Application of Immunohistochemistry in Breast Pathology

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of MEDICINE

Pathology & Laboratory Medicine

IHC in Breast Pathology

- Diagnostic
- Predictive

IHC in Breast Pathology

- Diagnostic
- Predictive

Prognostic v. Predictive

- Prognostic factors are used to know *which patients* should undergo treatment.
- Predictive factors are used to decide *what treatment* should be given to *which patients*.

Validated Prognostic/Predictive Factors in 2018

Tumor Size

Histologic Grade

Histologic Type

Nodal Status

Steroid Receptor Status

HER2-*neu*

Oncotype DX

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Validated Prognostic/Predictive Factors in 2018

Tumor Size

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Histologic Type

Nodal Status

Steroid Receptor Status

HER2-*neu*

Oncotype DX

Laboratory assay for ER and HER2

Standardization!

Standardization!

Standardization!

Standardization!

Standardization!

Standardization!

Jensen EV et al. Estrogen-receptor interactions in target tissues. Arch Anat Microsc Morphol Exp 56:547-69, 1967

McGuire WL. Estrogen Receptors in human breast cancer. J Clin Invest 52:73-77, 1973

Harvey JM el al. Estrogen receptor status by immunohistochemistry is superior to ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474-1481, 1999

ASCO/CAP Guidelines
for ER/PR testing in breast cancer
2010

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version)

M. Elizabeth H. Hammond; Daniel F. Hayes; Mitch Dowsett; D. Craig Allred; Karen L. Hagerty; Sunil Badve; Patrick L. Fitzgibbons; Glenn Francis; Neil S. Goldstein; Malcolm Hayes; David G. Hicks; Susan Lester; Richard Love; Pamela B. Mangu; Lisa McShane; Keith Miller; C. Kent Osborne; Soonmyung Paik; Jane Perlmutter; Anthony Rhodes; Hironobu Sasano; Jared N. Schwartz; Fred C. G. Sweep; Sheila Taube; Emina Emilia Torlakovic; Paul Valenstein; Giuseppe Viale; Daniel Visscher; Thomas Wheeler; R. Bruce Williams; James L. Wittliff; Antonio C. Wolff

Arch Pathol Lab Med—Vol 134, July 2010

“Up to 20% of current IHC determinations of ER and PR testing worldwide may be inaccurate”

Arch Pathol Lab Med—Vol 134, July 2010

- Preanalytic variables
- Thresholds for positivity
- Interpretation criteria

Immunohistochemistry

Results depend on:

- Antigen preservation
  - Fixation (adequacy and delays)
  - Fixative
- Antigen retrieval
- Antibody clone

Fixative and Fixation

Adequacy and Delays

Tumor removal to fixation ➡ ≤1 hour

Specimen sectioned at 5mm intervals

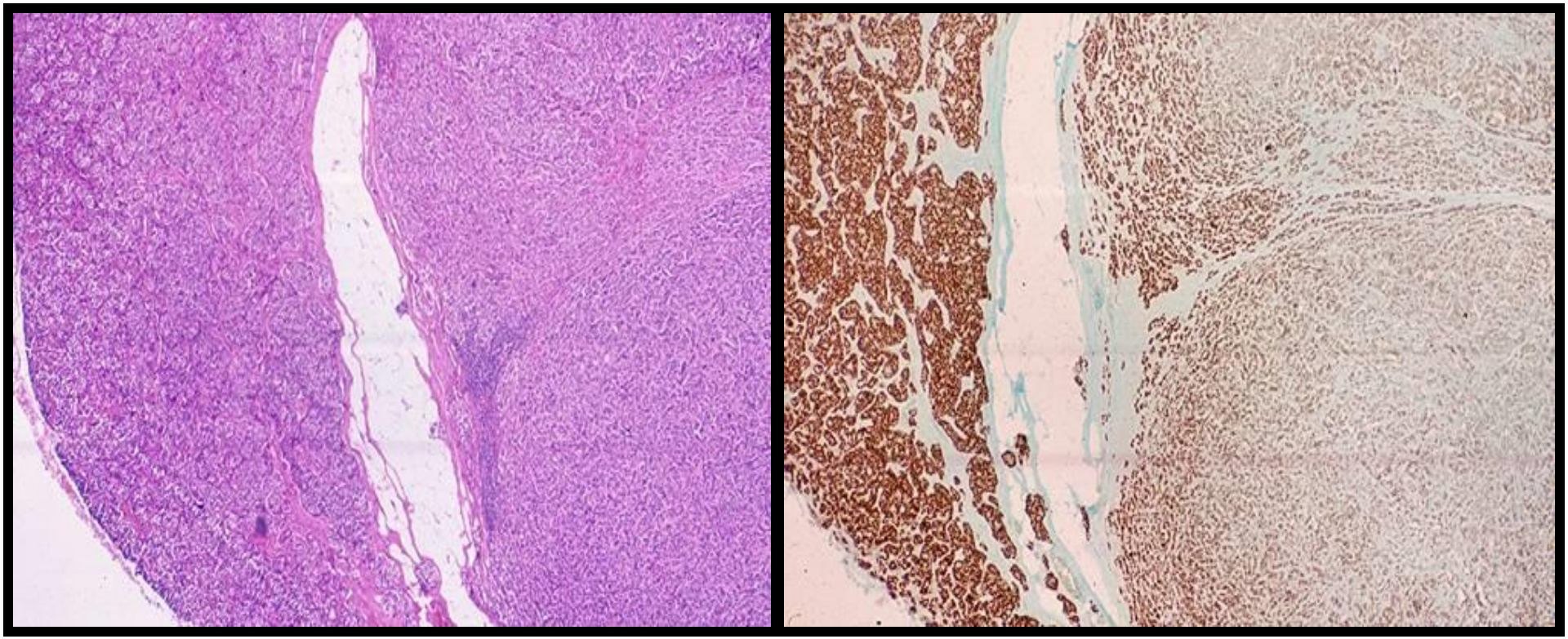
10% Neutral Buffered Formalin

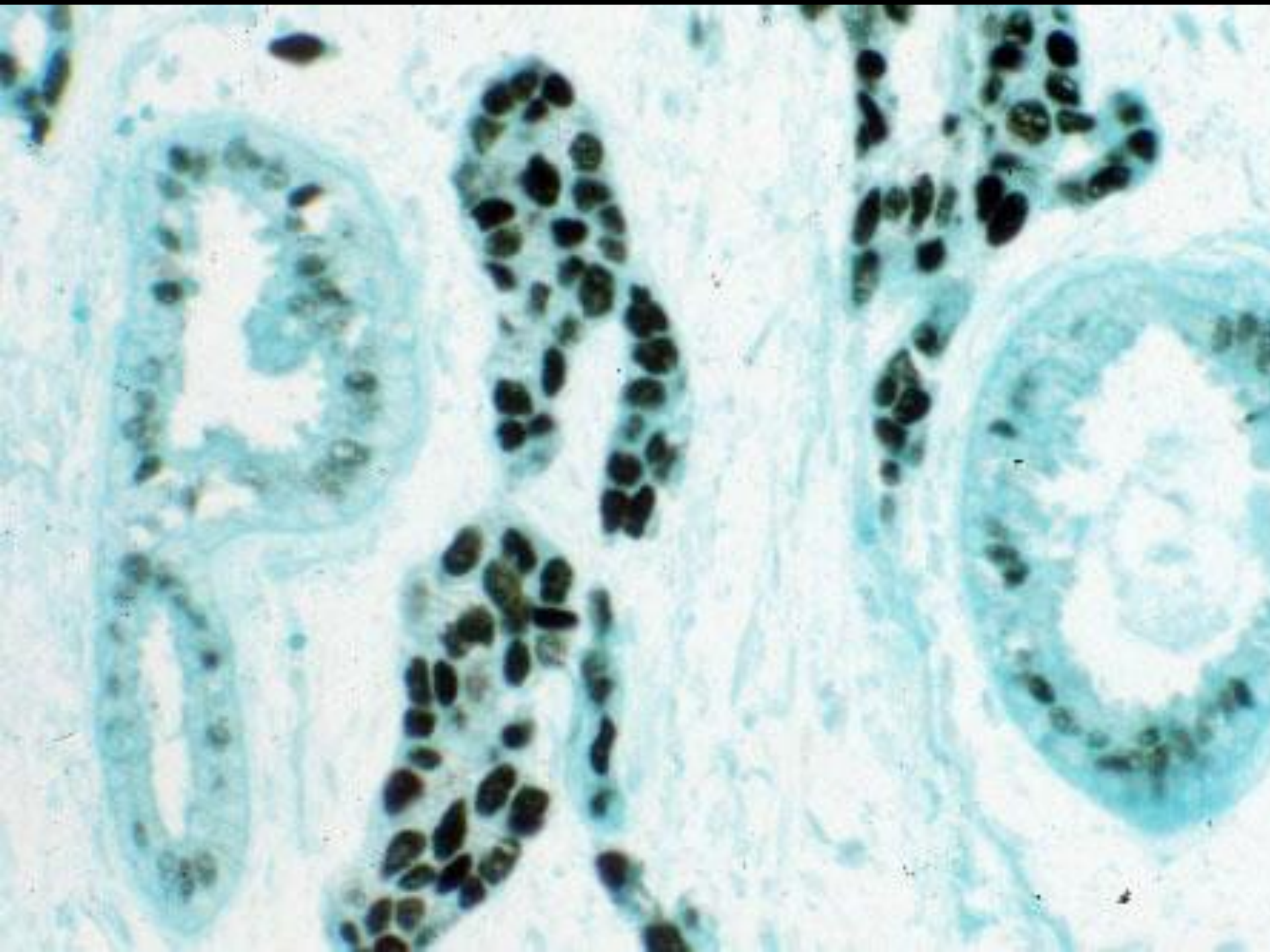
NBF volume 10x greater than specimen volume

