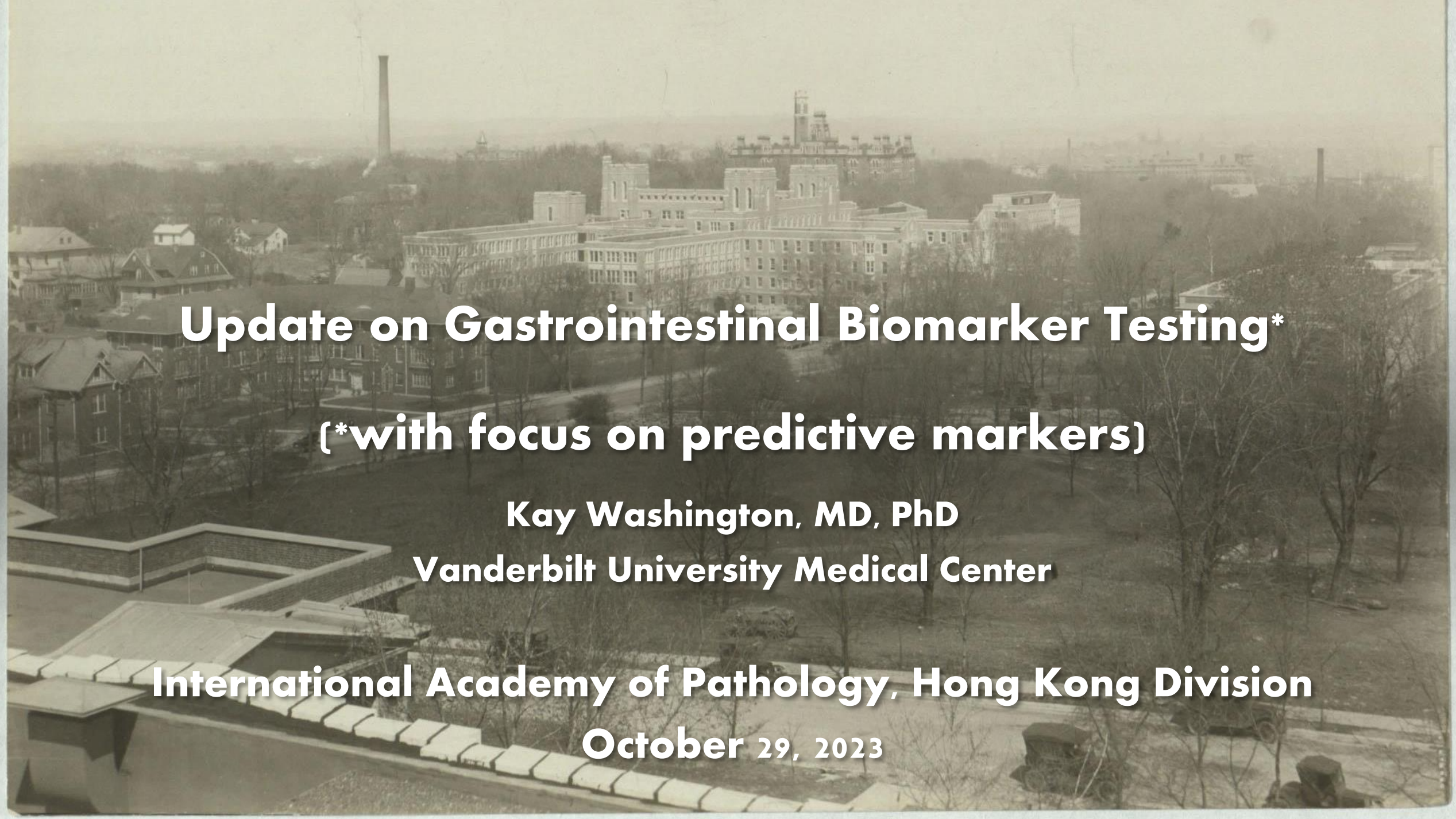


Update on Gastrointestinal Biomarker Testing*

(*with focus on predictive markers)

Kay Washington, MD, PhD
Vanderbilt University Medical Center

International Academy of Pathology, Hong Kong Division
October 29, 2023

Disclosures

- Chair of Center Guideline Committee for the College of American Pathologists
- Co-Chair of expert panel for the CAP/ASCP/ASCO guideline on HER2 testing in gastric and esophageal adenocarcinoma

Objectives

- Rationale for testing GI carcinomas (what tumors, why test, who)
- Current recommendations: when and how to test

- MSI/MMR testing
- PD-L1 testing
- Her2 (what about Her2 low?)

- Claudin 18.2 in gastric cancer

- Important but not IHC-based: RAS testing in colorectal carcinomas

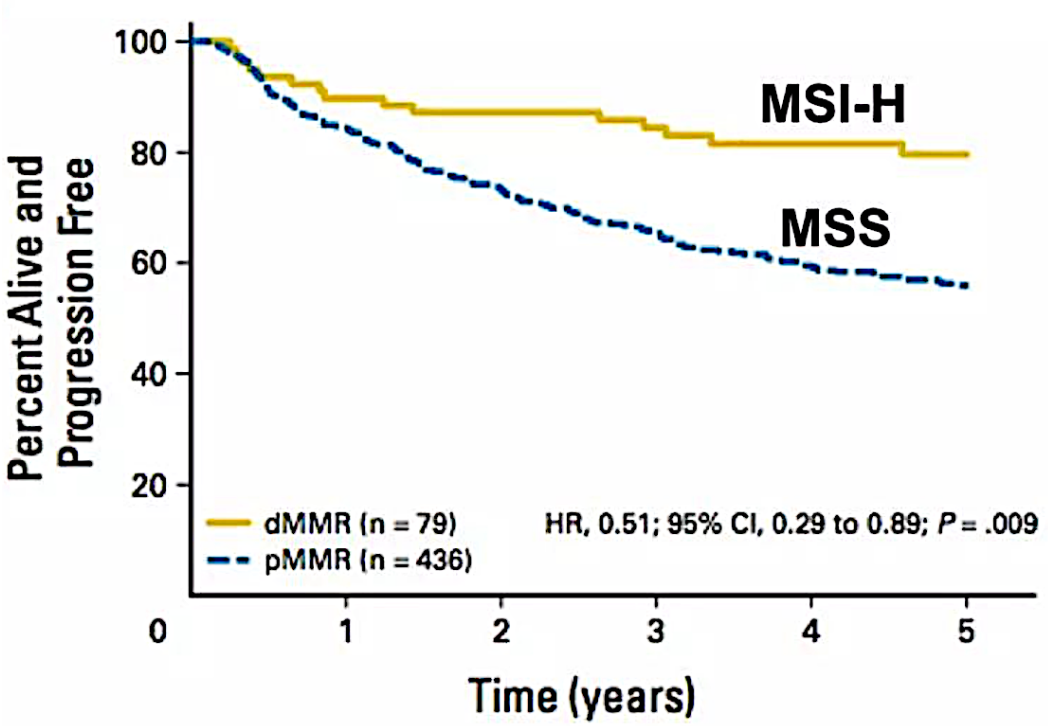
Consensus Molecular Subtypes of Colorectal Cancer

CMS1 MSI Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGFβ activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Guinney J, et al. Nat Med 2015;23(11): 1350-6.

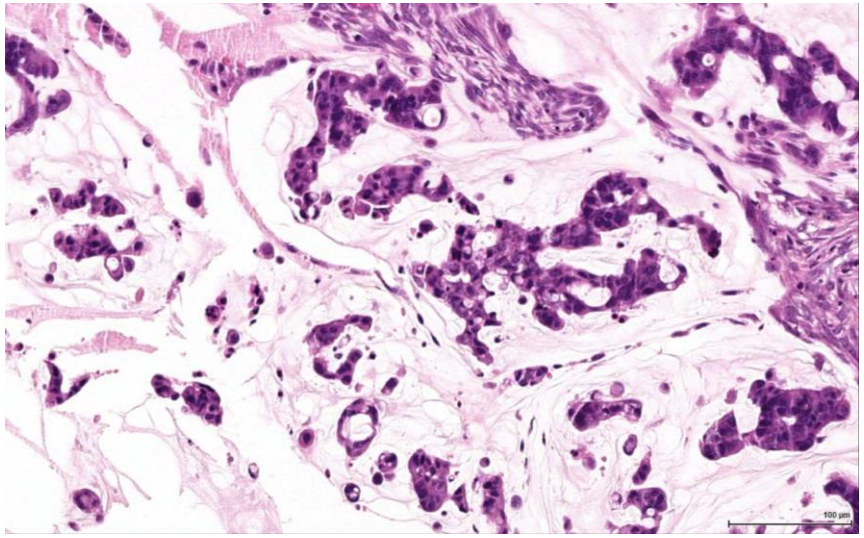
Microsatellite Instabililty Testing (CMS1)

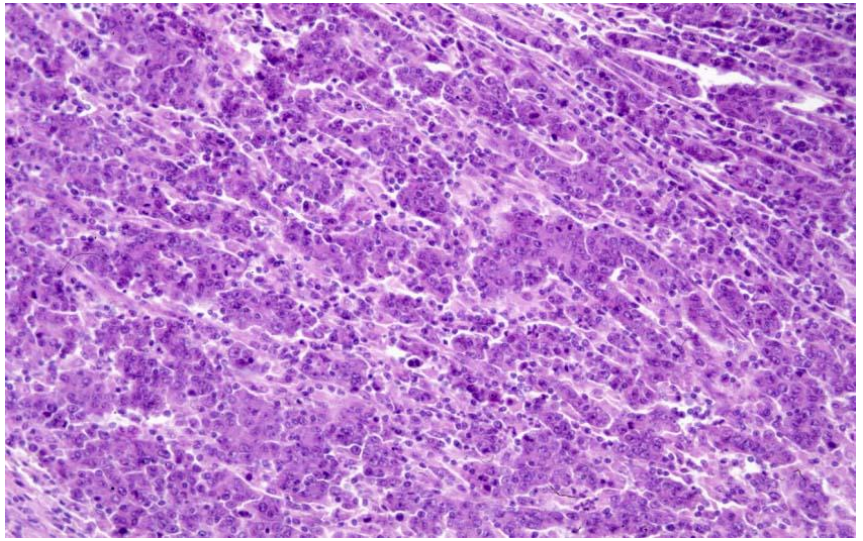
- Strong prognostic indicator
- Impacts treatment decisions
  - Less likely to receive adjuvant therapy for Stage II
  - Checkpoint inhibitor therapy
- Identify Lynch Syndrome patients



Pathology of MSI-H Cancers (Molecular Type CMS1)

- Unusual histologic subtypes
  - Medullary carcinoma
  - Mucinous carcinoma (57% vs 16% for MSS)
  - “Poorly differentiated” (47% vs 10% for MSS)
- Lack of Dirty Necrosis
- Numerous tumor-infiltrating lymphocytes





Microsatellite Instability Testing in Solid Cancers: Why?

- In 2017, the US FDA approved pembrolizumab immune checkpoint inhibitor therapy for unresectable or metastatic MSI-H or dMMR solid tumors with progression
- Applies to all digestive system tumors in the appropriate clinical setting
- Most commonly tested GI tumors are colorectal, small bowel, esophageal, and gastric carcinoma
  - Pancreatic, hepatobiliary, and hepatocellular carcinomas are rarely MSI-H or dMMR

Which test to use?

- Polymerase chain reaction microsatellite instability assays?
- Immunohistochemistry for DNA mismatch repair proteins?
- Next-generation sequencing based MSI analysis?
- NGS-based assessment of tumor mutation burden as a surrogate marker?

Should test choice be influenced by tumor site?

Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy

Guideline From the College of American Pathologists in Collaboration With the Association for Molecular Pathology and Fight Colorectal Cancer

(*Arch Pathol Lab Med.* 2022;146:1194–1210; doi: 10.5858/arpa.2021-0632-CP)

Recommendations: Colorectal Carcinoma

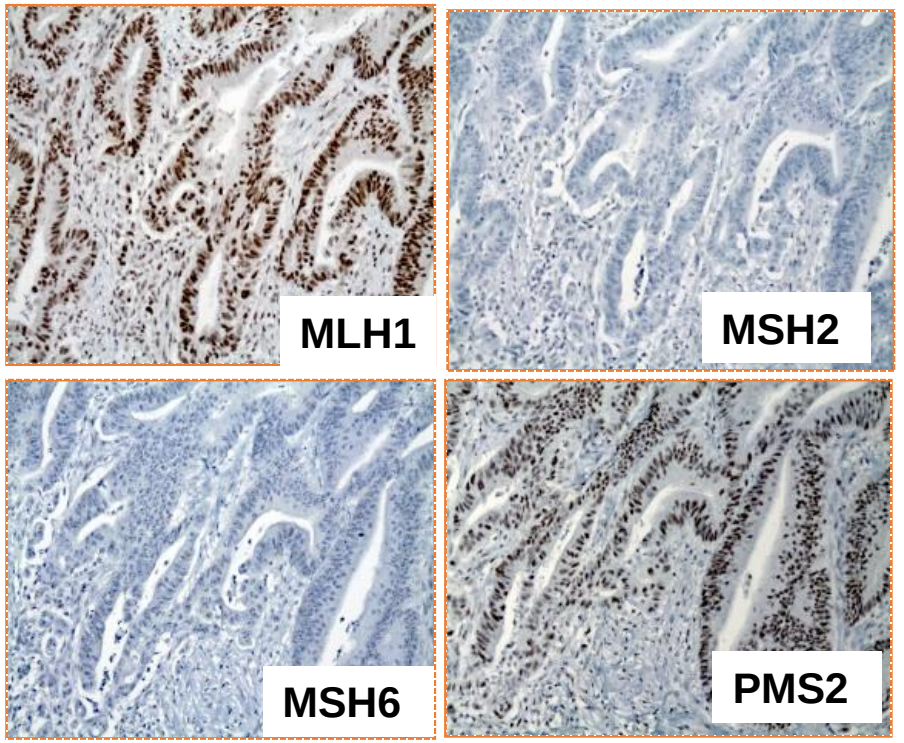
- For colorectal carcinoma, MMR-IHC or MSI by PCR should be used.
  - These methods have comparable performance in CRC
  - MMR-IHC can identify the probable gene defect
- Validated MSI by NGS assay may be used but is not preferred
  - NGS assays require more tissue and take longer to complete
- Tumor mutational burden should not be used as a surrogate marker
  - Also seen in POLE exonuclease-domain mutations in CRC

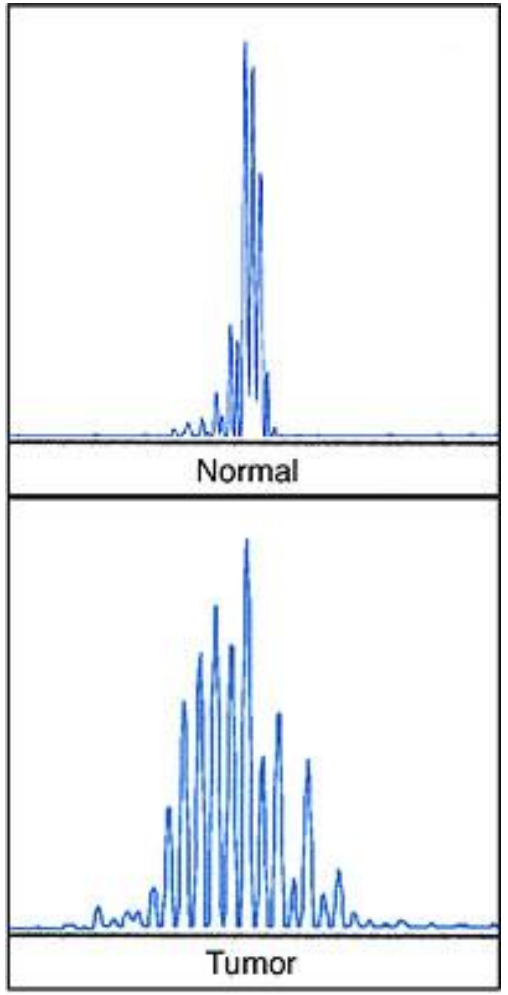
Recommendations (CAP)

- For gastroesophageal and small bowel cancers, NGS should not be used
- For other cancer types, the optimal approach has not been established.
- If results are discordant, any evidence of MMR-D or MSI-H should be interpreted as a positive result for treatment eligibility
- If results are indeterminate, an alternative method or a different tumor block should be used
- In the event of subclone loss by MMR-IHC, MSI by PCR should be performed in a dissected area of tumor with MMR protein loss

Microsatellite Instability/Mismatch Repair Testing

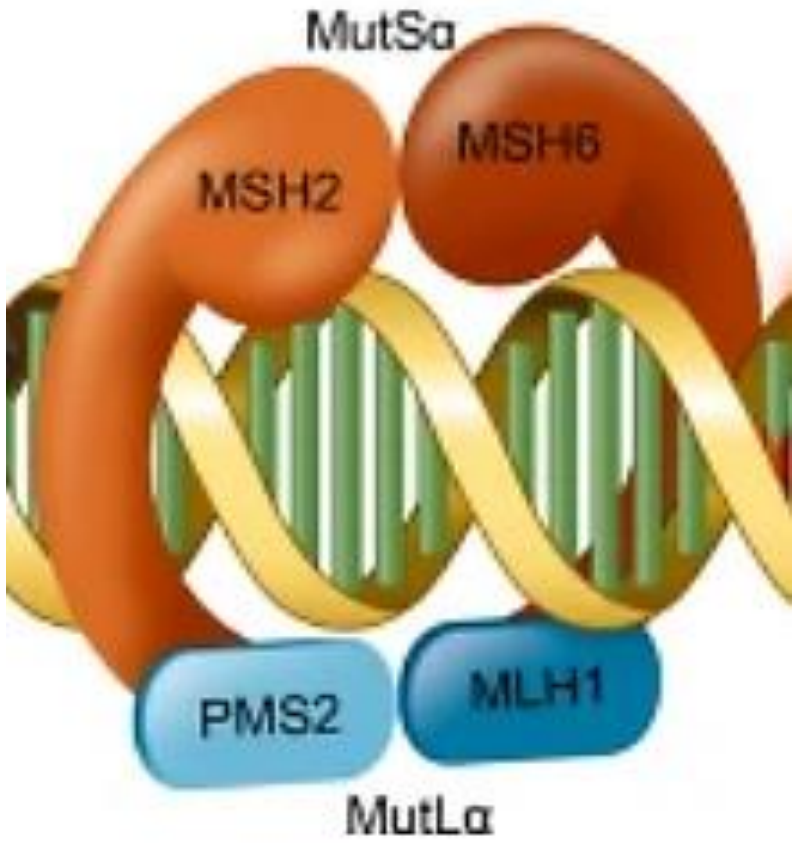
- Roughly 15% of CRCs arise from functional defect in mismatch repair genes
  - 12% sporadic (arise from SSA); typically BRAF mutated
  - 3% germline (Lynch syndrome)
- MLH1, MSH2, MSH6, PMS2
- ~ 95% concordance





MMR Panel Results: MLH1, PMS2, MSH2, MSH6

Finding	Interpretation	Lynch Syndrome?	Defective MMR gene
All 4 retained	MMR proficient	Unlikely	None implicated
MLH1, PMS2 lost	MMR deficient	Possible; usually sporadic	MLH1 promoter methylation
PMS2 lost	MMR deficient	Probable	PMS2
MSH2, MSH6 lost	MMR deficient	Probable	MSH2
MSH6 lost	MMR deficient	Probable	MSH6



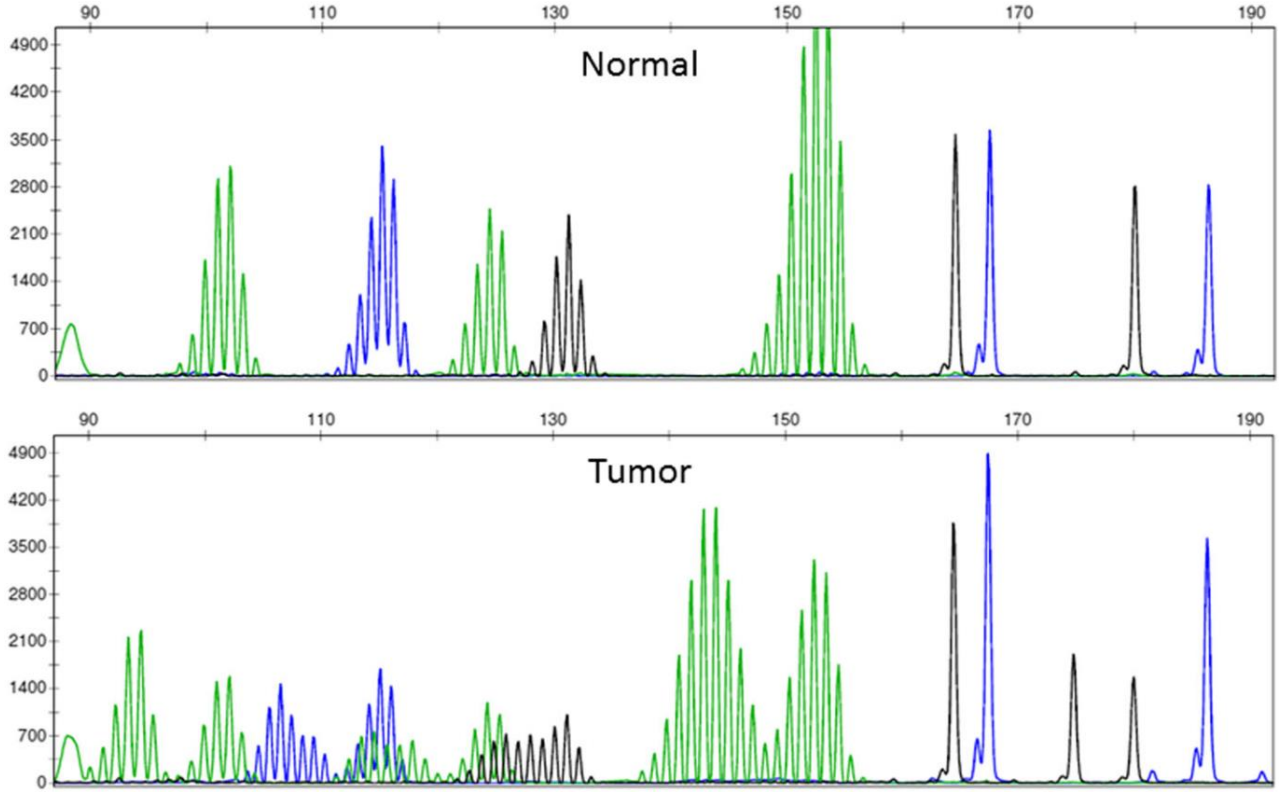
Sajjadi E, et al. Cancer Cell International 2021; 21: 266

