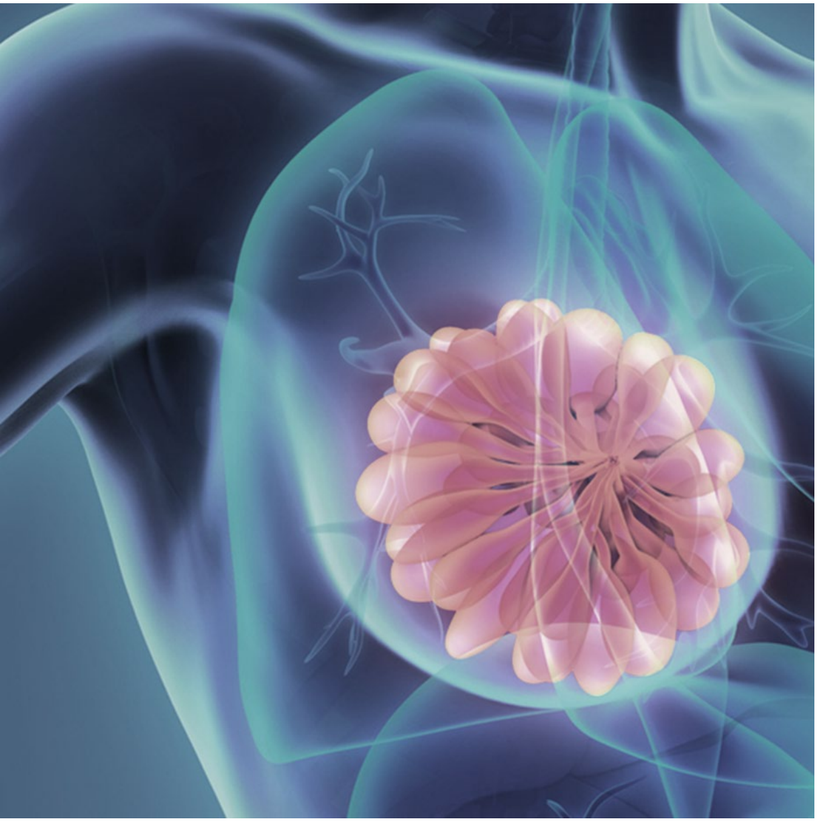
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Education



ER/PgR/HER2 Testing and Interpretation in Breast Cancer Overview

Huina Zhang, MD, PhD, FCAP

Associate Professor

University of Rochester Medical Center

Rochester, New York

Objectives

- To understand the significance of accurate interpretation of ER, PgR, and HER2 in breast cancer.
- Be familiar with the ER/PgR and HER2 ASCO/CAP testing guidelines in breast cancer and recent updates.
- To improve the assessment of ER, PgR, and HER2 in breast cancer.

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Outlines

- Introduction of biomarkers into clinical practice (ER, PgR, HER2 and beyond)
- Overview of ER/PgR and HER2 ASCO/CAP testing guidelines in breast cancer and recent updates
- Approaches to improve the assessment of ER, PgR and HER2 in breast cancer
  - Case presentations

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Part I

Introduction of Biomarkers Into Clinical Practice (ER, PgR, HER2, and beyond)

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Cancer Stat Facts – Female Breast Cancer

- The most common malignancy in women worldwide
- 310,720 new cases and 42,250 deaths are estimated in the U.S. in 2024.
- Five-year relative survival is ~91.2%
- >80% of breast cancer will be cured with the appropriate treatment.

How to identify the appropriate patients for appropriate treatment??

National Cancer Institute: Surveillance, Epidemiology, and End Results Program. Cancer Stat Facts: Female Breast Cancer. National Institutes of Health. Accessed May 23, 2024 https://seer.cancer.gov/statfacts/html/breast.html

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Biomarker in Breast Cancer

A measurable indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention

- ❑ **Prognostic biomarkers (ER, PgR, HER2, Ki-67):**
  - **Correlated to the disease outcome either without treatment or with standard treatment**
  - **Determine which cancers might need additional treatment**

- ❑ **Predictive biomarkers (ER, HER2, AR, PD-L1):**
  - **Determine potential benefit from a specific treatment**

❖ ***Biomarker testing in breast cancer has become standard of care to determine what therapies may be most effective for individual patients with breast cancer***

Allison KH. Ancillary Prognostic and Predictive Testing in Breast Cancer: Focus on Discordant, Unusual, and Borderline Results. *Surg Pathol Clin*. 2018;11(1):147-176. doi:10.1016/j.path.2017.09.006.

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Commonly-Tested Biomarkers by IHC in Breast Cancer

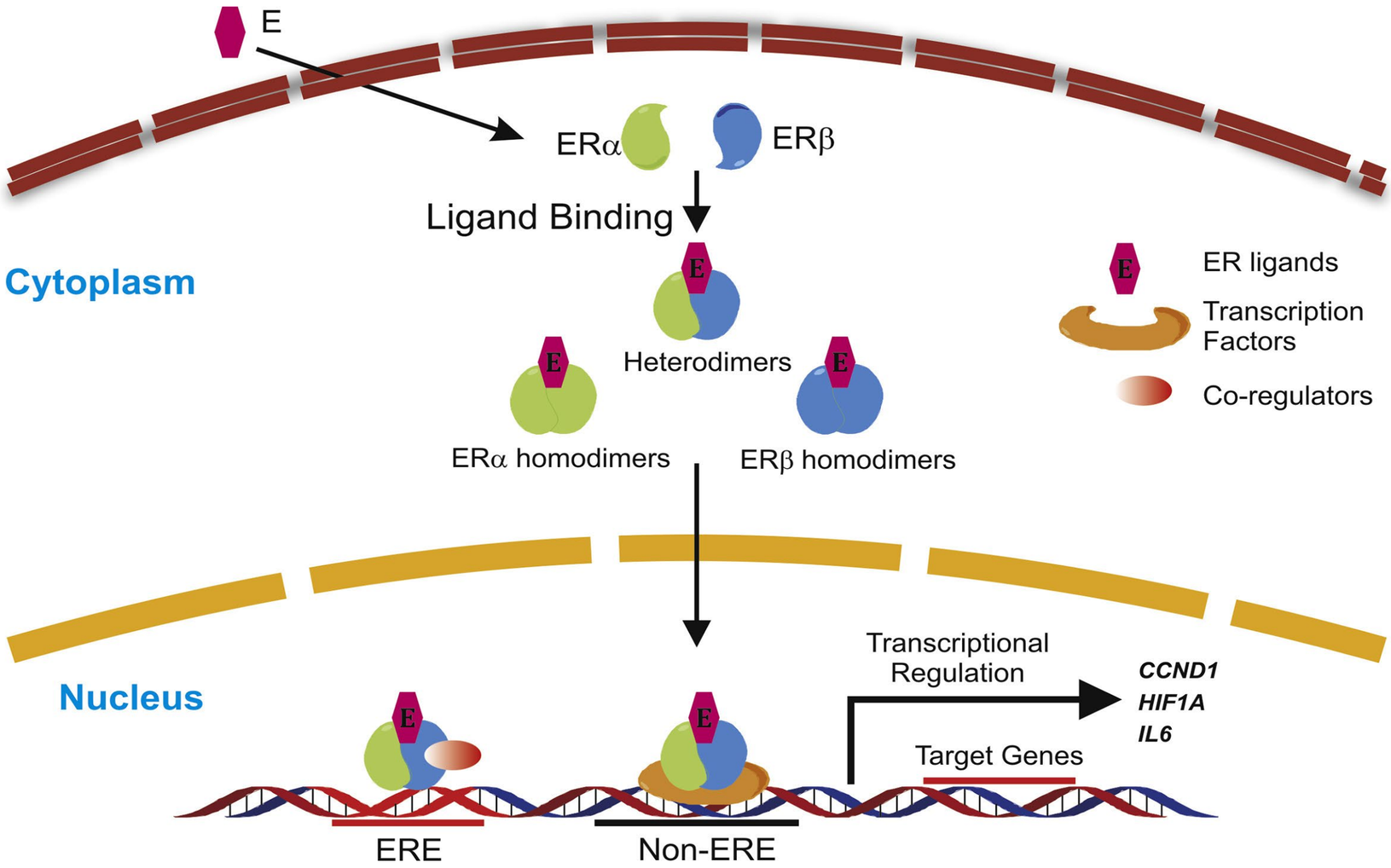
Newly diagnosed DCIS	Newly diagnosed invasive carcinoma	Metastatic breast carcinoma
• ER • PgR (optional) ***To determine the eligibility for endocrine therapy***	• ER • PgR • HER2 (by IHC and/or ISH) • Ki-67 (optional) ***To provide the prognostic values and determine the eligibility for endocrine therapy or HER2 targeted therapy***	• ER • PgR • HER2 (by IHC and/or ISH) **ER-/PR-/HER2-:** • PD-L1 (upon request) • AR (upon request) ***To determine the eligibility for endocrine therapy, HER2 targeted therapy or immunotherapy***

See molecular testing in Dr. Turner's lectures

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Estrogen Receptor (ER)



ER expressed in 79%-84% of breast cancer, increase by 0.75% per year

Feng Y, Spezia M, Huang S, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis.* 2018;5(2):77-106. Published 2018 May 12. doi:10.1016/j.gendis.2018.05.001

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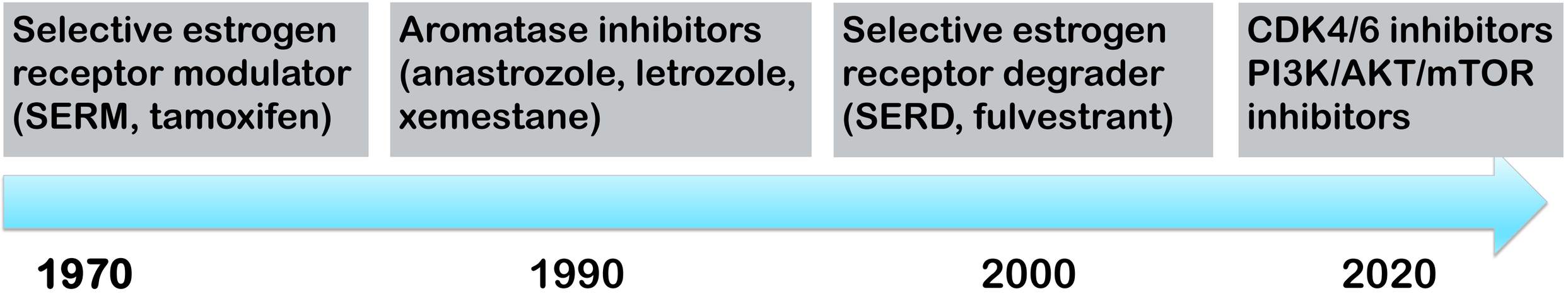
Role of ER Testing in Breast Cancer

- First predicative biomarker in cancer treatment
- Major classifier of breast cancer (ER+ versus ER-)
- Prognostic biomarker (weak) in breast cancer (AJCC 8th edition)
  - Favorable outcome in ER+ BC
- **Well-established predicative biomarker in breast cancer**