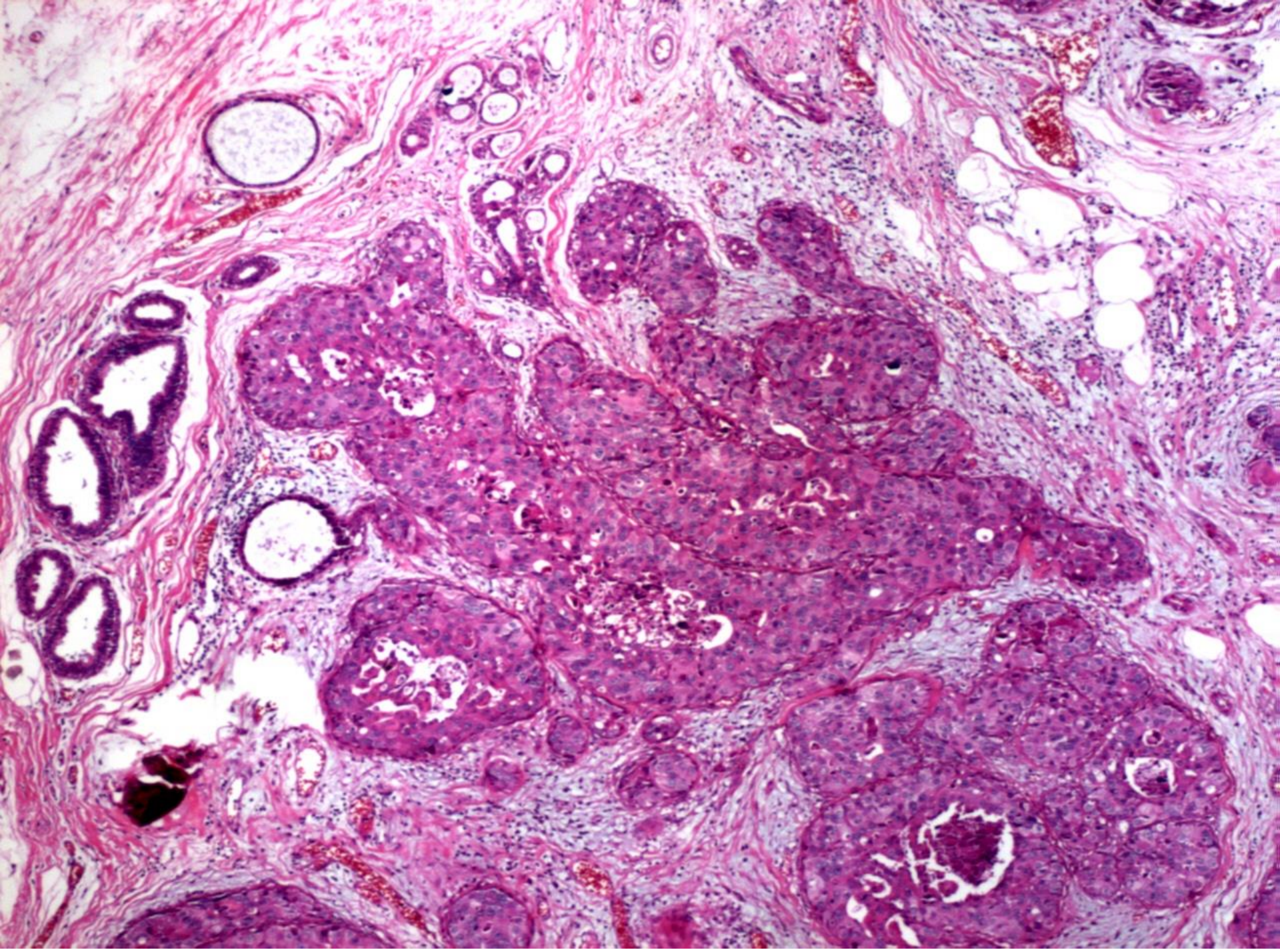
Fixation time min 6 hrs and max 72 hrs

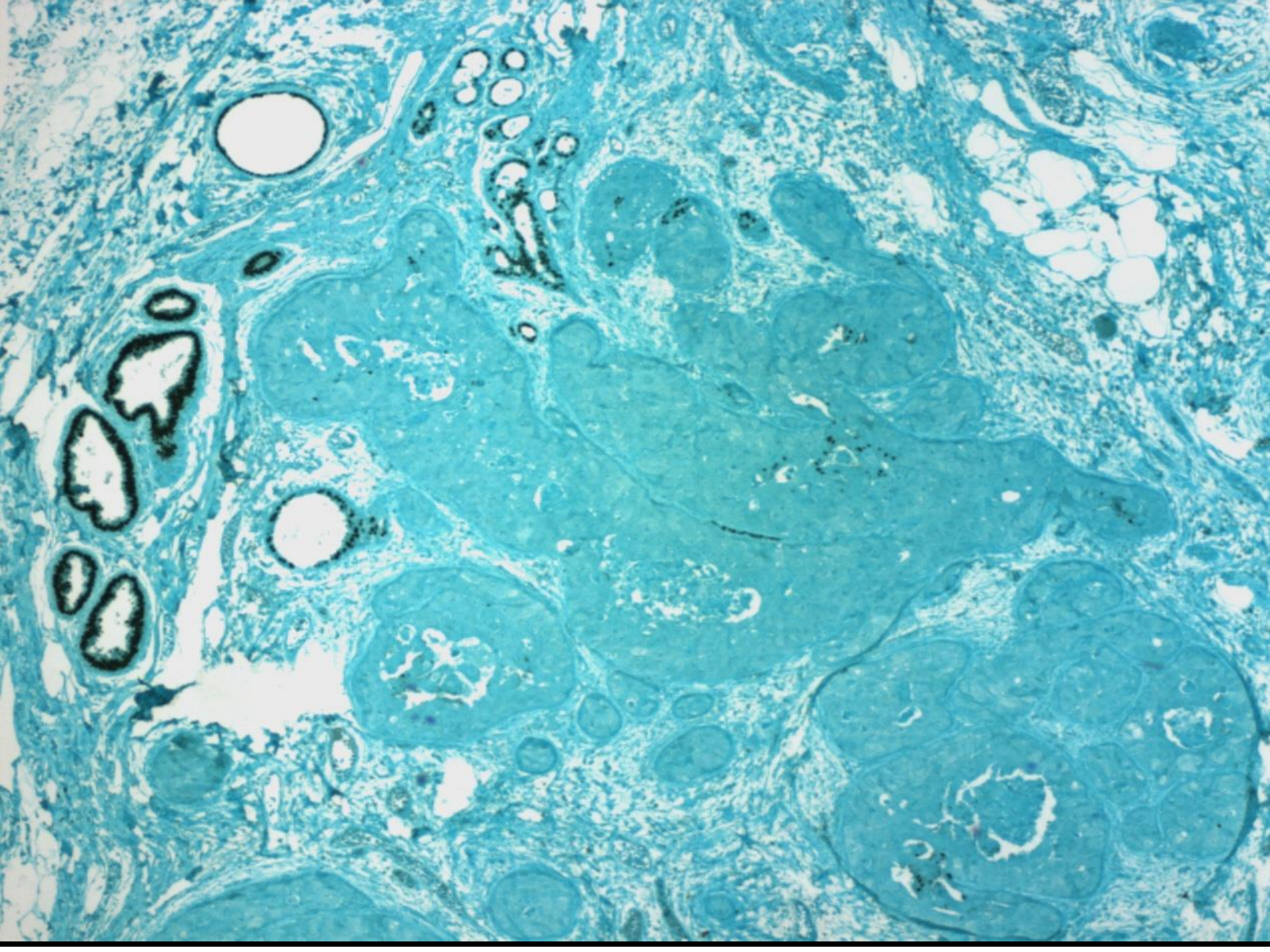

BIOHAZARD

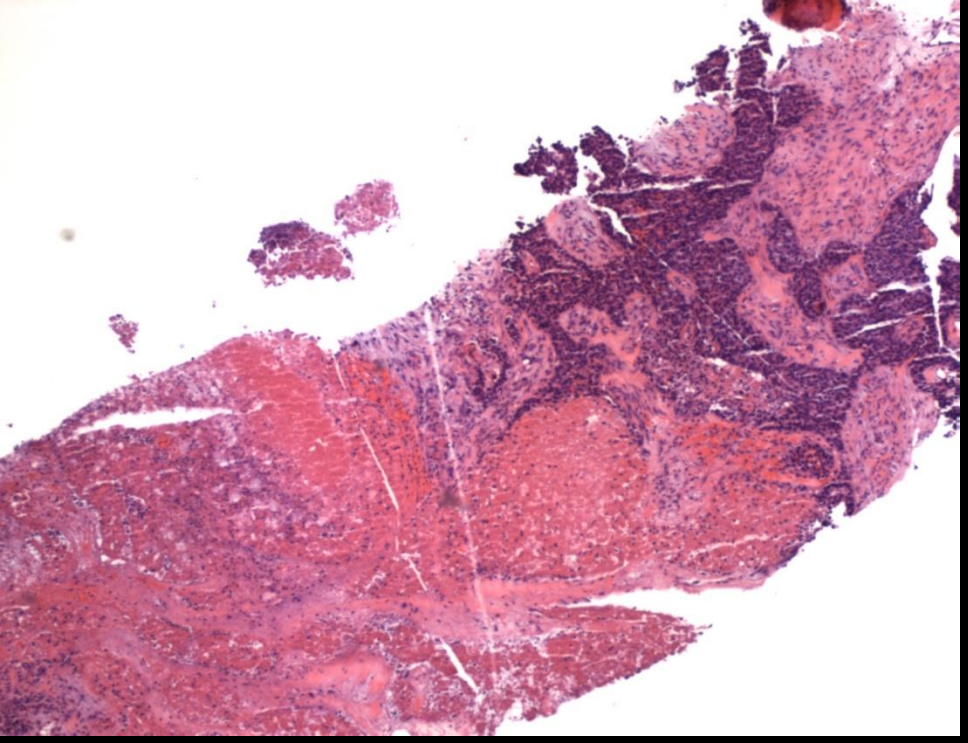
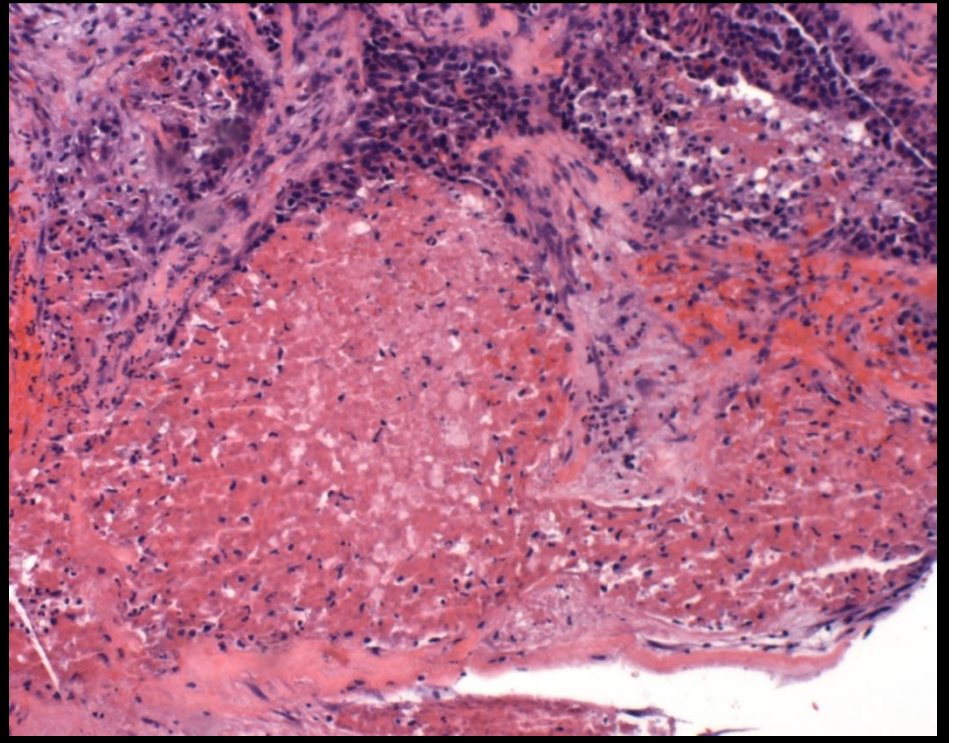
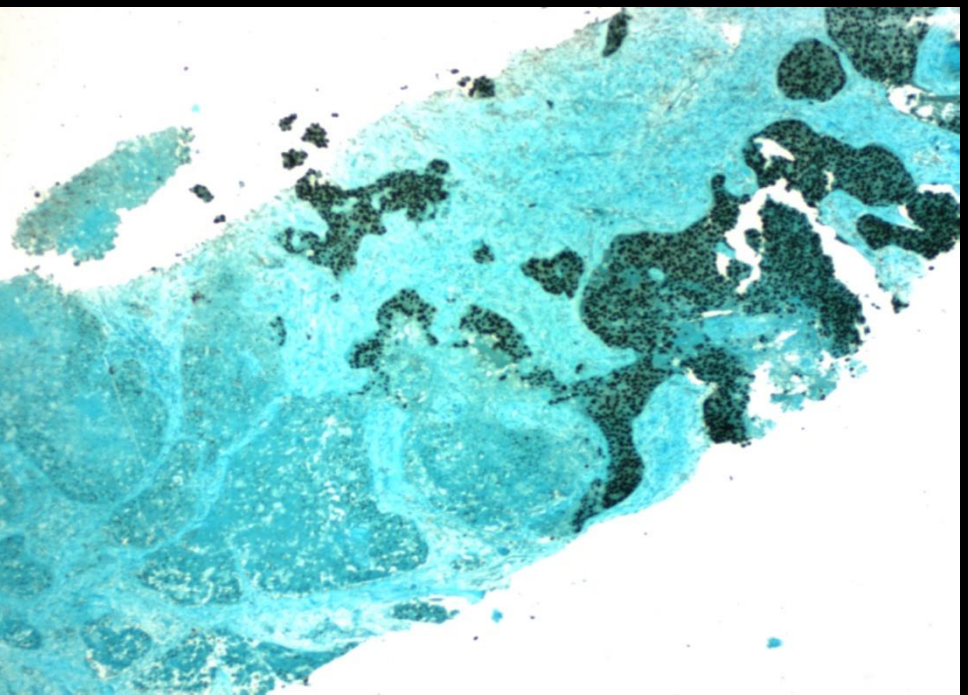
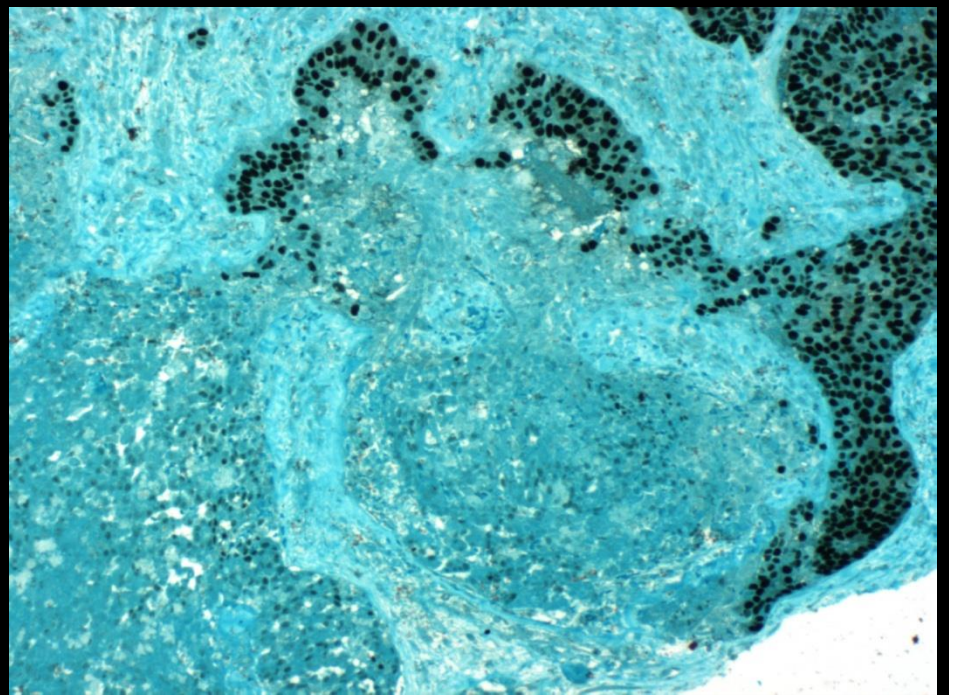
“Artifact of Fixation”









IHC for ER

Commercially available antibody clones:

6F11

1D5

SP1

ER in Breast Cancer

Using ER1D5:

- ER status is “all or none”
- ER in FNA/core biopsy = ER in excisional biopsy
- Do not find ER(-)/PR(+) results
- ER is a stable phenotype in recurrence/metastases

* Nadji M. Gomez-Fernandez C, Ganjei-Azar P et al. Immunohistochemistry of Estrogen and Progesterone Receptors Reconsidered. Experience with 5993 Breast Cancers. AJCP 2005; 123:21-7.

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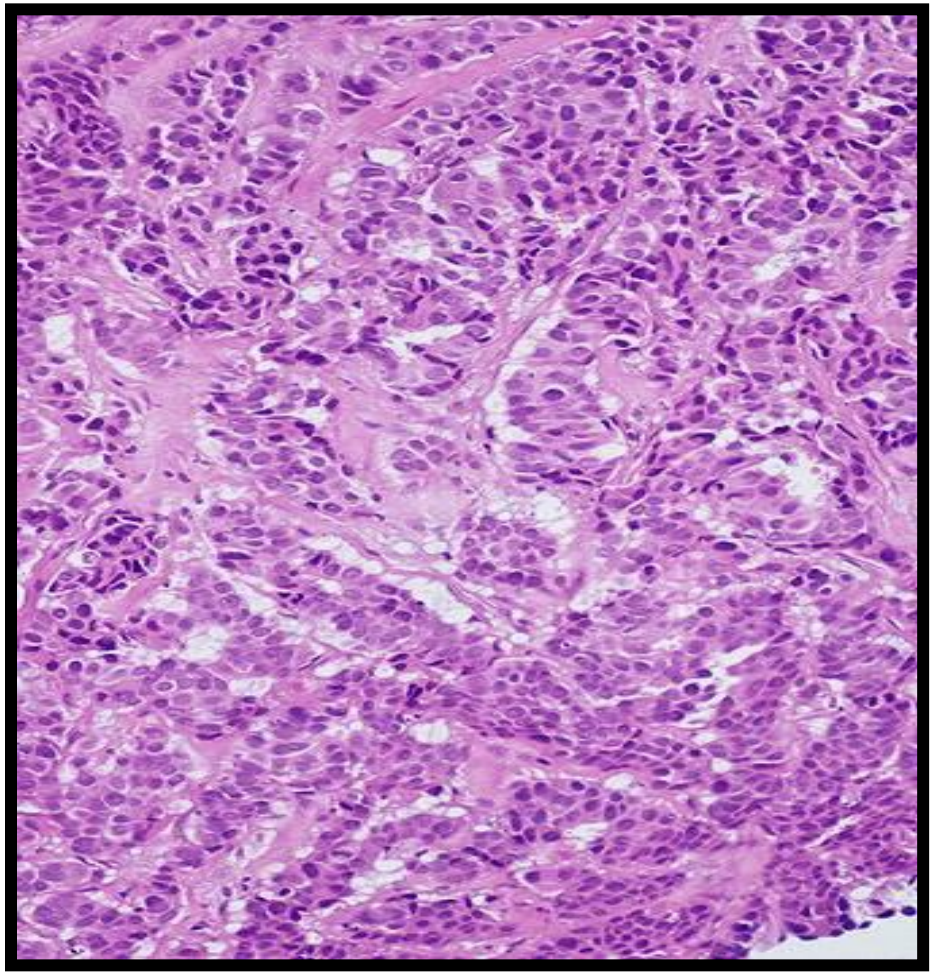
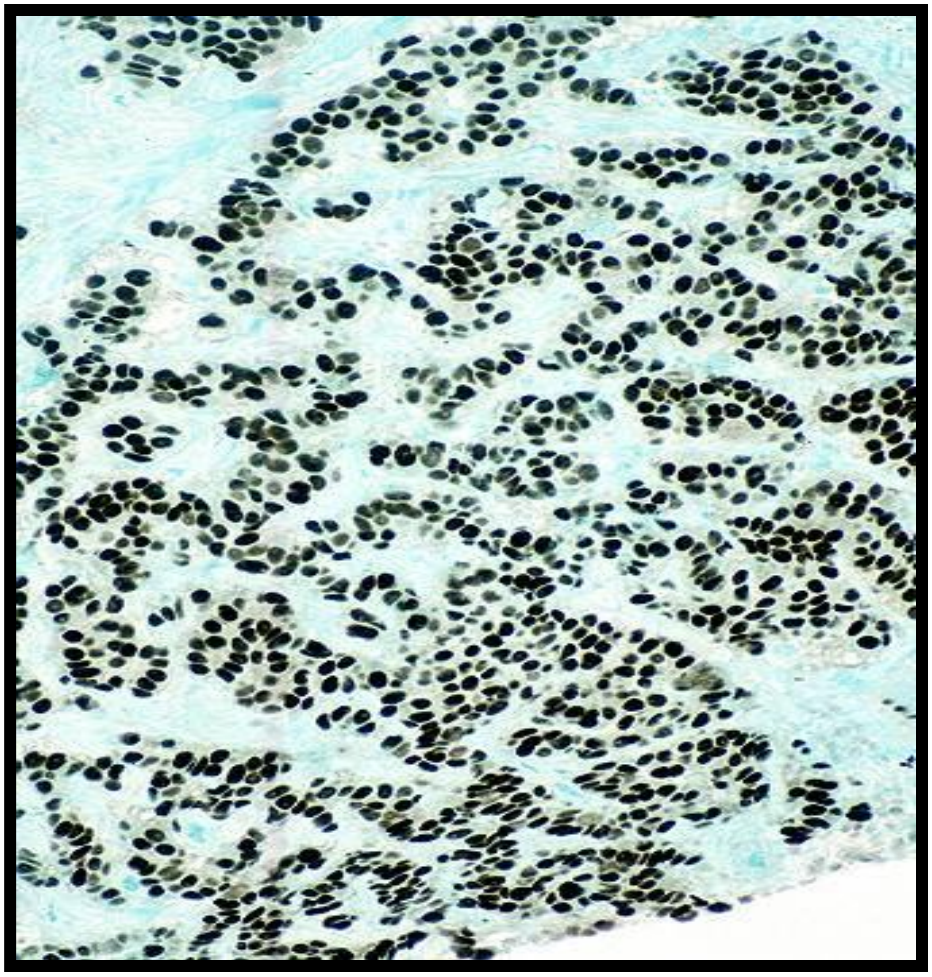
“All or None”





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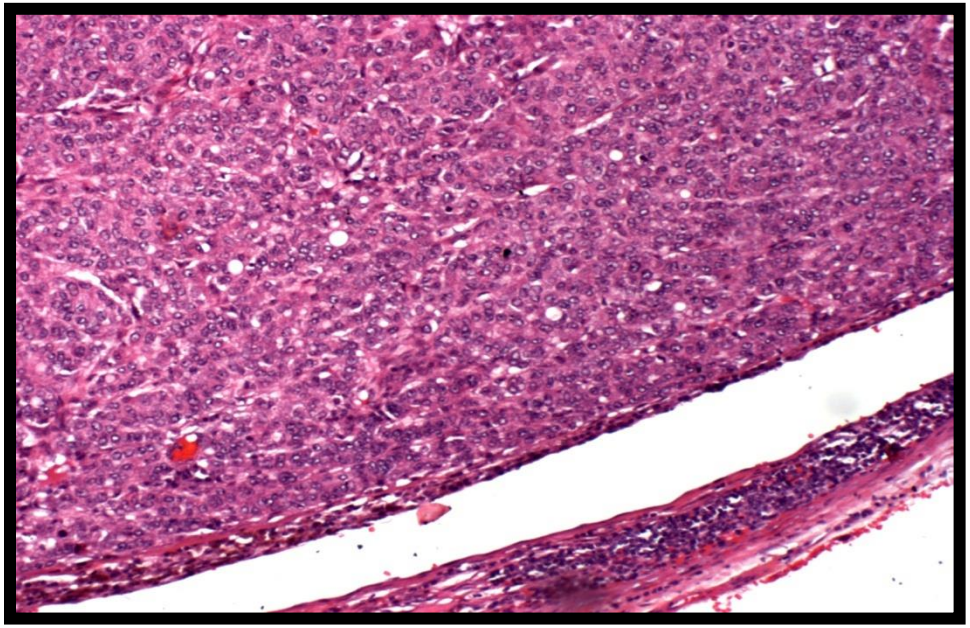
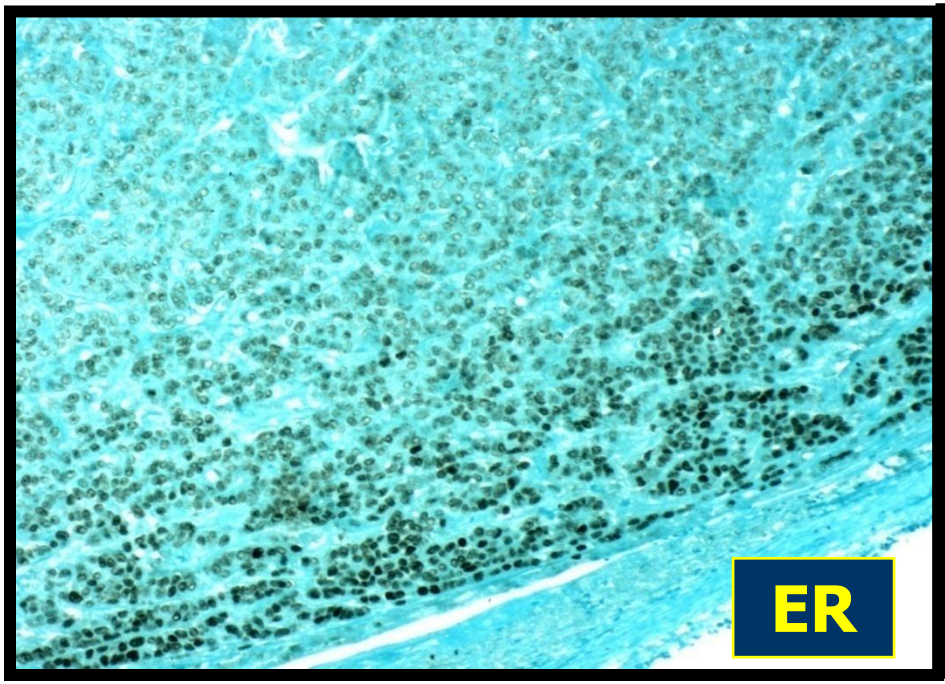
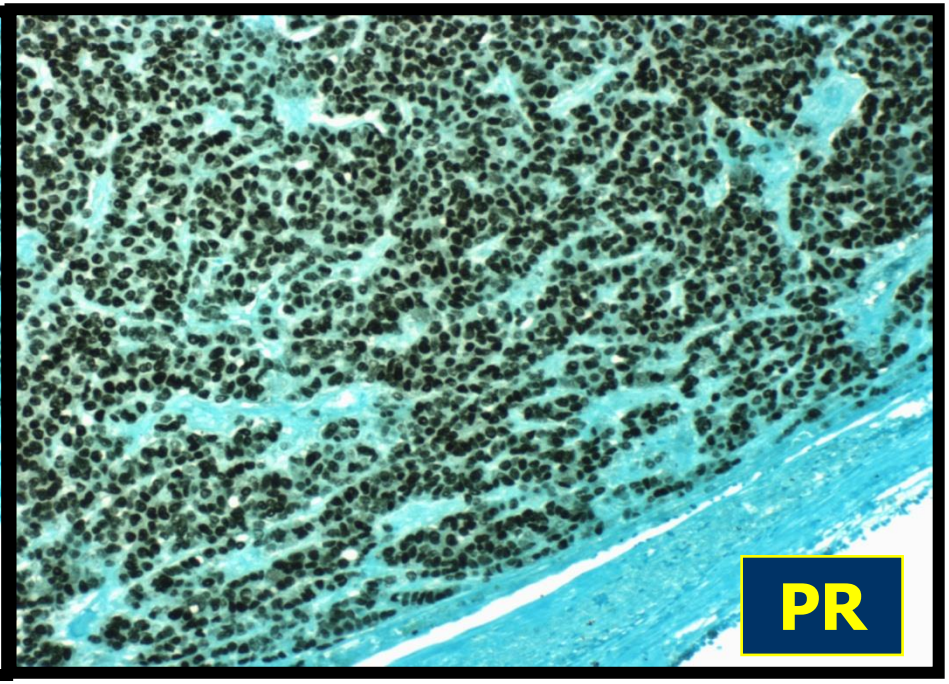
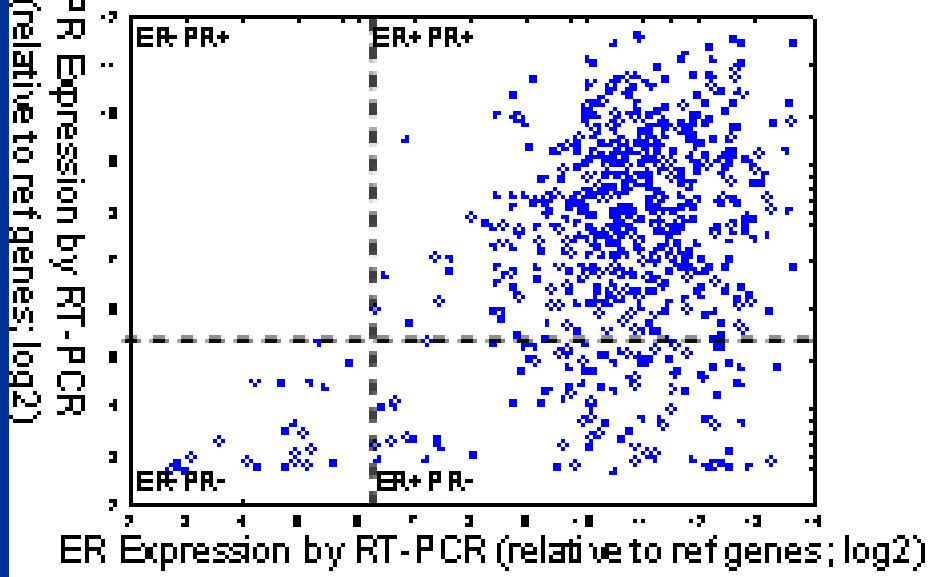



