34º Congresso Brasileiro de PATOLOGIA
27º Congresso Brasileiro de CITOPATOLOGIA
29/05 a 01/06/2024 Belém/PA

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KEY BIOMARKERS IN GASTRIC ADENOCARCINOMA - DISCUSSION BASED ON CHALLENGING CASES

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SBC
SOCIEDADE BRASILEIRA DE CITOPATOLOGIA

Disclosure of Relevant Financial Relationships

Dr. FASSAN declares he has
conflicts of interest to disclose:

Advisory boards or personal honoraria as invited speaker: Amgen, Astellas, Astra Zeneca, BMS, Diapath, Eli Lilly, GSK, Incyte, IQvia, Janssen Pharma, MSD, Novartis, Pierre Fabre, Roche

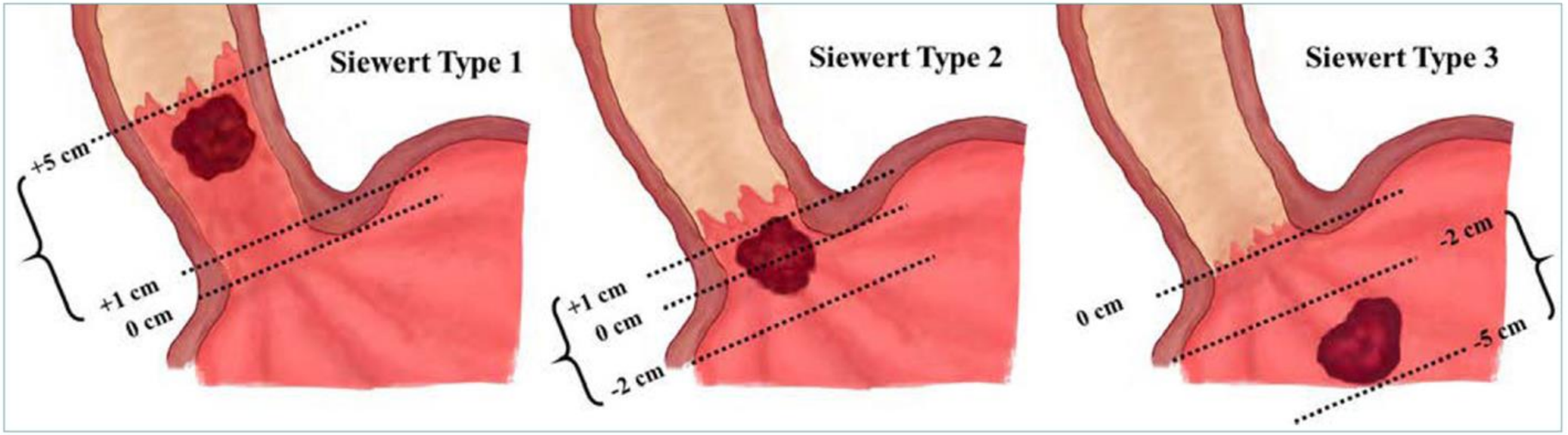
RDC Nº 96/08 da ANVISA

What we aim to cover during this presentation

- Importance of biomarker testing in GC
- Timing of GC biomarker testing
- Challenges in GC biomarker testing
- Novel biomarkers in the clinical scenario

WHO 2019

In this fifth-edition volume, adenocarcinomas of the esophagus and of the gastroesophageal junction are discussed **together** in a single section, because recent data suggest that these tumours share many etiological, histological, and biological features.



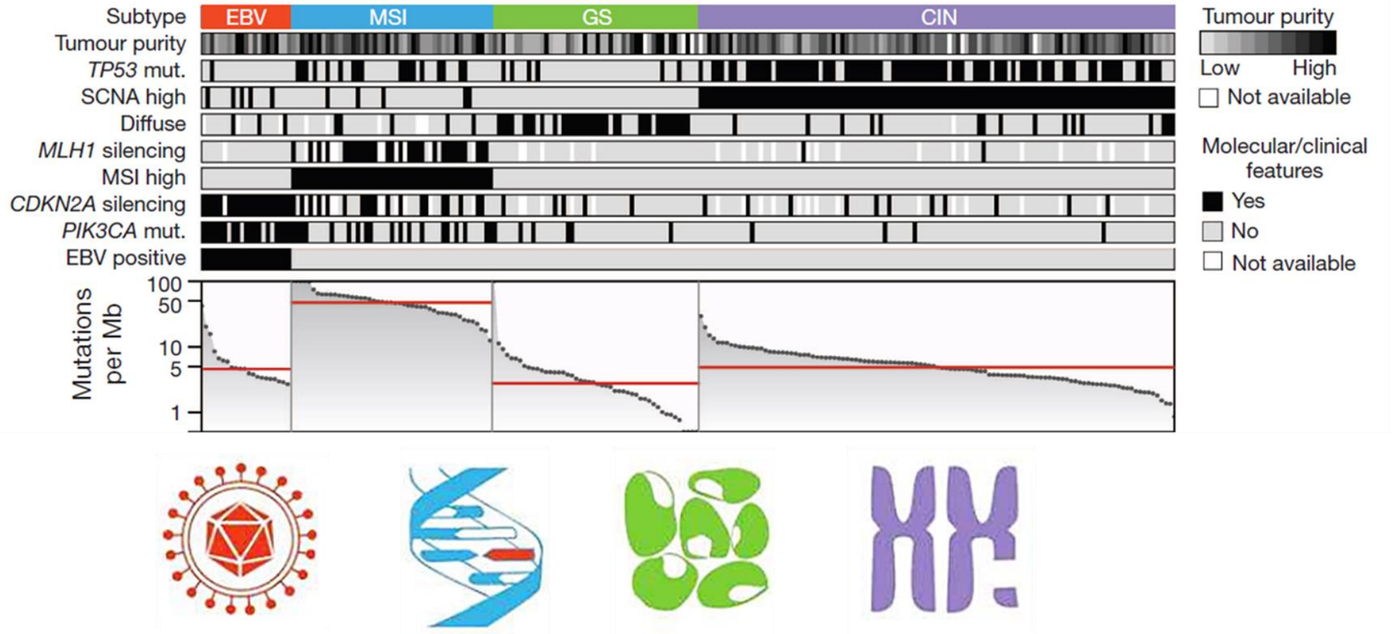
TYPE I	TYPE II	TYPE III
ADK of the distal esophagus	ADK of GEJ or cardia	ADK of subcardial stomach
ESOPHAGUS/GEJ		**GC**
G1 G2 G3		**Low High**
Glandular formation G1 >95%; G2 50-95%; G3 <50%		**Tubular and papillary**

Grillo F, *et al.*– Pathologica 2020

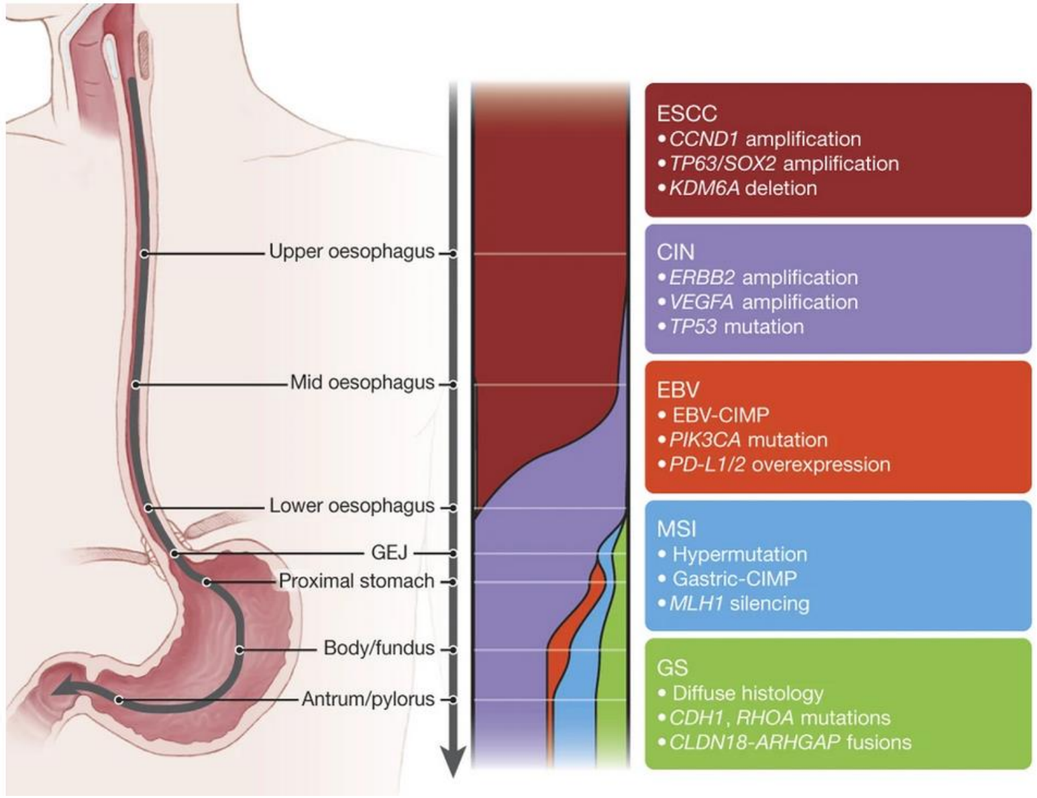
The molecular landscape of GC

NIH
THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

Whole-genome sequencing identifies four gastric cancer molecular subtypes



TGCA Consortium – Nature 2014



Cancer Genome Atlas Network – Nature 2017

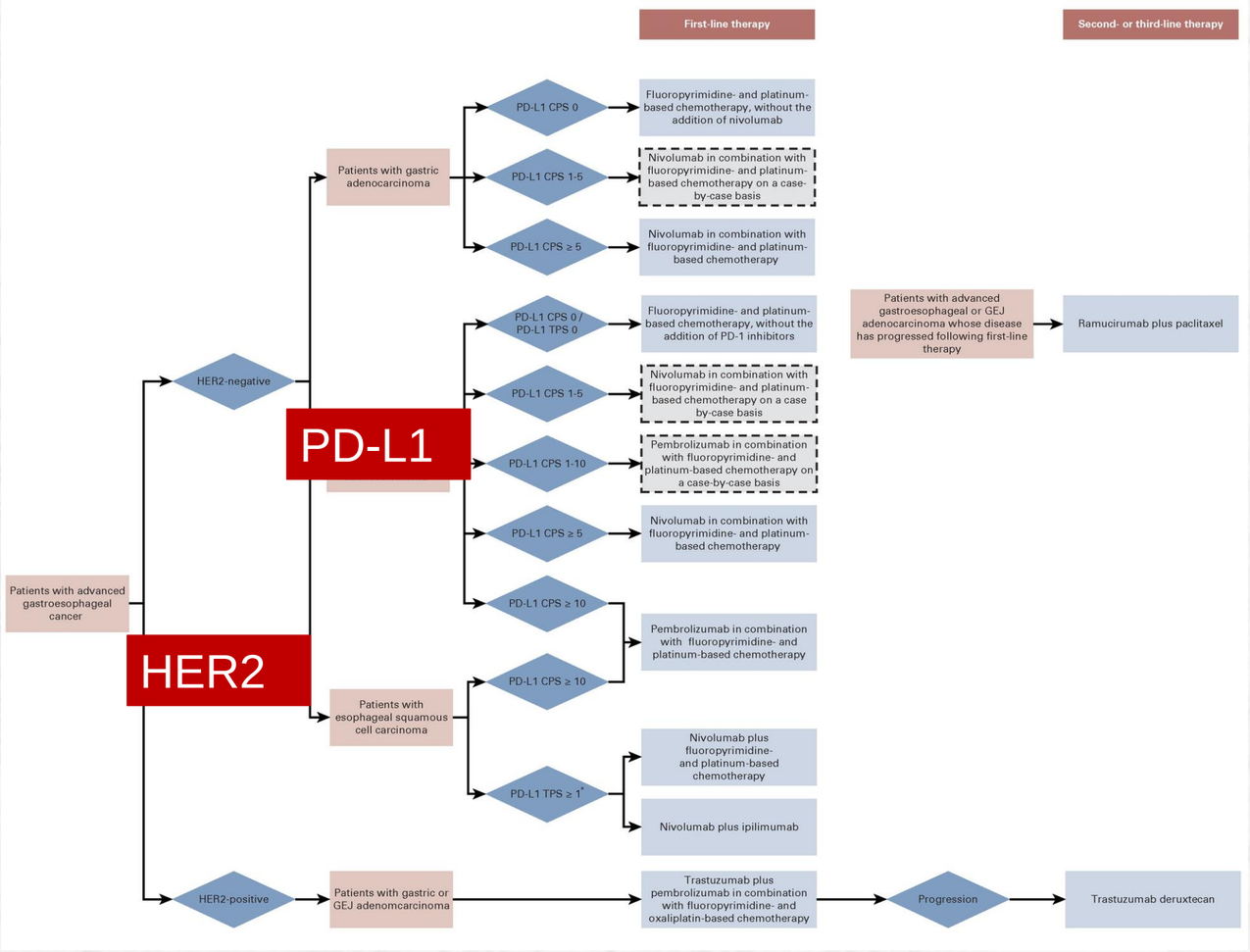
Four genomic subsets with therapeutic implications

MSI-high Immune checkpoint inhibitors	**Genomically unstable** HER2 only success
Ebstein-Barr virus Immune checkpoint inhibitors	**Genomically stable** Not clearly targetable

Four genomic subsets with therapeutic implications

MSI-high Immune checkpoint inhib MMR/ PD-L1	**Genomically uns** HER2 only success HER2
Ebstein-Barr vi Immune checkpoint inhib PD-L1	**Genomically sta** Not clearly targetable ?

HER2 and PD-L1 as an essential information for first line therapy in GC!



American Society of Clinical Oncology

Immunotherapy and targeted therapy for advanced gastroesophageal cancer: ASCO Guidelines

The information regarding HER2 and PD-L1 should be available at initial diagnosis!

Shah MA, *et al*. – J Clin Oncol 2023

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

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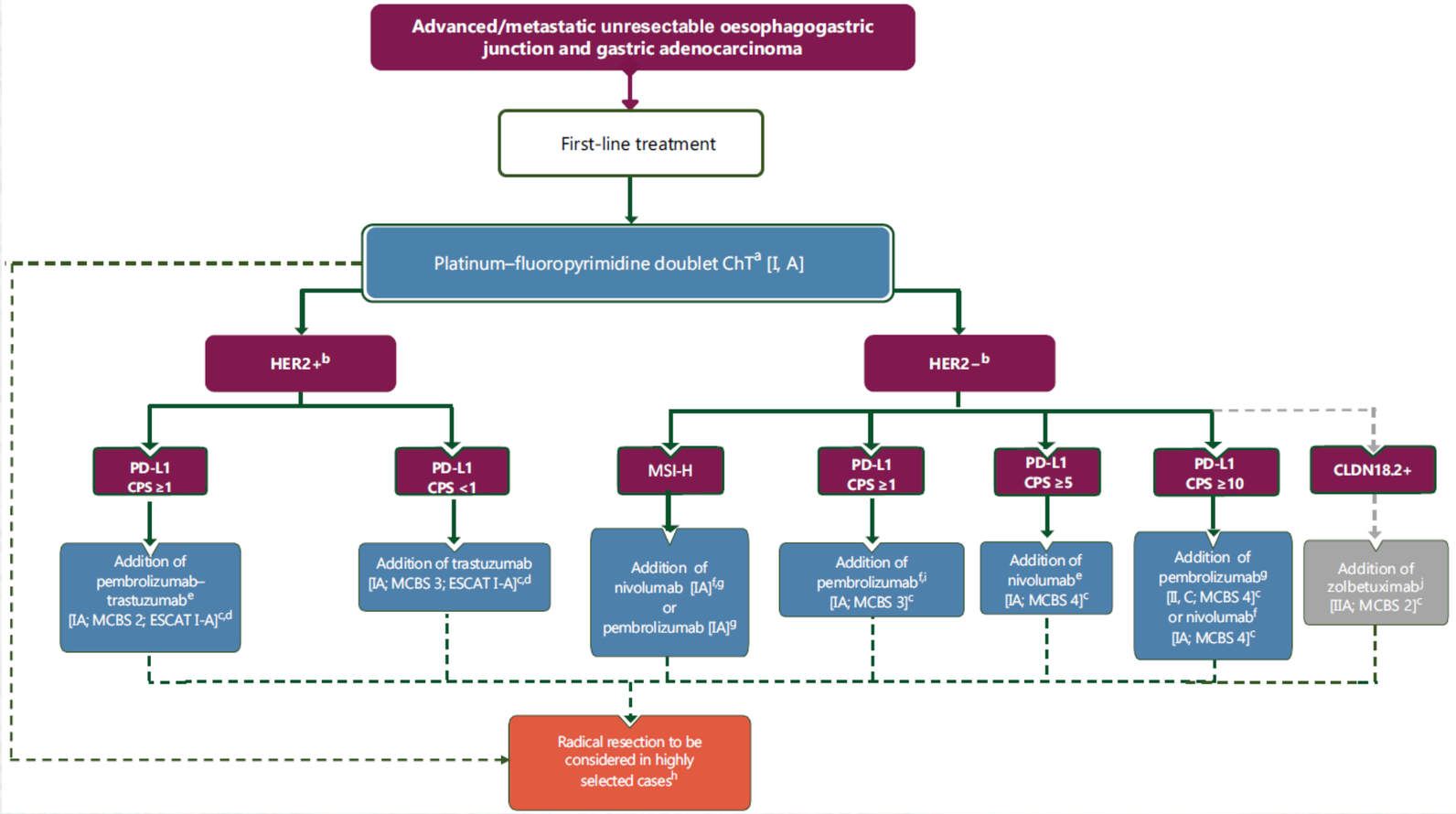
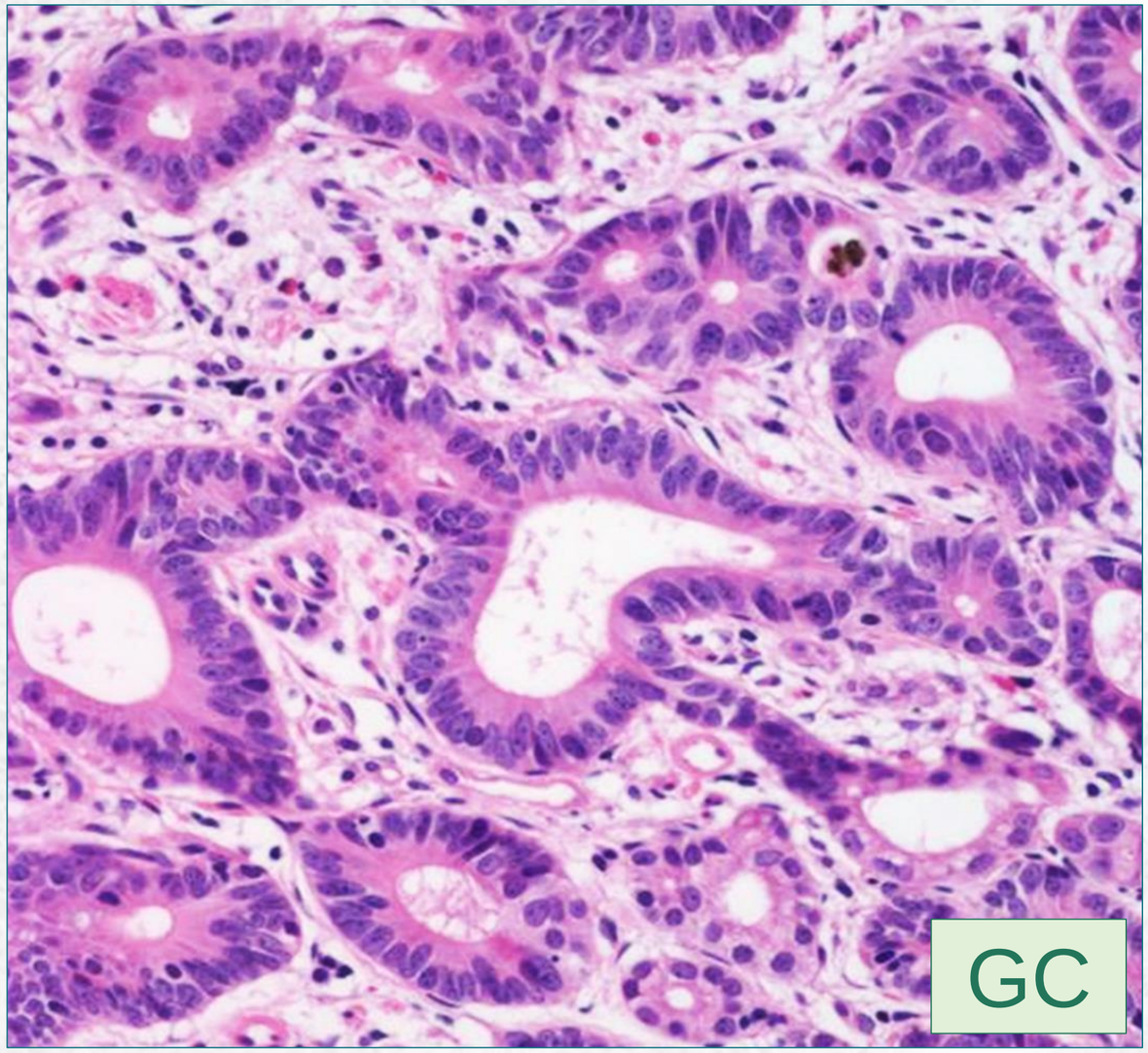
SPECIAL ARTICLE

Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with gastric cancer



- Diagnosis should be made from multiple (5-8) endoscopic biopsies to guarantee an adequate representation of the tumour [IV, B].
- The histological diagnosis should be reported according to WHO criteria [V, B].
- HER2 expression by IHC and/or amplification by in situ hybridisation [I, A; ESCAT score: I-A], PD-L1 by IHC according to CPS [I, A] and MSI-H/dMMR [II, A; ESCAT score: I-B] are validated predictive biomarkers. Claudin 18.2 expression by IHC [I, A; ESCAT score: I-A] may be examined, if available.

Shitara K, *et al*. – ESMO Open 2024



HER2
PD-L1
MMR/MSI
CLDN18.2

WORRISOME THINGS

1. Tissue is the issue – quality and quantity
2. Heterogeneity
3. Standardizing and prioritization of biomarker testing pathway
4. Reflex testing *vs* testing upon request

Tissue is the issue

quality and quantity

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surgical resections

lots of tissue ---- so no problems??

- May not be representative of clinical situation
- Neoadjuvant treatment (HER2 and PD-L1 can change)
- How long ago was the surgery?
- Potential pre-analytical problems

Surgical resections

Histochem Cell Biol (2015) 144:93–99
DOI 10.1007/s00418-015-1316-4

SHORT COMMUNICATION

Factors affecting immunoreactivity in long-term storage of formalin-fixed paraffin-embedded tissue sections

Federica Grillo · Simona Pigozzi · Paola Ceriolo · Paola Calamaro · Roberto Fiocca · Luca Mastracci

Cold ischemia time	Fewer than 30 minutes if possible, not exceeding 1 hour
Fixative	10% neutral buffered formalin
Time of fixation (biopsy)	6 to 48 hours
Time of fixation (resection)	24 to 48 hours
Preparation	Paraffin-embedded sections, cut at a thickness of 3 to 5 µm
Specimen storage	Tissue blocks
Storage time for blocks	Fewer than 3 years for PD-L1 IHC
Storage conditions for blocks	Prevented from light, heat, and humidity
Storage time for cut sections	Fewer than 2 months, particularly for testing with SP263 antibody
Decalcification	EDTA, if necessary

Table 1. FFPE-Related Preanalytical Factors Categorized by the Extent Each Has Been Investigated in the Literature for Potential Effects on DNA, RNA, Protein, and Morphology Analytes

Comprehensive (All 4 Analytes Have Been Evaluated)	Incomplete (Some but Not All Analytes Have Been Evaluated)	Unexplored (No Analytes Have Been Evaluated)
Cold ischemia	Postmortem interval	Pathology ink
Decalcification	Warm ischemia time	Fixative age
Fixation duration	Specimen size	Commercial versus in-house fixative
Duration of paraffin block storage	Prefixation handling	Use of recycled formalin
	Fixative buffer	Movement during fixation
	Tissue to fixative ratio	Light exposure during fixation
	Fixation temperature	Fixation container
	Fixative delivery method	Fixation alone or with other biospecimens
	Dehydration reagent and conditions	Postfixation wash solution and conditions
	Clearing reagent and conditions	Reagents and conditions of interim alcohol storage
	Paraffin embedding reagent and conditions	Use of recycled dehydration and clearing reagents
	FFPE block size or section thickness	Automated versus manual processing
	Type of slide or adhesive	Use of recycled paraffin for impregnation and embedding
	Slide drying duration and temperature	Embedding conditions
	Storage duration of slide-mounted FFPE sections	Slide pretreatment
		Equipment and conditions of sectioning and section transfer

Abbreviation: FFPE, formalin-fixed, paraffin-embedded.

Biopsies

3.8 Gastric cancer

RECOMMENDATIONS

ESGE recommends at least six biopsies in cases of suspected advanced gastric cancer.
Strong recommendation, moderate quality of evidence.

ESGE recommends taking only one to two targeted biopsies for lesions that are potentially amenable to endoscopic resection (Paris classification 0-II) to confirm the diagnosis and allow subsequent endoscopic resection.
Strong recommendation, low quality of evidence.

Biomarkers can be evaluated only on invasive carcinoma
Mucin, ulcer, granulation tissue, DYSPLASIA are not useful

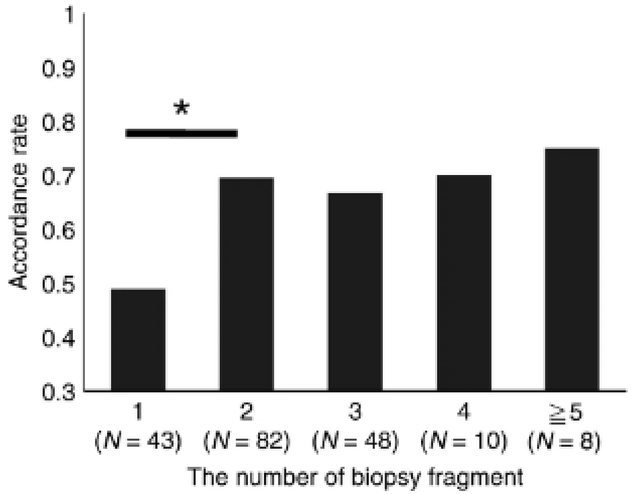
Endoscopists know they should take more than six neoplastic samples … right??

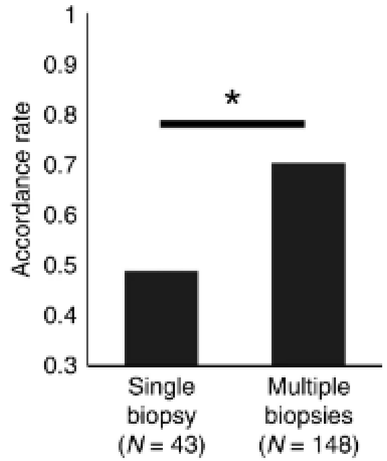
BJC British Journal of Cancer

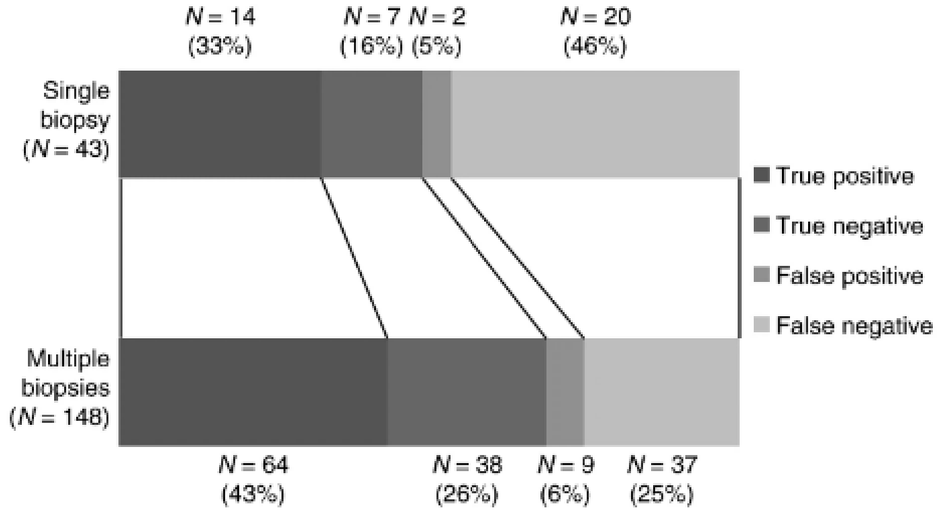
BRIEF COMMUNICATION
Cellular and Molecular Biology

Can PD-L1 expression evaluated by biopsy sample accurately reflect its expression in the whole tumour in gastric cancer?

Kohei Yamashita[1], Masaaki Iwatsuki[1,2], Kazuto Harada[1,2], Yuki Koga[1], Yuki Kiyozumi[1], Kojiro Eto[1], Yukiharu Hiyoshi[1], Takatsugu Ishimoto[1], Shiro Iwagami[1], Yoshifumi Baba[1], Yuji Miyamoto[1], Naoya Yoshida[1], Yoshihiro Komohara[3], Jaffer A. Ajani[2] and Hideo Baba[1]



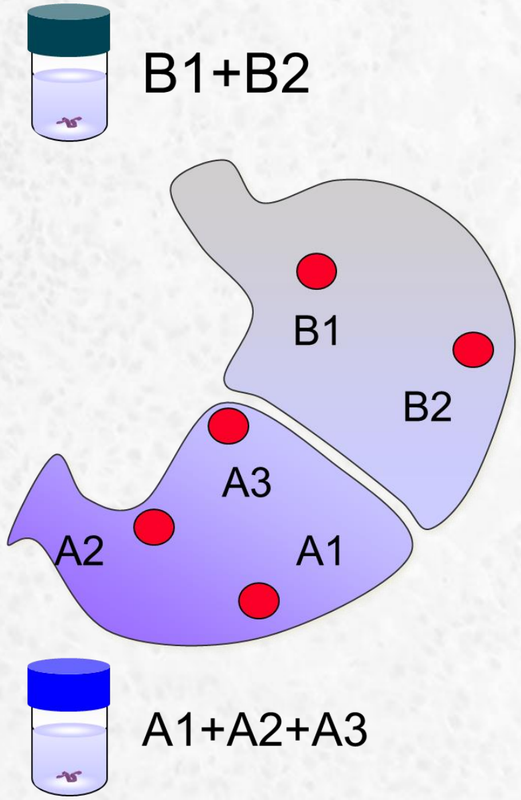


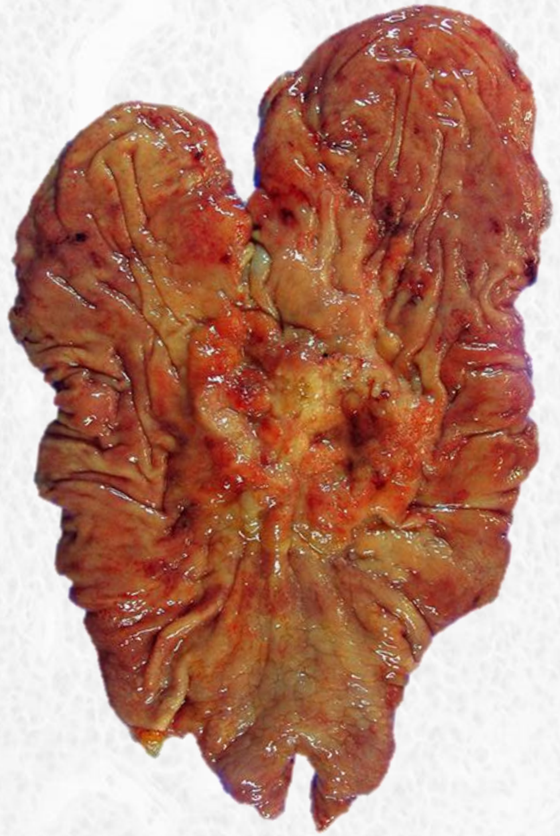


- The numbers of PD-L1-positive patients determined by biopsy and resected samples were 89 (46.6%) and 135 (70.1%), respectively
- The accordance rate was 64.4% ($\kappa = 0.31$)
- Single biopsy showed a lower accordance rate compared with multiple biopsies
- Our study revealed that single biopsy cannot fully reflect PD-L1 expression in the whole tumor in GC

Multiple biopsies are recommended for accurate diagnosis of PD-L1 expression in GC

Tissue-related influence on GC biomarkers' testing



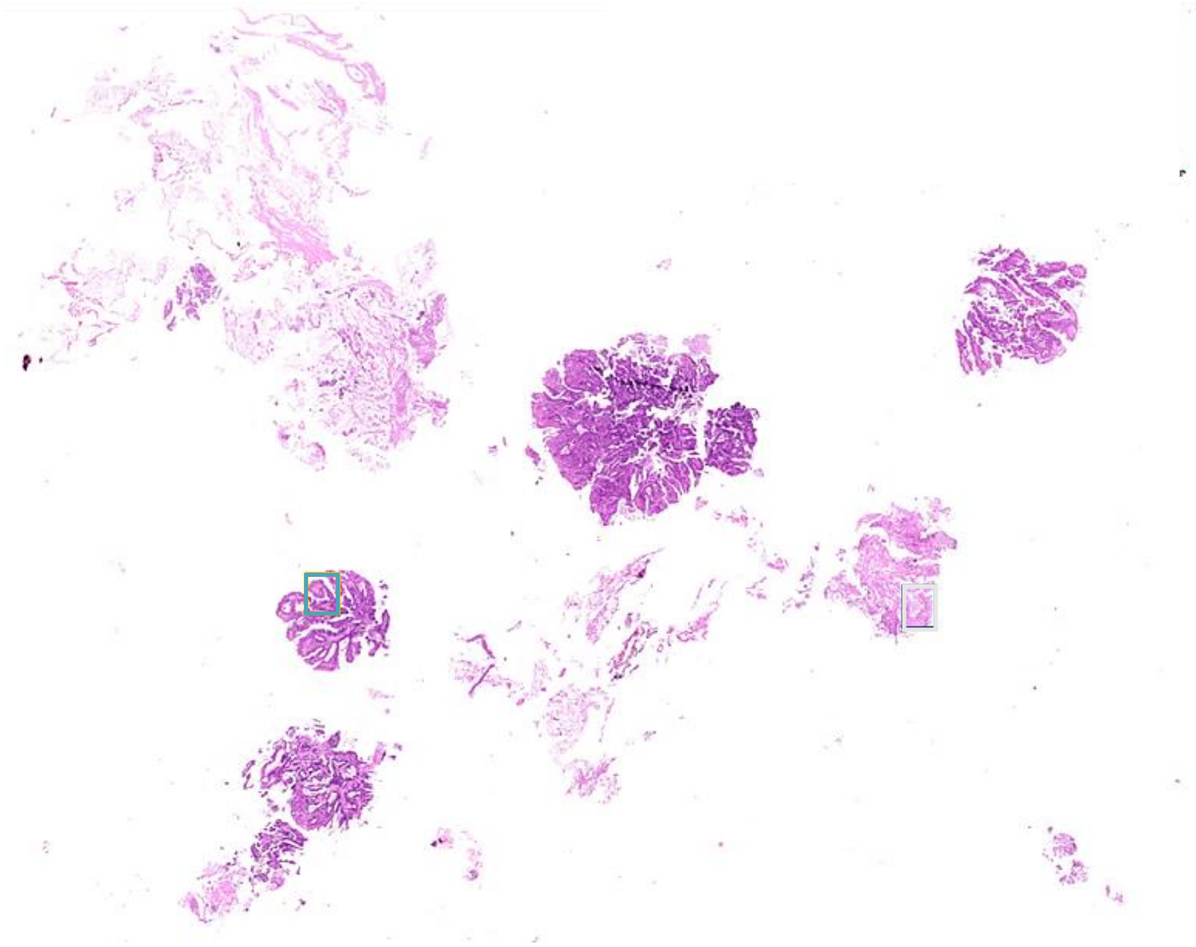


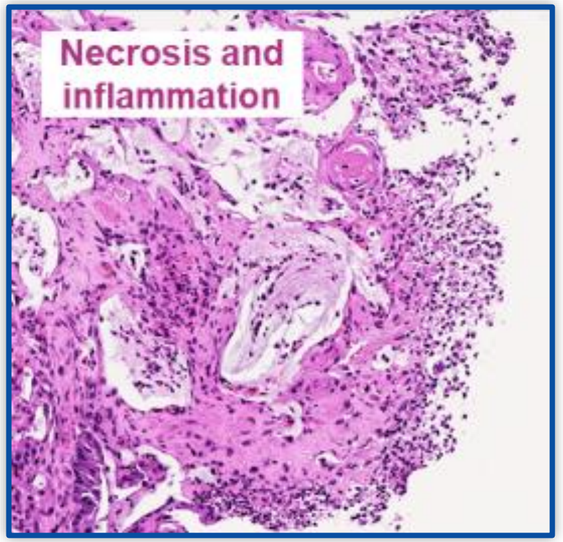
Biopsies ~70%

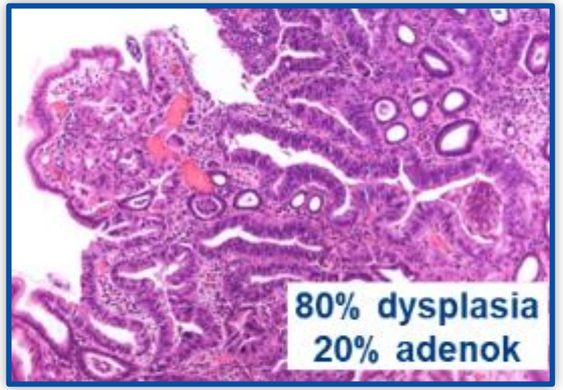
Surgery ~30%

Angerilli V, *et al.* – J Clin Pathol 2023

Not all biopsies are adequate for molecular testing!

