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Her2-neu testing in gastric cancer

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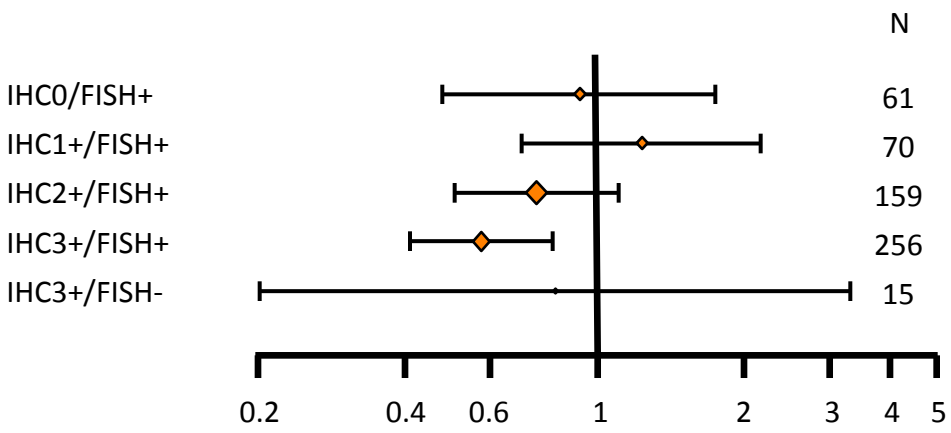
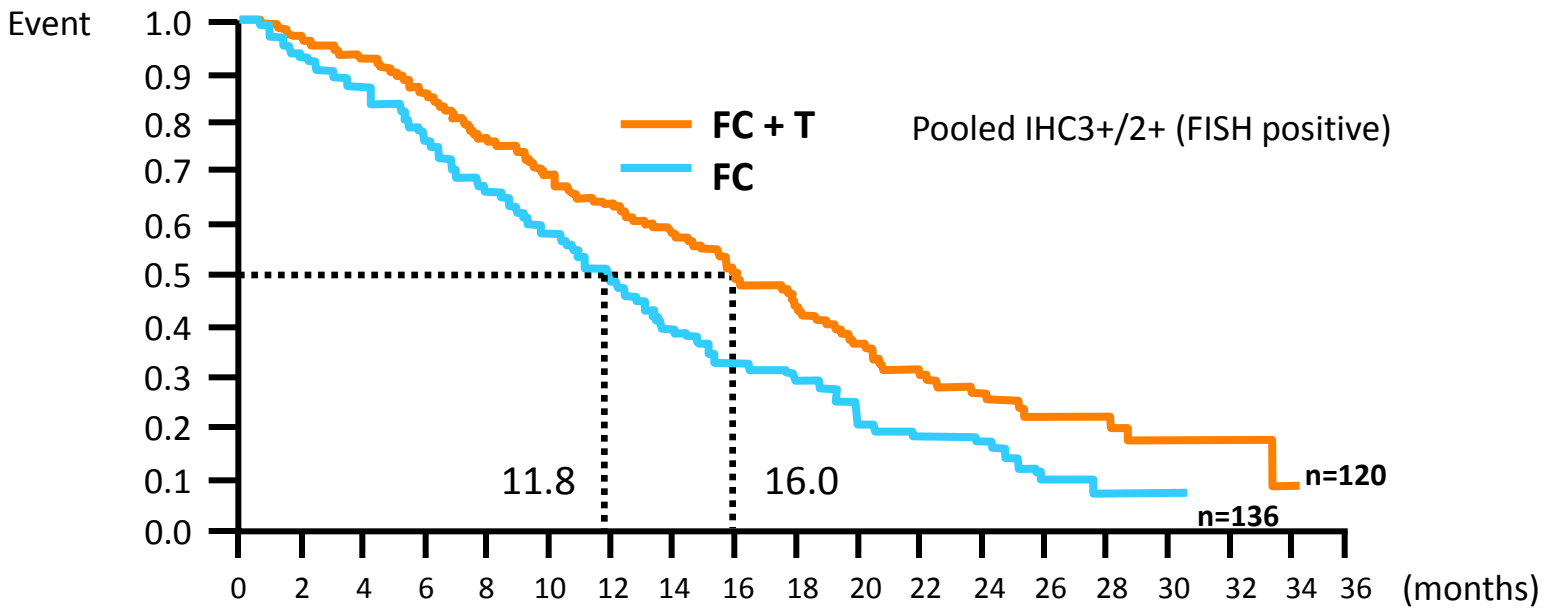
PATHOLOGIE
NORDHESSEN

Efficacy: OS by HER2 status





(ASCO 2009)

Recommended HER2 testing algorithm in gastric and GE junction cancer based upon ToGA results

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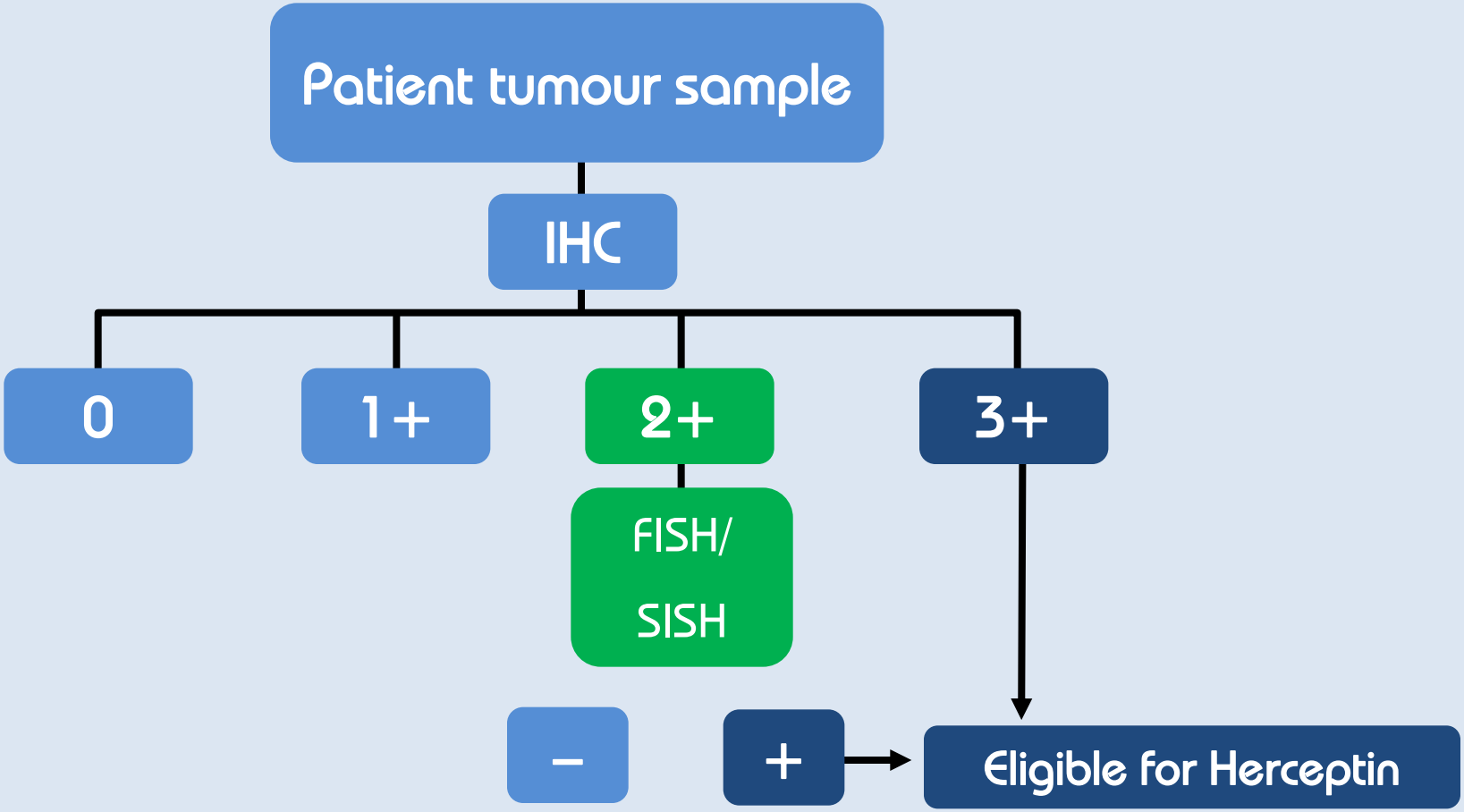

Herceptin EU SmPC: http://www.ema.europa.eu/humandocs/PDFs/EPAR/Herceptin/emea-combined-h278en.pdf

4B5 : Score 3+

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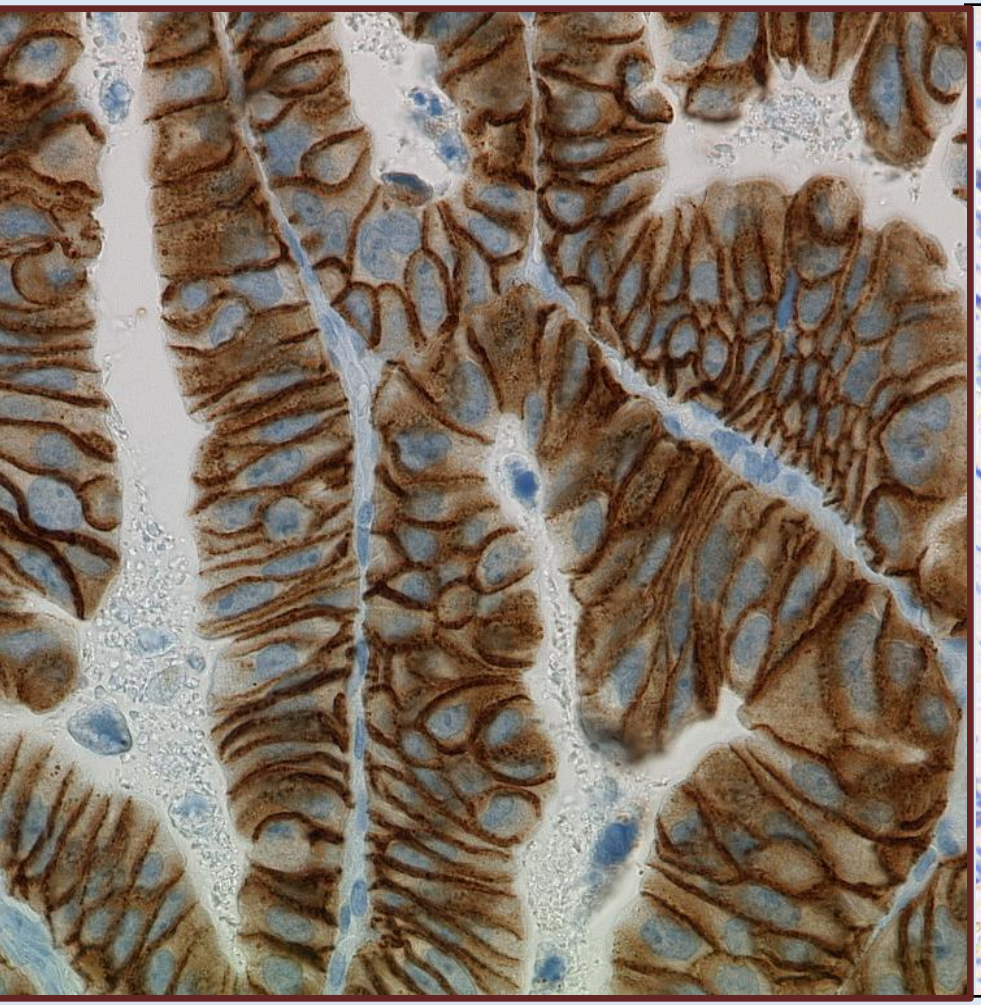
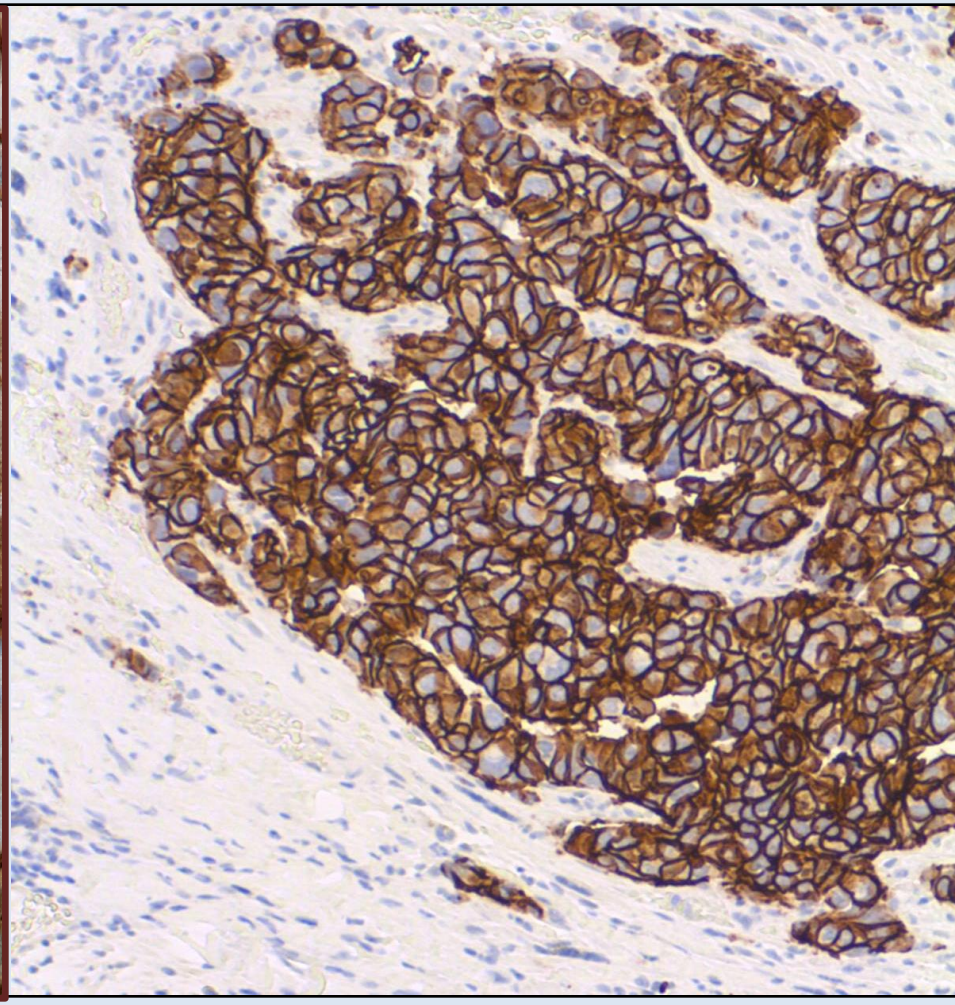
Gastric cancer