ER


PR

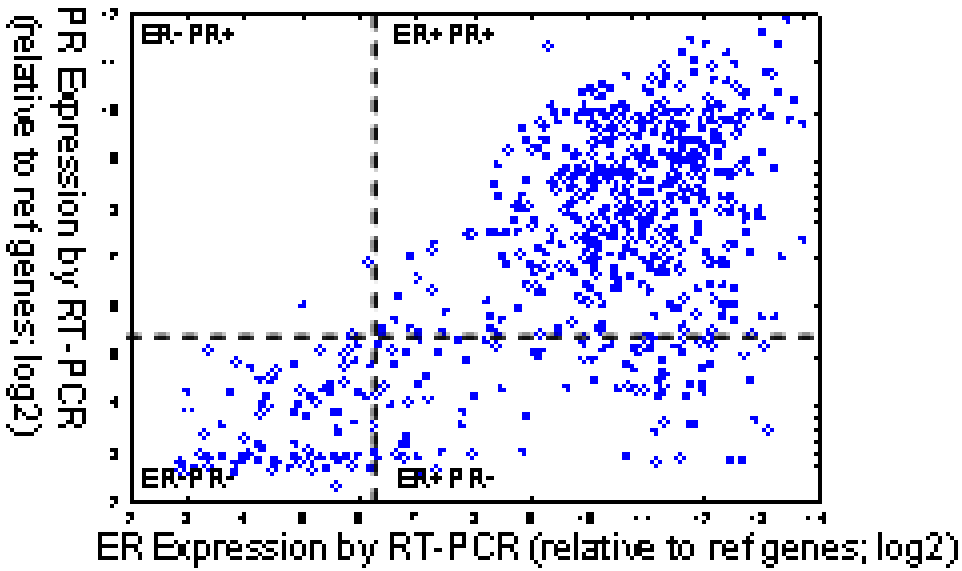
Clinical Studies: Quantitative Distribution of ER and PR

NSABP B-14
n = 645



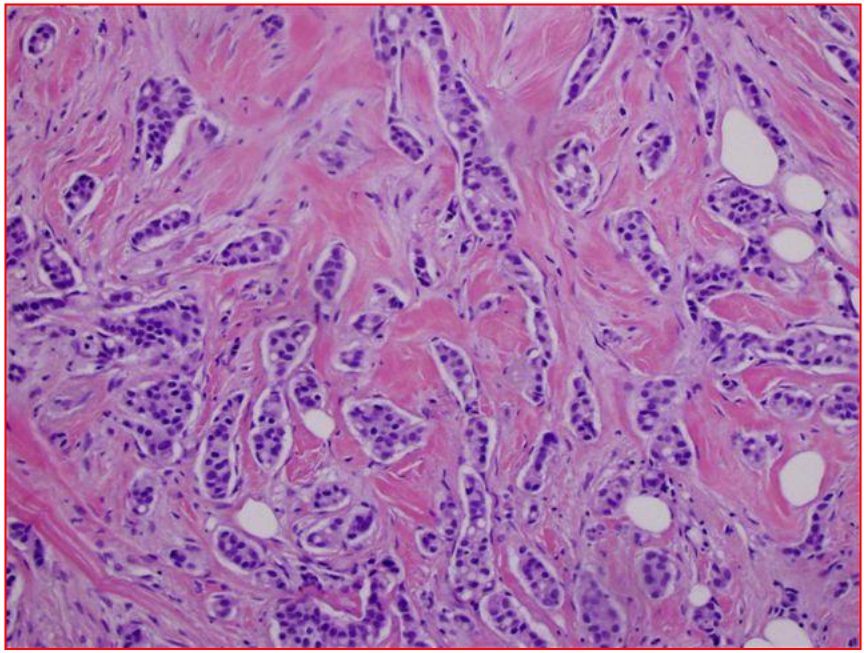
R = 0.38

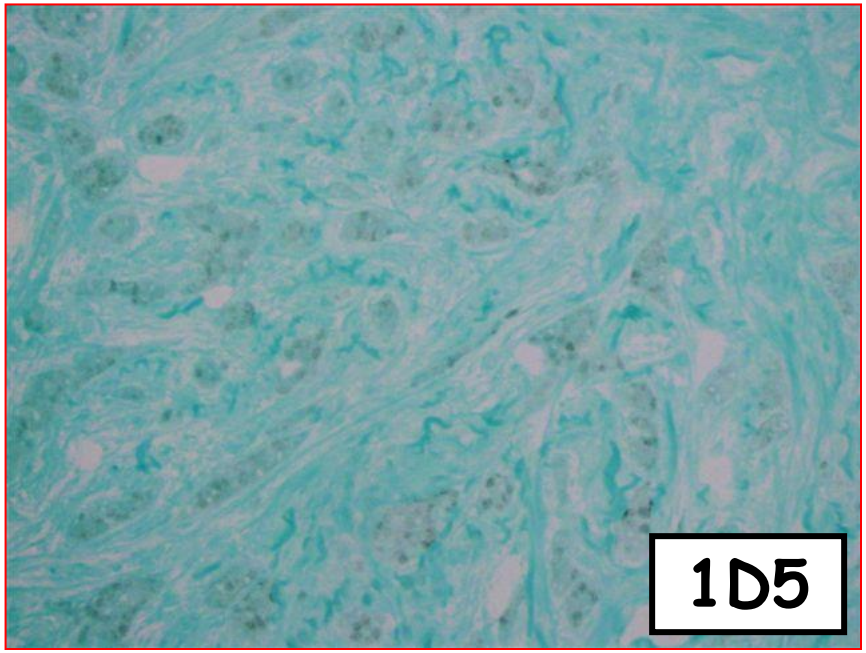
Kaiser Permanente
220 cases & 570 controls

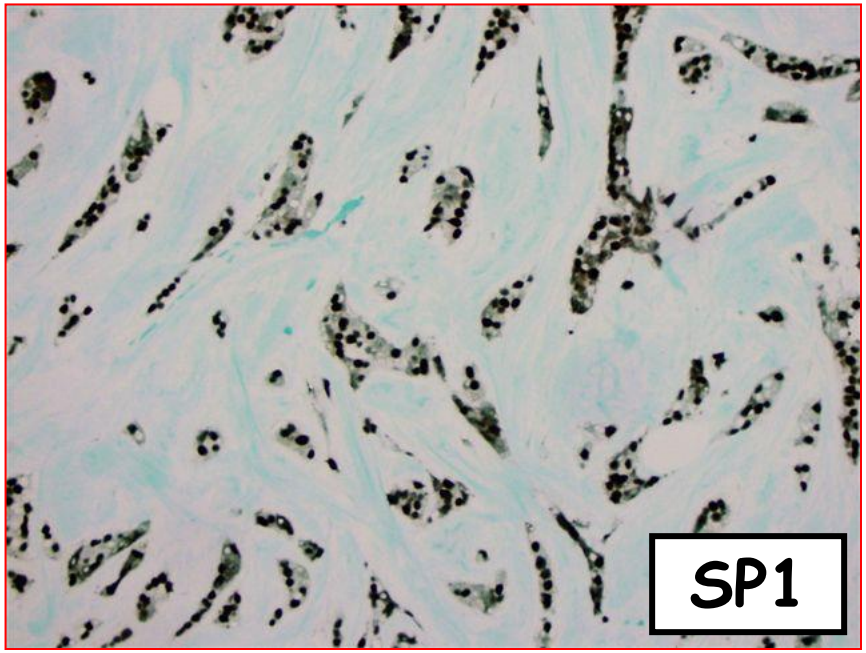


R = 0.66

ER and PR are modestly to moderately correlated




1D5


SP1

Immunohistochemical Expression of Estrogen Receptor in Adenocarcinomas of the Lung

Gomez-Fernandez C et al. AIMM, 2010

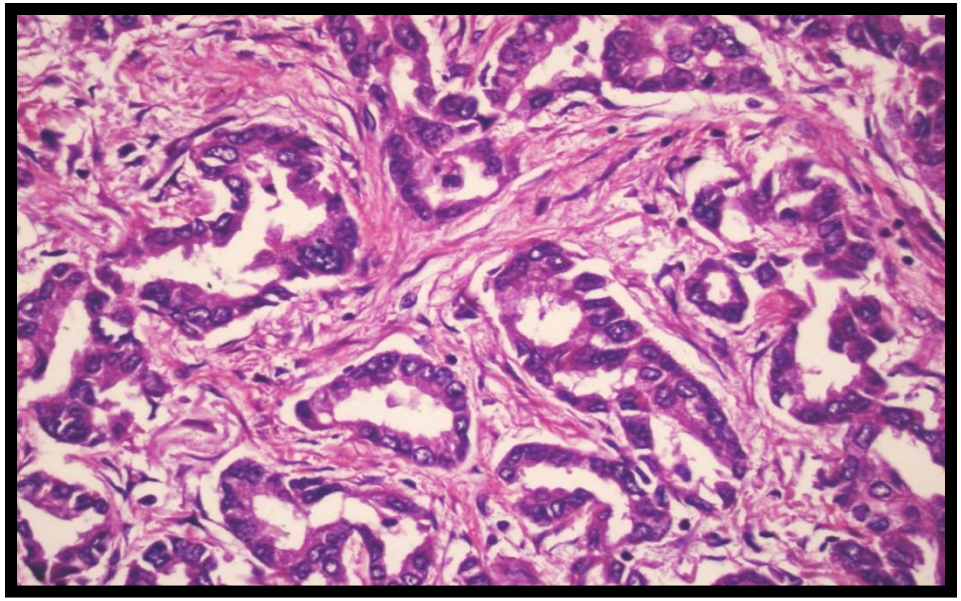
N (%) ER Positive Staining

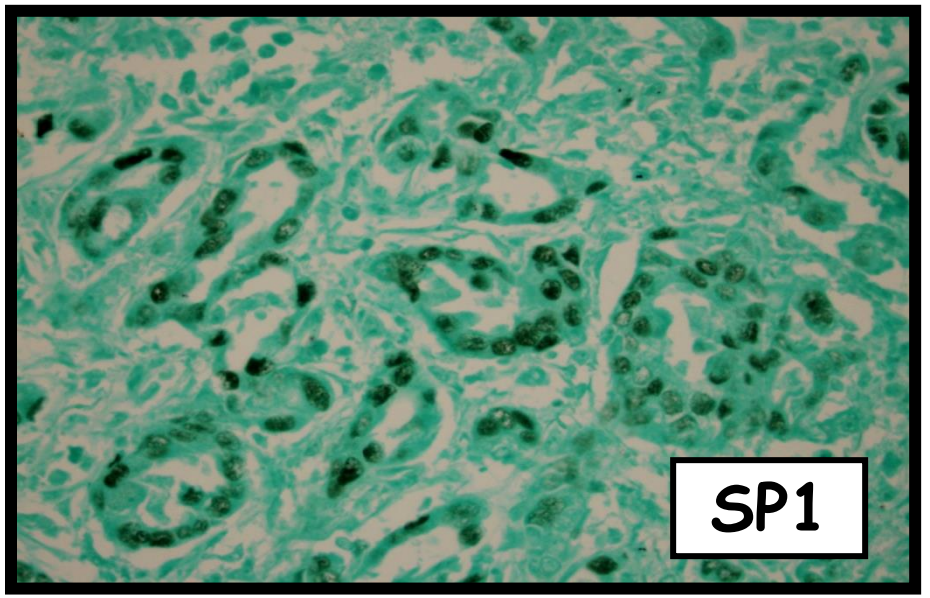
	N	SP1	6F11	1D5
ALL	70	20 (28.6)	11 (15.7)	6 (8.6)

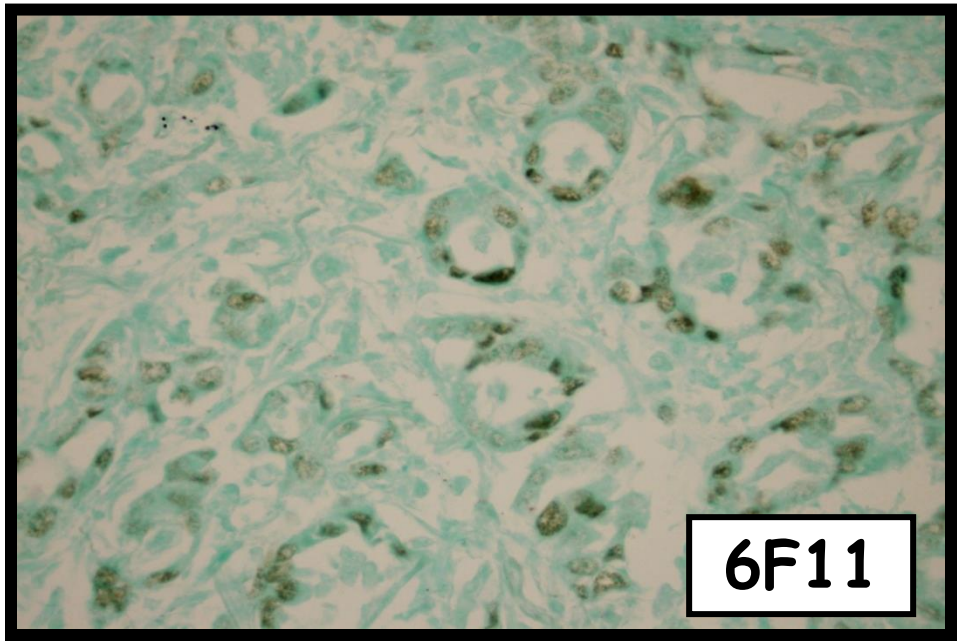
P=0.004

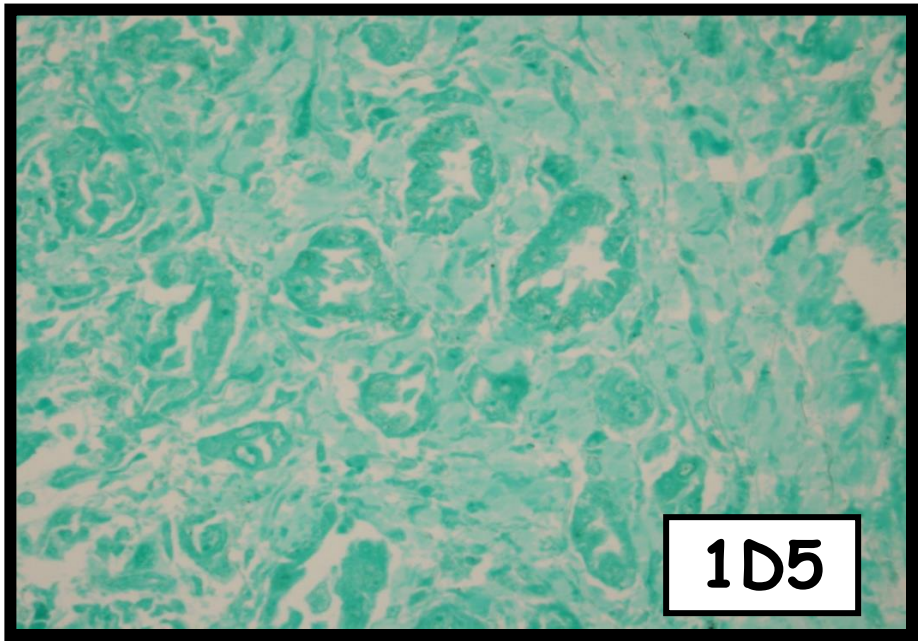
NS

P<0.001




SP1


6F11


1D5

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Using ER1D5:

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- ER is a stable phenotype in recurrence/metastases

Gomez-Fernandez C. et al. Am J Clin Pathol 2008

Immunohistochemically Determined Estrogen Receptor Phenotype Remains Stable in Recurrent and Metastatic Breast Cancer

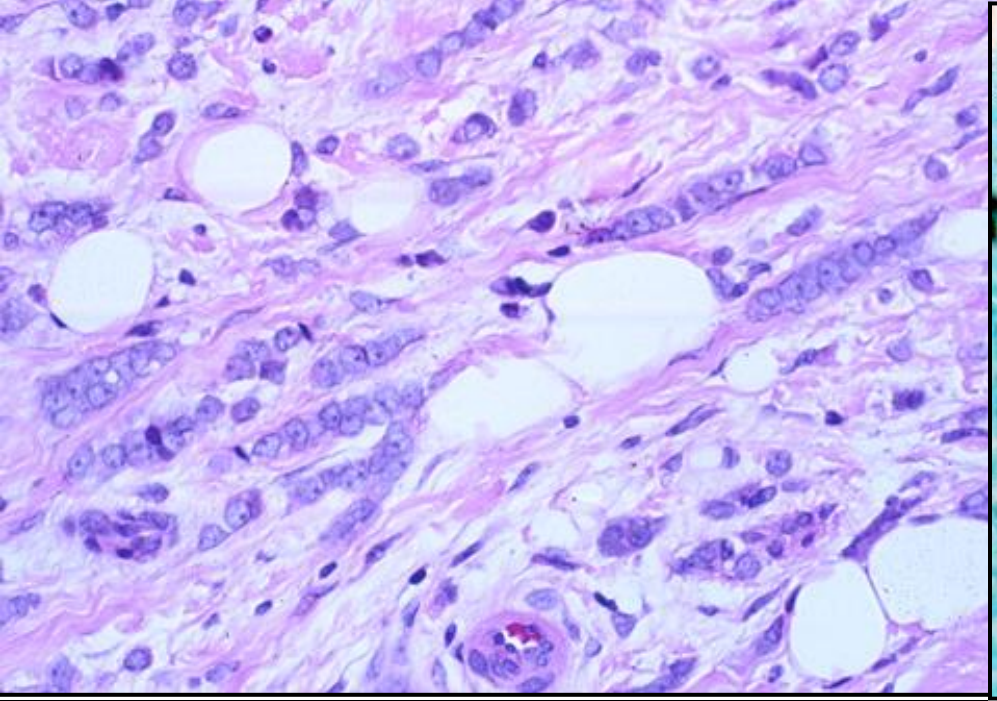
Carmen Gomez-Fernandez, MD,[1] Yahya Daneshbod, MD,[2] Mehdi Nassiri, MD,[1] Clara Milikowski, MD,[1] Consuelo Alvarez, MD,[1] and Mehrdad Nadji, MD[1]

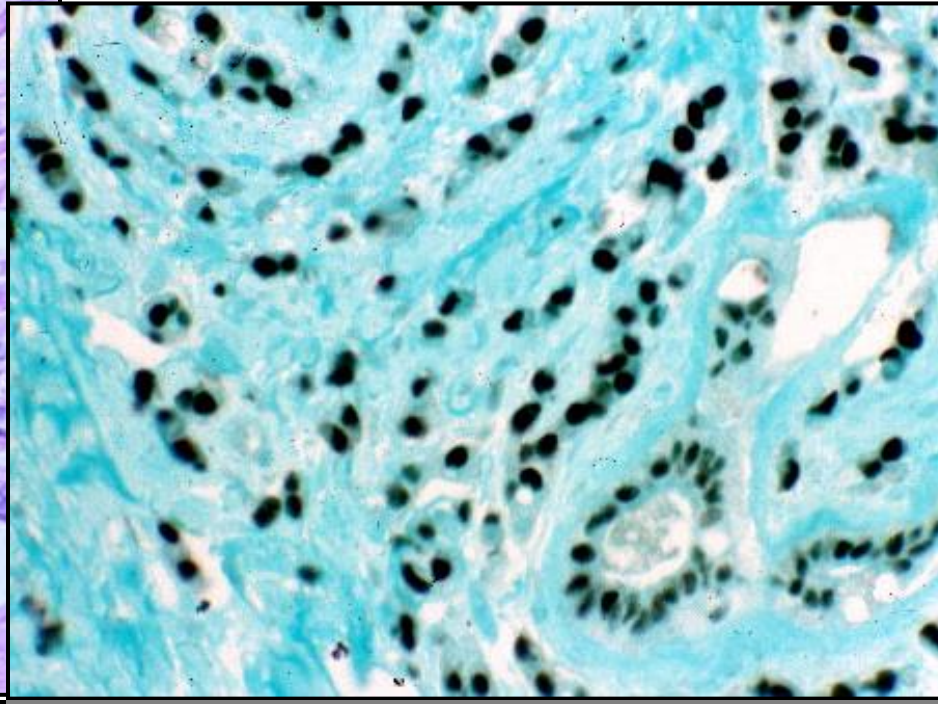
Key Words: Estrogen receptor; Immunohistochemistry; Breast cancer; Metastatic breast carcinoma