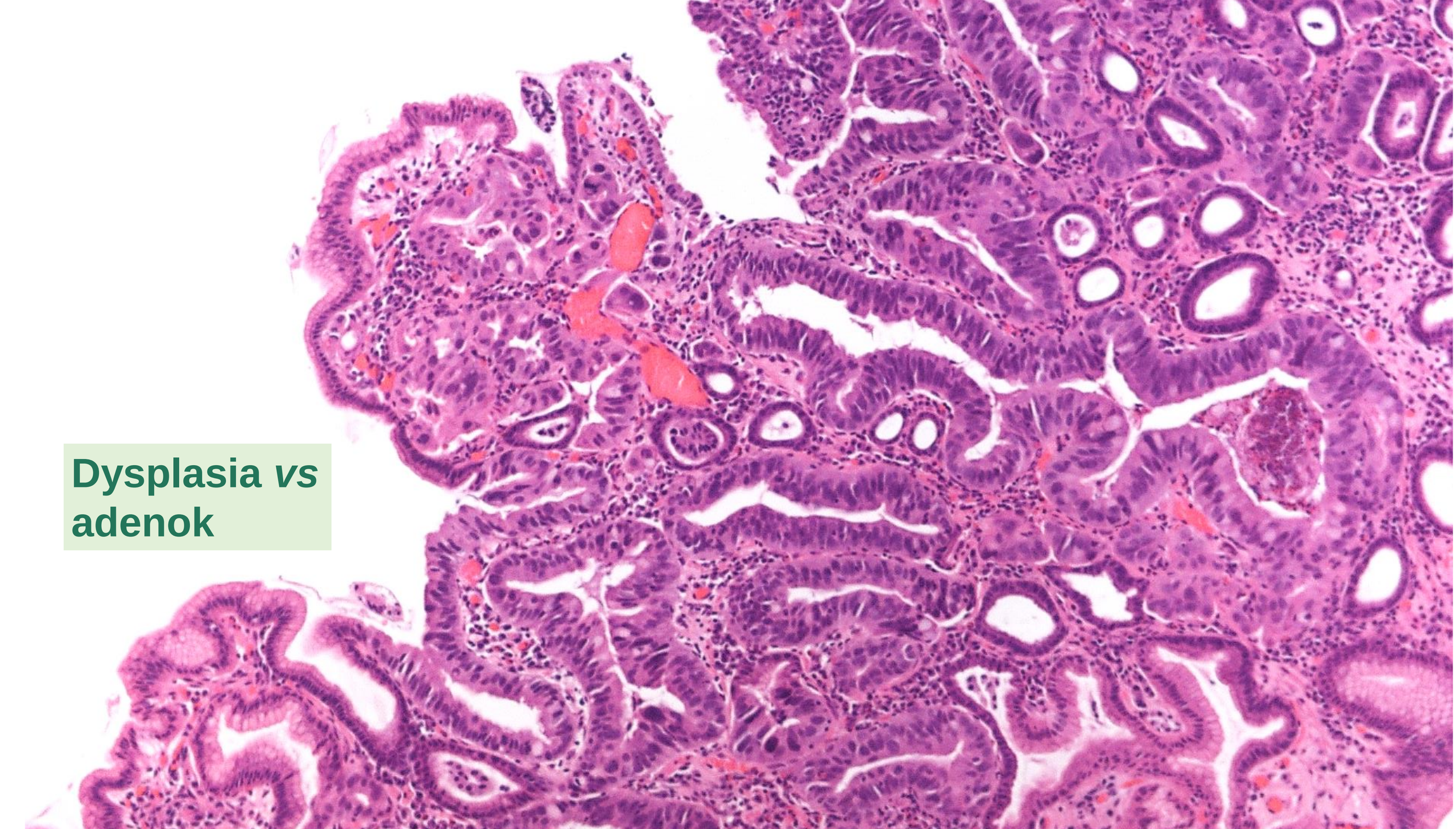




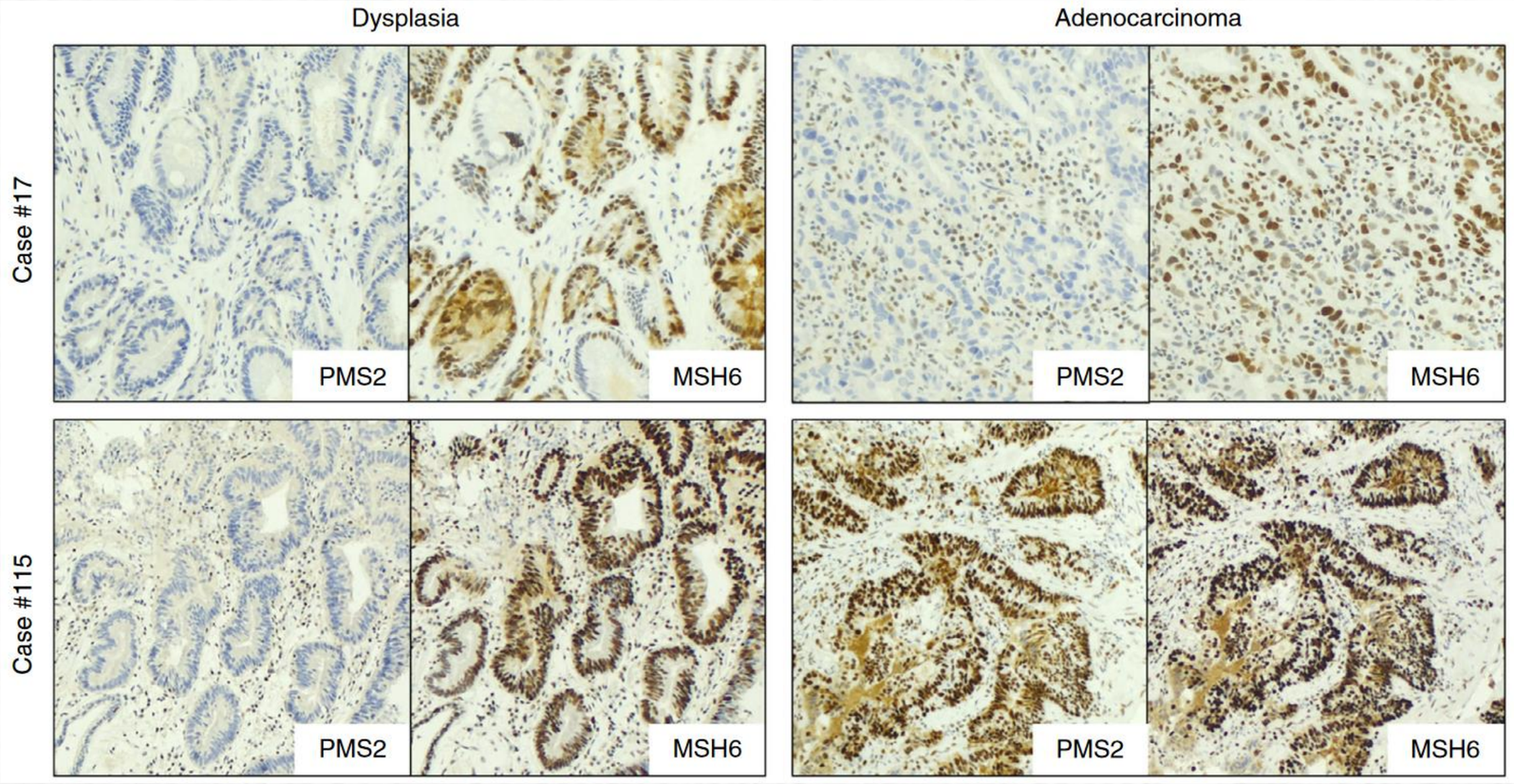


Parente P, *et al.* – Expert Rev Mol Diagn 2023

Dysplasia *vs* adenok



MMR status and GE dysplasia: need for a dedicated gastrointestinal pathologist?

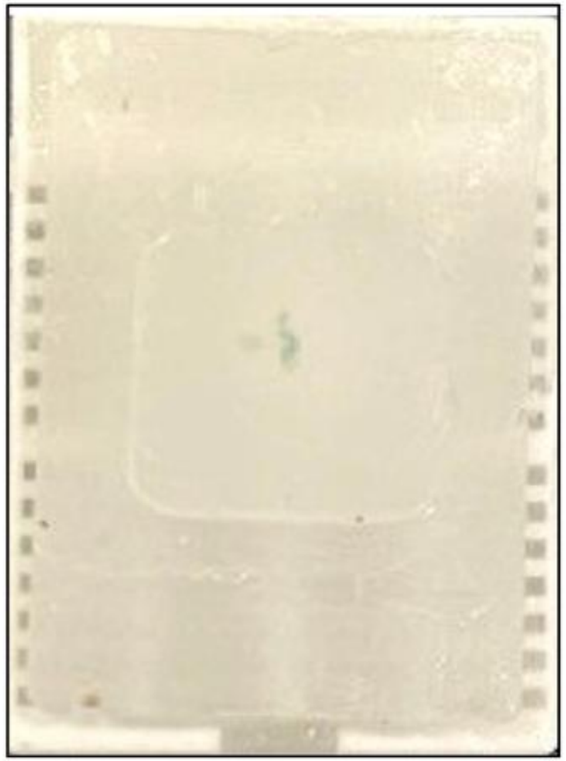


"…In fact, in certain circumstances, it may be challenging for non-expert gastrointestinal pathologists to make a proper distinction between preinvasive lesions and adenocarcinomas, thus hampering a correct biomarker status assessment, with important influences on the therapeutic decision-making process."

Angerilli V, *et al.* – Histopathology 2022

Dealing with the higher request of testing

Before IHC



After IHC

DIAGNOSIS

- 1 × 4 µm H&E
- 1 × 4 µm Giemsa
- 1 × 4 µm possible IHC (CK)
  + wastage 10–20 µm
  Total = around 20–30 µm

PREDICTIVE BIOMARKERS

- 1 × 4 µm HER2 (plus further 2 sections if 2+)
- 1 × 4 µm PD-L1
- 4 × 4 µm MMR
- 1 × 4 µm EBER
  + wastage 10–20 µm
  Total = around 30–50 µm

Parente P, *et al.* – Expert Rev Mol Diagn 2023

Heterogeneity

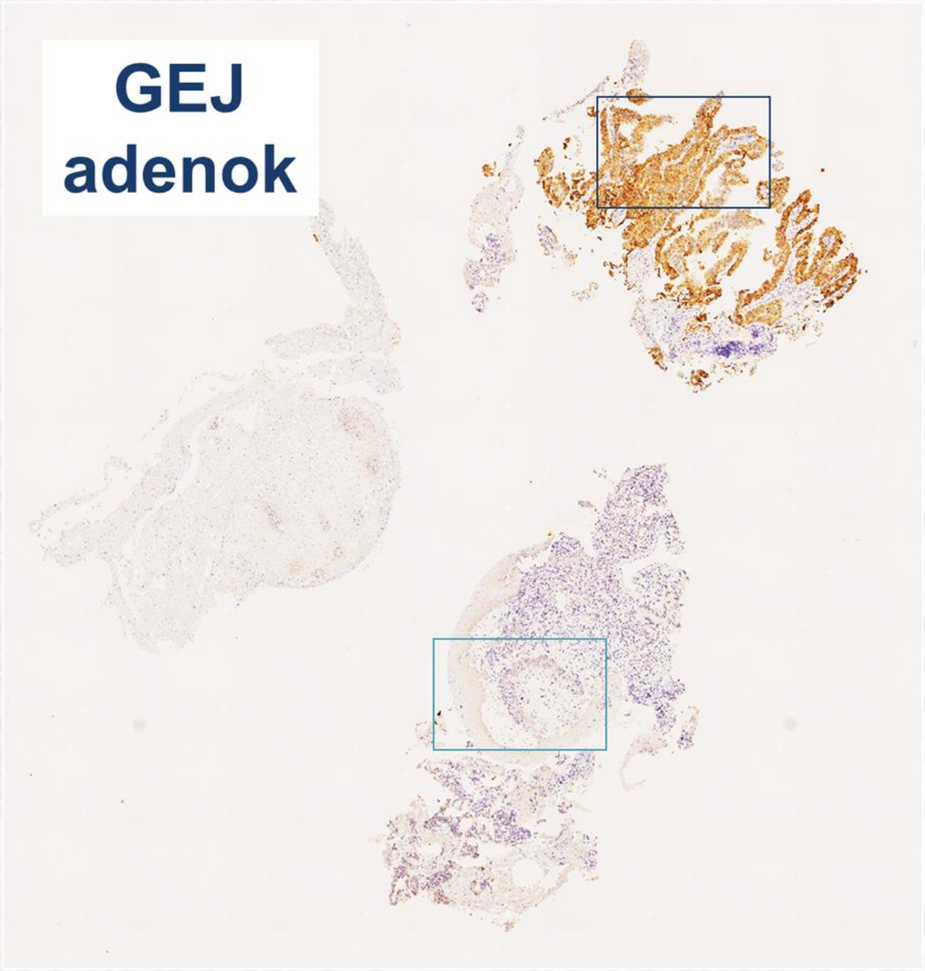
1. Within the tissue

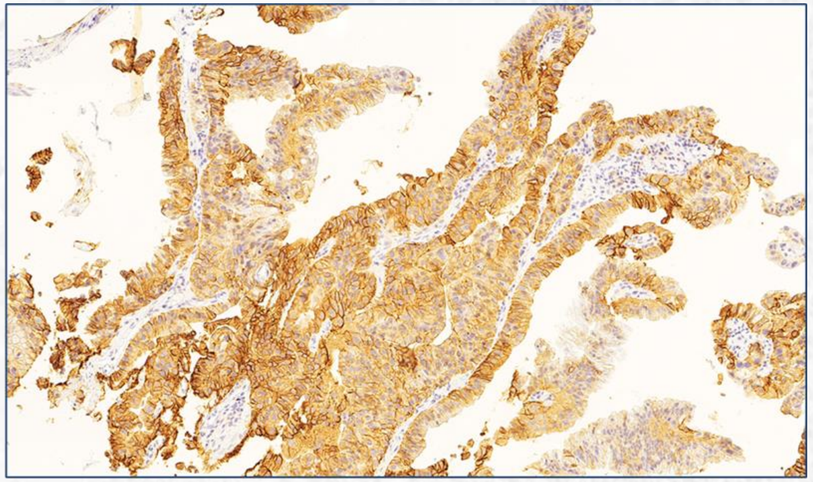
2. Between primary and metastases – between different metastatic sites – spatial

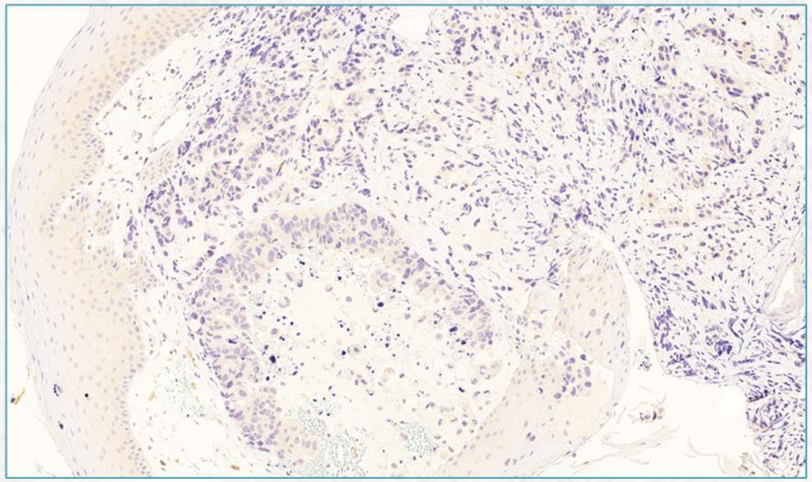
3. Differences in time – temporal

4. Treatment induced differences?

Gastric cancer as a heterogeneous disease







HER2
3+

HER2
0

HER2 status: spatial & temporal heterogeneity

HER2 status can change during the course of a patient's disease, differences between primary and metastases, between metastatic sites or in time

HER2 negative

Park et al. EurJCancer 2016

- **Repeat biopsy of primary (183 pts): 8.7%** of patients were shown to have HER2-positive gastric cancer
- **Repeat biopsy of metastatic and/or recurrent sites (175 pts): 5.7%** of patients turned out to have HER2-positive disease

HER2 positive

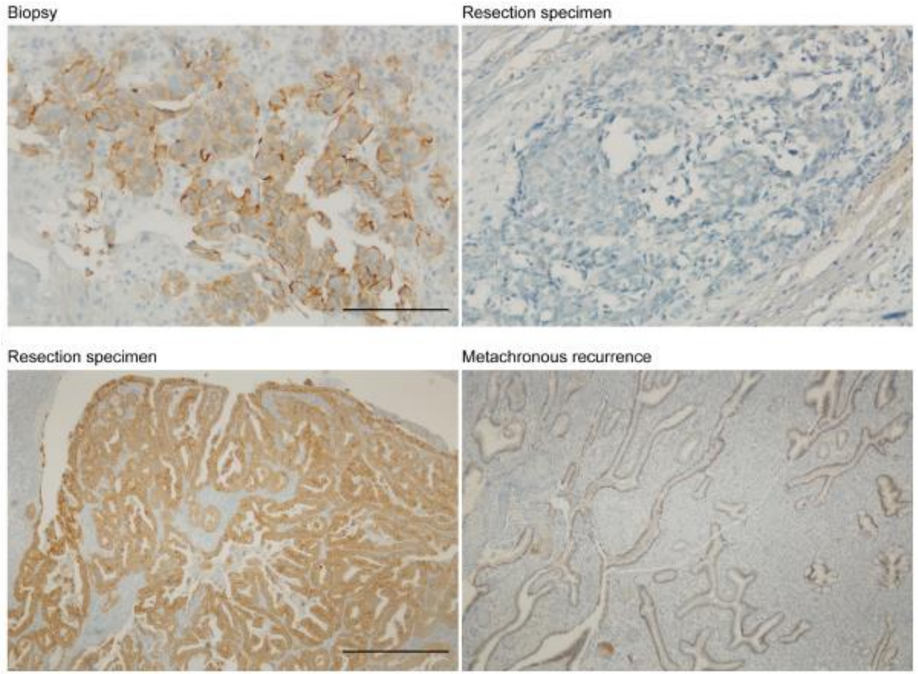
Seo et al. Gastric Cancer 2019

48 patients – baseline bx and bx at progression -

29.1% loss of HER2 at progression

- Patients with stable HER2 status - 44% RR, longer median PFS of 2.7

TREATMENT INDUCED CHANGES?



www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 42), pp: 26787-26799

Research Paper

The dynamics of HER2 status in esophageal adenocarcinoma

Aafke Creemers[1,2], Eva A. Ebbing[1,2], Gerrit K.J. Hooijer[3], Lisanne Stap[2], Rajni A. Jibodh-Mulder[1], Susanne S. Gisbertz[4], Mark I. van Berge Henegouwen[4], Maurits L. van Montfoort[3], Maarten C.C.M. Hulshof[5], Kausilia K. Krishnadath[6], Martijn G.H. van Oijen[2], Maarten F. Bijlsma[1], Sybren L. Meijer[3] and Hanneke W.M. van Laarhoven[1,2]

Gastric cancer as a heterogeneous disease

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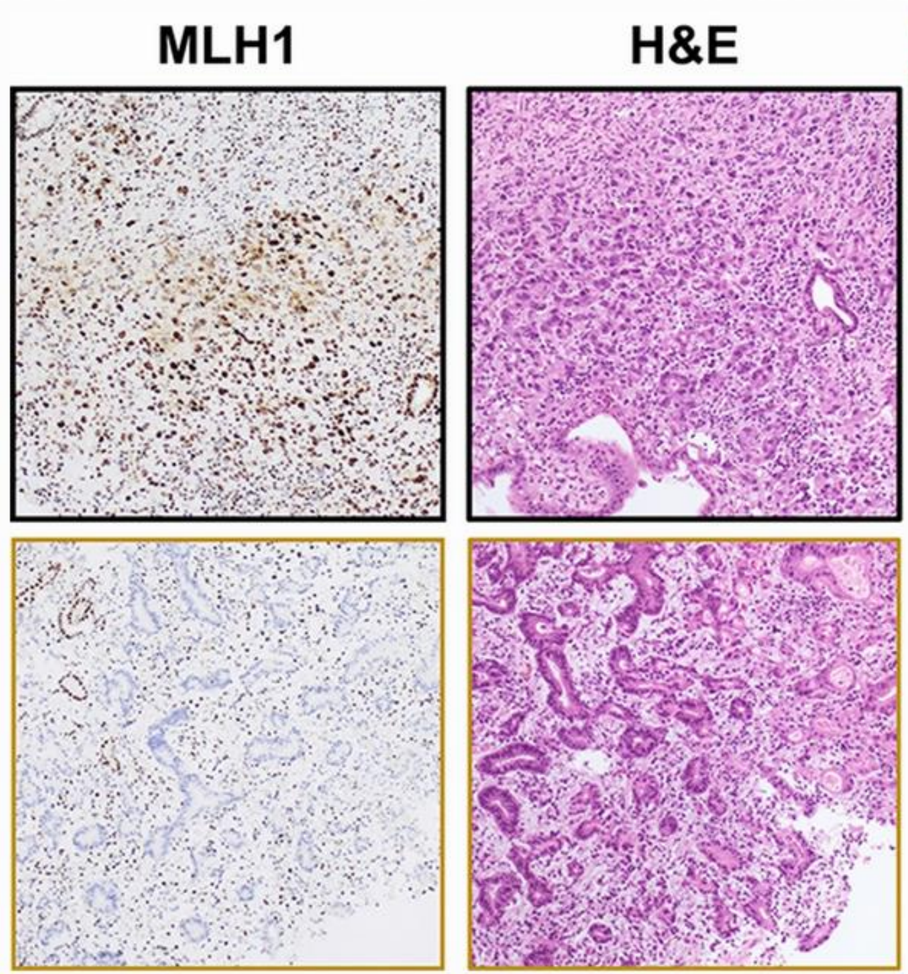
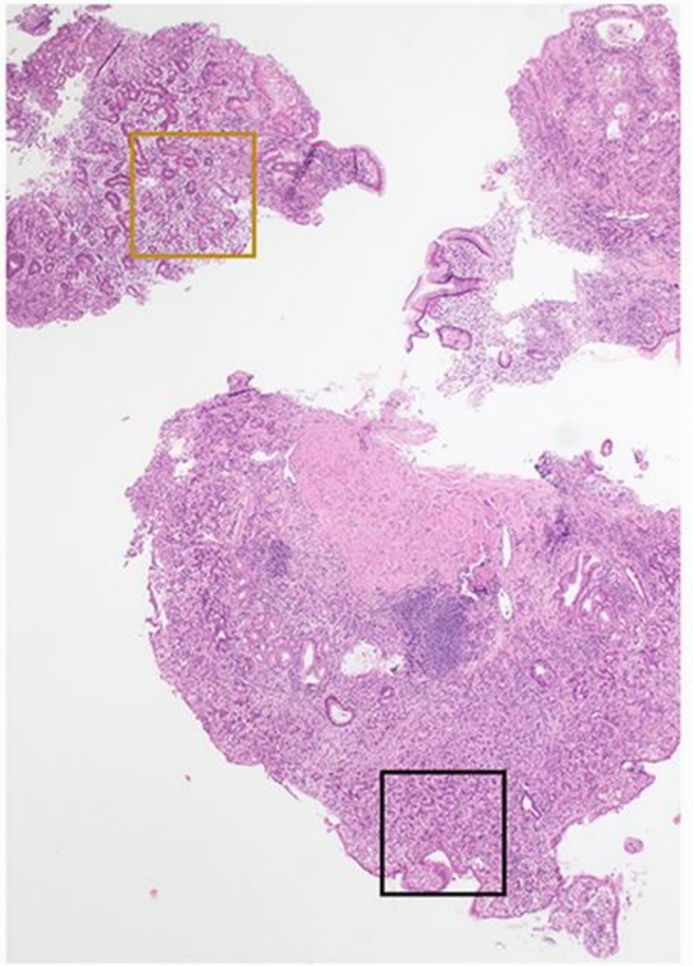
MLH1+
MMRp

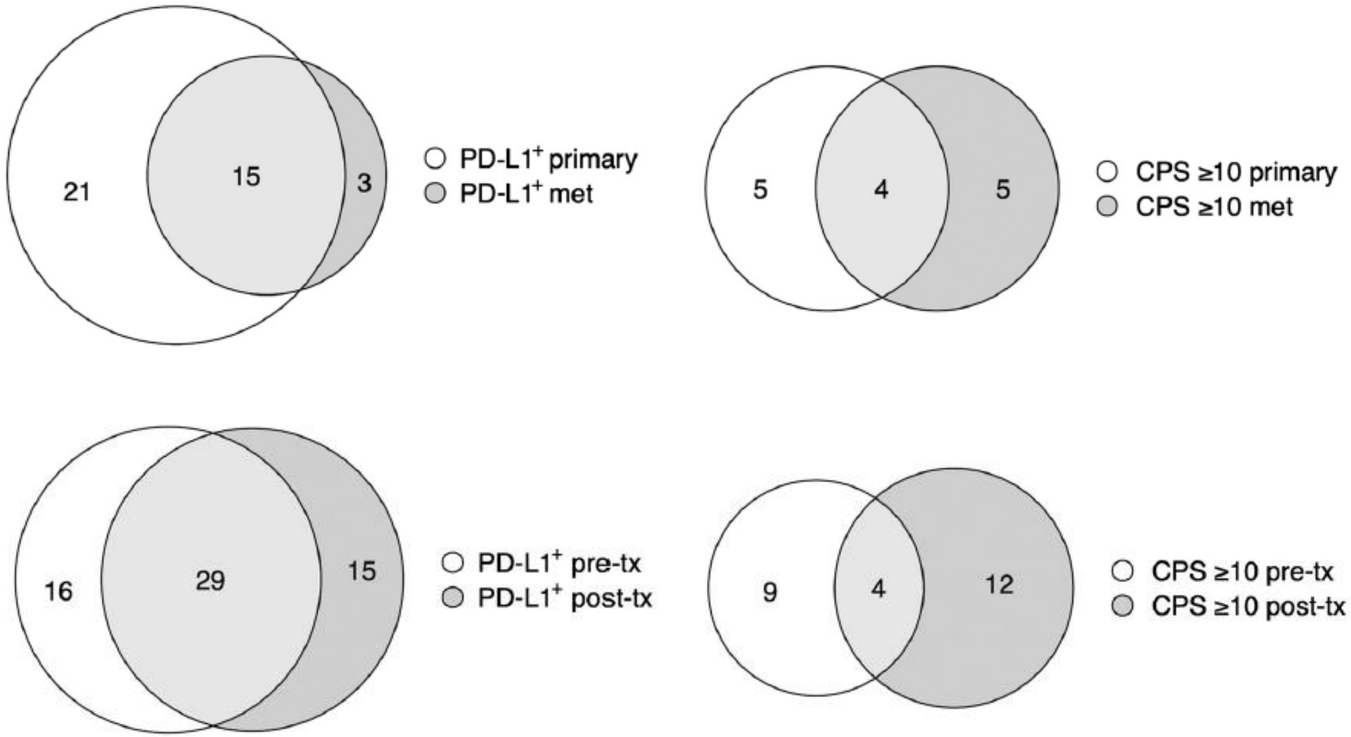
MLH1-
MMRd





Fornaro L, *et al.* – Gastric Cancer 2023

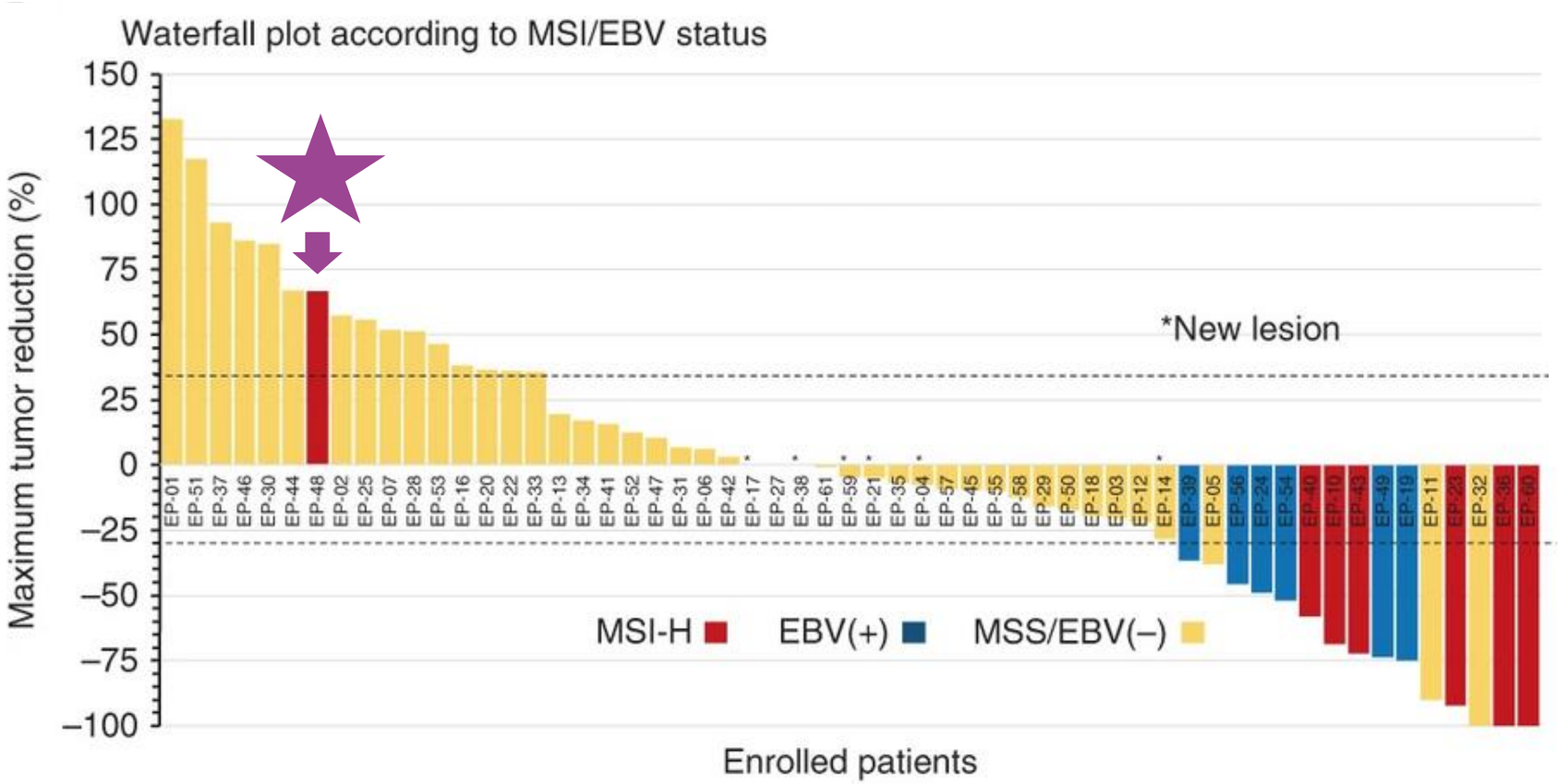
PD-L1: spatial and temporal heterogeneity

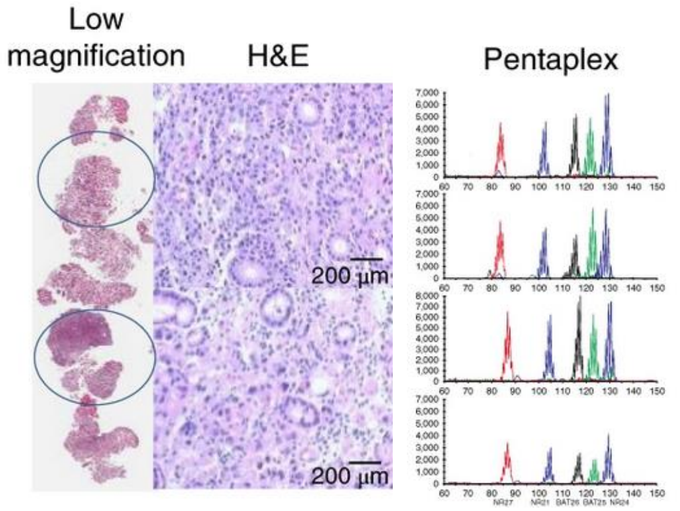


PD-L1 expression and TMB exhibit marked spatial and temporal heterogeneity in GEA. This heterogeneity should be considered when obtaining tumor samples for molecular testing and when deciding whether ICI therapy is appropriate.

Zhou KI, *et al.* – Clin Cancer Res 2020

Lack of response in an MSI-H patient associates with a heterogeneous MMR status!





MMR/MSI heterogeneity!

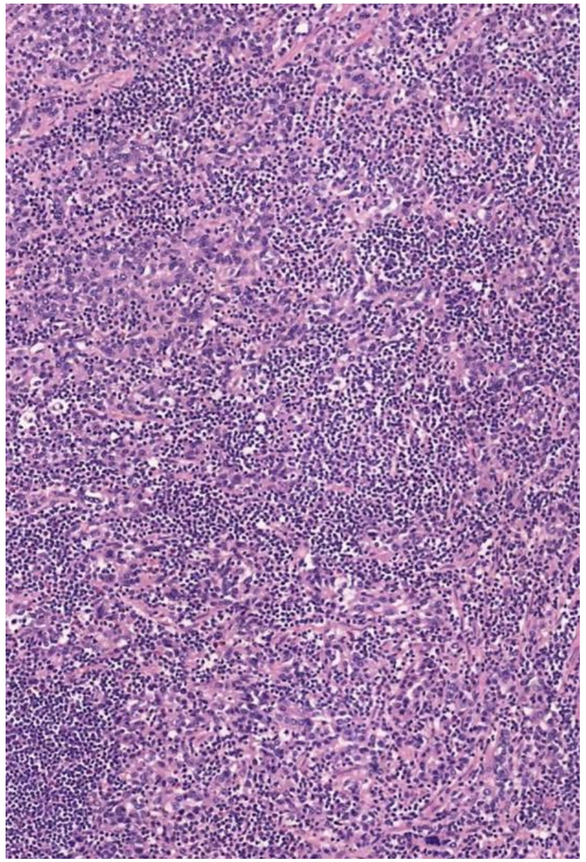
Kim ST, *et al.* – Nat Med 2018

Standardizing and prioritization of biomarker testing pathway

How do we prioritize?

Can we use site/morphology to choose?

HER2: > intestinal type, > GEJC
MMR: > lymphocyte rich, heterogeneous, solid poorly differentiated
Claudin18.2: > diffuse

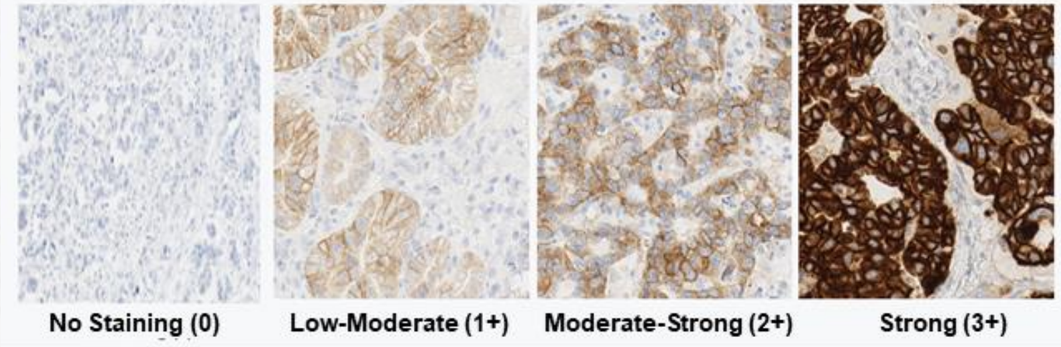


Novel diagnostics horizons

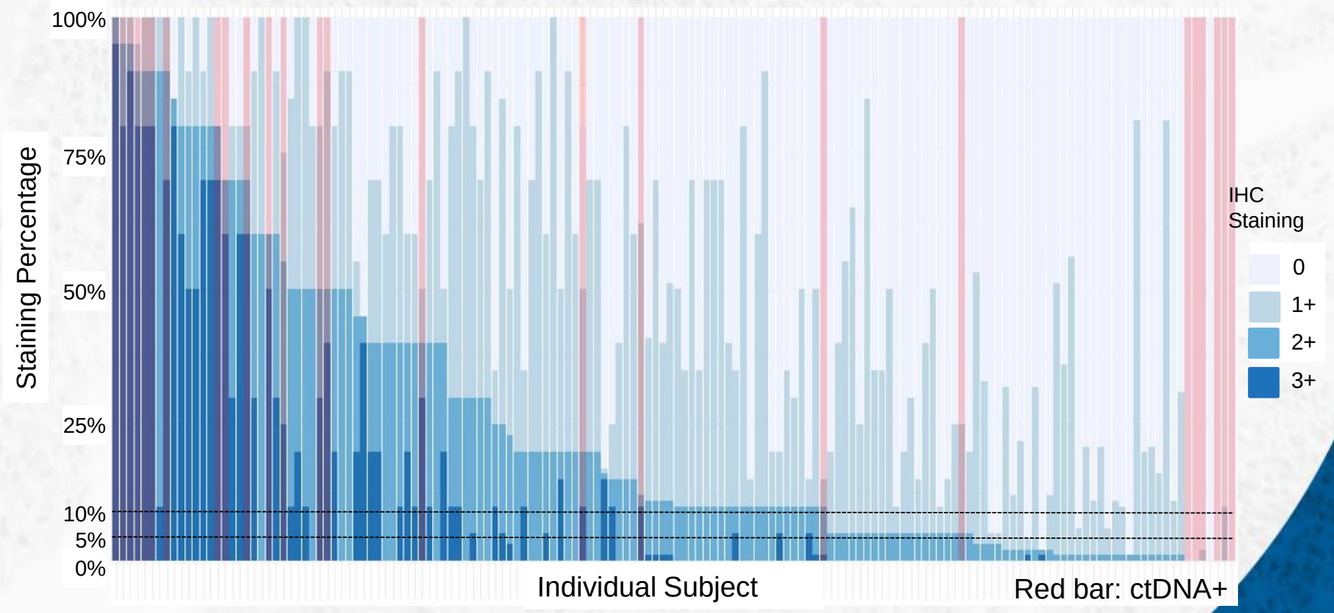
Despite a landscape clouded in complexity, emerging biomarkers are expanding our view of patient populations, and biomarker testing could provide a more comprehensive patient profile in the precision oncology era.