Breast cancer



Her2 Status in Gastric Cancer

heterogeneity &low level amplification/protein expression

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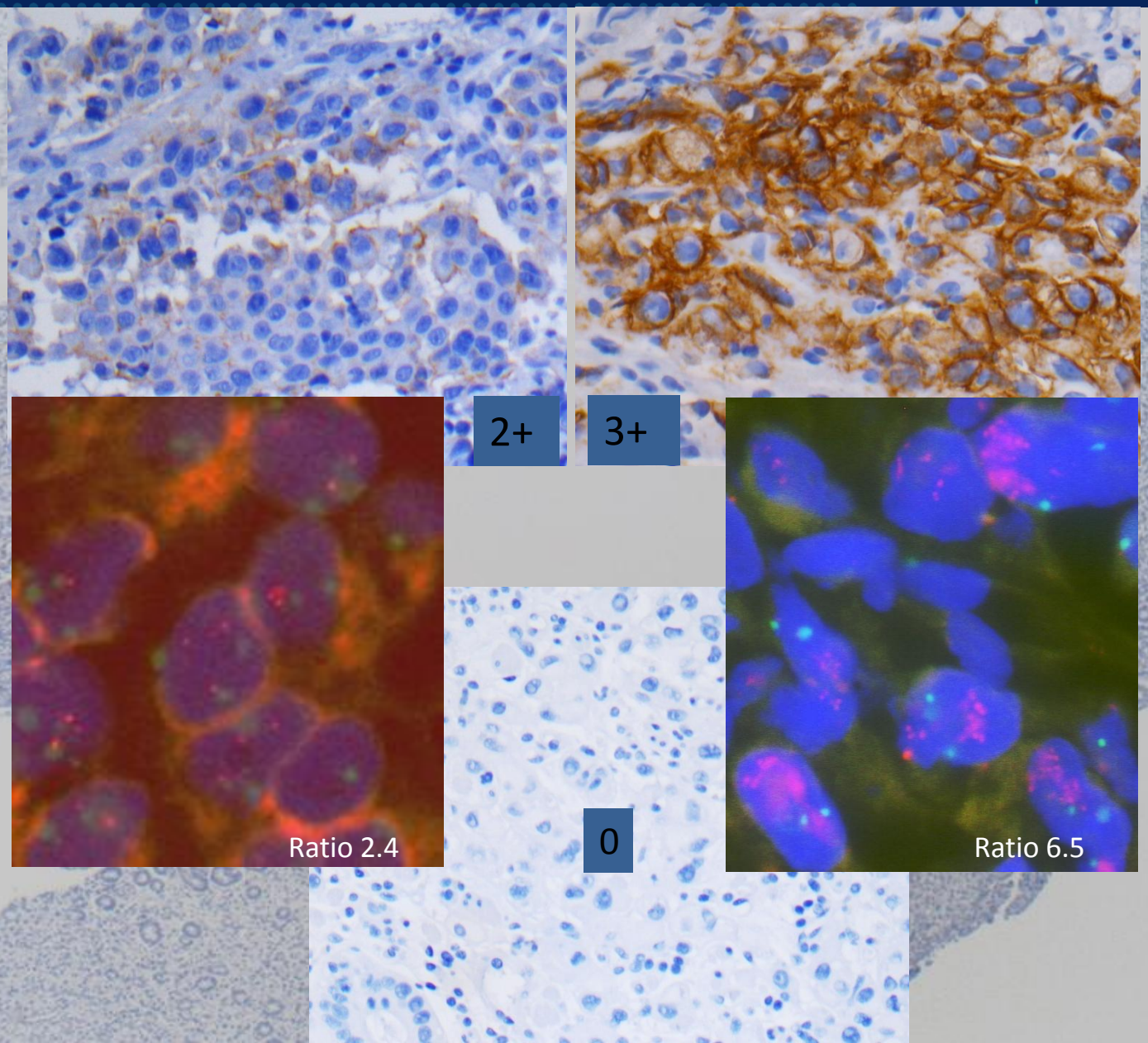

The breast cancer IHC testing criteria had to be modified for gastric cancer. Result: a **similar scheme** with **new definitions**

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ring shaped staining is **no** longer a scoring **criterium in gastric cancer**.

A **percentage cut off** is only used for resection specimens and **not for biopsies**.

Staining Intensity Score	Surgical specimens - staining pattern	Biopsy specimens - staining pattern	HER2 Overexpression Assessment
0	No reactivity or membranous reactivity in < 10% of tumour cells	No reactivity or membranous reactivity in any tmour cell	**Negative**
1+	Faint / barely perceptible membranous reactivity in ≥ 10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell clones with a faint / barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	**Negative**
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥ 10% of tumour cells	Tumour cell clones with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	**Equivocal**
3+	Strong complete, basolateral or lateral membranous reactivity in ≥ 10% of tumour cells	Tumour cell clones with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	**Positive**

(Rüschoff et al. Virch Arch 2010)

Her2 in gastric cancer (GC) is different from breast cancer (BC)

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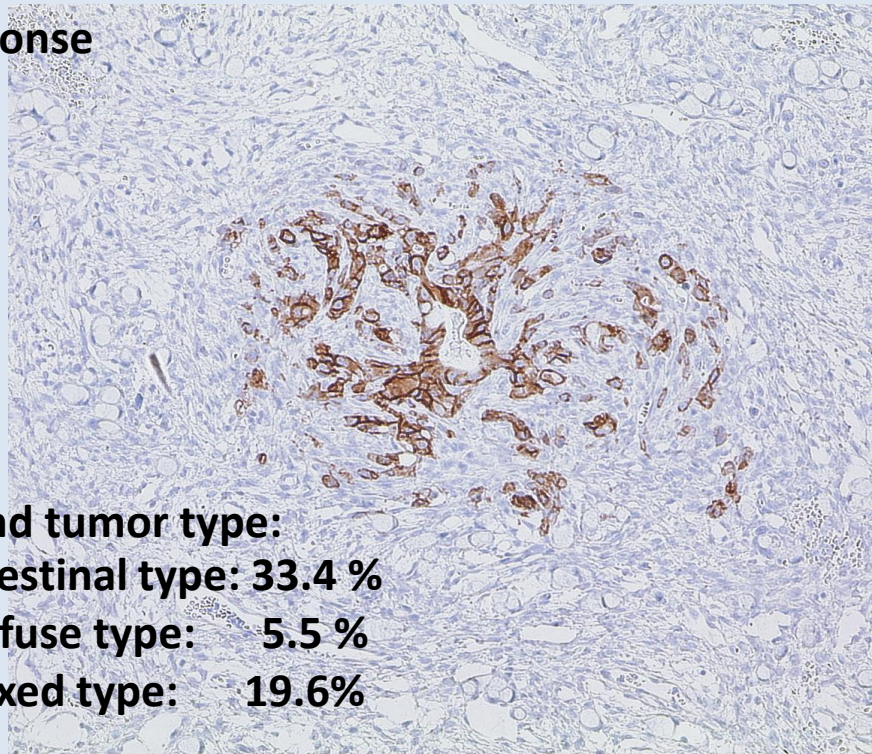
- **<u>Focal</u> Her2 expression & gene amplification in 33% of GC (<30% stained cells in IHC 3+)** [~1% in BC]

- **Membranous Her2 staining usually incomplete in GC**
  [= negative in BC]

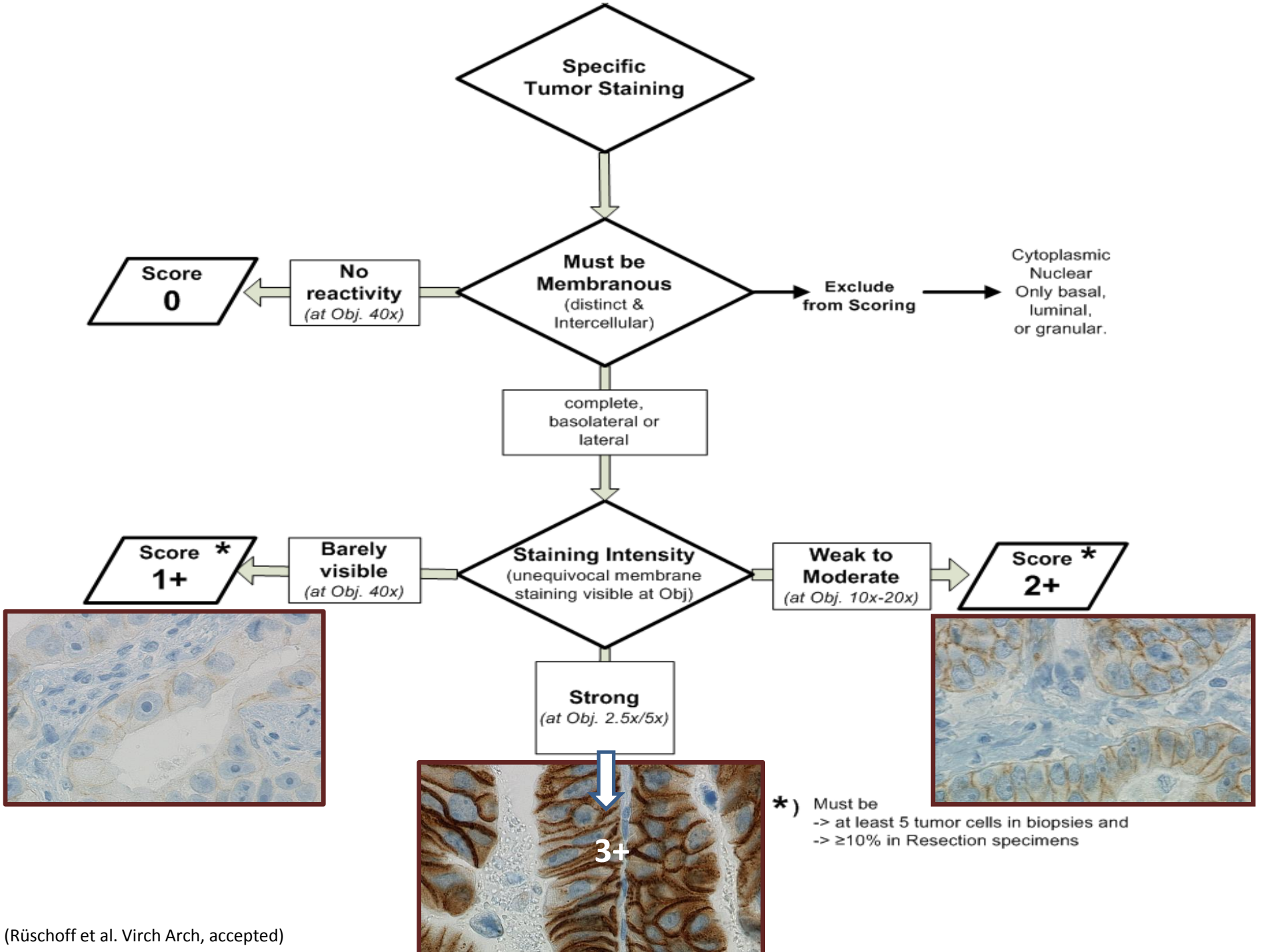
- **7.5% FISH positivity in IHC 0/1+ , but poor response to Herceptin therapy in this subgroup**
  [< 5% in BC]