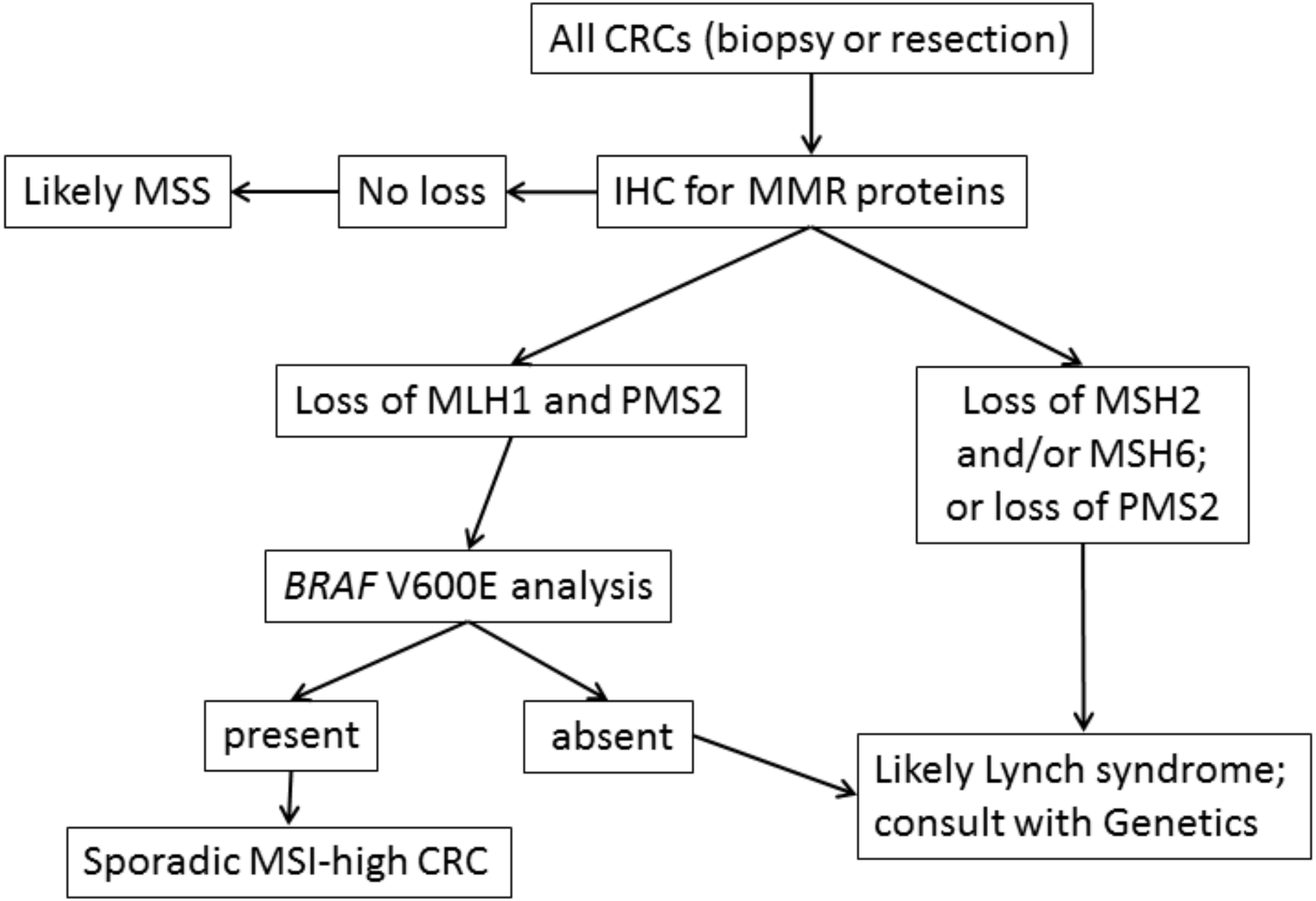
MMR IHC Interpretation

- Report as present/intact or absent/lost, not positive or negative
- Any convincing nuclear staining is intact- cutoffs vary, but many use 5% (1%, 5%, 10% positivity)
- Nuclear expression must be as strong as the control- use caution if weaker
- If the internal control is negative, the case is uninterpretable
- Tissue fixation and pre-analytical variables can affect the results
- Testing for 2 markers (MLH1/MSH2 or PMS2/MSH6) instead of 4 will miss cases

PCR-based testing for microsatellite instability

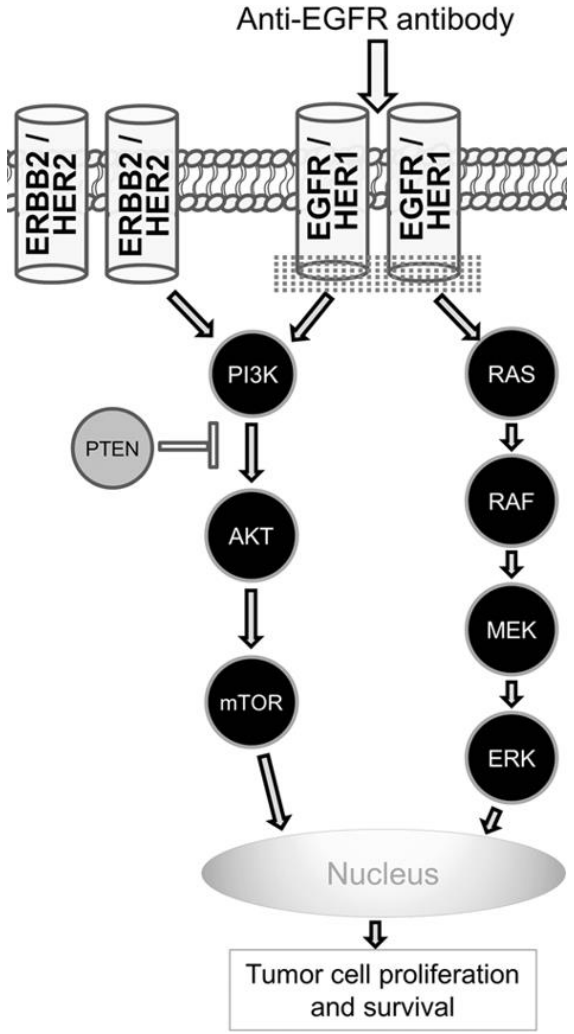
- Alternative to MMR IHC
- Detects ~90% of MSI-high CRC
- Five mononucleotide repeat markers tested for instability
  - BAT-25, BAT-26, NR-21, NR-24, MONO-27
  - ≥2 unstable: MSI-high
  - 1 unstable: MSI-low
  - 0 unstable: MSS

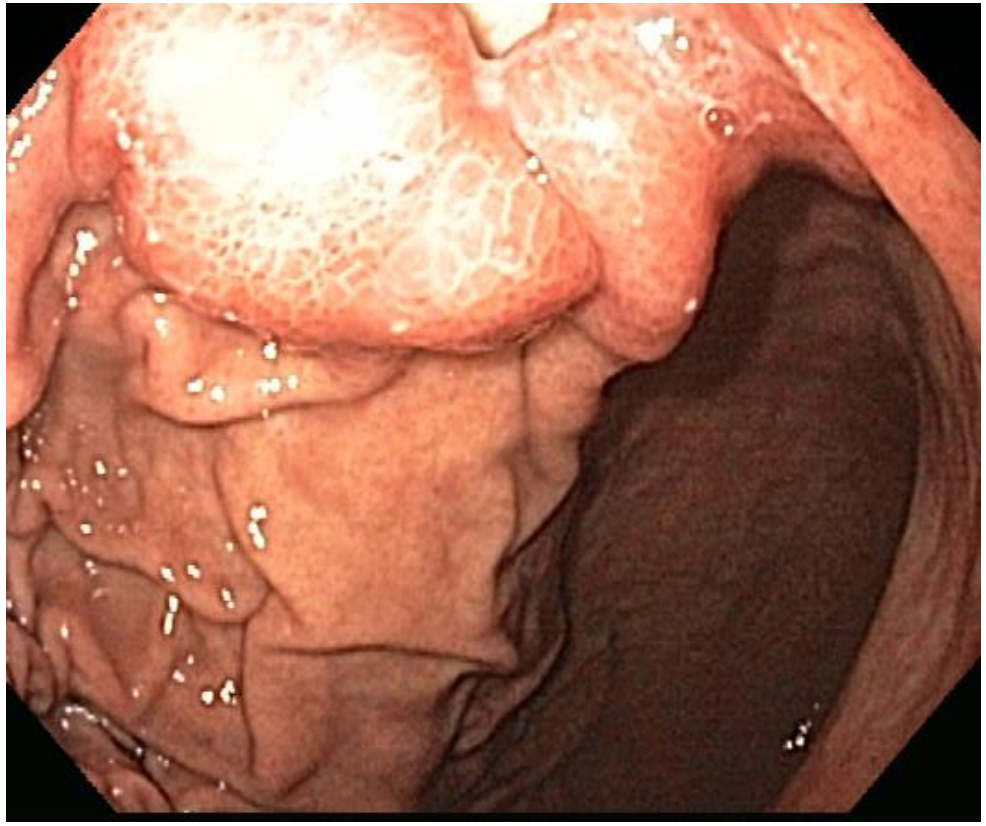


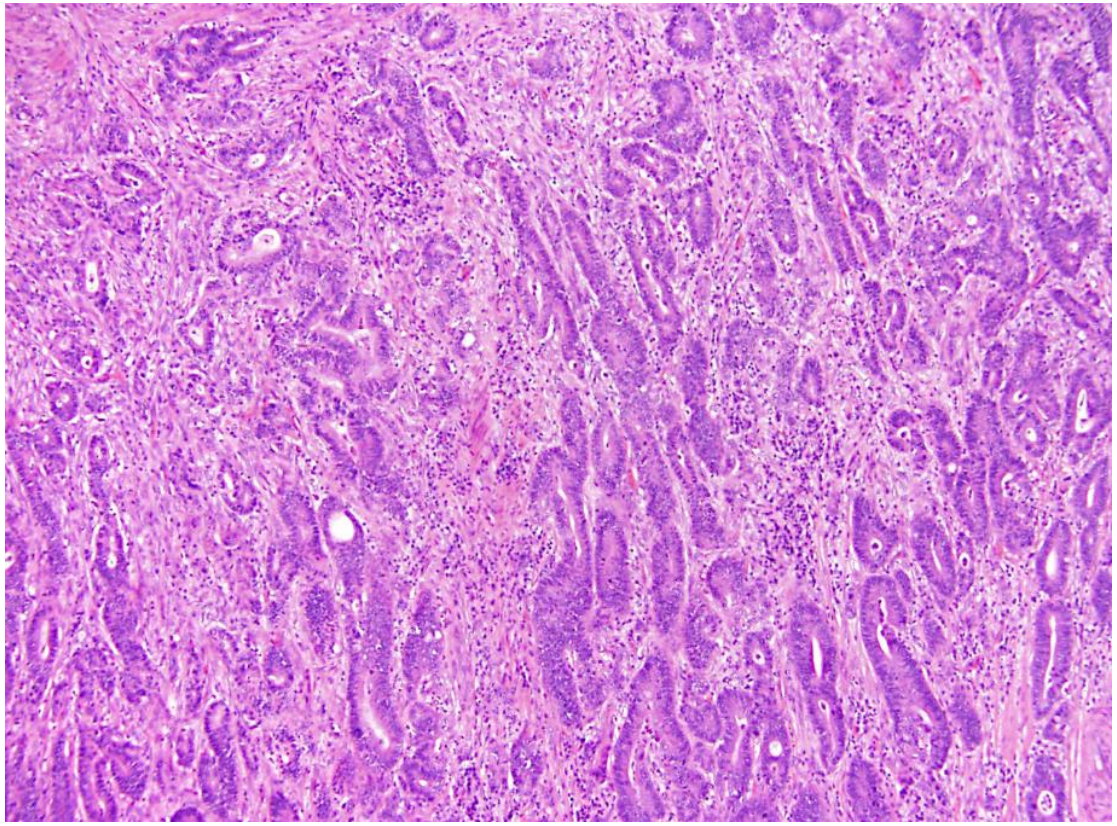

All CRCs (biopsy or resection)
IHC for MMR proteins
No loss
Likely MSS
Loss of MLH1 and PMS2
Loss of MSH2 and/or MSH6; or loss of PMS2
BRAF V600E analysis
present
absent
Sporadic MSI-high CRC
Likely Lynch syndrome; consult with Genetics

KRAS/NRAS testing: Predictive Markers

- Mutations in exons 2/3/4 of *KRAS* or *NRAS* indicate poor response to EGFR inhibitor therapy
  - Panitumumab, cetuximab
  - Same situation if *BRAF* is mutated
- Most mutations are in codons 12 or 13 of *KRAS* (exon 2)
- Current NCCN guidelines indicate this testing only for stage IV CRC
- Note: *PIK3CA* and *PTEN* mutations may occur in CRC
  - Not currently disqualifiers for EGFR inhibitors







What about MMR/MSI testing in upper GI cancers?

EBV (9%)

-PI3K, ARID1, and BCOR mutations
-JAK2 and HER2 amplification
-DNA promoter hypermethylation
-PDL1/2 overexpression
-IL-12 signaling

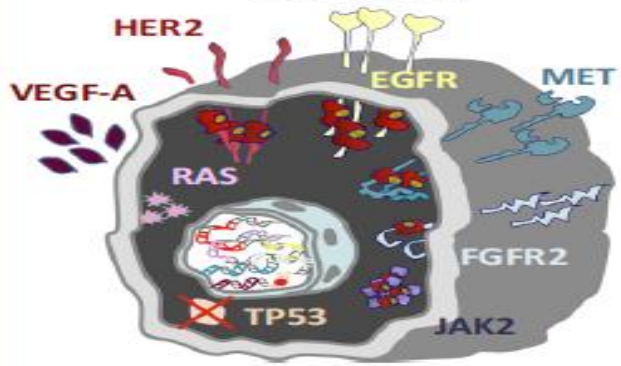
MSI (22%)

-Alterations in mismatched-repair genes (*MLH1* silencing)
-Hypermutation (mutations in PI3K, HER2, HER3, EGFR)

GS (20%)

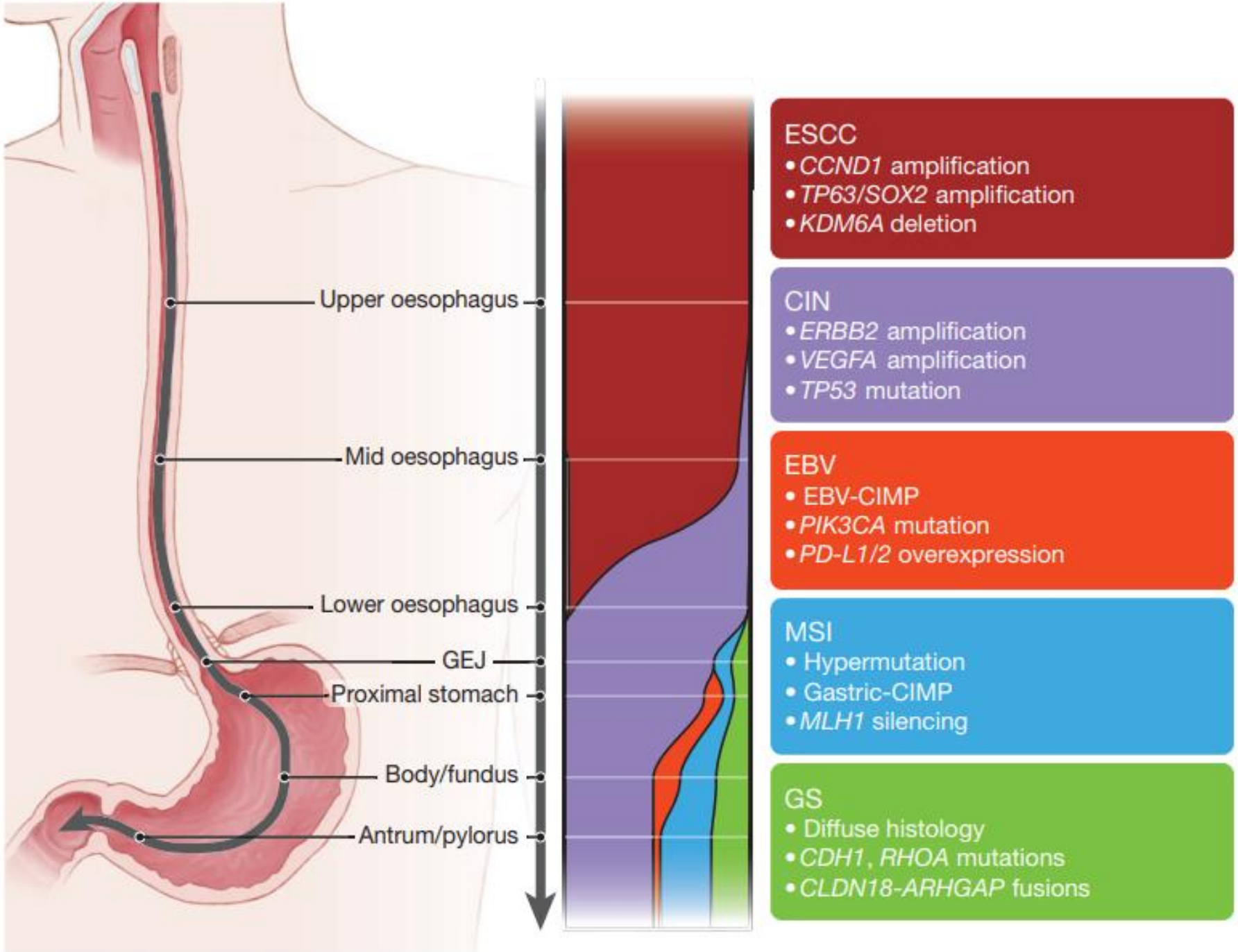
-Molecular alterations in cell adhesion/cell migration pathways (CDH1, RHOA mutations; CLDN18-ARHGAP fusion)
-ARID1, and BCOR mutations

CIN (50%)



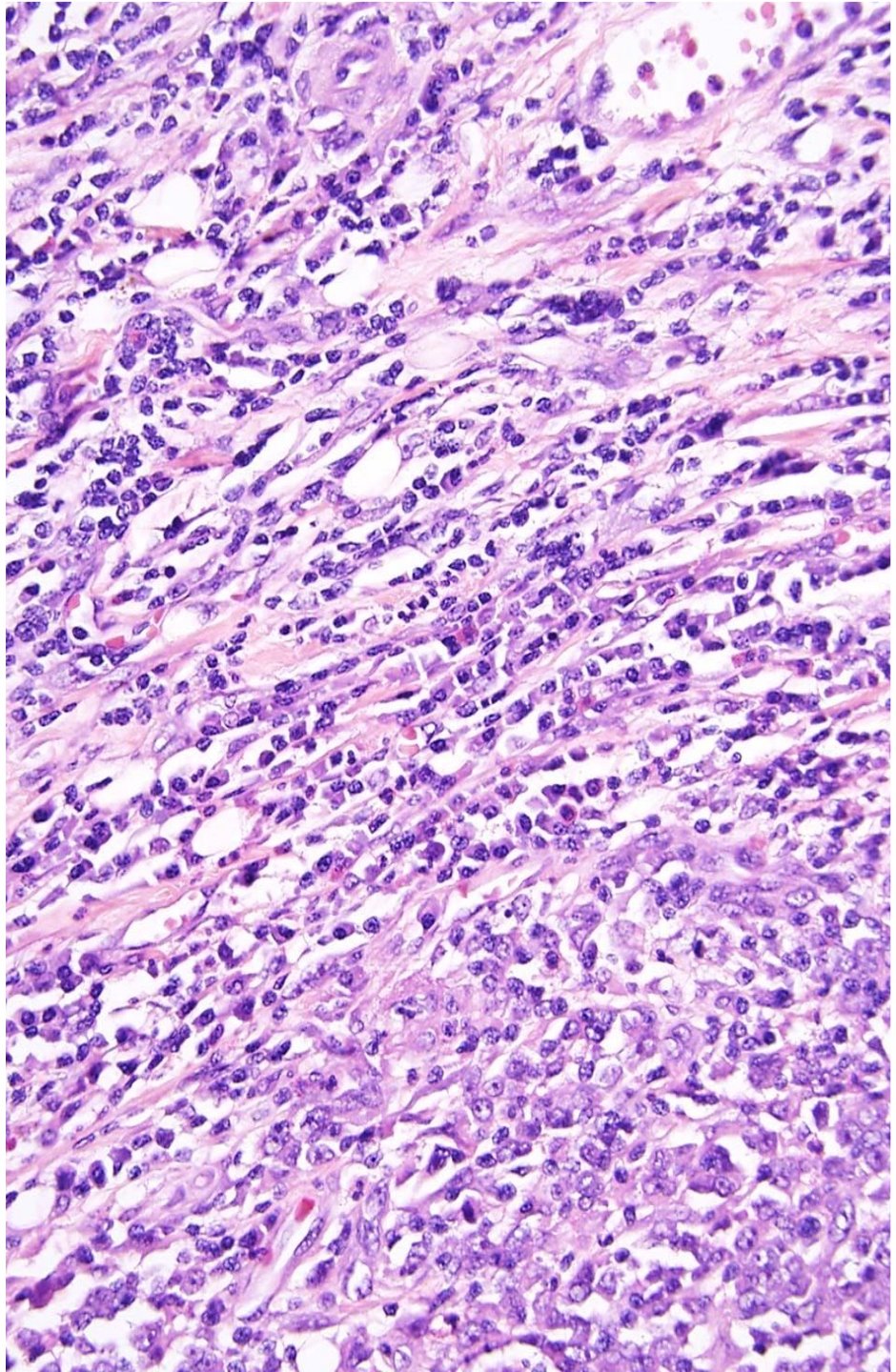
-TP53 mutations
-RTKs/RAS activation (EGFR, HER2, HER3, JAK2, FGFR2, MET, NRAS, KRAS amplification)
-VEGFA amplification
-Amplification of cell cycle mediators