FGFR2b as a new target in gastric cancer

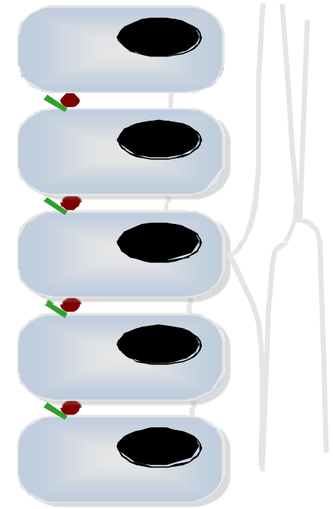
- 30.2% of metastatic G/GEJ adenocarcinomas were positive for FGFR2b expression
- FGFR2b genomic aberrations rarely overlap with other common GC genomic aberrations such as *HER2*, *MET and* other RTK biomarkers
- A Phase 2 study has demonstrated that the anti-FGFR2b mAb, bemarituzumab, plus mFOLFOX6 is generally well tolerated and has anti-tumour activity in G/GEJ cancer patients



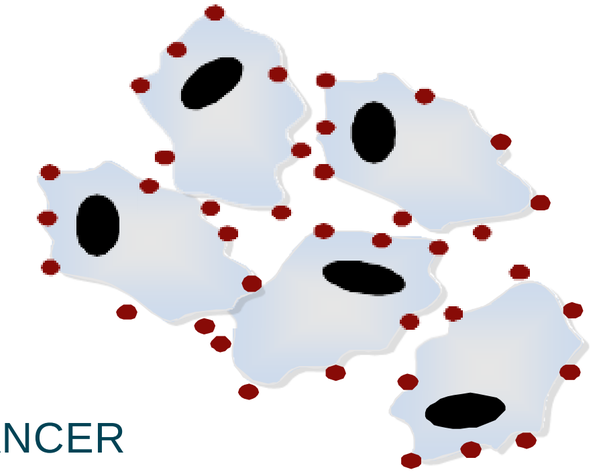
FGFR2b IHC+ defined as 2+/3+ staining



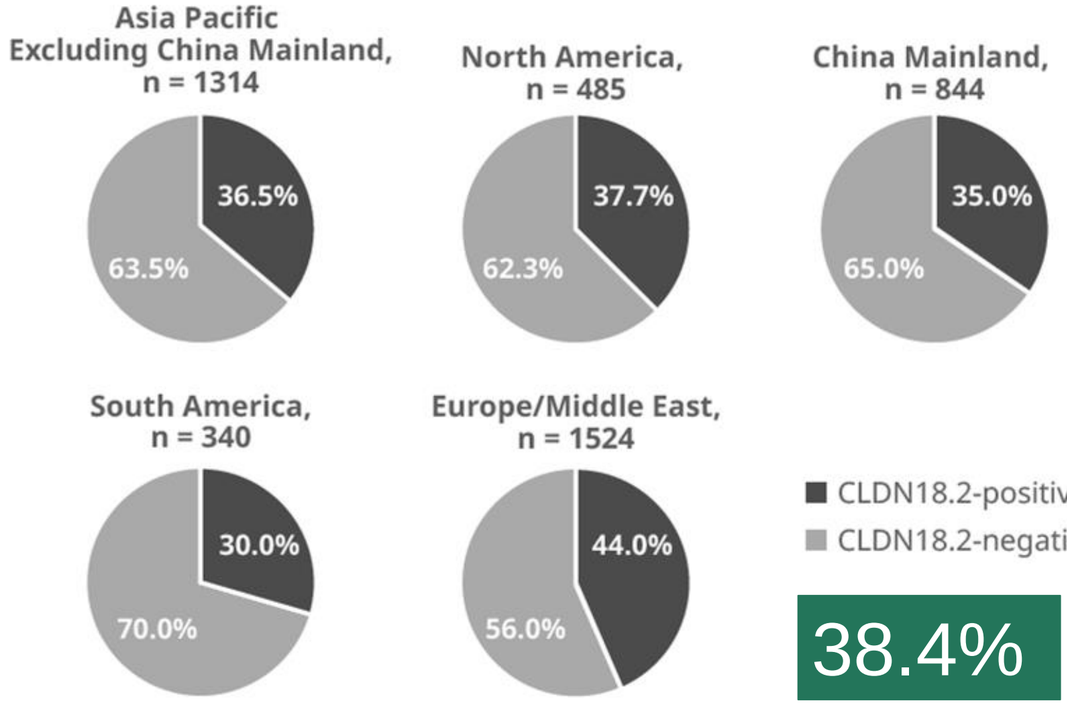
CLDN18.2 in gastric cancer cells



NORMAL



CANCER



Lauren classification			
Diffuse	553 (48.3)	592 (51.7)	0.0002
Intestinal	308 (38.8)	486 (61.2)	
Mixed	134 (42.9)	178 (57.1)	
Other	252 (37.2)	425 (62.8)	
Unknown	412 (35.0)	765 (65.0)	

Shitara K, *et al.* – Presented at 2023 ASCO Annual Meeting; June 2–6,

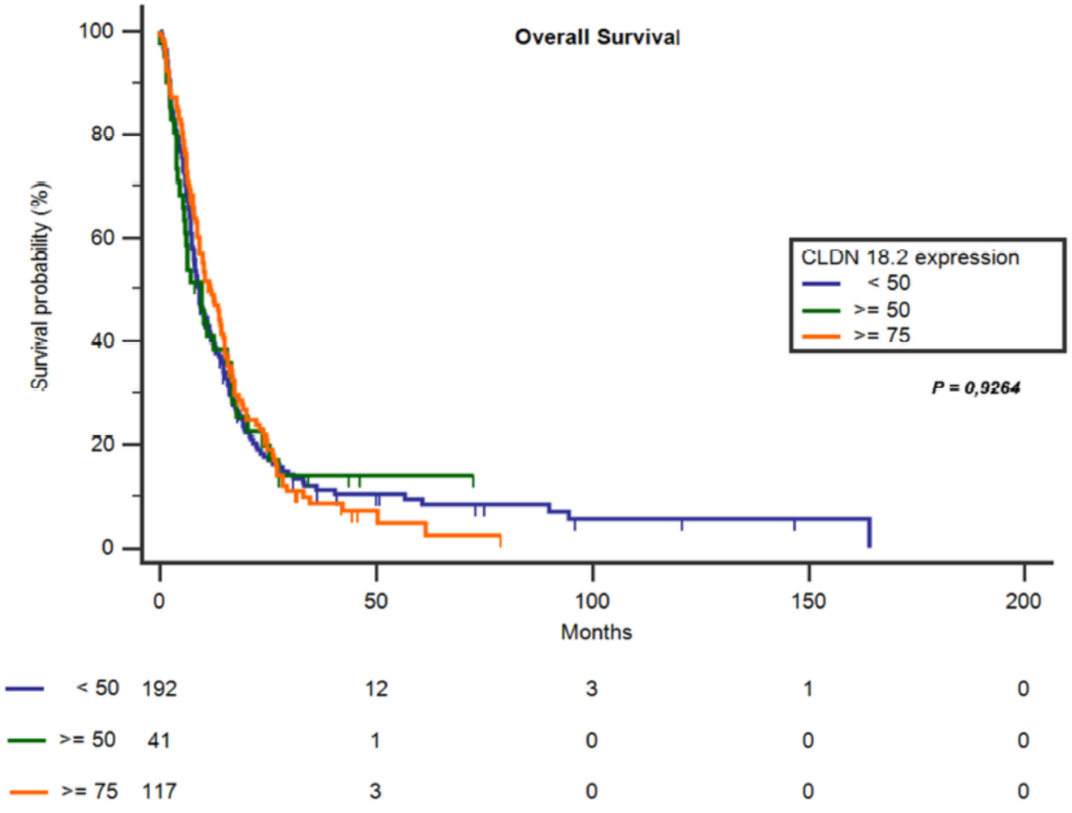
Journal of *Personalized Medicine*

MDPI

Article

Association of CLDN18 Protein Expression with Clinicopathological Features and Prognosis in Advanced Gastric and Gastroesophageal Junction Adenocarcinomas

Antonio Pellino [1,†], Stefano Brignola [2,3,†], Erika Riello [2], Monia Niero [3], Sabina Murgioni [1], Maria Guido [2,3], Floriana Nappo [1], Gianluca Businello [2], Marta Sbaraglia [2], Francesca Bergamo [1], Gaya Spolverato [4], Salvatore Pucciarelli [4], Stefano Merigliano [5], Pierluigi Pilati [6], Francesco Cavallin [7], Stefano Realdon [8], Fabio Farinati [9], Angelo Paolo Dei Tos [2], Vittorina Zagonel [1], Sara Lonardi [10,‡], Fotios Loupakis [1,‡] and Matteo Fassan [2,11,*,‡]



34º Congresso Brasileiro de PATOLOGIA
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29/05 a 01/06/2024 Belém/PA

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350 metastatic Caucasian GC/GEJ patients

- Nodal involvement (p = 0.0407)
- high stage disease (III, IV) at diagnosis (p = 0.019)
- age < 70 (p = 0.0035)
- peritoneal involvement (p < 0.001)
- lower incidence of liver metastases (p = 0.009)
- EBV positive status (p = 0.001)

CLDN18.2 expression can be detected using IHC

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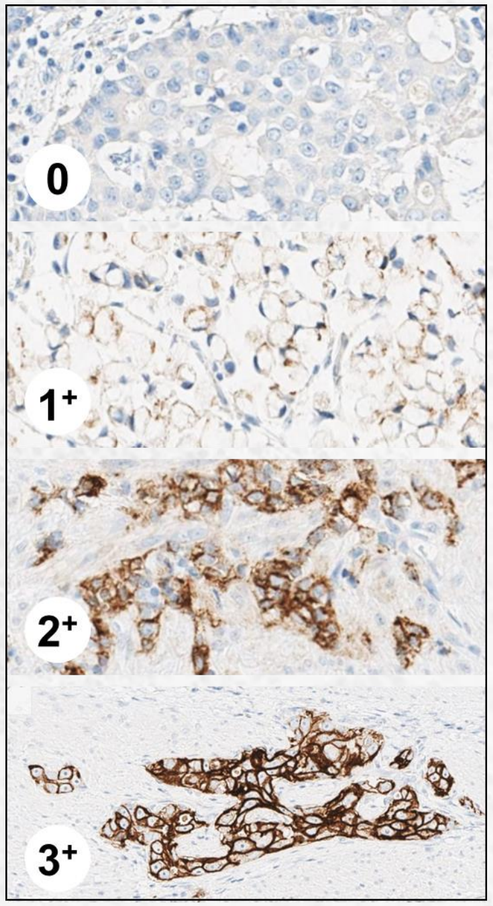


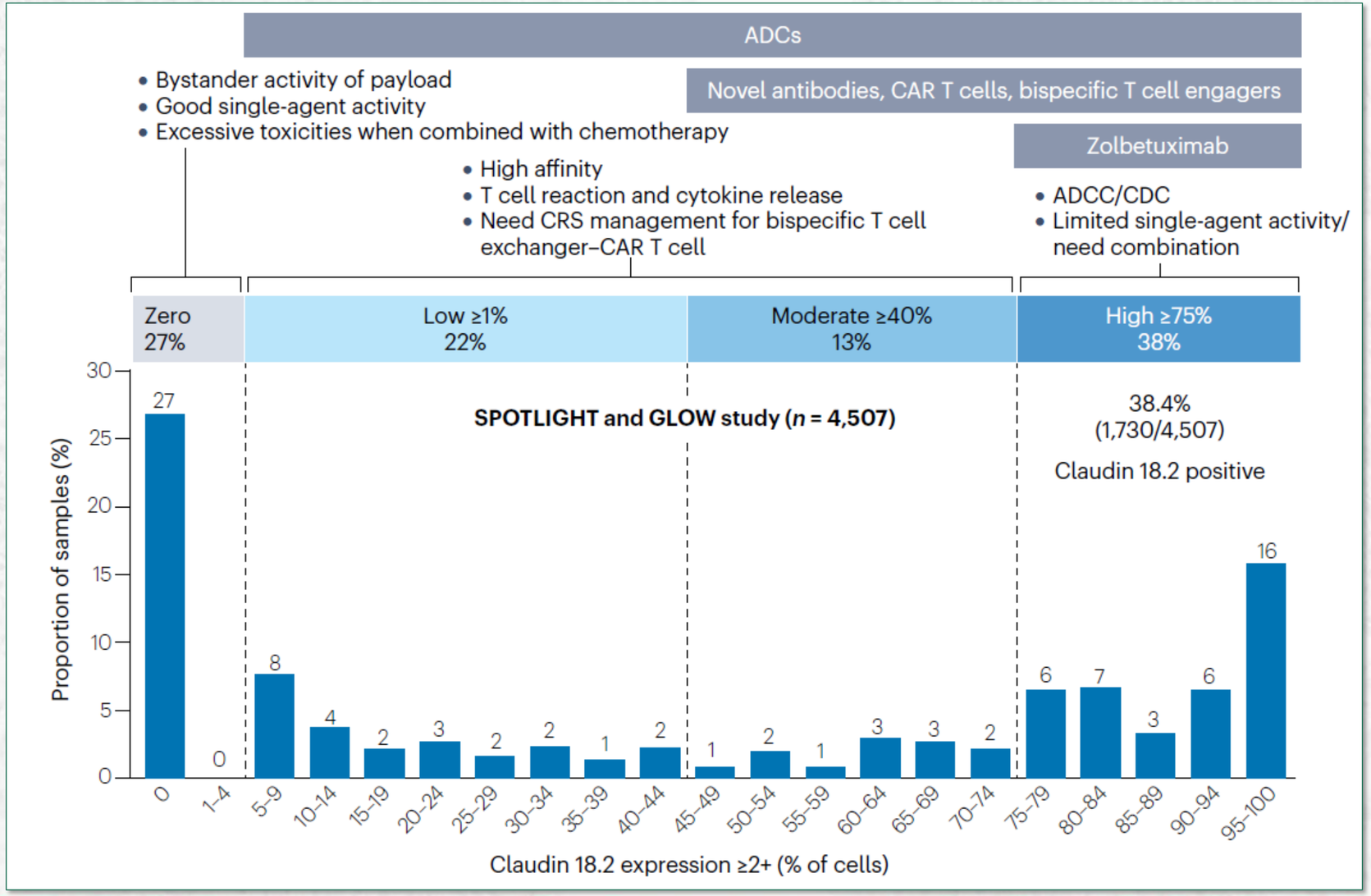
Membrane staining of tumour cells

- CLDN18 can be detected using IHC staining methods
- high expression is defined as 2+/3+ IHC staining in ≥75% of tumour cells

Today's cut-off, it can be modified according to next clinical trials' results

Angerilli V, *et al.* – Pathol Res Pract 2023; Fassan M, *et al.* – In submission

One biomarker, different therapeutic options

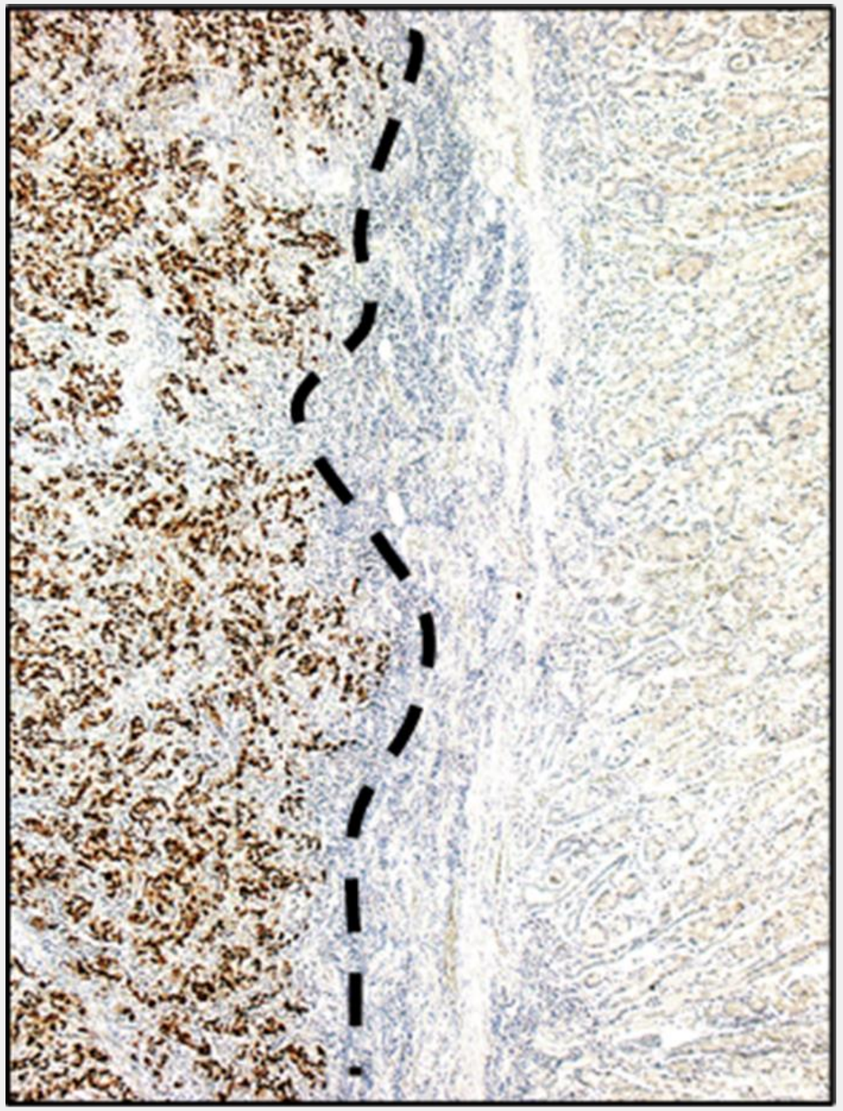


Nakayama I, *et al.* – Nat Rev Clin Oncol 2024

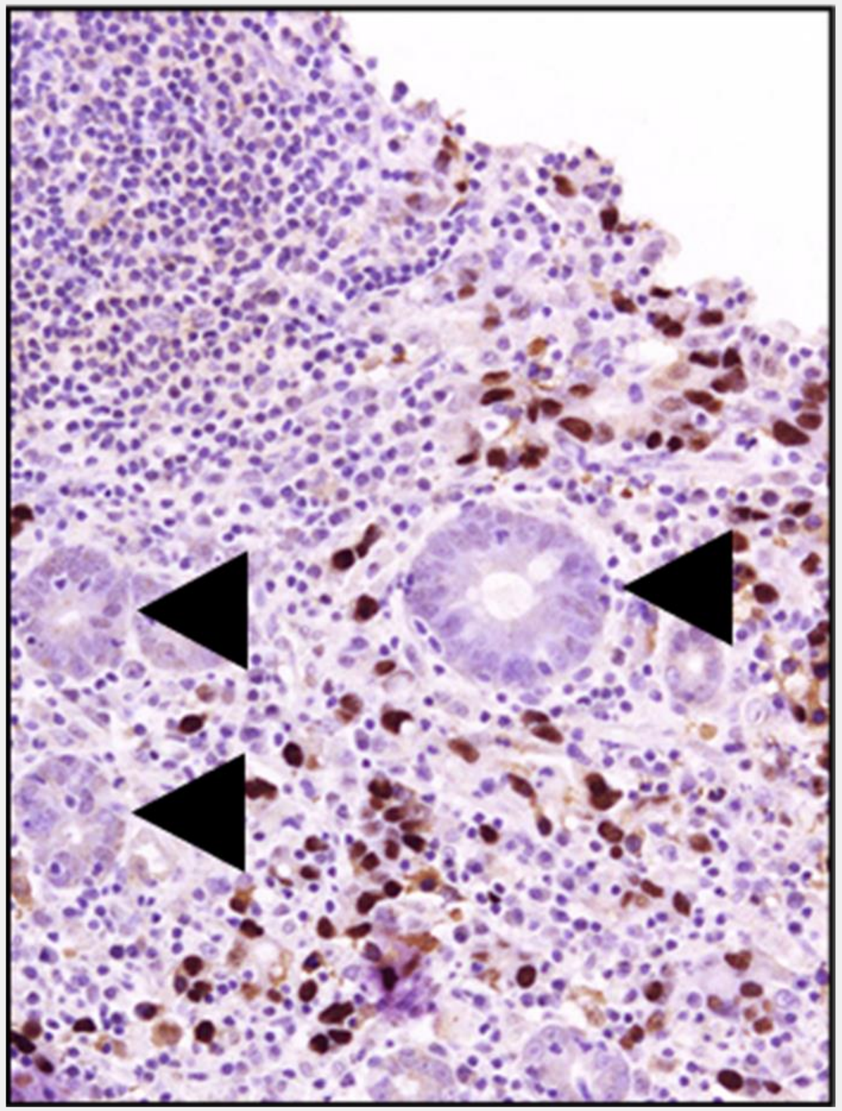
EBV

3-5%

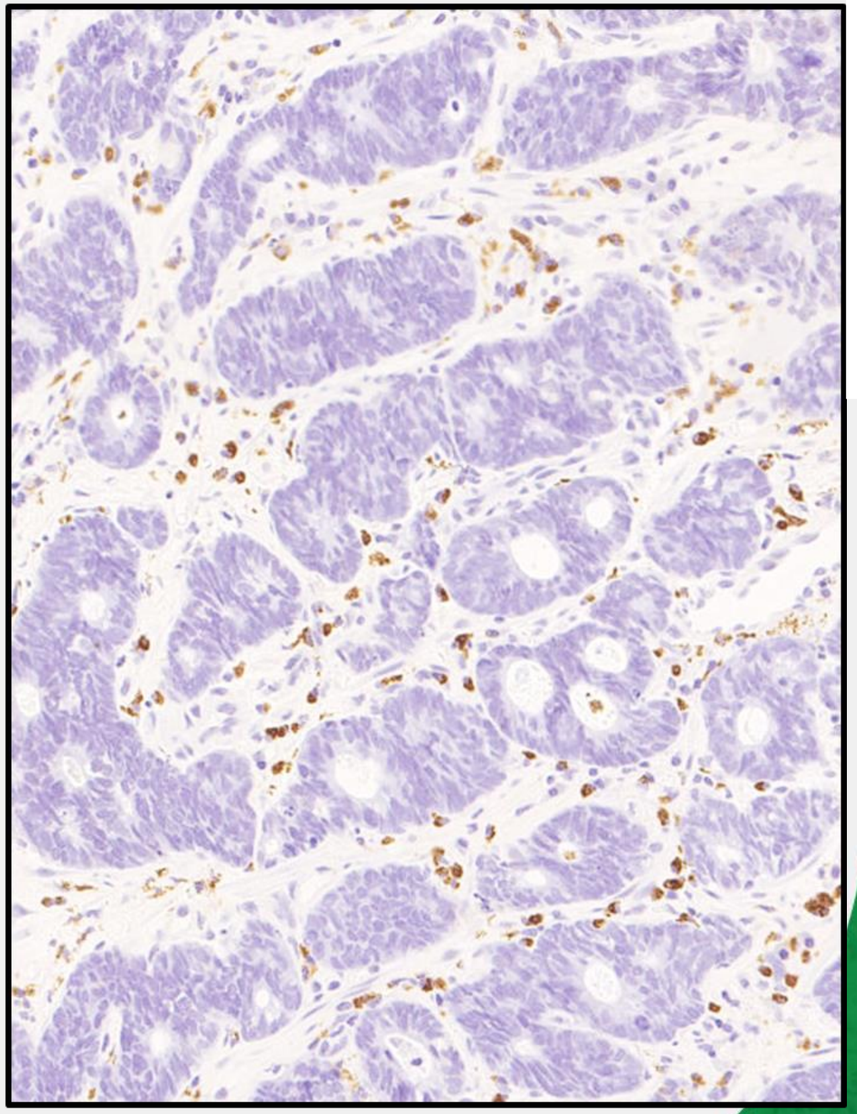
9-15%



EBV-GC and normal gastric mucosa



EBV-GC and atrophic gastritis



EBV-negative GC

GE biomarkers: today it is still a IHC story!

	HER2	PD-L1	MMR/ MSI	EBV	CLDN18	Fgfr2b
 IHC/ISH	✓	✓	✓	✓	✓	✓
 molecular	✗	✗	✓	✗	✗	✗

Barriers to universal implementation of predictive biomarkers in GE cancer

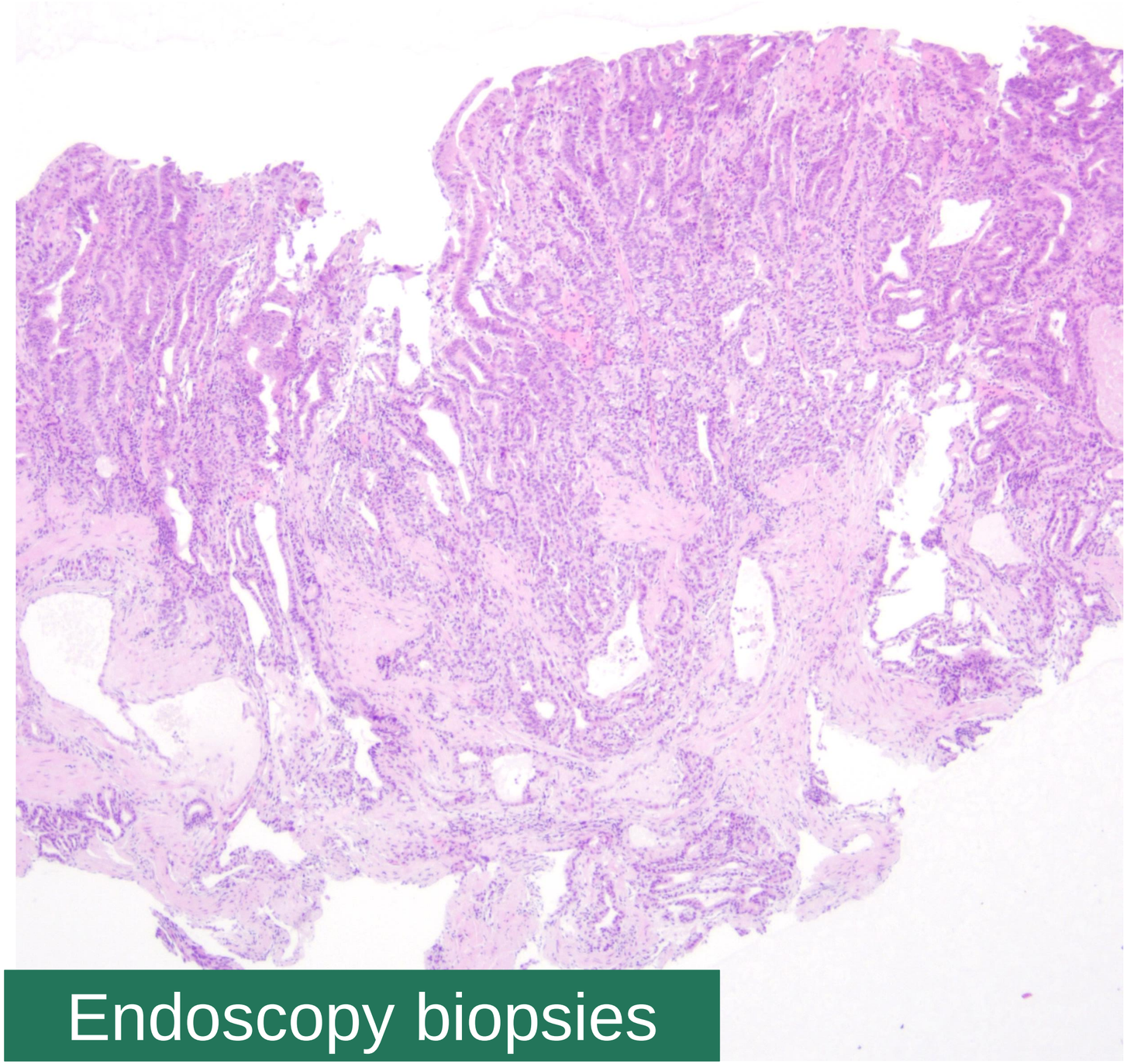
Limitations and barriers	PD-L1 by IHC	MMR by IHC	MSI by PCR	TMB	HER2
Prevalence in GC	•	•••	•••	•••	—
Diagnostic access	•	—	•	•••	—
Diagnostic cost	• (mainly a limitation in the United States)	—	•	•••	—
Regulatory/global ubiquity	•	••	••	•••	—
Correlation to clinical outcome	Label-specific	Label-specific	Label-specific	Label-specific	—
Poor uniformity with assays and scoring	•••	•	—	•••	—
Testing and communication timelines	•	•	••	••	—

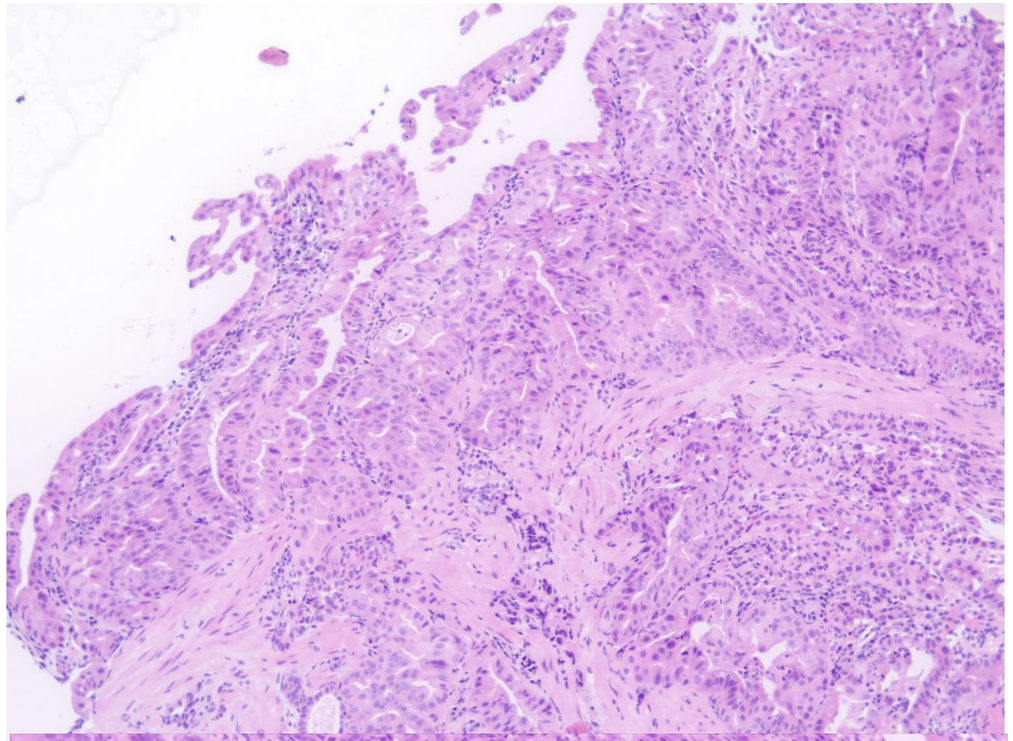
••• more limitations, • fewer limitations, — not a limitation

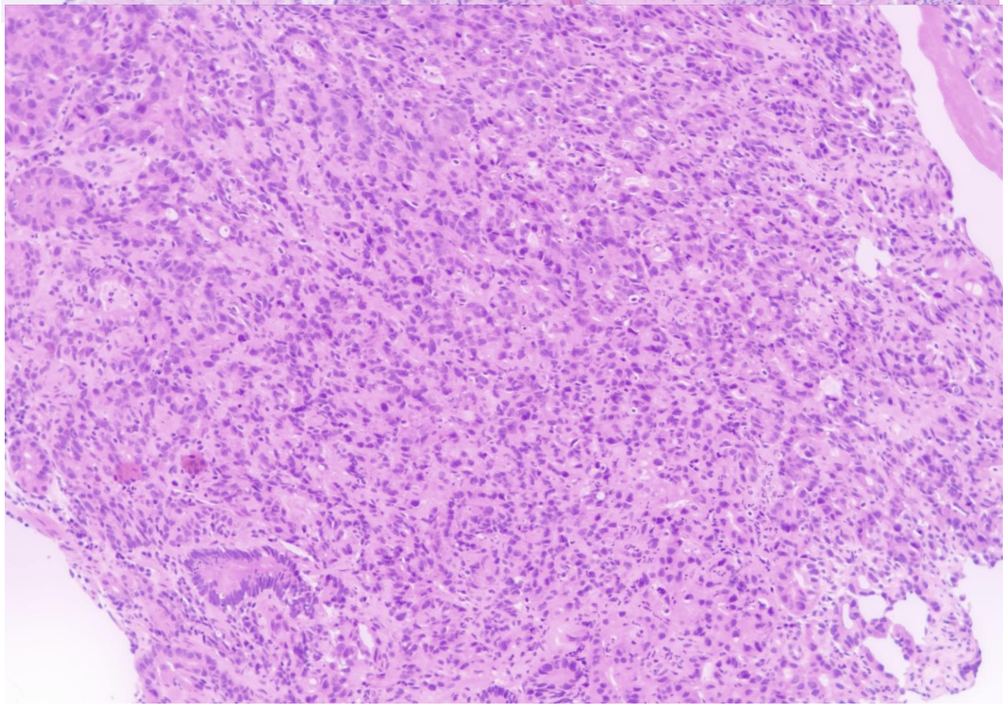
Myer NM, *et al.*– J Cancer Res Clin Oncol 2022

…just look at the microscope!

1. Don’t forget how to evaluate HER2!

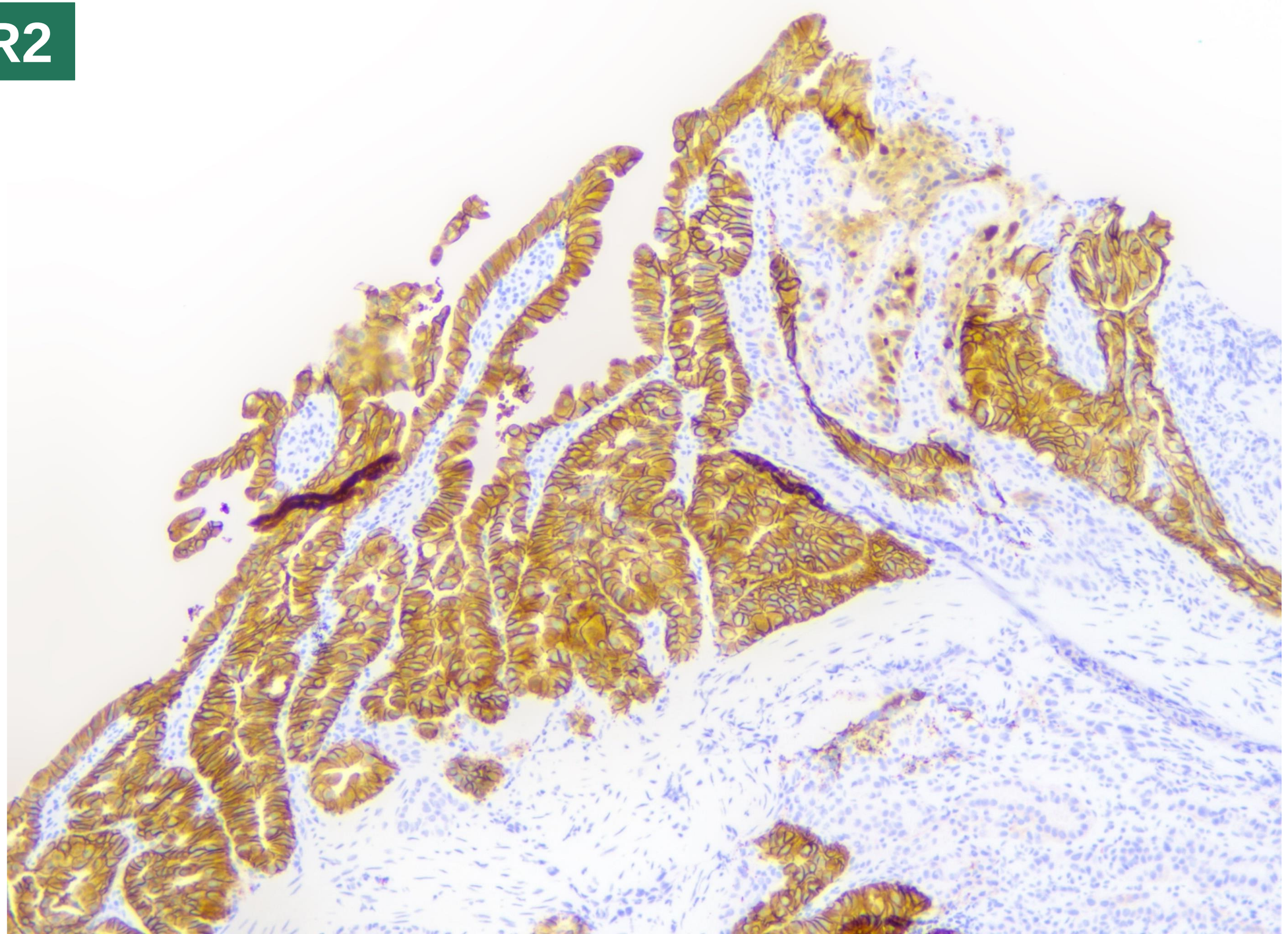




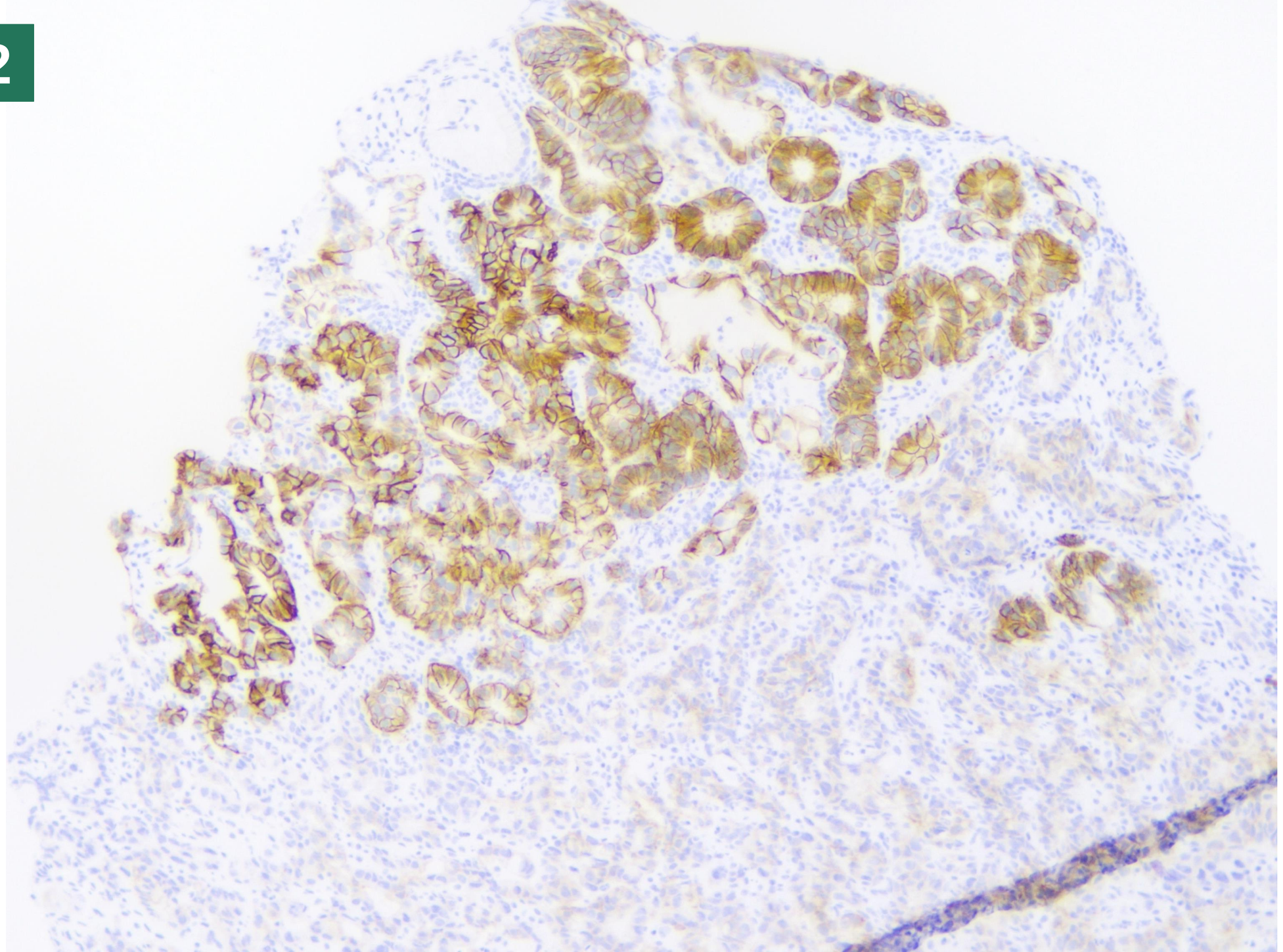


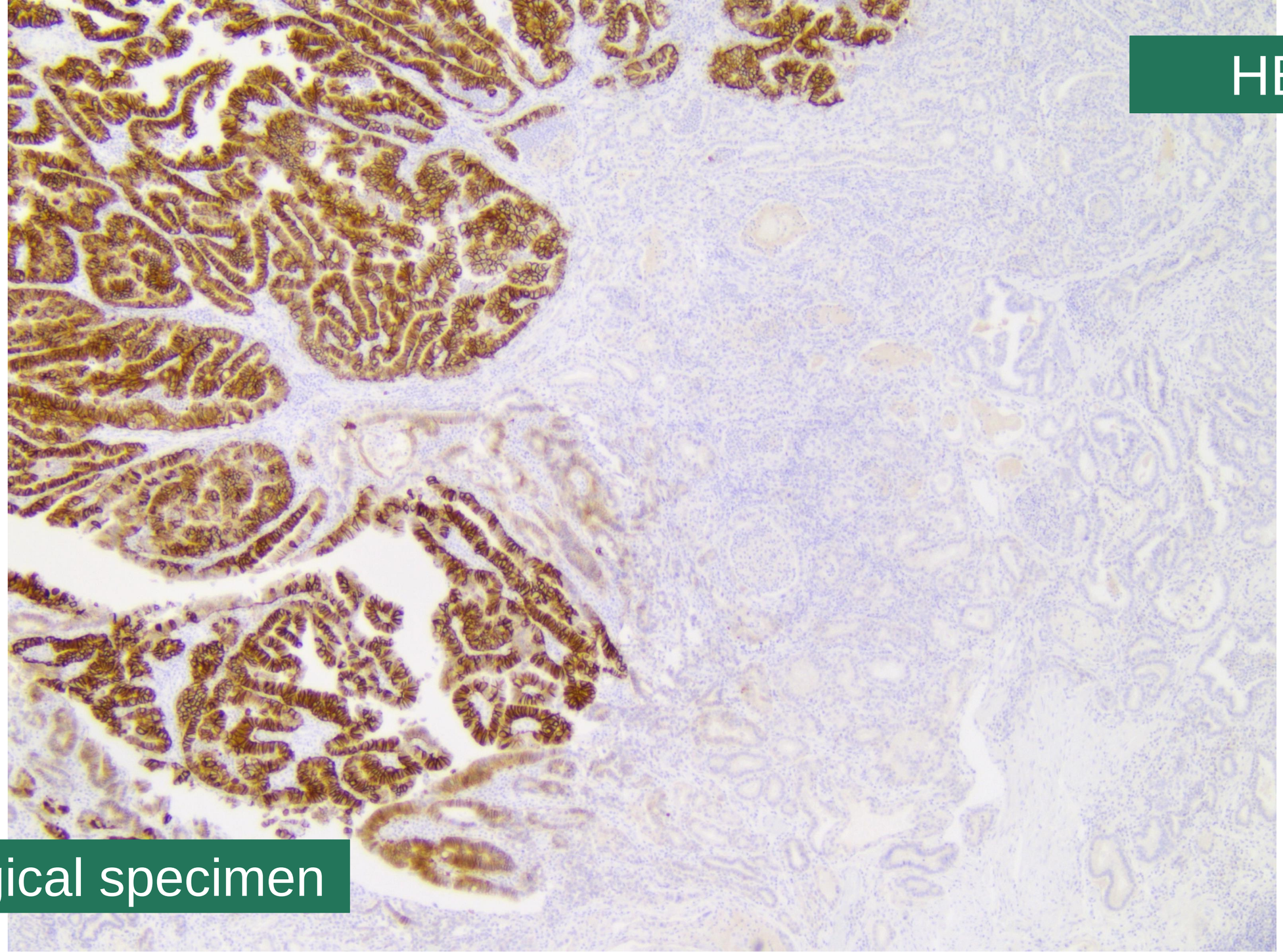
Endoscopy biopsies

HER2



HER2





HER2

Surgical specimen

How to evaluate HER2 in this case?

A) score 3 (positive) both in biopsy and in the surgical specimen

B) score 3 (heterogeneous positivity)

C) score 0 (it’s negative in the invasive component!)