- **Close relationship between protein expression level and degree of gene amplification**

- **Strong correlation with location:**
  GEJ cancer: 33.6%
  Gastric: 19.9%



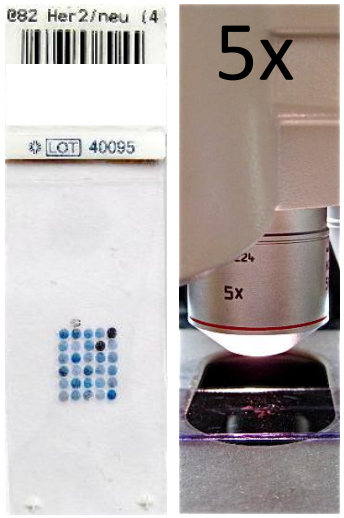
… and tumor type:
Intestinal type: 33.4 %
Diffuse type: 5.5 %
Mixed type: 19.6%



(Rüschoff et al. Virch Arch, accepted)

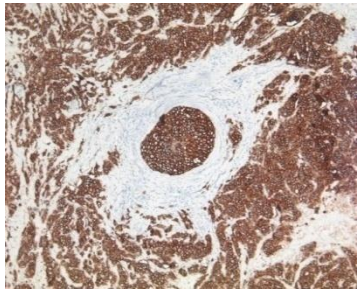
Her2-Score

3+

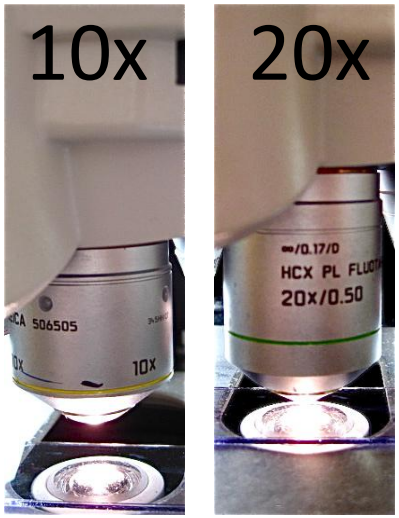


visible without a microscope!

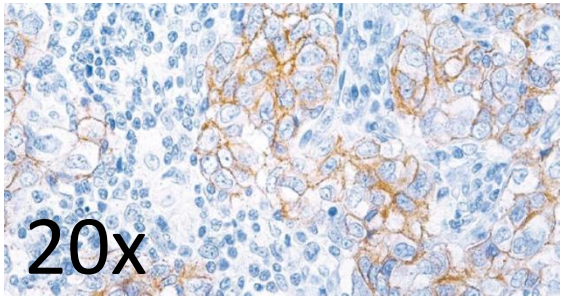
membranous staining visible in overview



2+

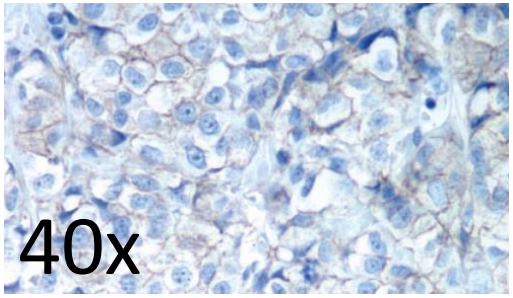


needs a more detailed magnification



1+

Needs high magnification



Recommended HER2 testing algorithm in gastric and GE junction cancer

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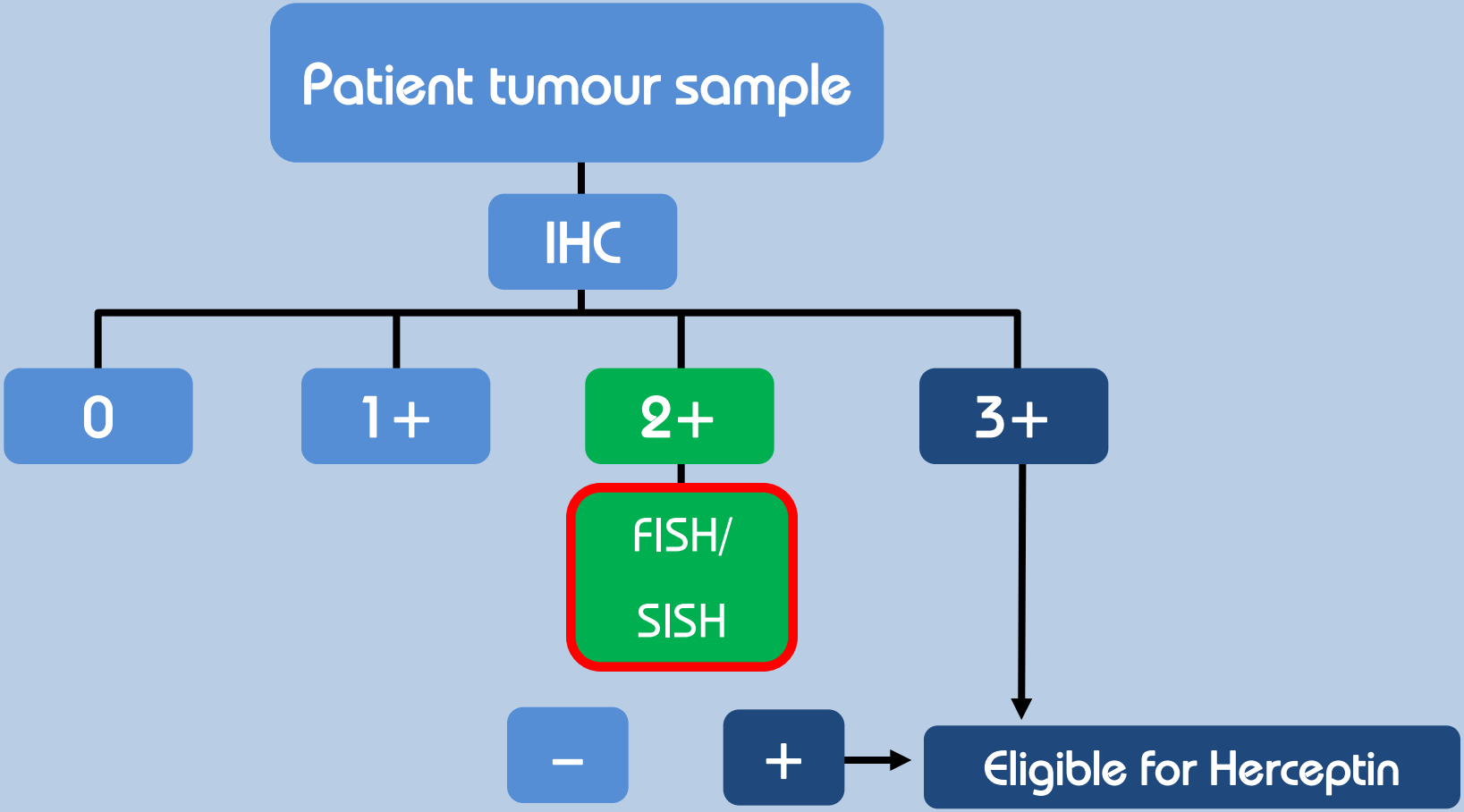
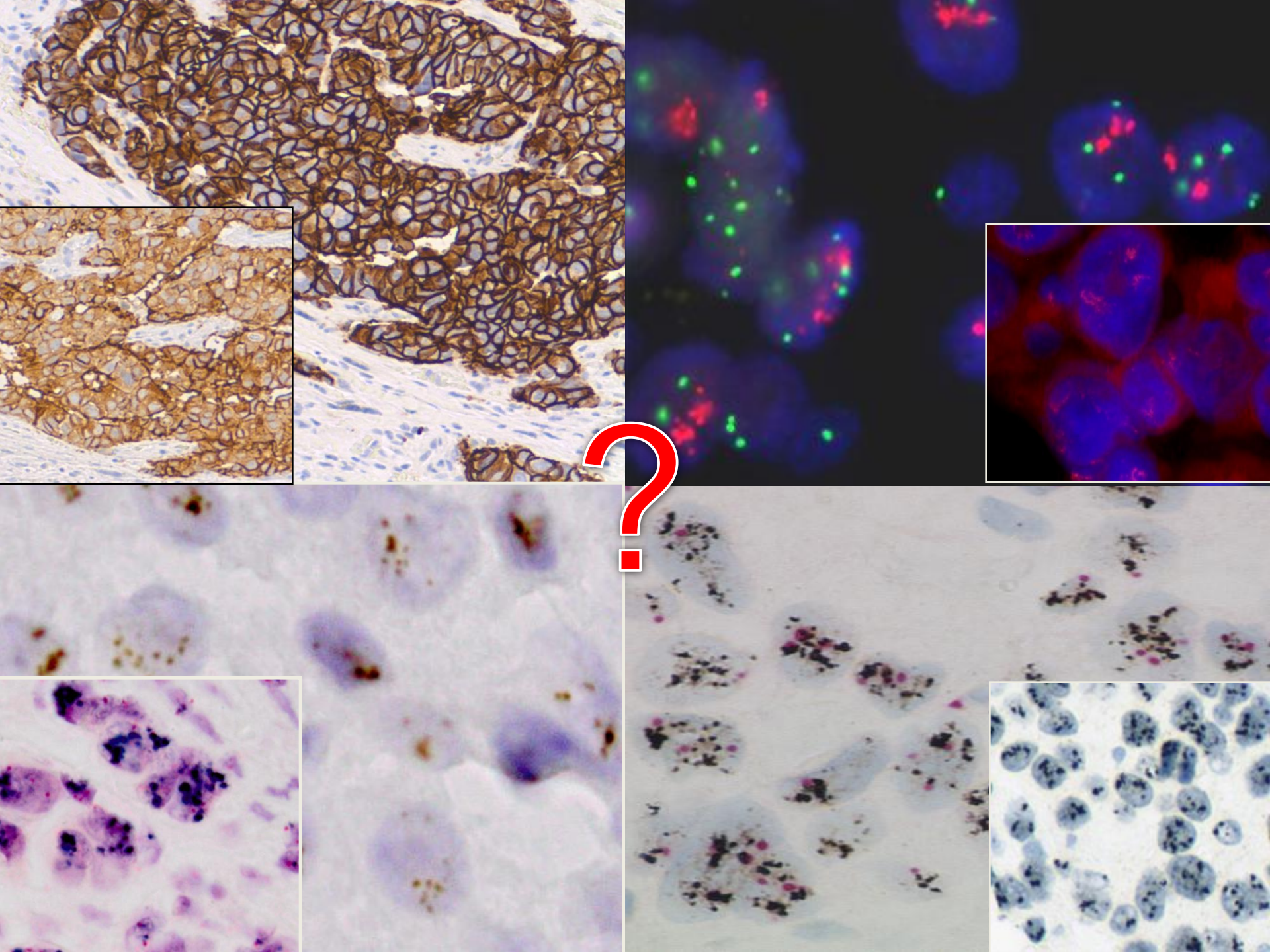


Herceptin EU SmPC: http://www.ema.europa.eu/humandocs/PDFs/EPAR/Herceptin/emea-combined-h278en.pdf



ISH principle

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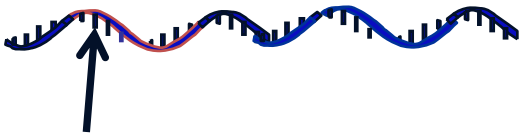
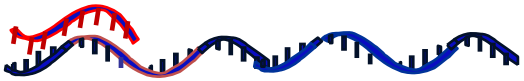
2-3 (4) µm sections of FFPE (formalin fixed paraffin embedded tissue).
Proper fixation time: 6-48 hrs.