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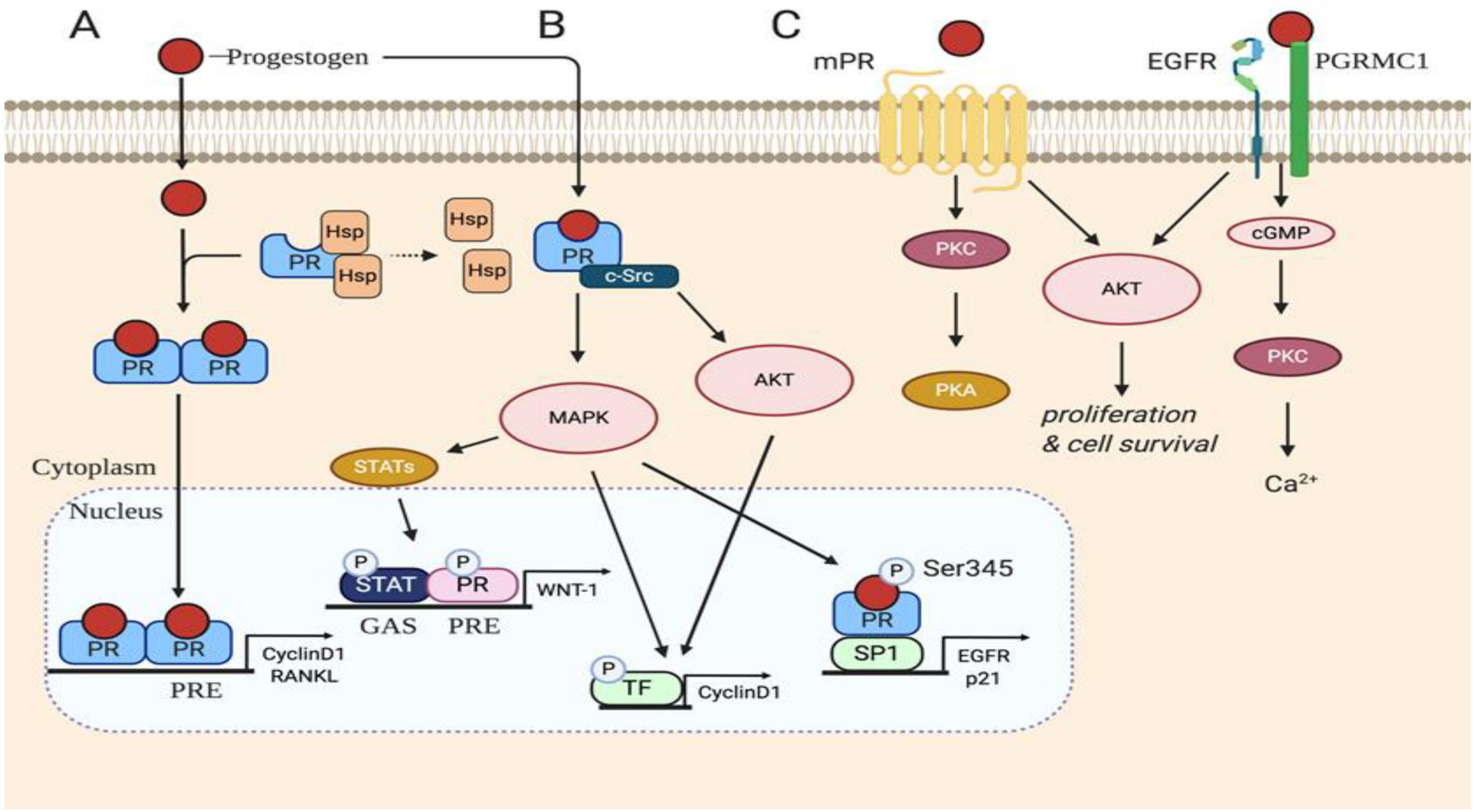
Targeted Endocrine Therapy in Breast Cancer



- ❑ Only effective in ER+ breast cancer
- ❑ Significantly improves survival and reduces risks of tumor recurrence

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Progesterone Receptor (PgR)



PgR expressed in ~65% of ER+ breast cancer

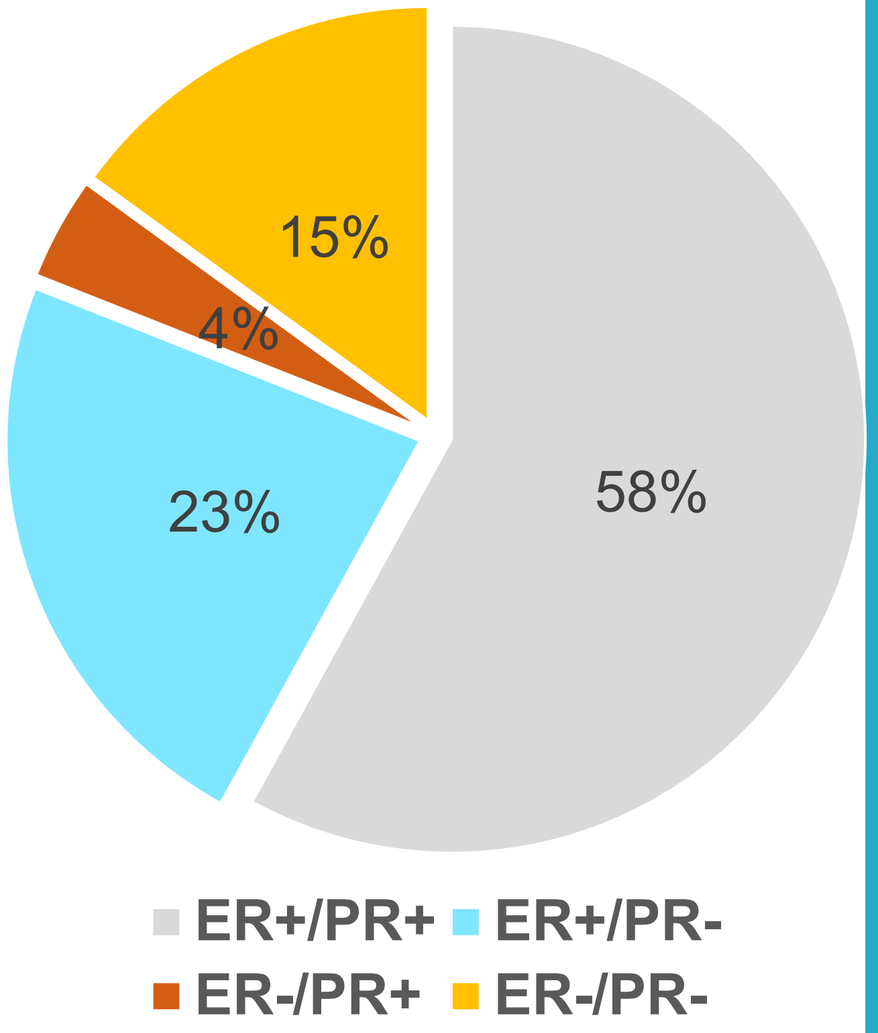
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Pedroza DA, Subramani R, Lakshmanaswamy R. Classical and Non-Classical Progesterone Signaling in Breast Cancers. *Cancers (Basel)*. 2020;12(9):2440. Published 2020 Aug 27. doi:10.3390/cancers12092440

Role of PgR Testing in Breast Cancer

- Not a predictor of benefit from adjuvant endocrine therapy
- PgR expression is regulated by ER, a surrogate for functional ER pathway
- PgR adds prognostic information to stratify outcomes in the ER+ population
  - ER+/PR- vs ER+/PR+: more likely to be luminal-B molecular subtypes, HER2+, higher histologic grade and TNM stage
  - Recurrence score is most strongly associated with PR negativity or low expression

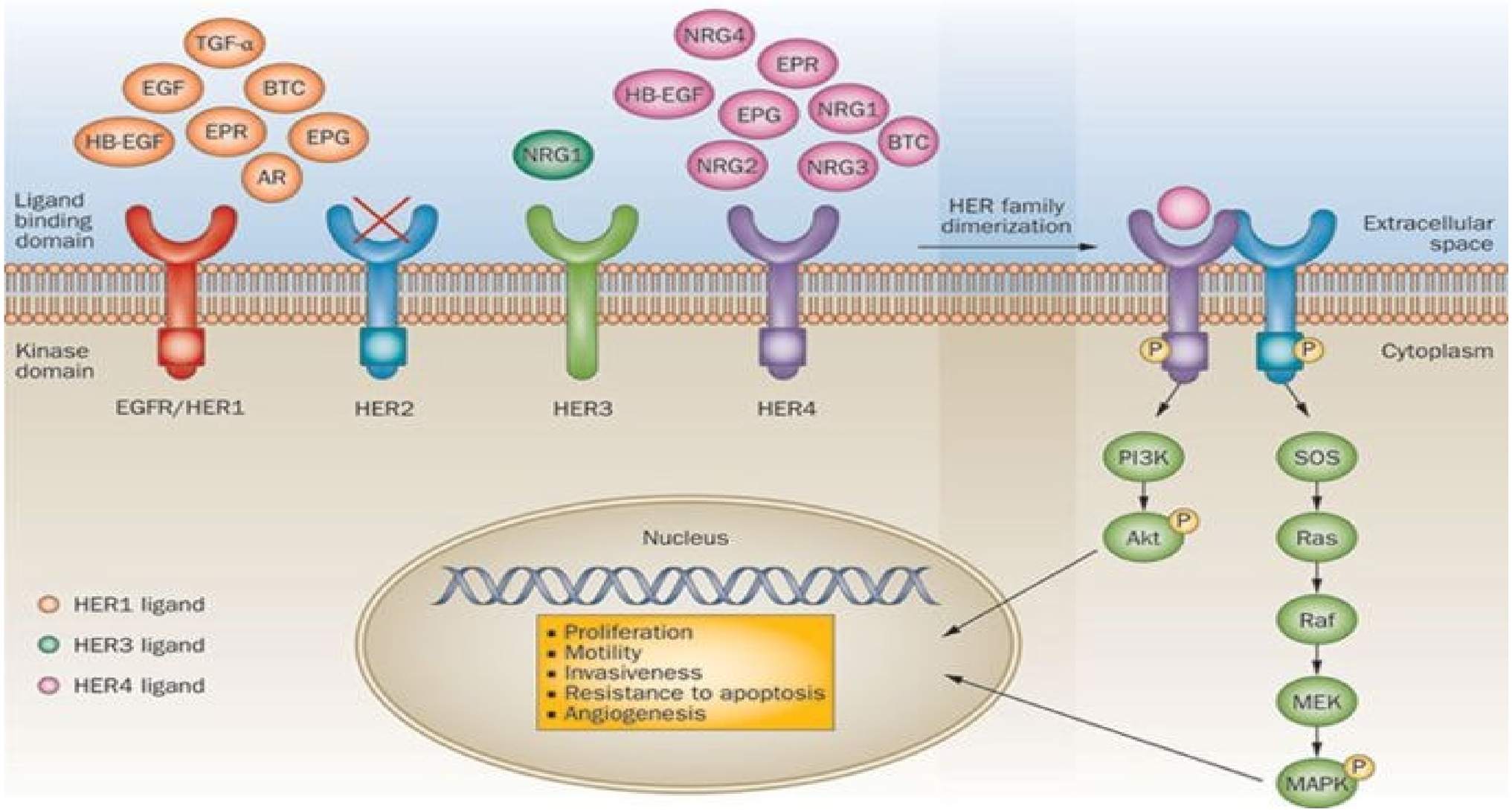


Prat A, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol. 2013;31(2):203-9
Tang P, et al. A lower Allred score for progesterone receptor is strongly associated with a higher recurrence score of 21-gene assay in breast cancer tumors Invest. 2010;28(9):978-82.