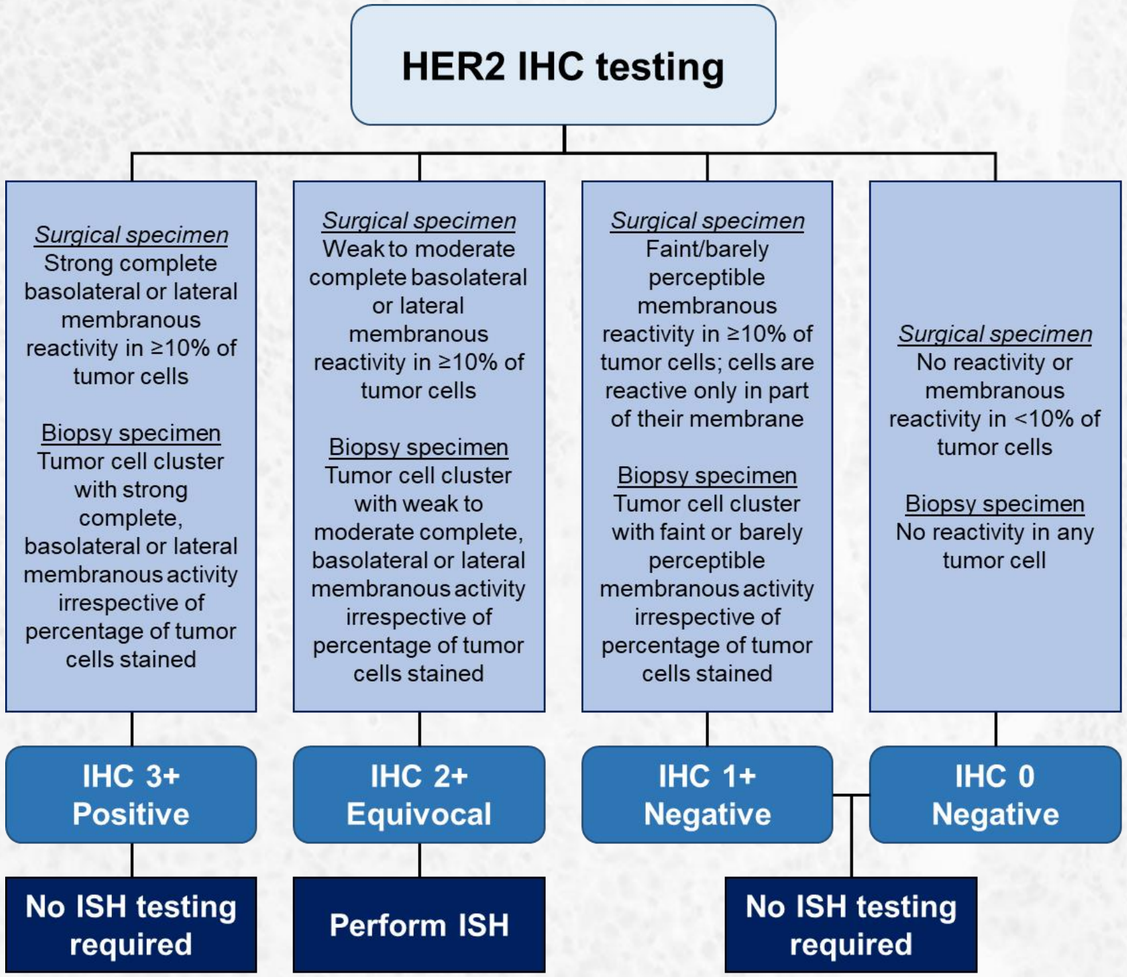
How to evaluate HER2 in this case?

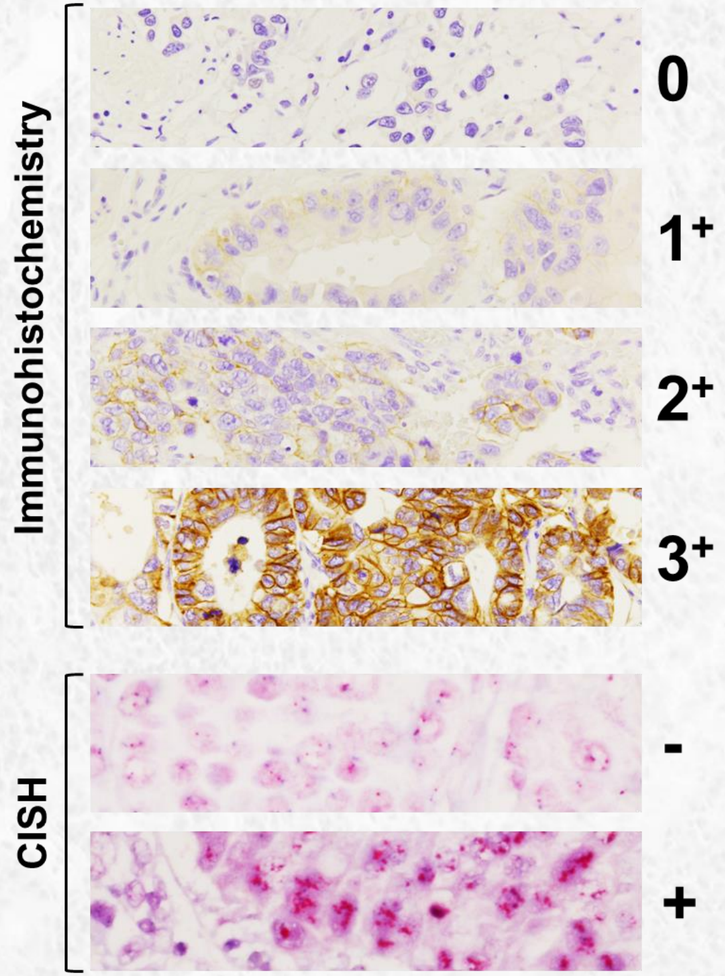
A) score 3 (positive) both in biopsy and in the surgical specimen

B) score 3 (heterogeneous positivity)

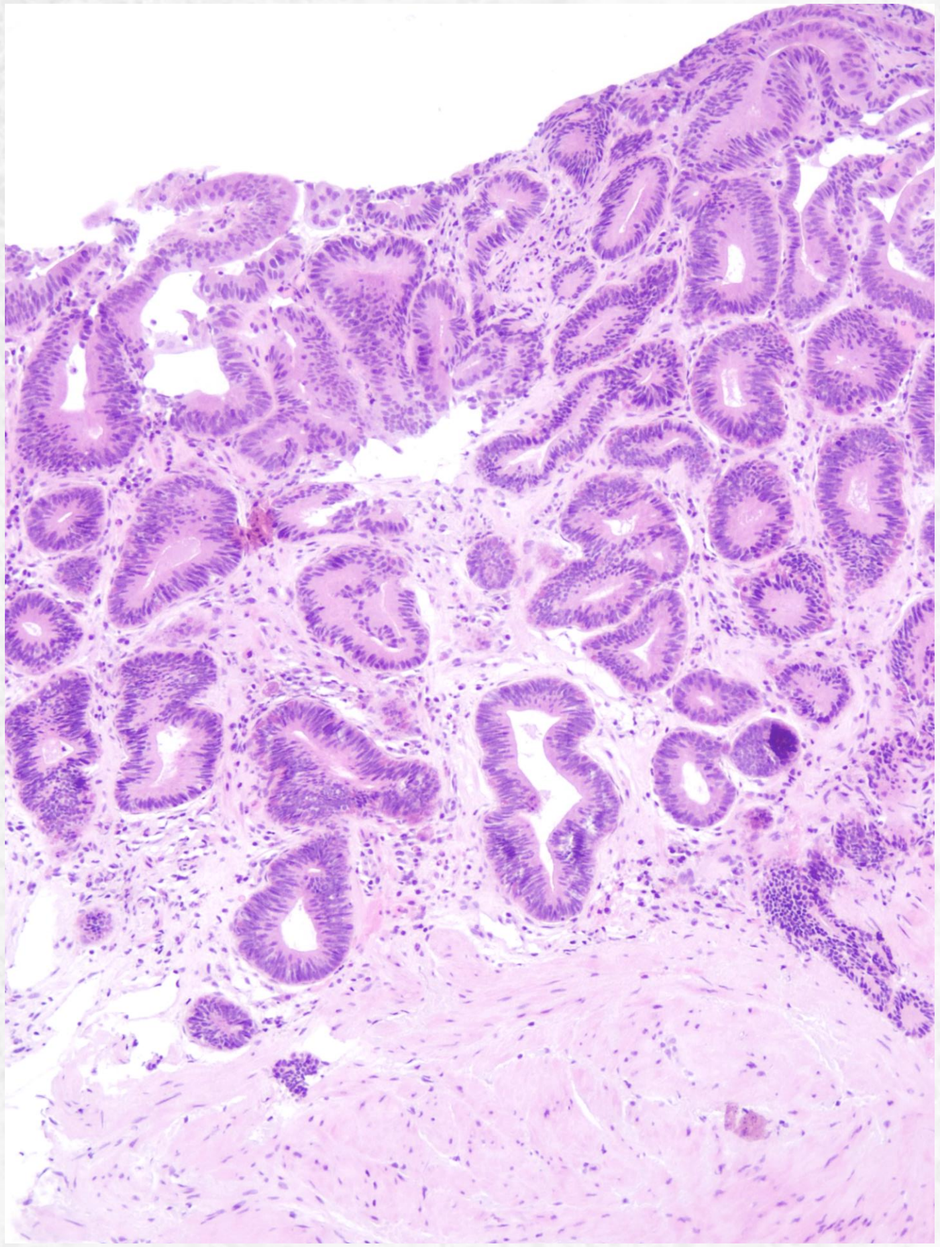
C) score 0 (it’s negative in the invasive component!)

HER2 testing in practice

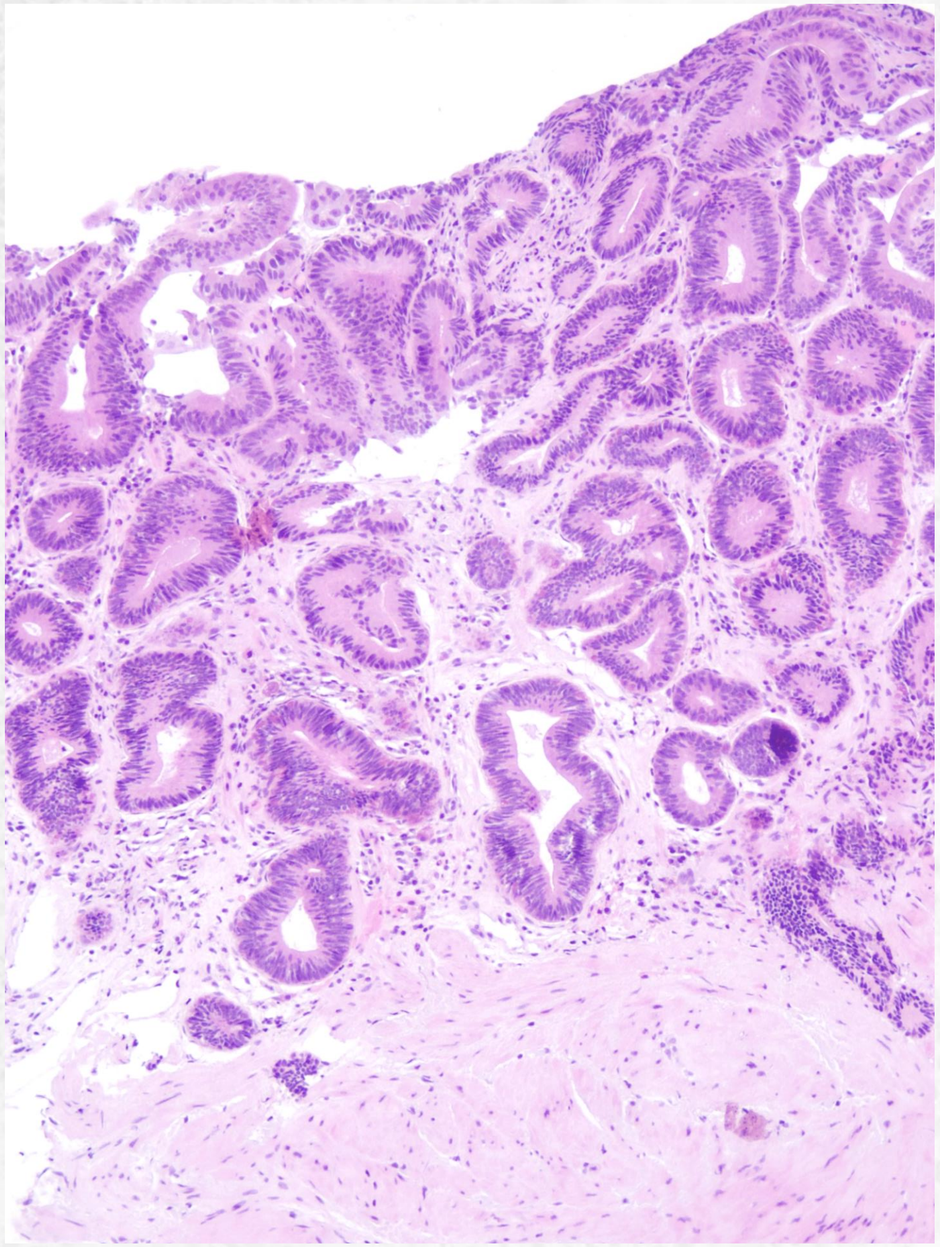


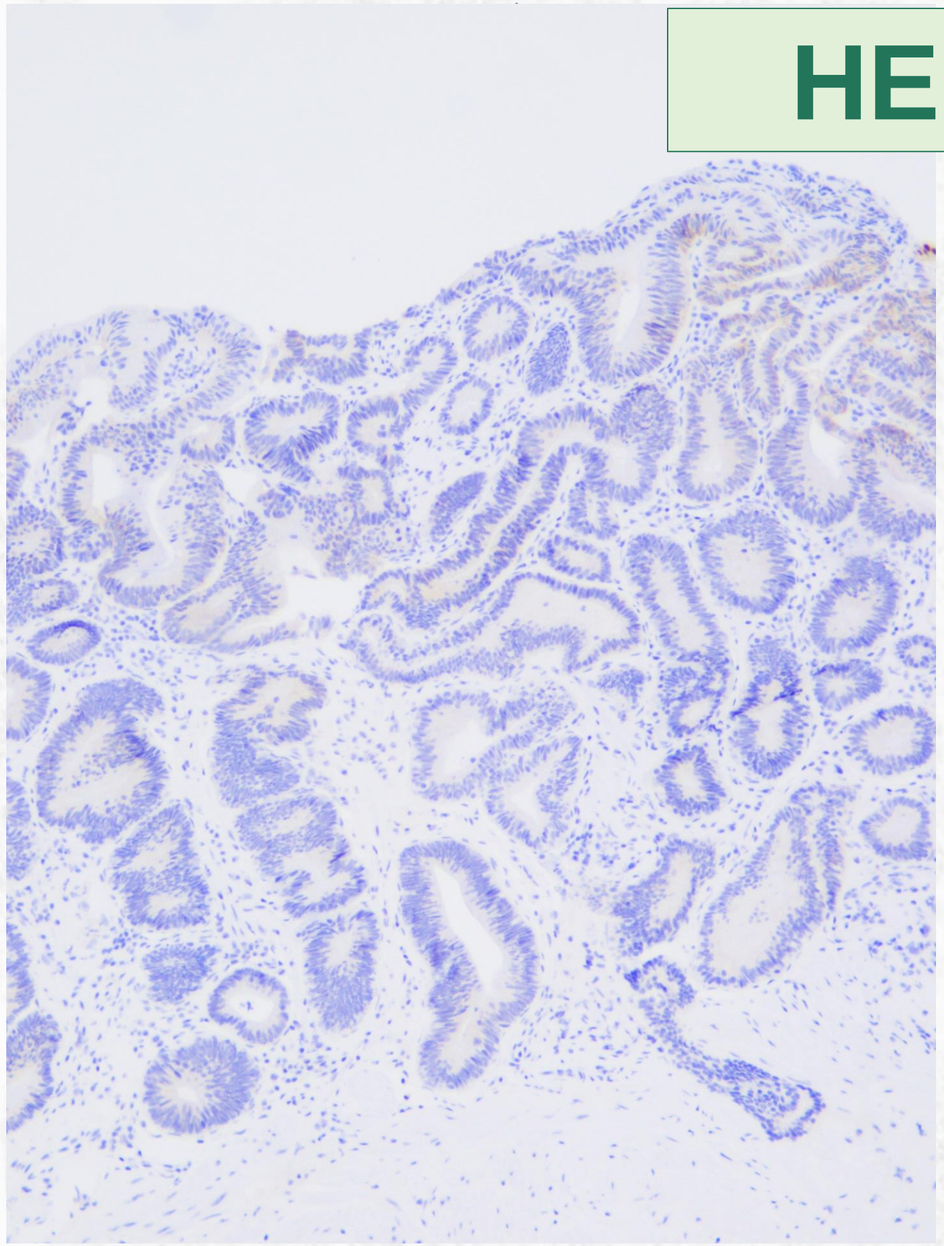


Fassan M, *et al.*– Pathologica 2020



What about HER2 status in this area?





HER2

How to evaluate HER2 in this case?

A) score 0

B) score 1+

C) It’s dysplastic tissue, and therfore I cannot evaluate HER2 in this area

How to evaluate HER2 in this case?

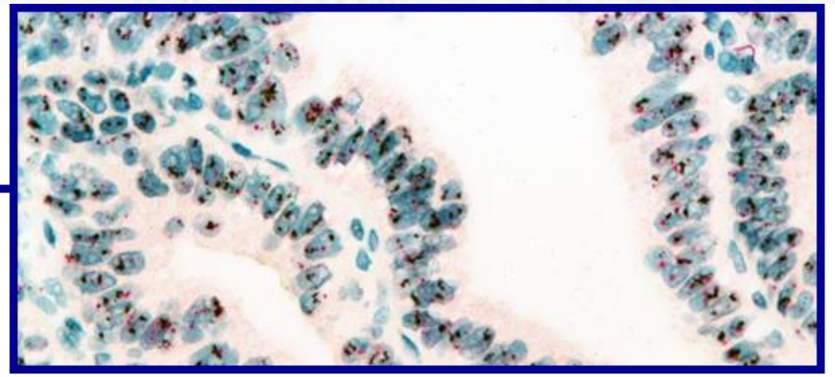
A) score 0

B) score 1+

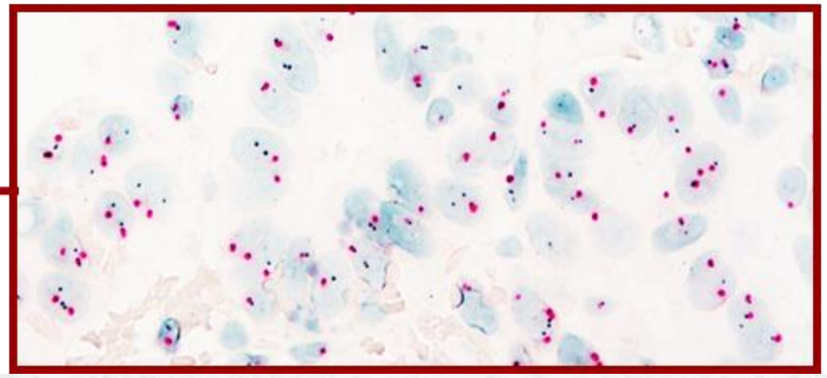
C) It’s dysplastic tissue, and therfore I cannot evaluate HER2 in this area

IHC HER2

LGD



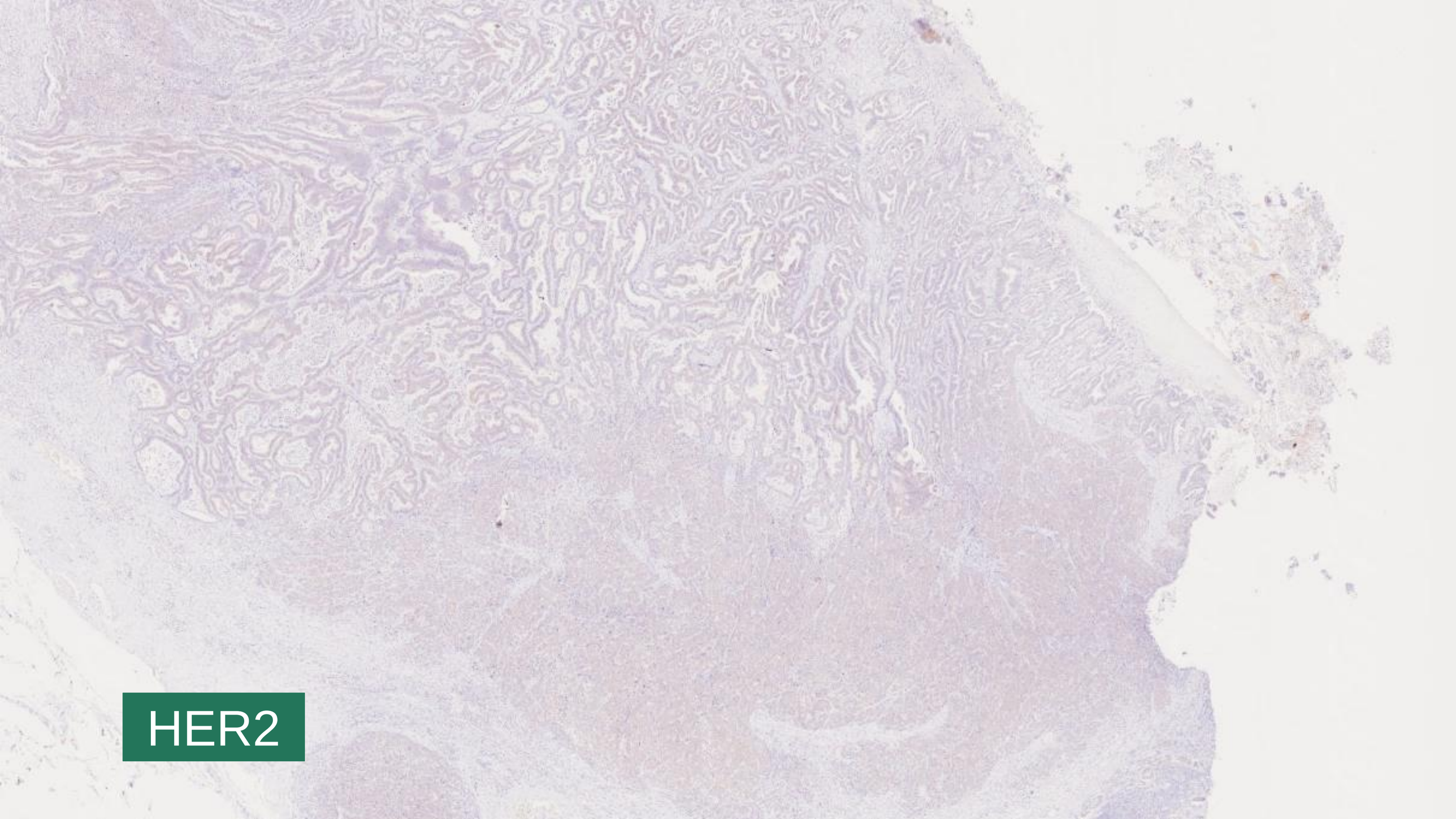
SISH
HER2

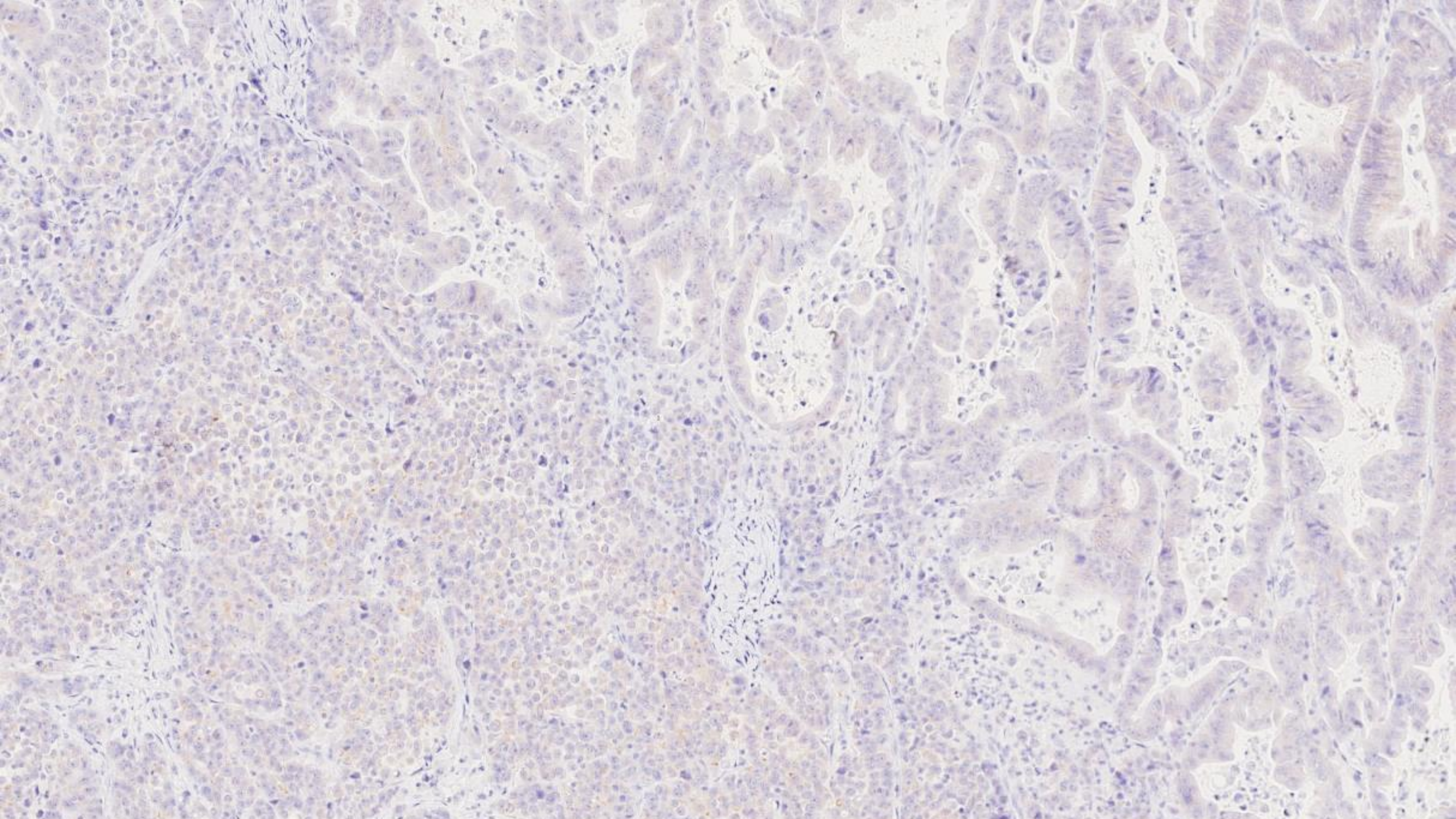


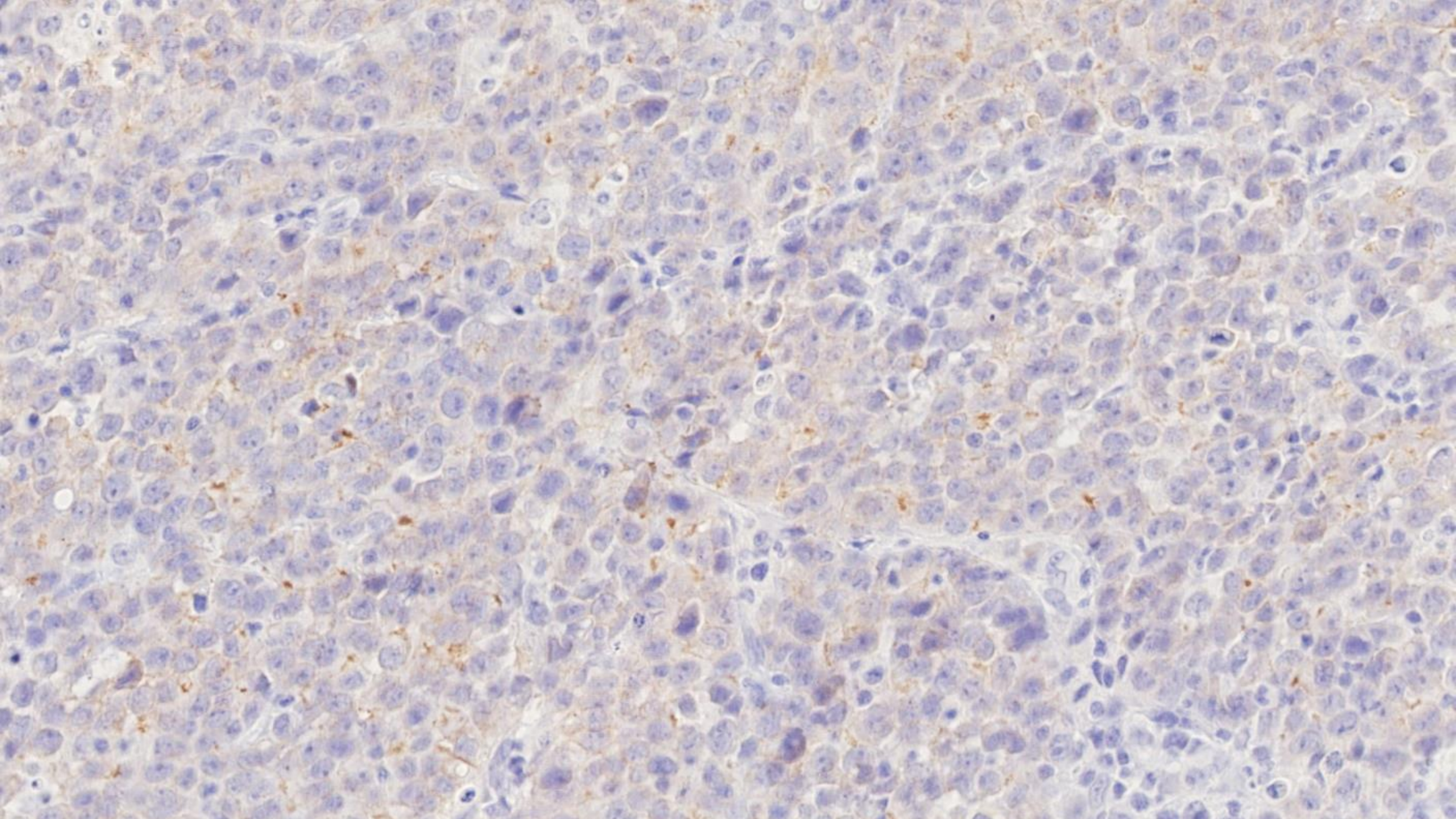
BIOMARKERS- H&E COMBINED EVALUATION IS REQUIRED IN BIOPSY MATERIAL

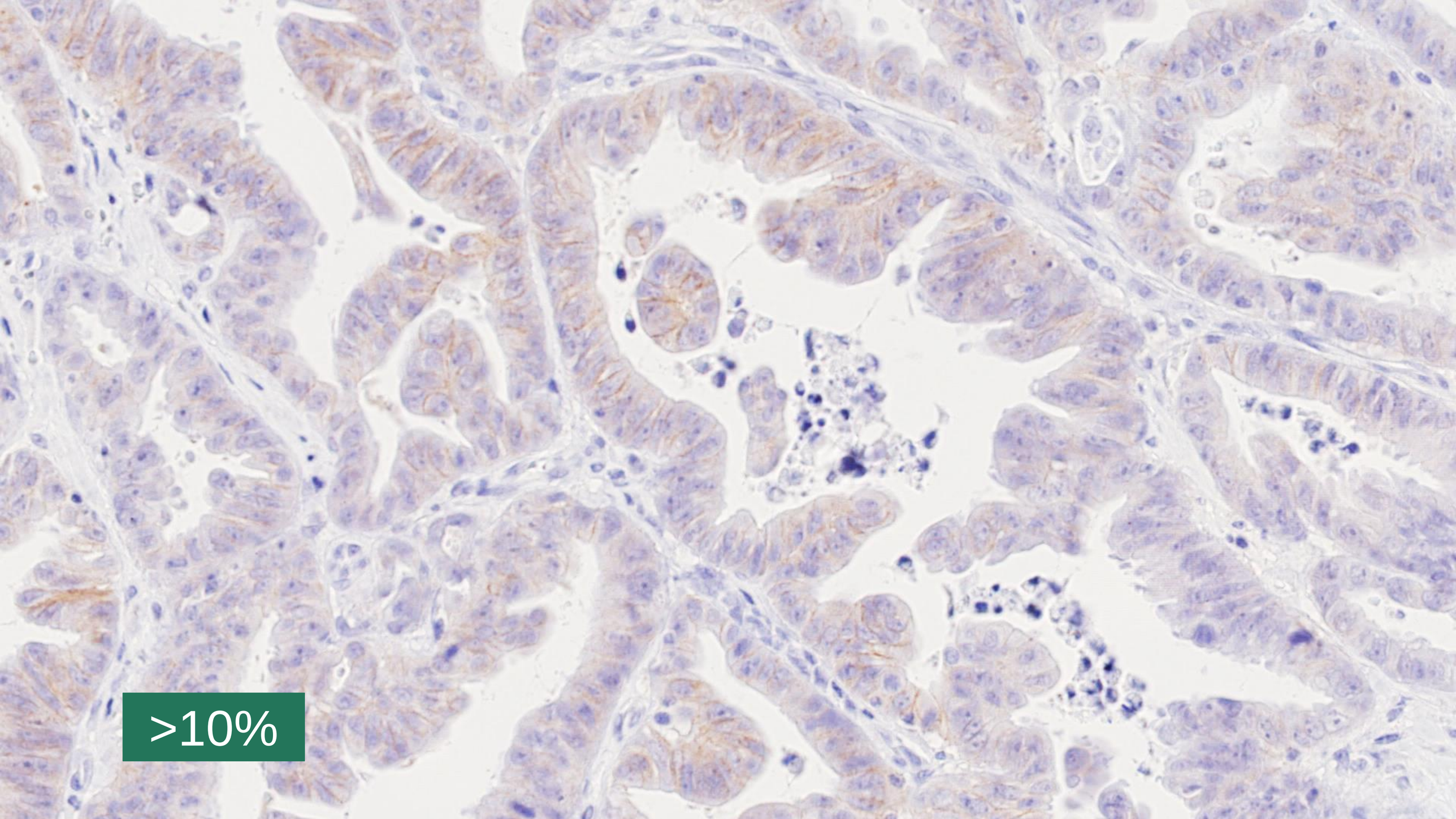
Fassan M, *et al.* – Histopathology 2012

2. HER2 negative?


HER2






>10%

How to evaluate HER2 in this case?

A) score 0

B) score 1+

C) inadequate

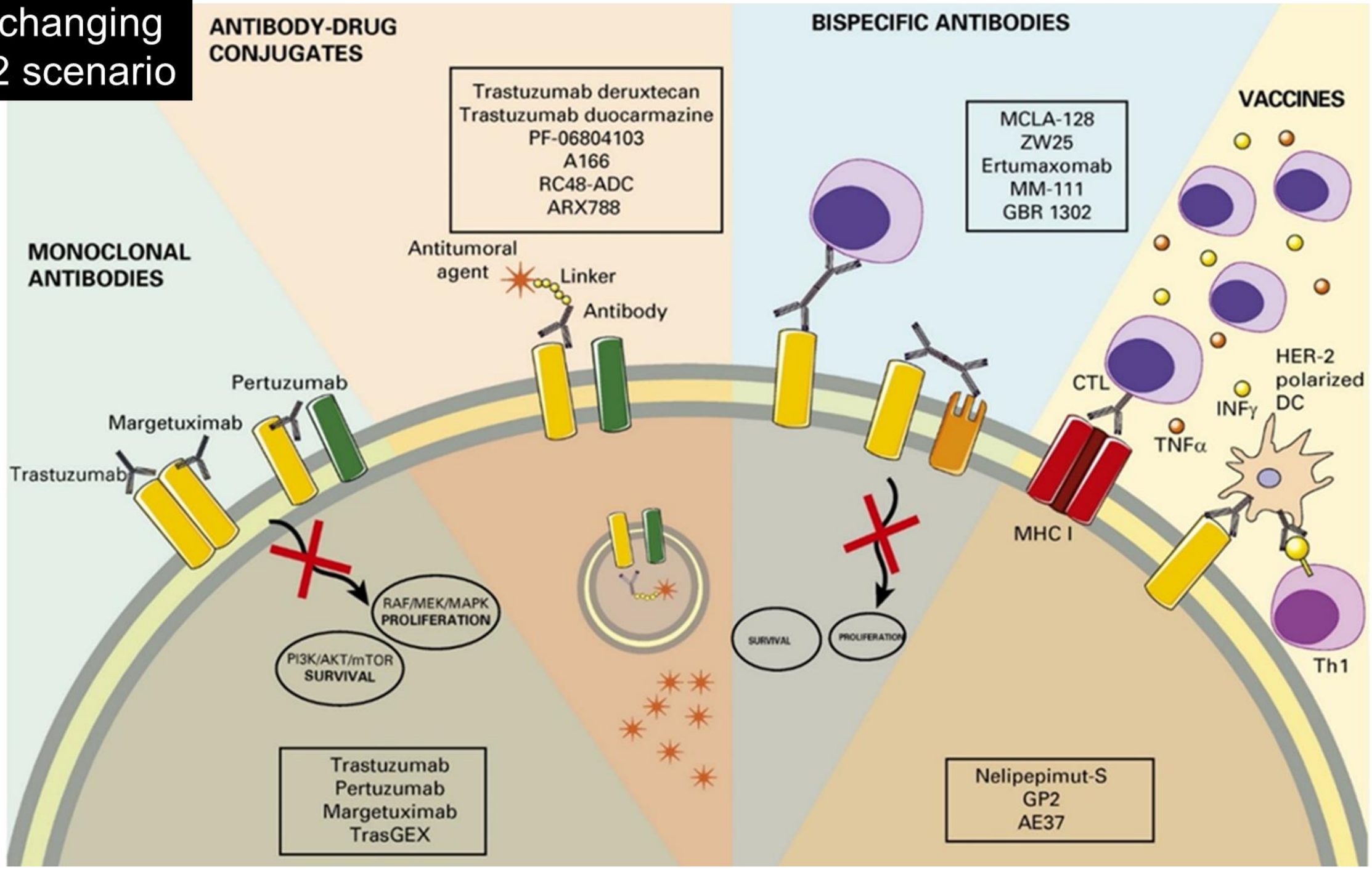
How to evaluate HER2 in this case?

A) score 0

B) score 1+

C) inadequate

The changing HER2 scenario



HER2-low in GC/GEJC: a real-world Italian perspective

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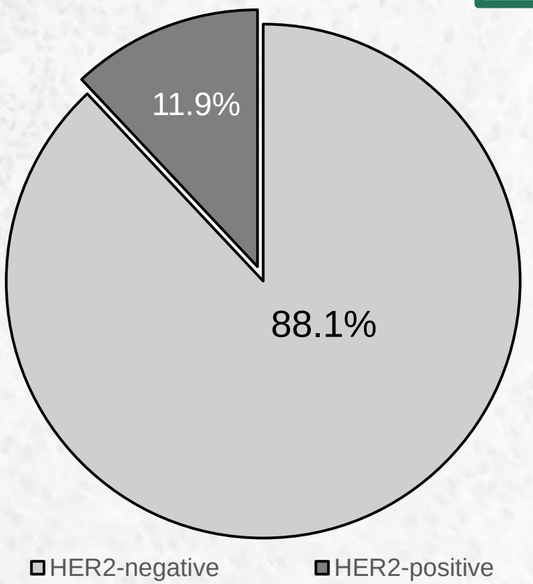
INOV AÇÃO INTEGR

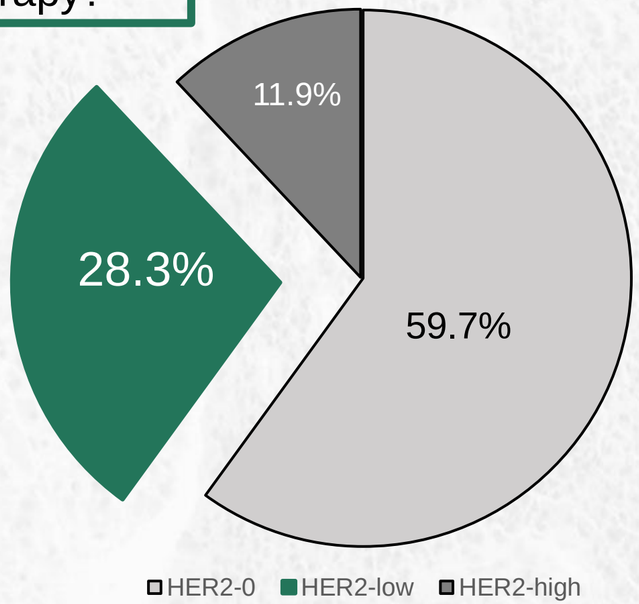
1,189 cases

Good candidate for Trastuzumab therapy

Not candidate for trastuzumab therapy



Benefit from T-DXd therapy?