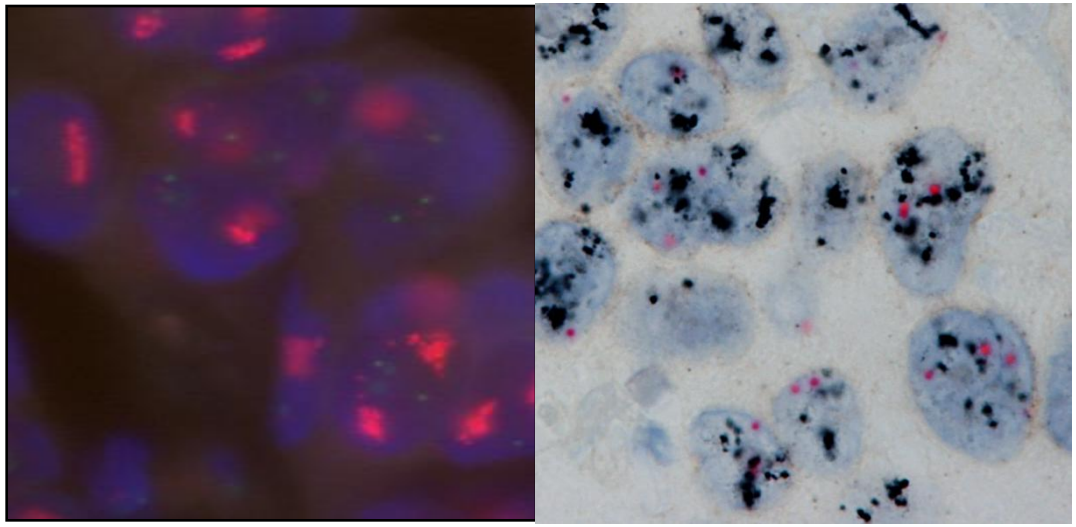
fluorescent/chromogene labeled probe(directly or secondary)



Her2/neu region on chromosome 17

Hybridisation of labeled probe to the site of the gene





analysis of signals with either brightfield microscope or fluorescence microscope

One step ahead of FISH: Brightfield dual color ISH (BDISH and DDISH, Ventana)

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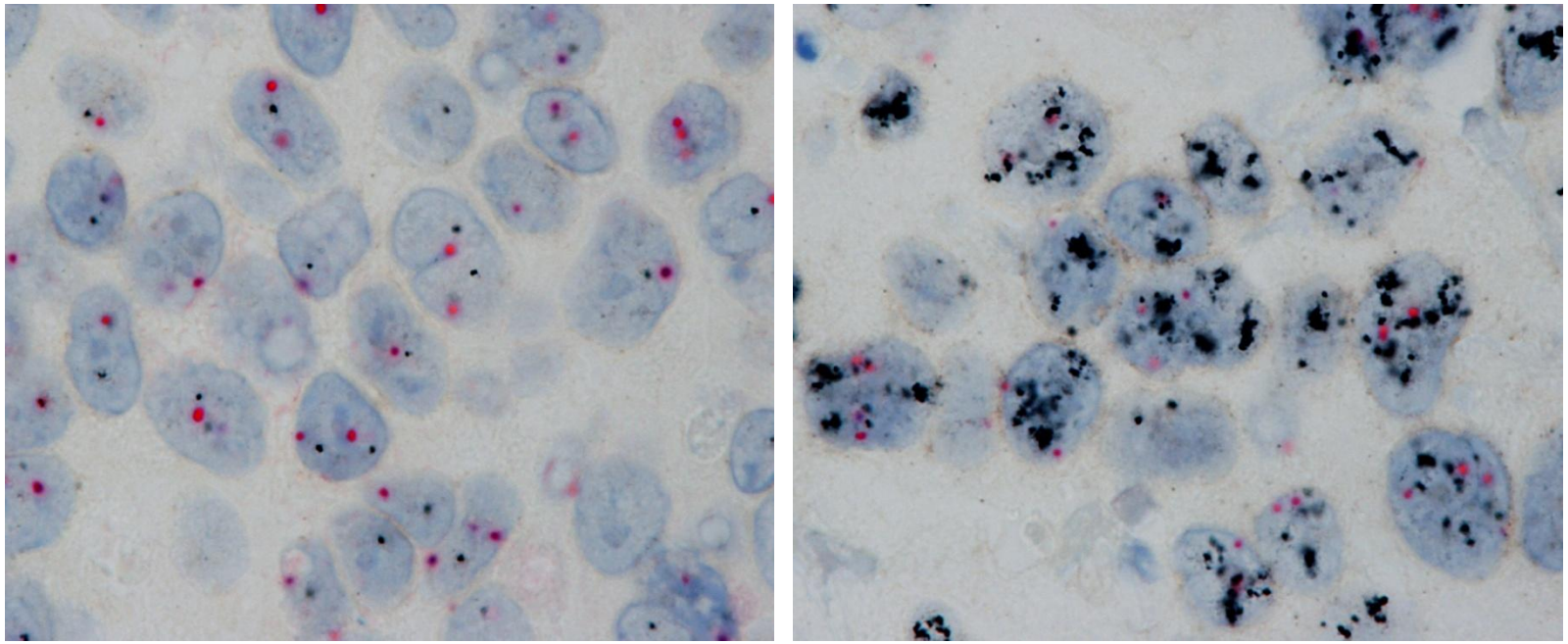

HER2 Gene
Chromosome 17 Centromere

BDISH: **b**rightfield **d**ual **i**n **s**itu **h**ybridisation : one hapten (DNP), serial hybridisation ofHer2 and Chr17 probe.

DDISH: **d**ual color **d**ual hapten **ISH**: two haptens (DNP and DIG), parallel hybridisation of Her2 and Chr17 probe (shorter hybridisation time, less artifacts, red signals are even more distinct!)

Her2 ISH analysis: risk factor heterogeneity

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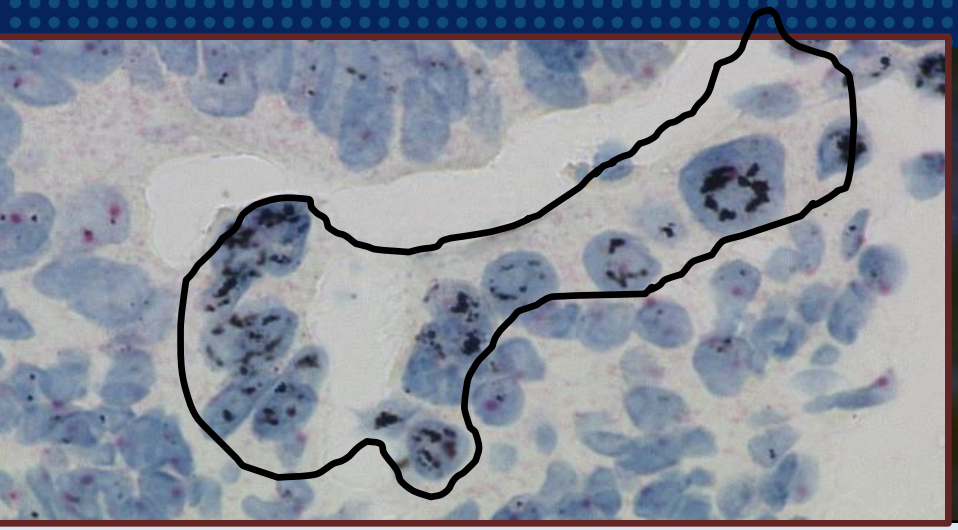

Scan all of the tumor tissue on the slide <u>for focal amplification or other gene count alteration</u> (e.g. polysomy, interspersed amplified cells). **The selection of the area makes the difference!**

Count signals in 20 *adjacent/neighboring* tumor cells.

In case of a ratio between 1.8 and 2.2 count additional cells in a different area. The final cut off is then **2.0**

BDISH allows a more thorough scanning of the tissue (20-40x magnification), and the counting can be done at 40x or 60x magnification as opposed to 100x in FISH.

Concordance between SISH and FISH

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- **A cohort study, including samples from the ToGA trial, showed concordance between SISH and FISH was 95.3% (241/253 cases)**

		PharmDx FISH		
		Negative	Positive	Total
INFORM HER2 DNA	Negative	201	8	209
	Positive	4	40	44
	Total	205	48	253

Powell WC, *et al*. ASCO 2010 Gastrointestinal Cancers Symposium, Orlando; Abstract 17.

Concordance between SISH and FISH

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Dual-colour HER2/Chromosome 17 chromogenic in situ hybridisation assay enables accurate assessment of HER2 genomic status in gastric cancer and has potential utility in HER2 testing of biopsy samples

Benedict Yan,[1] Ee Xuan Yau,[2] Shoa Nian Choo,[1] Chee Wee Ong,[2] Kol Jia Yong,[2] Brendan Pang,[1] Manuel Salto-Tellez[2,3]

100 % concordance between FISH and DDISH (Ventana) in 119 samples

Table 1 Concordance between HER2 fluorescence in situ hybridisation and dual-colour chromogenic in situ hybridisation results

		Fluorescence in situ hybridisation	
		Amplified	Non-amplified
Dual-colour chromogenic in situ hybridisation	Amplified	15	0
	Non-amplified	0	104

κ coefficient 1.0, $p<0.001$.

J Clin Pathol 2011;**64**:880—883. doi:10.1136/jclinpath-2011-200009

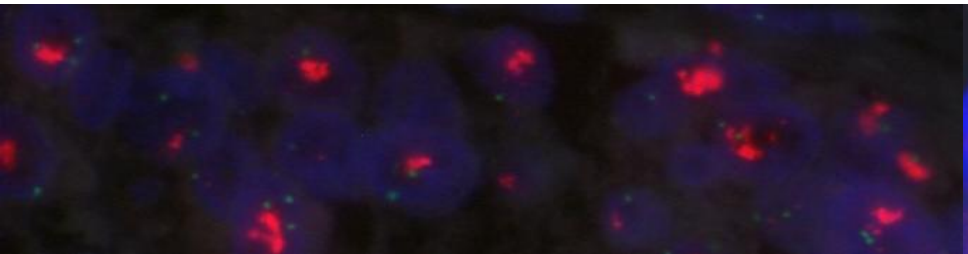
recommendations

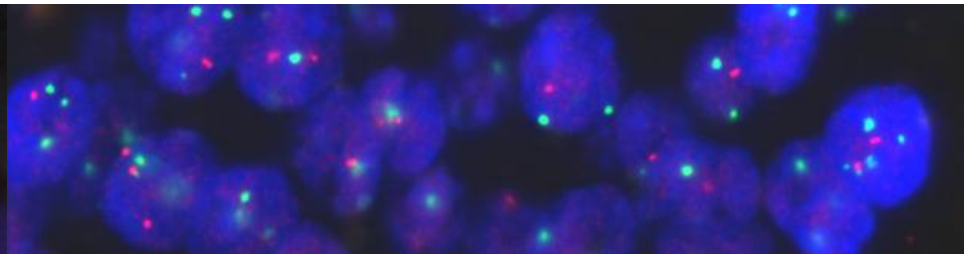
1. **<u>“polysomic” cases</u>:**
   in case of a negative ratio have a look at the average Her2 gene copy number: **more than 6 = Her2 positive**.

2. **IHC guided ISH is more reliable!** Have a look at the IHC slide and mark areas of interest (e.g. small foci), then find these areas on the ISH slide.

3. Brightfield ISH such as **SISH/BDISH is advantageous in heterogeneous cases** : better overview, easier to identify focal amplification.

Combining IHC and ISH makes both methods more powerful tools!