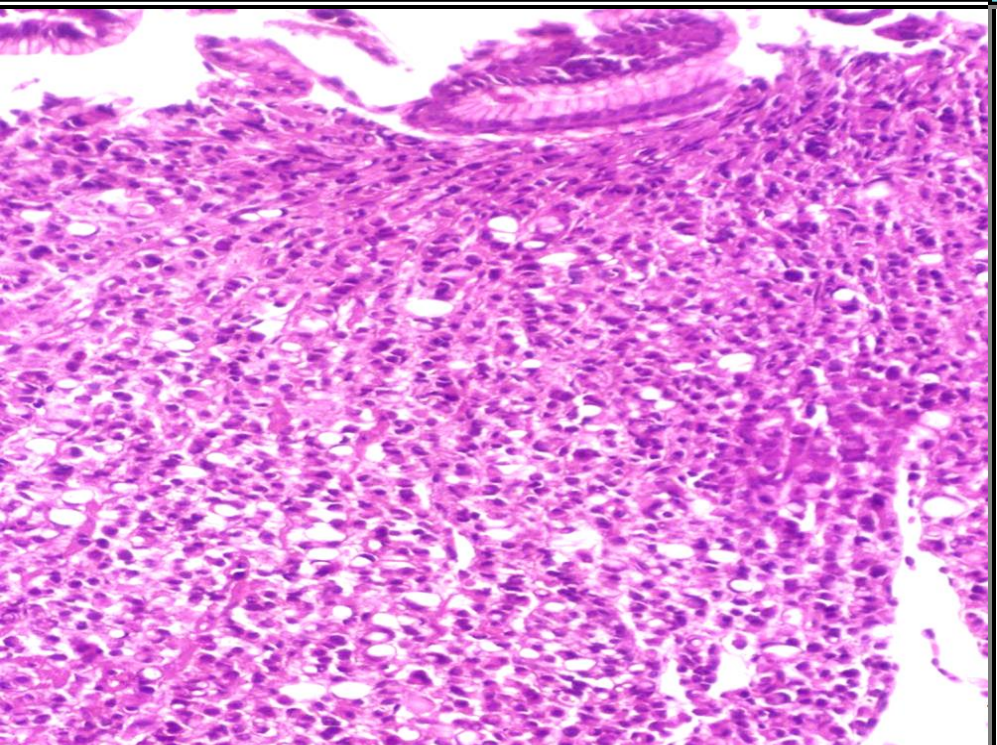
Am J Clin Pathol 2008;130:879-882

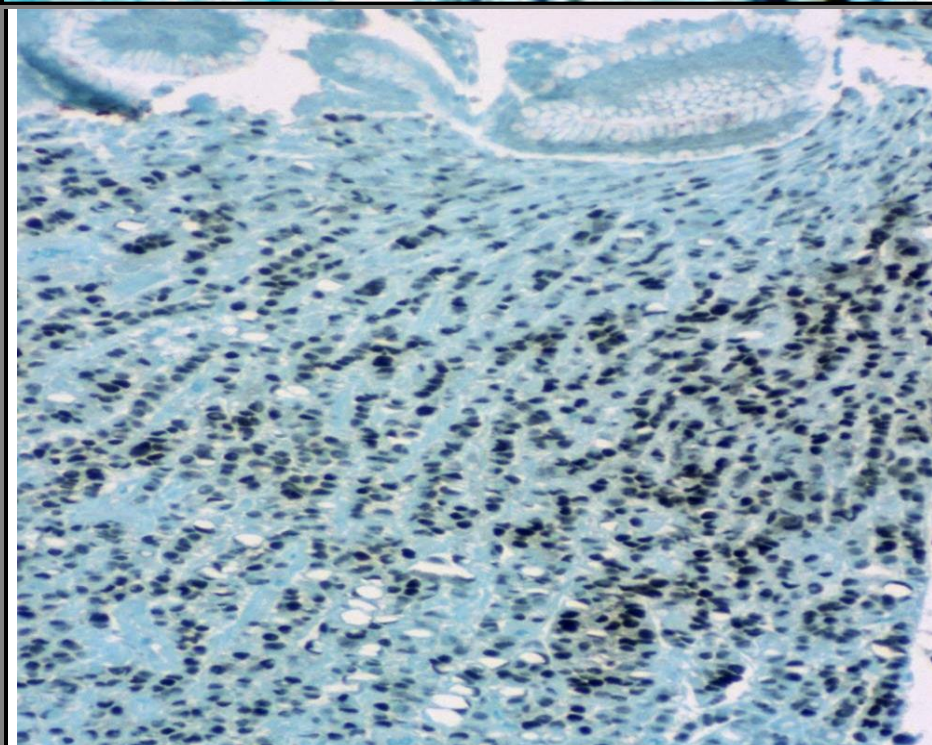
278 breast carcinomas with recurrence/metastasis
From 2 mos to 21 yrs
ER phenotype using 1D5

		Recurrence/Metastasis	
Primary		ER (+)	ER(-)
ER (+)	n=159	150	9
ER (-)	n=119	0	119









Threshold of positivity for ER and PR by IHC:

"Positive" for ER/PR → ≥ 1% immunoreactive cells

*Levels as low as 1% positive staining cells are associated with significant clinical response

Arch Pathol Lab Med—Vol 134, July 2010

ASCO/CAP Guideline Update for ER/PR Testing in Breast Cancer

Low Positive ER: 1%-10% of tumor cell nuclei

TABLE 2. Additional Recommended Reporting Comments for Specific Scenarios

Result	Additional Recommended Comment
1%-10% cells staining	The cancer in this sample has a low level (1%-10%) of ER expression by IHC. There are limited data on the overall benefit of endocrine therapies for patients with low level (1%-10%) ER expression, but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results are heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER-negative cancers.

J Clin Oncol 38:1346-1366. © 2020 by American Society of Clinical Oncology

IHC ER/PR Interpretation Criteria

Interpret assay as “positive”, “negative”, “uninterpretable”

Reject sample if non-neoplastic ducts fail to stain

Reject sample if improperly fixed

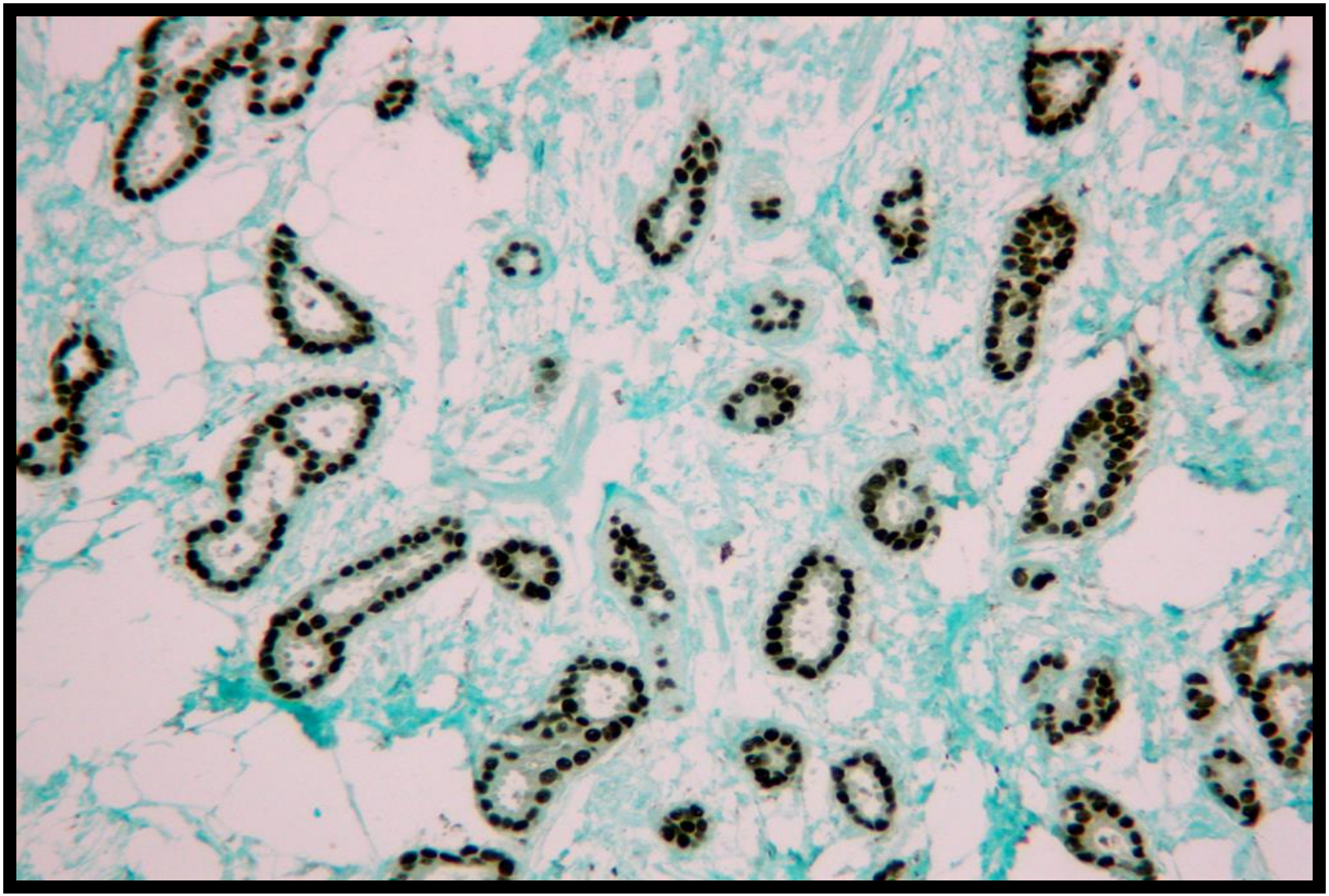
Reject sample if tumor section is necrotic

Results should correlate with subtype and grade

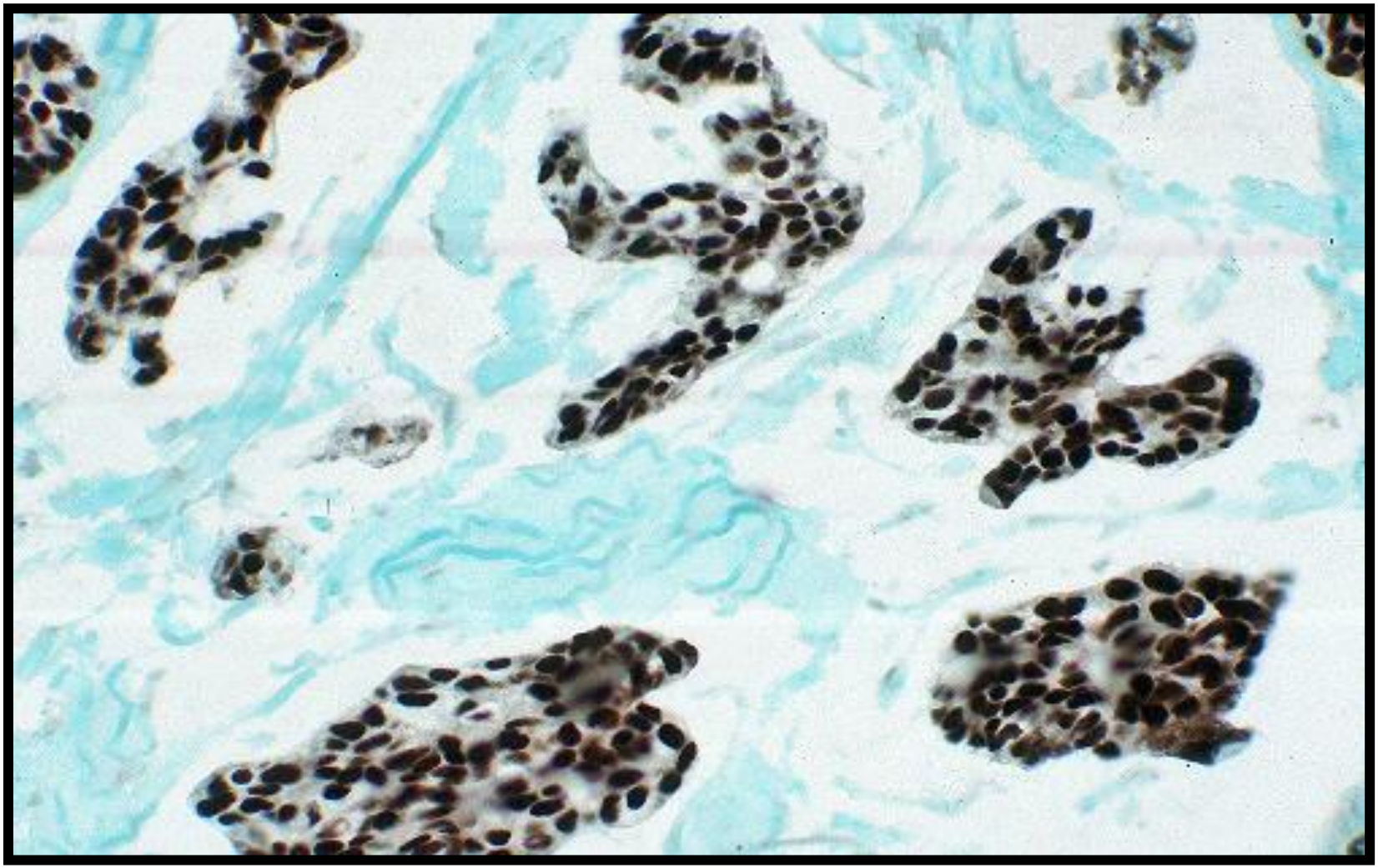
ER is predictable in certain subtypes

Tubular carcinoma	100%
Colloid carcinoma	100%
Papillary carcinoma	100%
Lobular carcinoma	100%
Metaplastic carcinoma	0%
Medullary carcinoma	0%