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Human Epidermal Growth Factor Receptor 2 (HER2)

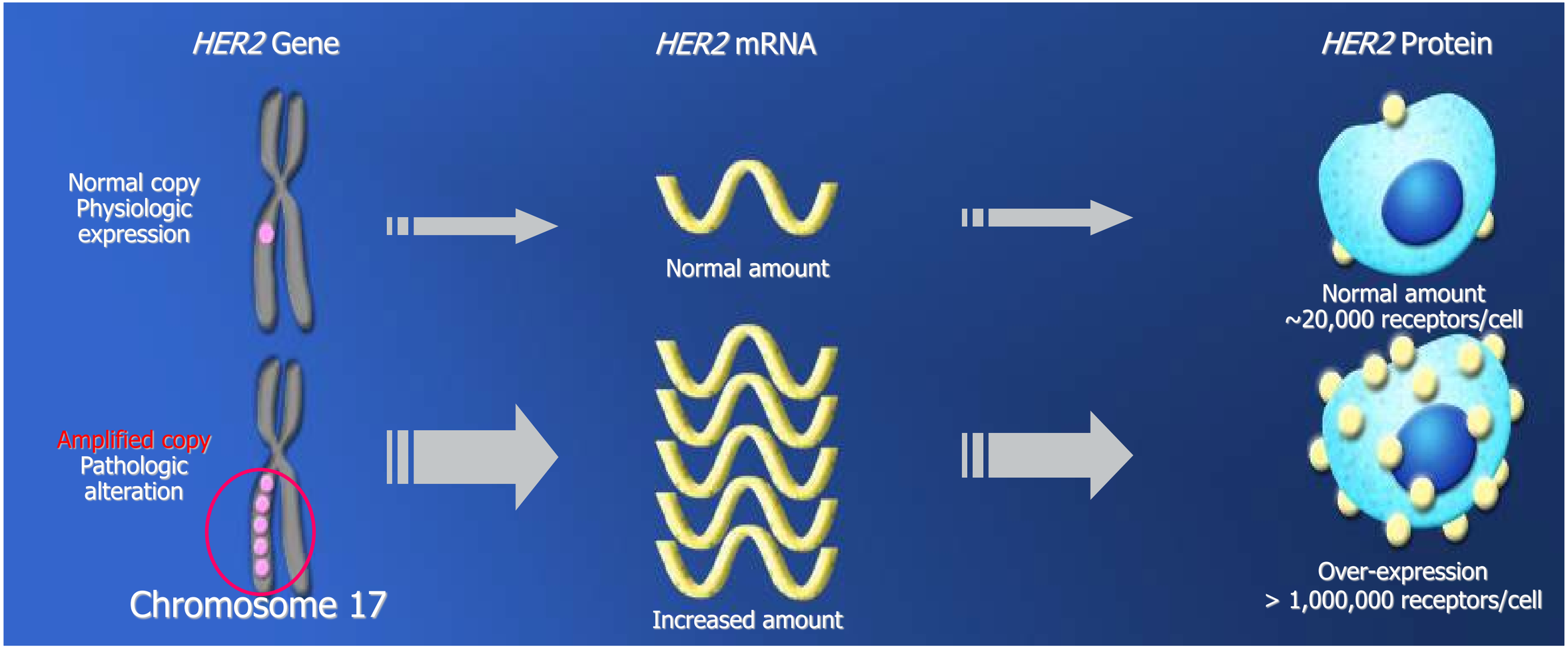


Arteaga, C., Sliwkowski, M., Osborne, C. et al. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat Rev Clin Oncol.* 2012; 9:16–32.

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HER2 Positive Breast Cancer



- *HER2* gene amplification in 15%-20% of early breast cancers
- *HER2* gene amplification drives overexpression of the *HER2* protein

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HER2 Positive Breast Cancer: Important Historical Finding

HER2 Gene Amplification by FISH

HER2 Protein Overexpression by IHC



Prognosis: Shortened median survival
Combined metastatic and adjuvant patients

HER2-positive	**3 years**
HER2-negative	**6-7 years**

HER2 amplified/overexpressing breast cancer:

- **HER2 receptor constitutively active**
- **HER2 contributed to more aggressive tumor behavior**

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177-182. doi:10.1126/science.3798106
Pauletti G, Dandekar S, Rong H, et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol*. 2000;18(21):3651-3664. doi:10.1200/JCO.2000.18.21.3651

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HER2 Targeted Therapy in Breast Cancer

Monoclonal Antibodies	Tyrosine kinase inhibitor	Antibody-drug conjugate
• Trastuzumab 1998 metastatic 2006 adjuvant • Pertuzumab 2012 metastatic 2013 adjuvant • Margetuximab 2020 metastatic	• Lapatinib 2007 metastatic • Neratinib 2016 adjuvant 2020 metastatic • Tucatinib 2020 metastatic	• T-DM1 2013 metastatic 2018 adjuvant after NA • T-DXd 2018 metastatic 2022 metastatic (HER2-low)**

- Only effective in HER2+positive breast cancers**
- HER2 targeted therapy + chemotherapy significantly reduce cancer recurrence and improves disease survival

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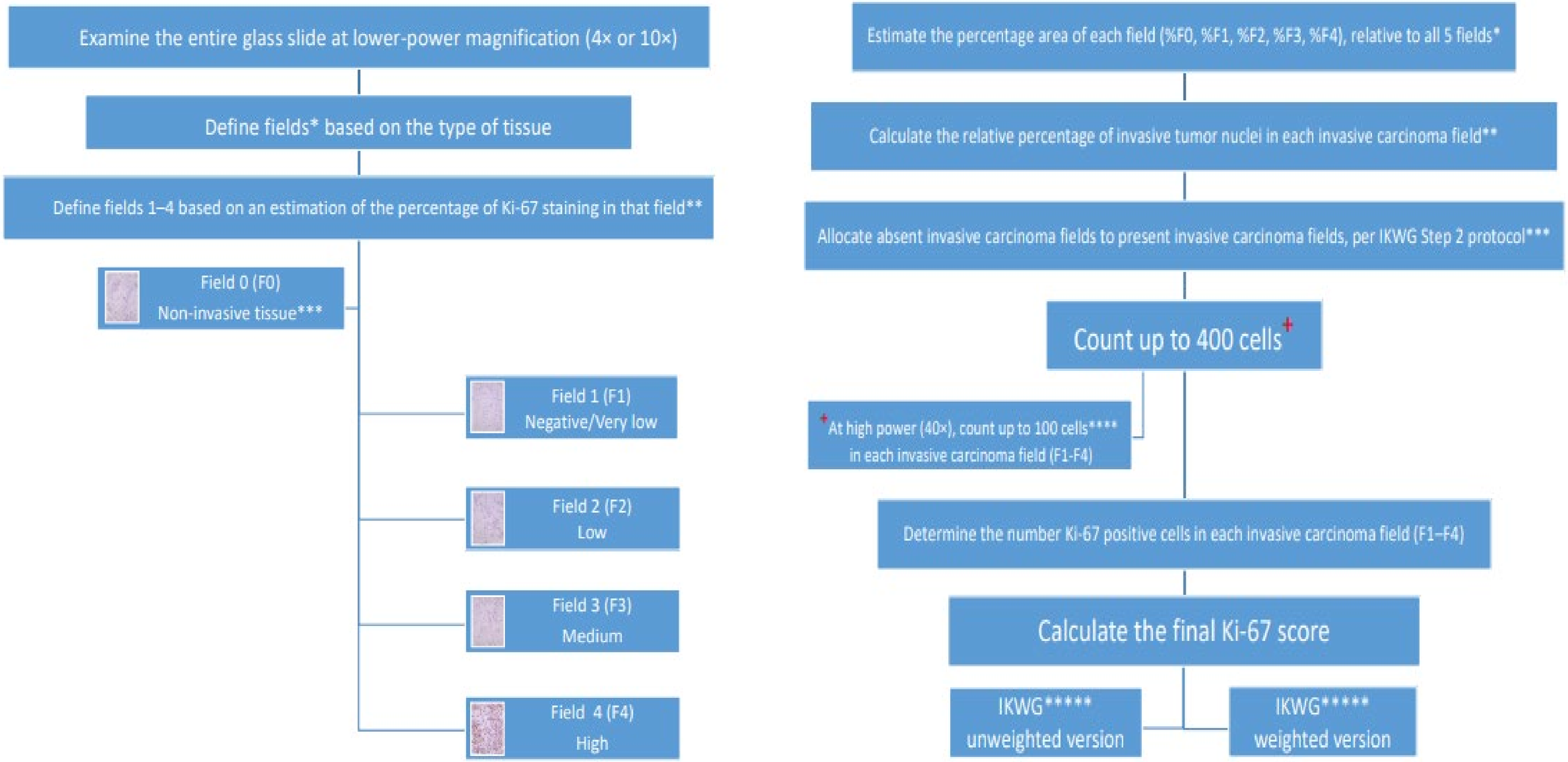
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Ki-67: The proliferation index

- Correlated with the proliferation status of the cells
- BC with high Ki-67 is associated with features of biological aggressiveness
- Different methods are used in the Ki-67 evaluation
- Major factors limiting the use of Ki-67 as prognostic and predictive biomarker in routine clinical practice:
  - Lack of consistently reproducible Ki-67 results
  - Lack of standardized and meaningful cut off

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IKWG's Global Assessment Method

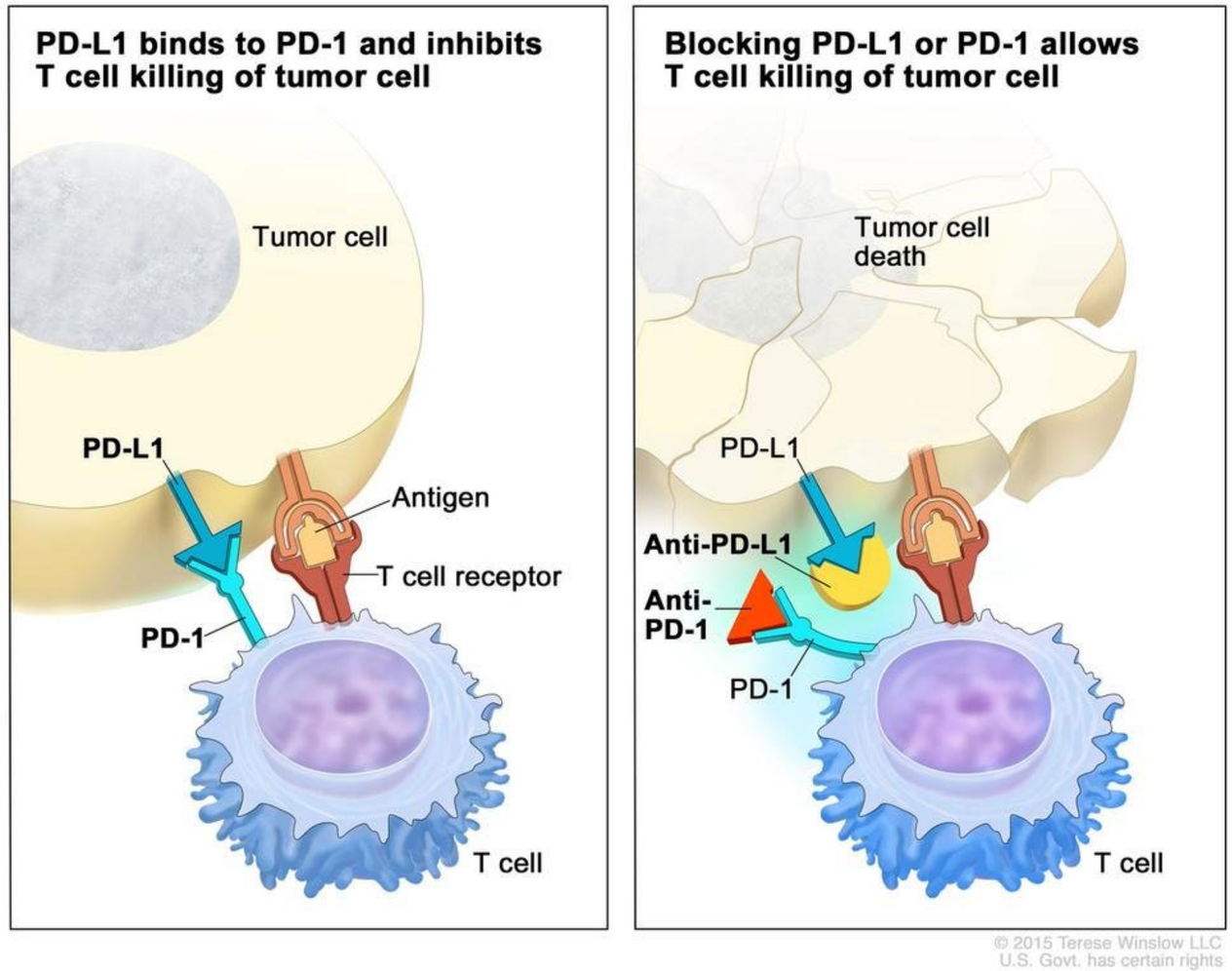


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PD-L1 (Programmed Death Ligand 1)