IHC 3+ case …
Equivocal ISH ratio of 2,13, gene copy number >6

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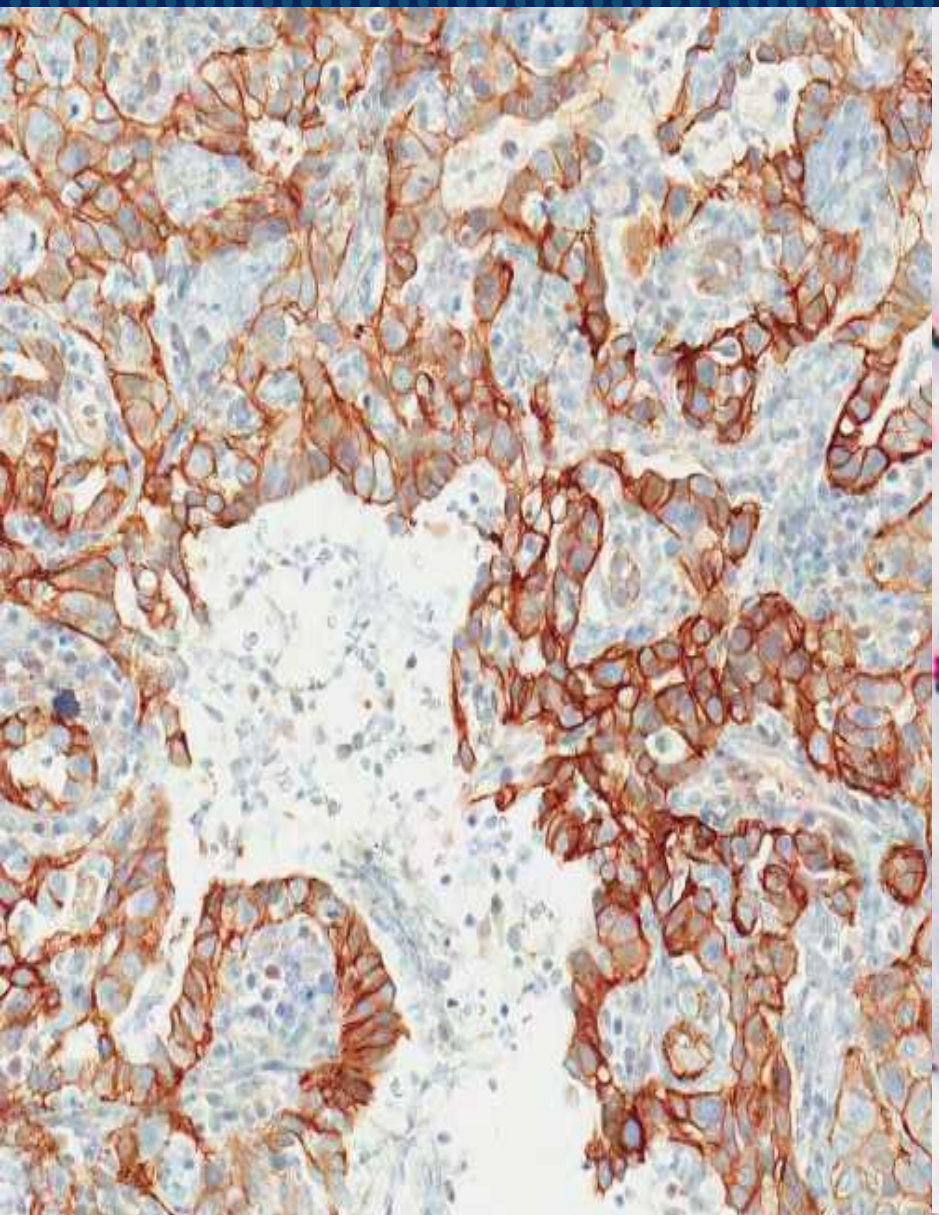

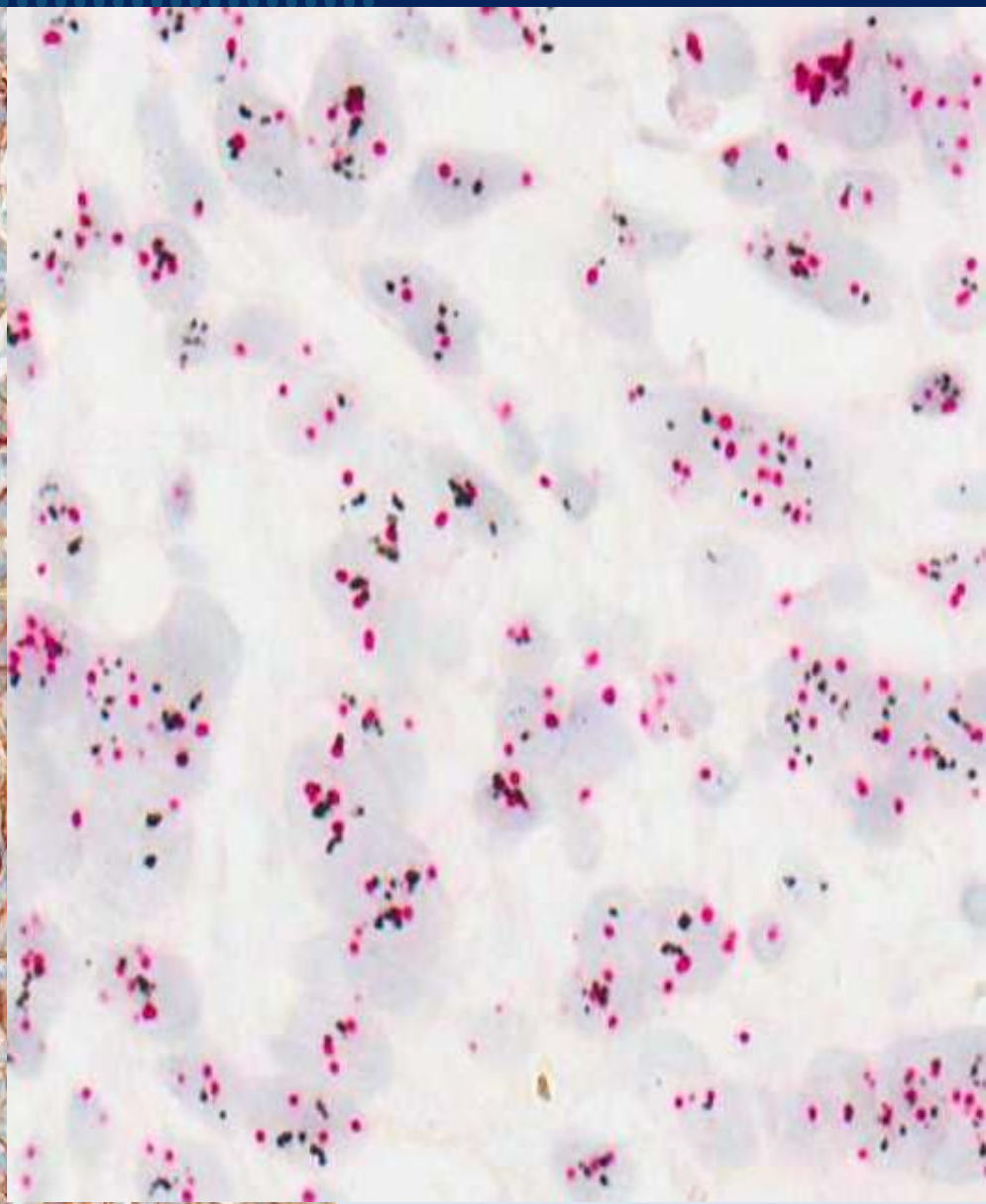

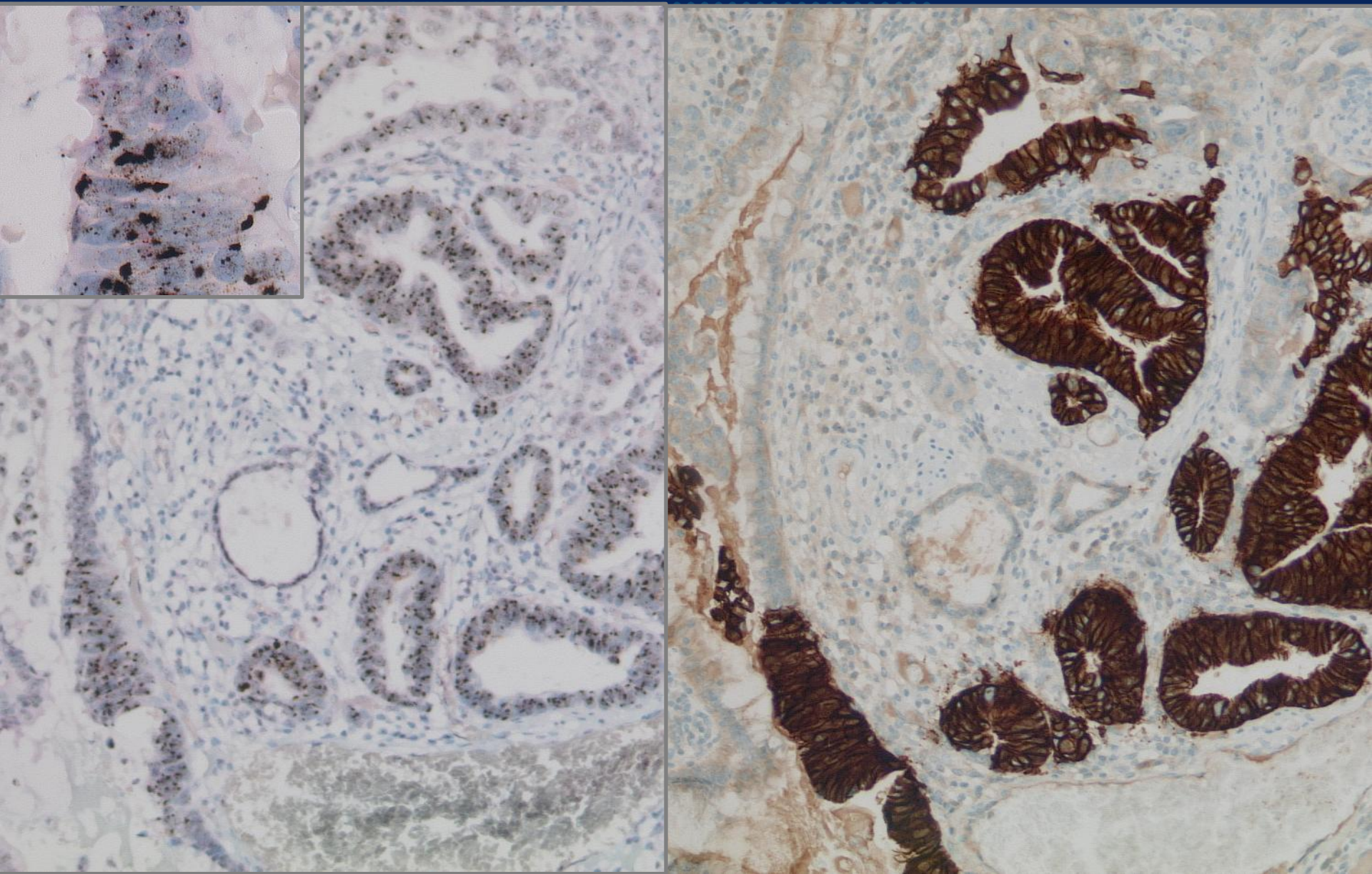

Fixation time and type of fixative have a great influence on ISH and IHC quality.

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- Formalin fixation without additives (don‘ t use AFA or Bouin‘s etc)

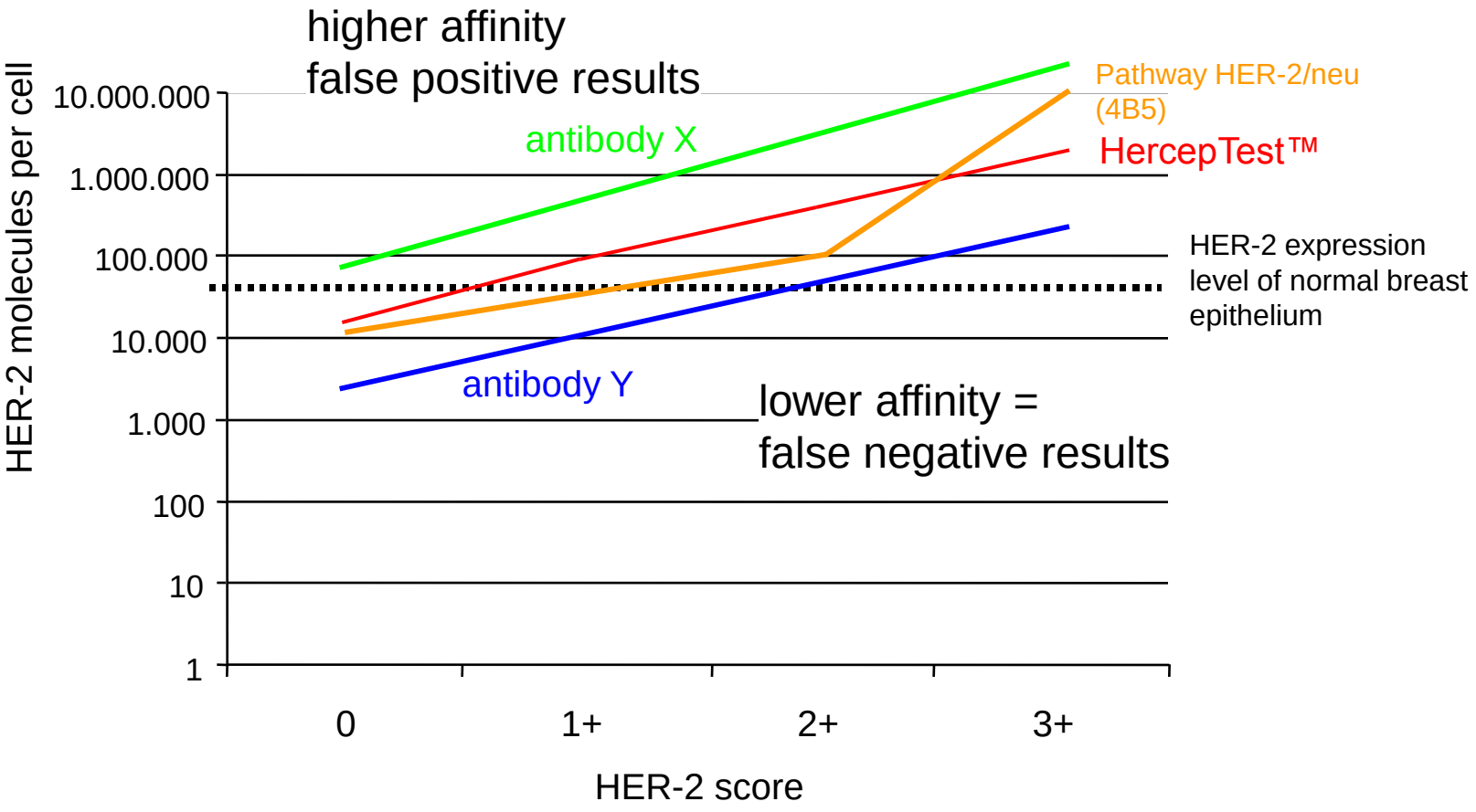
- Fixation time: 6-48 hrs (biopsy 6-24, resection specimen 12-48)

- overfixed tissue needs a longer digestion time in ISH

-Thin sections improve morphology + signal quality in ISH (2-4µm)

- **Appropriate fixation of gastric resection specimens is a problem because of delay of fixation**

How to choose the right antibody...