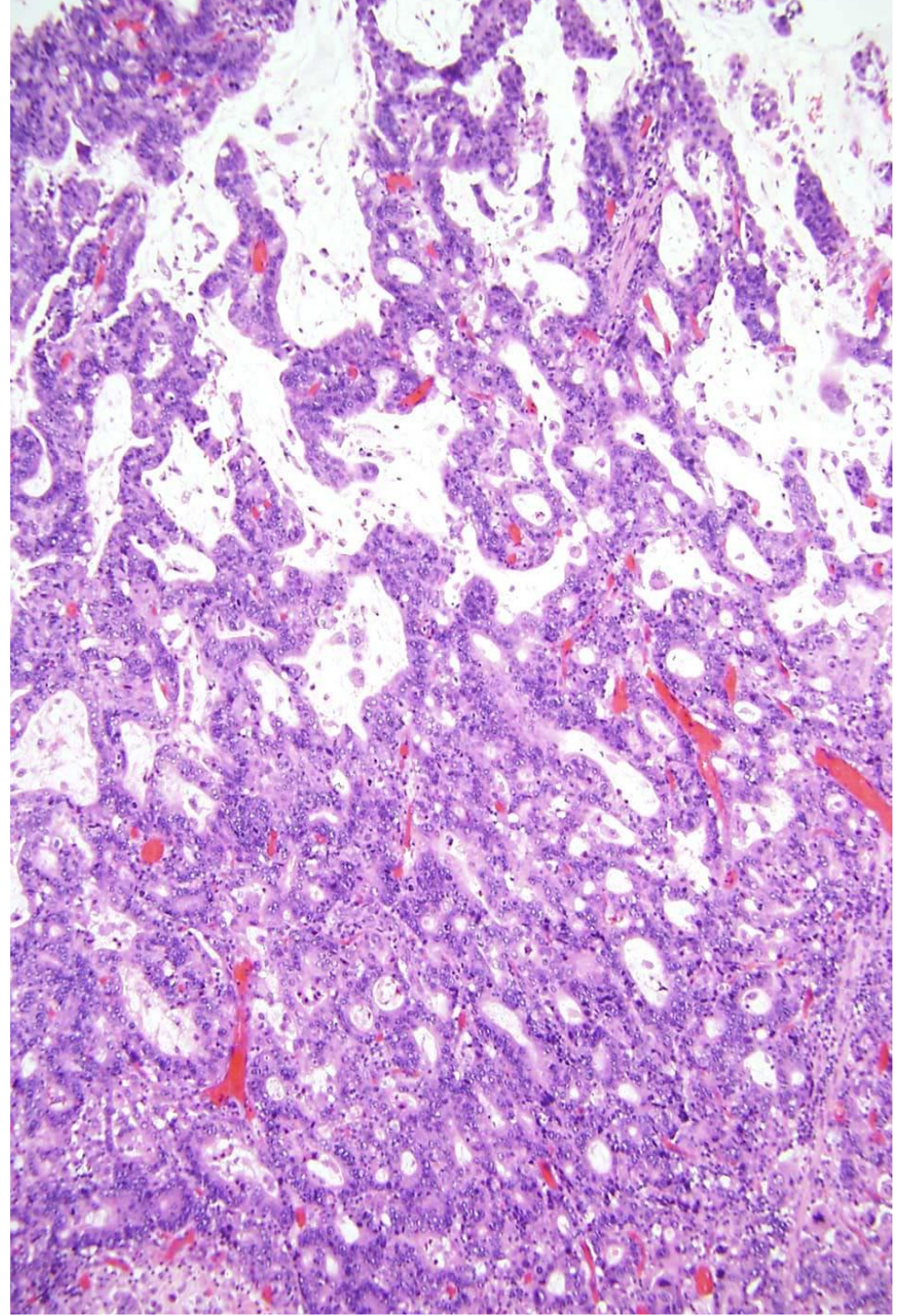
Trends in Molecular Medicine

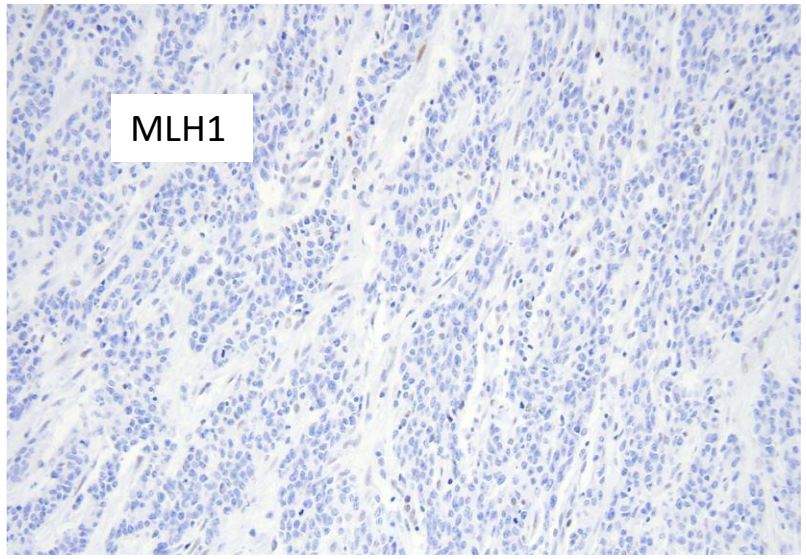

Upper oesophagus
Mid oesophagus
Lower oesophagus
GEJ
Proximal stomach
Body/fundus
Antrum/pylorus
ESCC
• CCND1 amplification
• TP63/SOX2 amplification
• KDM6A deletion
CIN
• ERBB2 amplification
• VEGFA amplification
• TP53 mutation
EBV
• EBV-CIMP
• PIK3CA mutation
• PD-L1/2 overexpression
MSI
• Hypermutation
• Gastric-CIMP
• MLH1 silencing
GS
• Diffuse histology
• CDH1, RHOA mutations
• CLDN18-ARHGAP fusions

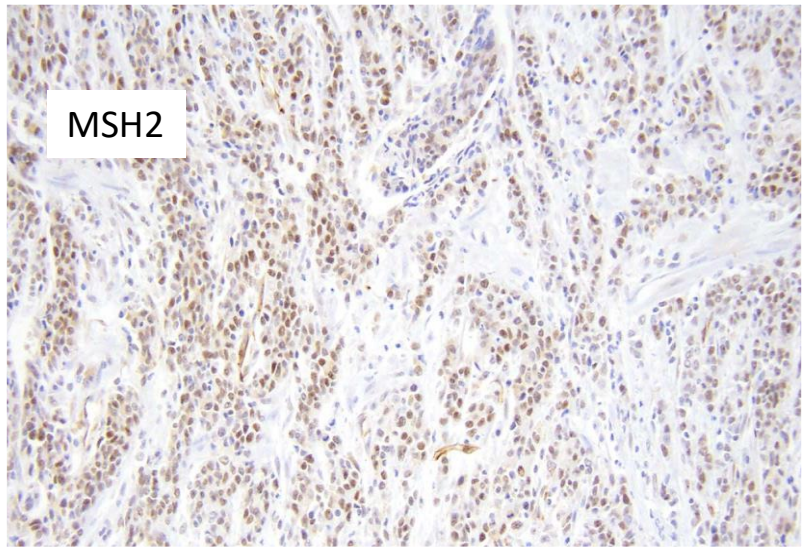
MSI-H Gastric Carcinomas

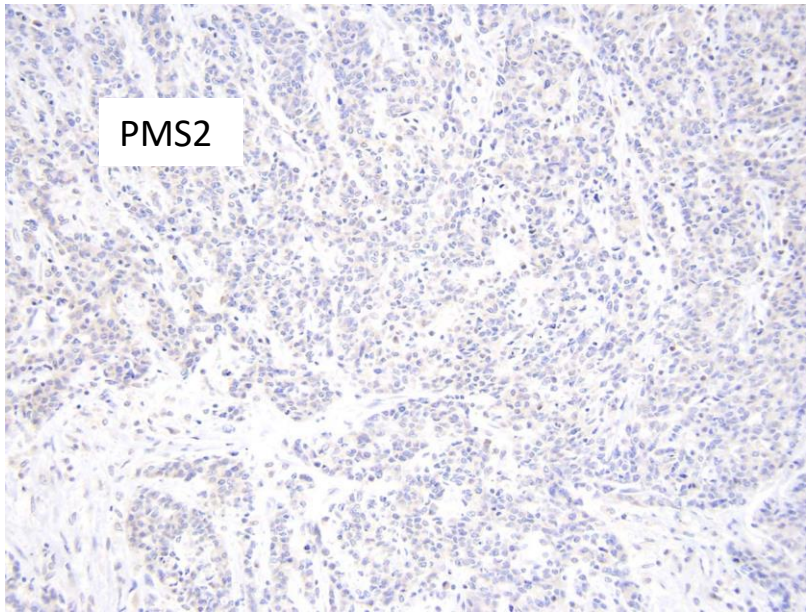
- 15-30% of gastric carcinomas
- 17% of GEJ carcinomas
- Older women, distal stomach, fewer positive lymph nodes
- High tumor mutation burden, including MHC class I genes
- Mostly hypermethylation of *MLH1* promoter.
- Better survival than genomically stable subtype but worse than EBV cancers
- Strong over-expression of PD-L1 but may not show benefits from adjuvant chemotherapy

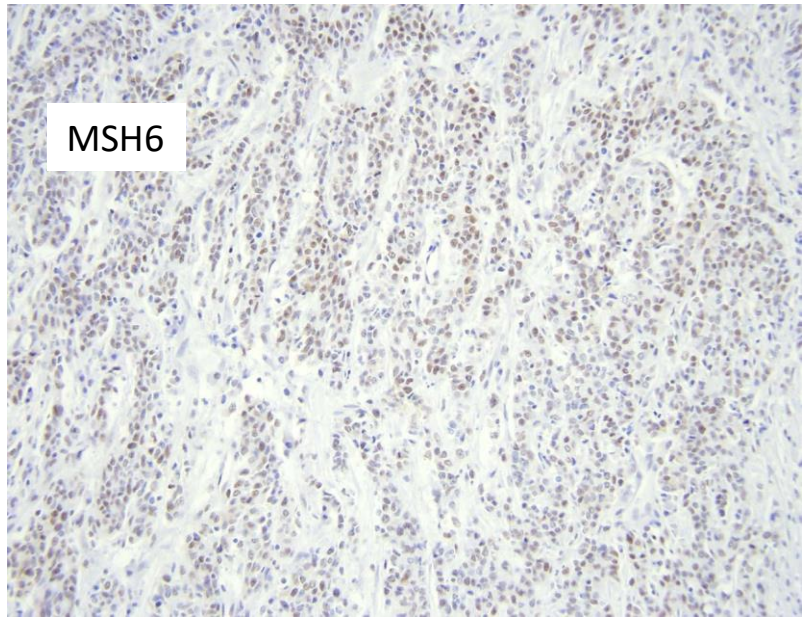





MLH1


MSH2

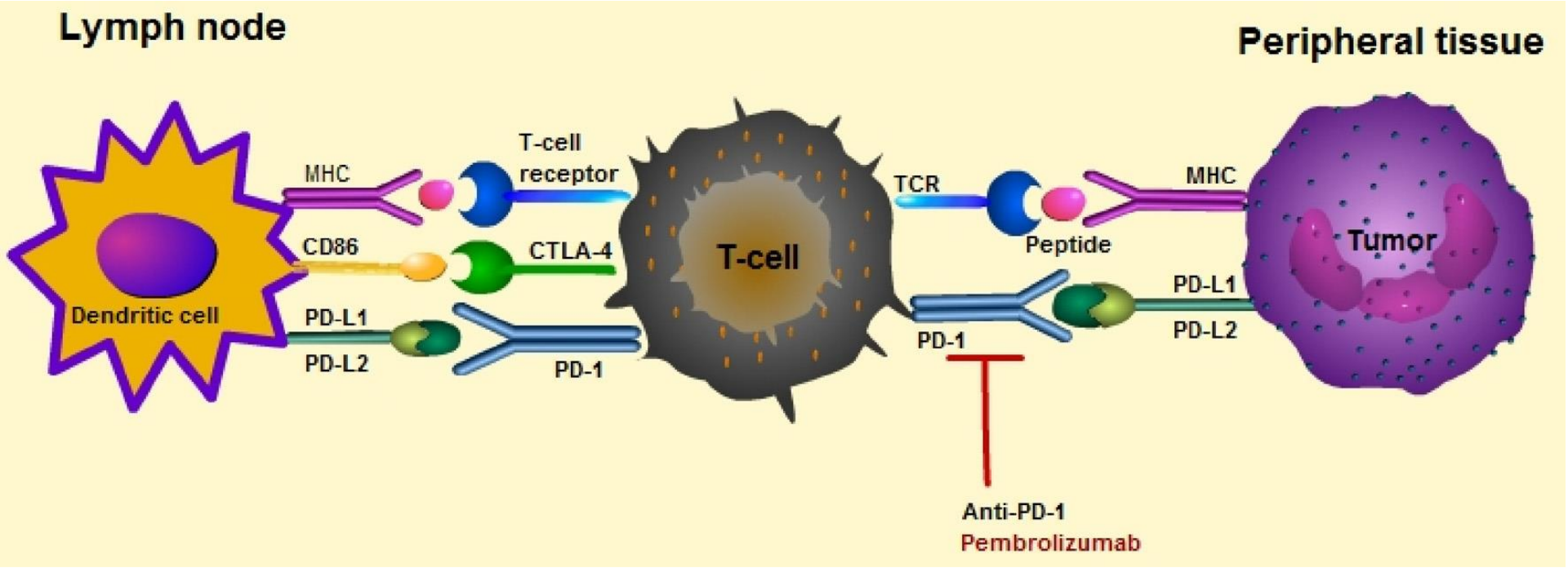

PMS2


MSH6

Current US NCCN Guidelines

- MMR or MSI testing should be done on **all newly diagnosed** gastric carcinomas
- PD-L1 testing may be considered on locally advanced, recurrent, or metastatic gastric carcinomas in patients who are candidates for treatment with PD-1 inhibitors.
  - Combined positive score of at least 1 is considered PD-L1 positive.
  - An FDA-approved companion diagnostic is available.
- PD-L1 is positive in about 50% of gastric cancers

PD



- PD-1 is an immune checkpoint receptor regulating T cell function in immunity and tolerance; inhibits cytolytic activity of T cells.
- 2 ligands, PL-L1 and PD-L2, expressed in some solid tumors.
- Tumor cells can escape immune surveillance.
- Pembrolizumab, monoclonal antibody directed against PD-1, has shown activity in gastric cancer.

US FDA approved for Gastric or GEJ ACA: PD-L1 IHC 22C3 pharmDx

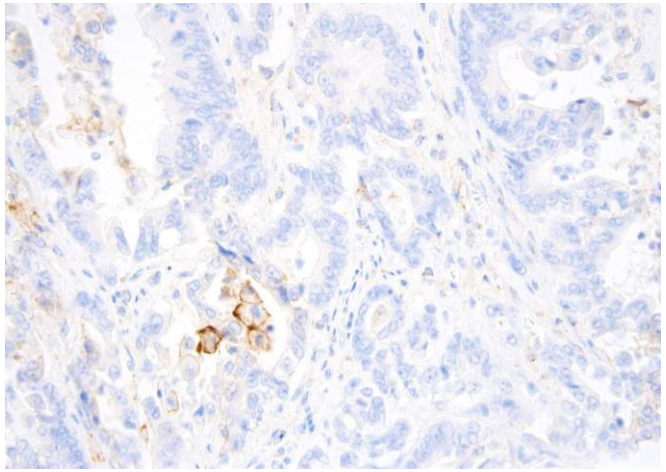
- Clinical trials of pembrolizumab used 22C3 clone
- Some trials of other PD-1 and PD-L1 inhibitors used SP142 clone

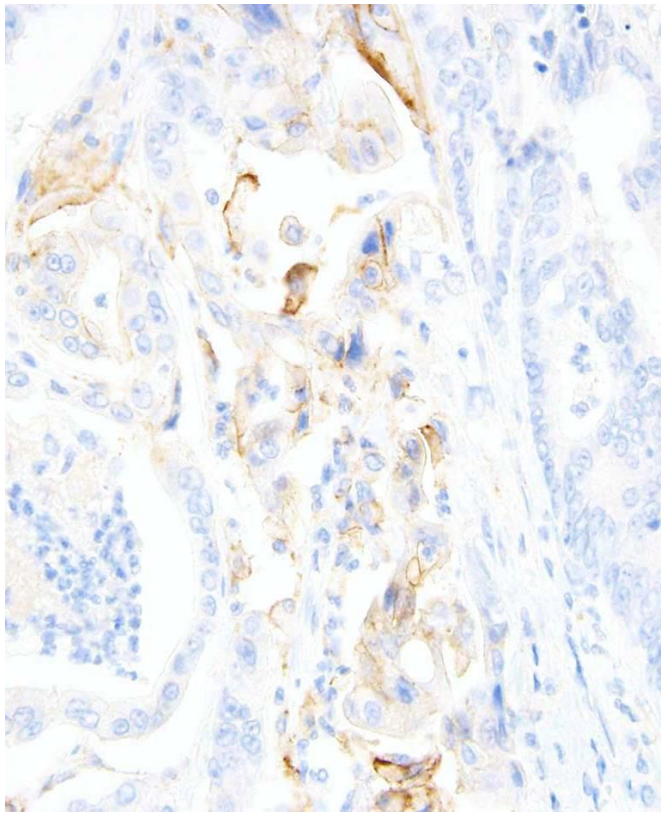
Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma

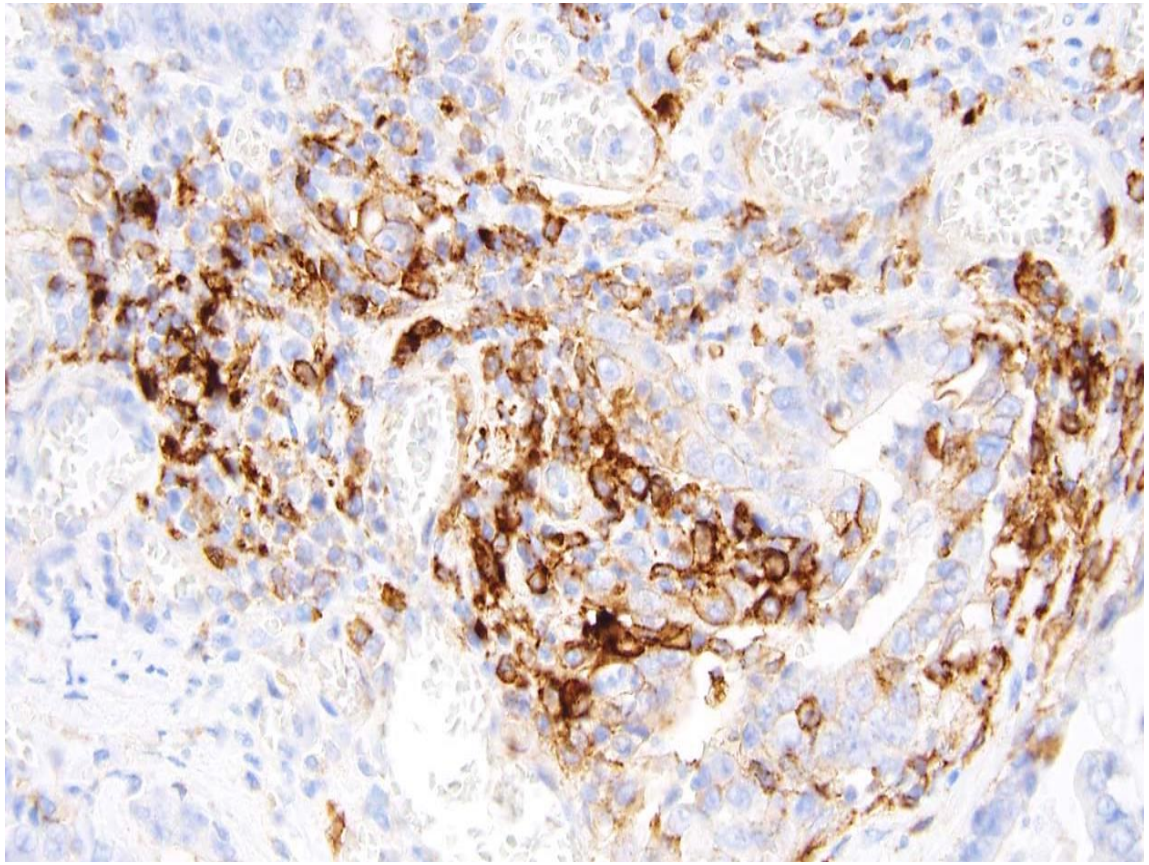
PD-L1 protein expression in gastric or GEJ adenocarcinoma is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS ≥ 1. PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with KEYTRUDA® (pembrolizumab).