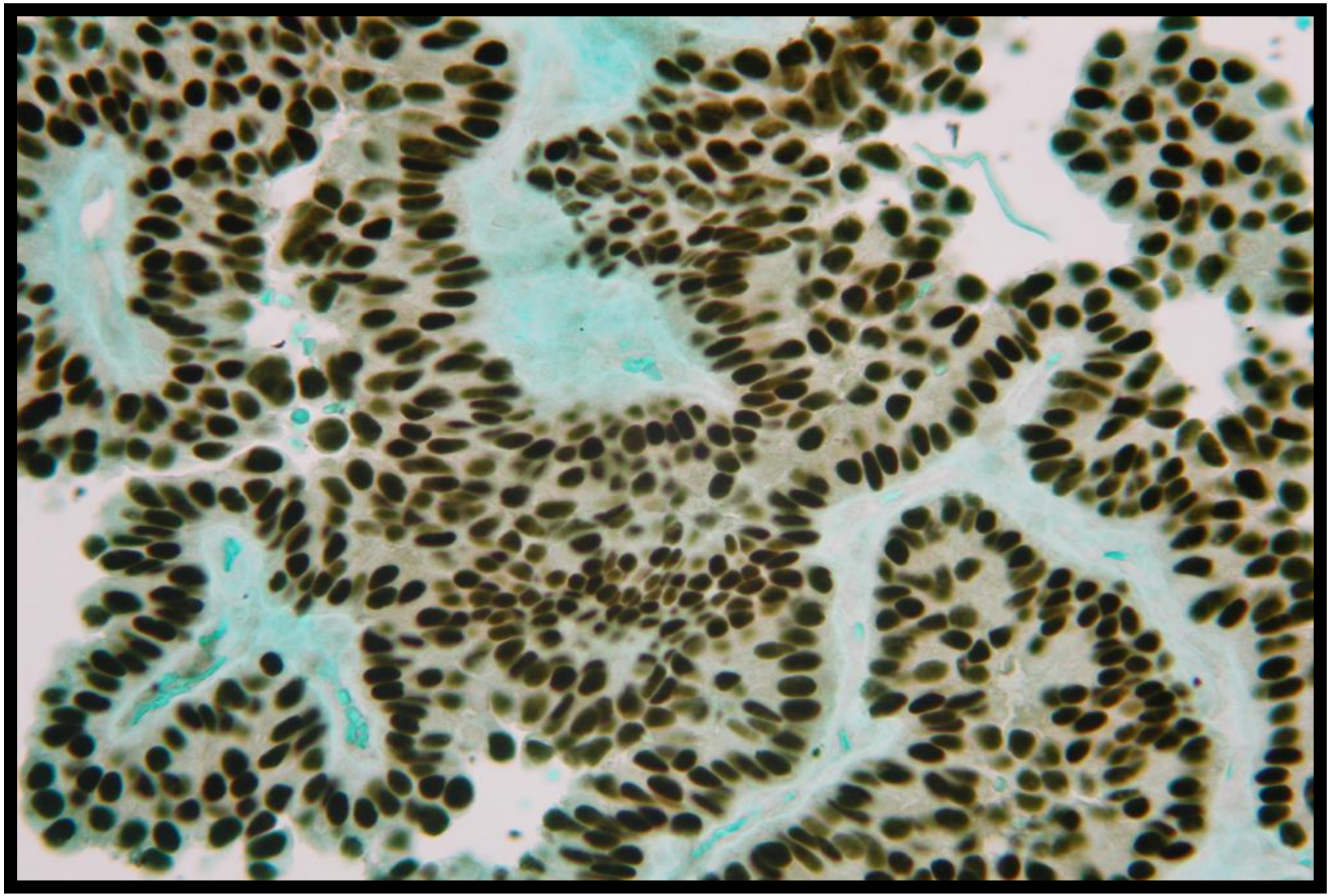
Tubular Carcinoma



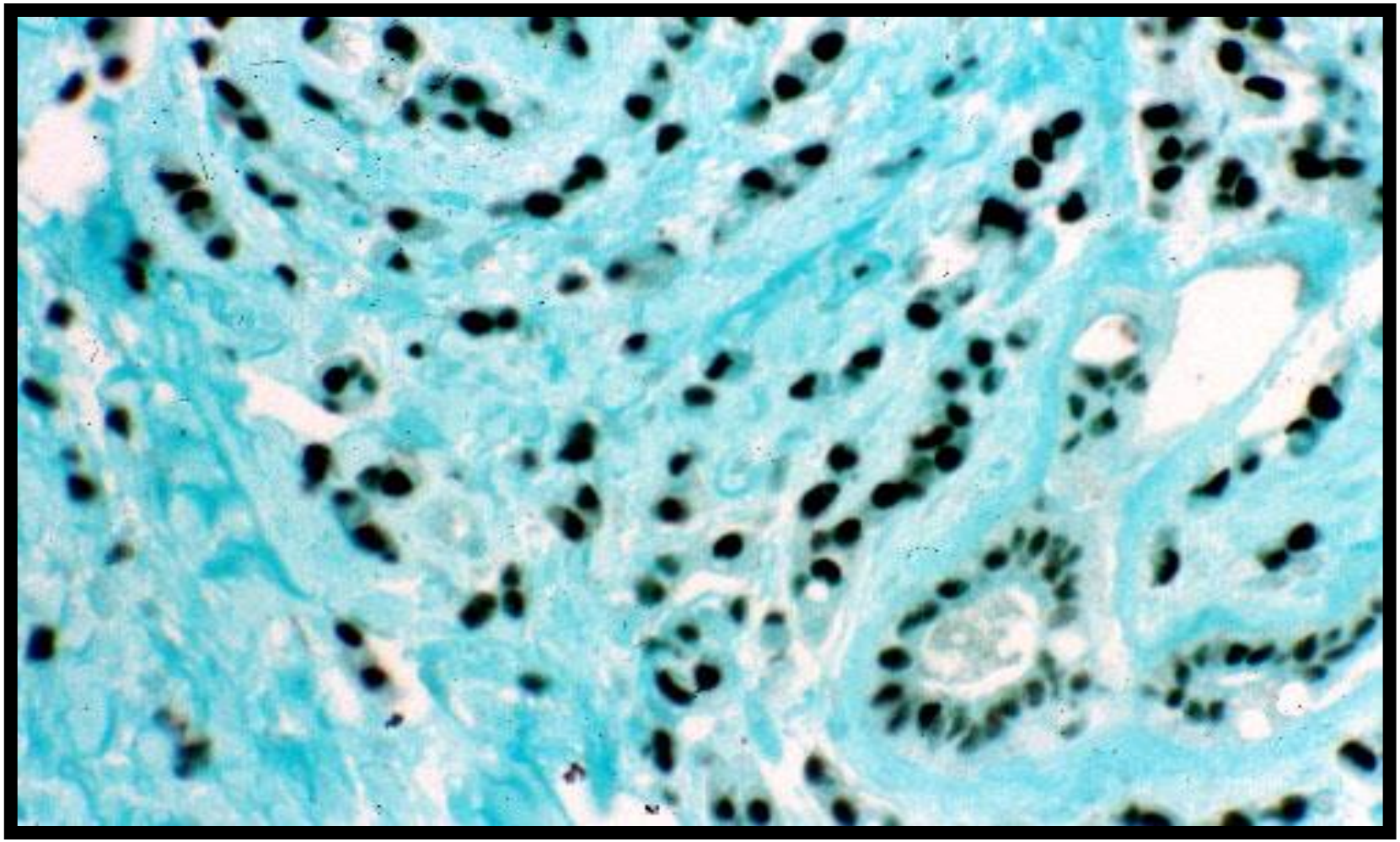
Mucinous (Colloid) Carcinoma



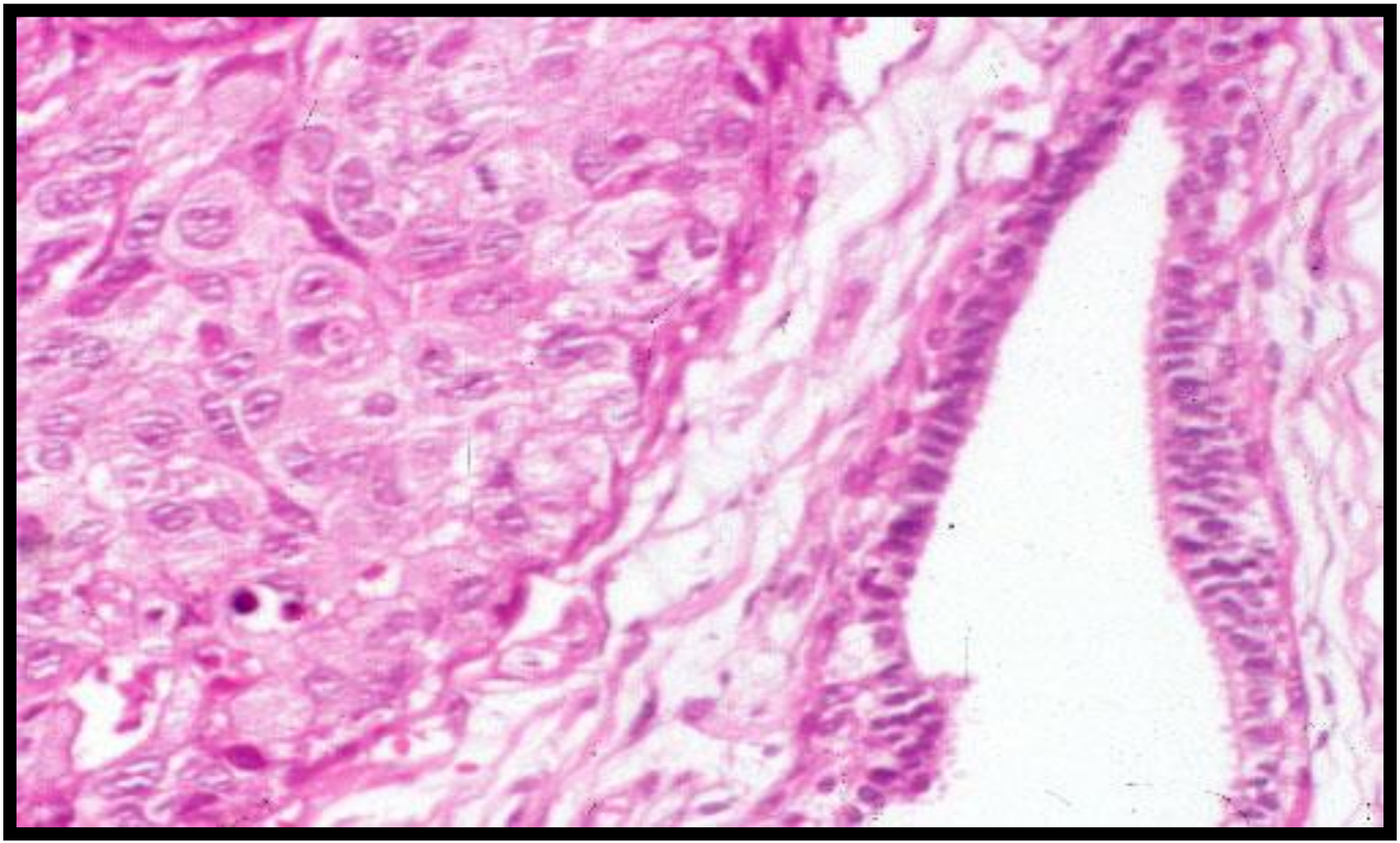
Papillary Carcinoma



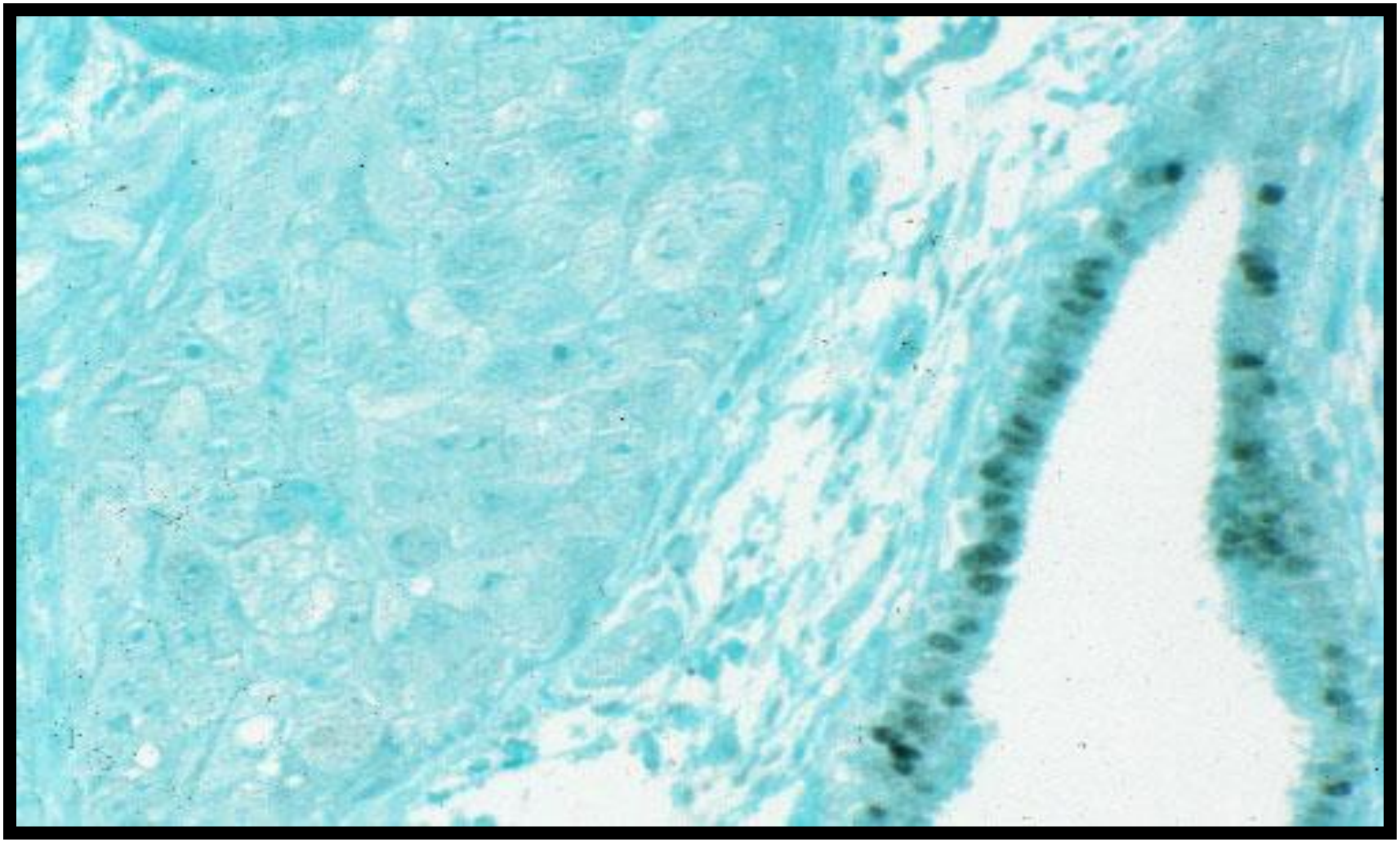
Classic Lobular Carcinoma



Medullary Carcinoma



Medullary Carcinoma

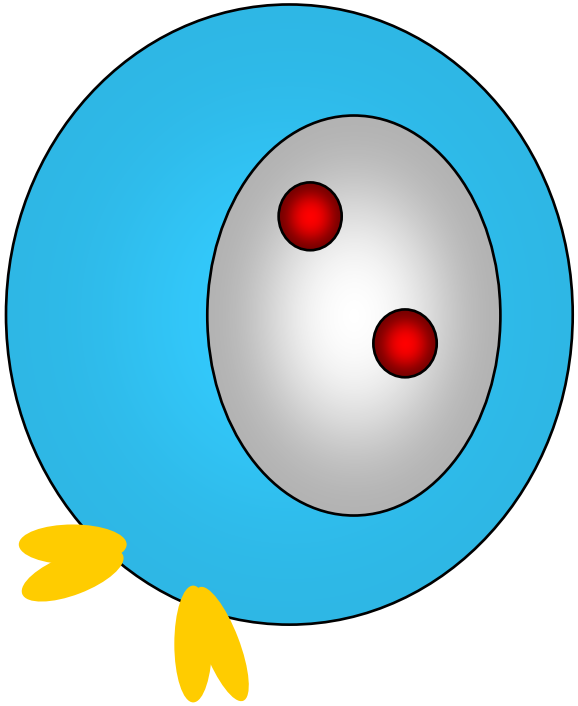


ER can be predicted from the nuclear grade

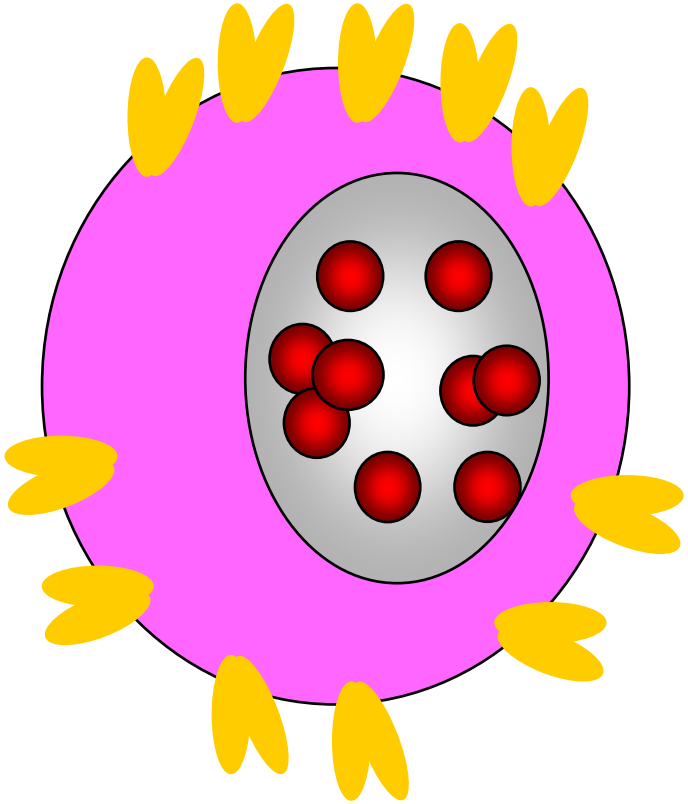
Nuclear grade 1	+
Nuclear grade 2	+/-
Nuclear grade 3	-

HER2 in Breast Cancer

Normal Cell

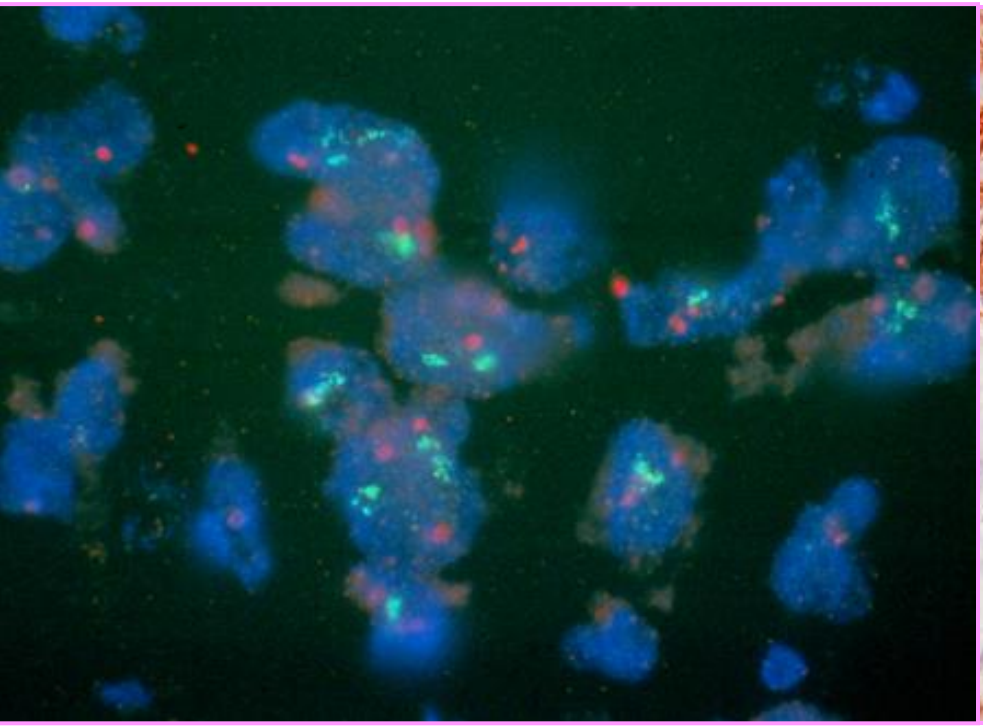


Overexpressing Cell

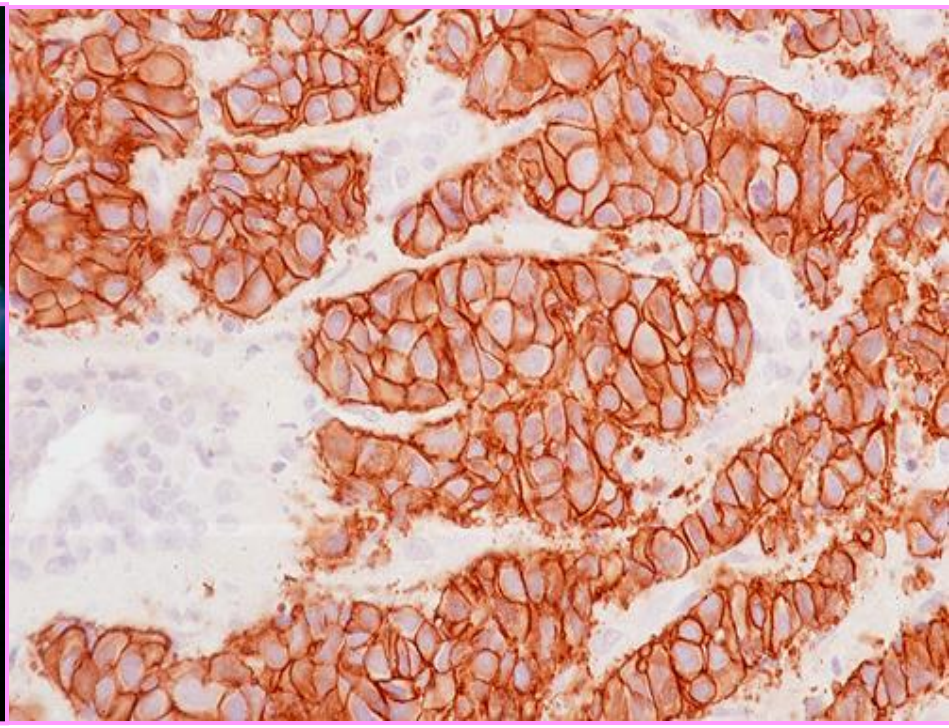


HER2 Testing

FISH v. IHC



FISH(+) HER2/CEP17>2.0



IHC (+) HecepTest 3+

HER2 in Breast Cancer

- 1998: HERCEPTIN FDA-approved for the treatment of metastatic disease

- 1998: HercepTest FDA-approved as the diagnostic assay for HER2 status

- *2007: ASCO/CAP Guidelines for HER2 testing in breast cancer*

2007 ASCO/CAP Guidelines for HER2 Testing

Positive HER2

- IHC 3+ (>30% of tumor cells)
- FISH ratio HER2/CEP17>2.2; HER2 gene copy >6

Equivocal HER2

- IHC 2+
- FISH ratio 1.8-2.2; HER2 gene copy 4-6

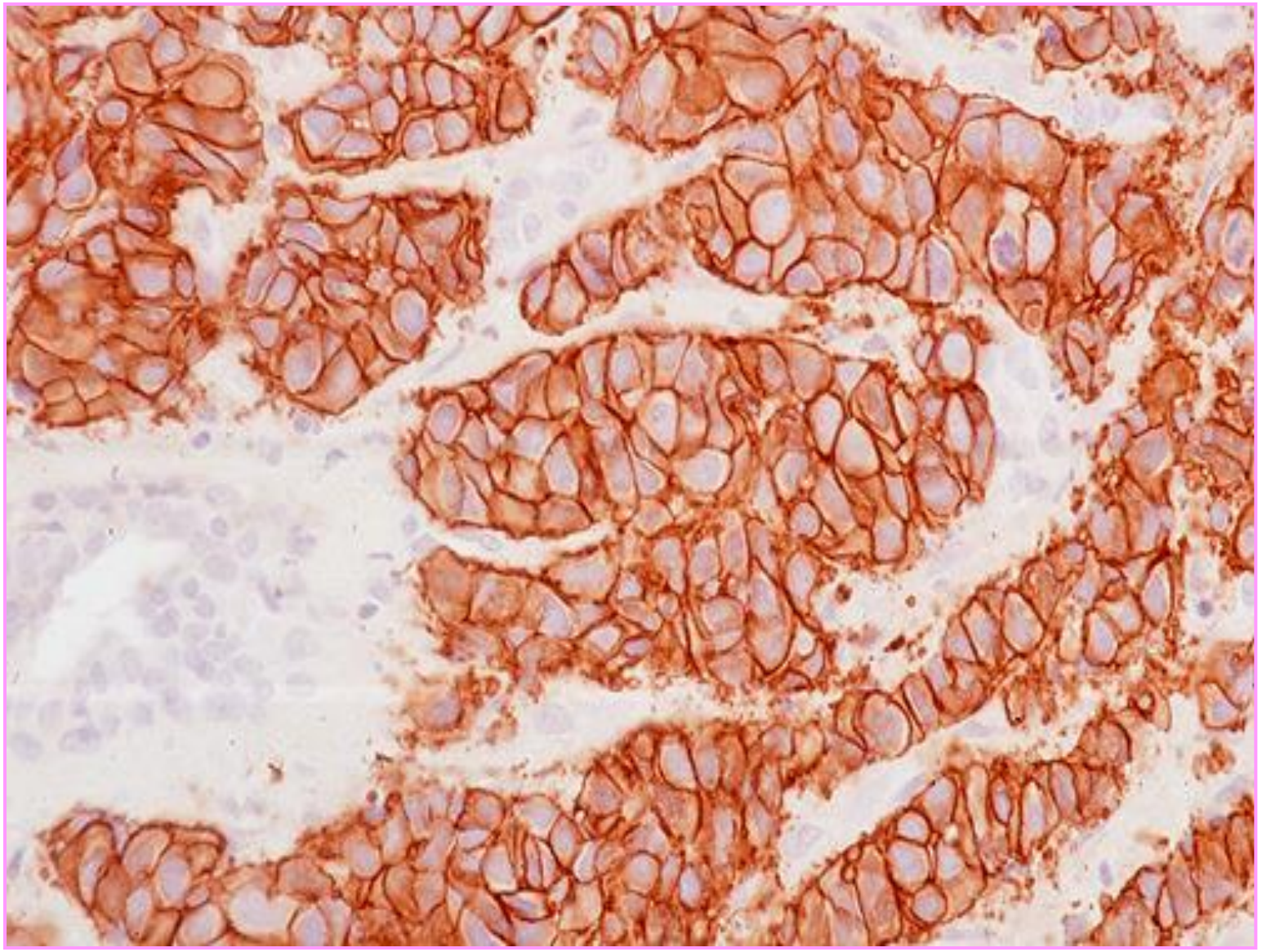
Negative HER2

- IHC 0-1+
- FISH ratio <1.8; HER2 gene copy <4

ASCO/CAP Guidelines for HER2 Testing

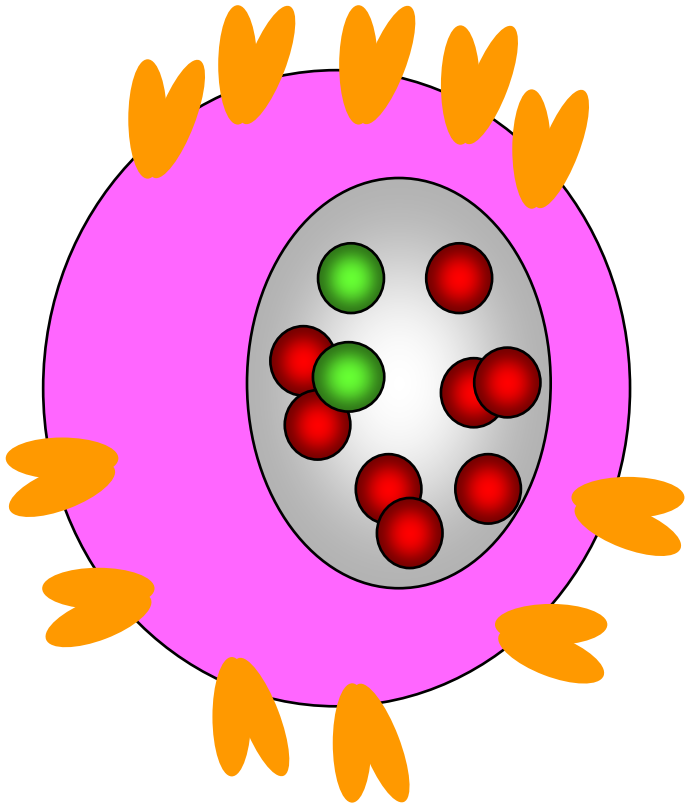
Positive, negative, or reflex!