NordiQC breast cancer module 2011

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Table 1. The IHC systems/Abs used and the assessment marks given:

FDA approved HER-2 systems	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	56	Ventana	55	0	0	1	98 %	98 %
CONFIRM™, rmAb clone **4B5, 800-2996**	30	Ventana	28	2	0	0	100 %	100 %
HercepTest™ **SK001**	27	Dako	22	2	0	3	89 %	96 %
HercepTest™ **K5204**	6	Dako	3	1	0	2	67 %	75 %
HercepTest™ **K5207**	17	Dako	15	0	1	1	88 %	94 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	8	Leica	7	1	0	0	100 %	100 %
Abs for in-house HER-2 systems, concentrated Ab	**N**	**Vendor**	**Optimal**	**Good**	**Borderline**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
pAb clone **A0485**	35	Dako	16	6	3	10	63 %	63 %
mAb clone **CB11**	5 1 1 1	Leica/Novocastra BioGenex Monosan NeoMarkers	2	1	2	3	38 %	60 %
mAb clone **e-2-4001+3B5**	1	NeoMarkers	0	1	0	0	-	-
rmAb clone **SP3**	14 2 1 1 1	NeoMarkers Zytomed Master Diagnostica Spring Vector	8	2	0	9	53 %	64 %
rmAb clone **EP1045Y**	1	Epitomics	1	0	0	0	-	-

http://www.nordiqc.org/Run-32-B11/Assessment/assessment-B11-HER2_IHC.htm

NordiQC gastric cancer pilot module 2011

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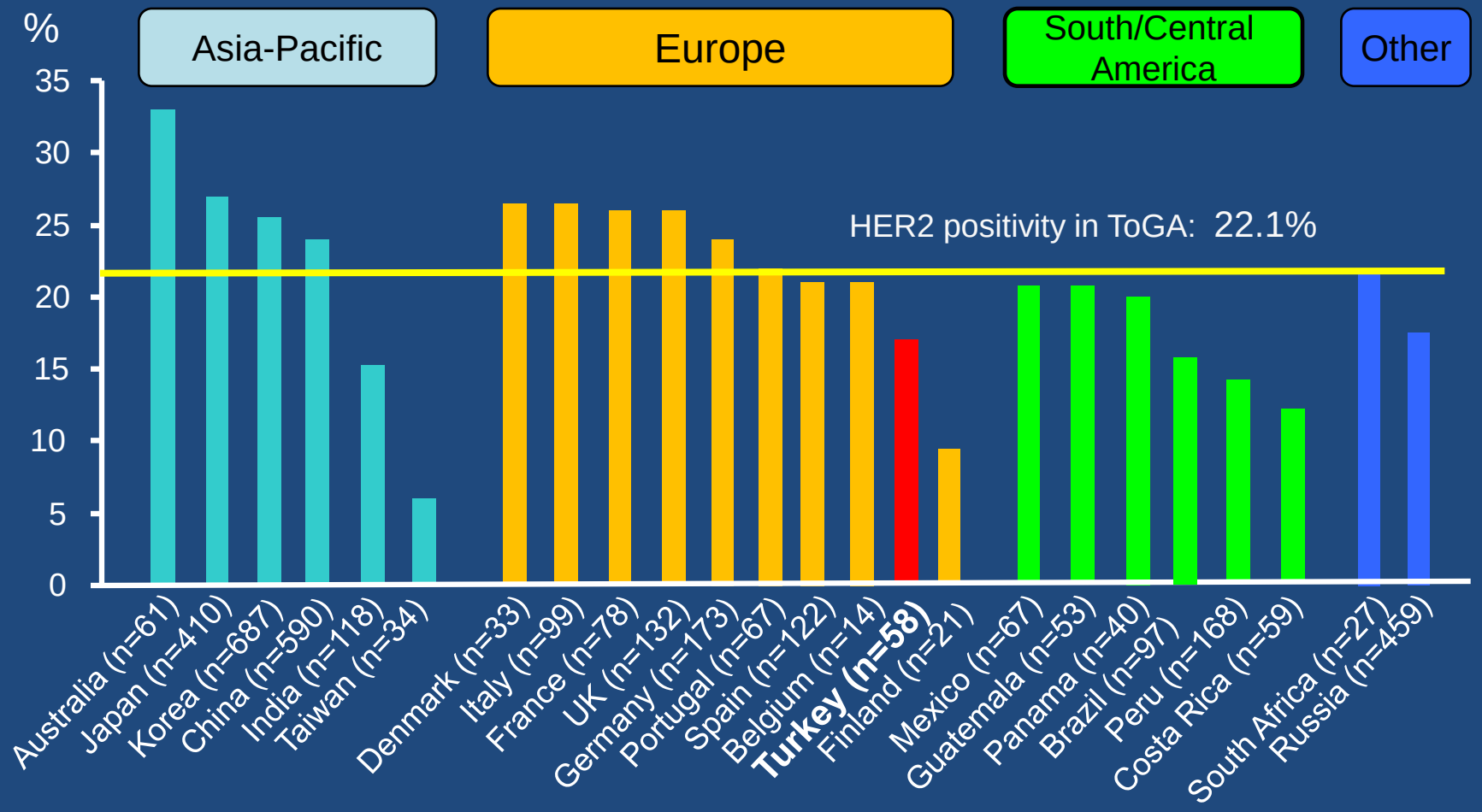
http://www.nordiqc.org/Run-31-G1/Assessment/assessment-G1-HER2.htm

Table 1. The IHC systems/Abs used and the assessment marks given:

FDA approved HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**, CONFIRM™, rmAb clone **4B5, 800-2996**	28	Ventana	20	8	0	0	100 %	100 %
HercepTest™ **K5204, K5207, SK001**	12	Dako	6	6	0	0	100 %	100 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	3	Leica/Novocastra	0	3	0	0	-	-
Abs for in-house HER-2 systems, conc. Ab.								
pAb **A0485**	7	Dako	3	3	0	1	86 %	80 %
rmAb clone **SP3**	4	NeoMarkers	0	2	0	2	-	-
rmAb clone **EP1045Y**	1	Epitomics	0	0	0	1	-	-
Total	55		29	22	0	4	-	-
Proportion			53 %	40 %	-	7 %	93 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below. 3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.

HER2-positivity rate in ToGA



Virchows Arch (2011) 459:283–289
DOI 10.1007/s00428-011-1132-8

ORIGINAL ARTICLE

Quality assessment of HER2 testing by monitoring of positivity rates

Harald Choritz · Guntram Büsche · Hans Kreipe · On behalf of the Study Group HER2 Monitor

Received: 17 May 2011 / Revised: 25 June 2011 / Accepted: 11 July 2011 / Published online: 2 August 2011

Keeping an eye on the Her2 positivity rate is a tool to control testing performance.

Keep statistics on your test results.

Table 1 HER2 positivity rates in breast and gastric cancer

	Breast cancer	Gastric cancer
Number of HER2 assay results	18,081	982
Participating institutes of pathology	42	15
Average HER2 assay number per institute±standard deviation	430±705	65±118
Mean HER2 positivity rate[a]±standard deviation	16.7±3.2%	23.2±5.7%
Range[a]	11.8–23.1%	17.6–28.1%
Outliers	6 (14.3%)	1 (6.7%)
HER2 2+ (mean rate[a]±standard deviation)	18.7±14.0%	28.7±12.7%
Range[a]	0.0–54.0%	13.9–54.6%
HER2 gene amplification rate among HER2 2+ cases	17.9±17.0%	30.5±12.1%
Range[a]	0.0–75.0%	8.3–52.2%

[a] Institutes with outlying positivity rates were not included in the calculation of the mean value and range; institutes with ≤20 gastric cancer cases and <35 breast cancer cases were merged and considered as a single group

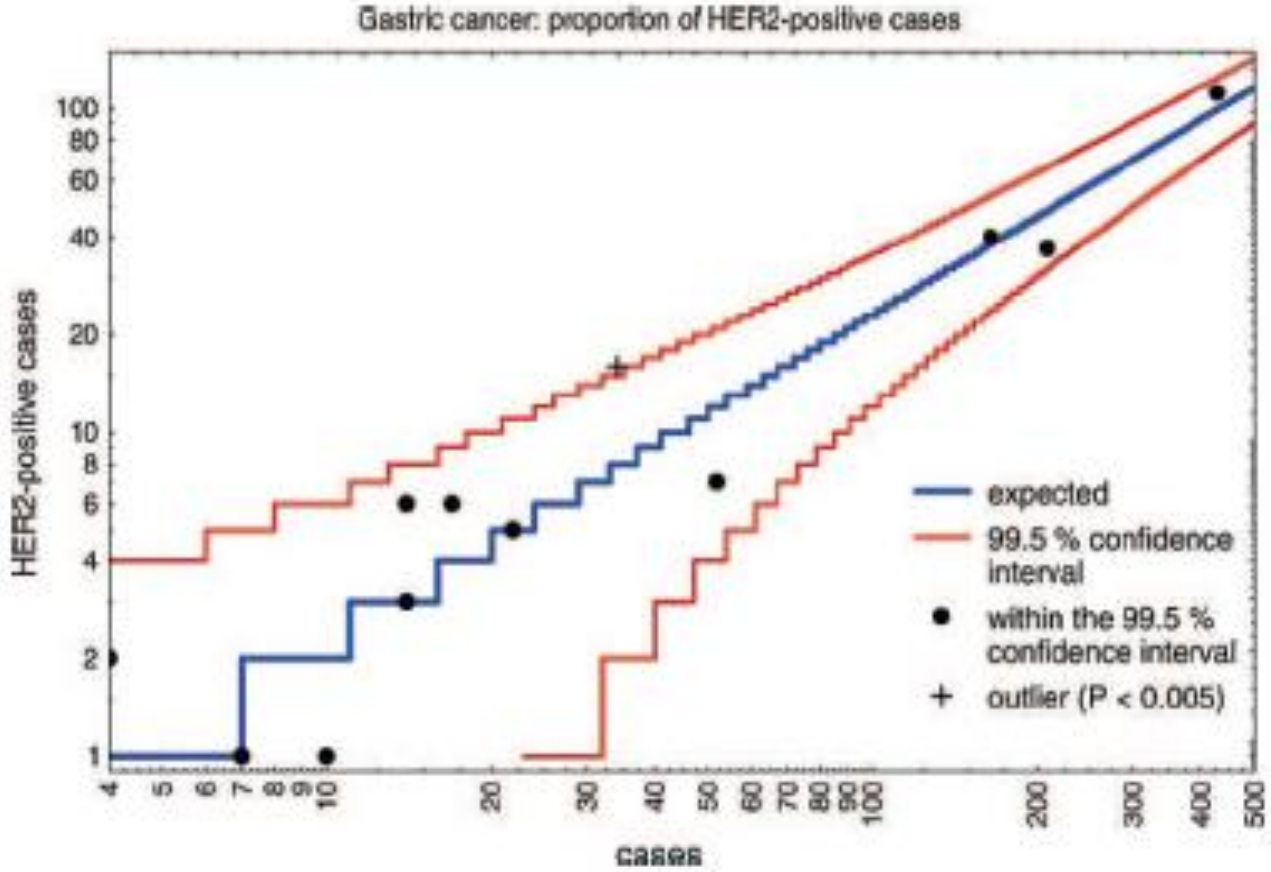


Fig. 2 The number of HER2-positive gastric cancer cases (HER2 3+, HER2 2+/amplified) per institute of pathology in relation to the number of cases investigated was plotted on a logarithmic scale. The 99.5% confidence interval is indicated by *red lines*. In institutions with a low number of assessments, the confidence interval is broader. The expected rate calculated from the mean value of 14 institutions within the 99.5% confidence interval is demonstrated by a *blue line*. There is one institution outside the 99.5% confidence interval (indicated by a *black cross*). Institutes with a positivity rate within the 99.5% confidence interval are represented by *black points*. The confidence interval might narrow over time when more assessments are available for consideration

