (32.3%)	
	CPS ≥ 10	260	155 (59.6%)	105 (40.4%)	
PD-L1					.011
	CPS < 1	322	229 (71.1%)	93 (28.9%)	
	CPS ≥ 1	678	427 (63.0%)	251 (37.0%)	
HER2					.005
	Negative	933	601 (64.4%)	332 (35.6%)	
	Positive	67	55 (82.1%)	12 (17.9%)	
FGFR2					.007
	Negative	846	570 (67.4%)	276 (32.6%)	
	Positive	154	86 (55.8%)	68 (44.2%)	
EBV					<.001
	Negative	936	638 (68.2%)	298 (31.8%)	
	Positive	64	18 (28.1%)	46 (71.9%)	
MSI					.647
	MSS/MSI-L	901	589 (65.4%)	312 (34.6%)	
	MSI-H	99	67 (67.7%)	32 (32.3%)	

ORIGINAL ARTICLE

Clinicopathological analysis of claudin 18.2 focusing on intratumoral heterogeneity and survival in patients with metastatic or unresectable gastric cancer

T.-Y. Kim^{1†}, Y. Kwak^{2†}, S. K. Nam³, D. Han⁴, D.-Y. Oh^{1,3,5}, S.-A. Im^{1,3,5} & H. S. Lee^{2,3,5*}

Departments of ¹Internal Medicine; ²Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul; ³Department of Interdisciplinary Program in Cancer Biology, Seoul National University College of Medicine, Seoul; ⁴Medical Affairs, Astellas Pharma Korea Inc., Seoul; ⁵Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea

Background: This study aimed to investigate the prevalence of claudin 18.2 (CLDN18.2) positivity, with a particular focus on intratumoral heterogeneity, and its association with clinicopathological features in metastatic or unresectable gastric cancer (GC).

Patients and methods: We investigated 400 patients who received systemic chemotherapy for unresectable, metastatic, or recurrent GC. Immunohistochemistry for CLDN18 (43-14A), human epidermal growth factor receptor 2 (HER2), programmed death-ligand 1 (PD-L1), and fibroblast growth factor receptor 2, as well as HER2 silver *in situ* hybridization (ISH), Epstein–Barr virus (EBV) ISH, and microsatellite instability testing were carried out. CD3+, CD8+, CD4+, and Foxp3-positive immune cell densities were calculated using digital image analysis.

Results: In GC cases with any CLDN18.2 expression, more than half of the cases (61.3%) showed different expression results between four different tissue microarray (TMA) cores. When comparing CLDN18.2 status between whole tissue sections and the combined results from the four TMA cores, discrepancies were observed in only 2 out of 85 GC cases (2.4%), with 1 false positive and 1 false negative. After considering intratumoral heterogeneity, a CLDN18.2 positivity rate of 31.3% was observed among the 400 GC patients. CLDN18.2 positivity was rare in GCs located in the antrum (or lower third) and in HER2-positive cases but was common in EBV-positive GCs ($P < 0.05$). No differences in overall survival (OS) were observed according to CLDN18.2 positivity ($P = 0.116$). Additionally, there was no association between OS and CLDN18.2 positivity in patients treated with fluoropyrimidine plus platinum, chemotherapy plus trastuzumab, paclitaxel with or without ramucirumab, and immuno-oncologic agents. CLDN18.2-positive/PD-L1-high GCs showed statistically significantly longer OS than others ($P = 0.025$) and higher CD8+ T-cell densities in both the tumor center and periphery ($P < 0.001$).

Conclusions: Characterizing unresectable, metastatic, or recurrent GC with positive CLDN18.2 expression and evaluating intratumoral heterogeneity and prognostic implications of various therapeutics help advance treatment strategies and develop new therapies for patients with GC.

Key words: gastric cancer, claudin 18.2, immunohistochemistry, intratumoral heterogeneity, immune microenvironment

Table 1. Correlation between CLDN18.2 expression status and other GC biomarkers

		CLDN18		Total	P value
		Negative	Positive		
FGFR2	Negative	237	99	336	0.077
		70.5%	29.5%	84.0%	
	Positive	38	26	64	
		59.4%	40.6%	16.0%	
HER2	Negative	245	119	364	0.048
		67.3%	32.7%	91.0%	
	Positive	30	6	36	
		83.3%	16.7%	9.0%	
MSI	MSS/MSI-L	259	113	372	0.169
		69.6%	30.4%	93.0%	
	MSI-H	16	12	28	
		57.1%	42.9%	7.0%	
EBV	Negative	270	114	384	0.001
		70.3%	29.7%	96.0%	
	Positive	5	11	16	
		31.3%	68.8%	4.0%	
PD-L1 (cut-off: CPS 1)	Negative	99	43	142	0.757
		69.7%	30.3%	35.5%	
	Positive	176	82	258	
		68.2%	31.8%	64.5%	
PD-L1 (cut-off: CPS 5)	Negative	193	83	276	0.448
		69.9%	30.1%	69.0%	
	Positive	82	42	124	
		66.1%	33.9%	31.0%	
PD-L1 (cut-off: CPS 10)	Negative	224	99	323	0.596
		69.3%	30.7%	80.8%	
	Positive	51	26	77	
		66.2%	33.8%	19.3%	
Total		275	125	400	
		68.8%	31.3%	100.0%	

CLDN18.2, claudin 18.2; CPS, combined positive score; EBV, Epstein–Barr virus; FGFR2, fibroblast growth factor receptor 2; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; PD-L1, programmed death-ligand 1.

Results: Among 65 patients who received zolbetuximab-containing therapy, CLDN18.2 status was assessable at both baseline and disease progression in 15 patients. At disease progression, 53.3% of cases converted to CLDN18.2-negative. CLDN18.2 positivity was retained in 66.7% and 73.3% of patients when applying 40% and 25% cut-off levels, respectively.

Conclusions: CLDN18.2 expression above the $\geq 75\%$ cut-off declined after zolbetuximab, but lower-level expression was often preserved, supporting the potential for subsequent targeted therapy.