- Ligand of PD-1
- Expressed on various tumors and immune cells
- Binding of PD-L1 to PD-1 mediates the escape of tumor cells from immune surveillance
- Pembrolizumab approved in adjuvant and neoadjuvant treatment of TNBCs

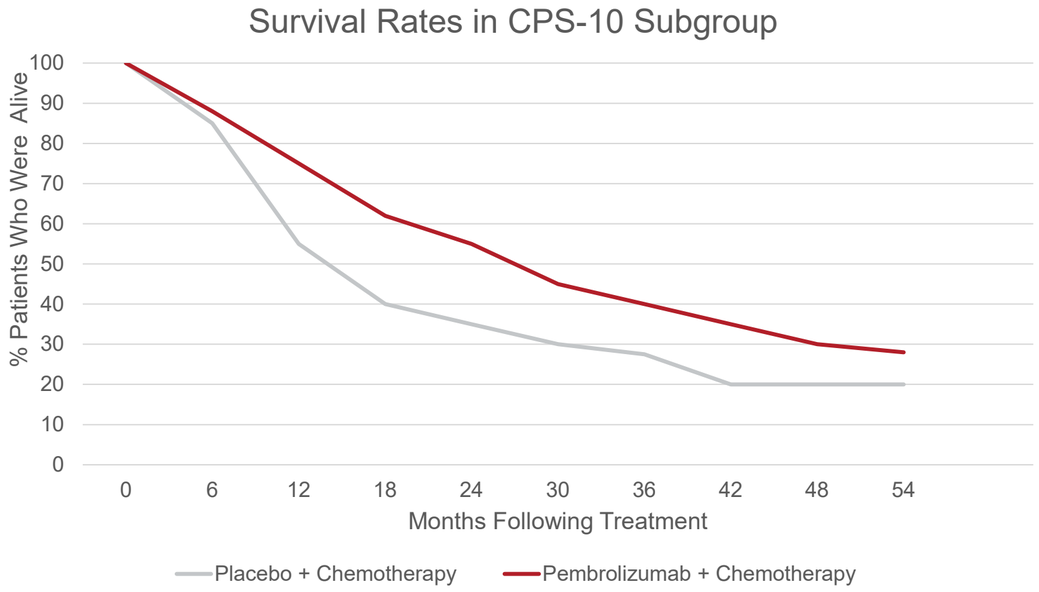


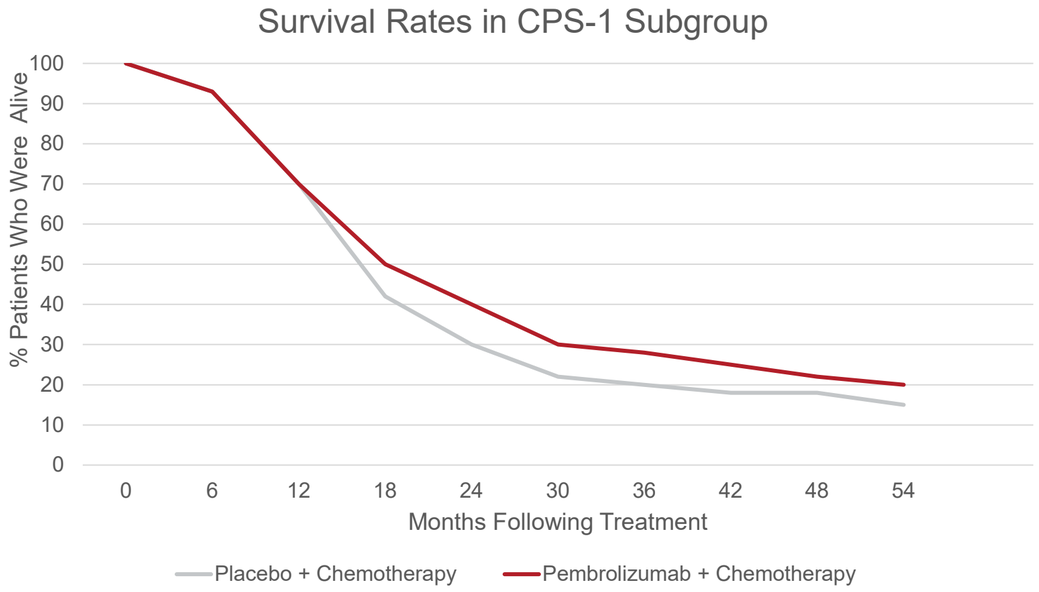
Credit: © Terese Winslow

National Cancer Institute. Immune checkpoint inhibitors. National Cancer Institute. Accessed September 19, 2023. https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

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Keynote 355

- **847 advanced TNBC patients received placebo ± pembrolizumab plus chemotherapy.**
- **Overall survival: significantly longer with pembrolizumab–chemotherapy (23 months) than with chemotherapy alone (16.1 months) in the CPS-10 subgroup.**
- **In the CPS-1 subgroup, the between-group difference was not significant.**

Locally advanced unresectable or metastatic TNBC for pembrolizumab treatment

----Need PD-L1 testing

Adapted from Cortes J, Rugo HS, Cescon DW, et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. *N Engl J Med*. 2022;387(3):217-226. doi:10.1056/NEJMoa2202809

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Variable	Pembrolizumab-Chemotherapy (N = 401)	Pacebo-Chemotherapy (N = 201)	Estimated Treatment Difference % points (95% CI)	P Value
Pathological stage ypT0/Tis yp N0				
No of pts	260	103		
% of pts with response (95% CI)	64.8 (59.9-69.5)	51.2 (44.1-58.3)	13.6 (5.4-21.8)	P<0.001
Pathological stage ypT0 ypN0				
No of pts	240	91		
% of pts with response (95% CI)	59.9 (54.9-64.7)	45.3 (38.3-52.4)	14.5 (6.2-22.7)	

Early stage TNBC for pembrolizumab treatment in NAC
----Do not need PD-L1 testing

Keynote 522

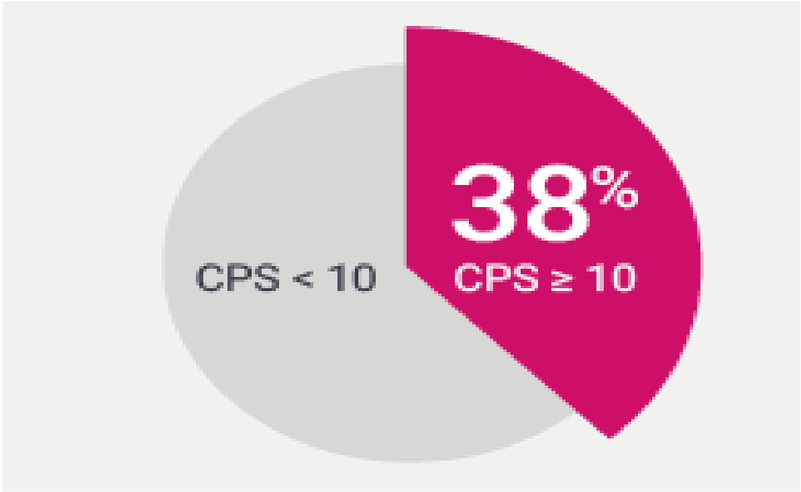
- 1174 early stage TNBC patients received placebo ± pembrolizumab plus chemotherapy in NAC setting.
- Interim analysis (602 patients)
  - pCR rate was significantly higher with pembrolizumab–chemotherapy (64.8%) than with chemotherapy alone (51.2%).
- The benefits of pembrolizumab–chemotherapy with respect to pCR were generally consistent across subgroups, including PD-L1–expression subgroups.

Adapted from Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med*. 202027;382(9):810-821. doi: 10.1056/NEJMoa1910549.

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PD-L1 Testing in Advanced TNBC

- Determine eligibility for pembrolizumab in aTNBC
- FDA-approved companion diagnostic test: PD-L1 IHC 22C3 pharmDx
- Can be performed on either new metastatic tumor biopsies or archival primary tumor samples
  - Metastases tend to decrease the chances of having immune cells present to express PD-L1 compared to primary tumor; **primary tumor is preferred**
  - Liver and brain tends to have fewer TILs; **preferable to avoid PD-L1 testing on liver samples if possible**.
  - Not validated for decalcified bone specimens, cytology cell blocks or smears, or circulating tumor cells



$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# viable tumor cells}} \times 100$$

- CPS <10: Negative
- CPS ≥10: Positive

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Androgen receptor (AR)

- Steroid receptor superfamily
- AR is expressed in 60%-90% of BCs
  - ER+ tumors (~70%) and TNBCs (~ 40%)
- AR IHC testing is well-established
  - Different cut-offs (1% or 10% cells with nuclear staining) proposed to classify AR-positive cases in BC
- Prognostic and predicative values of AR in BC are still uncertain
  - AR positive TNBC patients may respond to AR targeting agents
- No formal recommendation on AR testing in BC

Ravaioli S, Maltoni R, Pasculli B, et al. Androgen receptor in breast cancer: The "5W" questions Front Endocrinol (Lausanne). 2022 Aug 30;13:977331
Androgen Receptor in Breast Cancer-Clinical and Preclinical Research Insights. Molecules. 2020 Jan 15;25(2):358. Anestis A, Zoi I, Papavassiliou AG, Karamouzis MV.

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Part II

ASCO/CAP Testing Guidelines in Breast Cancer and Recent Updates

- ER/PgR (2010, 2020)
- HER2 (2007, 2013, 2018, 2023)

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General guidelines for ER/PgR and HER2 testing

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When to Test ER/PgR/HER2

- All newly-diagnosed invasive breast cancer: ER, PgR, and HER2
- Metastatic/recurrent invasive breast cancer (if material available): ER, PgR, and HER2
  - ER/PgR/HER2 status may change from neg to pos with disease progression (9-16% of cases)
- DCIS: ER (recommended), PgR (optional)
  - Endocrine therapy: significant risk reduction in recurrence restricted to ER-positive DCIS, but no significant impact on overall survival
  - No data supporting the prognostic or predictive value of PgR testing in DCIS independent of ER
  - HER2 testing is not applicable due to in situ disease and systemic treatment is not indicated

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Material Chosen for ER/PgR/HER2 Testing

- Multiple synchronous tumors
  - Testing should be performed on at least one of the tumors, preferably the largest if morphologically similar
- Histologically distinct ipsilateral carcinomas
- Bilateral breast cancers
- Large (preferably multiple) core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection
  - Excellent concordant results between core biopsy and excisional specimen
  - Well-fixed
  - Ensure timely availability of results for treatment planning discussion

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Pre-analytic Variables:
---- Accurate Results Dependent on Standardization

Preanalytical variables

- Time to fixation
- Type of fixative
- Time in fixative
- Method of tissue processing

- **Tissue handling**
  - Avoid delay in start of fixation (within 1 hour)
  - Sample sliced at 5-mm intervals after gross evaluation and margin designation
  - If remote location, sample bisected through the tumor and immersed in sufficient fixation

Type of fixation used in laboratory
- 10% NBF

- **Fixation time**
  - 6-72 hours

- **Unstained slides cut more than six weeks before analysis is not recommended**

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Controls for ER/PgR/HER2 Test run

- **Judicious use of controls are important for ensuring accurate results.**
  - Ensures the assay performed properly
  - Allows monitoring for analytical drift of assay performance.
  - External controls: include negative and positive samples as well as samples with lower percentages of tested biomarker expression
    - ER/PgR: Tonsil, cervix, endometrium, engineered material
    - HER2: Tissue-based controls with validated HER2 status or cell line preparations containing multiple samples of known HER2 status
  - Internal controls: assess the quality of the tissue for biomarker analysis.
  - On-slide controls: ensure the reagents have properly dispensed on the slide.
- **Tissue handling is important and should be monitored.**
  - Use of controls help to identify an “issue with the tissue.”