Virchows Arch (2011) 459:283–289

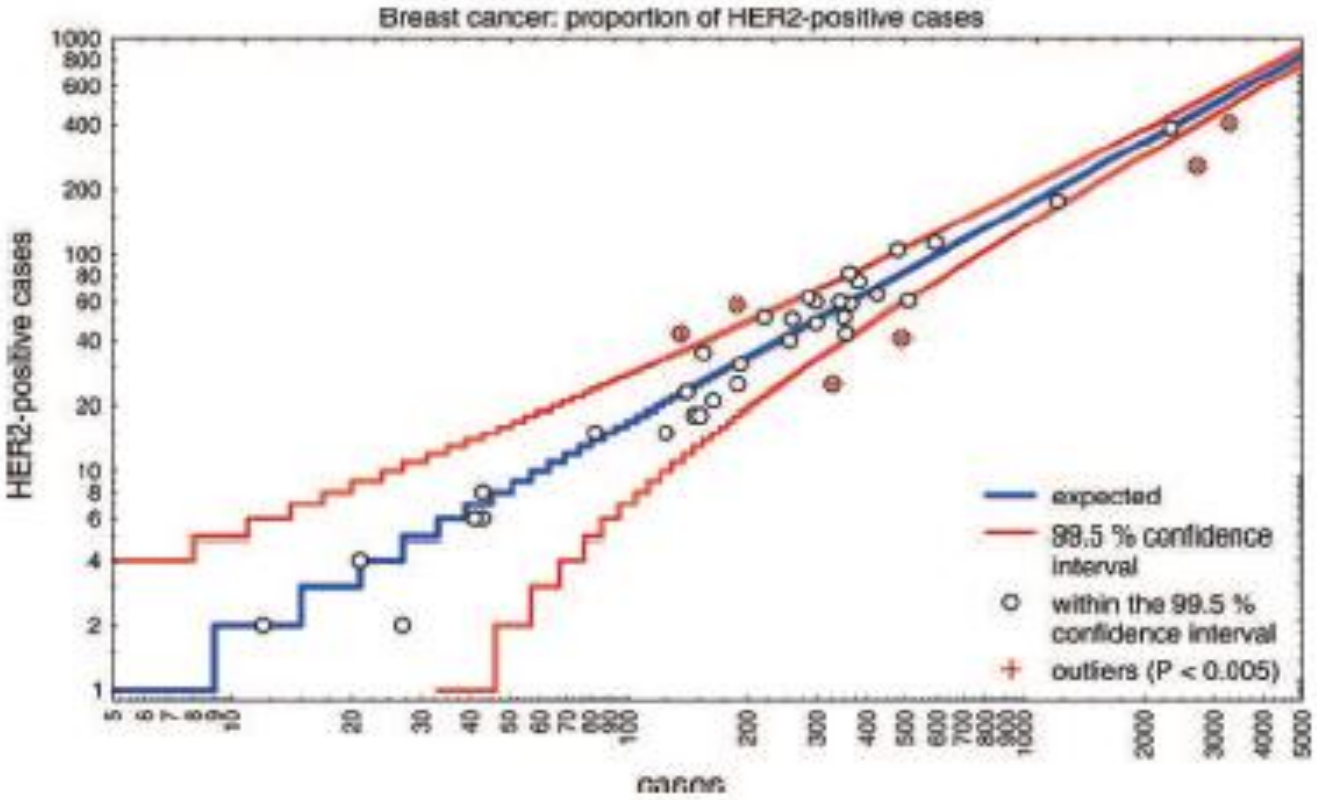


Fig. 1 The number of HER2-positive breast cancer cases (HER2 3+, HER2 2+/amplified, amplified) per institute of pathology in relation to the number of cases investigated was plotted on a logarithmic scale. The 99.5% confidence interval is indicated by *red lines*. In institutions with a low number of assessments, the confidence interval is broader. The expected rate calculated from the mean value of 36 institutions within the 99.5% confidence interval is demonstrated by a *blue line*. There are six institutions outside the 99.5% confidence interval (indicated by *red crosses*). Four of these potentially underestimate HER2 and two have a higher positivity rate than could have been expected. Institutes with a positivity rate within the 99.5% confidence interval are represented by *white circles*

FAQ quality

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Choice of antibody: standardized test platforms show much better perfomance than home brewed systems, less day to day variation.

Control your results: keep a statistical overview and search for reasons of drastic deviations from the average positivity rate (test platform, observer, fixation, etc.)

Participate in external quality control programs, such as Nordiqc (www.nordiqc.org) or UK Nequas (www.uknequas.org.uk)

Discuss and share your Her2 cases with your colleagues to „keep everybody in training“

FAQ

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Which tissue block for testing?
Pick the block with the highest percentage of intestinal differentiation.

Should all GC types be tested?
Yes, as we do in breast cancer.

Biopsy or resection specimen?
Biopsies: better fixation; appropriate for testing (ToGA data).
Retest : borderline result in biopsy; intestinal differentiation in resection specimen that was not present in biopsy.

Did patients with focal Her2 overexpression/amplification respond to therapy?
Yes, they did! (data not published yet).

Severe cytoplasmic staining should be called doubtful and controlled by ISH

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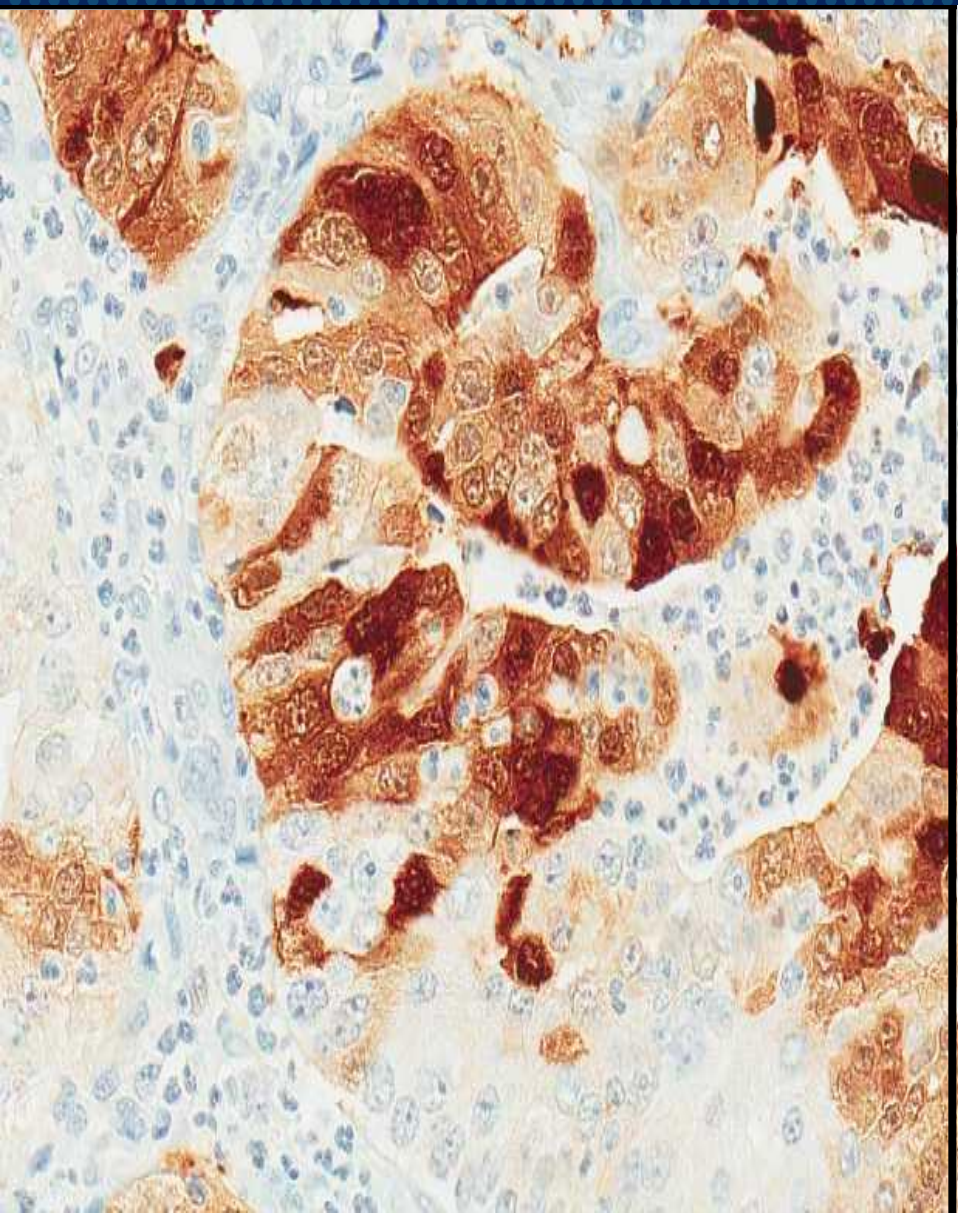

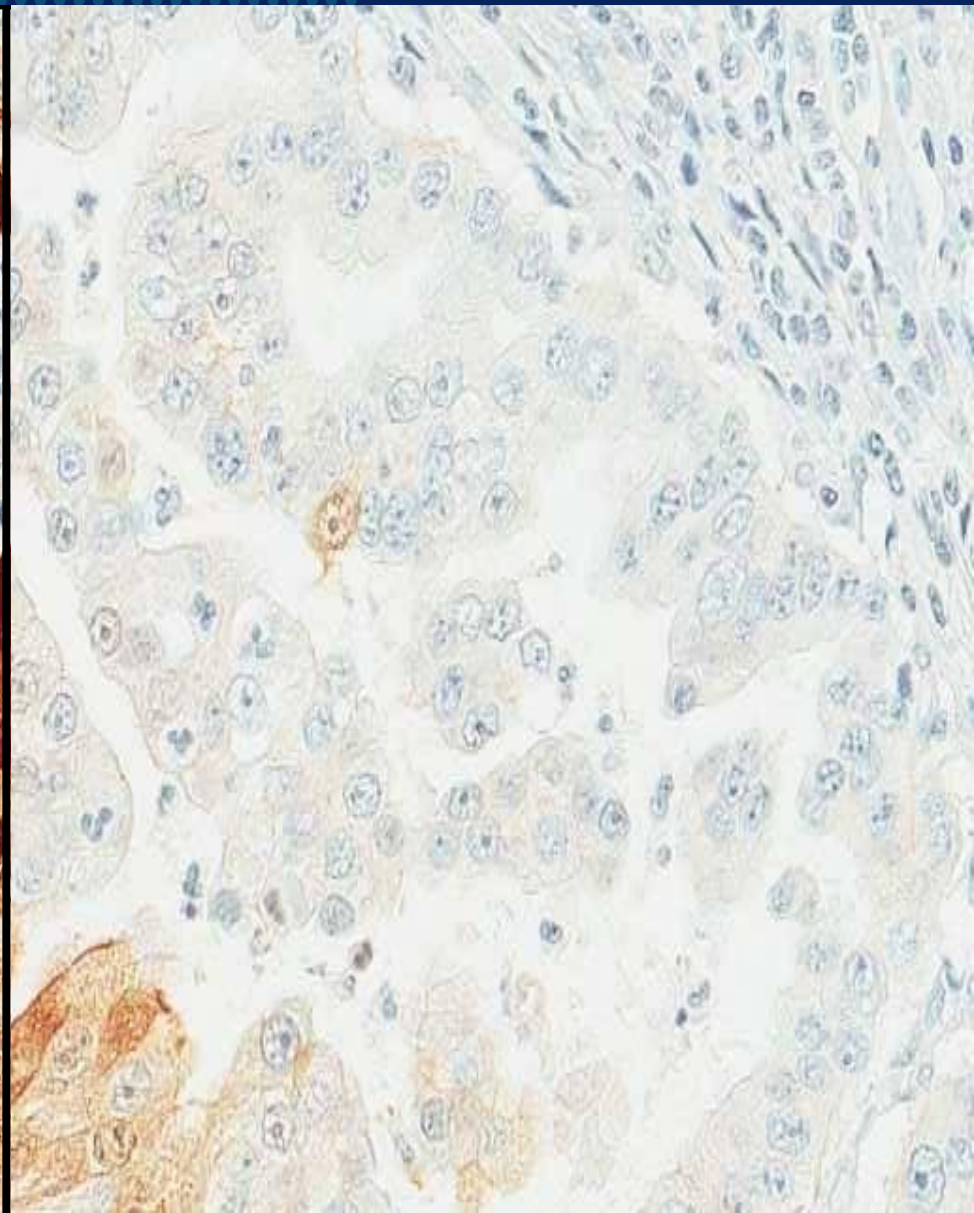

Be aware of granular basal staining!