Gastric ACA and PD-L1

- Asian/non-Asian cases have different CD68/CD3 ratios; less favorable outcome for non-Asian ACA
- PD-L1 is expressed in tumor and stroma across all stages and histologies; slightly more common in HER2 negative cases.
- OR for EBV cases and PD-L1 expression is 15.50; for MSI-H, 6.09.
- *H. pylori* induces increased PD-L1 expression in gastric mucosa.







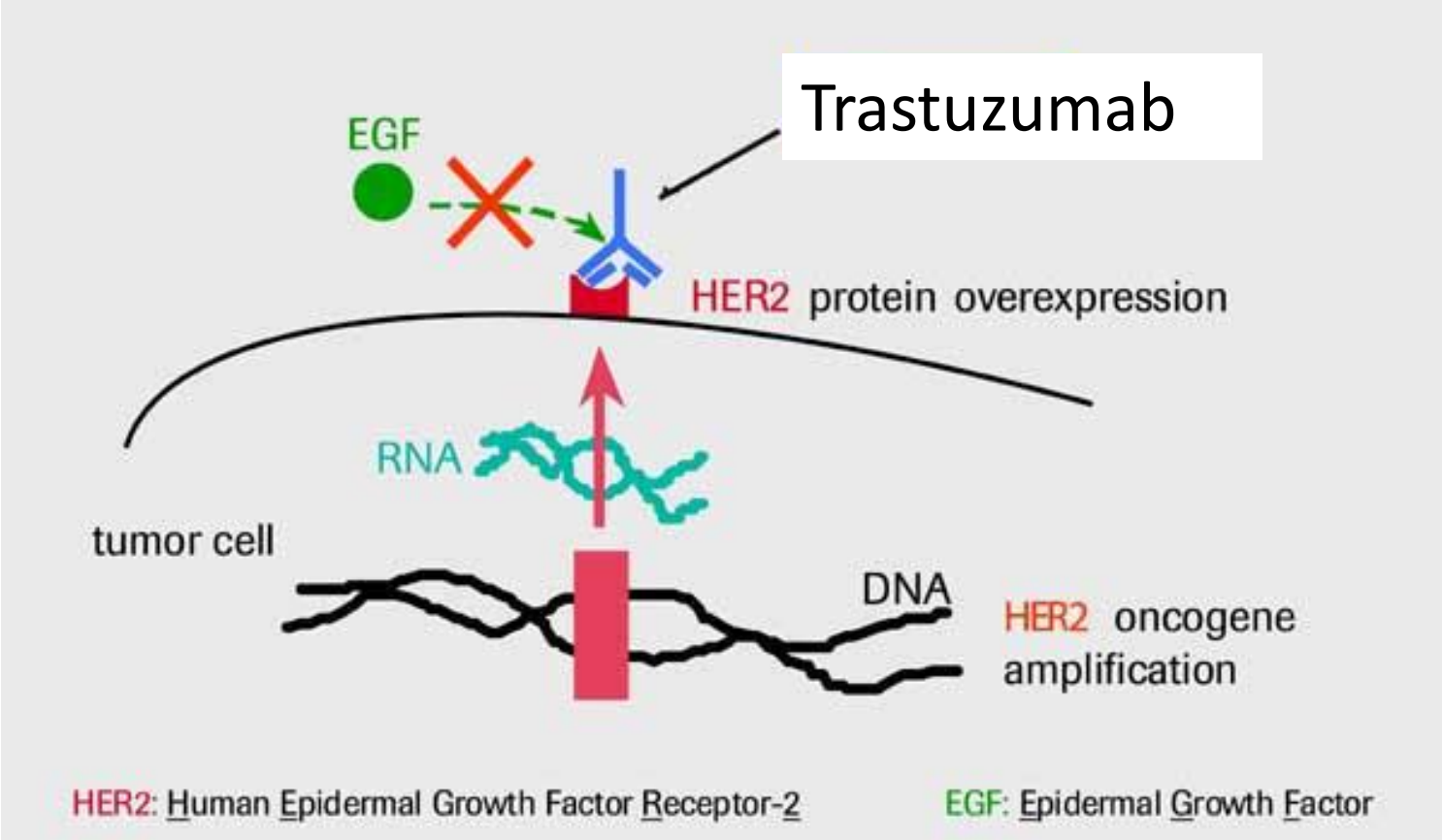
PD-L1 in Gastric Adenocarcinoma

PD-L1 in Colorectal Cancer

- No mandate to perform PD-L1 testing, as checkpoint inhibitors may be used to treat MSI-H/MMR-D tumors
- A subset of CRC show strong to moderate PD-L1 expression, depending on case selection
- Little data on response to treatment of PD-L1 +, MSS tumors- some studies suggest little response
- Interaction of MS status, PD-L1 expression, CD8+ tumor infiltrating lymphocytes is unclear

HER2 Testing in GI Cancers

- Established in gastric and esophageal adenocarcinomas
- Emerging in colorectal carcinoma



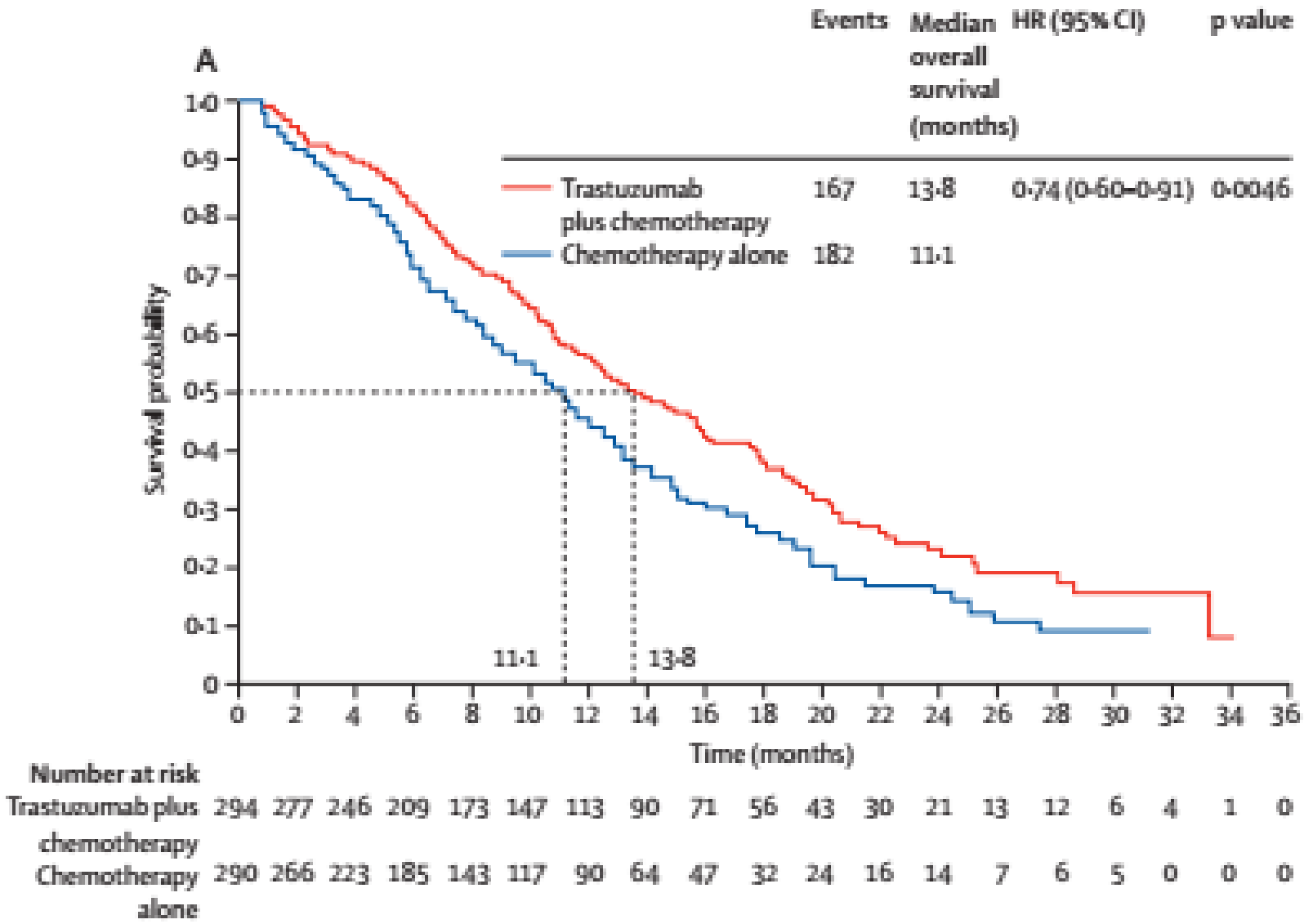
THE LANCET

Volume 376, Issue 9742, 28 August–3 September 2010, Pages 687–697

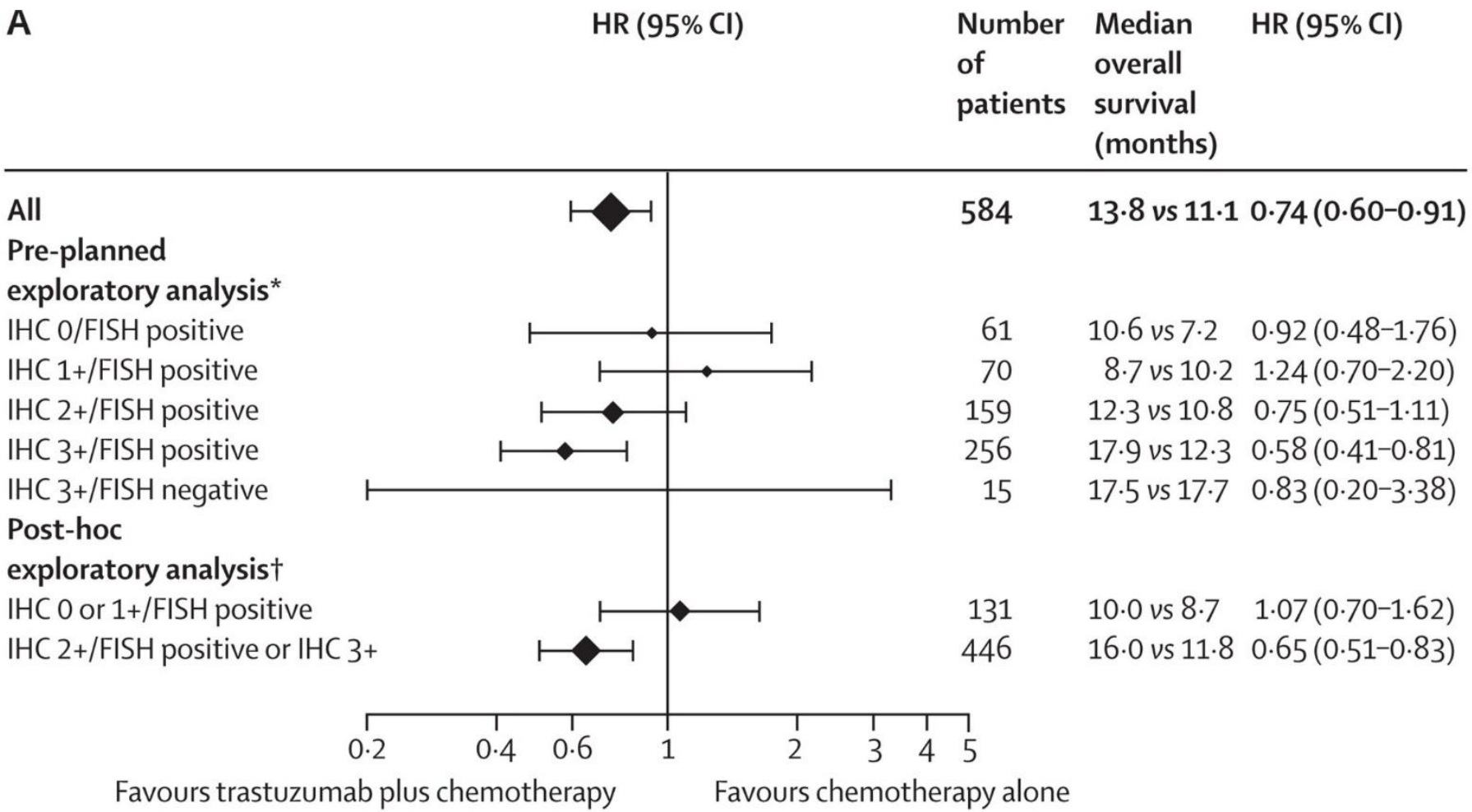
Articles

Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial

Prof Yung-Jue Bang, MD[a, *], , , Prof Eric Van Cutsem, MD[b, *], Andrea Feyereislova, MD[c], Prof Hyun C Chung, MD[d], Prof Lin Shen, MD[e], Akira Sawaki, MD[f], Florian Lordick, MD[g], Atsushi Ohtsu, MD[h], Yasushi Omuro, MD[i], Taroh Satoh, MD[j], Giuseppe Aprile, MD[k], Evgeny Kulikov, MD[l], Julie Hill, PhD[m], Michaela Lehle, PhD[c], Prof Josef Rüschoff, MD[n], Prof Yoon-Koo Kang, MD[o], for the ToGA Trial Investigators[†],



Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376(9742):687-697.



Source: Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376(9742):687-697.

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JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Arch Pathol Lab Med—Vol 140, December 2016

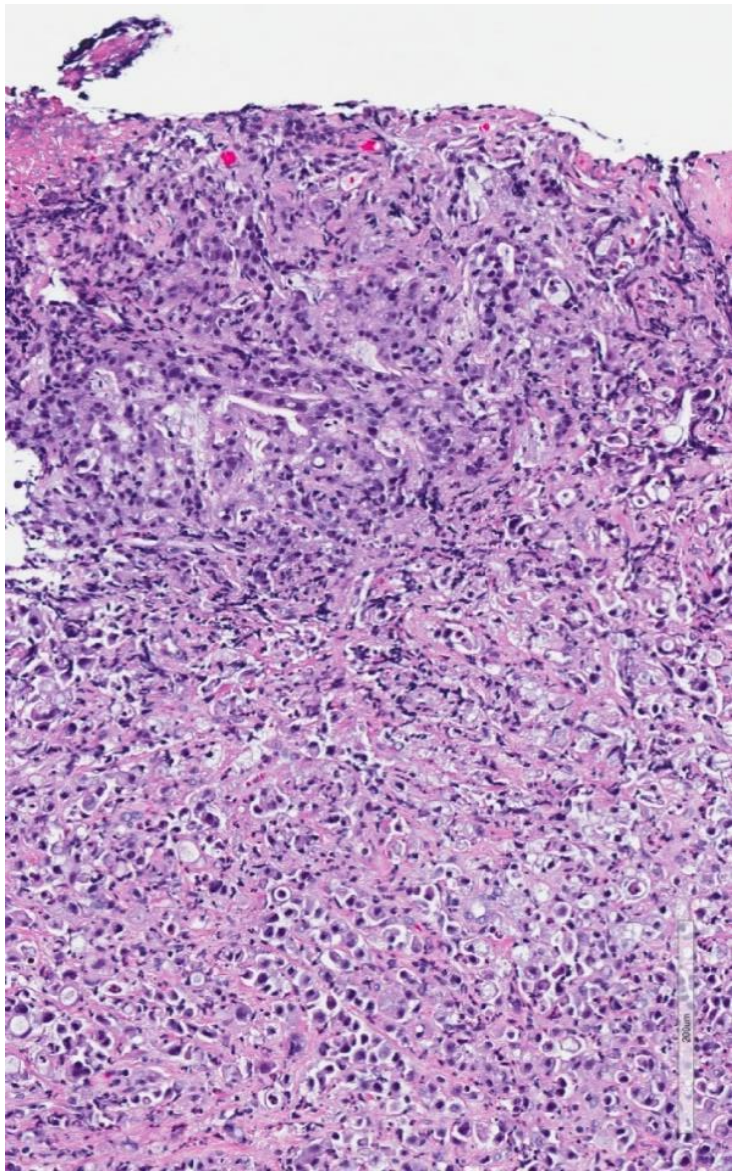
Am J Clin Pathol December 2016;146:647-669

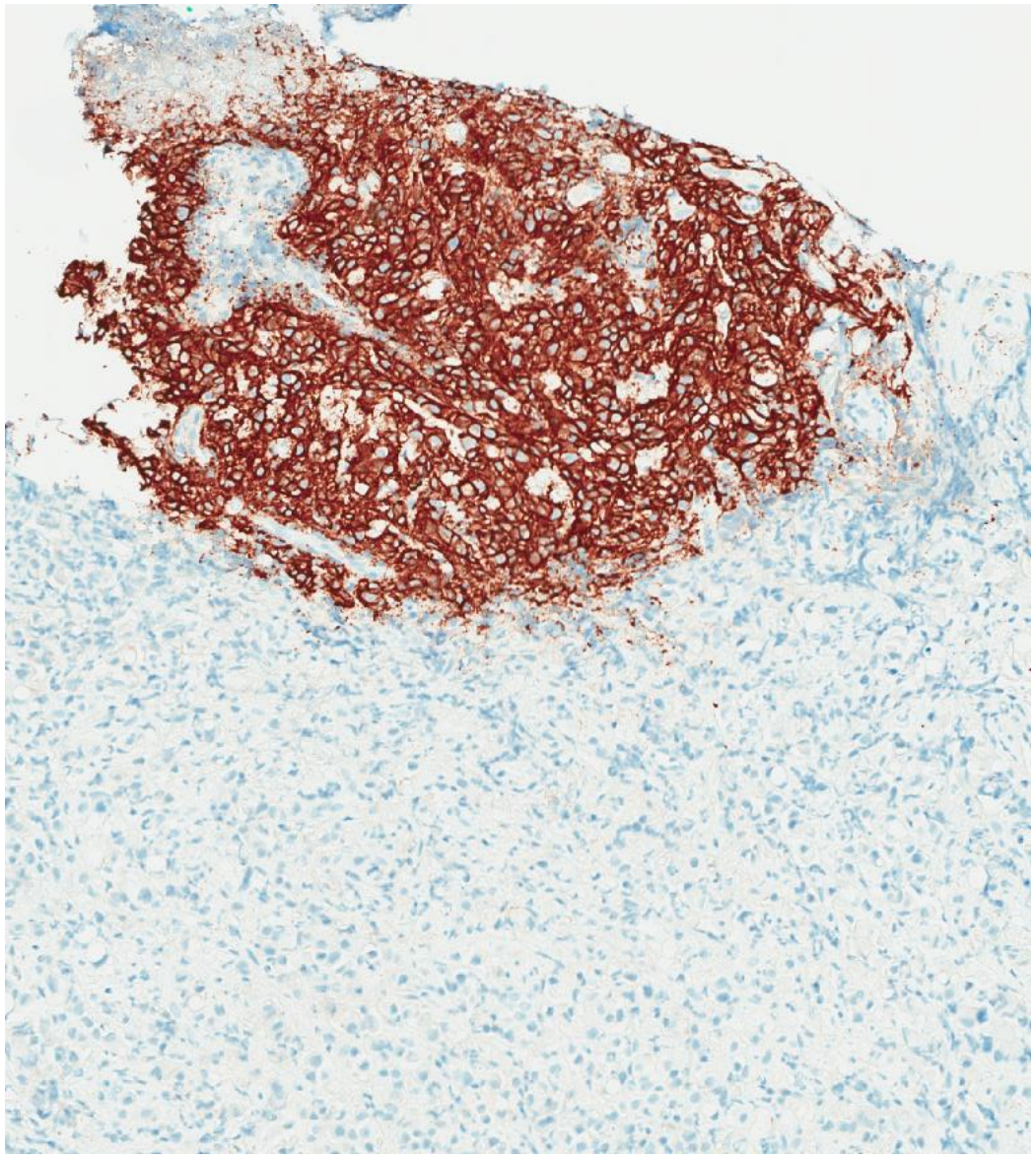
HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma

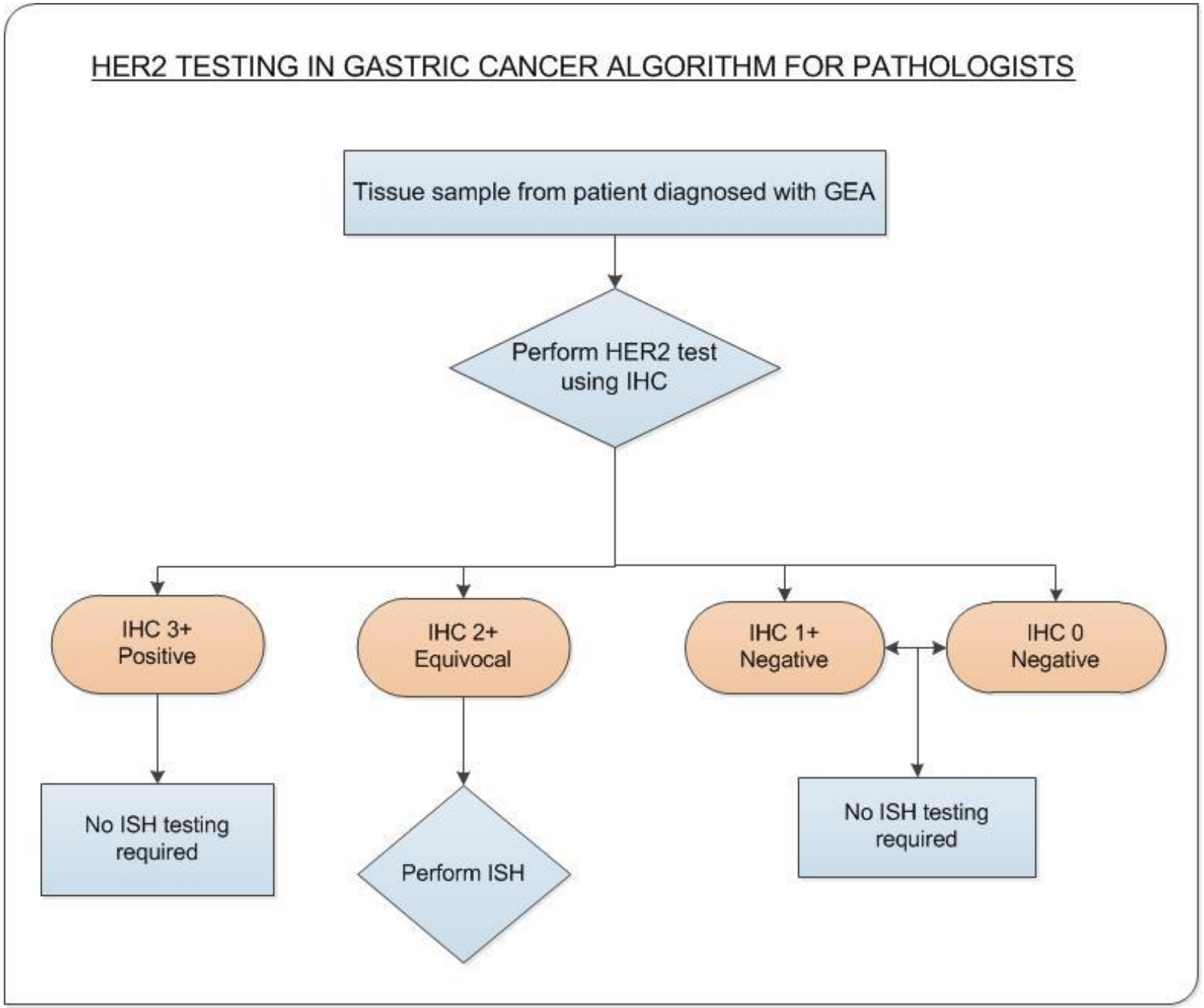
Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology

Angela N. Bartley, MD; Mary Kay Washington, MD, PhD; Christina B. Ventura, MT(ASCP); Nofisat Ismaila, MD; Carol Colasacco, MLIS, SCT(ASCP); Al B. Benson III, MD; Alfredo Carrato, MD, PhD; Margaret L. Gulley, MD; Dhanpat Jain, MD; Sanjay Kakar, MD; Helen J. Mackay, MBChB, MD; Catherine Streutker, MD; Laura Tang, MD, PhD; Megan Troxell, MD, PhD; Jaffer A. Ajani, MD

HER2 expression by IHC






HER2 TESTING IN GASTRIC CANCER ALGORITHM FOR PATHOLOGISTS
Tissue sample from patient diagnosed with GEA
Perform HER2 test using IHC
IHC 3+ Positive
IHC 2+ Equivocal
IHC 1+ Negative
IHC 0 Negative
No ISH testing required
Perform ISH
No ISH testing required

Basis for Recommendation of IHC

- Patients with amplification by ISH without overexpression by IHC did not benefit from trastuzumab in ToGA
- Benefit of therapy appears to correlate with protein overexpression
- No need to test 0 and 1+ IHC with ISH (amplification rate is low (14-24%))
- IHC 3+ and ISH positivity concordance is high (>90%)

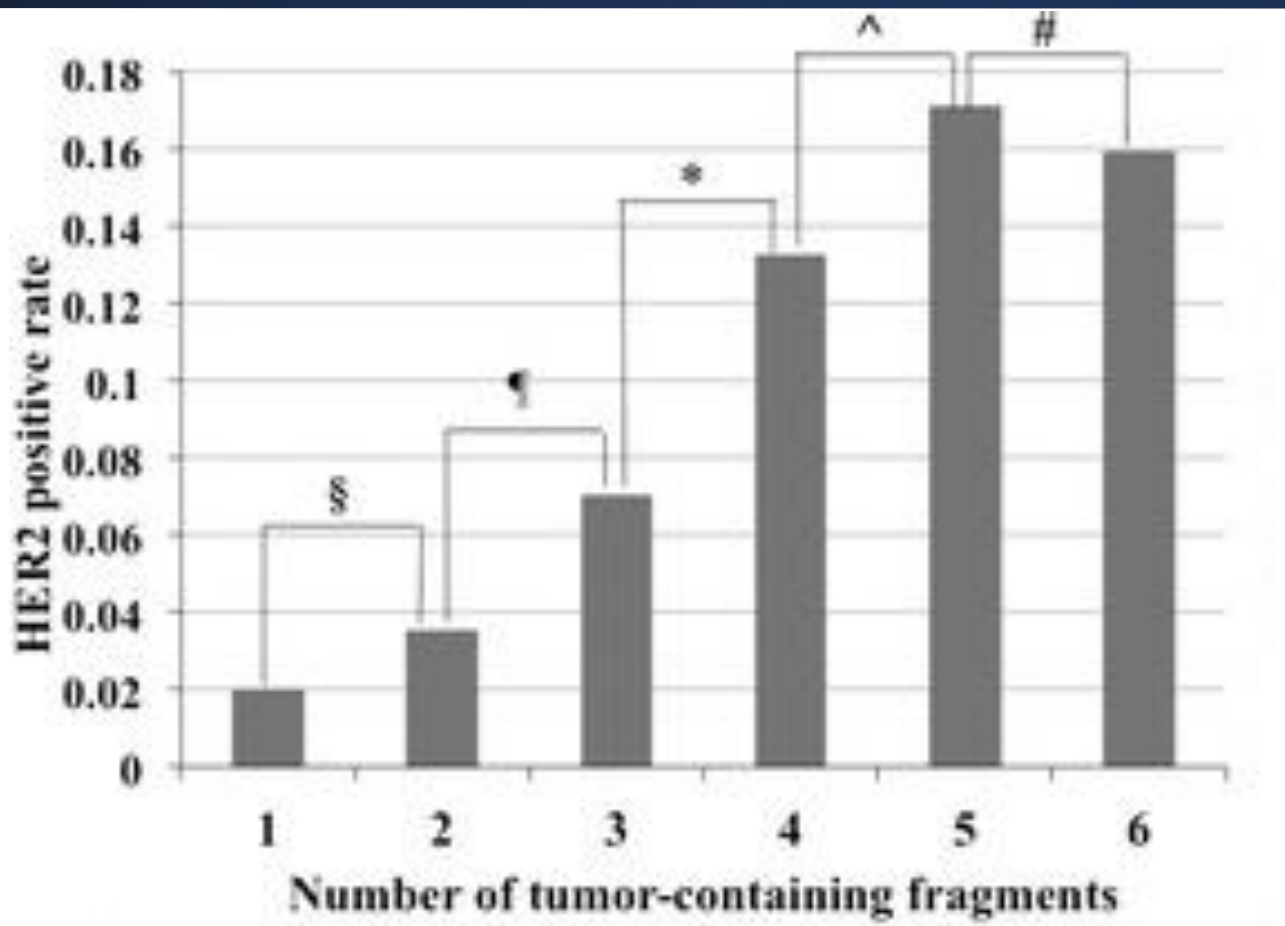
Who should be tested?

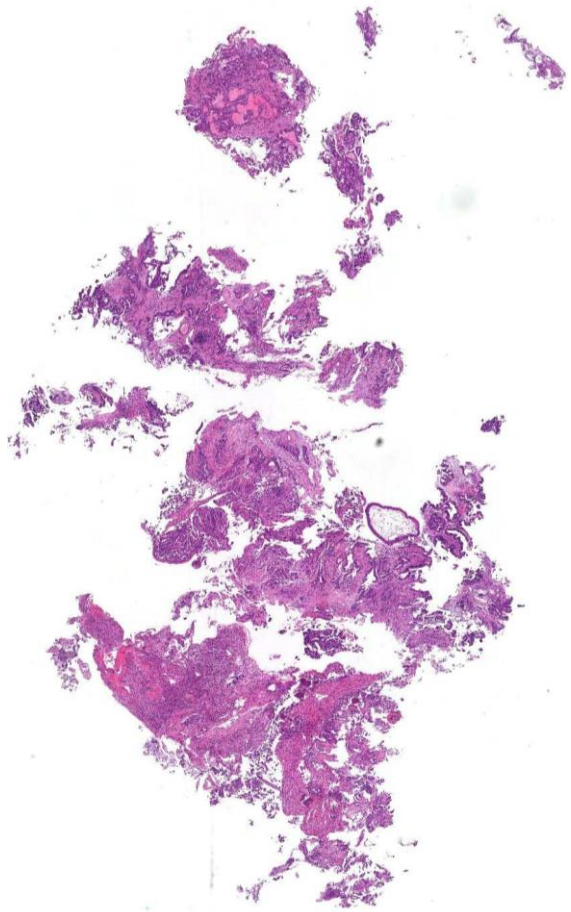
- Tumors from patients who have locally advanced, metastatic, or recurrent gastric, esophageal, or GEJ adenocarcinomas
- Reflex versus oncologist-requested test depends on local needs

What tissue should be tested?

- Choose best block
  - >90% concordance between biopsy and resection, and primary versus metastasis
  - At least 5 biopsy fragments, preferably 6 to 8 to overcome tumor heterogeneity
- Intestinal subtype more likely to be positive
- Select better differentiated tumor areas, or if highly heterogeneous, consider testing more than one block

3+ IHC Rate Correlates with Number of Biopsies





Xu C, et al. Diagnostic Pathology 12(1):41, 2017.

HER2 and Histologic Subtype

Site	% HER2 +
Esophagus/GEJ	32%
Stomach	21%

Histologic Type	% HER2 +
Intestinal	~ 25%
Diffuse	0-6%
Mixed	0-20%
Rare subtypes	Limited data (hepatoid type frequently positive)

HER2 and Grade

Grade	% HER2 +
Low grade	15%-45%
High grade	6% to 28%

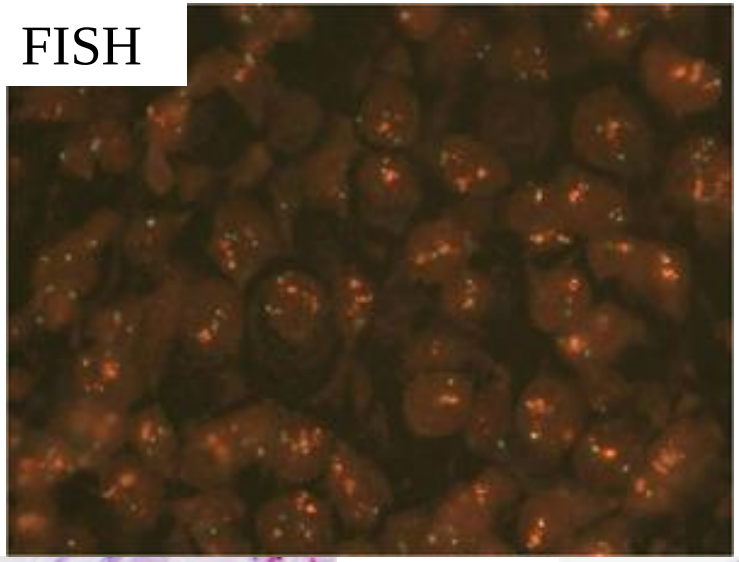
Recommendation: Select the block with the lowest grade tumor morphology. More than one tissue block may be selected if different morphologic patterns are present.

- Most studies do not specify grading criteria
- Select better differentiated tumor areas, or if highly heterogeneous, consider testing more than one block

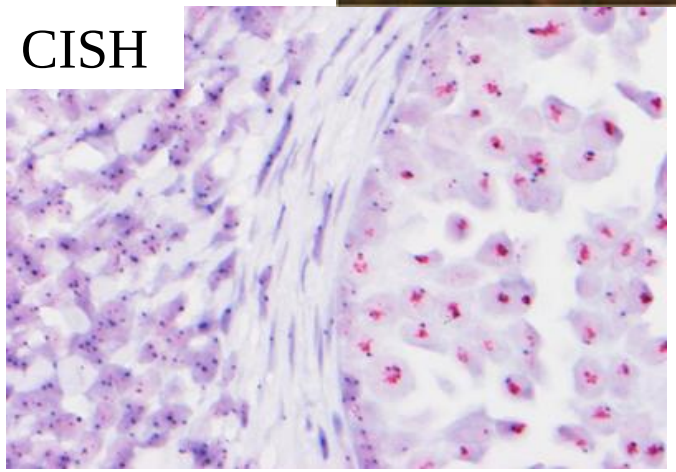
IHC 2+ cases should be tested with ISH

- 30-50% of IHC 2+ cases will show amplification (considered eligible for treatment)
- If there is uncertainty over whether score is 1+ or 2+, consider ISH (goal is to avoid false negatives)

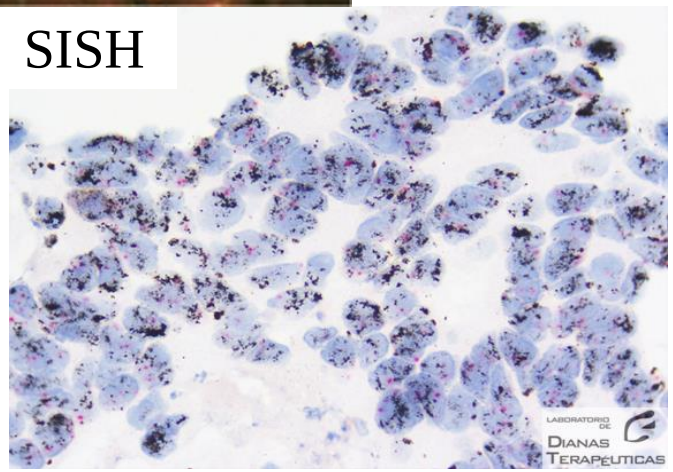
FISH



CISH



SISH



DAKO

Antibody Options

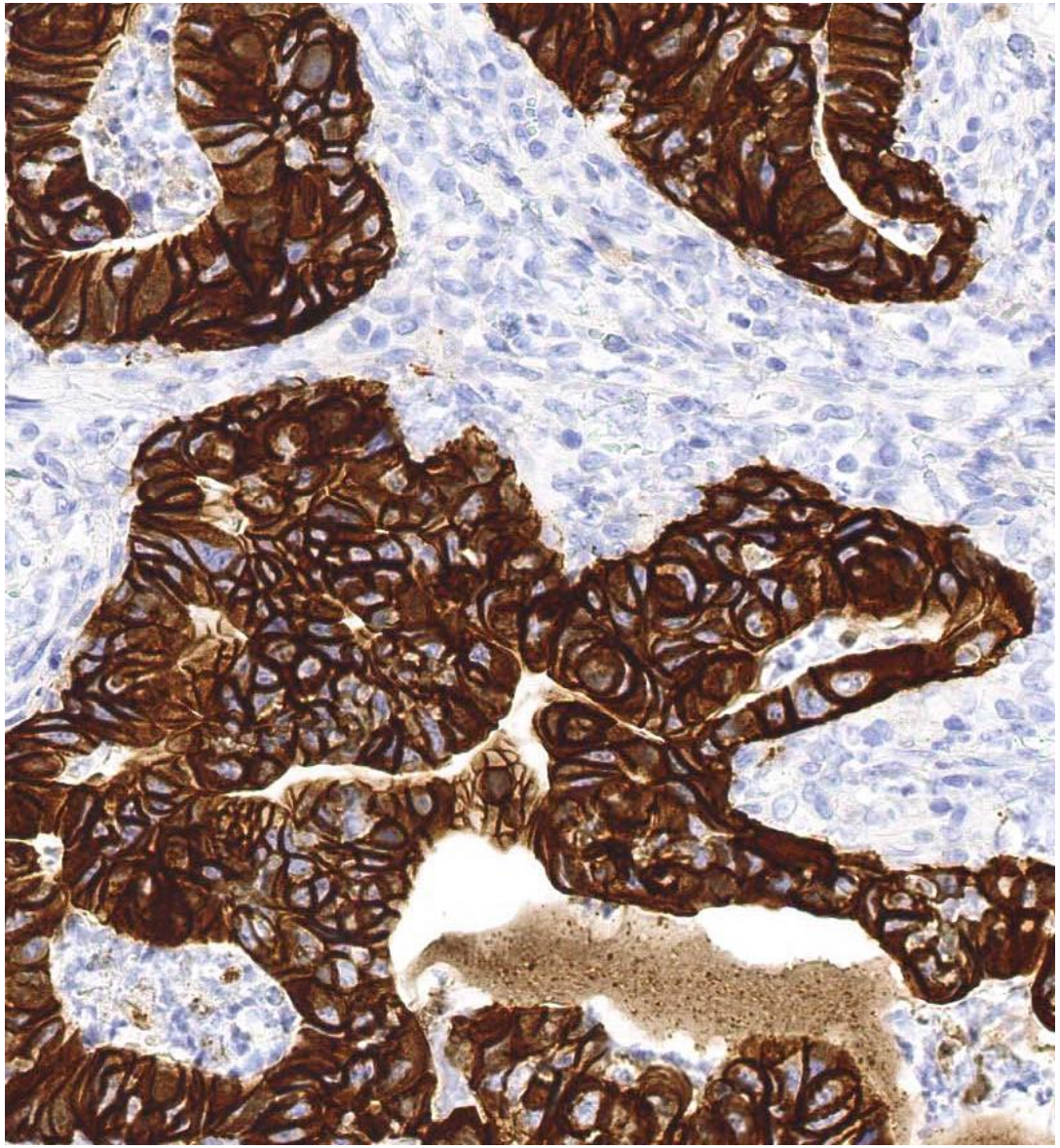
- Multiple antibodies available
  - ToGA trial used HercepTest
  - Many studies have used Ventana 4B5 or Thermo Fisher Scientific CB11
  - Others are available
- Generally moderate to good concordance among antibodies
- No specific recommendation

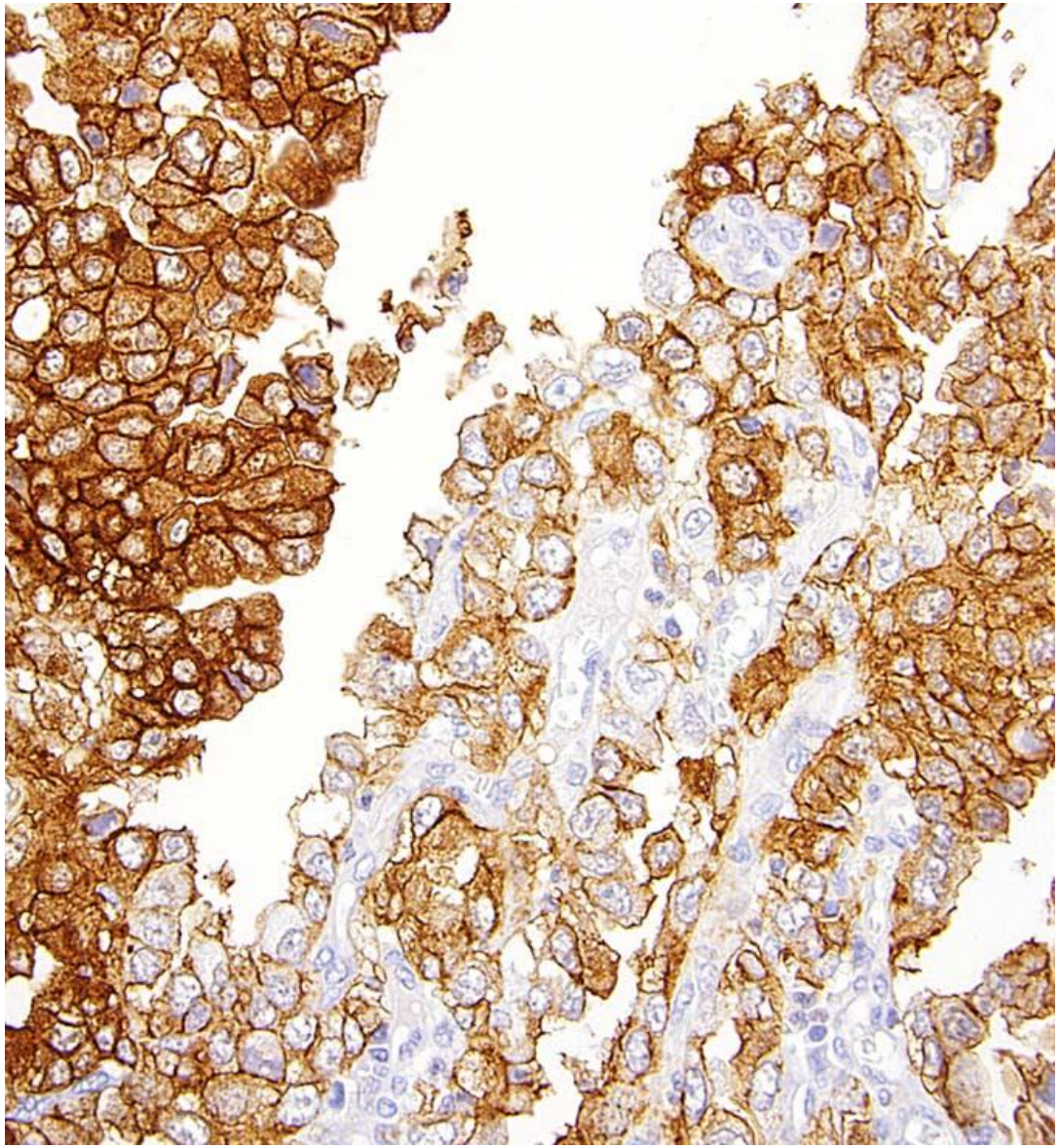
Score	Whole-tissue sections		
	HercepTest	4B5	SP3
0	179 (90.9)	125 (63.1)	128 (65.2)
1+	7 (3.5)	30 (15.2)	17 (8.5)
2+	1 (0.5)	20 (10.1)	34 (17.2)
3+	10 (5.1)	23 (11.6)	18 (9.1)
Total	197 (100.0)	198 (100.0)	197 (100.0)