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ER/PgR Testing and Evaluation

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ER/PgR Testing Methods

- **Ligand binding assay (quantitative, 1970s-1990s)**
  - Most of the clinical trial of endocrine therapy

- **Immunohistochemistry (semi-quantitative, 1990s-)**
  - Multiple methods, different primary Abs (clone 6F11, 1D5, and SP1)
  - Varying sensitivity and specificity
  - Most assays developed by comparison to LBA
  - Few assays validated using patient outcome
  - Varying degrees of assay standardization in many laboratories

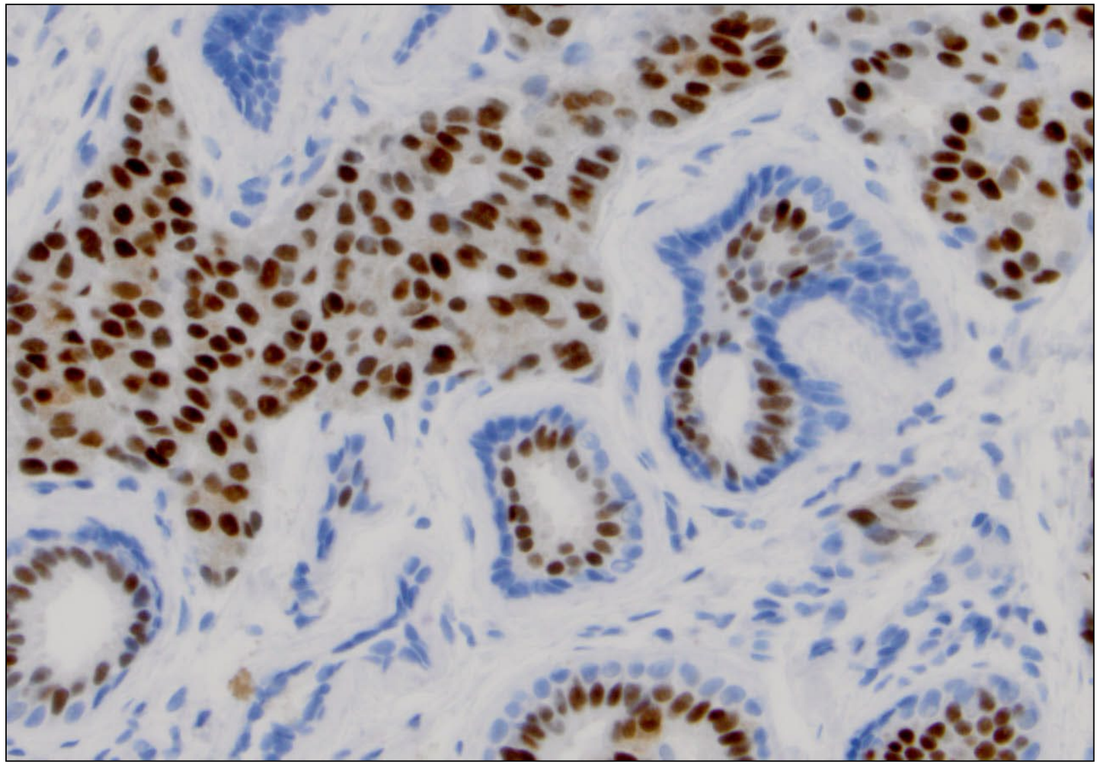
- **RNA expression or other molecular assay**
  - Research only or as a part of multigene expression assay
  - ER/PgR protein and mRNA: discordance rate of 9% and 12%
  - Ability to predict endocrine therapy benefit as an initial screening test?
  - Intermixed non-cancer tissues may affect results
  - Premature to recommend these individual measures for assay standardization and validation

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IHC for ER and PgR

Validated IHC is the recommended standard test for predicting benefit from endocrine therapy

- **Advantages of IHC for ER/PgR:**
  - Morphologic visualization
  - Lower cost, rapid TAT
  - Ability to test small quantities of tissue
  - Good correlation with biochemical method assay results
  - Equivalent or superior to LBA in predicting benefit from adjuvant endocrine therapy



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Evaluate Control—External control
Unacceptable Staining: Need to Repeat

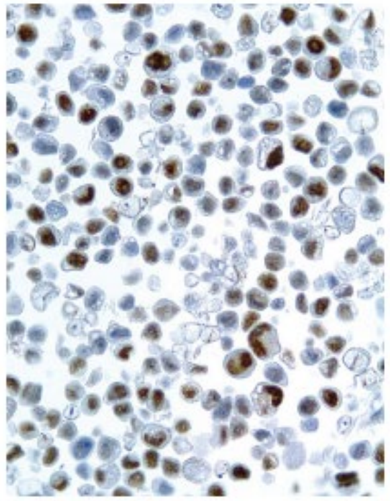


Figure 10
Unacceptable staining.
Unacceptable weak staining intensity (too light);
Weak staining may result in false-negative results;
40x magnification

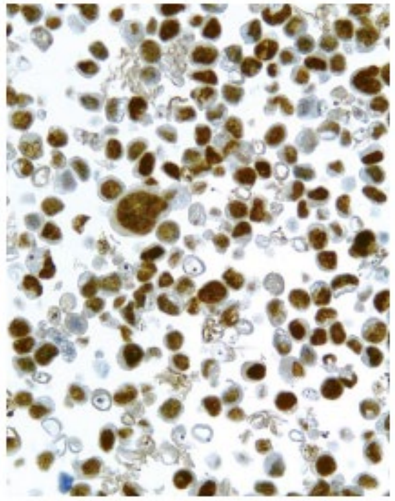


Figure 11
Acceptable staining;
40x magnification

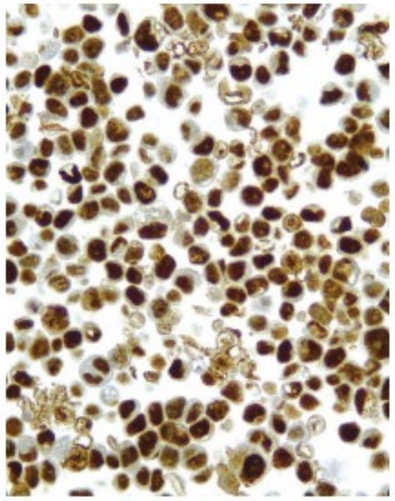


Figure 12
Unacceptable staining.
Unacceptable strong staining intensity (too dark);
Excessively strong staining may result in
false-positive results;
40x magnification

The controls are too weak or too strong

Unacceptable Background

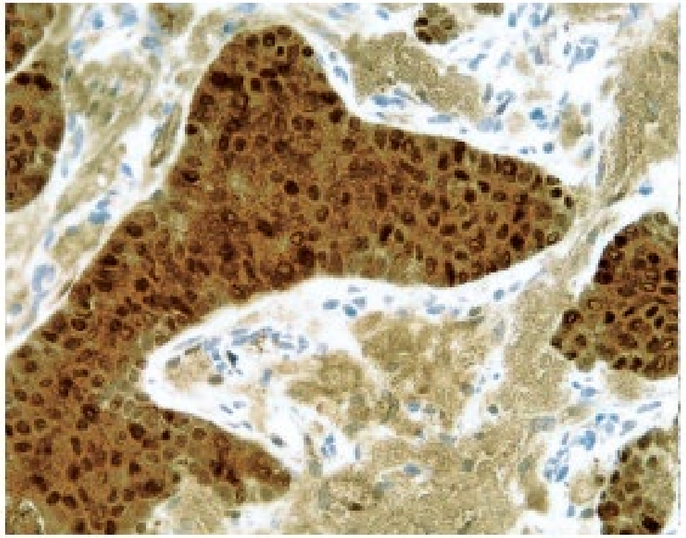


Figure 40
Breast cancer stained with ER; Example of unacceptable background staining
Invalid test; 40x magnification

Background staining

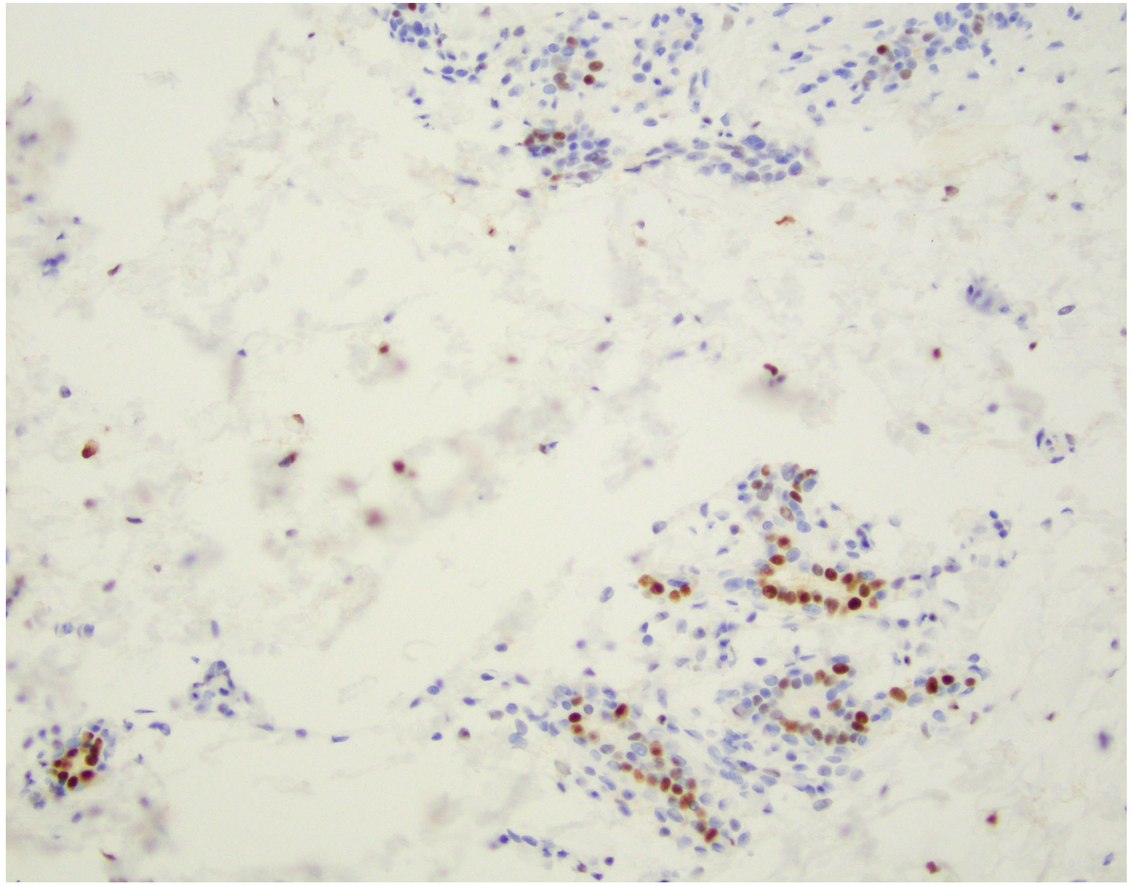
- Diffuse, nonspecific staining
- Issues during fixation and processing, incomplete removal of paraffin and rinsing