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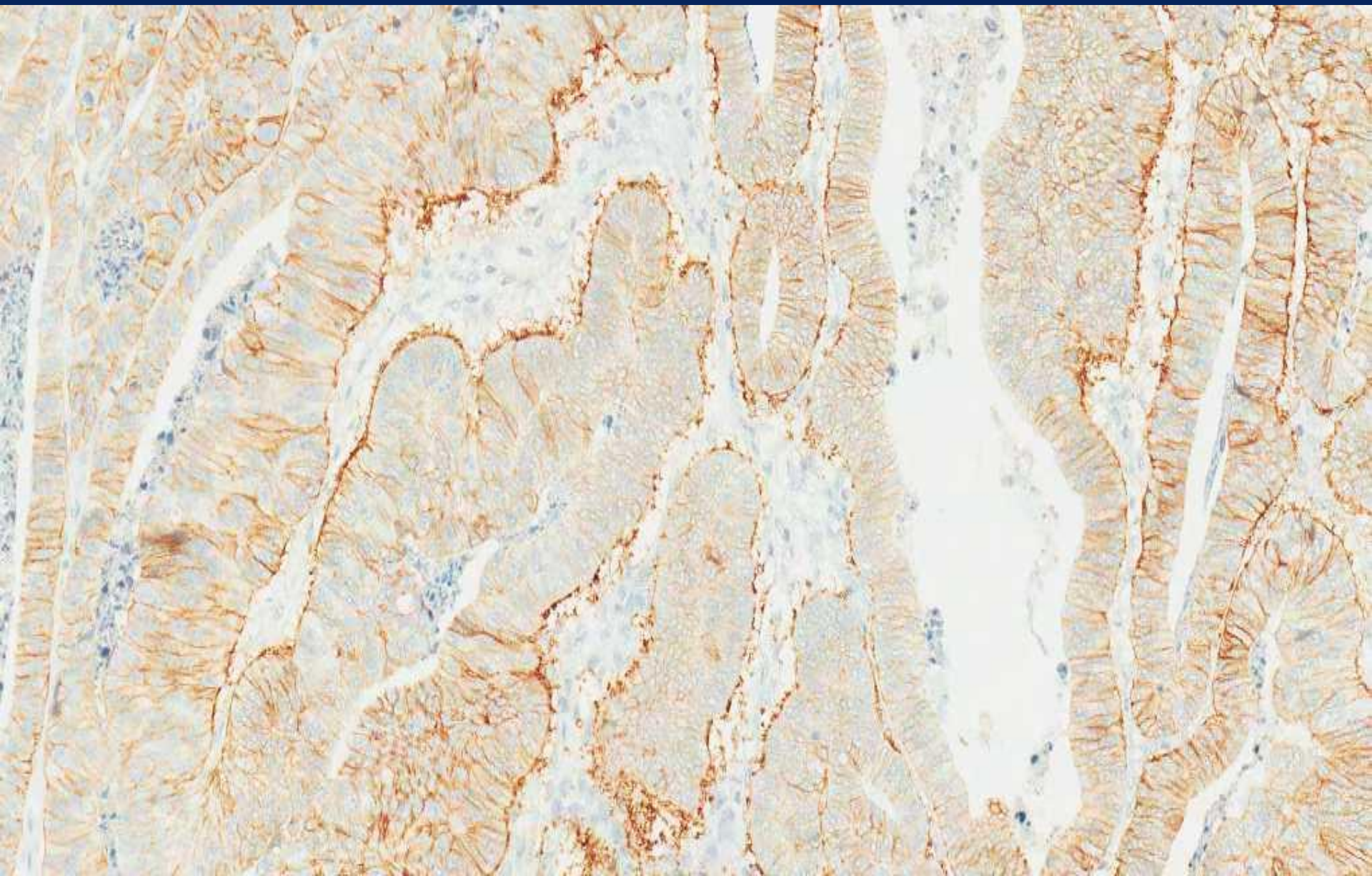

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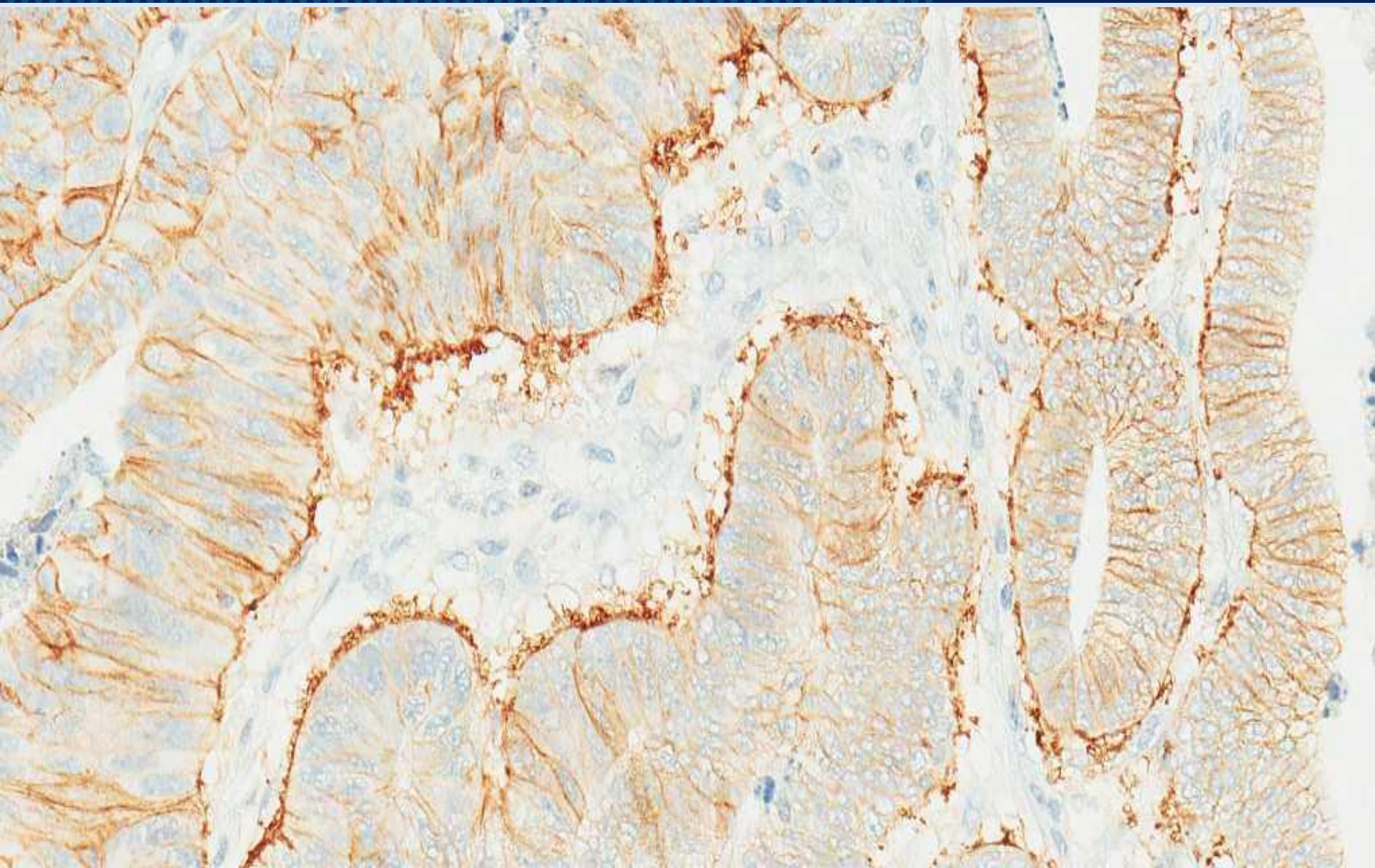

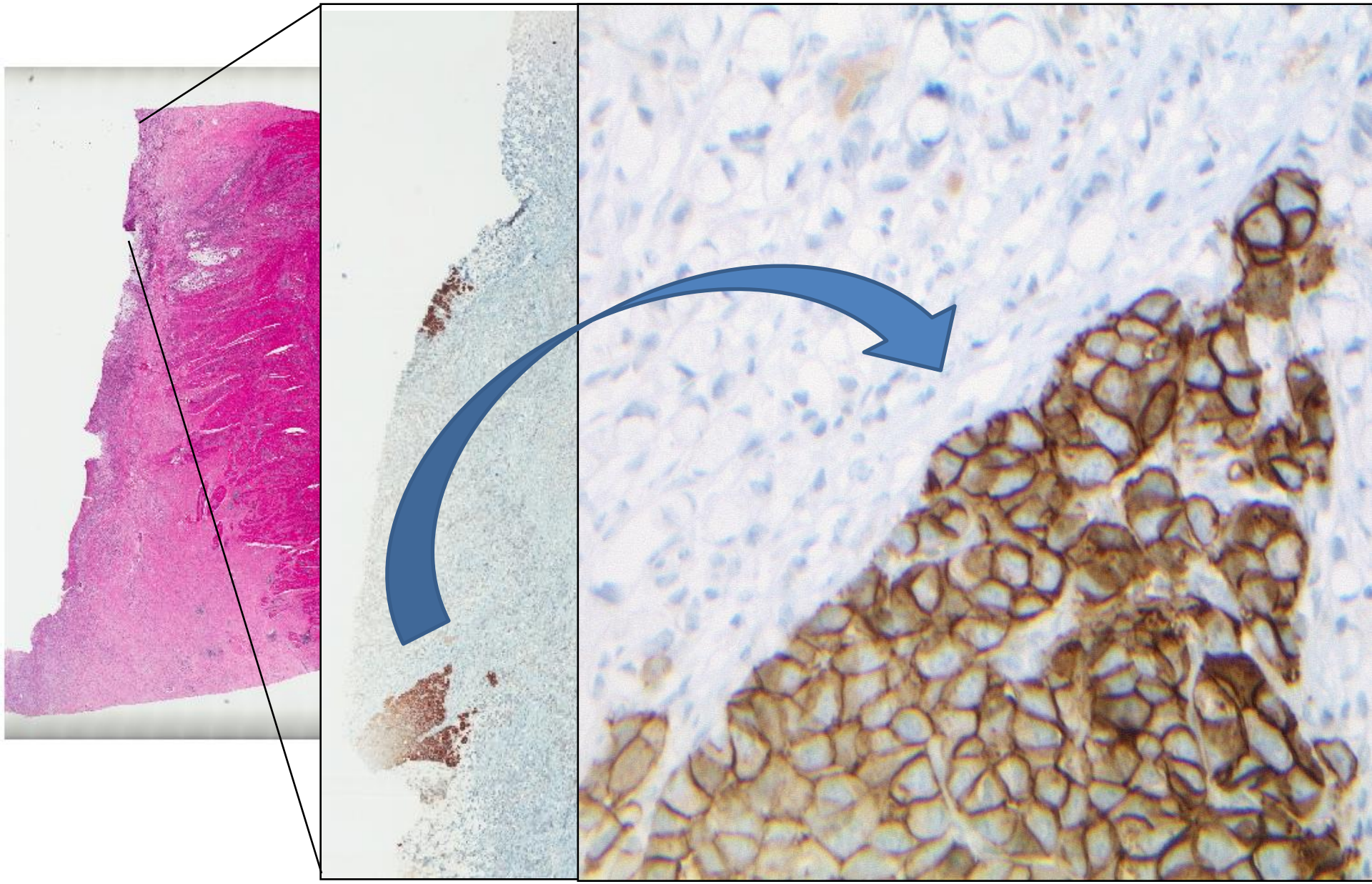

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