32.2% of HER2-negative tumours were HER2-low, and may be targetable by T-DXd (Destiny - GASTRIC01 trial)

- Higher prevalence in biopsy specimens (p>0.0001)
- High inter-center variability (p=0.0005)

Angerilli V, *et al.* – J Clin Pathol 2023

HER2-low in GC/GEJC: a real-world Italian perspective

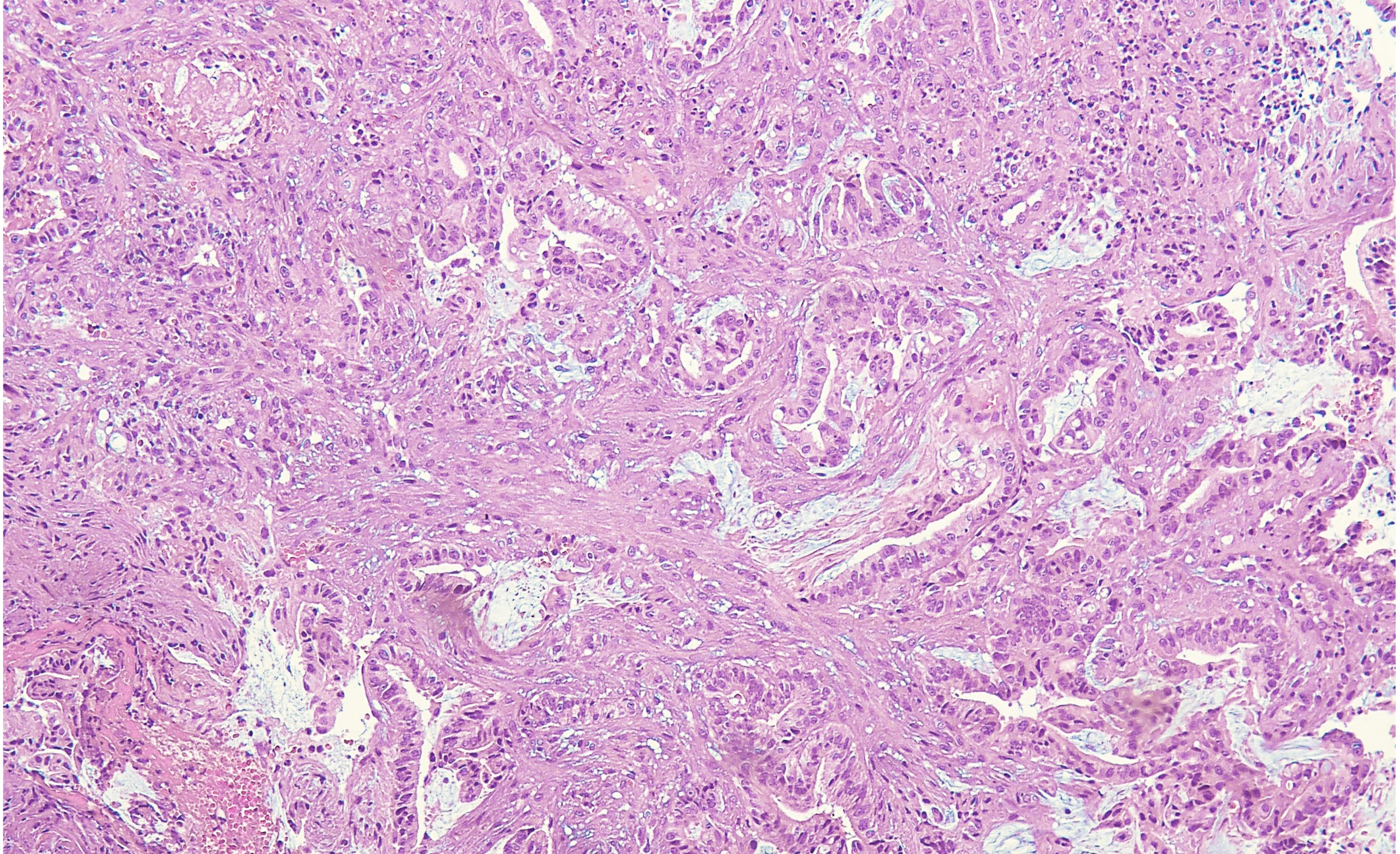
Table 2 Association between HER2 status and the other tested biomarkers

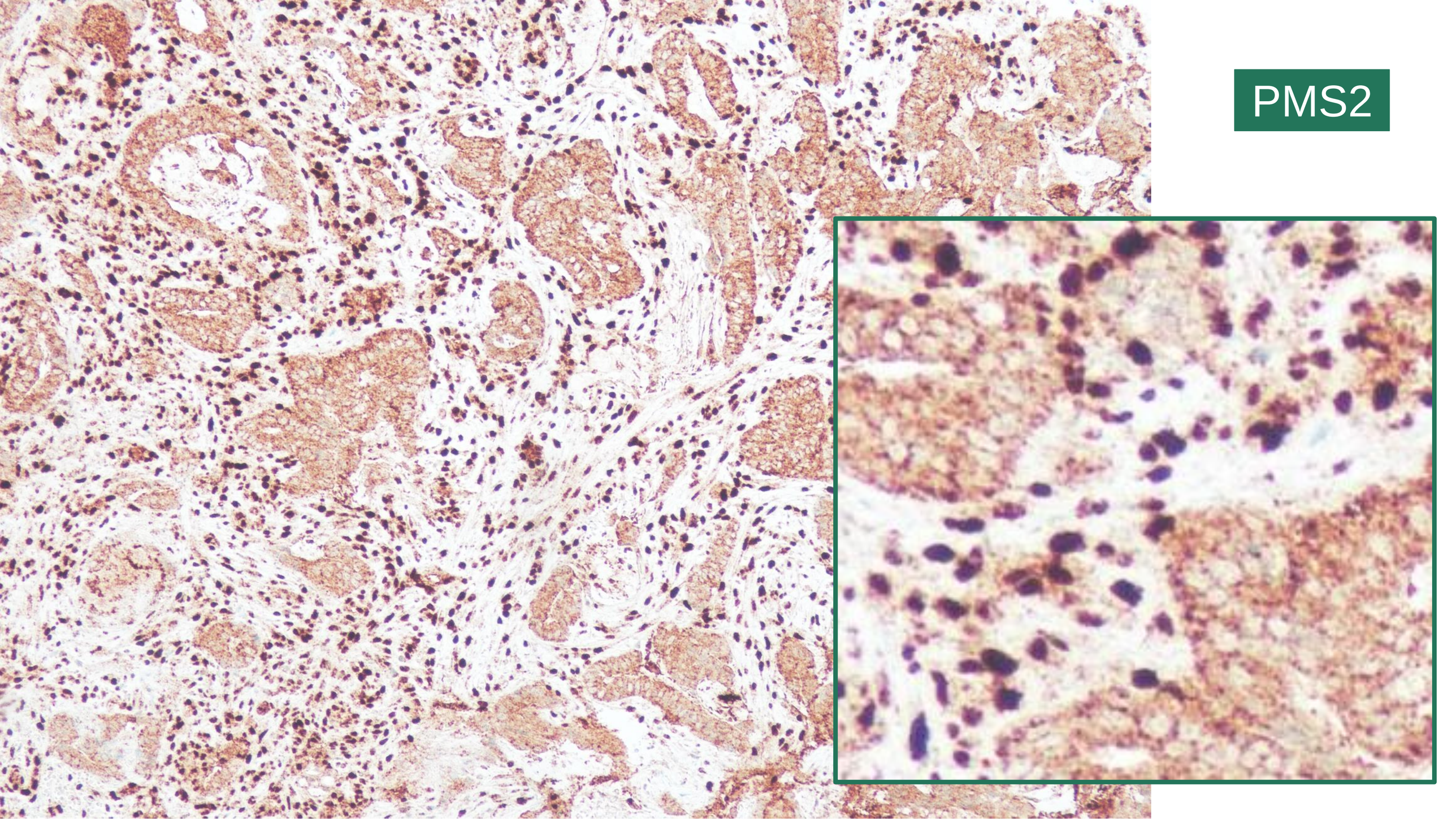
Strata	Total (n=1189)	HER2 0 (n=710)	HER2-low (n=337)	HER2-high (n=142)	Comparison of HER2-low prevalence in the strata (p value)
PD-L1 (CPS) (n=250):*					0.62
CPS<1	47 (18.8%)	33 (70.2%)	10 (21.3%)	4 (8.5%)	
1≤CPS<10	77 (30.8%)	54 (70.2%)	17 (22.1%)	6 (7.8%)	
CPS≥10	126 (50.4%)	73 (57.9%)	34 (27.0%)	19 (15.1%)	
EBER (n=223):†					0.99
Negative	213 (95.5%)	128 (58.4%)	65 (29.7%)	26 (11.9%)	
Positive	10 (4.5%)	5 (50.0%)	3 (30.0%)	2 (20.0%)	
MMR/MSI status (n=612):‡					0.75
MMRp, MSS, MMRp/MSS	540 (88.2%)	306 (56.7%)	166 (30.7%)	68 (12.6%)	
MMRd, MSI, MMRd/MSI	72 (11.8%)	43 (59.7%)	24 (33.3%)	5 (7.0%)	

Notes: Data summarised as n (%). Percentages are calculated by column for the whole series and by row for the HER2 groups.
MMRd, MSI, MMRd/MSI included MLH1/PMS2 loss (n=62), MSH2/MSH6 loss (n=3), MLH1/MSH6 loss (n=1), PMS2 loss (n=1) and MSI (n=5).
*Data not available in 939.
†960.
‡577 cases.
CPS, combined positive score; EBER, Epstein-Barr encoding region; MMRd, mismatch repair deficient; MMRp, MMR proficient; MSI, microsatellite instability; MSS, microsatellite stable.

Angerilli V, *et al.* – J Clin Pathol 2023

3. Can be MMR cytoplasmic?



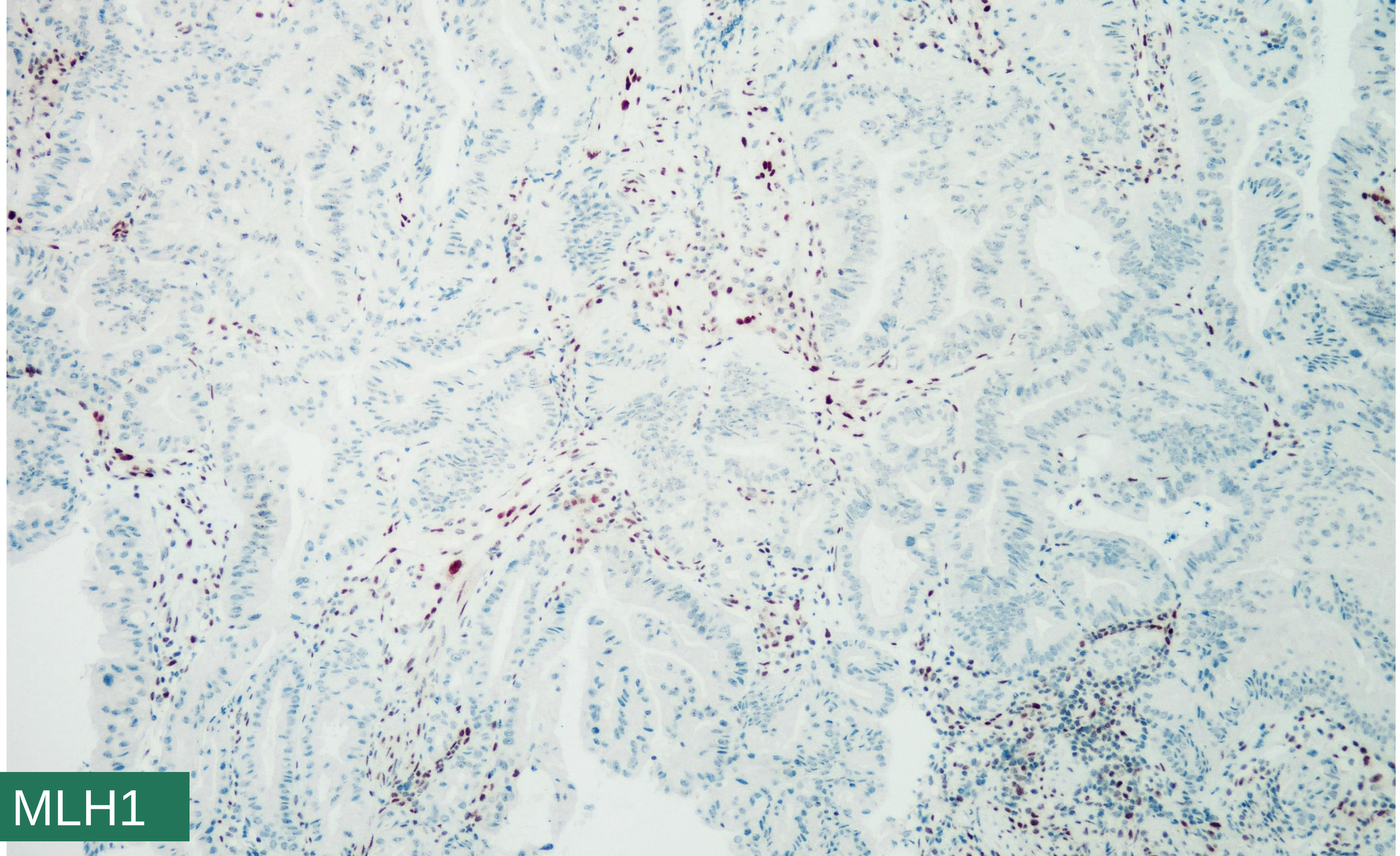

PMS2

What is the MMR status?

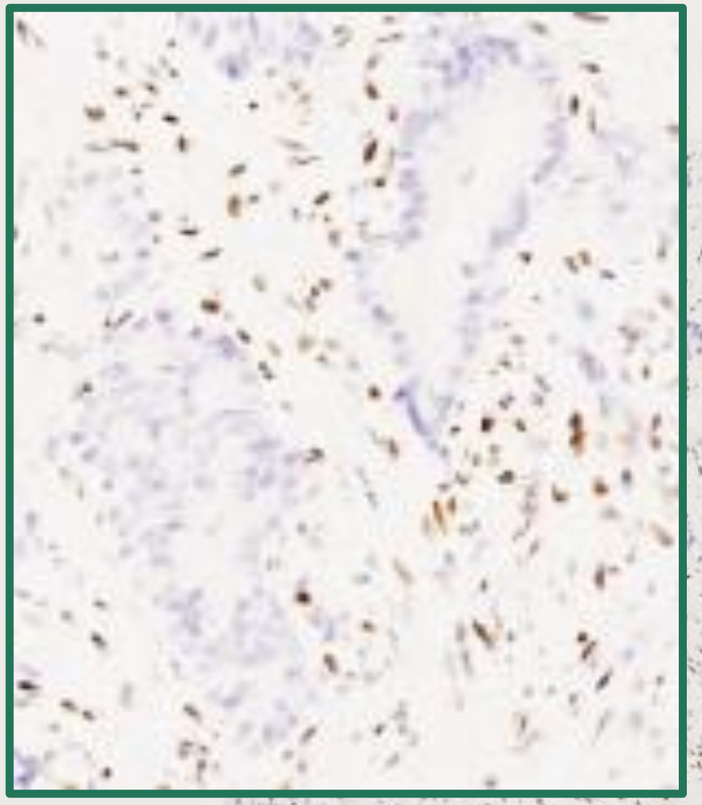
1) MMRp
2) MMRd
3) MMRind and think about MSI testing

What is the MMR status?

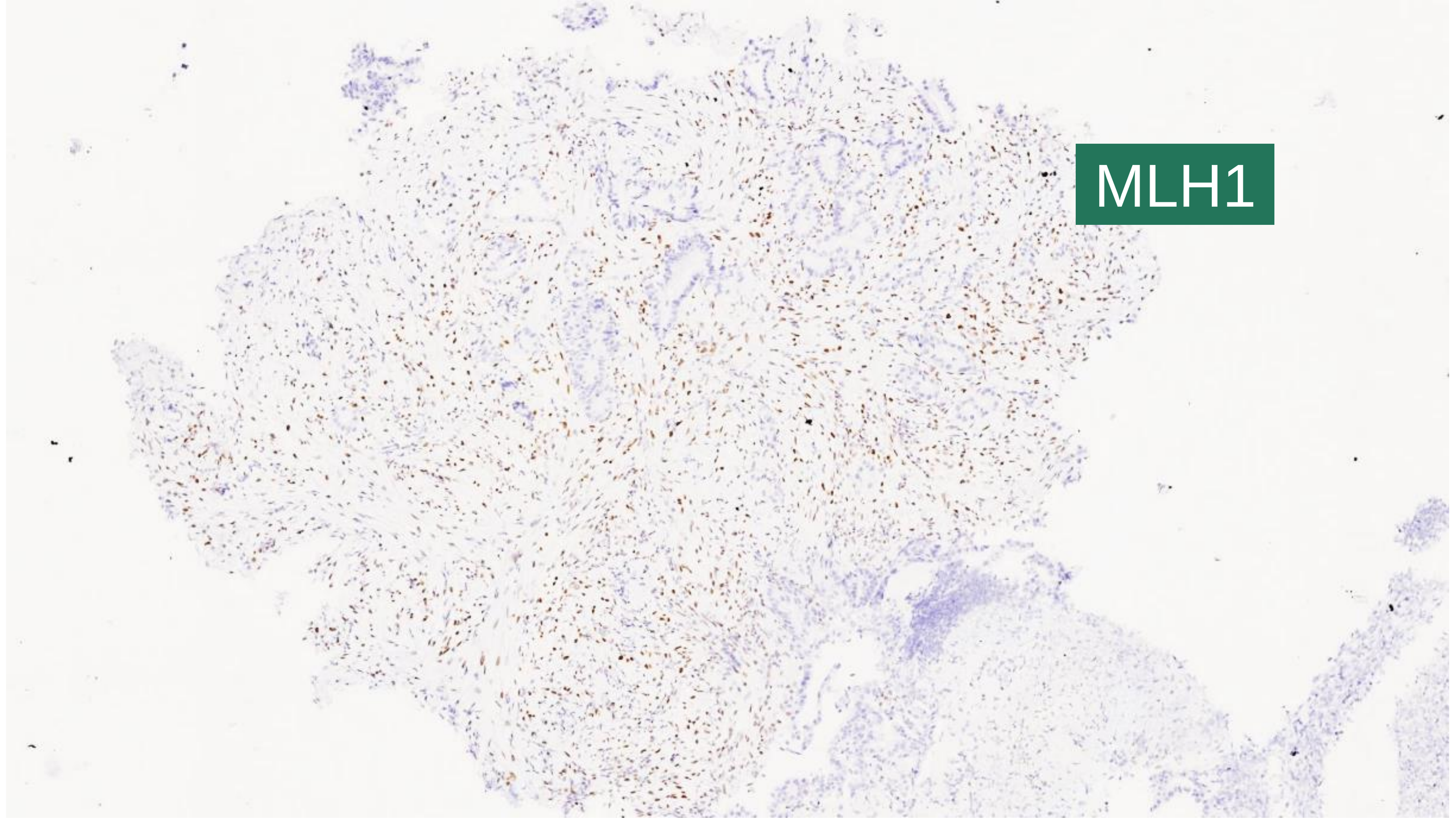
1) MMRp
2) MMRd
3) MMRind and think about MSI testing

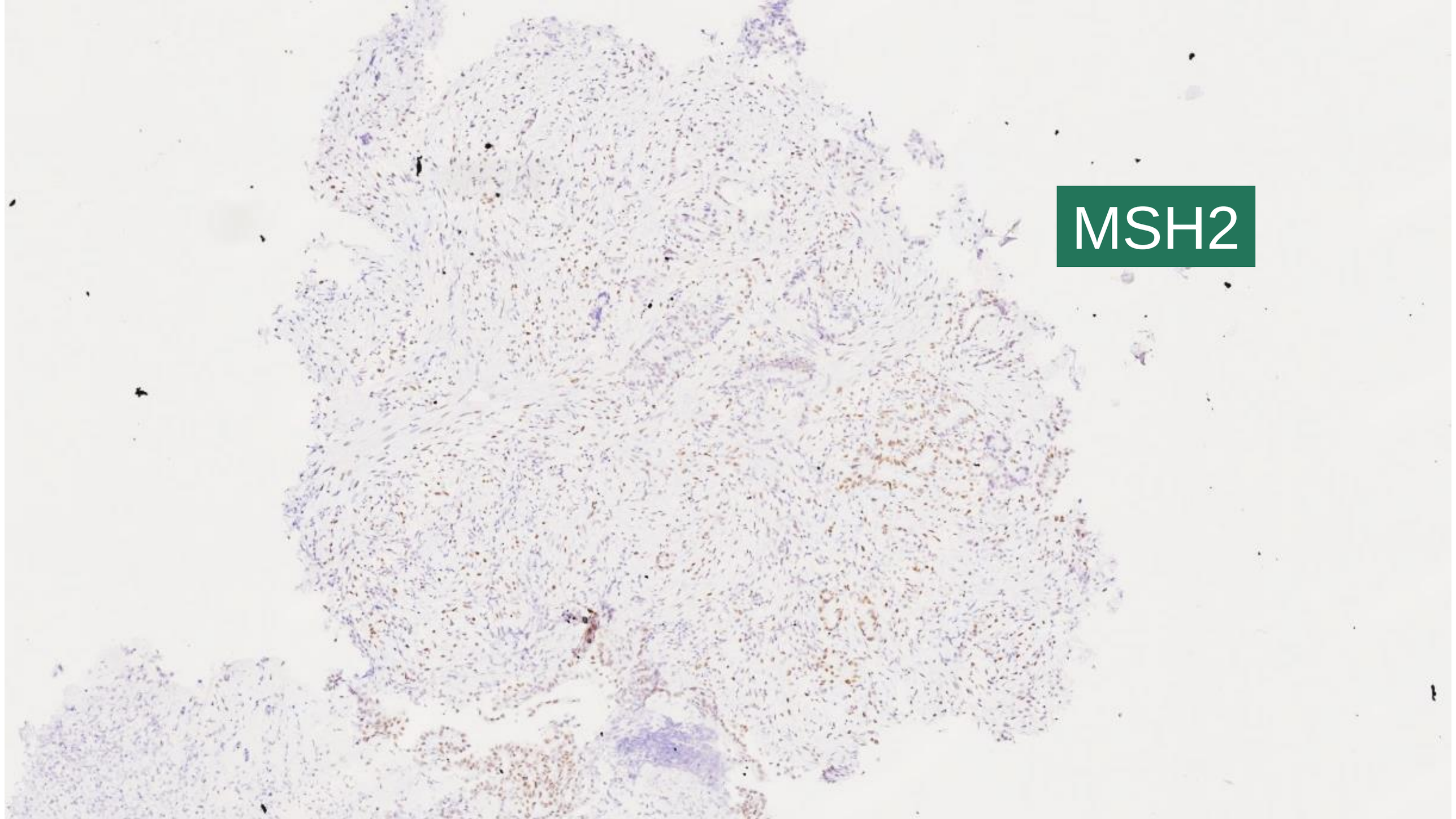

MLH1

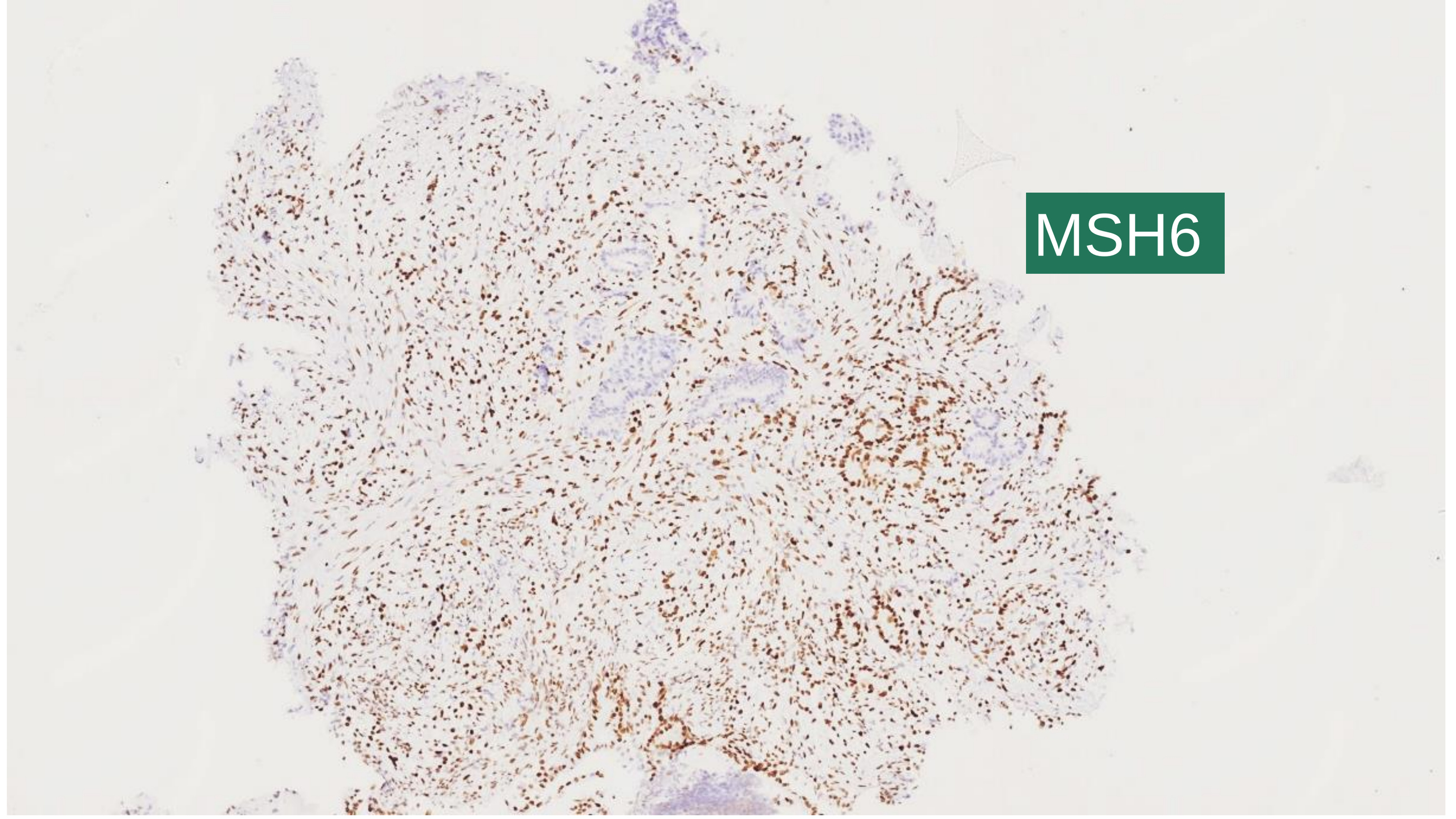
4. MMR heterogeneity

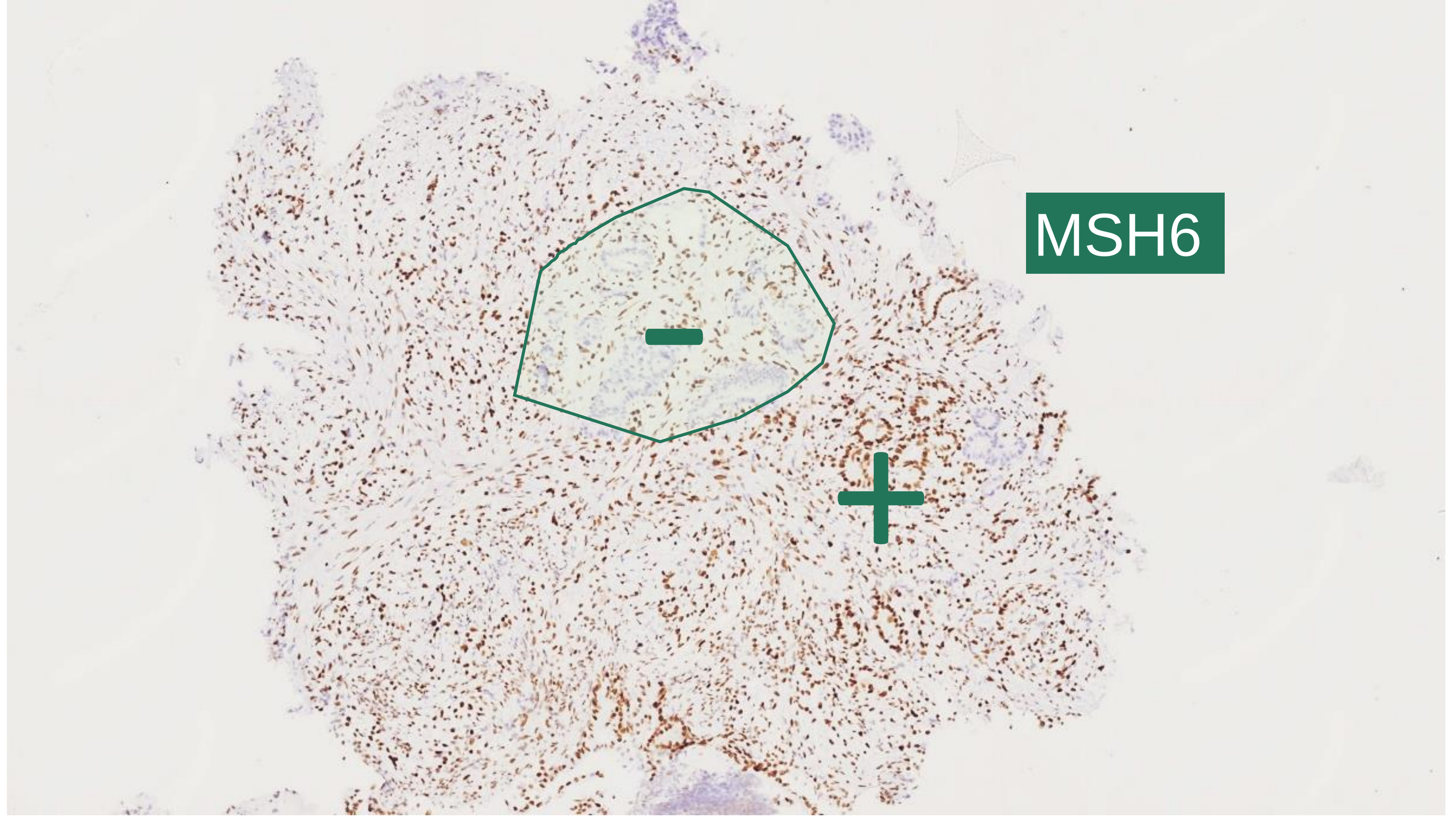


PMS2


MLH1


MSH2


MSH6


MSH6
-
+

What is the MMR status?

1) MMRp
2) MMRd
3) MMRind and think about MSI testing
4) MMRhet and think about MSI testing

What is the MMR status?

1) MMRp
2) MMRd
3) MMRind and think about MSI testing
4) MMRhet and think about MSI testing

Is MMR heterogeneity in GC real?

Virchows Archiv (2023) 482:517–523
https://doi.org/10.1007/s00428-023-03506-9

ORIGINAL ARTICLE

Check for updates

An exploration of gastric cancer with heterogeneous mismatch repair status

Xinyu Wang[1,2] · Kang Jiang[1] · Yajie Hu[1] · Xinya Zhao[1] · Lisha Yin[1] · Xinting Diao[1] · Xiuli Ma[1] · Yu Xu[3] · Yuezong Bai[3] · Yan Zhang[4] · Ziyu Li[4] · Yu Sun[1]

- 3723 patients with GC – retrospective review
- Radical surgical resection or gastric mucosa biopsy
- Retained MMR expression in one geographic region and complete loss of nuclear staining in the other
- Heterogeneous MMR protein staining: **12 cases (0.3%)**

Is MMR assessment on biopsy reliable in GC?

Oncology

Assessment of the reliability of MSI status and dMMR proteins deficiency screening on endoscopic biopsy material in esophagus and gastric adenocarcinoma ☆

Nicolas Asesio[a,*], Nozha Mhamdi Aloui[b], Julie Bonnereau[c], Jacqueline Lehmann-Che[d], Fatiha Bouhidel[b], Rachid Kaci[e], Hélène Corte[f], Magali Svrcek[g], My Linh Tran Minh[a], Jean Marc Gornet[a], Pierre Cattan[f], Matthieu Allez[a], Philippe Bertheau[b], Thomas Aparicio[a]

[a] Gastro-enterology department, Saint-Louis Hospital, APHP, Université Paris Cité, Paris, France
[b] Pathology department, Saint-Louis Hospital, APHP, Université Paris Cité, Paris, France
[c] INSERM U1160, Institut de Recherche Saint-Louis, Saint Louis Hospital, Université de Paris Cité, Paris, France
[d] Molecular oncology department, Saint-Louis Hospital, APHP, Université Paris Cité, Paris, France
[e] Pathology department, Lariboisière Hospital, APHP, Université Paris Cité, Paris, France
[f] Digestive Surgery department, Saint Louis Hospital, APHP, Université Paris Cité, Paris, France
[g] Pathology department, Saint Antoine Hospital, APHP, Sorbonne Université, Paris, France

- 2.2%: MMR IHC discordance rate EB/SS (1/47)
- Intratumoral heterogeneity (in SS)
- 12.7%: non-conclusive results (in EB)

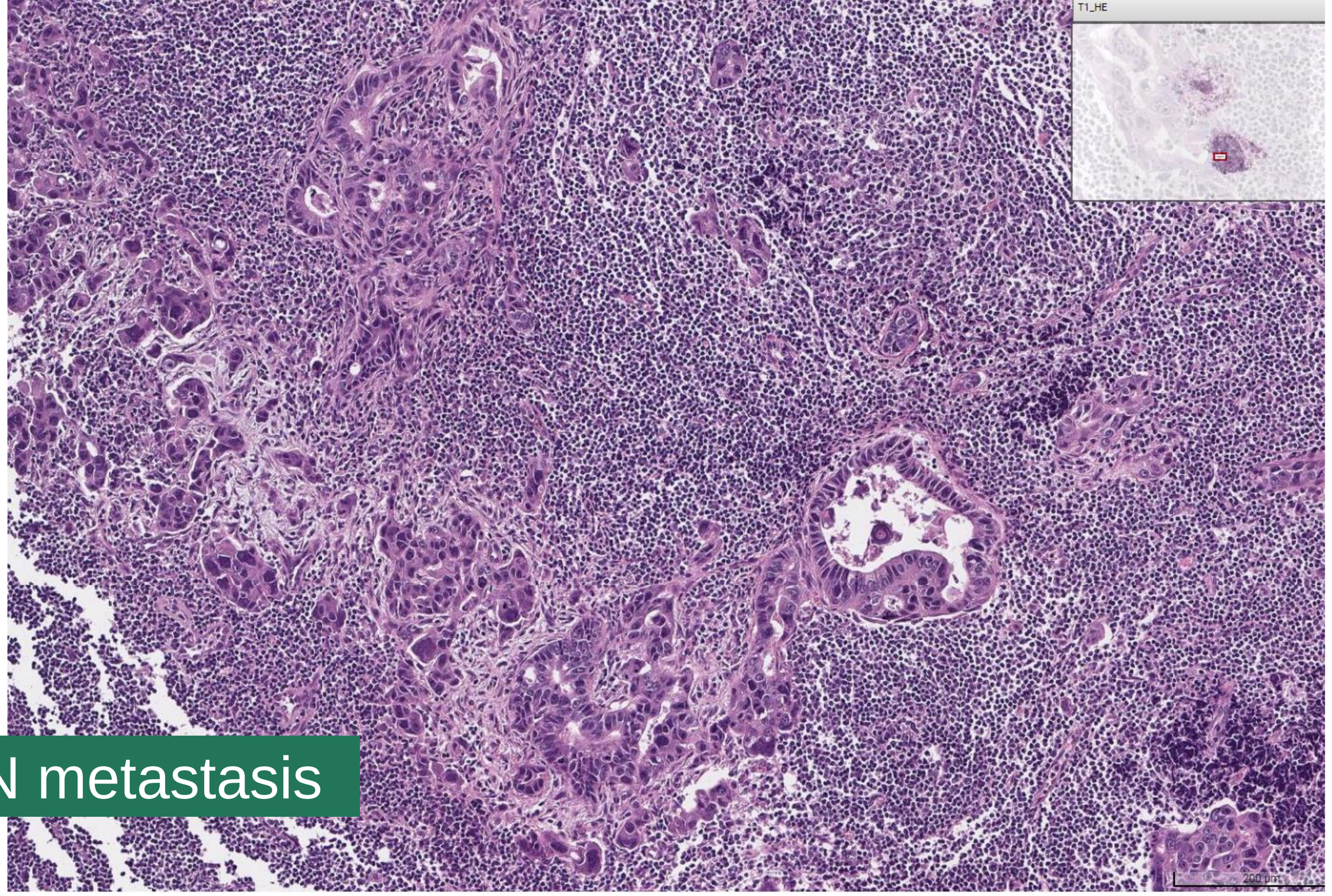
Role of Endoscopic Biopsies and Morphologic Features in Predicting Microsatellite Instability Status in Gastric Cancer

A Multicenter Comparative Study of Endoscopic Biopsies and Surgical Specimens

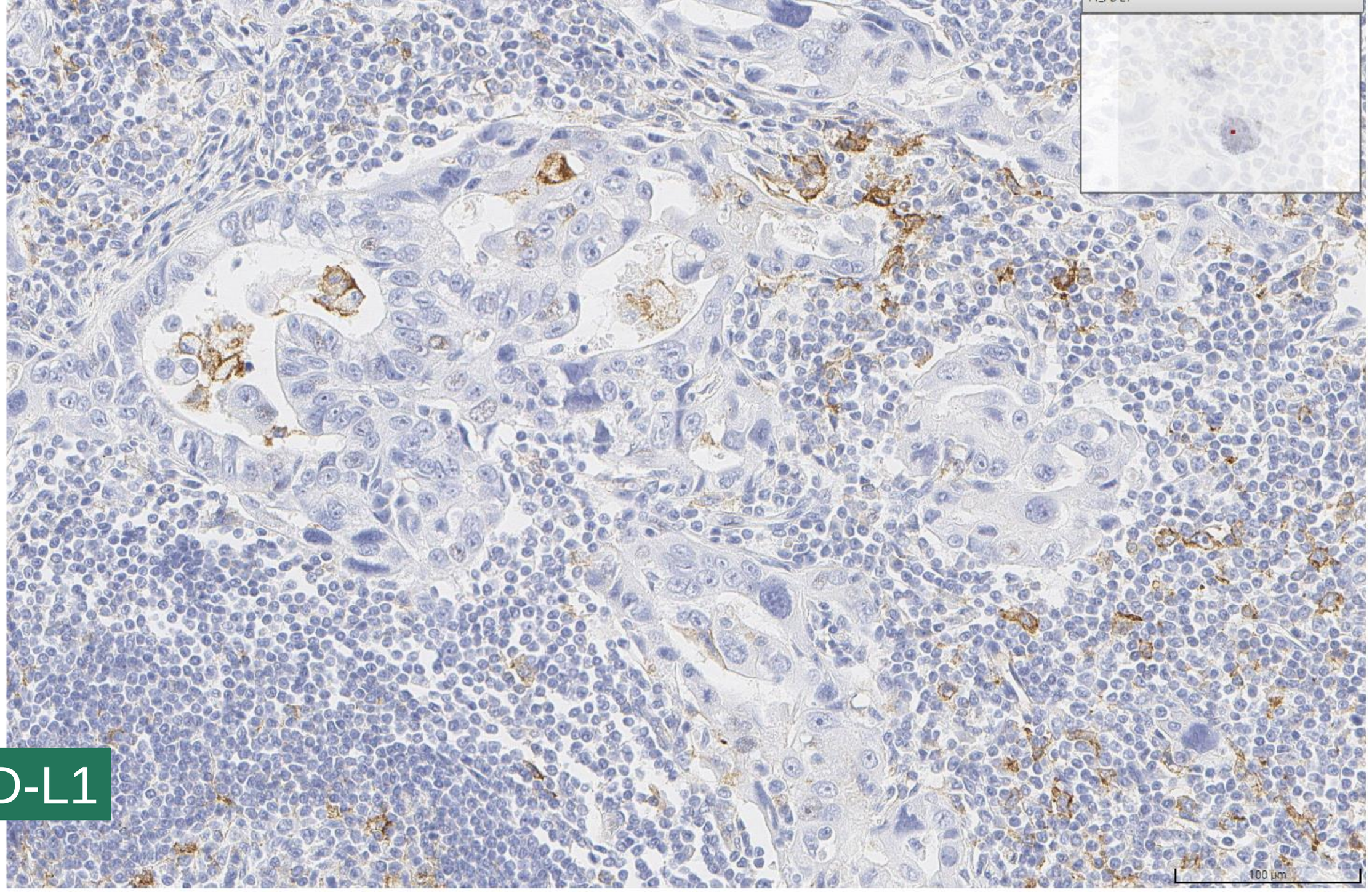
João R. Silva, BSc, Luís Mascarenhas-Lemos, MD,†‡§ Catarina Neto do Nascimento, MD,‖ Diogo Sousa Marques, BSc,* Xiaogang Wen, MD,¶# Lídia Pinho, BSc,¶ Rui Maio, MD, PhD,§** Patrícia Pontes, BSc,†† Luís Cirnes, BSc,¶ Marília Cravo, MD, PhD,‡‡§§ Fátima Carneiro, MD, PhD,¶††‖‖ and Irene Gullo, MD, PhD¶††‖‖*

- 2.2%: discordant results (3/135)
- EB were able to accurately predict the overall MMR status in SS, with a sensitivity of 97.3% and a specificity of 98.0%
- High concordance rates between EB and SS (Cohen κ =94.5%).

5. PD-L1 the difficult case



LN metastasis



PD-L1

How to evaluate PD-L1 in a LN?

A) evaluate all the lymphoid tissue if it is activated
B) evaluate only areas close to the metastatic tissue at 20x
C) it’s a sample inadequate for scoring
D) evaluate only intraglandular macrophages

How to evaluate PD-L1 in a LN?