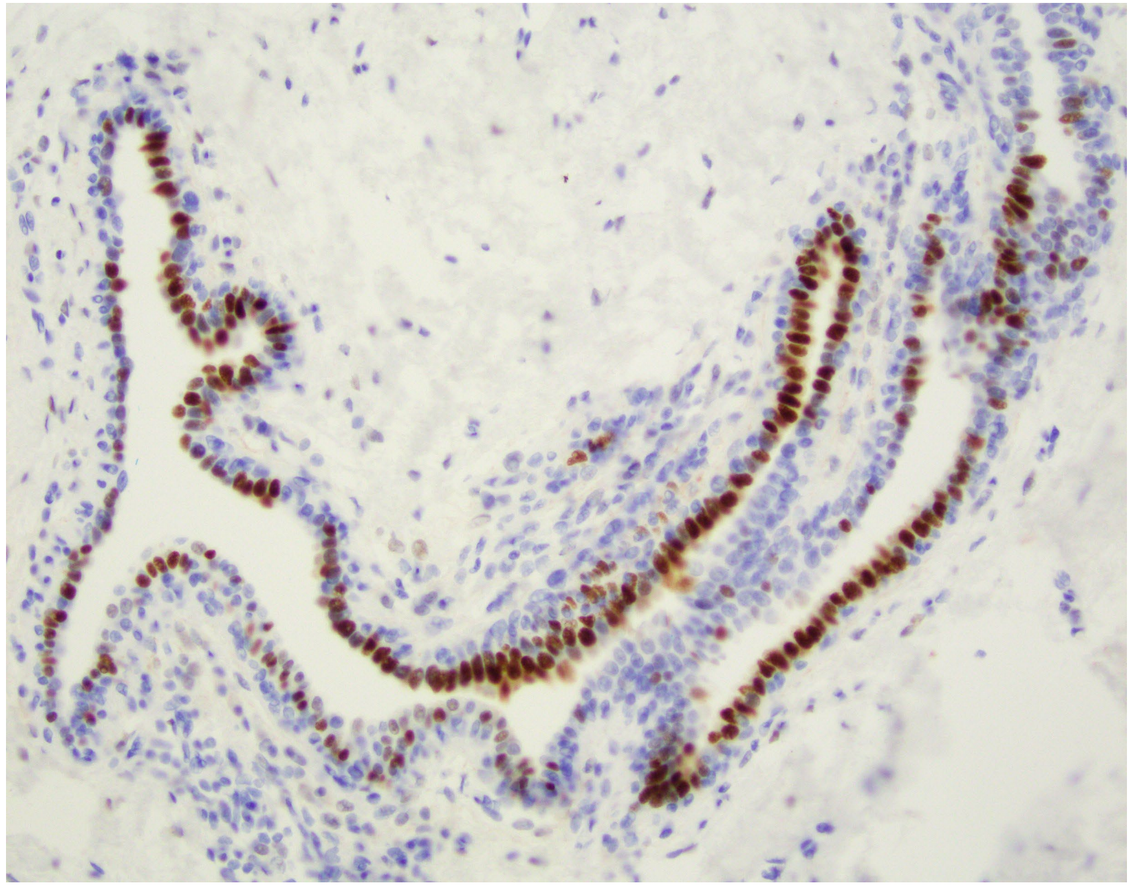
ER/PR pharmDx™Interpretation Manual: Pathology. Dako. Accessed September 19, 2023. https://www.agilent.com/cs/library/usermanuals/public/28252_er-pr_pharmdx_interpretation_manual.pdf

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Evaluate Control—Internal control

-- Normal Breast Epithelium Staining





- Positive in ~15%-30% of normal luminal epithelial cells
- ER increase in columnar cell change (less optimal in evaluating cases with low levels of ER expression)
- Negative ER expression in apocrine metaplasia

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Standardize ER/PgR Interpretation and reporting

- Evaluate the entire tumor area of the section on the IHC slide
- % evaluated by estimation or quantification
- Cytology: At least 100 cells
- Record:
  - Testing result: Positive or negative
    - Positive: ≥1% of any staining
    - Negative:<1% of any staining in the presence of appropriately stained extrinsic and intrinsic controls
  - % of ER+/PgR+ invasive tumor cells
  - Average intensity of positive staining

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Cut-off Point for ER/PgR Positive Versus Negative

Threshold for ER/PgR positive: ≥1% of cells with any intensity of staining

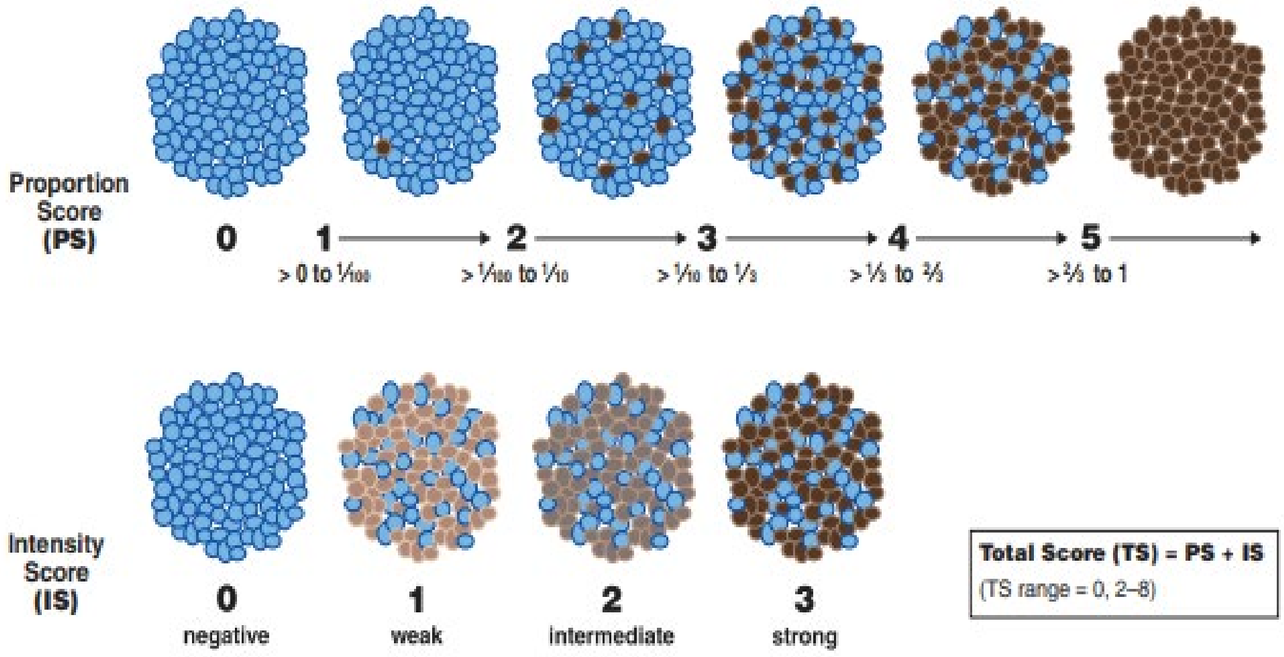
- **Levels as low as 1% positive staining carcinoma cells are associated with significant clinical response**

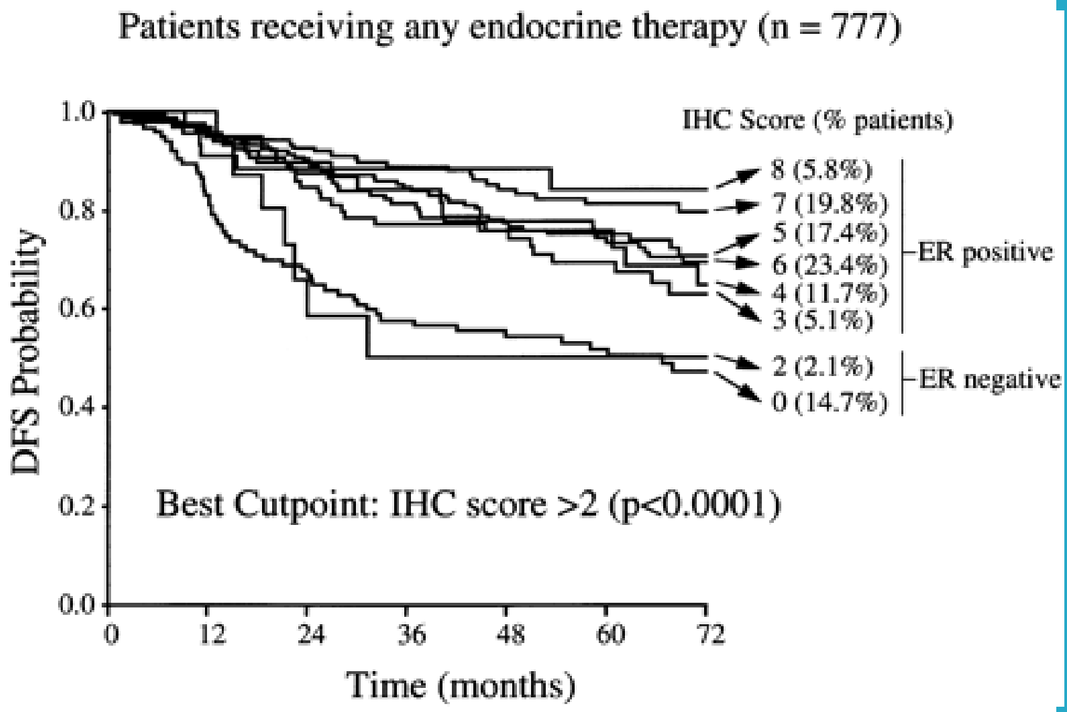
- **Substantial impact of tamoxifen and other endocrine therapies on mortality reduction and relatively low toxicity profile**

Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer [published correction appears in J Clin Oncol. 2010 Jul 20;28(21):3543]. *J Clin Oncol*. 2010;28(16):2784-2795. doi:10.1200/JCO.2009.25.6529

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Quantitative Interpretation: ER and PgR Scoring IHC Slides