Overexpression of HER2 occurs in the absence of gene amplification in <5% of the cases



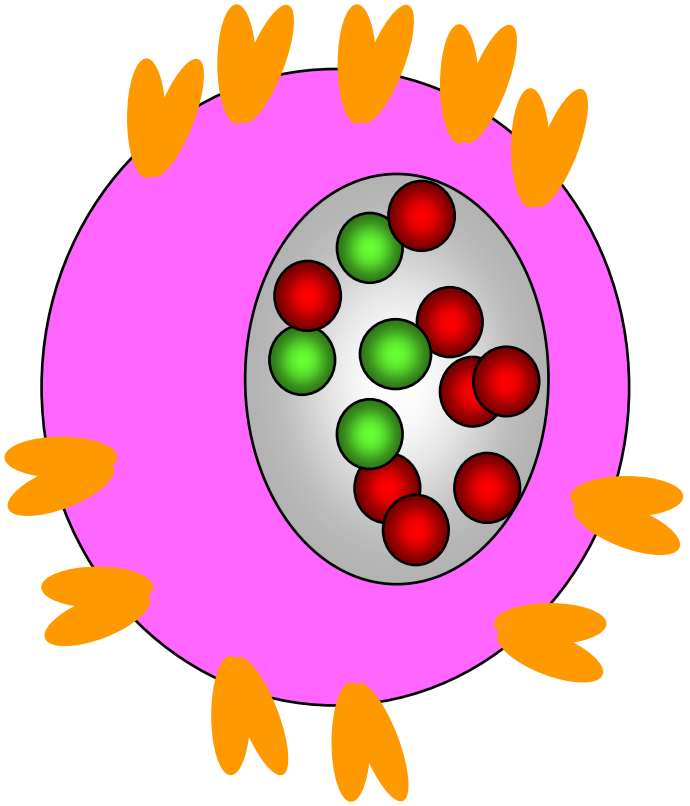
HER2 in Breast Cancer

Diploid for chrm 17

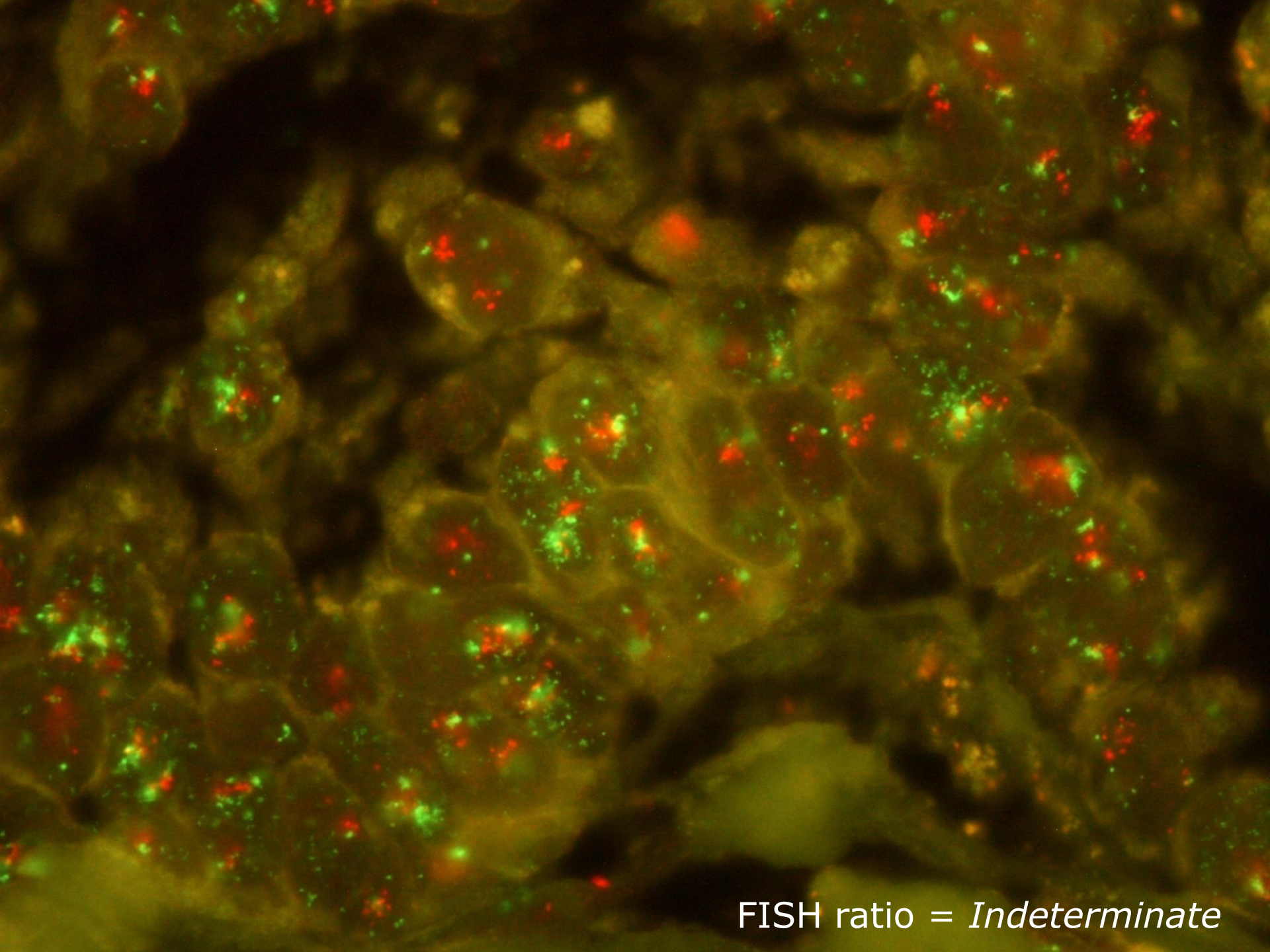


HER2 gene/centromere = 4

Polysomic for chrm 17



HER2 gene/centromere = 2


FISH ratio = *Indeterminate*

VOLUME 31 · NUMBER 31 · NOVEMBER 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update

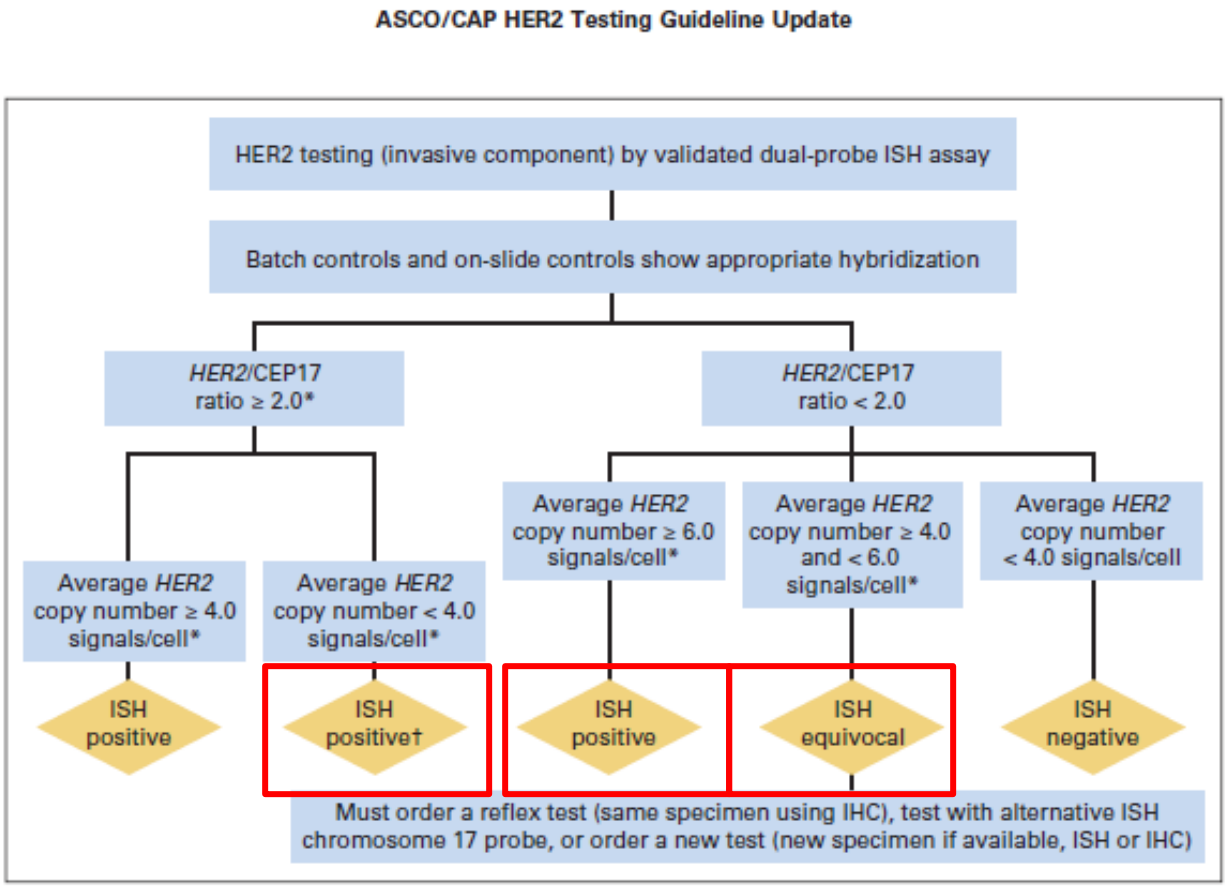
Antonio C. Wolff, M. Elizabeth H. Hammond,* David G. Hicks,* Mitch Dowsett,* Lisa M. McShane,* Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes**

HER2 in Breast Cancer

2013 ASCO/CAP Clinical Practice Guidelines for IHC

- HER2 (3+) positive ⇨ >10% of tumor cells with homogenous, dark circumferential staining
- HER2 (2+) equivocal ⇨ incomplete and/or weak to moderate staining in >10% of the tumor cells or complete and dark staining in <10% of the tumor cells

2013: ASCO/CAP Clinical Practice Guideline Update for HER2 testing in breast cancer



HER2 in Breast Cancer

Key Differences between 2013 and 2007 ASCO/CAP Clinical Practice Guidelines for FISH

Major Difference: Increased number of HER2 FISH positive and equivocal results

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MILLER SCHOOL of MEDICINE
Pathology & Laboratory Medicine

Breast Cancer Res Treat (2016) 155:457–462
DOI 10.1007/s10549-016-3717-z

PRECLINICAL STUDY

HER2 FISH classification of equivocal HER2 IHC breast cancers with use of the 2013 ASCO/CAP practice guideline

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Table 1 Summary of reflex HER2 FISH results in 172 HER2 IHC 2+ equivocal cases with use of the 2013 versus the 2007 guideline

Result	# of cases reported with 2013 guideline	# of cases classified with 2007 guideline	Difference (%)
FISH positive	49 (28.5 %)	40 (23.3 %)	+5.3
FISH negative	95 (55.2 %)	125 (72.7 %)	−17.5
FISH equivocal	28 (16.3 %)	7 (4.1 %)	+12.2
Qualified for HER2-targeted therapy	77 (44.8 %)	44 (25.6 %*)	+19.2

* Four of seven equivocal cases were eligible for HER2-targeted therapy based on the 2007 recommendations

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, Mitchell Dowsett

Arch Pathol Lab Med 2018

- What is the most appropriate definition for IHC HER2 (2+)?
- **Must** HER2 testing be repeated on surgical specimen if initially negative on a core biopsy?
- What is the optimal algorithm for the less common patterns of HER2 results with dual-probe FISH?

What is the most appropriate definition for IHC HER2 (2+)?

ASCO/CAP 2013

- HER2 (2+) equivocal ⇨ incomplete and/or weak to moderate staining in >10% of the tumor cells or complete and dark staining in <10% of the tumor cells

ASCO/CAP 2018

- HER2 (2+) equivocal ⇨ **complete** weak to moderate staining in >10% of the tumor cells

Must HER2 testing be repeated on surgical specimen if initially negative on a core biopsy?

ASCO/CAP 2013

- HER2 testing must be repeated on surgical specimen if initially negative on a core biopsy

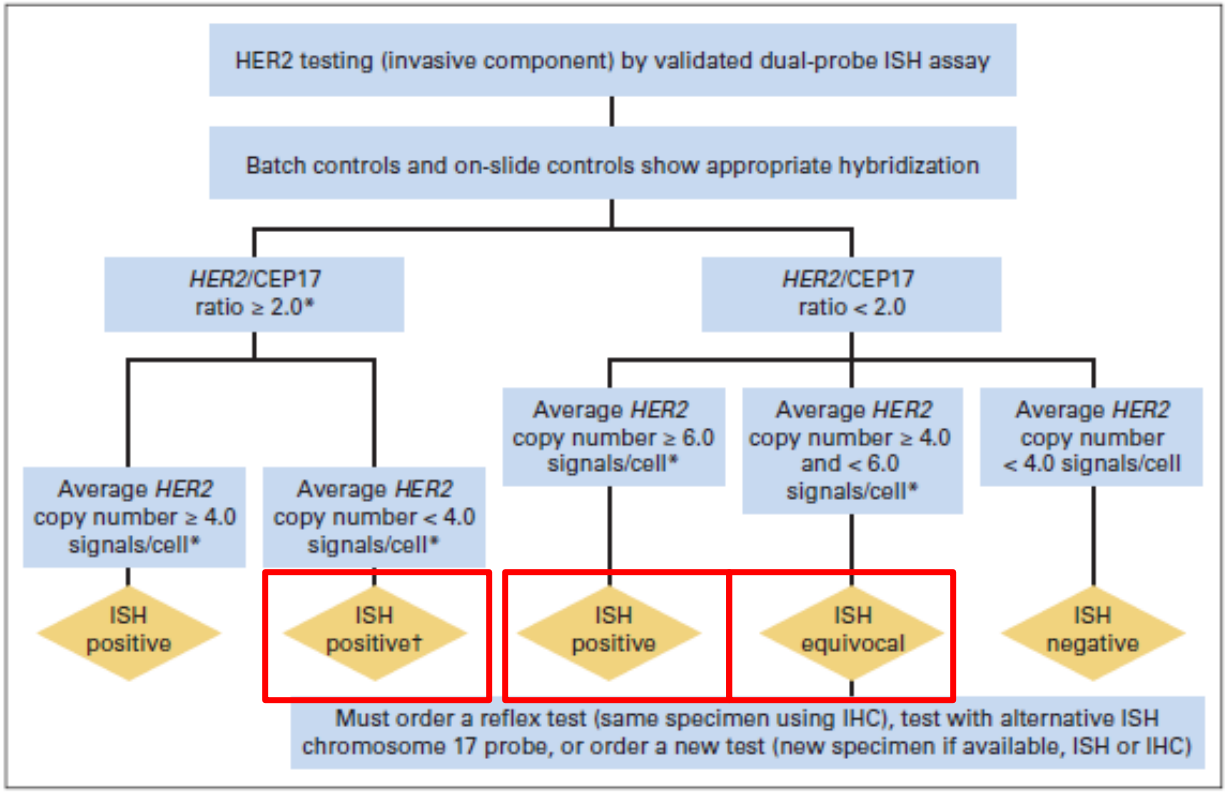
ASCO/CAP 2018

- HER2 testing **may** be repeated on surgical specimen if initially negative on a core biopsy

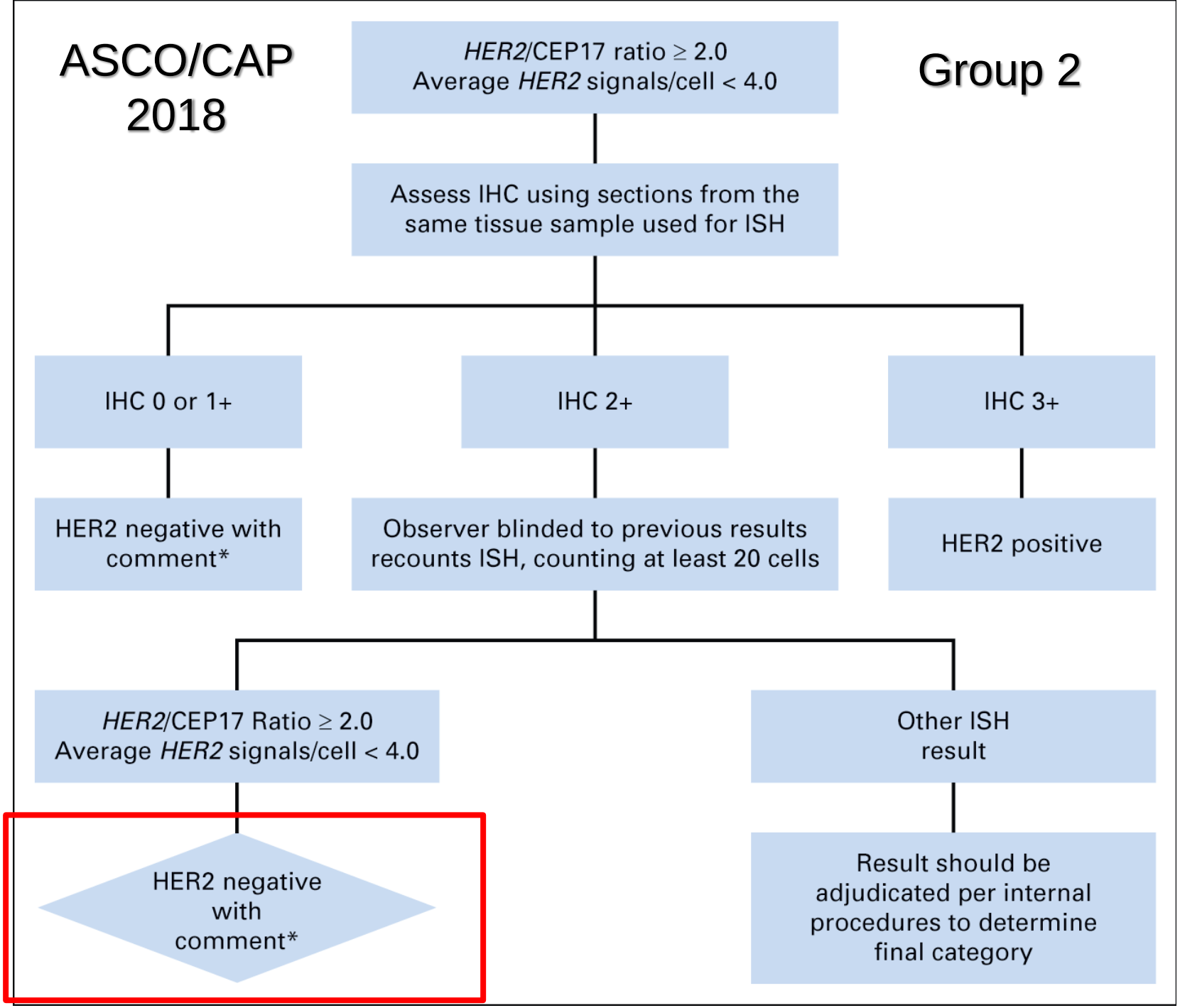
What is the optimal algorithm for the less common patterns of HER2 results with dual-probe FISH?