A) evaluate all the lymphoid tissue if it is activated

B) evaluate only areas close to the metastatic tissue at 20x

C) it’s a sample inadequate for scoring

D) evaluate only intraglandular macrophages

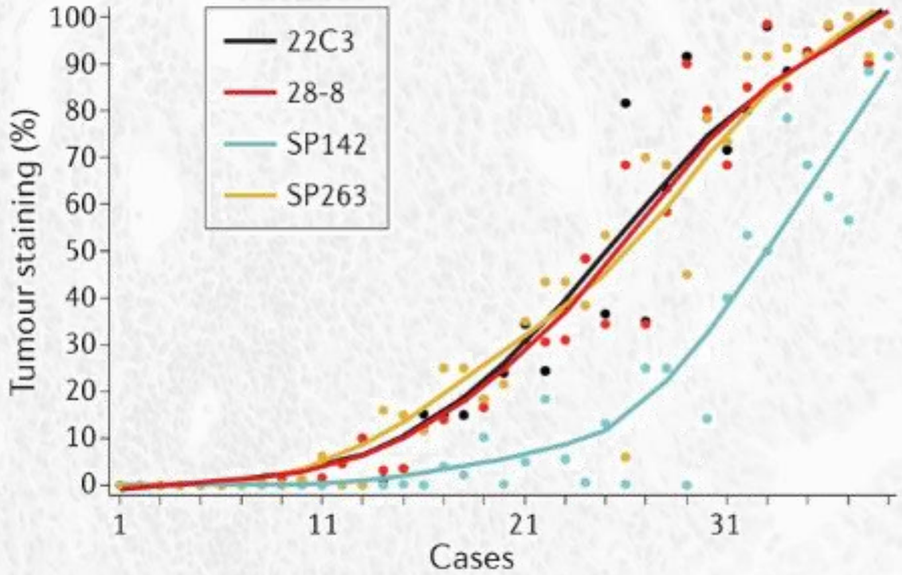

What about PD-L1 evaluation?
Chemistry

PD-L1 is a drug specific biomarker: multiple tests and approaches

	MERCK	Bristol Myers Squibb	AstraZeneca	Roche	Pfizer MERCK	BeiGene
Lead Rx asset	Pembrolizumab KEYTRUDA (anti-PD-1)	Nivolumab OPDIVO (anti-PD-1)	Durvalumab IMFINZI (anti-PD-L1)	Atezolizumab TECENTRIQ (anti-PD-L1)	Avelumab BAVENCIO (anti-PD-L1)	Tislelizumab (anti-PD1)
Diagnostic partner	Dako	Dako	Ventana	Ventana	Dako	Ventana
Clones	22C3	28-8	SP263	SP142/SP263	73-10	SP263
Machines Utilized	Link 48	Link 48	BenchMark series	BenchMark series	Link 48	BenchMark series
Compartment	TM	TM	TM/IC	TC/IC	TC	TM/IC
Scoring	TPS/CPS	TPS/TC/CPS	TC/ICP/IC	TC/IC	TC	TC/TAP

PD-L1: different antibodies with specific diagnostic performances

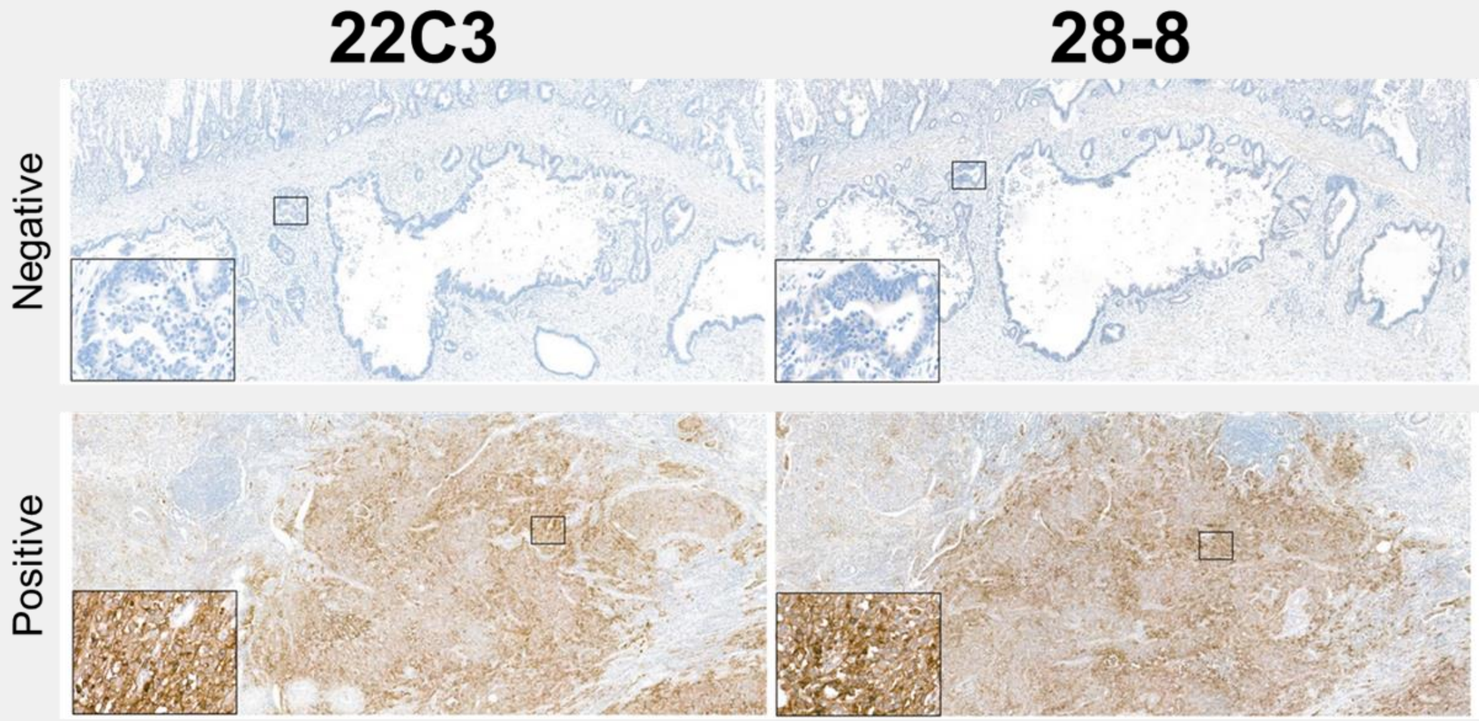
Assay	Anti-PD-1 or anti-PD-L1 antibody	Interpretive scoring	Instrument and detection systems required
28-8 (Dako)	Nivolumab	Tumour cell membrane	EnVision Flex-Autostainer Link 48
22C3 (Dako)	Pembrolizumab	Tumour cell membrane	EnVision Flex-Autostainer Link 48
SP142 (Ventana)	Atezolizumab	Tumour cell membrane and infiltrating immune cells	OptiView Detection and Amplification-Benchmark ULTRA
SP263 (Ventana)	Durvalumab	Tumour cell membrane	OptiView Detection and Benchmark ULTRA

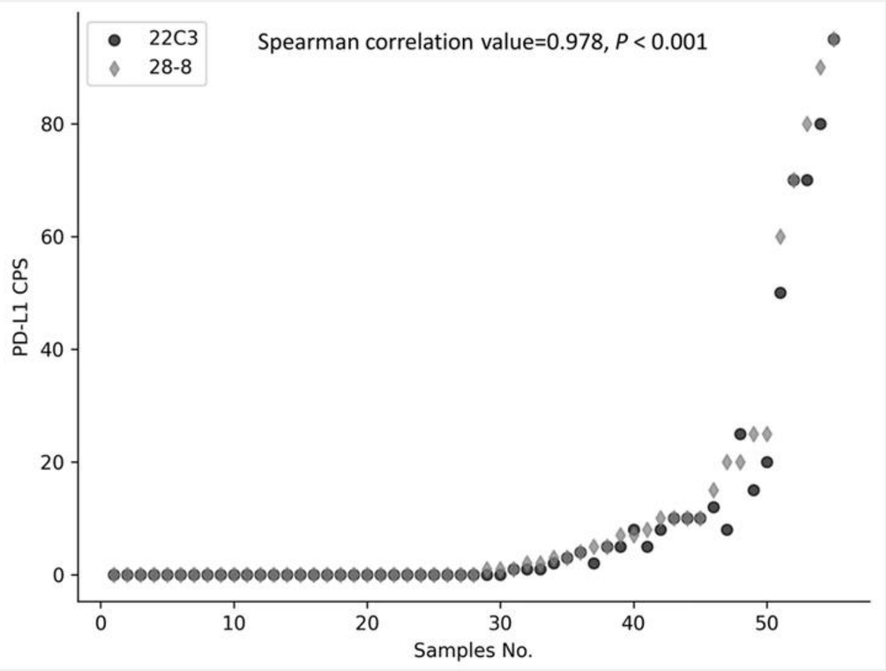


During the development of anti-PD-1 and anti-PD-L1 antibodies, each pharmaceutical company pursued its own unique diagnostic antibody and corresponding protocol for PD-L1 staining and interpretation. This disjointed approach created a major challenge in the development of PD-L1 expression as a universally predictive biomarker across different tumour types

Doroshow DB, *et al.* – Nat Rev Clin Oncol 2021

PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28-8 pharmDx assays for responses to immunotherapy





The two assays are highly comparable at various CPS cutoffs. This study provides evidence for the potential interchangeability of these two assays in gastric cancer.

Ahn S and Kim KM – Mod Pathol 2021

PD-L1: choice of antibody and IHC platform

Modern Pathology (2021) 34:1719–1727
https://doi.org/10.1038/s41379-021-00823-9

ARTICLE

PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28-8 pharmDx assays for responses to immunotherapy

Soomin Ahn[1] · Kyoung-Mee Kim[1]

DAKO 22C3, DAKO 28-8

Comparative Study › Appl Immunohistochem Mol Morphol. 2021 Jul 1;29(6):462-466.
doi: 10.1097/PAI.0000000000000902.

PD-L1 Expression Harmonization in Gastric Cancer Using 22C3 PharmDx and SP263 Assays

Tamara Z Dabbagh [1], Maher A Sughayer

Affiliations + expand

PMID: 33480602 DOI: 10.1097/PAI.0000000000000902

DAKO 22C3, VENTANA SP 263, VENTANA 22C3

Analytical performance of 22C3 and SP263 clones on the Ventana platform was close to that of the reference assay (Dako 22C3 assay), suggesting that the 2 LDTs can be utilized interchangeably.

Gastric Cancer (2022) 25:741–750
https://doi.org/10.1007/s10120-022-01301-0

DAKO 22C3, DAKO 28-8, VENTANA SP142

ORIGINAL ARTICLE

Choice of PD-L1 immunohistochemistry assay influences clinical eligibility for gastric cancer immunotherapy

Joe Yeong[1,2] · Huey Yew Jeffrey Lum[3] · Chong Boon Teo[4] · Benjamin Kye Jyn Tan[4] · Yiong Huak Chan[5] · Ryan Yong Kiat Tay[4] · Joan Rou-En Choo[6] · Anand D. Jeyasekharan[6,7] · Qing Hao Miow[6] · Lit-Hsin Loo[8] · Wei Peng Yong[6,7,11] · Raghav Sundar[4,6,9,10,11]

Pathology (August 2021) 53(5), pp. 586–594

DAKO 22C3, VENTANA SP 263, VENTANA 22C3

ANATOMICAL PATHOLOGY

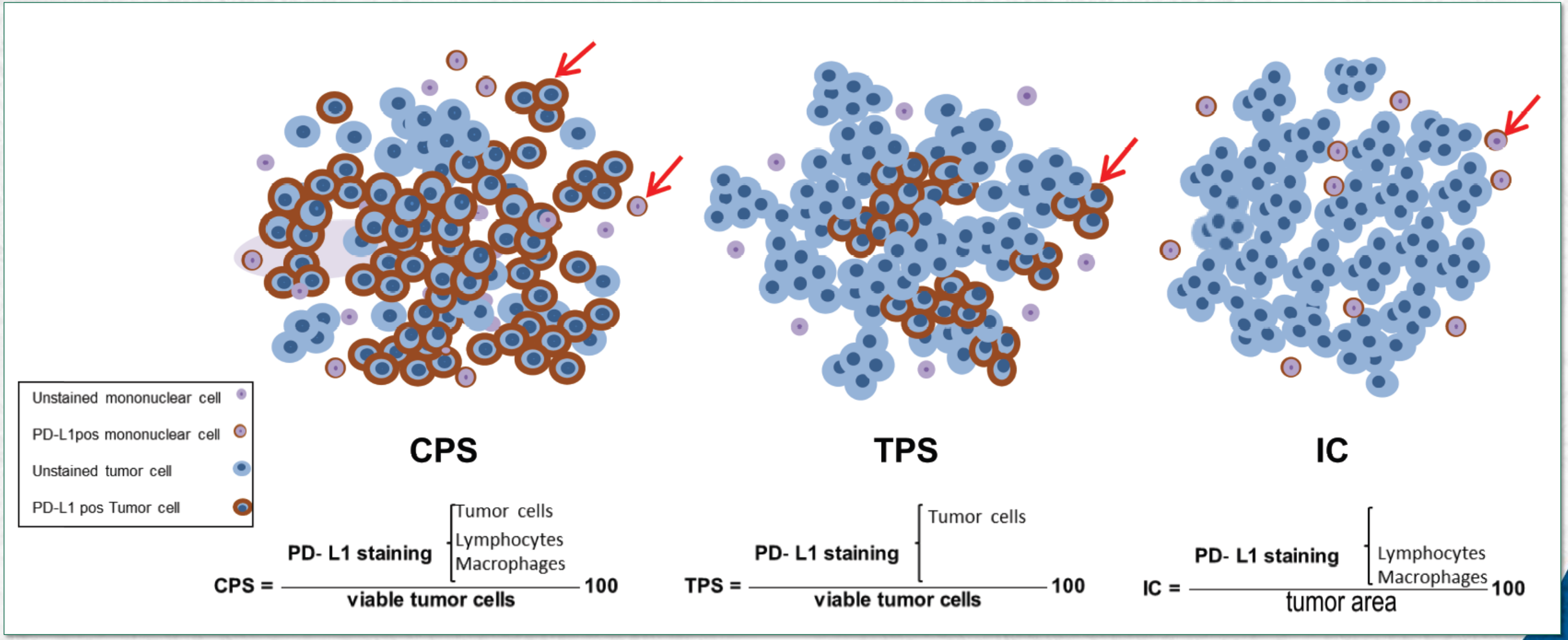
Comparison of PD-L1 immunohistochemical assays in advanced gastric adenocarcinomas using endoscopic biopsy and paired resected specimens

So-Woon Kim[1,2], Gowun Jeong[3], Min-Hee Ryu[4], Young Soo Park[1]

Until stronger evidence of inter-assay concordance is found, we urge caution in treating the various assays as equivalent.

DO NOT USE SP142 FOR GC/GEJ/EC

PD-L1 scoring algorithms



Sajjadi E, *et al.* - Ecancermedicalscience 2020

PD-L1 inter-pathologists' agreement (yes, they are reproducible)

Arch Pathol Lab Med. 2019;143:330–337; doi: 10.5858/

Clinical Utility of the Combined Positive Score for Programmed Death Ligand-1 Expression and the Approval of Pembrolizumab for Treatment of Gastric Cancer

Karina Kulangara, PhD; Nancy Zhang, MD; Ellie Corigliano, PhD; Lindsay Guerrero, MS; Stephanie Waldroup, BSc; Dipeshkumar Jaiswal, MS; Malinka Jansson, MS; Supriya Shah, PhD; Debra Hanks, MD; Jiangdian Wang, PhD; Jared Lunceford, PhD; Mary J. Savage, PhD; Jonathan Juco, MD; Kenneth Emancipator, MD

External reproducibility assessments demonstrated:

- Inter-pathologist overall agreement of 96.6%
- Intra-pathologist overall agreement of 97.2%

PD-L1 inter-pathologists' agreement (no, they aren't reproducible)

Mod Pathol 36 (2023) 100154

MODERN PATHOLOGY

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

Journal homepage: https://modernpathology.org/

Research Article

High Interobserver Variability Among Pathologists Using Combined Positive Score to Evaluate PD-L1 Expression in Gastric, Gastroesophageal Junction, and Esophageal Adenocarcinoma

Marie E. Robert[a,*], Josef Rüschoff[b], Bharat Jasani[b], Rondell P. Graham[c], Sunil S. Badve[d], Manuel Rodriguez-Justo[e], Liudmila L. Kodach[f], Amitabh Srivastava[g], Hanlin L. Wang[h], Laura H. Tang[i], Giancarlo Troncone[j], Federico Rojo[k], Benjamin J. Van Treeck[c], James Pratt[l], Iryna Shnitsar[l], George Kumar[l], Maria Karasarides[l,*], Robert A. Anders[m,n,*]

[a] *Yale University School of Medicine, New Haven, Connecticut;* [b] *Discovery Life Sciences, Hesse, Germany;* [c] *Mayo Clinic, Rochester, Minnesota;* [d] *Emory University School of Medicine, Atlanta, Georgia;* [e] *University College London, London, UK;* [f] *The Netherlands Cancer Institute, Amsterdam, Netherlands;* [g] *Memorial Sloan Kettering Cancer Center, New York, New York;* [h] *University of California Los Angeles, Los Angeles, California;* [i] *Memorial Sloan Kettering Cancer Center, New York, New York;* [j] *University of Naples, Naples, Italy;* [k] *IIS-Fundacion Jimenez Diaz CIBERONC (Madrid), Madrid, Spain;* [l] *Bristol Myers Squibb, Princeton, New Jersey;* [m] *John Hopkins University, Convergence Institute, Baltimore, Maryland;* [n] *Bloomberg-Kimmel Intitute for Cancer Immunotherapy, Baltimore, Maryland*

Mod Pathol 36 (2023) 100128

MODERN PATHOLOGY

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

Journal homepage: https://modernpathology.org/

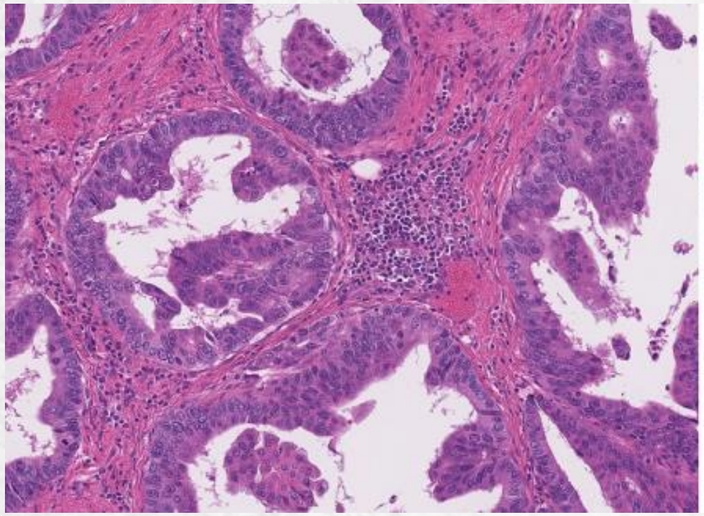
Research Article

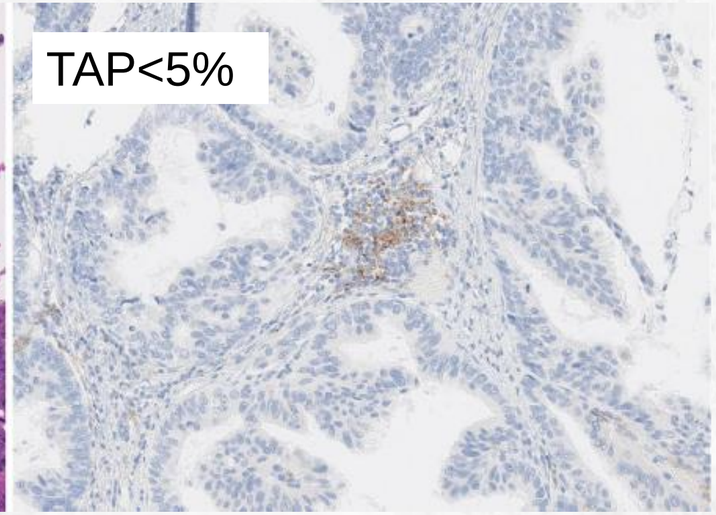
Multi-Institutional Study of Pathologist Reading of the Programmed Cell Death Ligand-1 Combined Positive Score Immunohistochemistry Assay for Gastric or Gastroesophageal Junction Cancer

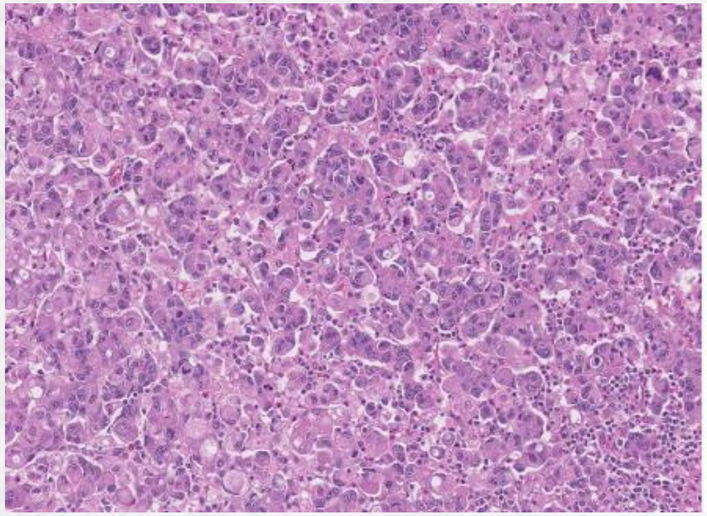
Aileen I. Fernandez[a], Charles J. Robbins[a], Patricia Gaule[a], Diana Agostini-Vulaj[b], Robert A. Anders[c], Andrew Bellizzi[d], Wei Chen[e], Zongming Eric Chen[f], Purva Gopal[g], Lei Zhao[h], Mikhail Lisovsky[i], Xiuli Liu[j], Jinru Shia[k], Huamin Wang[l], Zhaohai Yang[m], Leena McCann[n], Yvonne G. Chan[n], Jodi Weidler[o], Michael Bates[o], Xuchen Zhang[a], David L. Rimm[a,p,*]

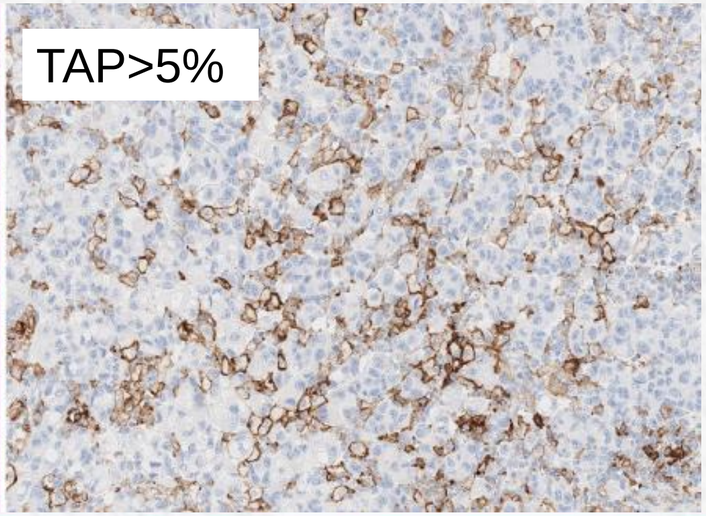
Tumor Area Positivity (TAP) score of PD-L1: a novel visual estimation method for combined tumor cell and immune cell scoring

34º Congresso Brasileiro de PATOLOGIA
27º Congresso Brasileiro de CITOPATOLOGIA
29/05 a 01/06/2024 Belém/PA
INOV AÇÃO INTEGR



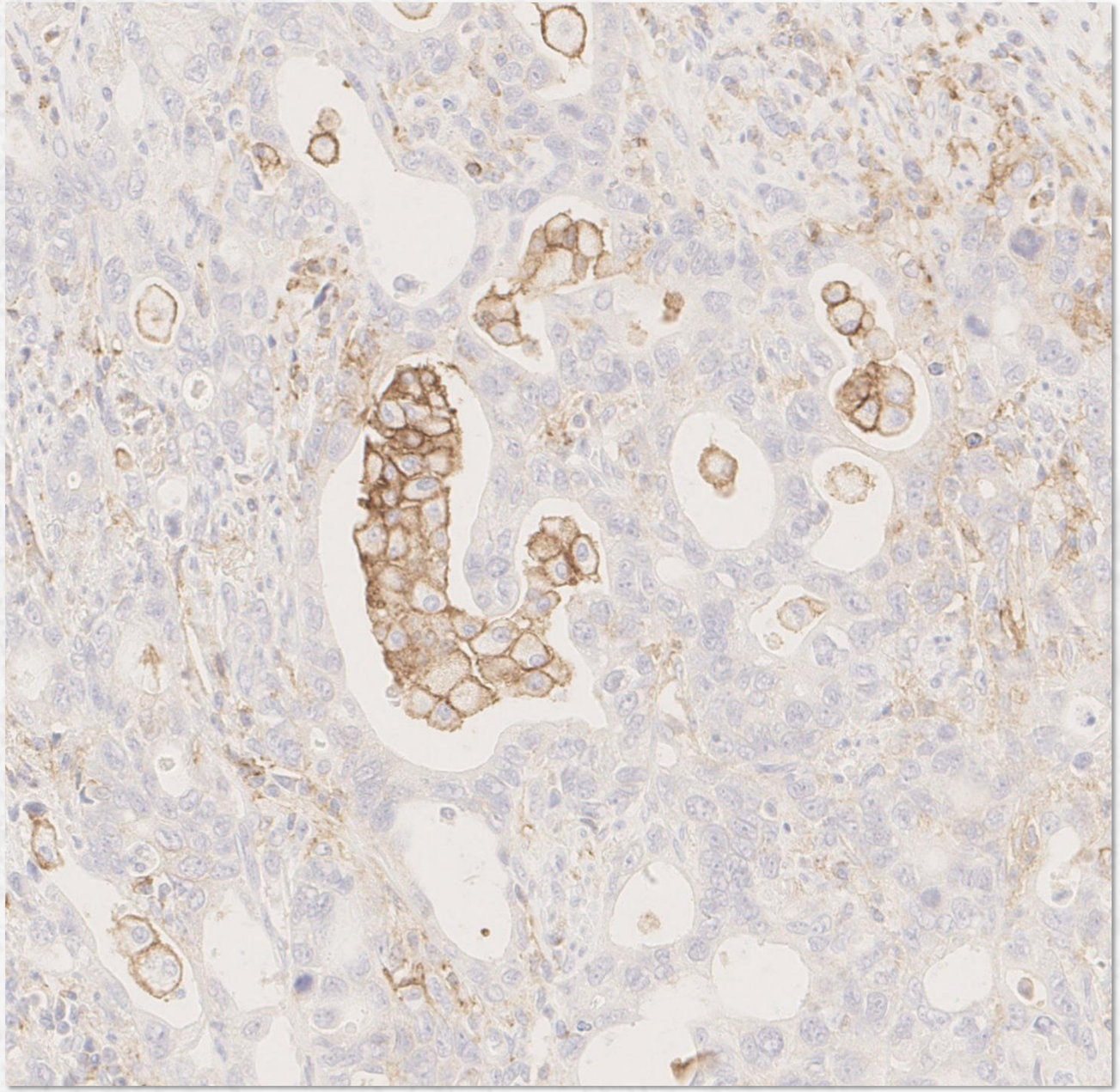






- The average positive agreement, average negative agreement, and overall percent agreement between and within readers were all above 85% for both internal and combined external reader precision studies.
- TAP score had high concordance rate at 5% cutoff compared with CPS at cutoff 1.
- TAP scoring method to be straightforward, significantly less time-consuming, and highly reproducible with a high concordance rate between TAP score and CPS.

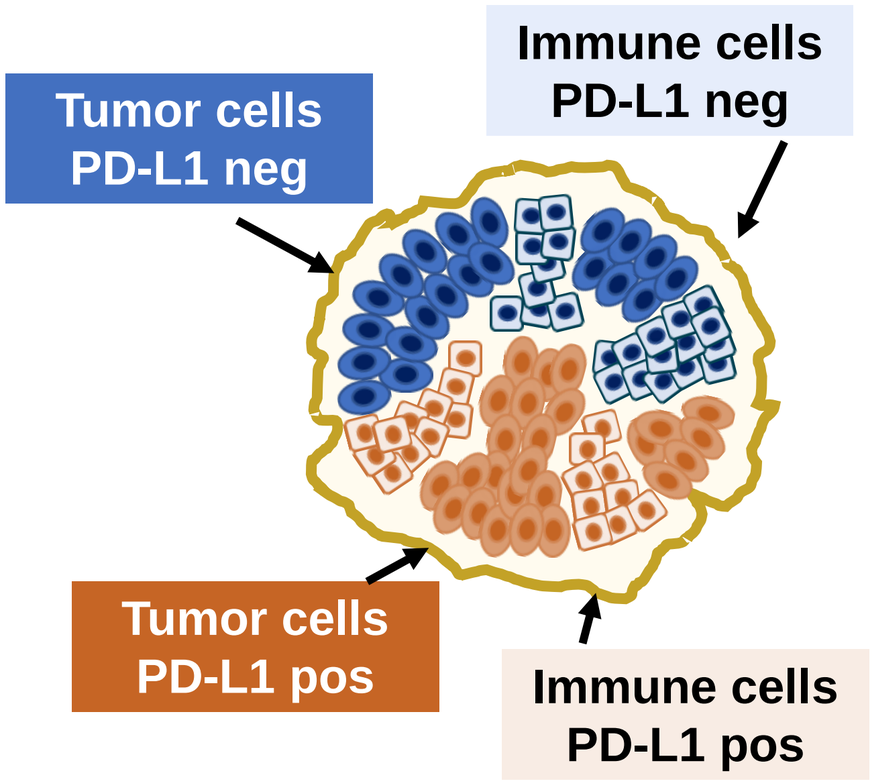
Liu C, *et al.* – Diagn Pathol 2023



- **<u>Intra-luminal macrophage</u>** staining is not included in the TAP score unless the macrophages completely fill the luminal space and are in direct contact with the TC.

- Staining of **<u>multi-nucleated giant cells, granulomas, and IC located within blood vessels</u>** and lymphatics are not included in the TAP score.

Liu C, *et al.* – Diagn Pathol 2023



Cell counting

CPS*

Number of TCs with PD-L1 expression + Number of ICs with PD-L1 expression

Total number of viable TCs

*ICs= lymphocytes, macrophages

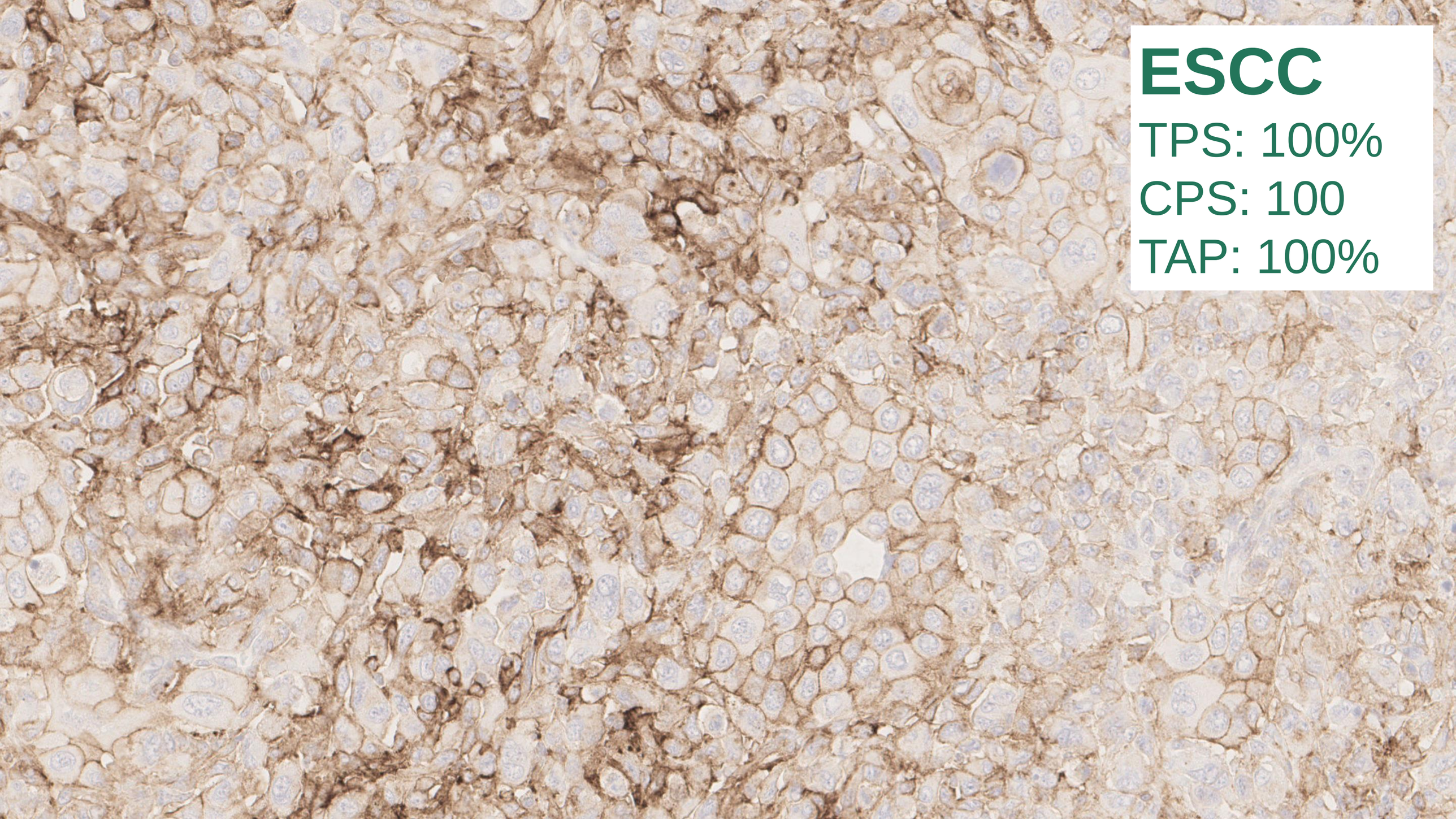
Visual estimation

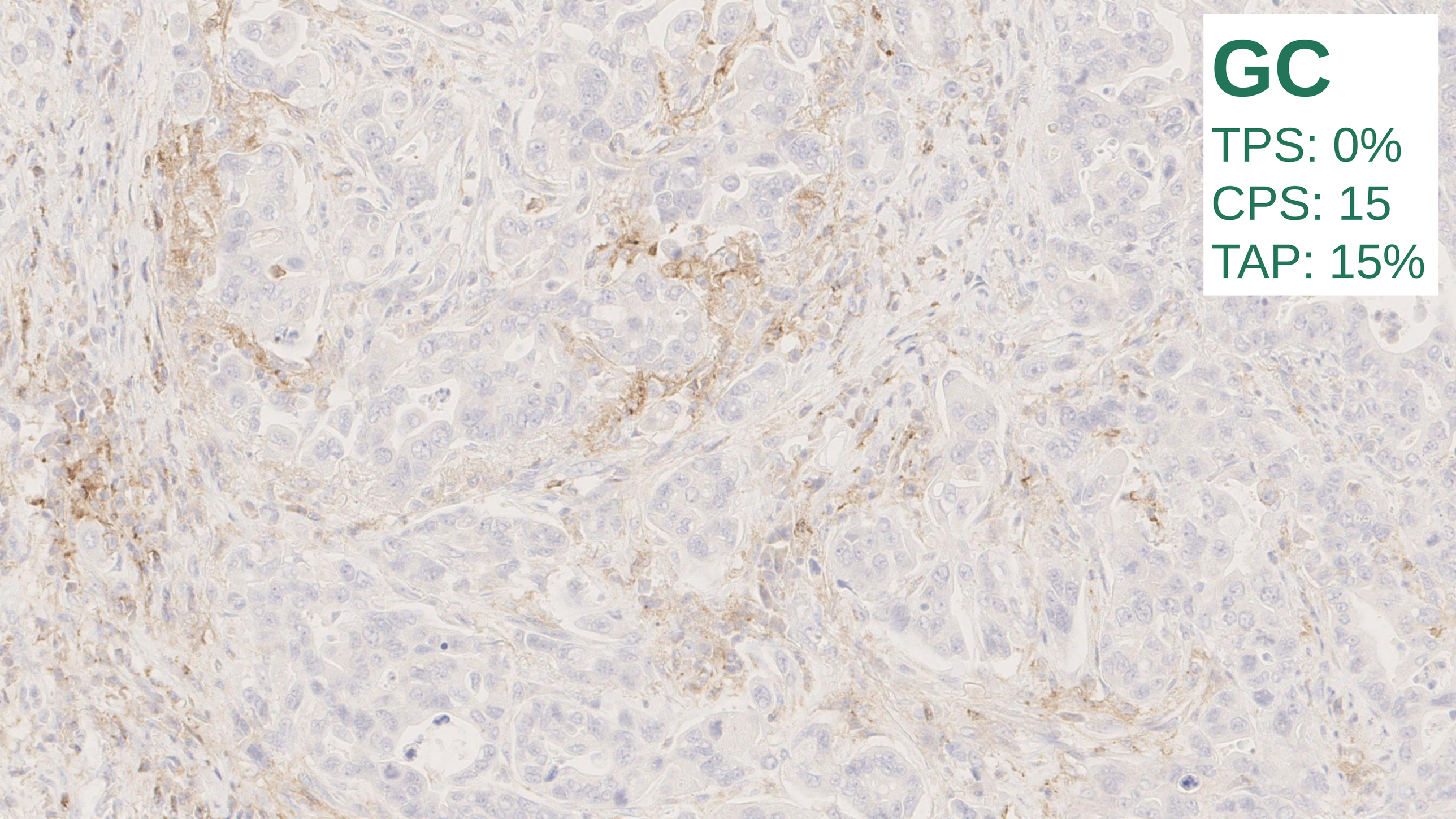
TAP§

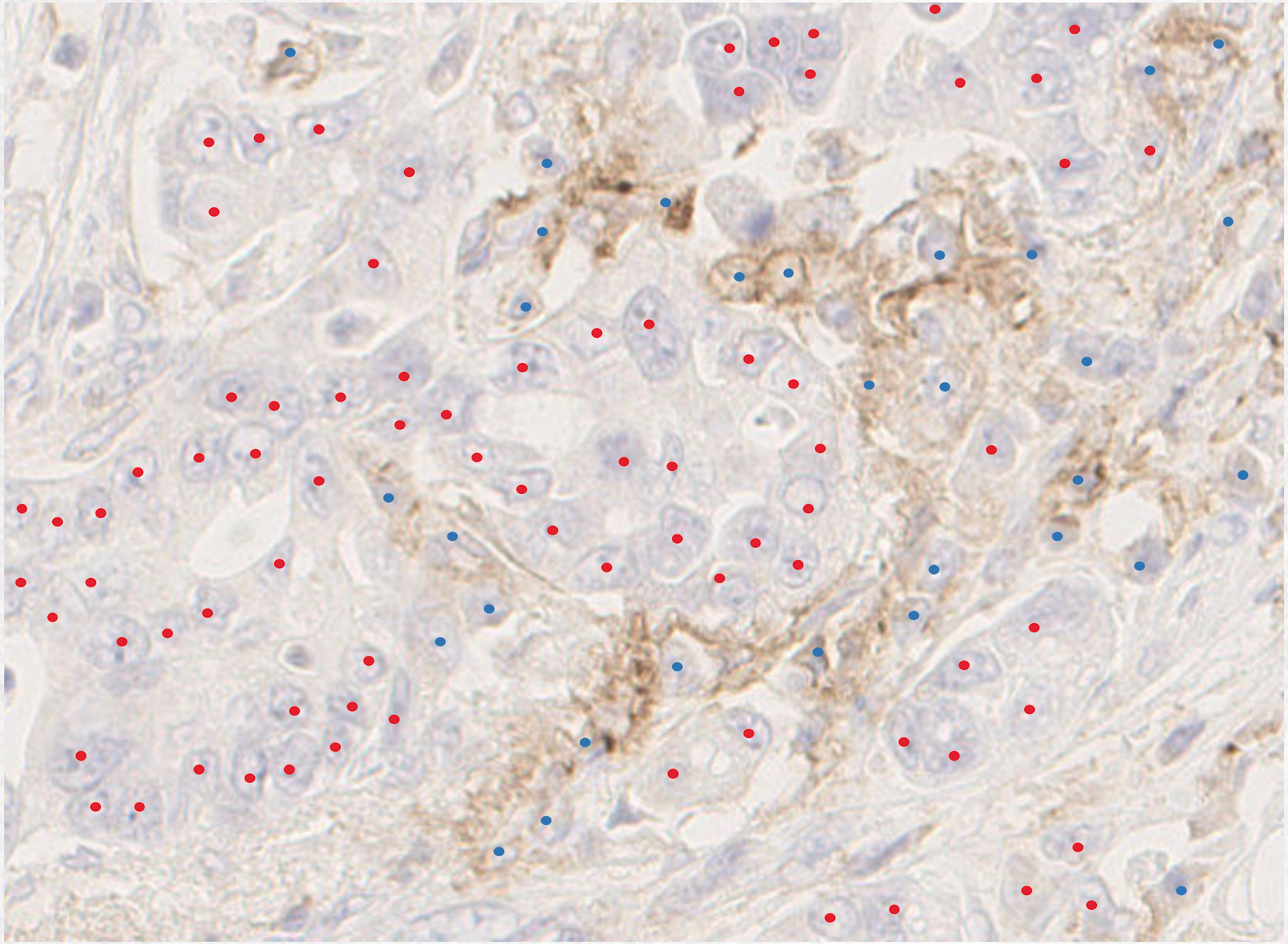
Area occupied by TCs with PD-L1 expression + Area occupied by ICs with PD-L1 expression