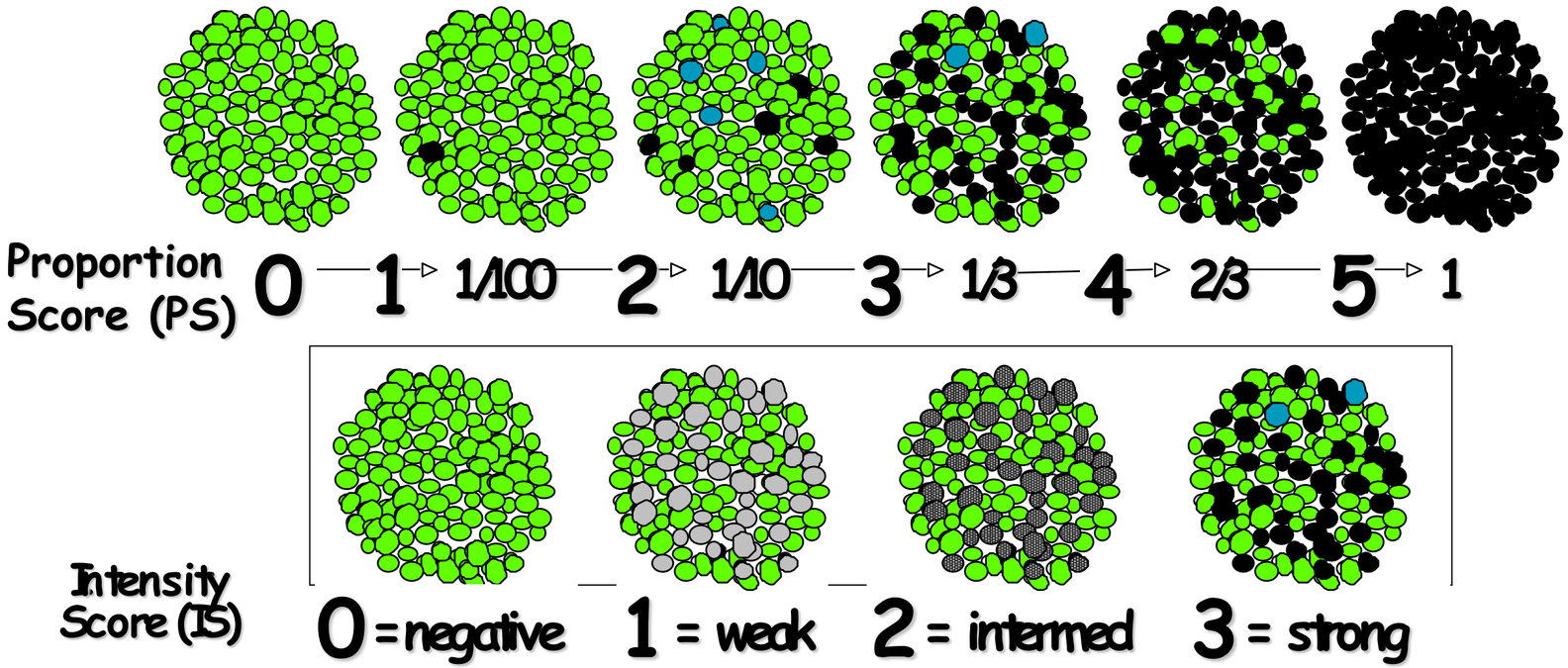




Allred score (range 0-8): Total score (TS) = PS + IS

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Quantitative Interpretation: ER and PgR Scoring IHC Slides



H score (range 0-300): Multiplying the percentage of cells demonstrating each intensity (scored from 0 to 3) and adding the result

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Highly Unusual ER/PgR Findings/Discordance

- **Highly unusual ER-negative results**
  - Invasive ductal carcinoma, histologic grade 1
  - Invasive lobular carcinoma, classic type
  - Pure tubular carcinoma, cribriform carcinoma or mucinous carcinoma
  - Encapsulated or solid papillary carcinoma
- **Highly unusual ER-Negative Results**
  - Metaplastic carcinoma
  - Adenoid cystic carcinoma, secretory carcinoma, or other salivary-gland like breast carcinoma
  - Invasive carcinoma with apocrine differentiation

When encountered, additional steps should be taken:

- **Check the accuracy of the histologic type or grade**
- **Check the preanalytic and analytic testing factors**
- **Consider second reviews and repeat testing**
- **If all results appear valid, the result can be reported with a comment noting that the findings are highly unusual, and testing of additional samples may be of value to confirm the findings**

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Recent Updates on ER/PgR Testing Guidelines

2020 ASCO/CAP: ER-Low Positive Breast Cancer

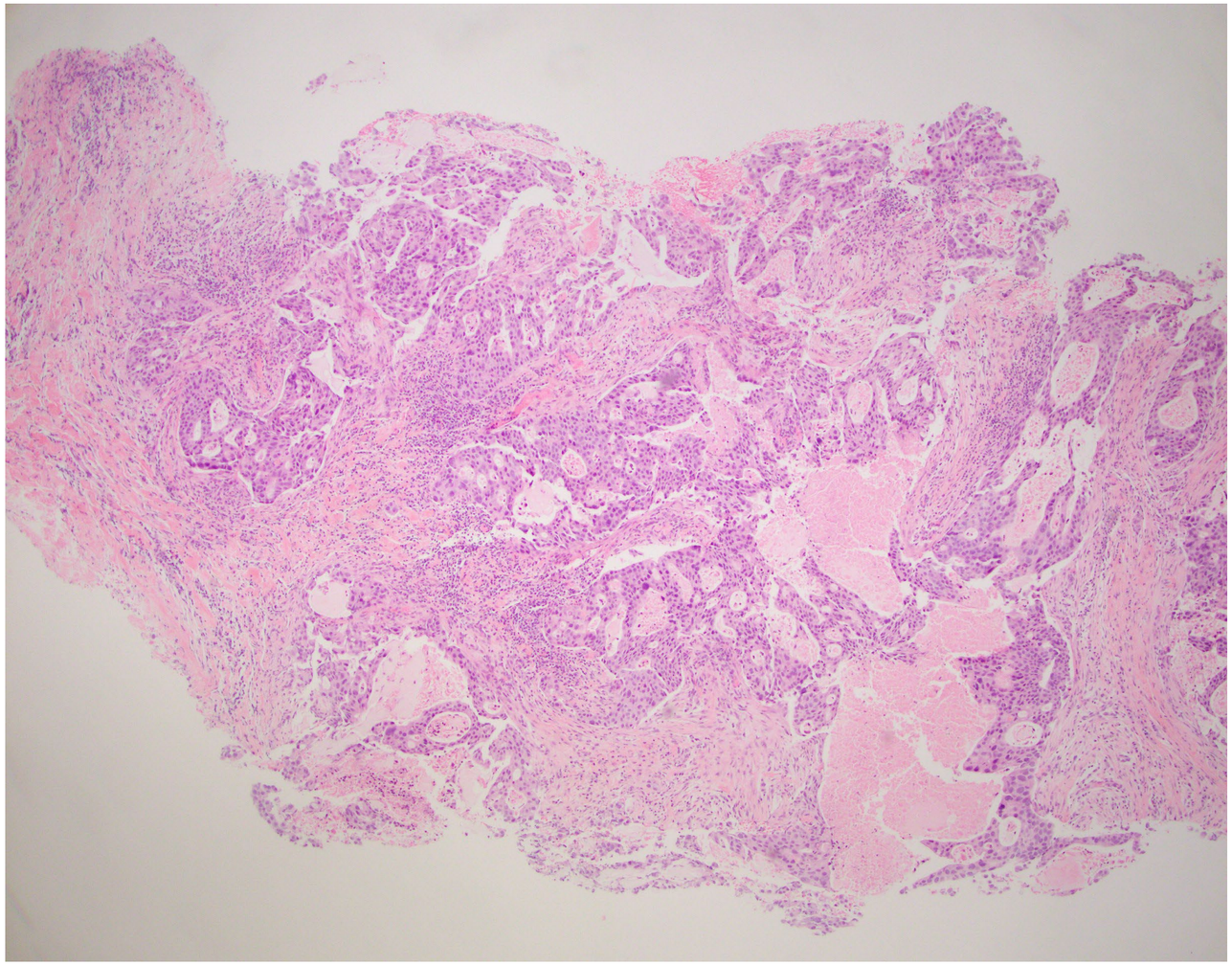
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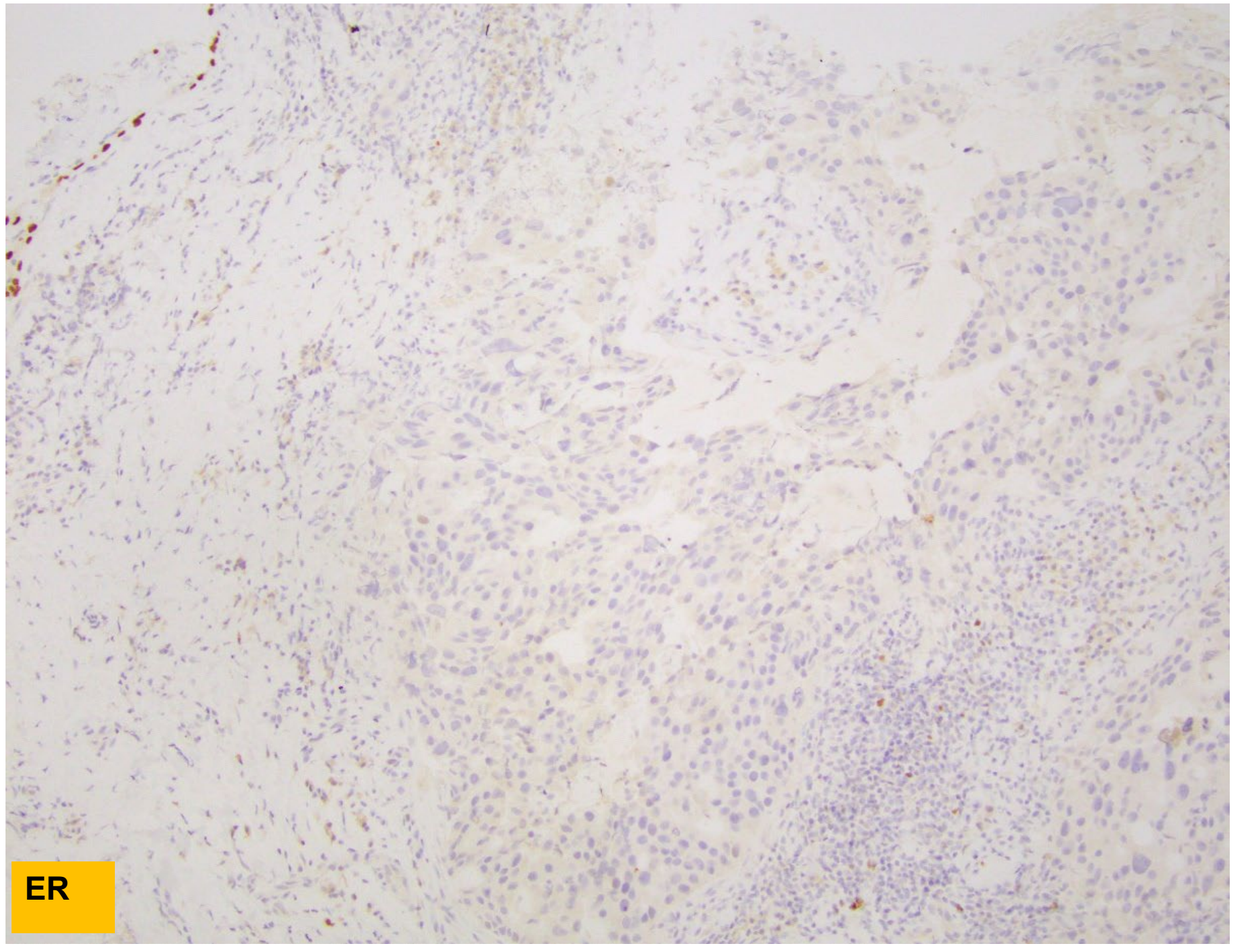
55-year-old woman: 3.3-cm breast mass