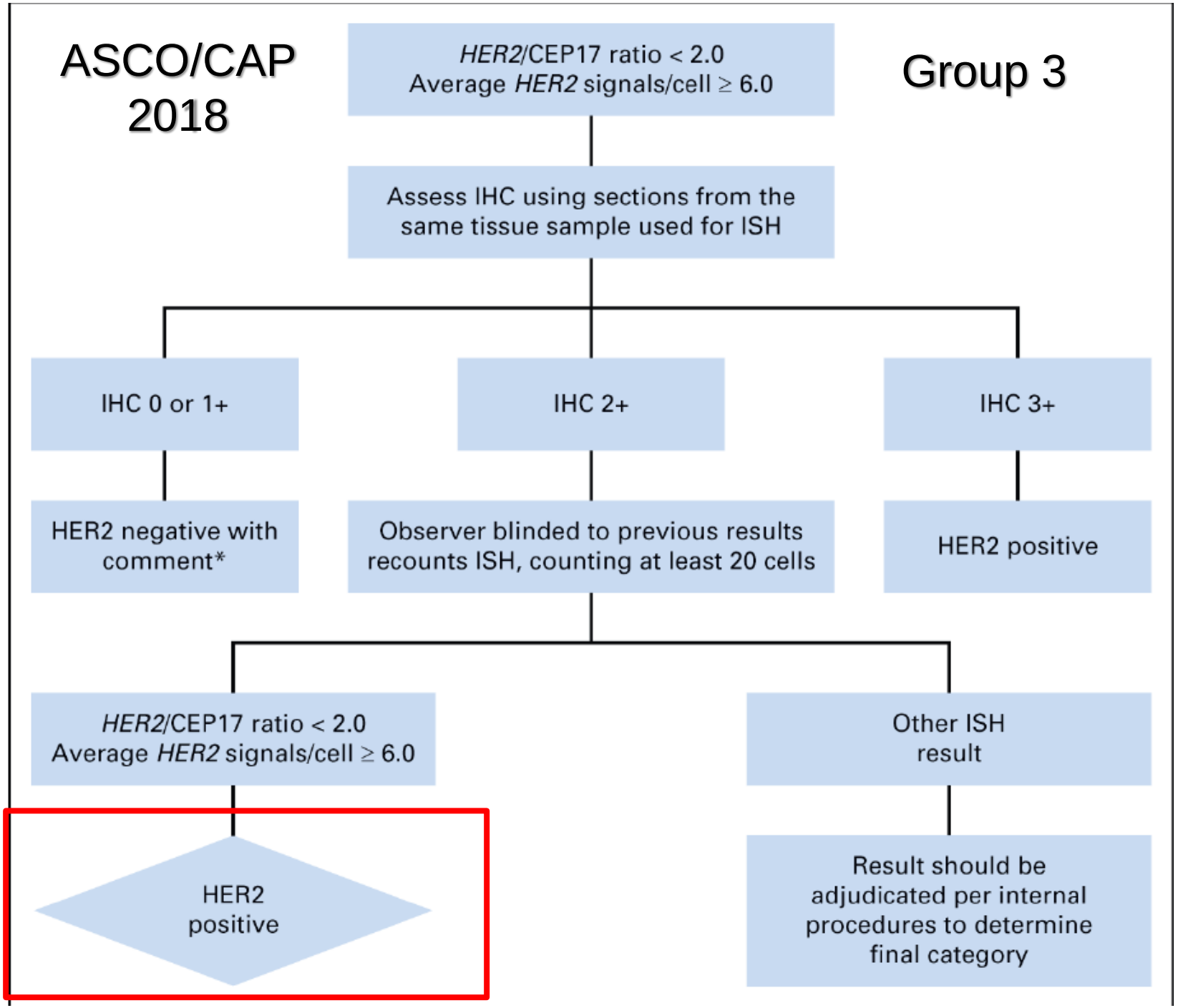
2013

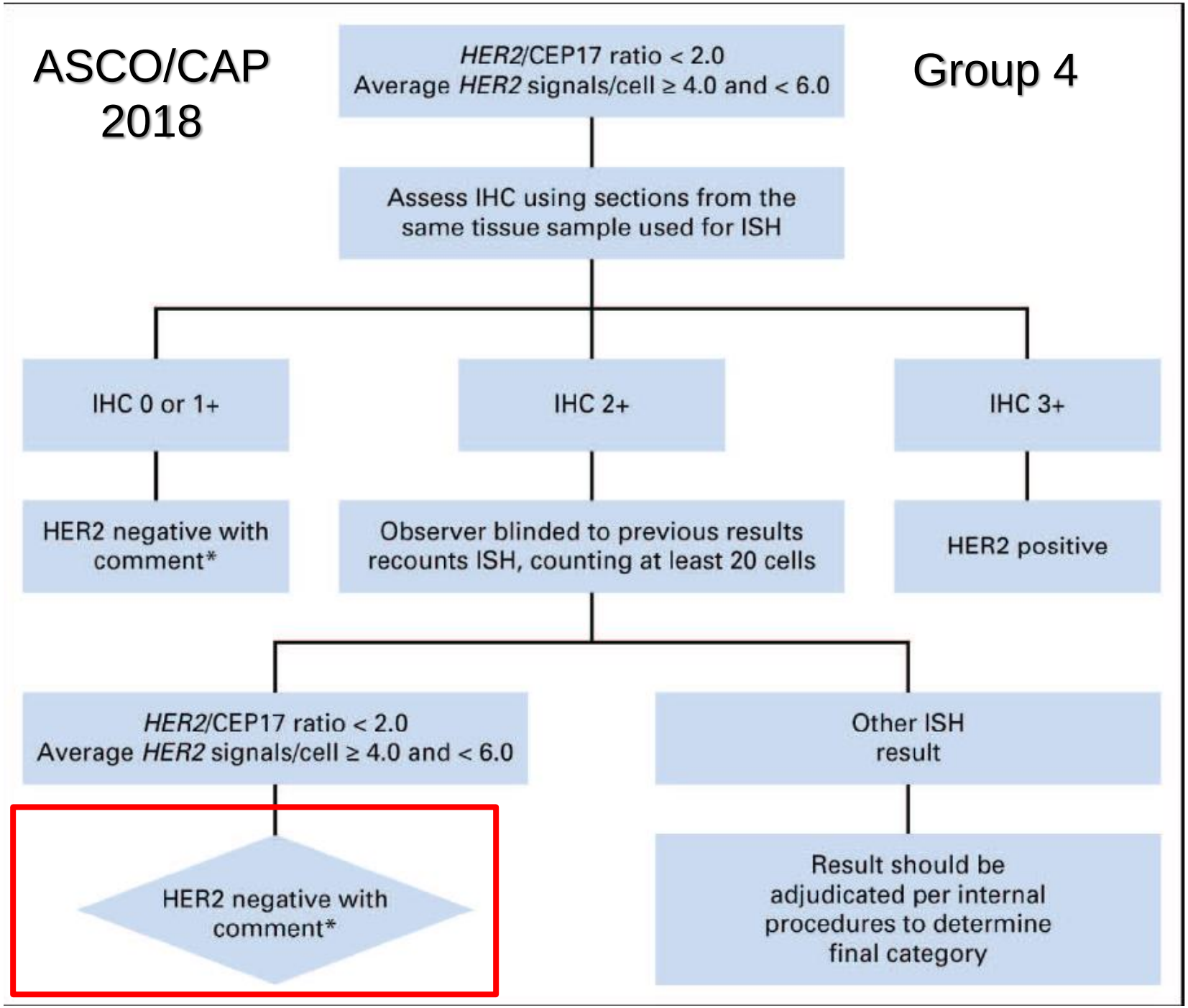
ASCO/CAP HER2 Testing Guideline Update



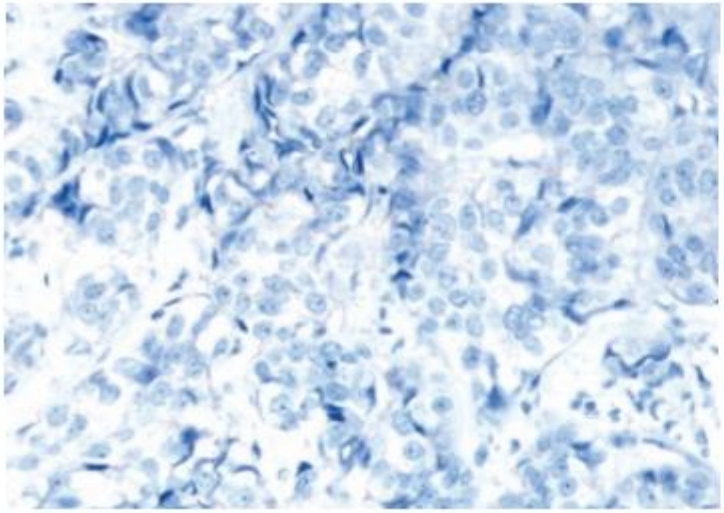
Group 1 Group 2 Group 3 Group 4 Group 5


ASCO/CAP 2018
Group 2
HER2/CEP17 ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 Ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

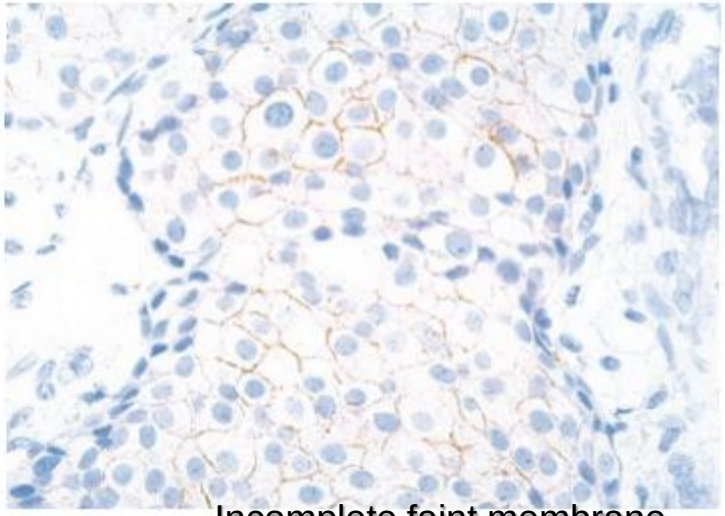

ASCO/CAP 2018
Group 3
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Other ISH result
HER2 positive
Result should be adjudicated per internal procedures to determine final category


ASCO/CAP 2018
Group 4
HER2/CEP17 ratio < 2.0
Average HER2 signals/cell ≥ 4.0 and < 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average HER2 signals/cell ≥ 4.0 and < 6.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

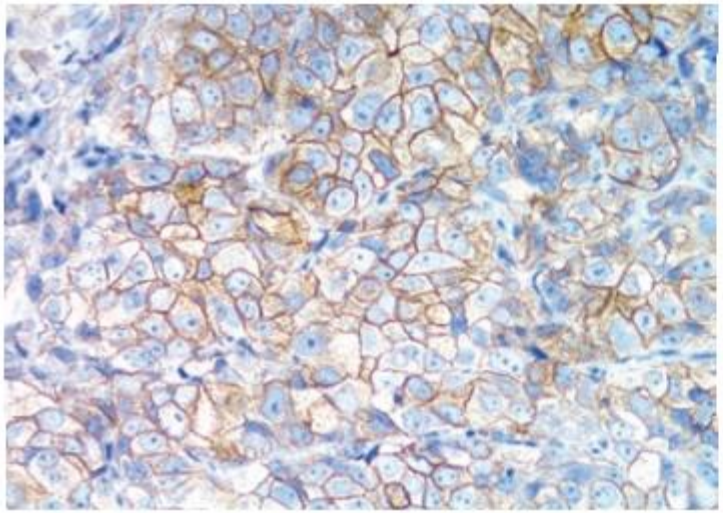
2018 ASCO/CAP Interpretation of HER2 Testing by Immunohistochemistry



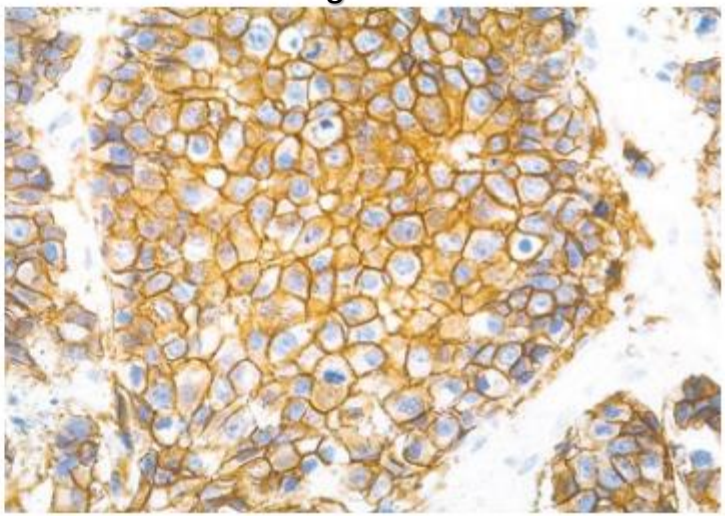
Score: 0 (20x) Absence of staining



Score: 1+ (20x) Incomplete faint membrane staining in >10% of tumor cells



Score: 2+ (20x) Complete weak membrane staining in >10% of tumor cells



Score: 3+ (20x) Complete intense membrane staining in >10% of tumor cells

2018: ASCO/CAP Clinical Practice Guideline Update for HER2 Testing by IHC in Breast Cancer

- Must report a HER2 result as Indeterminate if:
  - Inadequate specimen handling and not keeping to:
    - Cold ischemic time <1 hr.
    - Fixed in 10% neutral buffered formalin
    - Fixation time is ≥ 6 and ≤ 72 hours
  - Lab does not conform to standards set by CAP and/or does not participate in ongoing external proficiency testing
  - Histopathologic discordance
  - Technical artifacts, e.g. crush or edge artifacts, cytoplasmic staining, staining of non-neoplastic epithelial cells