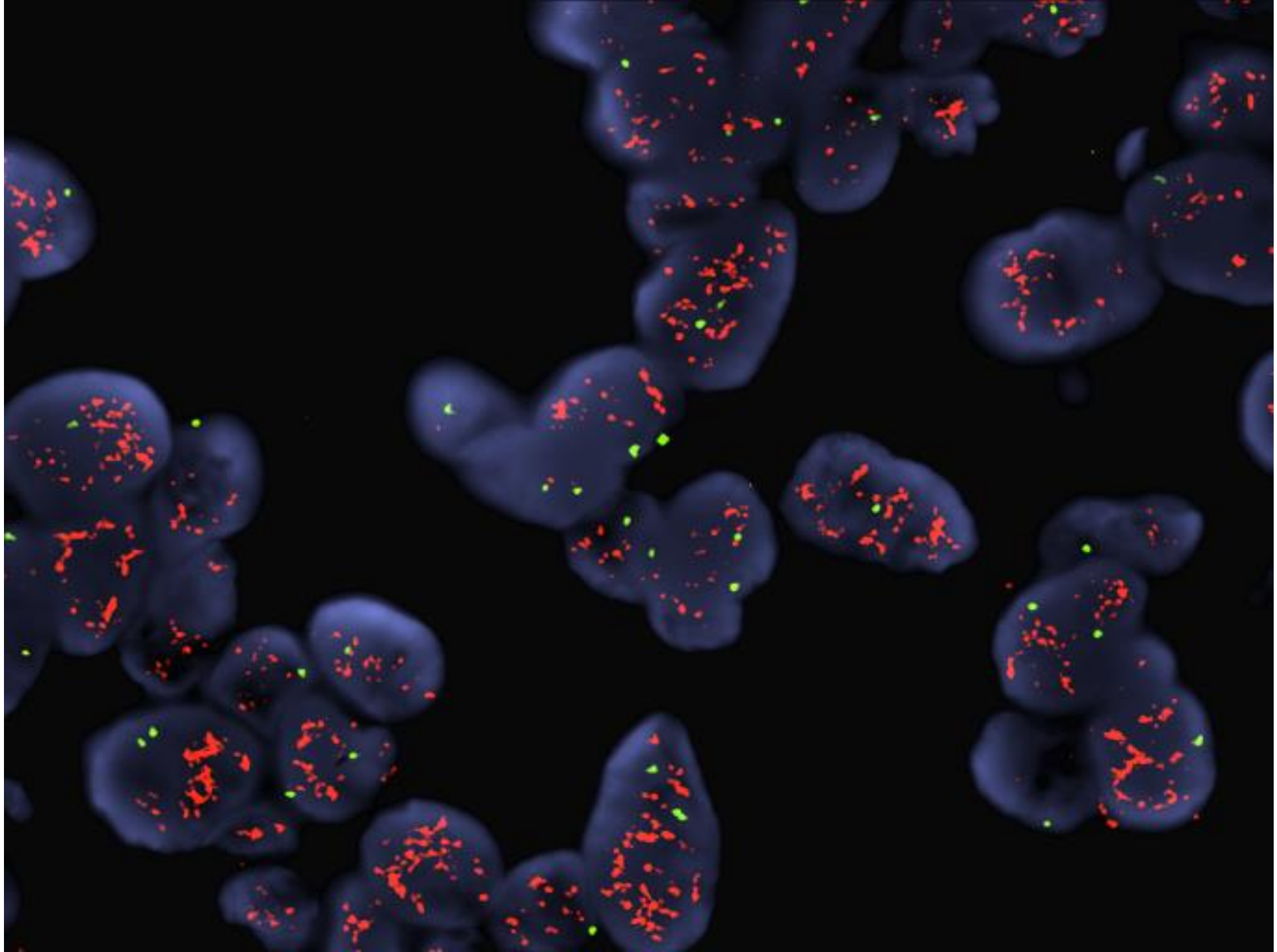
Score	HercepTest	4B5	SP3
0 or 1+	94.5%	78.3%	73.7%
2+	0.5%	10.1%	17.2%
3+	5.1%	11.6%	9.1%

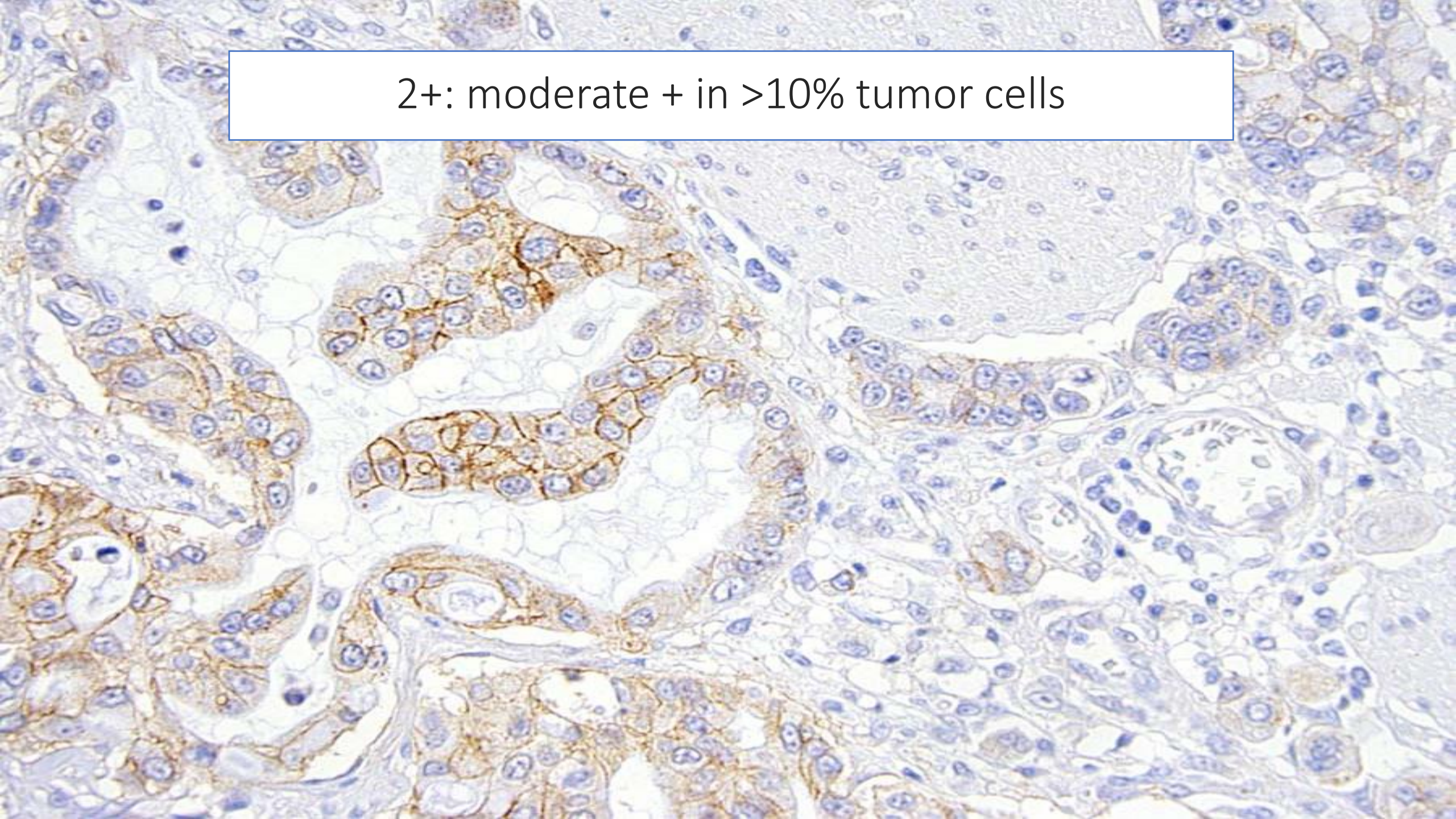
Source: Abrahao-Machado LF, Jacome AA, Wohnrath DR, et al. HER2 in gastric cancer: comparative analysis of three different antibodies using whole-tissue sections and tissue microarrays. World J Gastroenterol. 2013;19(38):6438-6446.

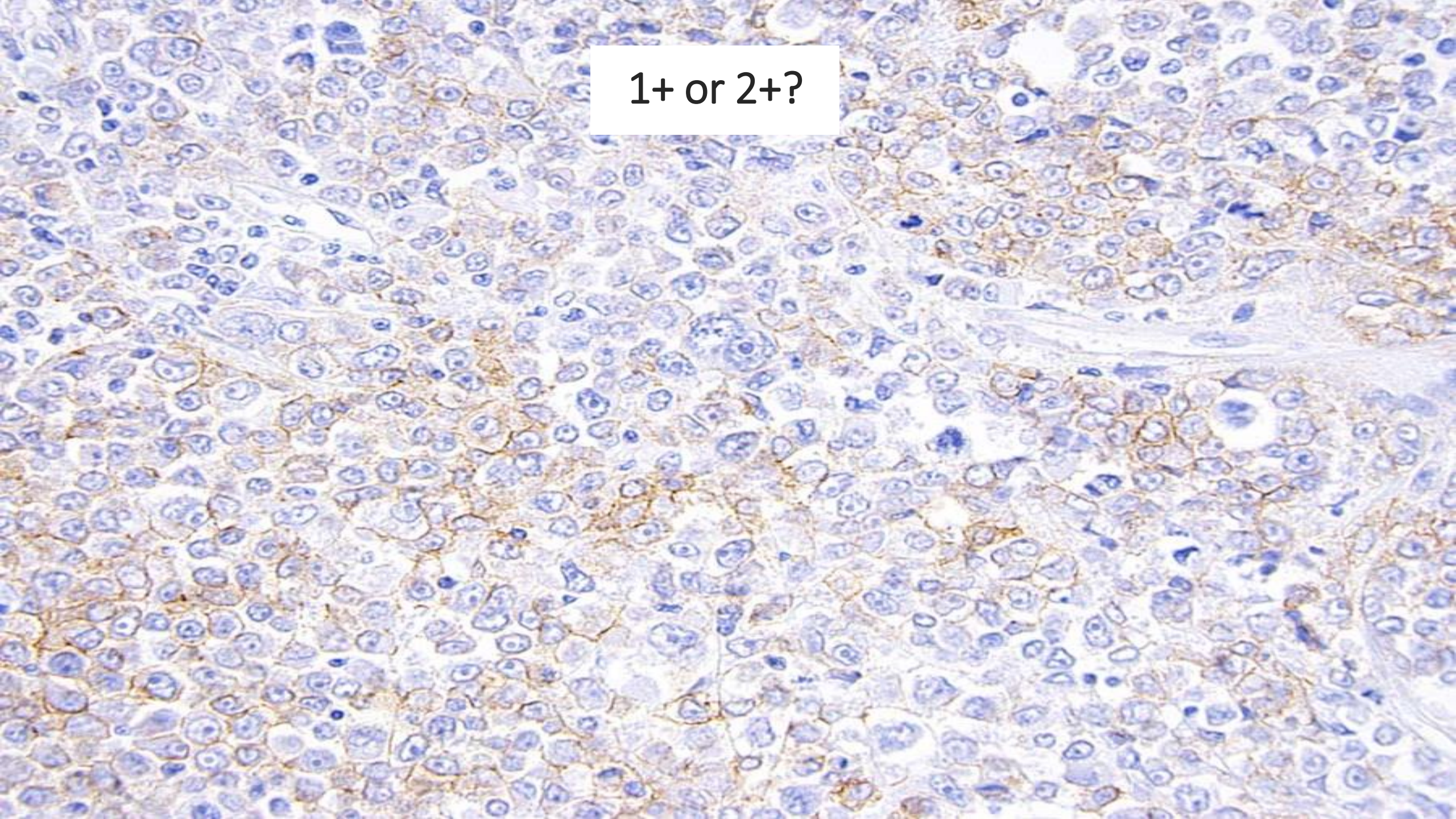
HER2 IHC Score	HER2 IHC Pattern in Surgical Specimen	HER2 IHC Pattern in Biopsy Specimen	HER2 Expression Assessment
0	No reactivity or membranous reactivity in <10% of cancer cells	No reactivity or no membranous reactivity in any cancer cell	Negative by IHC
1+	Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are reactive only in part of their membrane	Cancer cell cluster* with a faint or barely perceptible membranous reactivity irrespective of percentage of cancer cells positive	Negative by IHC
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in >10% of tumor cells	Cancer cell cluster* with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Equivocal by IHC
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of cancer cells	Cancer cell cluster* with a strong complete basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Positive by IHC

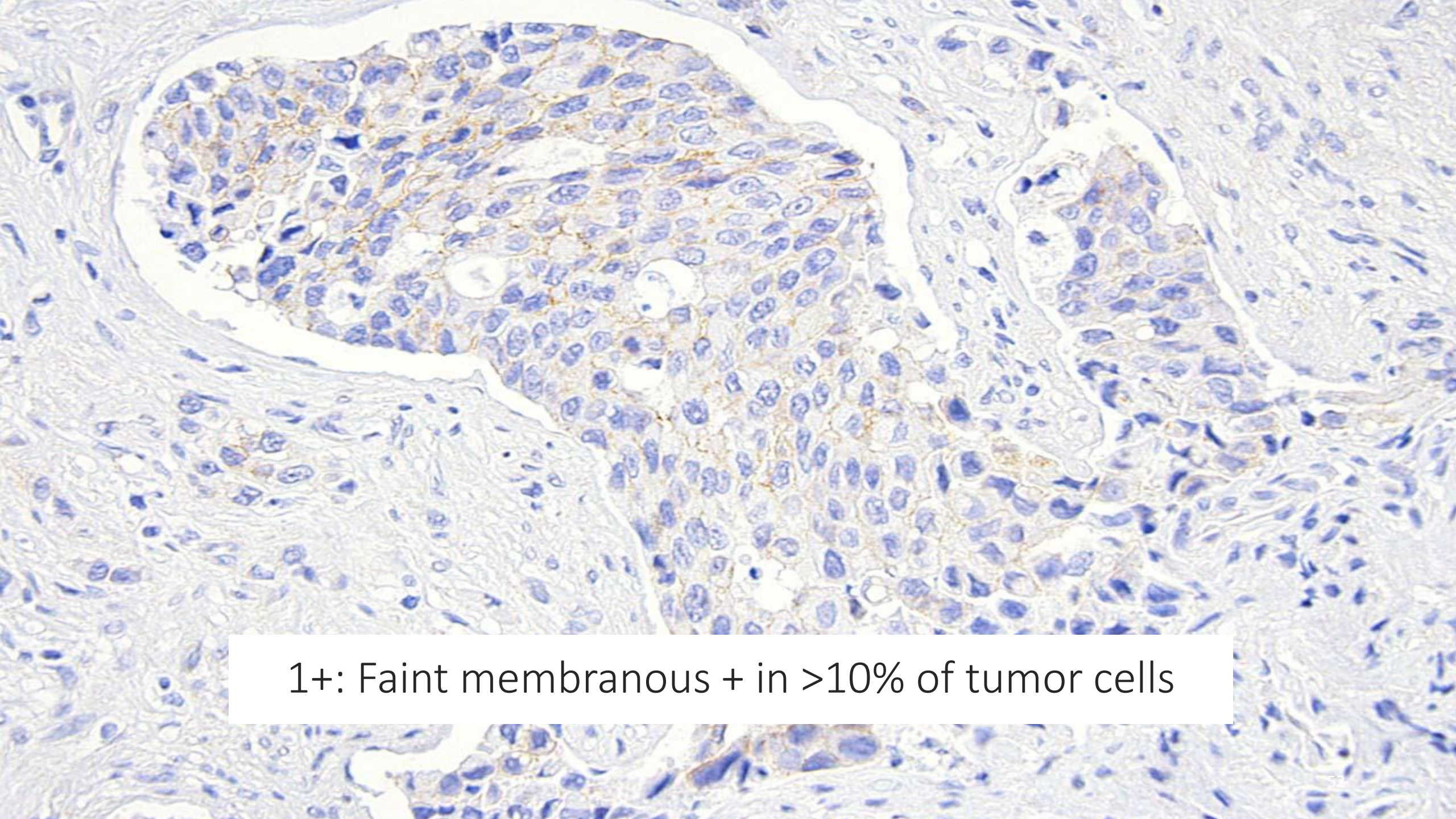




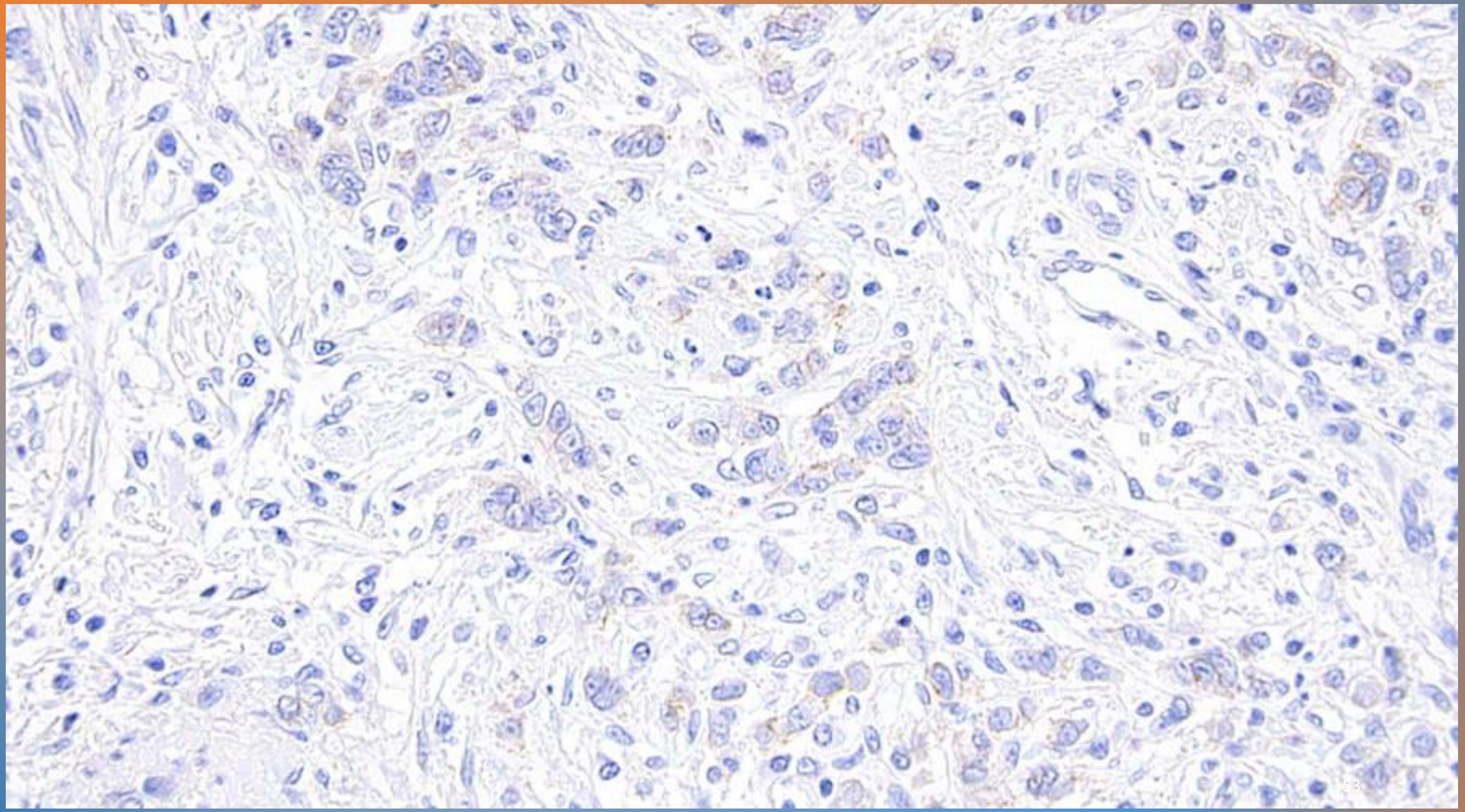


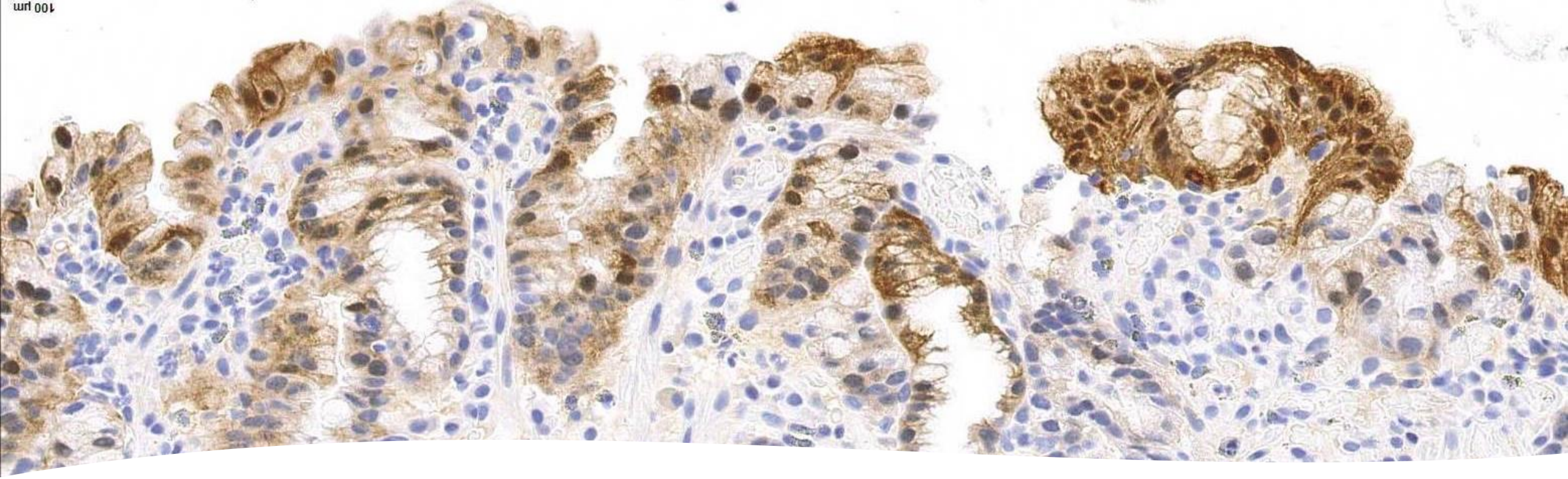

2+: moderate + in >10% tumor cells


1+ or 2+?