Entire tumor area

§inclusive of all types of ICs


ESCC
TPS: 100%
CPS: 100
TAP: 100%

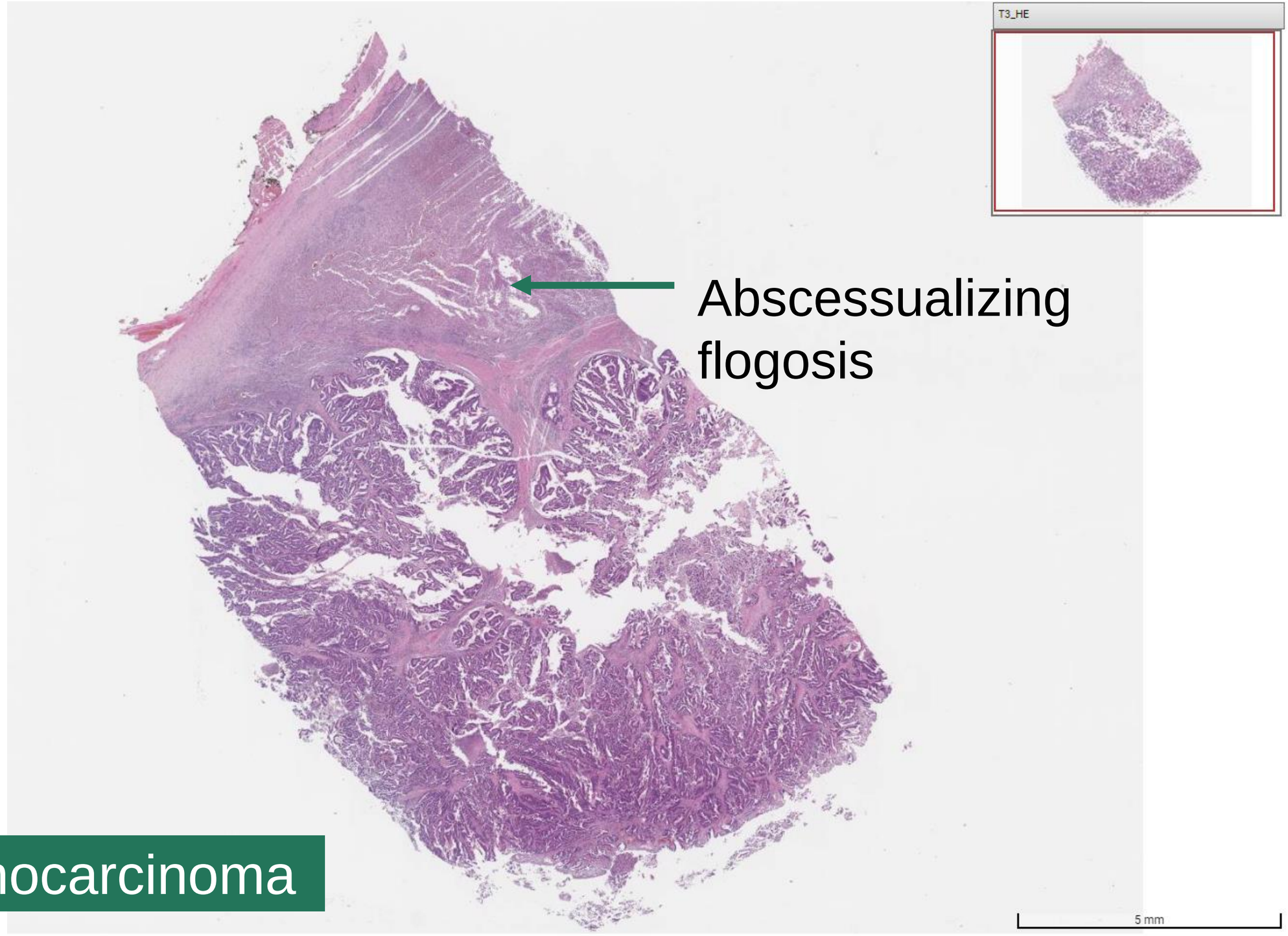

GC
TPS: 0%
CPS: 15
TAP: 15%



TC: RED 100

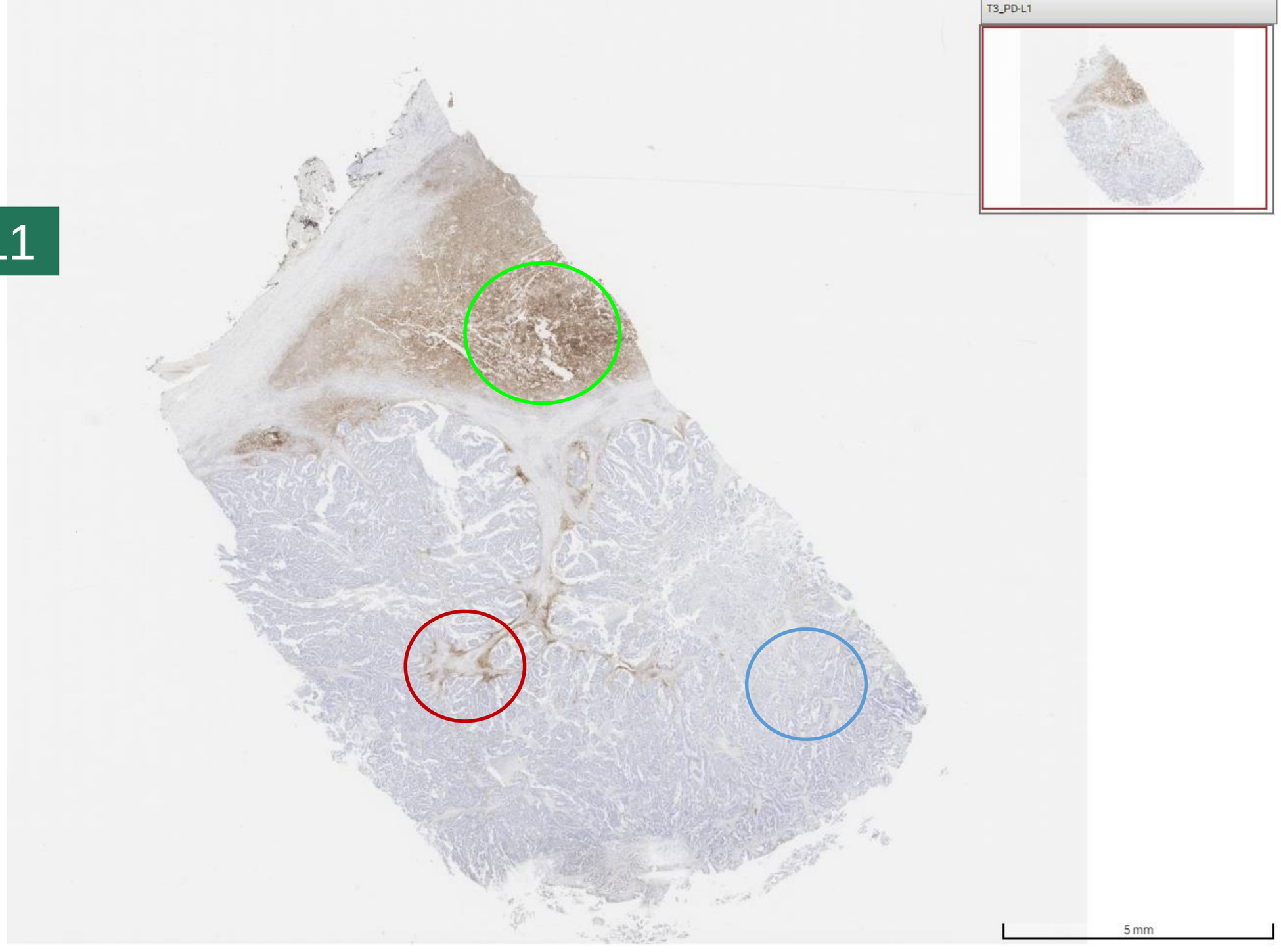
IC: BLUE 25

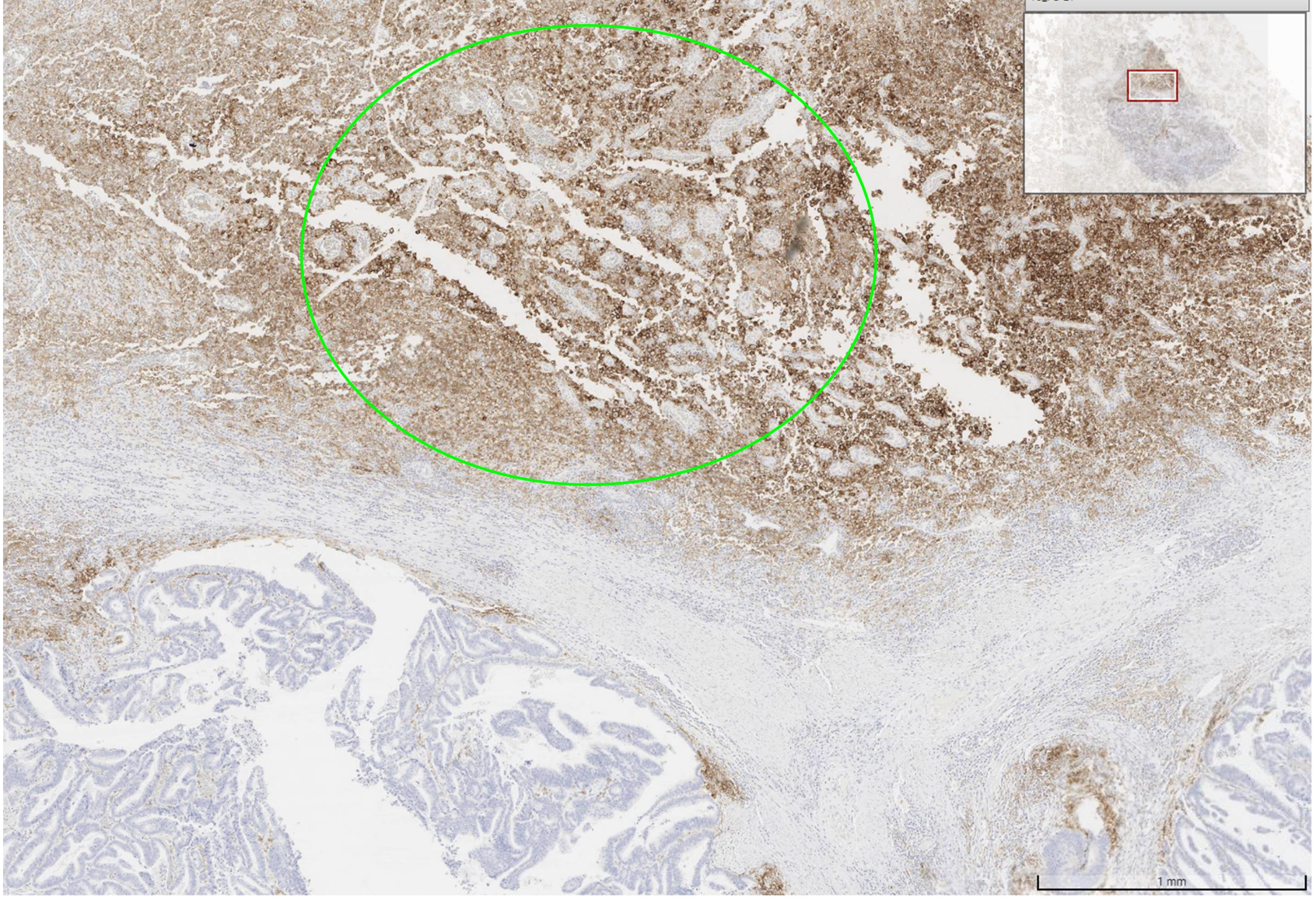
6. PD-L1: the importance of H&E



Adenocarcinoma

PD-L1




1 mm

Where evaluate PD-L1 in this case?

A) In the red cicle as hotspot
B) In the blue circle as hotspot
C) In the green cicle as hotspot
D) As an average between blue and red cicle, avoiding the abscessualized area

Where evaluate PD-L1 in this case?

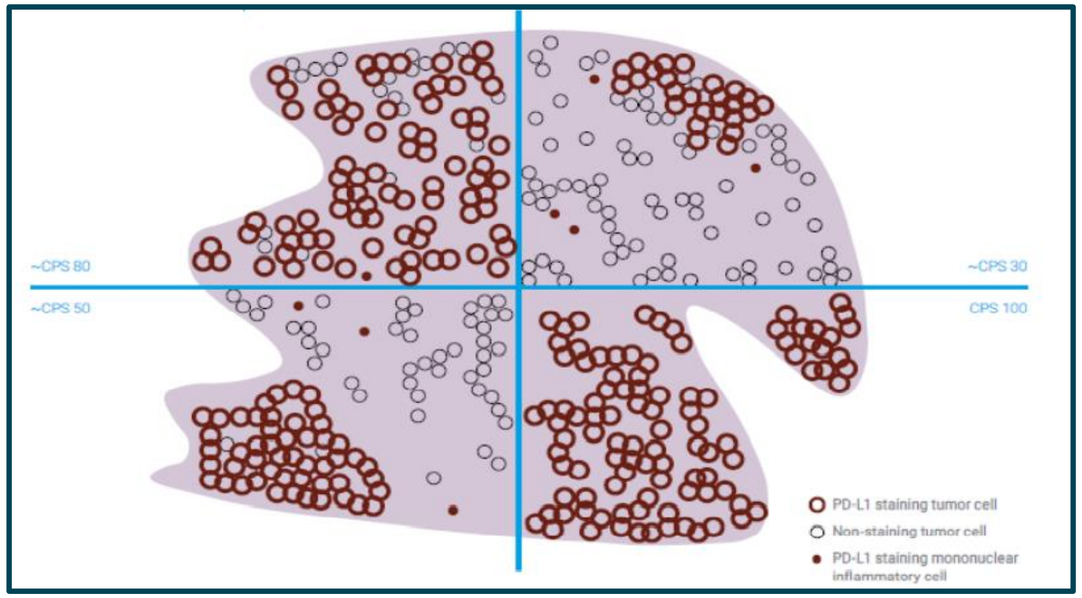
A) In the red cicle as hotspot

B) In the blue circle as hotspot

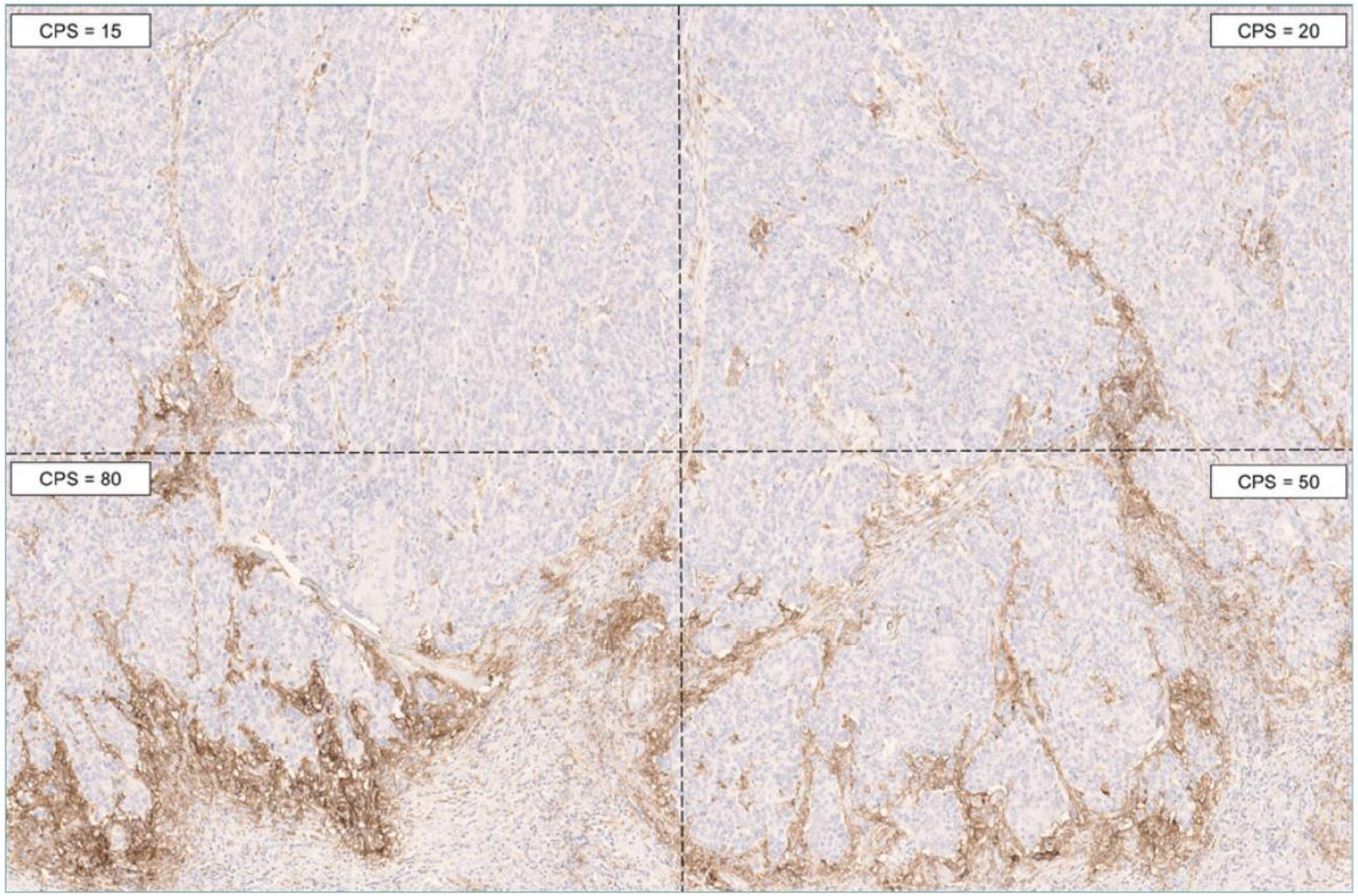
C) In the green cicle as hotspot

D) As an average between blue and red cicle, avoiding the abscessualized area

PD-L1: how to count?



It is <u>not</u> a hot-spot evaluation!

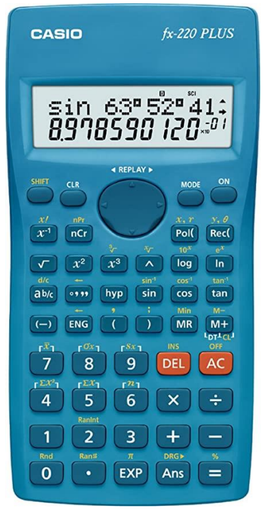


Heterogeneous PD-L1 Staining Area

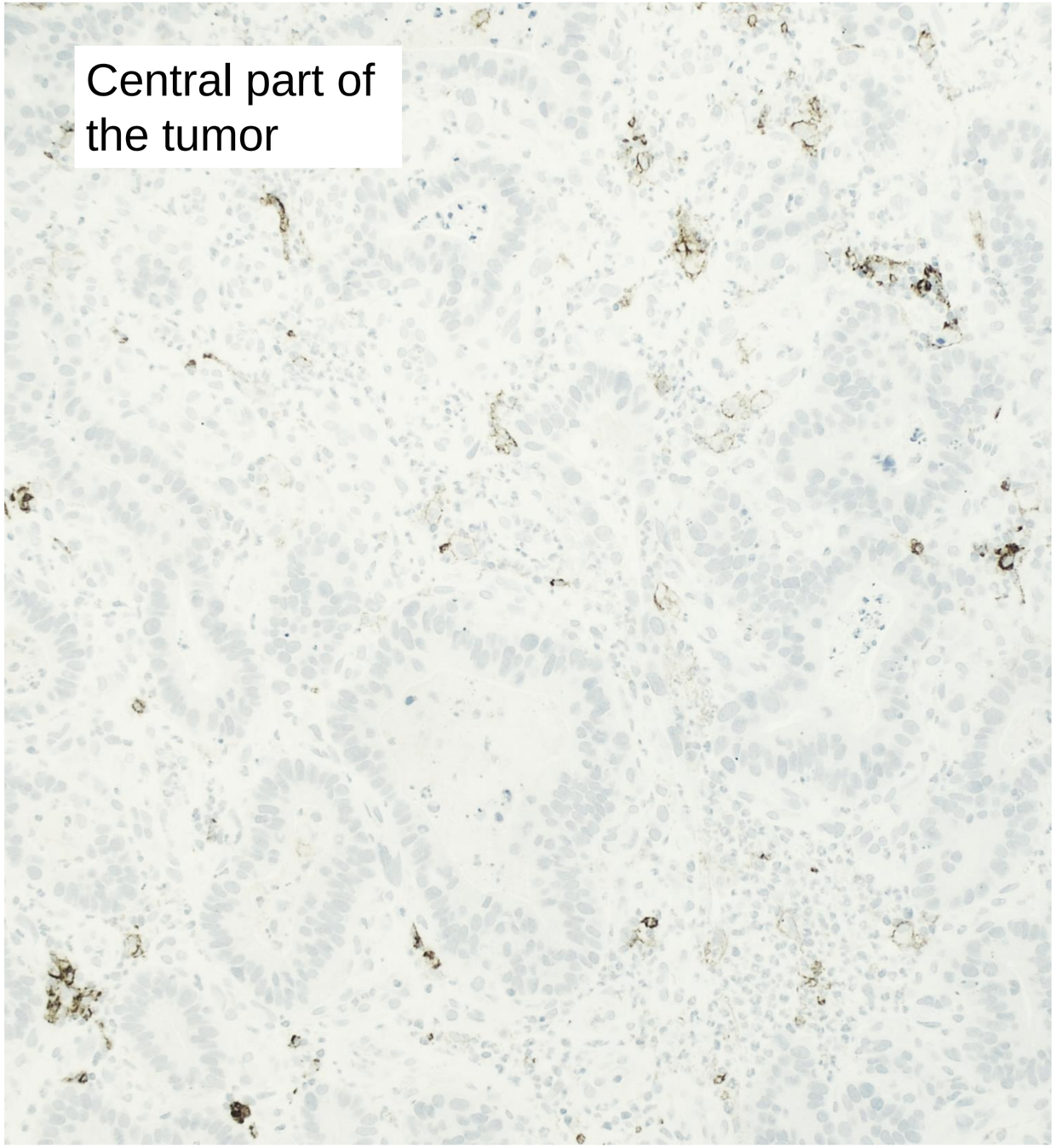
Combined Positive Score:

((80 + 30 + 50 + 100) / 4) ≅ 65

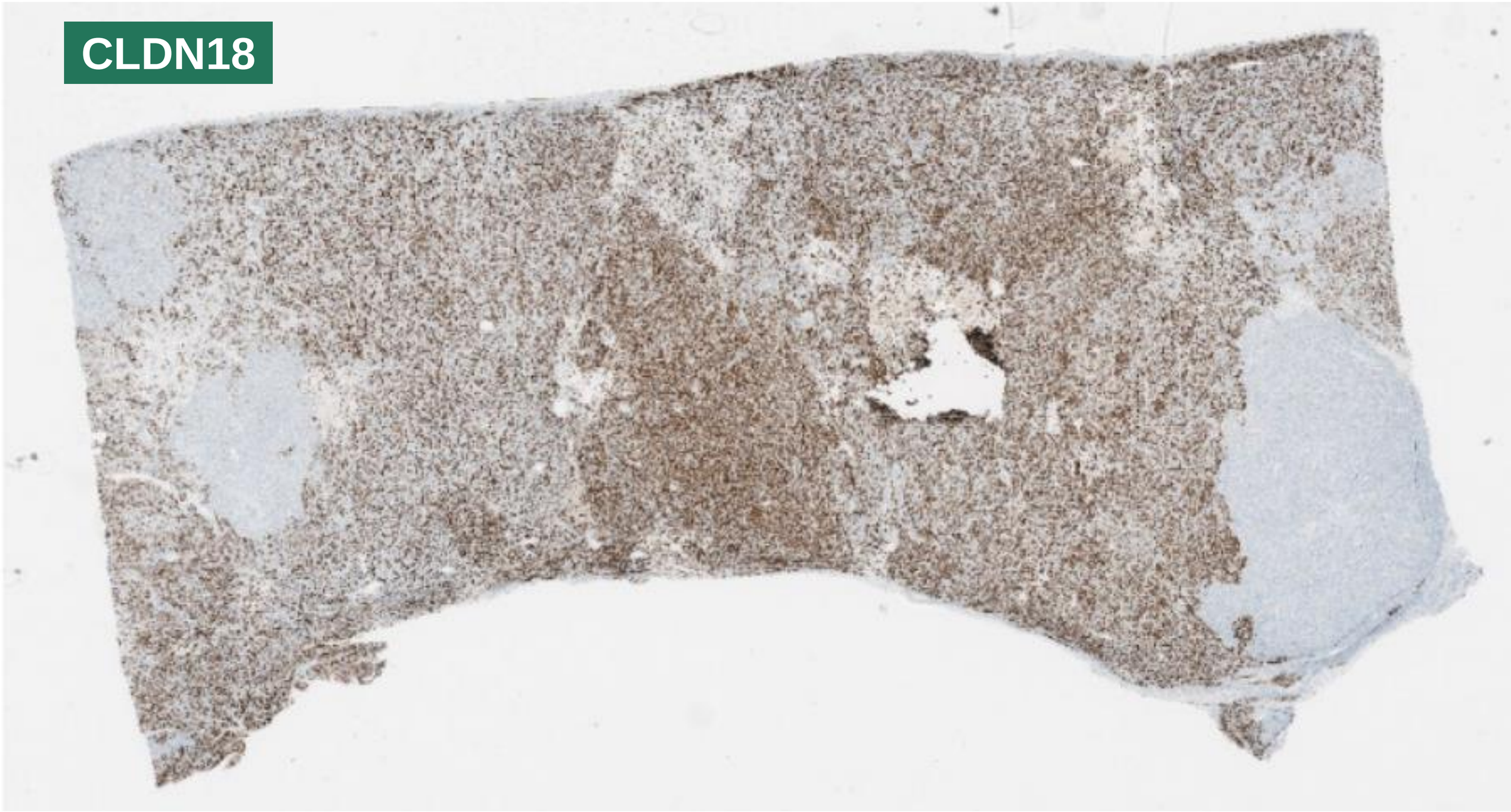
CPS 65, Clinical Interpretation: CPS≥10

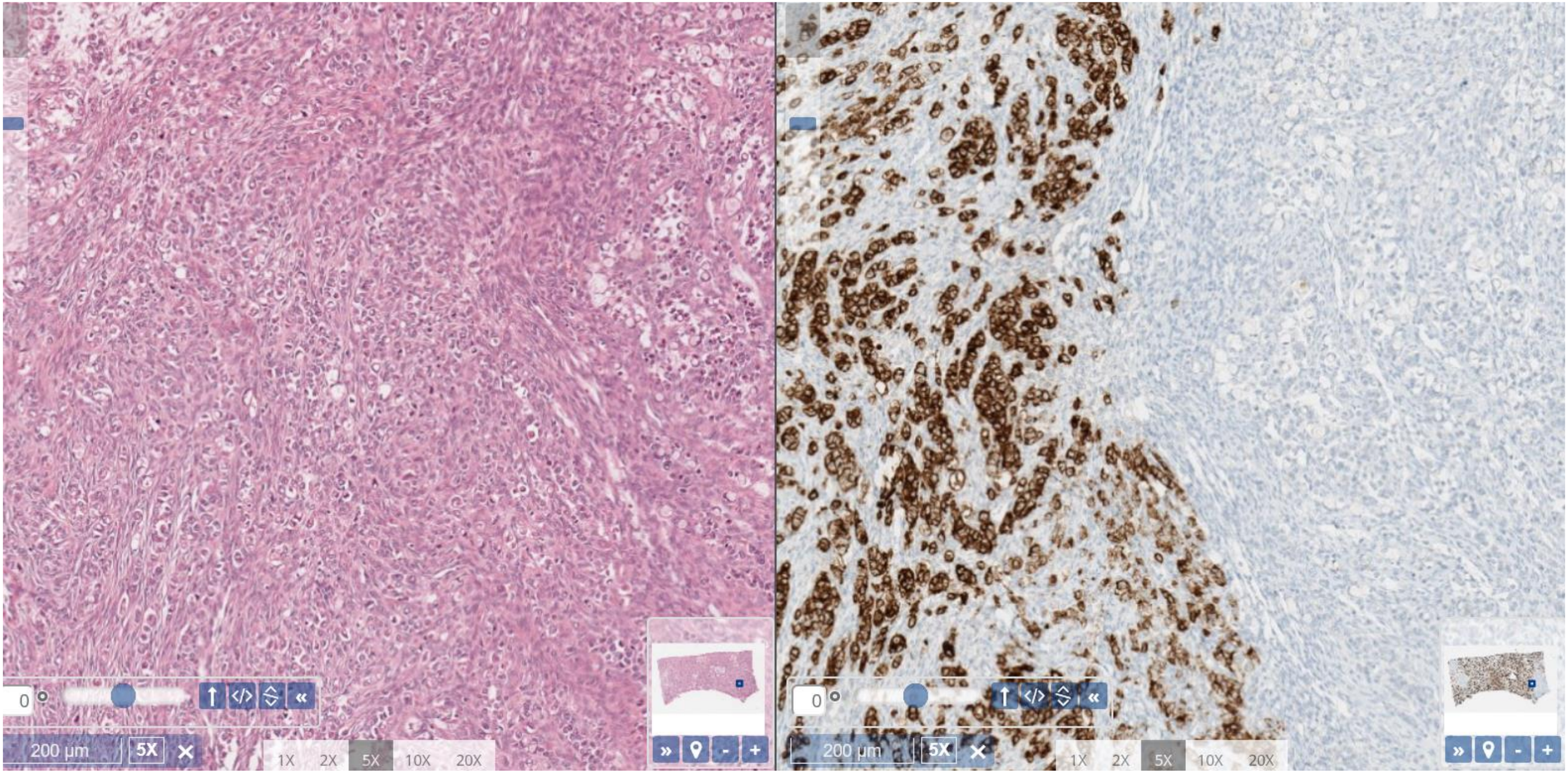


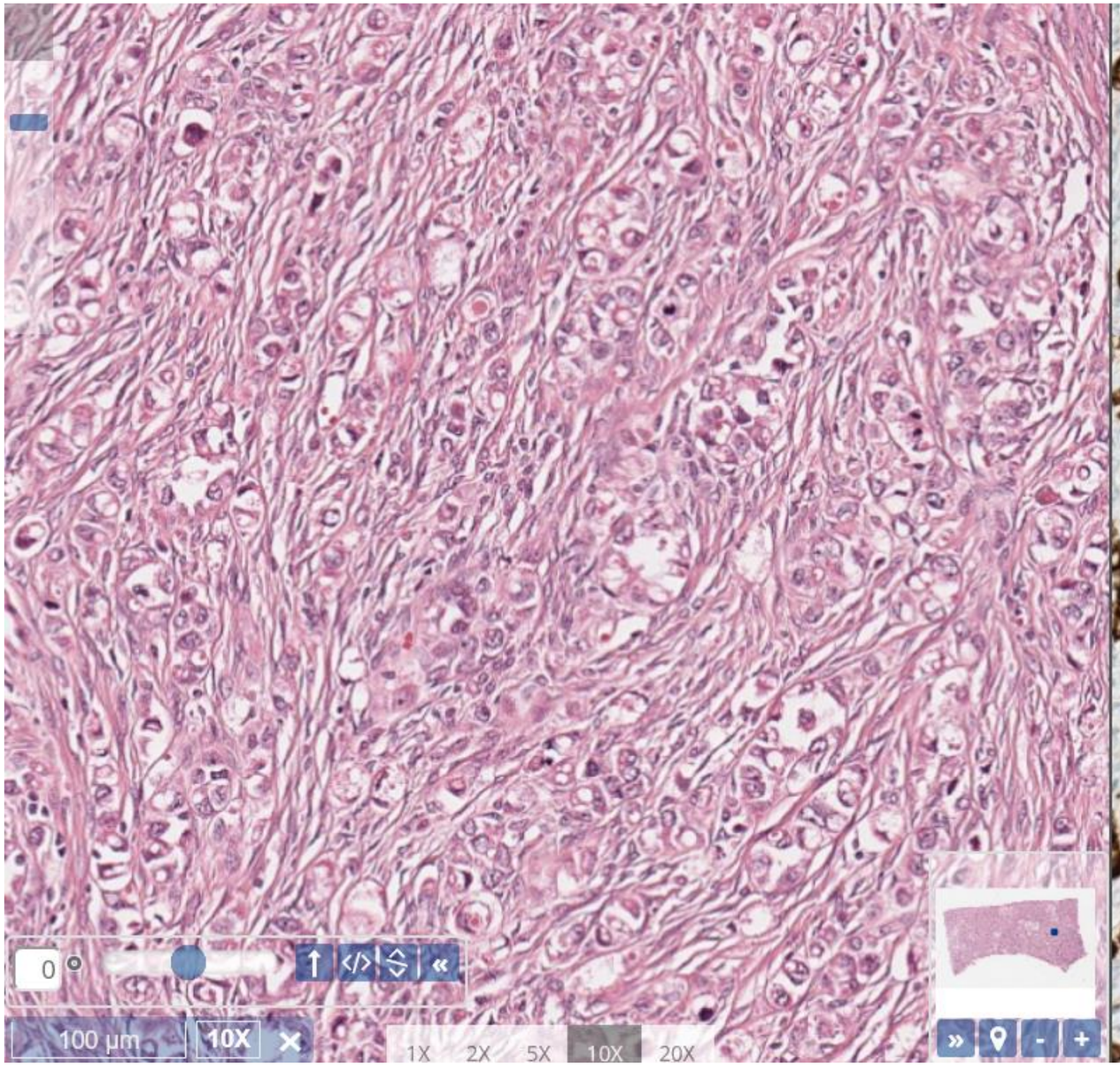

Ulcerated part
of the tumor

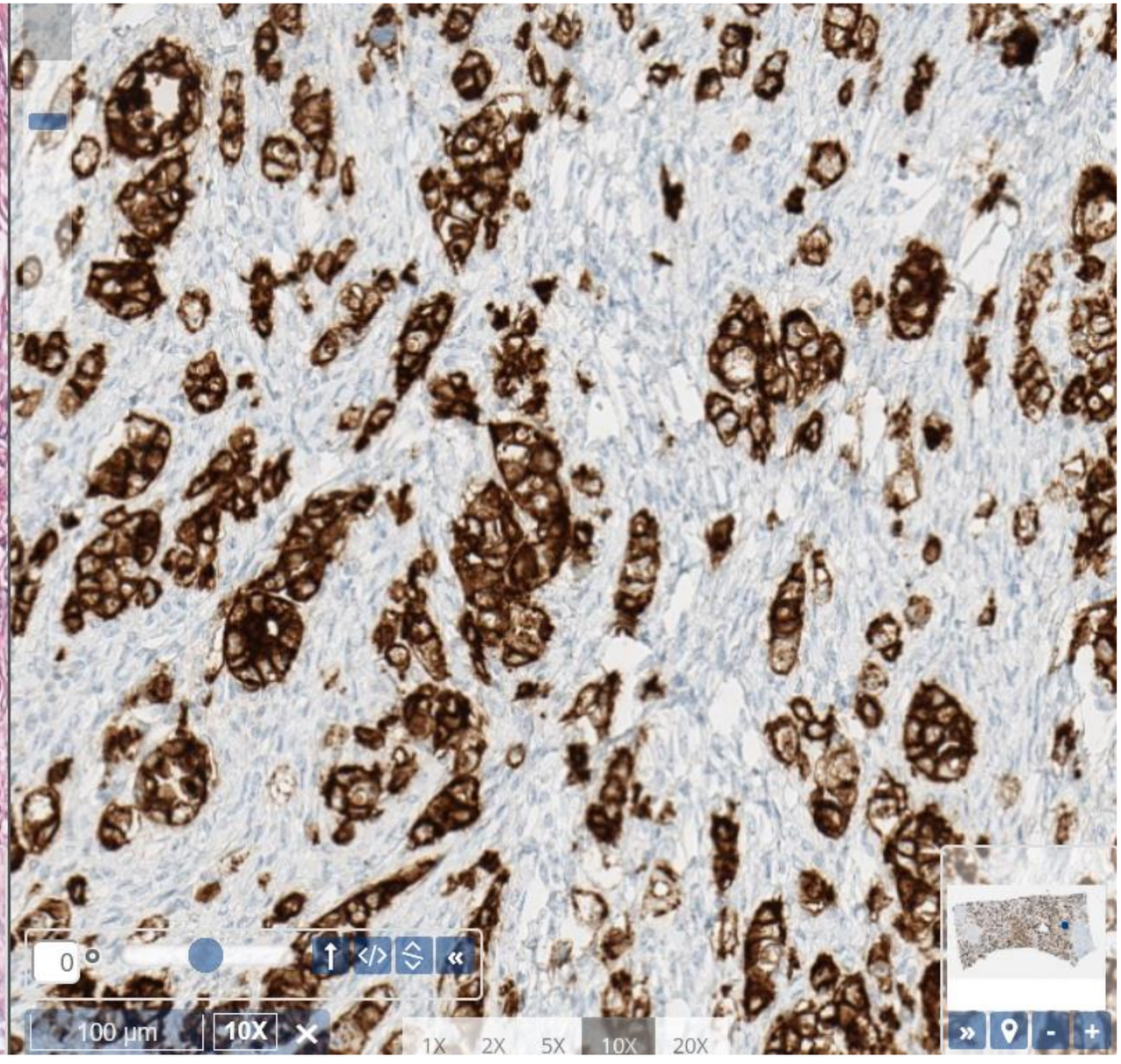

Central part of
the tumor

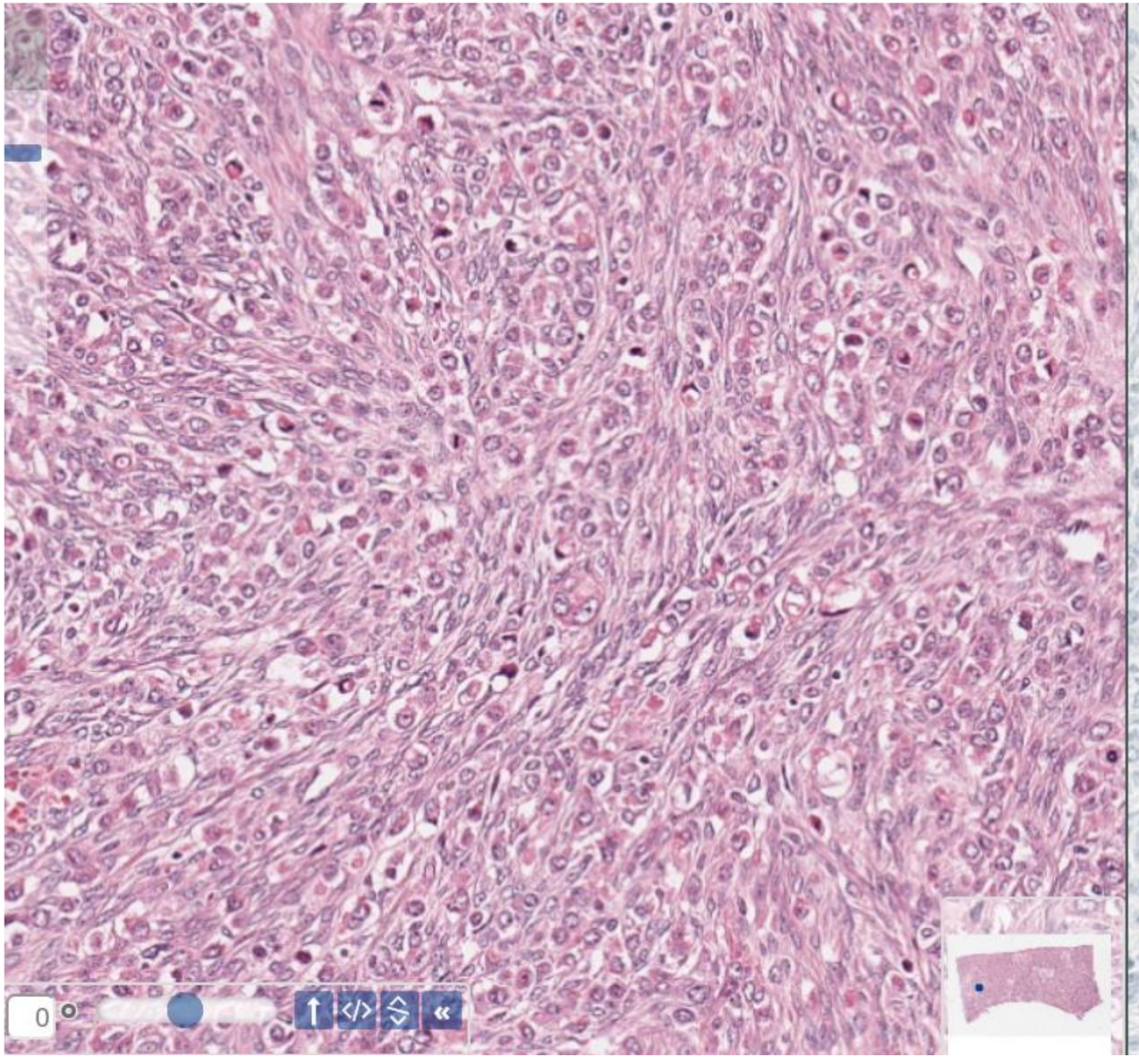
7. Can CLDN18.2 be heterogeneous?