Usually HER2 Negative

Tubular Carcinoma

Mucinous (Colloid) Carcinoma

Papillary Carcinoma

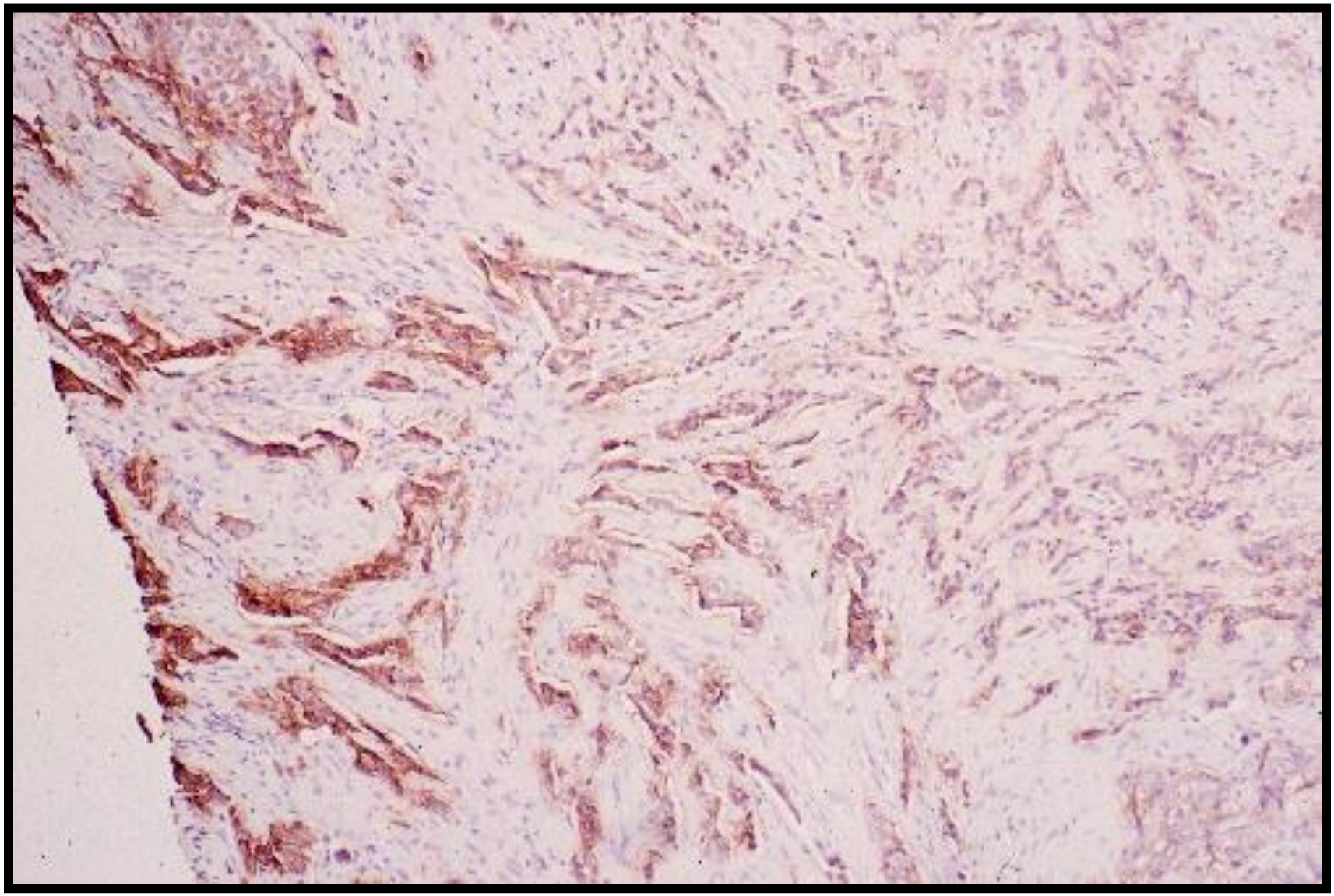
Ductal Carcinoma, low grade

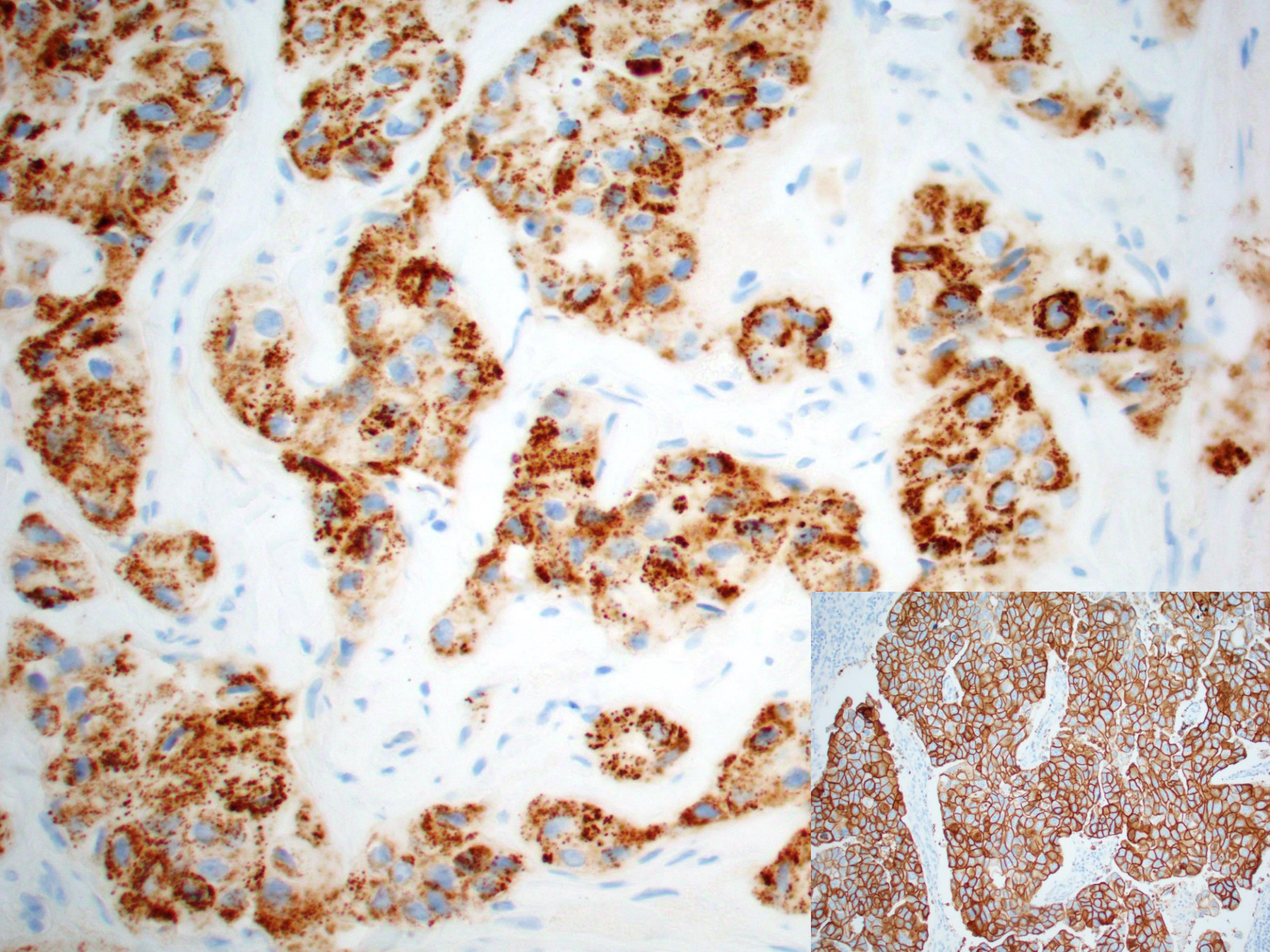
Classic Lobular Carcinoma

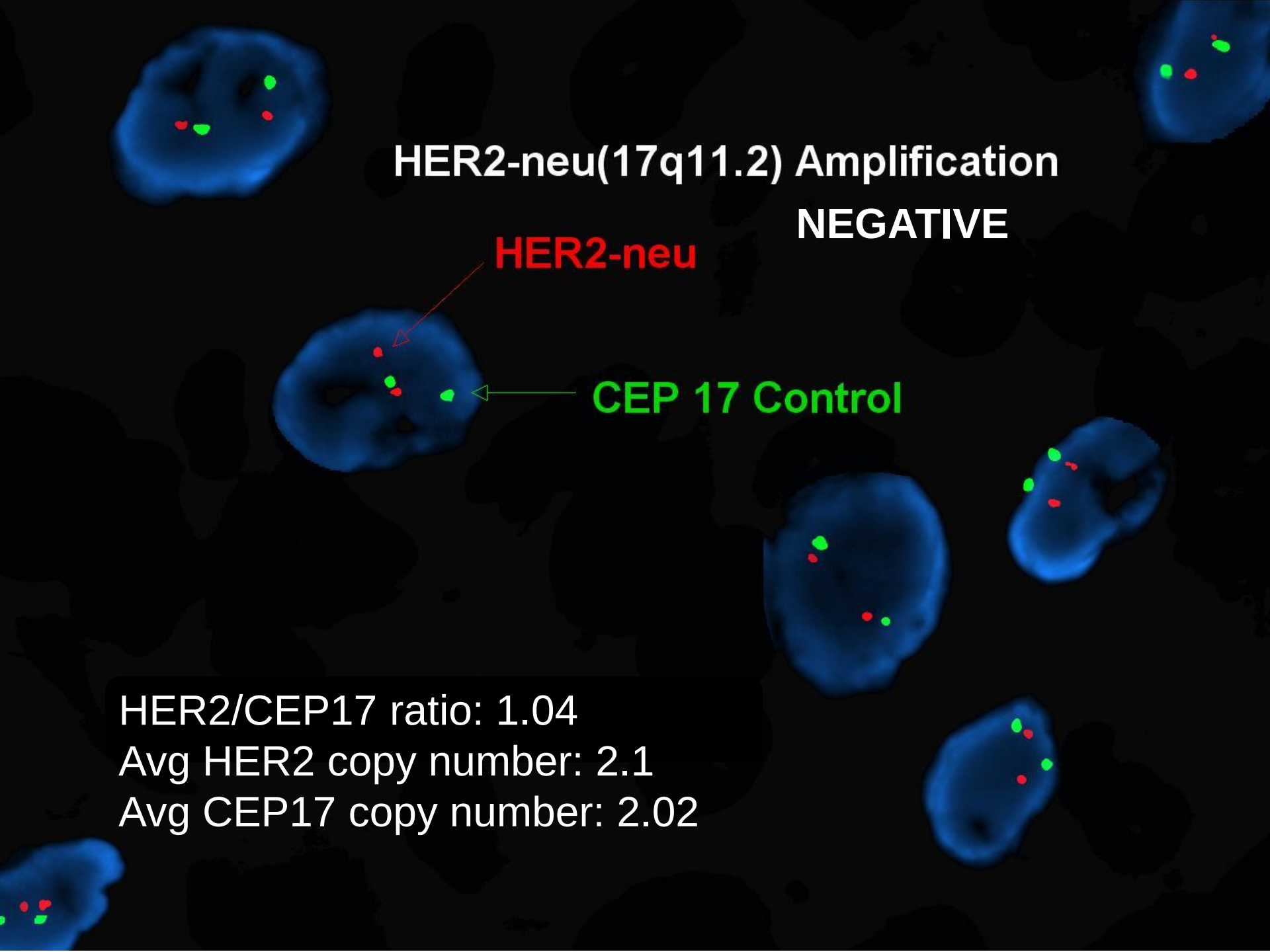
Medullary Carcinoma

Metaplastic Carcinoma

“Indeterminate”

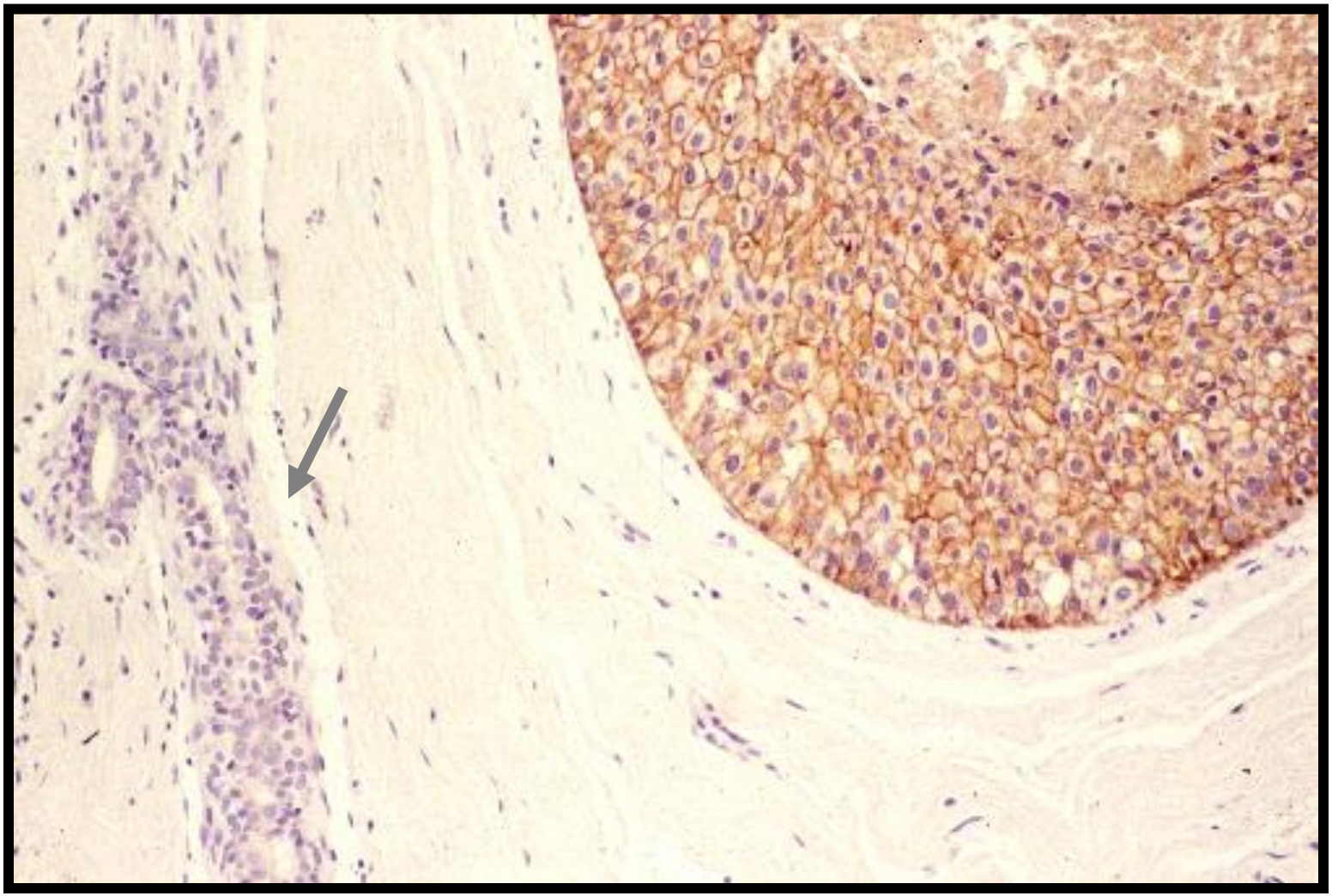






HER2/CEP17 ratio: 1.04
Avg HER2 copy number: 2.1
Avg CEP17 copy number: 2.02

Non-neoplastic ductal cells…



…should be HER2 negative.

Interpretation of HER2 by FISH

Potential problems:

- Under/over digestion
- Correct cell identification
- In Situ vs Invasive Ca
- Chromosomal polysomy

ORIGINAL CONTRIBUTION

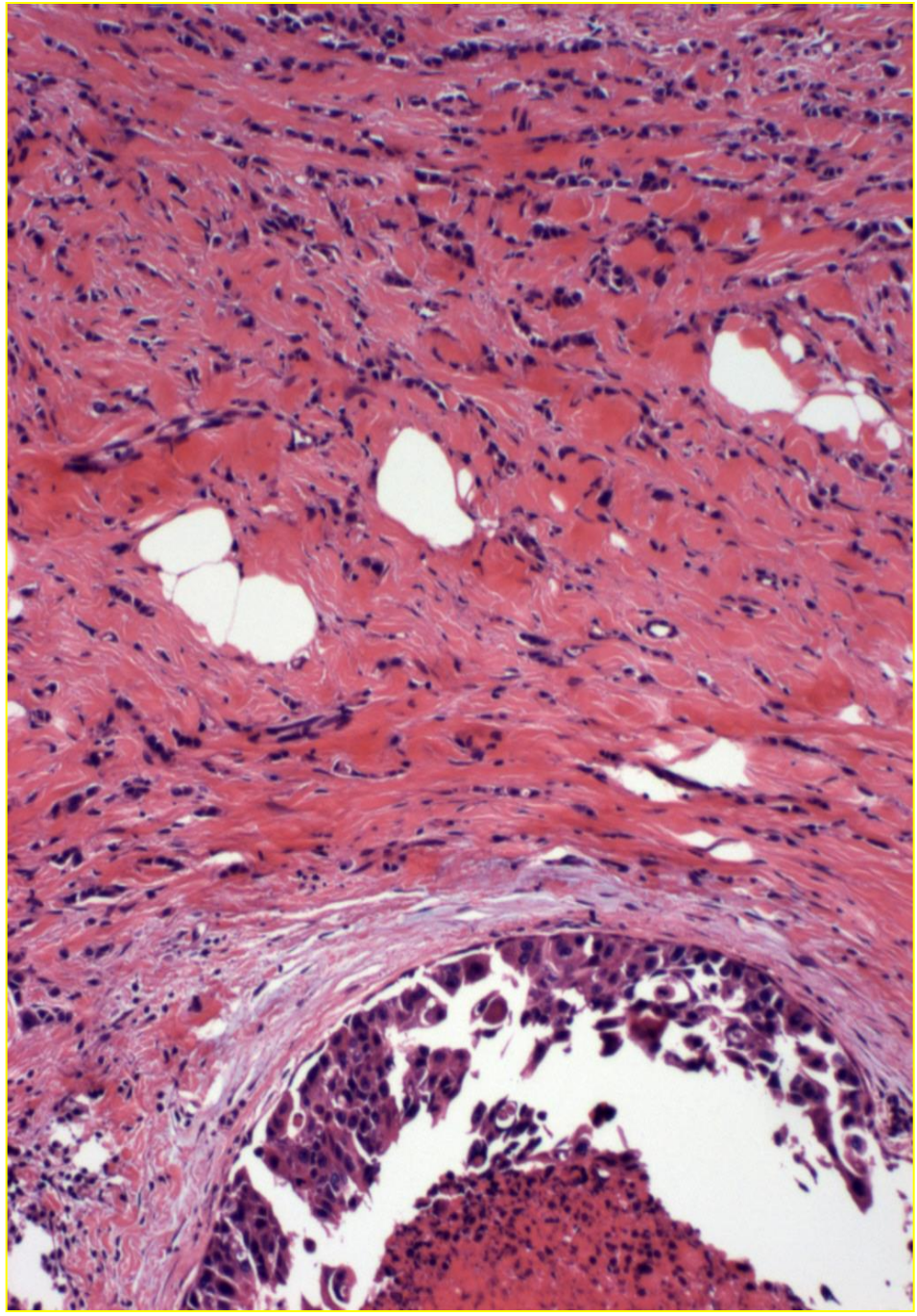
JAMA, APRIL, 2004

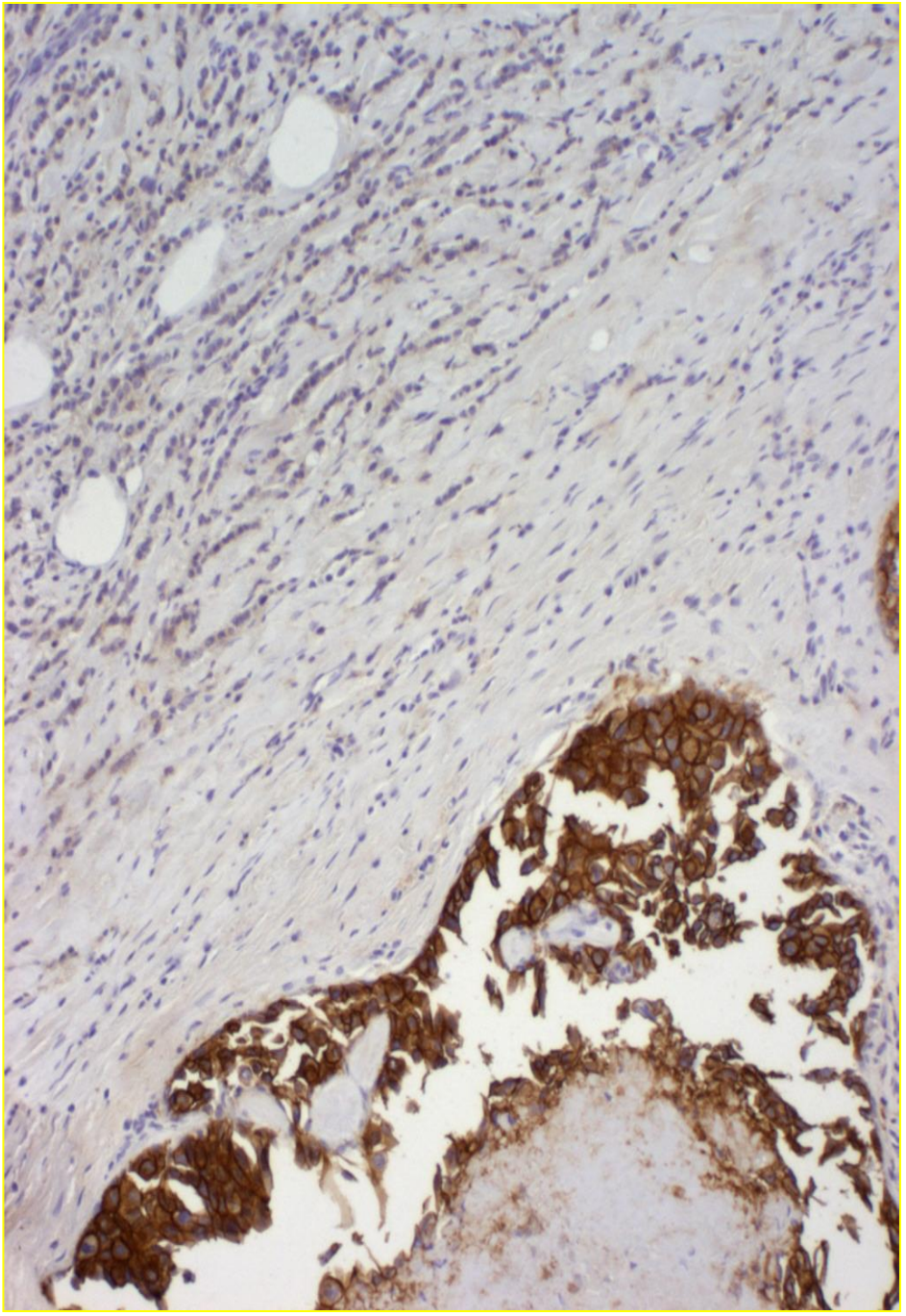
HER-2 Testing in Breast Cancer Using Parallel Tissue-Based Methods

Hadi Yaziji, MD
Lynn C. Goldstein, MD
Todd S. Barry, MD, PhD
Robert Werling, MD
Harry Hwang, MD

Context Testing for HER-2 oncogene in breast cancer has increased because of its role as a prognostic and predictive factor. Some advocate gene testing by fluorescence in situ hybridization (FISH) vs protein testing by immunohistochemistry as the method which most accurately evaluates and predicts response to the anti–HER-2 antibody, trastuzumab. However, critical examination of FISH on a screening basis has yet to be performed.

nohistochemistry score (0 or 1+) was 97.2%. The sensitivity of immunohistochemistry tests, including tumor sections with scores of 2+ or 3+, was 92.6% and the specificity of immunohistochemistry tests with scores of 3+ was 98.8%. The FISH test had a significantly higher failure rate (5% vs 0.08%) and reagent cost ($140 vs $10), and longer testing (36 hours vs 4 hours) and interpretation times (7 minutes vs 45 seconds) vs immunohistochemistry tests.

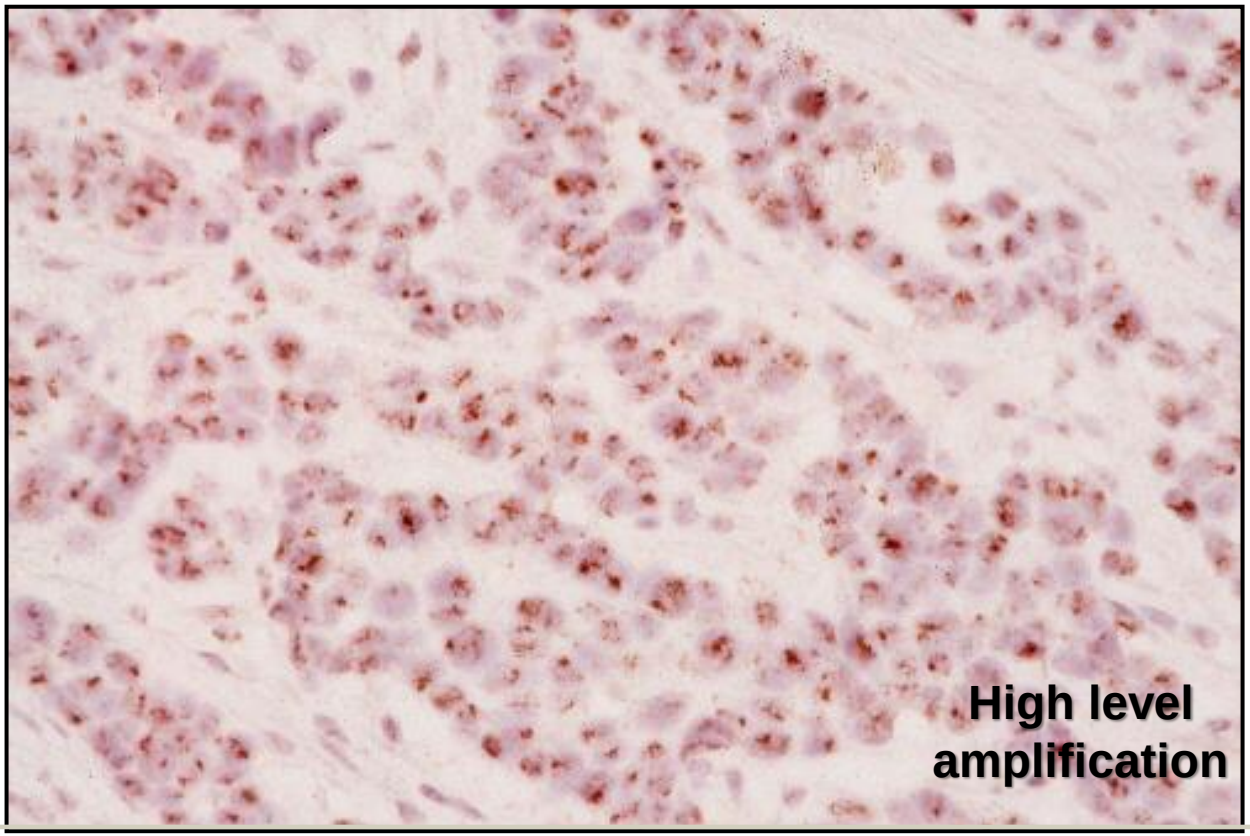




Chromogenic In Situ Hybridization (CISH)

HER2

- Heat and enzyme digestion
- HER2 DNA probe
- DAB chromogen



Current Status of HER2 in Breast Cancer

- 10-15% of all breast cancer cases
- After lymph node status, ER and HER2 status are the most important prognostic/predictive factors
- In tumors that overexpress HER2, the entire machinery of the malignant cells is driven and dependent upon HER2
- **It is critical to measure HER2 in all invasive breast cancers**
- All HER2 positive breast cancers must be treated with HER2 directed therapy

Current Status of HER2 in Breast Cancer

- The addition of trastuzumab to chemotherapy in the metastatic setting improved PFS by 9 mos.
- More recently, the addition of trastuzumab to adjuvant chemotherapy has improved the **cure** rate by **50%**
- In the neoadjuvant setting, dual HER2 blockade with trastuzumab and pertuzumab has improved the pCR rate from 30% to 45%

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