1+: Faint membranous + in >10% of tumor cells

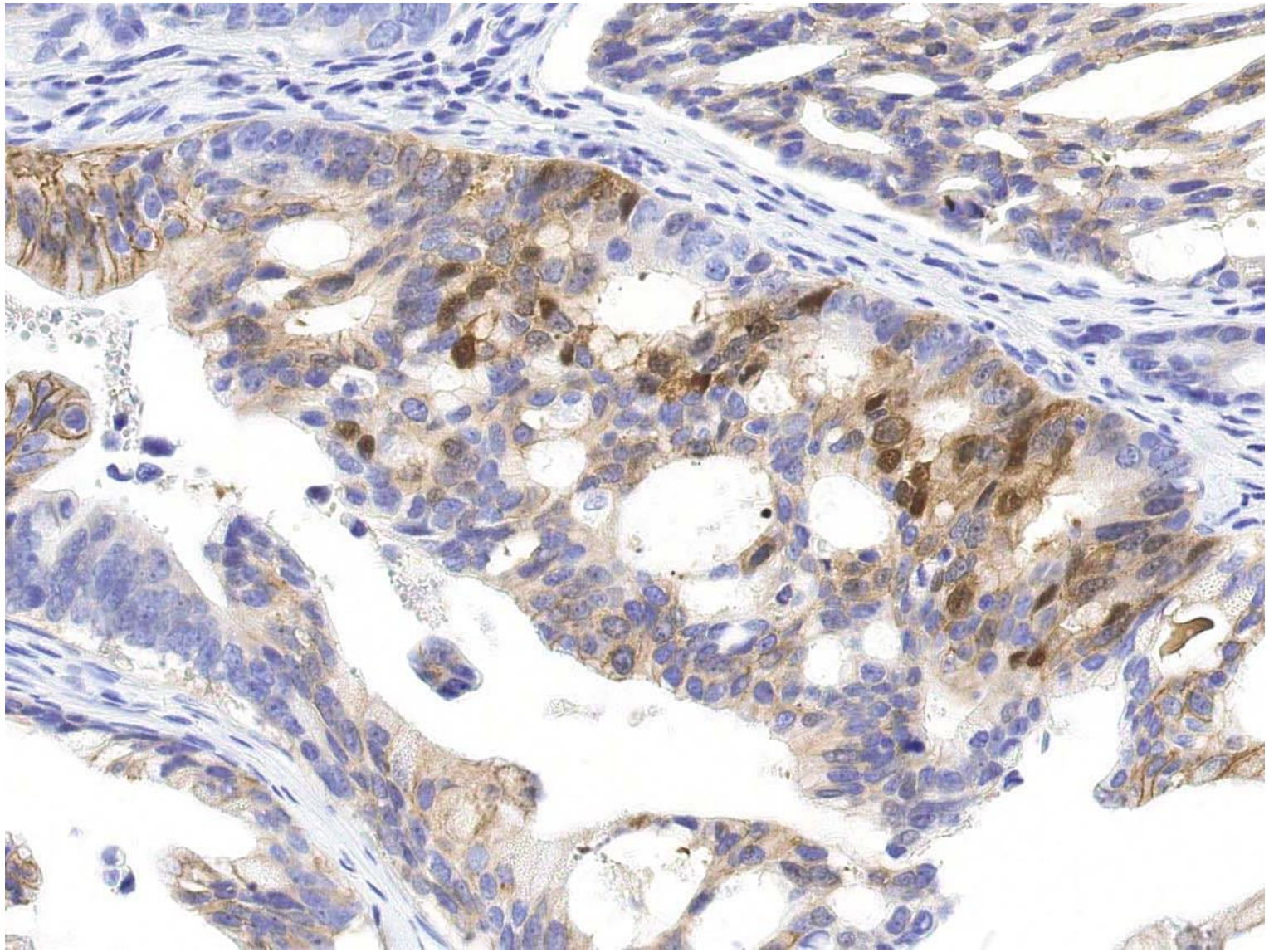




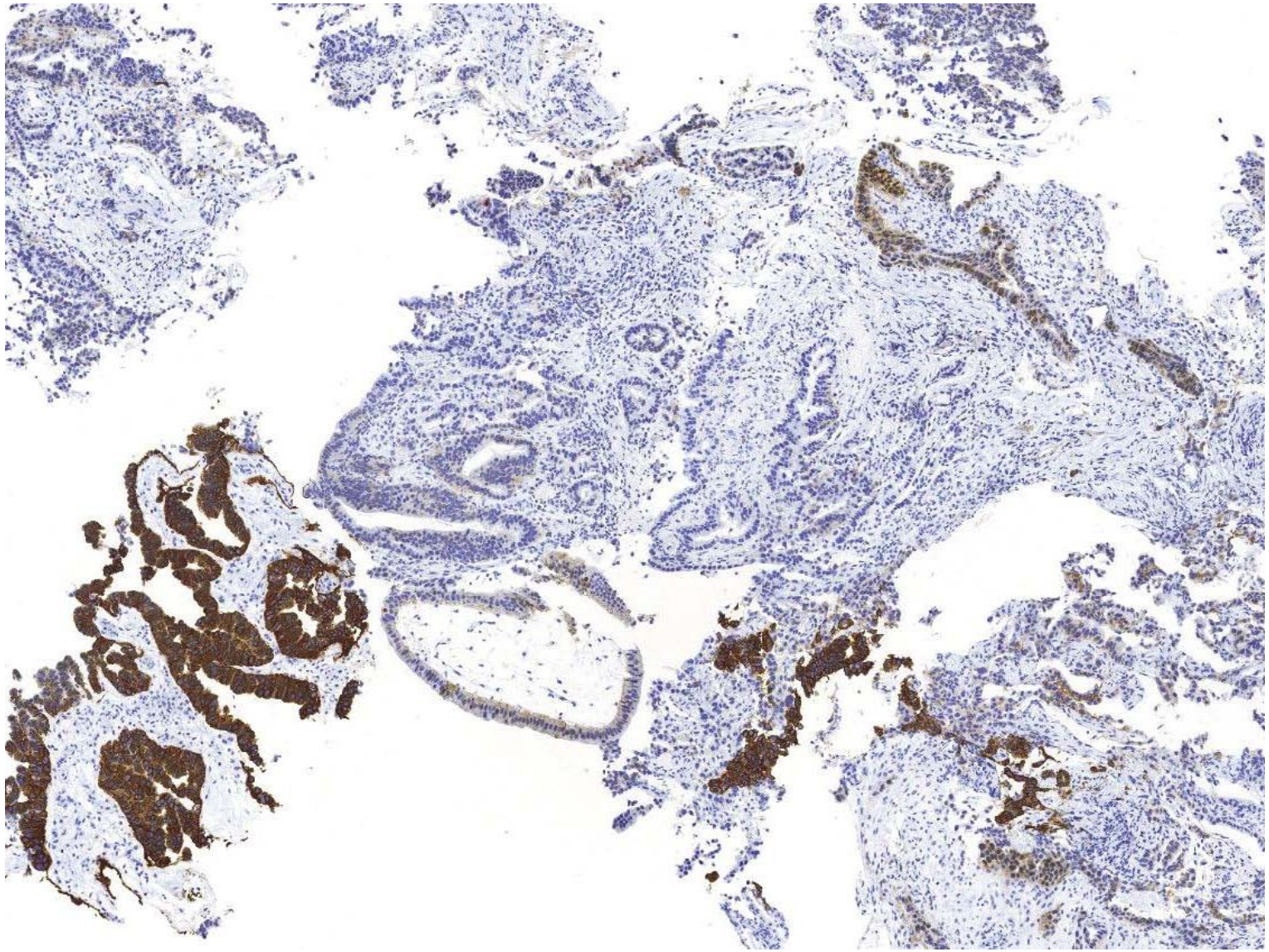
Pitfalls in IHC Assessment

- Gastric intestinal metaplasia and epithelium next to ulcers
- Edge effect
- Non-specific granular and pericellular staining
- Diffuse cytoplasmic and/or nuclear staining
- Non-specific staining in marginated cytoplasm in signet ring cells

Tumor Heterogeneity and Non-specific Nuclear staining



Extreme degree of heterogeneity



Tips to Increase Interobserver Agreement

Magnification rule overcomes problem of scoring based entirely on intensity

- 3+: May be visible to naked eye; strong membrane + at low magnification using up to 5x objective
- 2+: Membrane expression first apparent using 10x objective
- 1+: Membrane expression at 40x objective

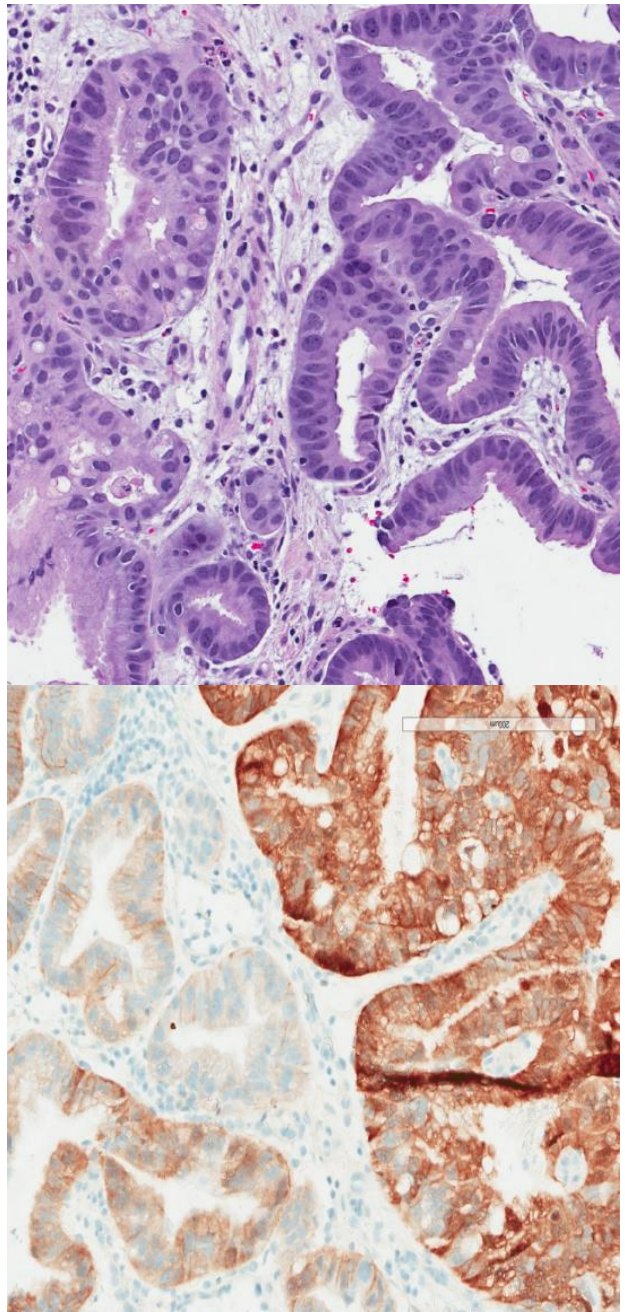
Membrane expression: distinct linear complete, basolateral, or lateral (not granular) expression at cell-cell contact sites

Source: Ruschoff J, Dietel M, Baretton G, et al. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch. 2010;457(3):299-307.

ISH Interpretation Pearls

Accurate ISH results scoring depends on localizing:

- Areas of invasive tumor
- Areas of intense HER2 overexpression by IHC
- Morphology of the malignancy to select cells



HER2 testing of gastro-oesophageal adenocarcinoma: a commentary and guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Committee

Newton A C S Wong,[1] Fernanda Amary,[2] Rachel Butler,[3] Richard Byers,[4] David Gonzalez,[5] Harry R Haynes,[6] Mohammad Ilyas,[7] Manuel Salto-Tellez,[8] Philippe Taniere[9]

- **Recommends that testing be performed on tissue obtained after treatment, because of selective pressure on tumors**
- **Recommends resection over biopsy**
- **Recommends only freshly cut sections based on breast carcinoma data**
- If there is equivocation between 1+ and 2+, perform FISH
- Calls for minimum of 20 cases in each category for validation
- **May prefer 4B5 antibody because it will produce a positive internal control- foveolar epithelium**

Wong NACS, *et al*. *J Clin Pathol* 2018;**71**:388–394. doi:10.1136/jclinpath-2017-204943

HER2 in Colorectal Carcinoma

- Rare- Expressed or amplified in only 3% to 5% (IHC and ISH)
- Occurs in RAS wild-type, BRAF wild-type tumors
  - No need to test in CRCs with RAS or BRAF mutations

HER2 Trials in Colorectal Cancer

Regimen	Trial	Comments	Criteria	Objective Response Rate
Trastuzumab + lapatinib	HERACLES-A	Lapatinib is a dual HER1/HER2 TKI; this regimen is now in NCCN guidelines for mCRC	HERACLES	30%
Traztuzumab +pertuzumab	MyPathway	Pertuzumab is a HER2/HER3 dimerization inhibitor	Breast criteria	30%
+Trantuzumab-deruxtecan	DESTINY	T-DXd is an antibody drug conjugate of an anti-HER2 monoclonal antibody linked to a topoisomerase I inhibitor.	GEA criteria	45% in IHC 3+ or IHC 2+/ISH+ patients *
Pertuzumab + T-DM1	HERACLES-B	Transtuzumab emtansine (T-DM1) is an antibody drug conjugate to microtubule inhibitor	HERACLES	10%; did not meet trial endpoint of ≥30%

* No ORRs were seen in DESTINY with IHC 2+/ISH- or IHC 1+ tumors

HER2 IHC- GEA	ToGA	Action	HERACLES- CRC	Action
No reactivity	Negative (0)	Not eligible	No staining (0)- Negative	Not eligible
Membranous reactivity in <10% of tumor cells	Negative (0)	Not eligible	Faint staining (1+) in any cellularity- Negative	Not eligible
Faint membranous reactivity in ≥10% of tumor cells	Negative (1+)	Not eligible	**Moderate (2+) in <50% of cells- Negative**	**Not eligible**
Weak to moderate membranous reactivity in >10% of tumor cells	Equivocal (2+)	ISH testing	Moderate (2+) in ≥50% of cells- Equivocal	Retest IHC to confirm ≥50% of cells; perform ISH. Eligible if amplified
Strong membranous reactivity in >10% of tumor cells	**3+**	**Eligible**	**Intense (3+) in ≤10% of cells- Negative**	**Not eligible**
			Intense (3+) in >10% to <50% of cells	Retest IHC to confirm >10% of cells; perform ISH. Eligible if amplified
			Intense (3+) in ≥50% of cells	Eligible

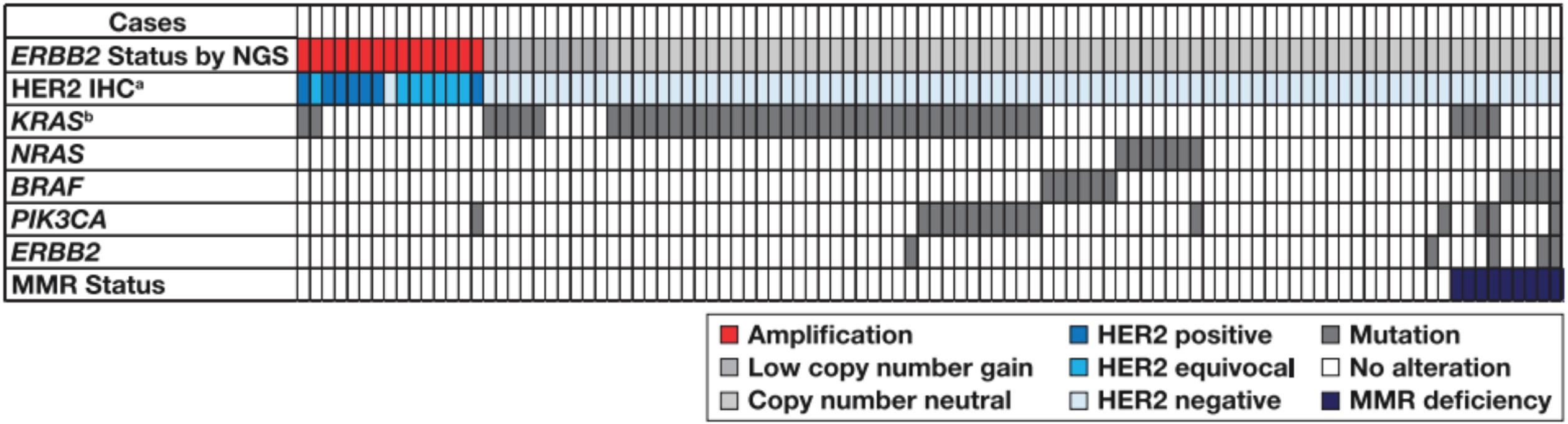
ISH Criteria: CAP/ASCP/ASCO versus HERACLES (GEA versus CRC)

CAP/ASCP/ASCO (Based on ToGA)	HERACLES
HER2:CEP17 RATIO ≥2 in 10% of tumor cells	HER2:CEP17 RATIO ≥2 in 50% of tumor cells
HER2 count >6 per cell in >10% of tumor cells	Good concordance between CISH and FISH for both
For cases with HER2:CEP17 ratio <2.0 and HER2 count per cell of 4 to 6, count another 20 cells - May use ancillary techniques such as multiplex ligation-depending probe amplification	
Additional options for indeterminate ISH scores - Use an alternative probe for chromosome 17 - Select a different tumor block - Use genomics or alternate method (PCR, SNP chip, CGH array, RNAseq, targeted/exome/whole genome sequencing)	

HER2: Consensus HERACLES IHC Criteria[17]

Intensity	Pattern	CLASSIFICATION
No staining, or staining in < 10% of cells	-	Negative
Faint staining (1+), any cellularity	Segmental or granular	
Moderate staining (2+), < 50% of cells	Any	
Intense staining (3+), ≤ 10% of cells	Circumferential, basolateral, or lateral	
Moderate staining (2+), ≥ 50% of cells	Circumferential, basolateral, or lateral	Equivocal
Intense staining (3+), > 10% of cells	Circumferential, basolateral, or lateral	Positive

- Membrane-bound HER2 expression is associated with *ERBB2* gene amplification
- Do not include cytoplasmic staining



Cenaj O, et al. Am J Clin Pathol 2019;152(1):97-108

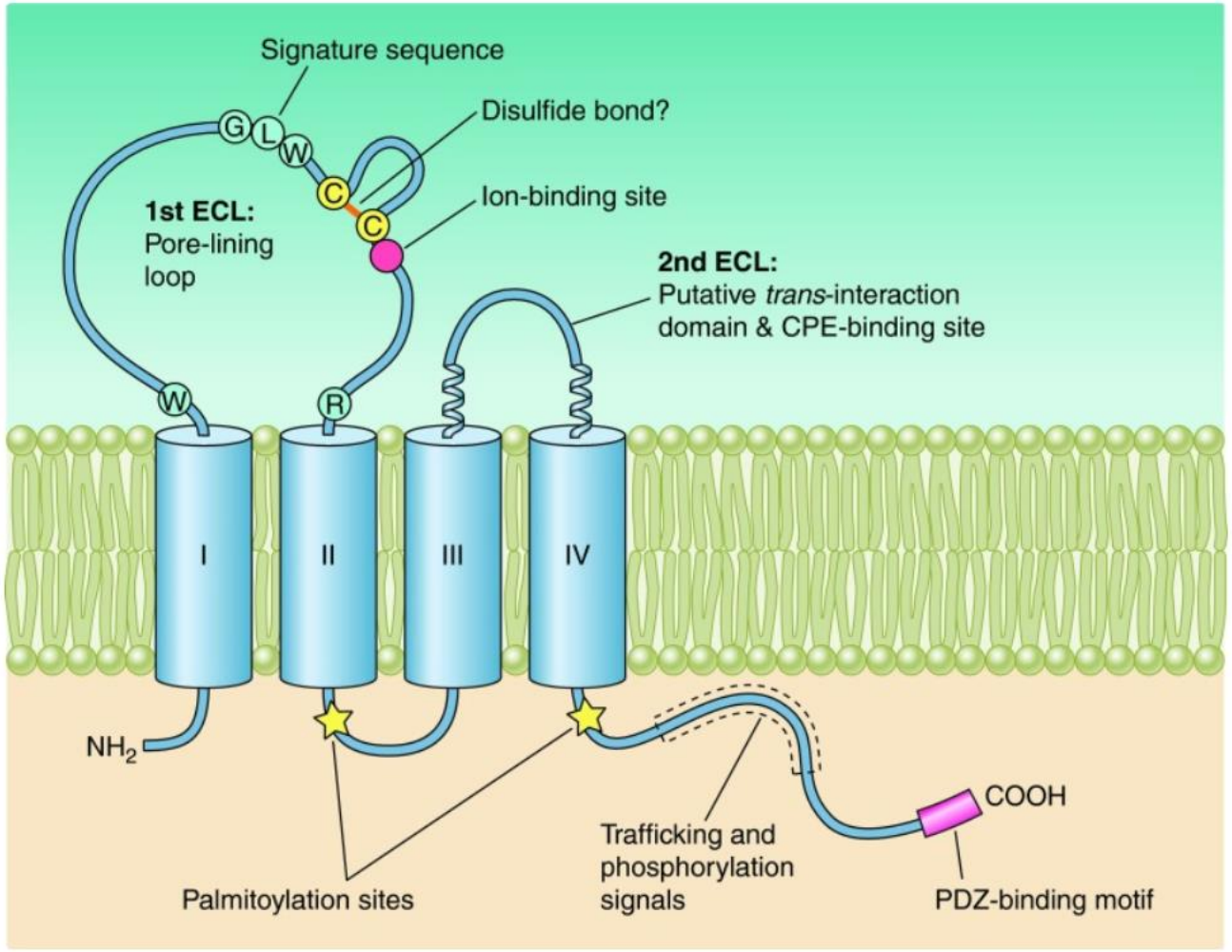
HER2 in Colorectal Carcinoma

- Low prevalence of overexpression/amplification means that reflex testing of HER2 in CRC is not warranted
- US NCCN guidelines allow for treatment of HER2-positive or amplified metastatic CRCs with targeted therapies
  - Amplification usually detected as part of NGS or targeted panel
  - If known to be RAS or RAF mutated, no need to test HER2
  - NCCN guideline specifies HERACLES criteria
- OK to test either metastasis or primary (use best block)
- I report using both the GEA resection criteria and the HERACLES criteria

HER2 Low?