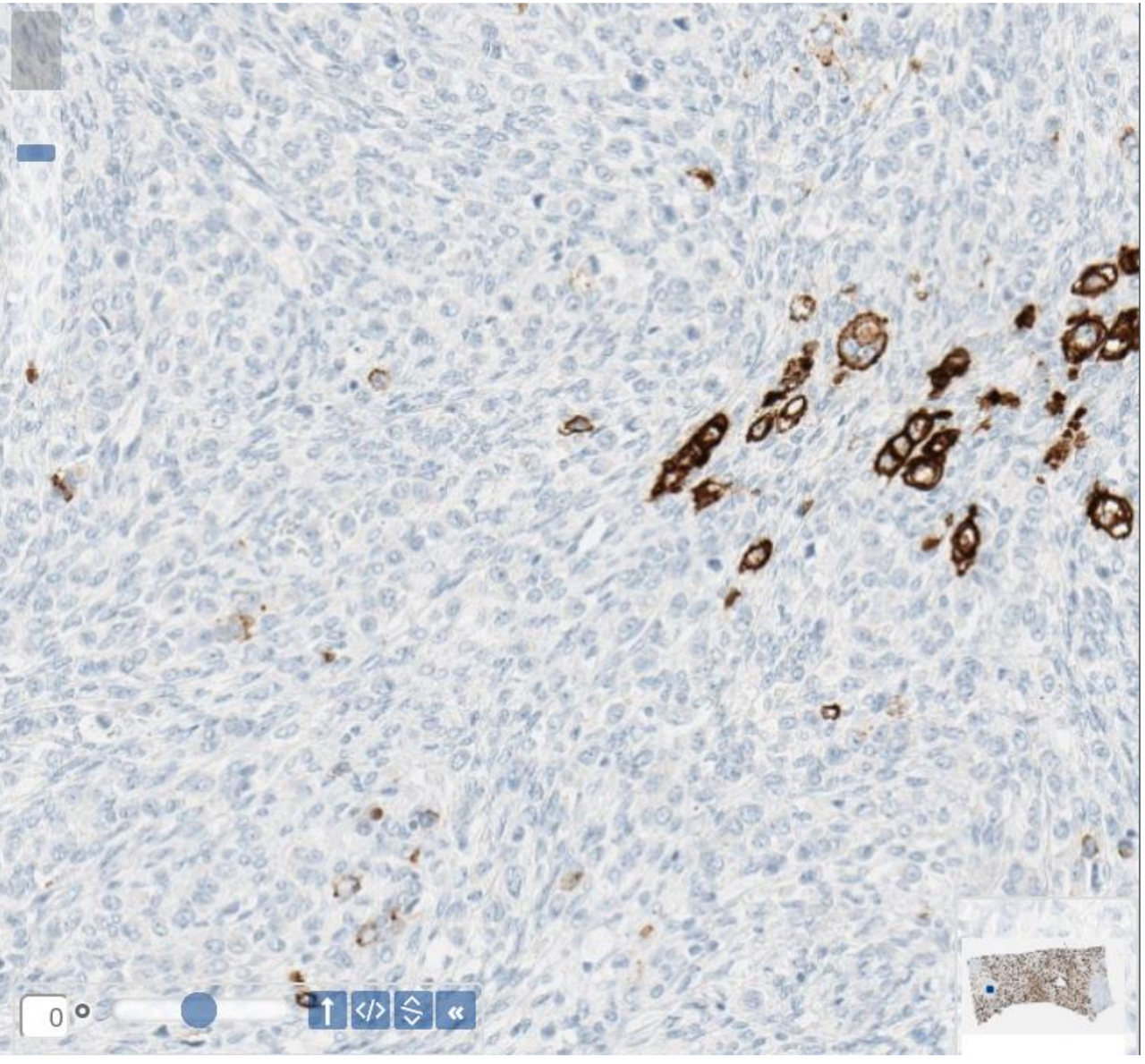

CLDN18


0
200 µm
5X
1X
2X
5X
10X
20X
0
200 µm
5X
1X
2X
5X
10X
20X

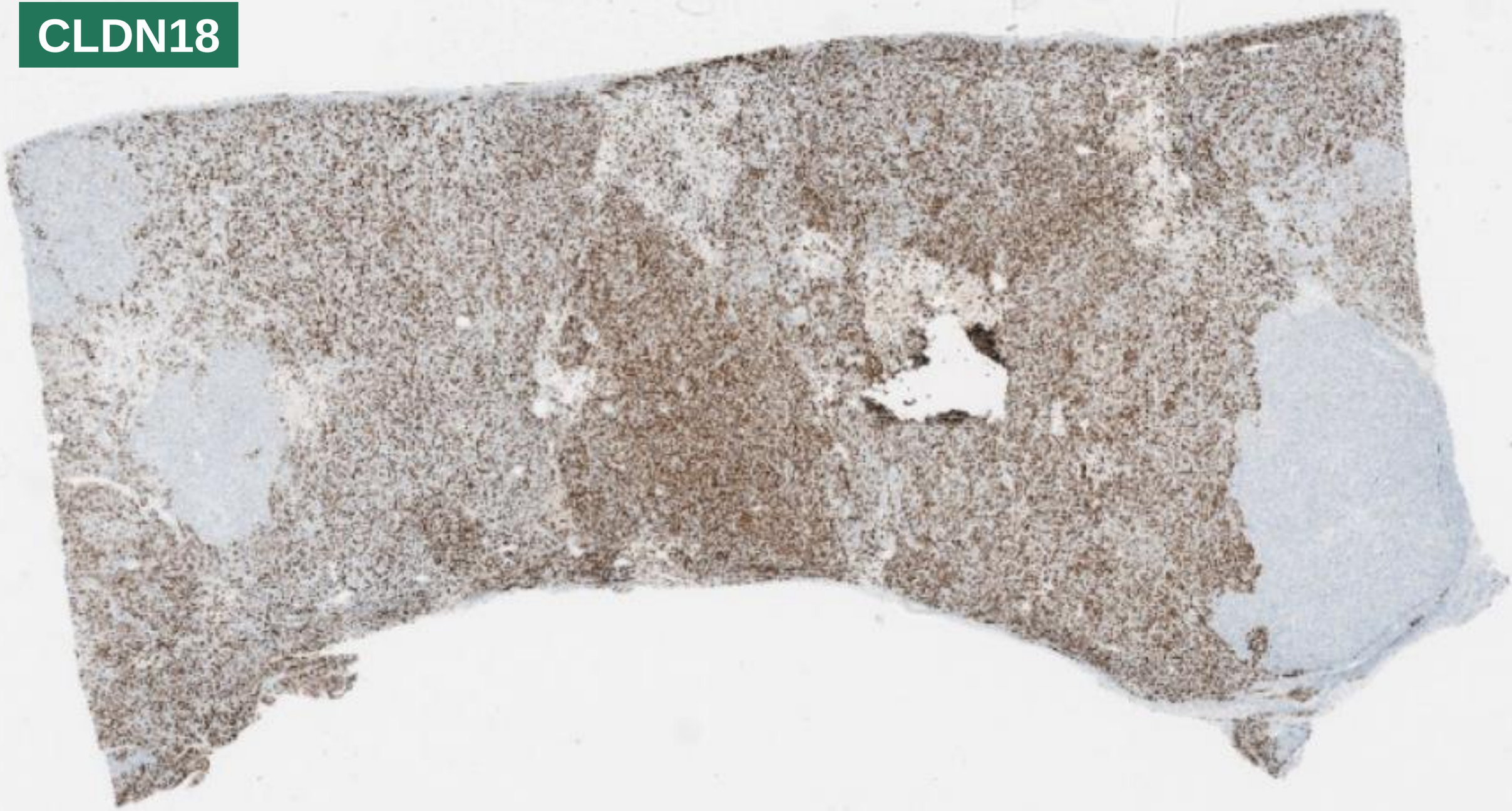

0
100 µm
10X
1X
2X
5X
10X
20X


0
100 µm
10X
1X
2X
5X
10X
20X





CLDN18



What about CLDN18.2 status in this case?

A) ≥75%
B) 75-65%
C) 55-65%
D) <55%

What about CLDN18.2 status in this case?

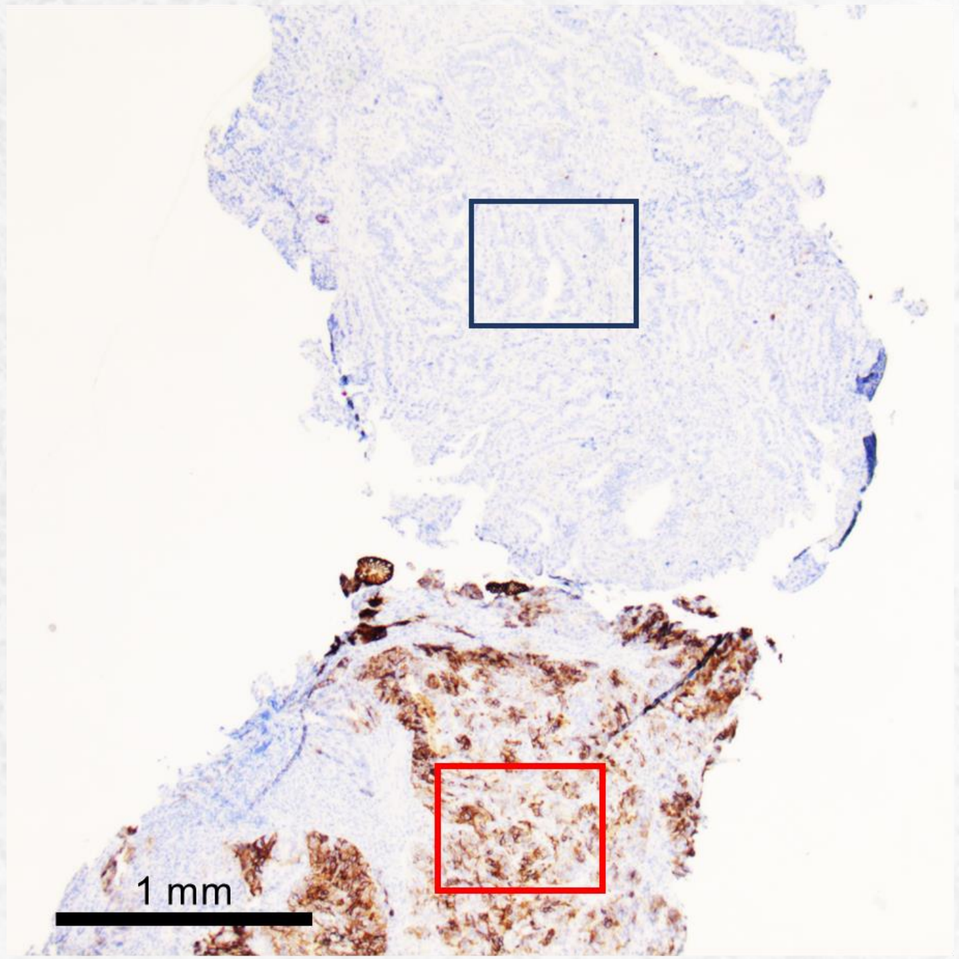
A) ≥75%

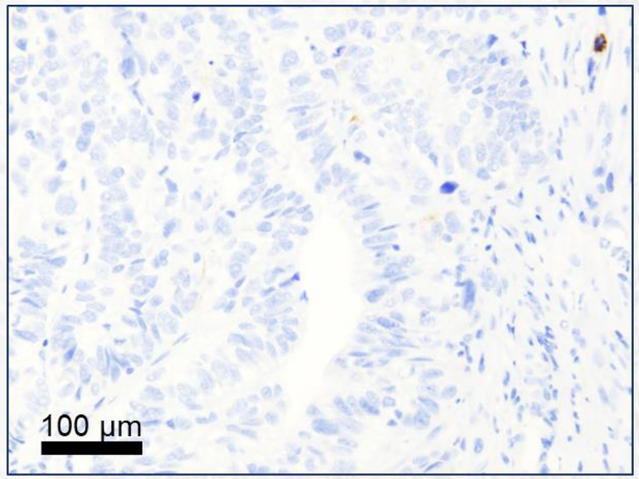
B) 75-65%

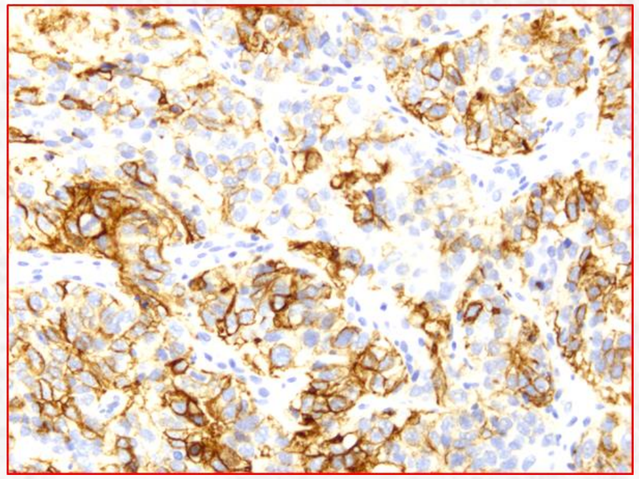
C) 55-65%

D) <55%

Gastric cancer as a heterogeneous disease





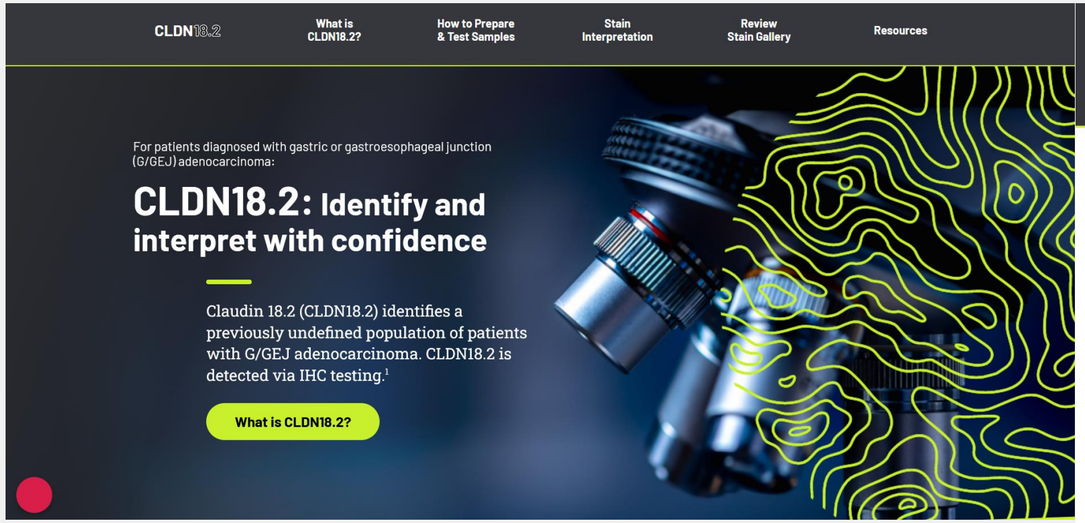


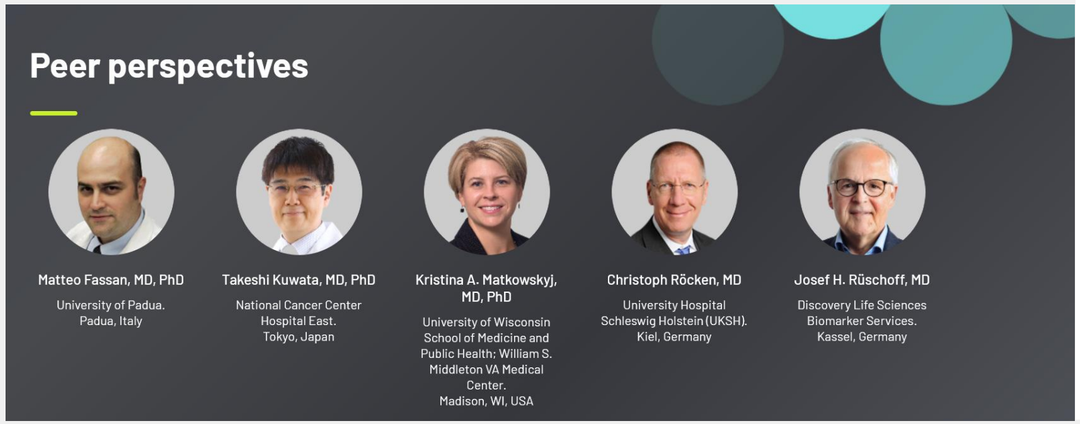
CLDN18.2
0

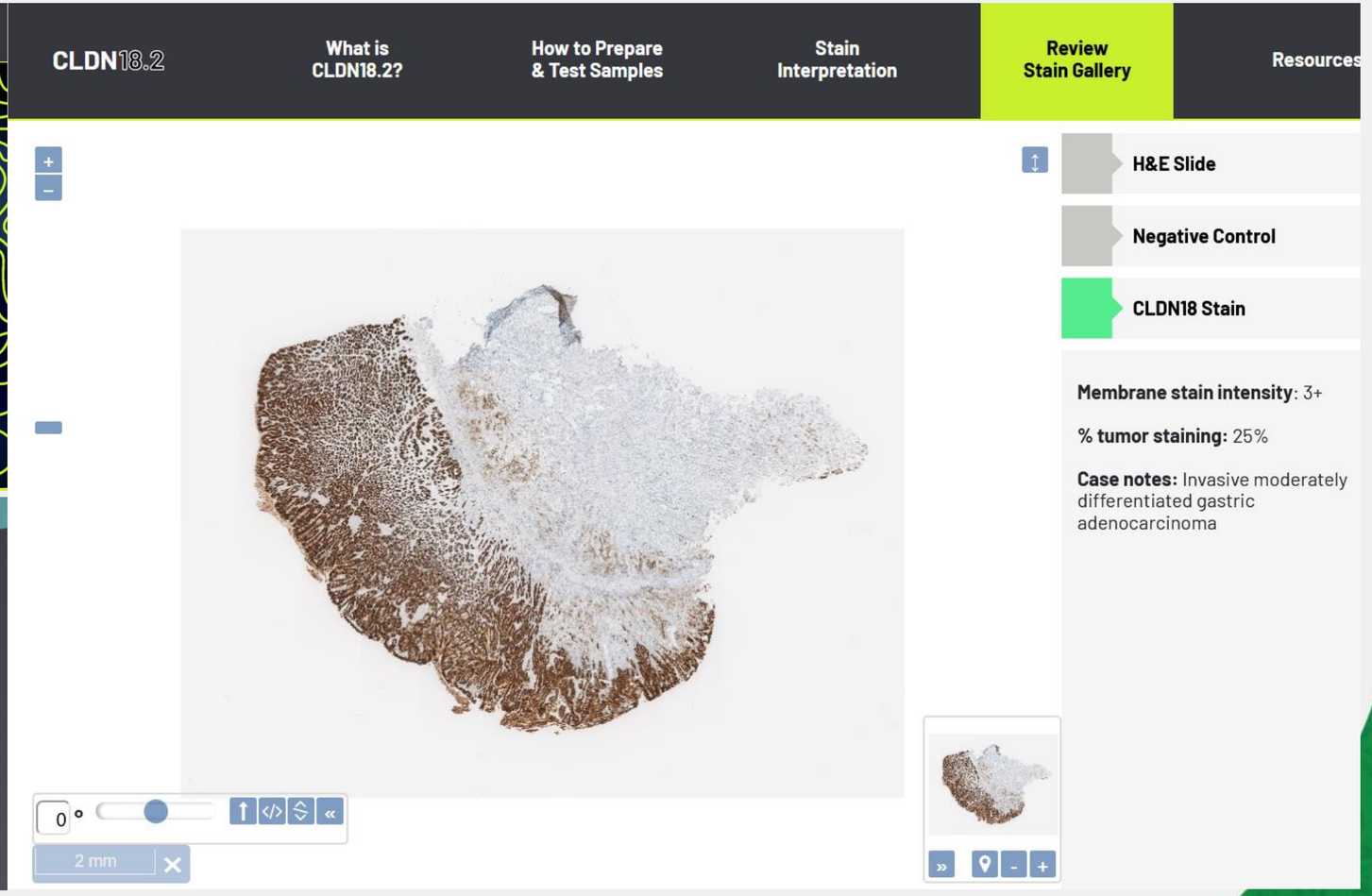
CLDN18.2
2/3+

Pellino A, *et al.* - J Pers Med 2021

The CLDN18.2 pathology hub experience: an interpretation guide and tutorial



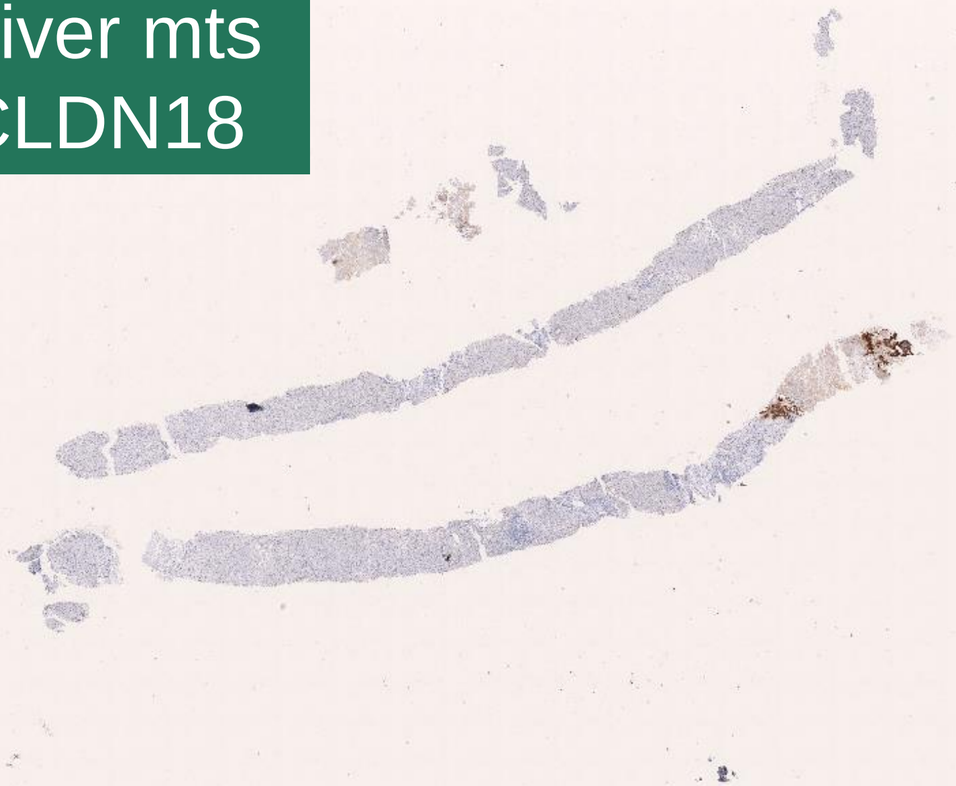




https://www.claudin182.com/ (in updating process)

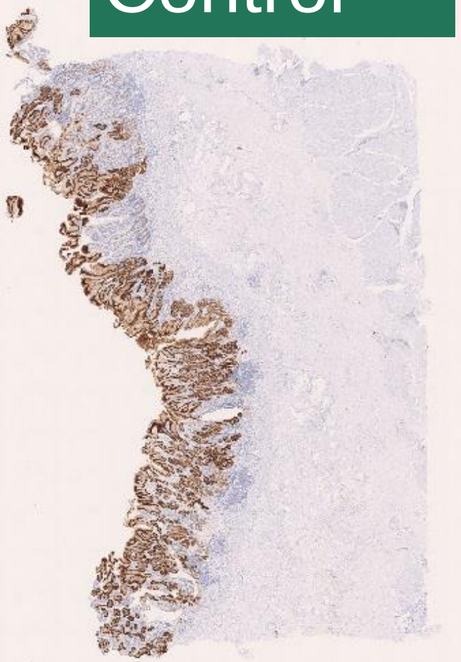
8. CLDN18.2 positive tissue

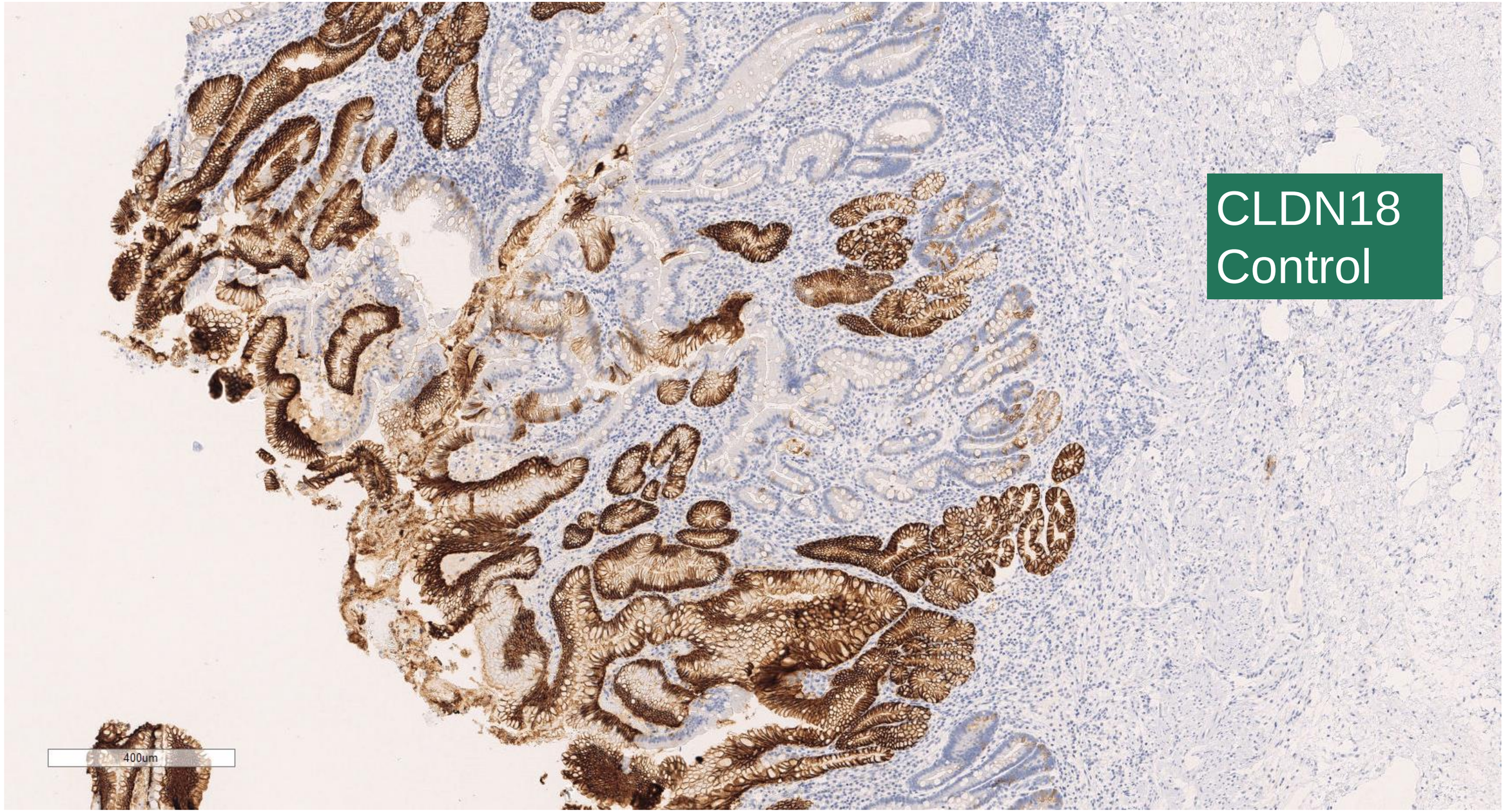
Liver mts
CLDN18



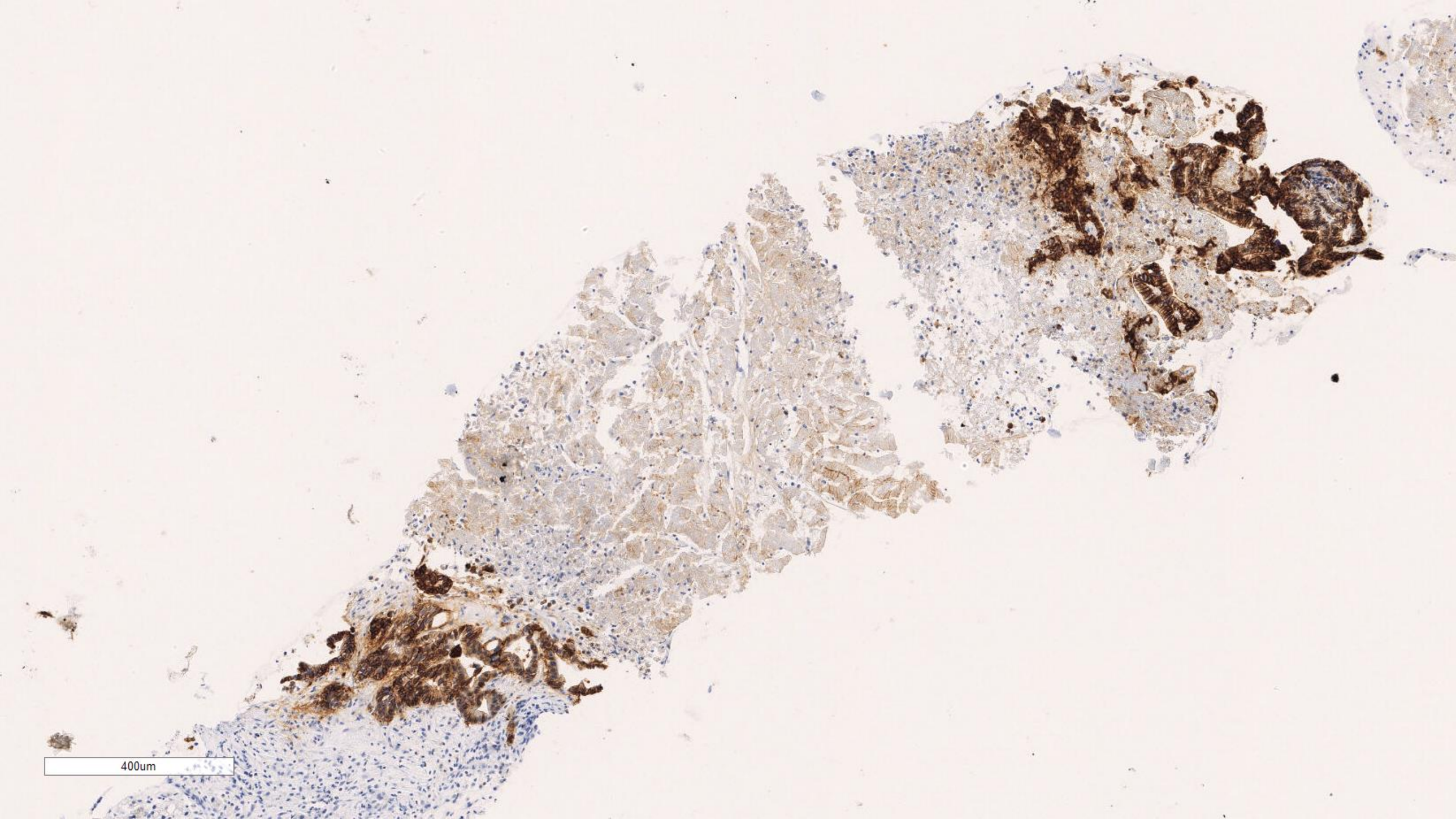
5mm

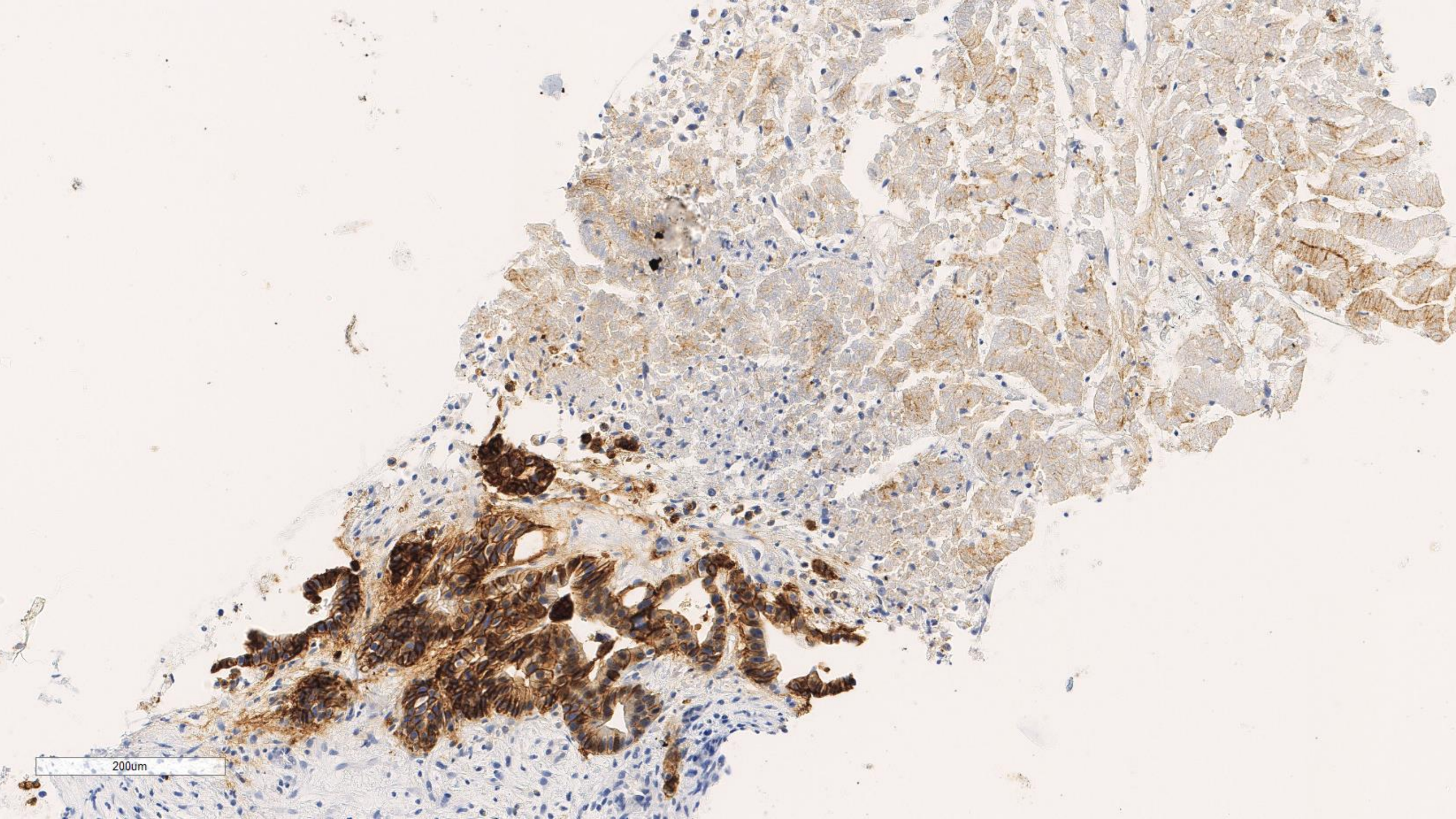
CLDN18
Control





CLDN18
Control


400um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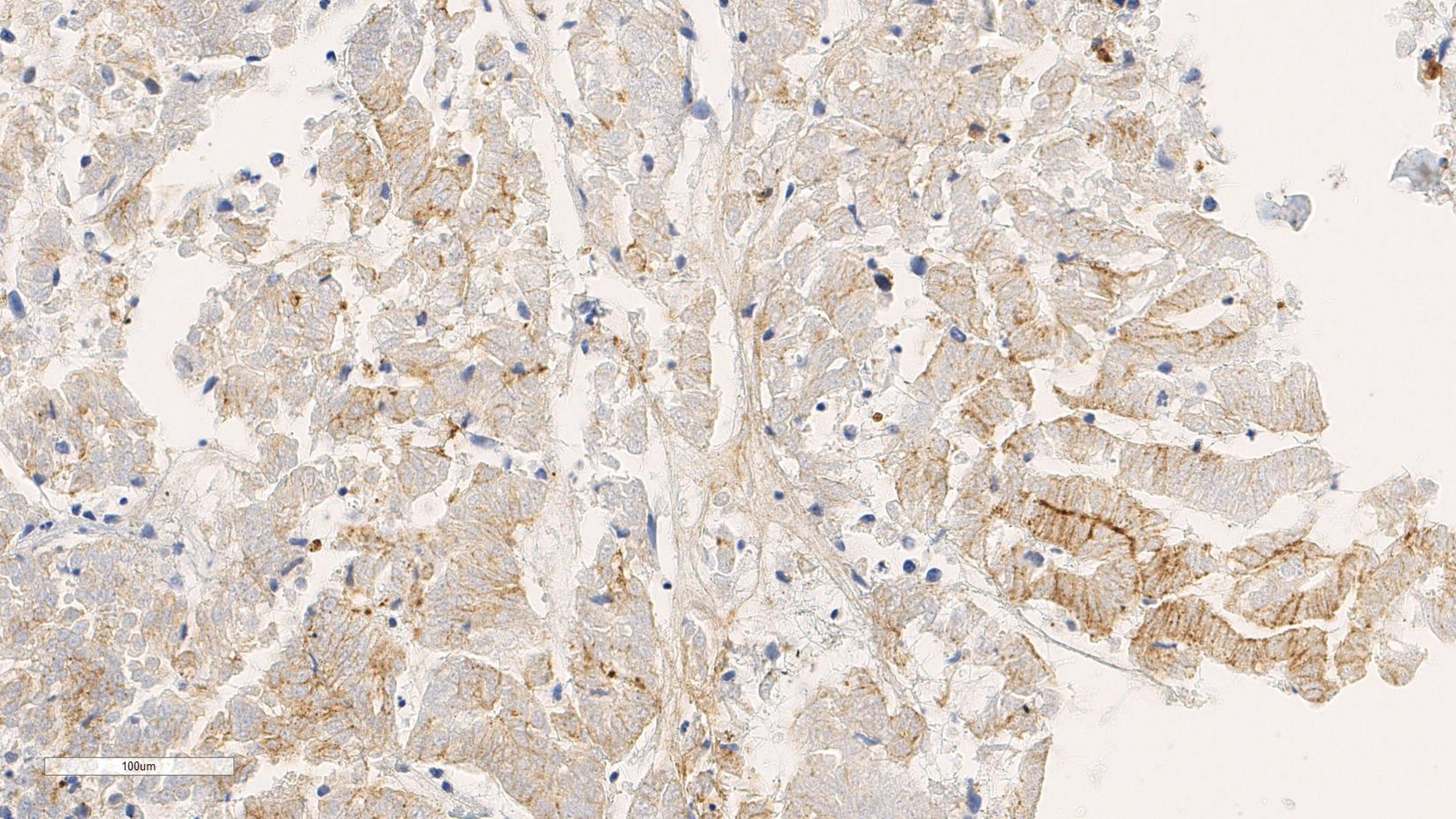
100um

What about CLDN18.2 status in this case?

A) ≥85%
B) 85-75%
C) 75-55%
D) <55%

What about CLDN18.2 status in this case?

A) ≥85%

B) 85-75%

C) 75-55%

D) <55%

Take home messages

- The pathologist should be aware of the clinical impact of the histology report
- Increasing number of biomarkers' portfolio: request for a more adequate tumor sampling/sample's sparing strategies
- Need for educational programs involving pathologists, lab technicians, oncologists, surgeons and gastroenterologists (es. PD-L1, preneoplastic lesions, MMR, CLDN18.2)!

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