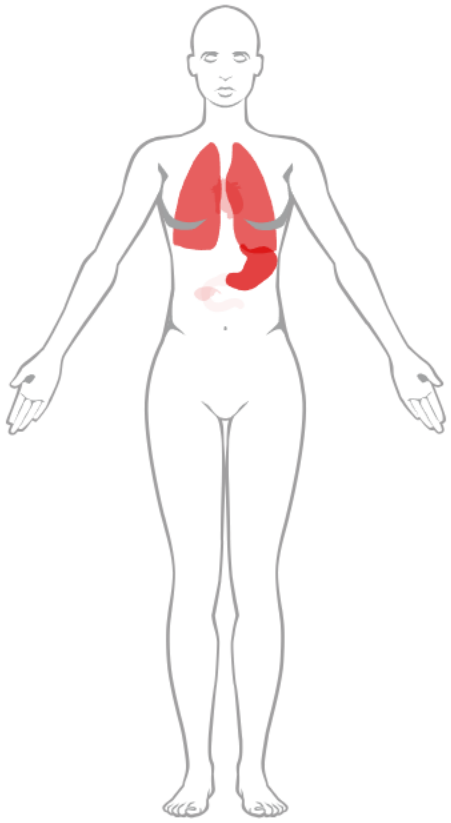
- Fam-trastuzumab deruxtecan-nxki is approved for breast, lung, gastric, and gastroesophageal junction carcinomas in the US, pending outcome of confirmatory studies for GI sites
- DESTINY-Gastric03 (Phase 1b/2) defines HER2 low as IHC 2+/ISH negative, or IHC 1+
- Other trials are open, including basket trials
- For now, use accepted criteria and report IHC as 0, 1+, 2+, 3+, including percentage of positive cells

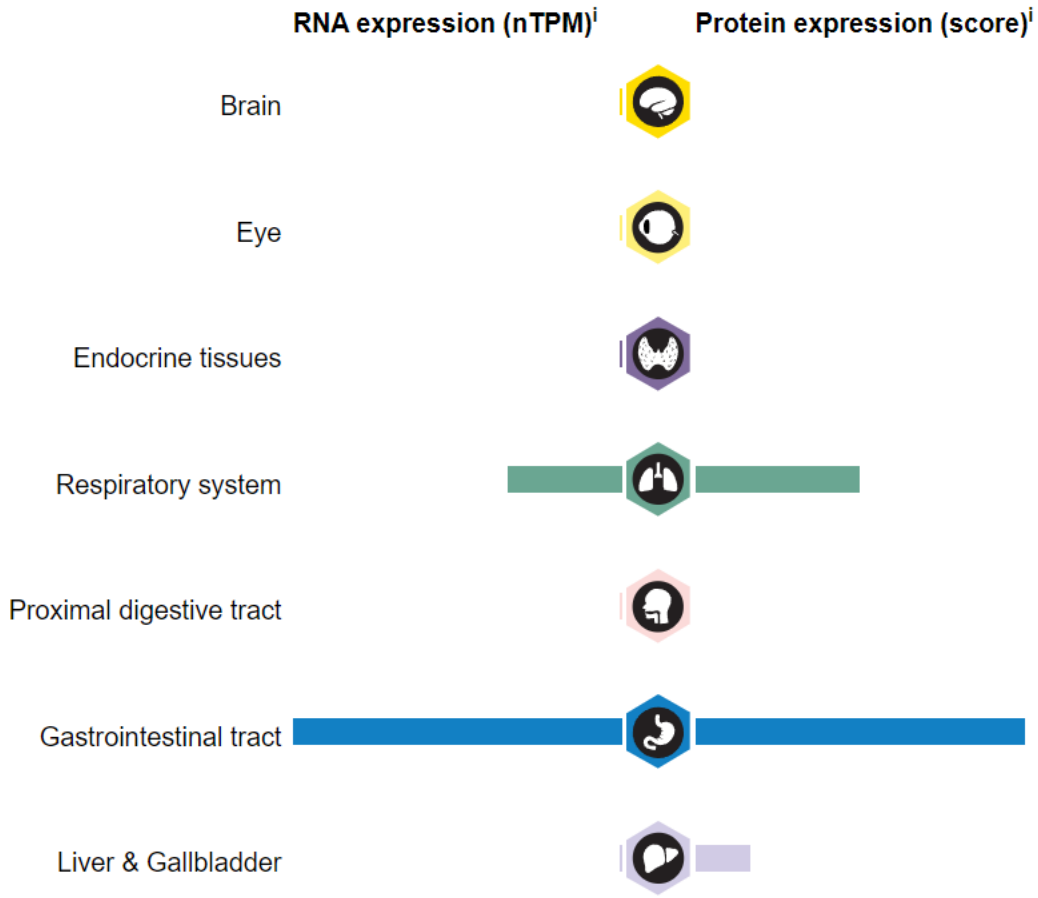
Claudin 18.2



Gunzel D, Yu ASL. Physiol Rev 2013 Apr;93(2):525-69.
doi: 10.1152/physrev.00019.2012.

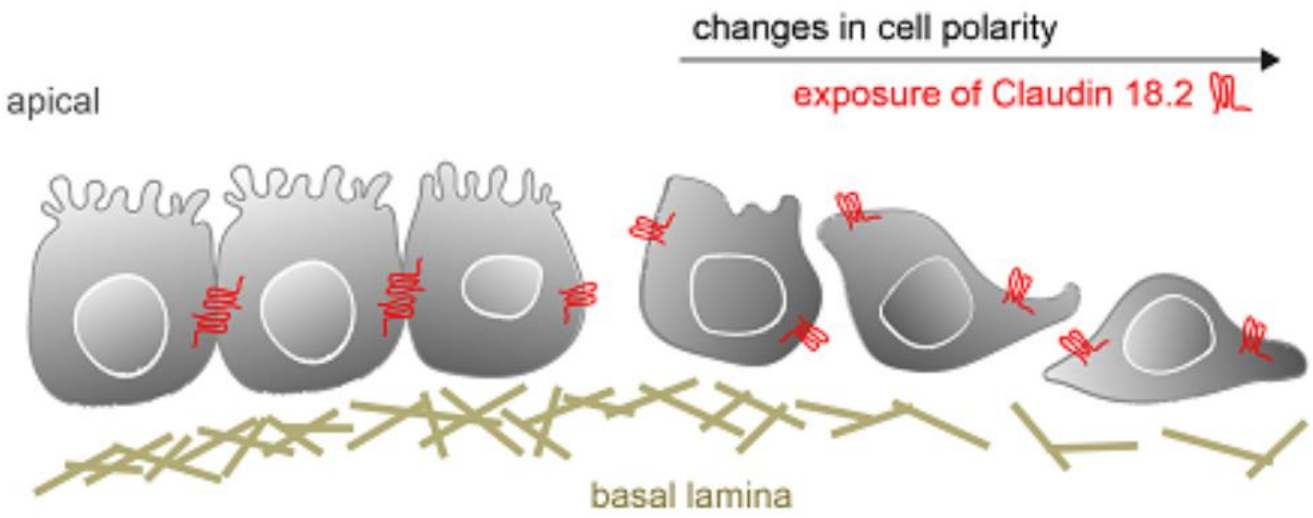
Expression of Claudin 18 in Normal Organs

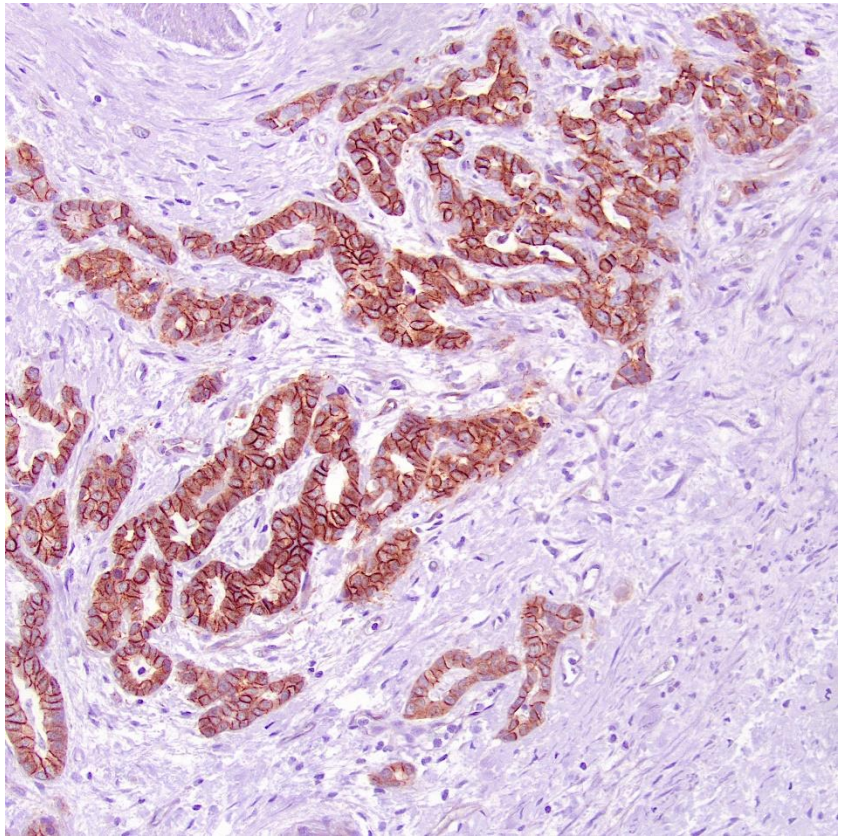




https://www.proteinatlas.org/ENSG00000066405-CLDN18/tissue

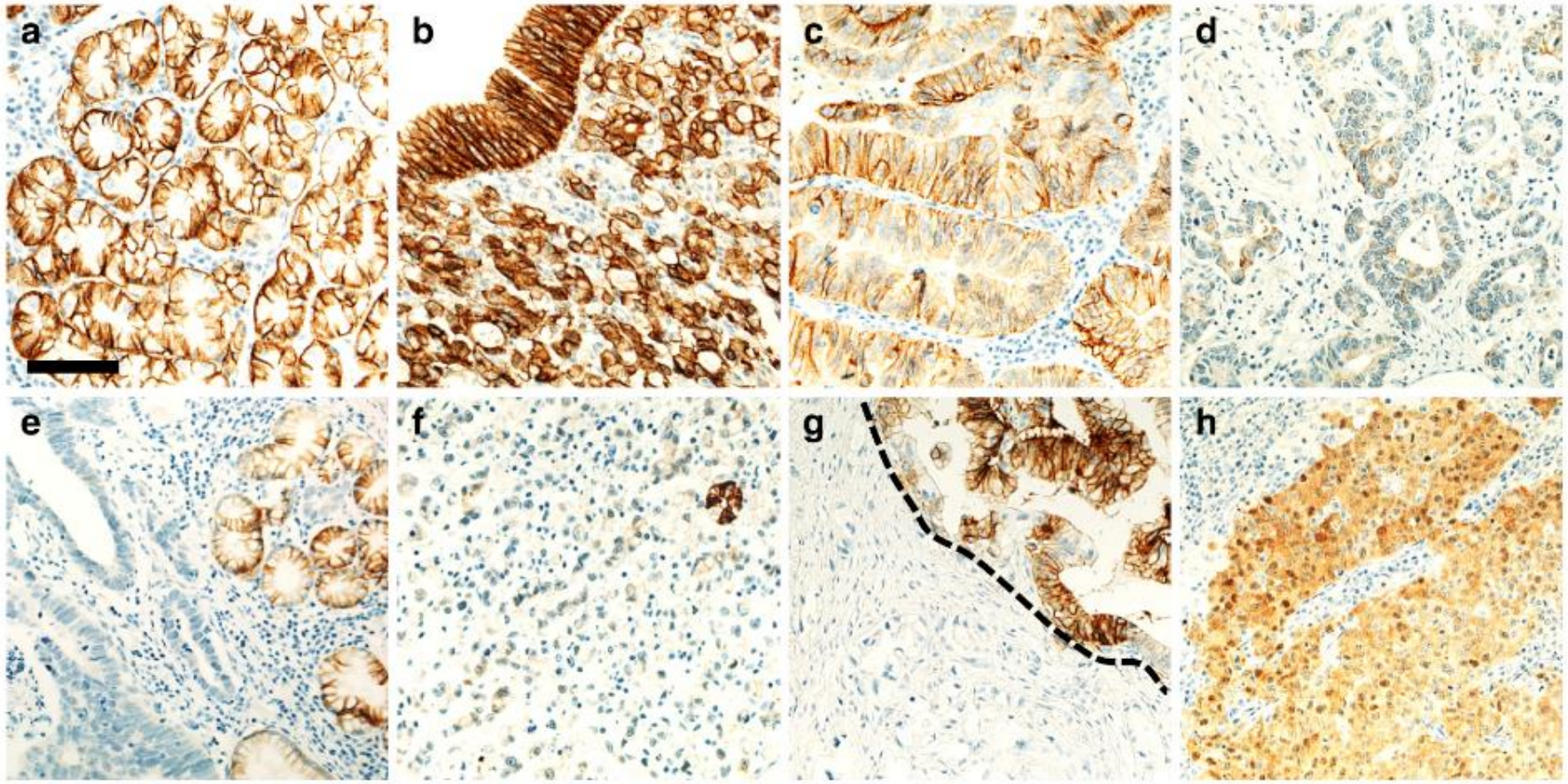
Claudin 18.2 in Gastric Carcinoma





https://www.arigobio.com/news/claudin-18.2

Claudin 18.2 Expression in Stomach



Coati I, et al. British Journal of Cancer (2019) 121:257–263; https://doi.org/10.1038/s41416-019-0508-4

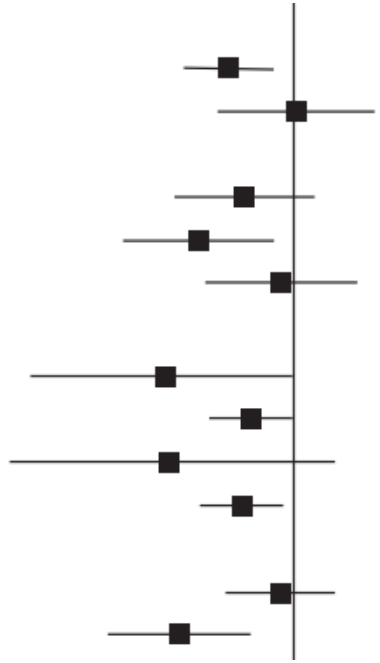
Claudin 18.2

- About 38% of gastric cancers express claudin 18.2
- Expressed on differentiated cells in normal stomach, not in the stem cell compartment
- US FDA has granted priority review to the zolbetuximab application (target date January 12, 2024)

Drug name	Phase	Company	Indications	Types
Zolbetuximab IMAB362	Phase III	Astellas Pharma Global Development Inc	Esophageal cancer; gastric cancer; adenocarcinoma; pancreatic cancer; gastrointestinal disease; cystic lymphadenoma; pain; solid tumors	Monoclonal antibody
LCAR-C18S CAR-T cell therapy	Phase I	Shanghai Oriental Hospital	Gastric cancer	CAR-T

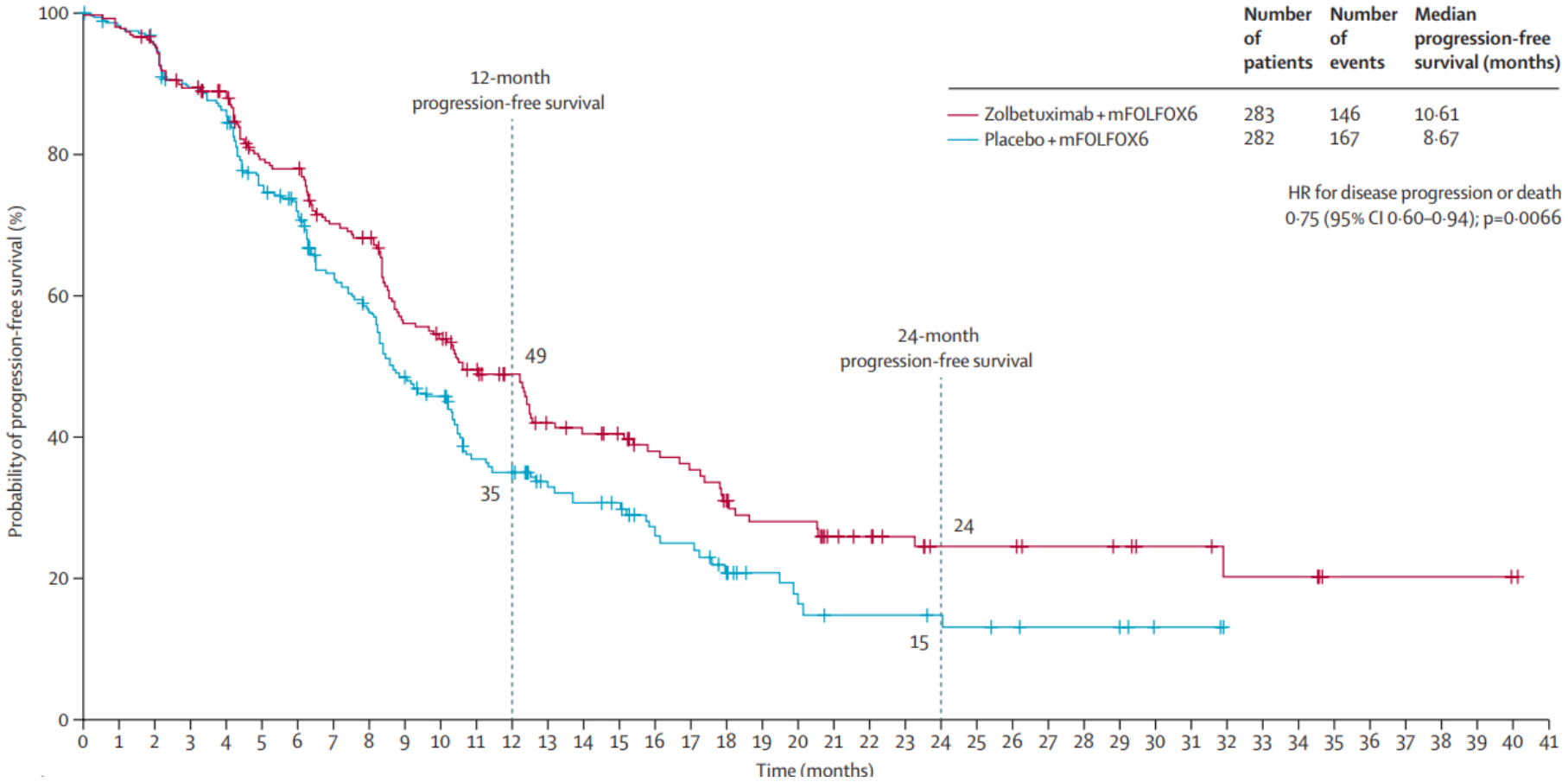
SPOTLIGHT Trial: Patient Characteristics

Primary site					
Stomach	109/219	12·22	126/210	8·38	0·69 (0·53–0·89)
Gastro-oesophageal junction	37/64	8·77	41/72	8·94	1·02 (0·65–1·59)
Lauren classification					
Diffuse	40/82	12·48	64/117	10·28	0·76 (0·51–1·13)
Intestinal	41/70	10·28	46/66	6·57	0·58 (0·38–0·89)
Mixed or other	49/81	9·79	35/55	8·67	0·93 (0·60–1·43)
Country					
Japan	12/32	18·07	17/33	8·28	0·48 (0·23–1·01)
Non-Japan	134/251	10·41	150/249	8·74	0·79 (0·63–1·00)
China	12/19	8·54	10/17	6·24	0·50 (0·20–1·26)
Non-China	134/264	11·04	157/265	9·07	0·75 (0·60–0·95)
Race					
White	77/140	8·94	82/134	10·15	0·93 (0·68–1·27)
Asian	47/96	13·96	51/97	8·21	0·53 (0·35–0·79)



Positivity defined as >75% of tumor cells with moderate to strong expression
Shitara K, et al. Lancet 2023;401:1655-68

Progression-Free Survival: SPOTLIGHT trial



Shitara K, et al. Lancet 2023;401:1655-68

Thoughts on Biomarker Testing

- MMR/MSI on all gastric, colorectal cancers
- NGS and molecular panels may identify actionable targets that we are asked to assess by IHC
- HER2 on gastric and esophageal adenocarcinomas
  - Report using accepted CAP/ASCP/ASCO criteria, don't worry about labeling tumors as "HER2 low"
- No need to perform HER2 IHC on all colorectal carcinomas- prevalence is too low
  - Criteria per HERACLES trial are different from upper GI
- Stay tuned for more information on claudin 18.2 in gastric carcinoma

Questions?