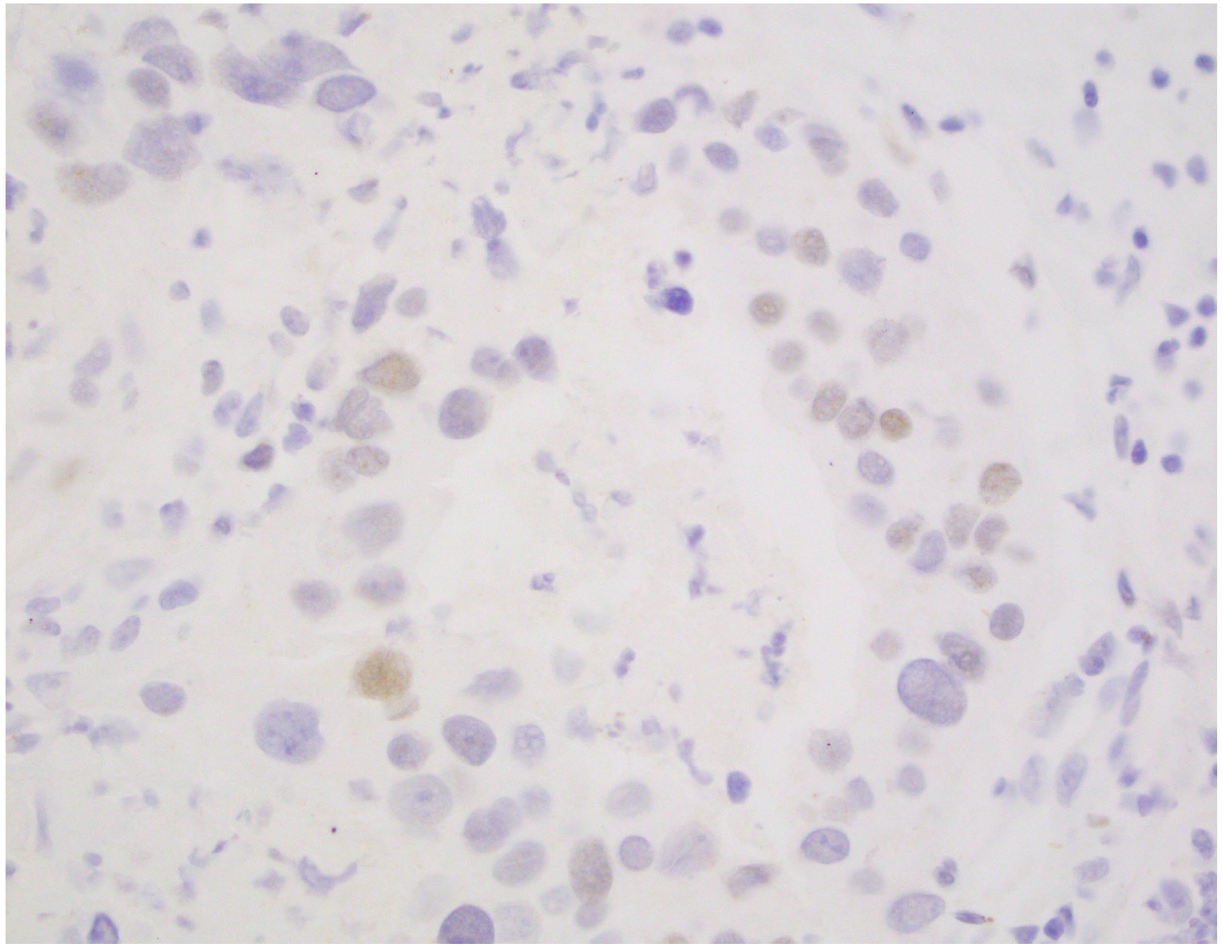
Breast, core biopsy:

- **Invasive ductal carcinoma, grade 3**
  - **ER: See next slide**
  - **PgR: Negative**
  - **HER2: Positive**



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ER: ~8% of invasive tumor cells with weak positivity

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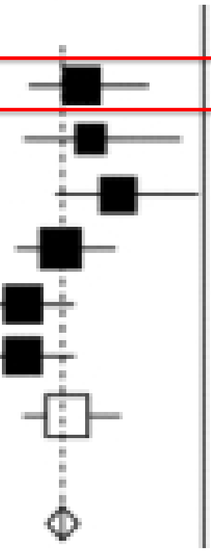
✓ **Potential benefit of endocrine therapy in patients with breast cancers with >10% ER expression or the lack of potential benefit for cancers with <1% ER expression.**

? **Limited data on low ER expression (most often defined as 1%-10% ER-positive cells by IHC) and the potential benefit from endocrine therapy.**

2020 ASCO/CAP ER Testing guideline:
What additional strategies can promote optimal performance, interpretation, and reporting of immunohistochemistry (IHC) assays, particularly in cases with low estrogen receptor (ER) expression?

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Category	Events/woman-years (rate [% per year])		Tamoxifen events		Ratio of annual event rates
	Allocated tamoxifen	Allocated control	Log-rank O-E	Variance of O-E	Tamoxifen : control
(b) ER-positive by ER measurement					
ER 10–19	232/8173 (2·8)	316/7252 (4·4)	-47·4	120·6	0·67 (SE 0·08)
ER 20–29	158/5104 (3·1)	197/4630 (4·3)	-27·3	76·4	0·70 (SE 0·10)
ER 30–49	235/8107 (2·9)	260/6952 (3·7)	-29·0	112·1	0·77 (SE 0·08)
ER 50–99	293/10650 (2·8)	361/8973 (4·0)	-69·6	144·8	0·62 (SE 0·07)
ER 100–199	211/8429 (2·5)	344/7376 (4·7)	-80·4	122·8	0·52 (SE 0·07)
ER≥200	216/8279 (2·6)	325/6672 (4·9)	-78·2	119·0	0·52 (SE 0·07)
Other ER+	308/7868 (3·9)	415/6898 (6·0)	-72·9	161·3	0·64 (SE 0·06)
(b) Subtotal	**1653/56610 (2·9% per year)**	**2218/48753 (4·5% per year)**	**-404·8**	**856·9**	**0·62 (SE 0·03) 2p<0·0000**



- **Effects of ER-targeted therapies correlated with ER levels**
- **ER weakly positive disease (10-19 fmol/mg cytosol protein): Substantial benefit**

Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*. 2011; 378(9793)): 771–784

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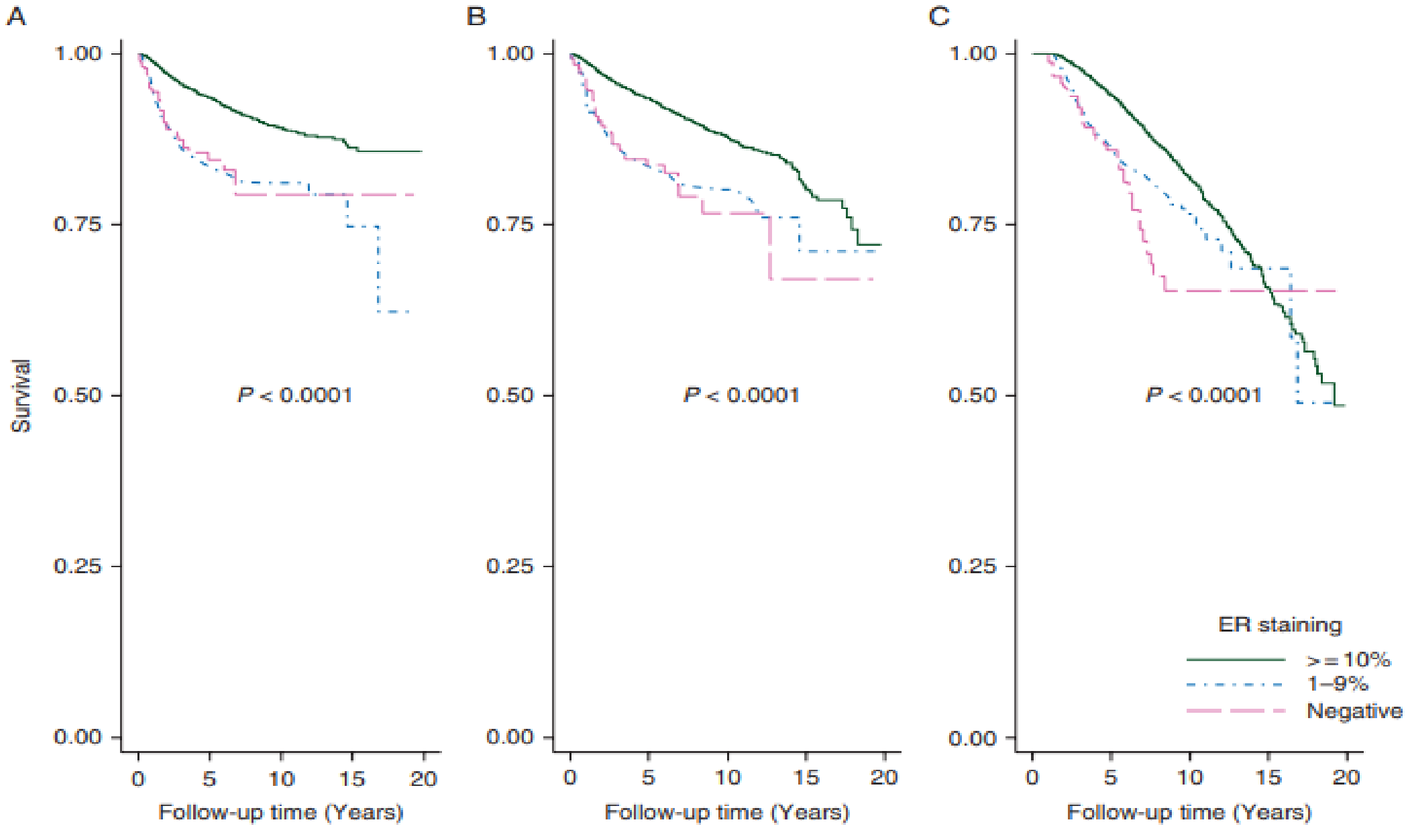


Figure 1. Comparison of survival outcomes among patients with three different levels of ER expression in the primary tumor: (A) distant recurrence-free survival, (B) recurrence-free survival, (C) overall survival.

Patients with ER 1%-9% tumors:

- Worse survival outcomes than ER+ tumors
- Similar survival outcome as those with ER- tumors

Yi M, Huo L, Koenig KB, et al. Which threshold for ER positivity? a retrospective study based on 9639 patients. *Ann Oncol*. 2014;25(5):1004-1011. doi:10.1093/annonc/mdu053

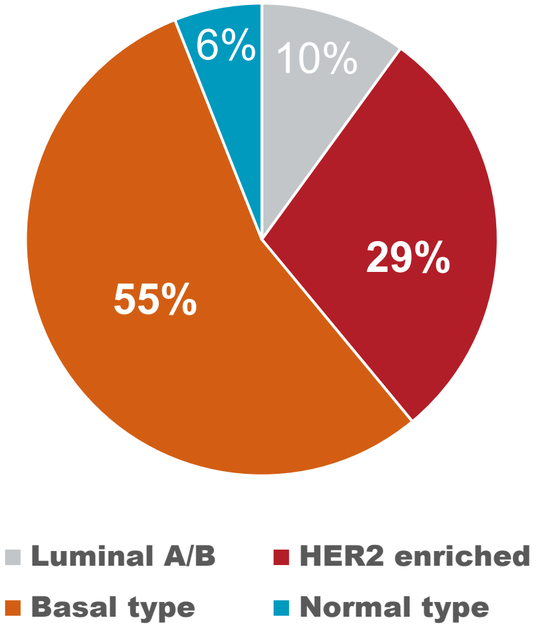
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ER-Low Positive Breast Cancer

- Breast cancers with 1% to 10% cells staining for ER
- 2% to 3% of ER positive breast caner
- Heterogeneous group
- Clinical outcomes and biologic/molecular profiles more similar to those of ER-negative cancers
- Additional steps should be taken to confirm or adjudicate the validity of the results, and correlation with histology should be performed

The low positive category does not apply to PgR

Distribution of molecular subtypes of ER-low positive breast cancer



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2020 ASCO/CAP ER/PgR Testing Guidelines Recommendation

Additional Recommended Reporting Comments for Specific Scenarios	
Result	**Additional Recommended Comment**
1-10% cell 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.
No internal controls and ER is 0-10%	No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

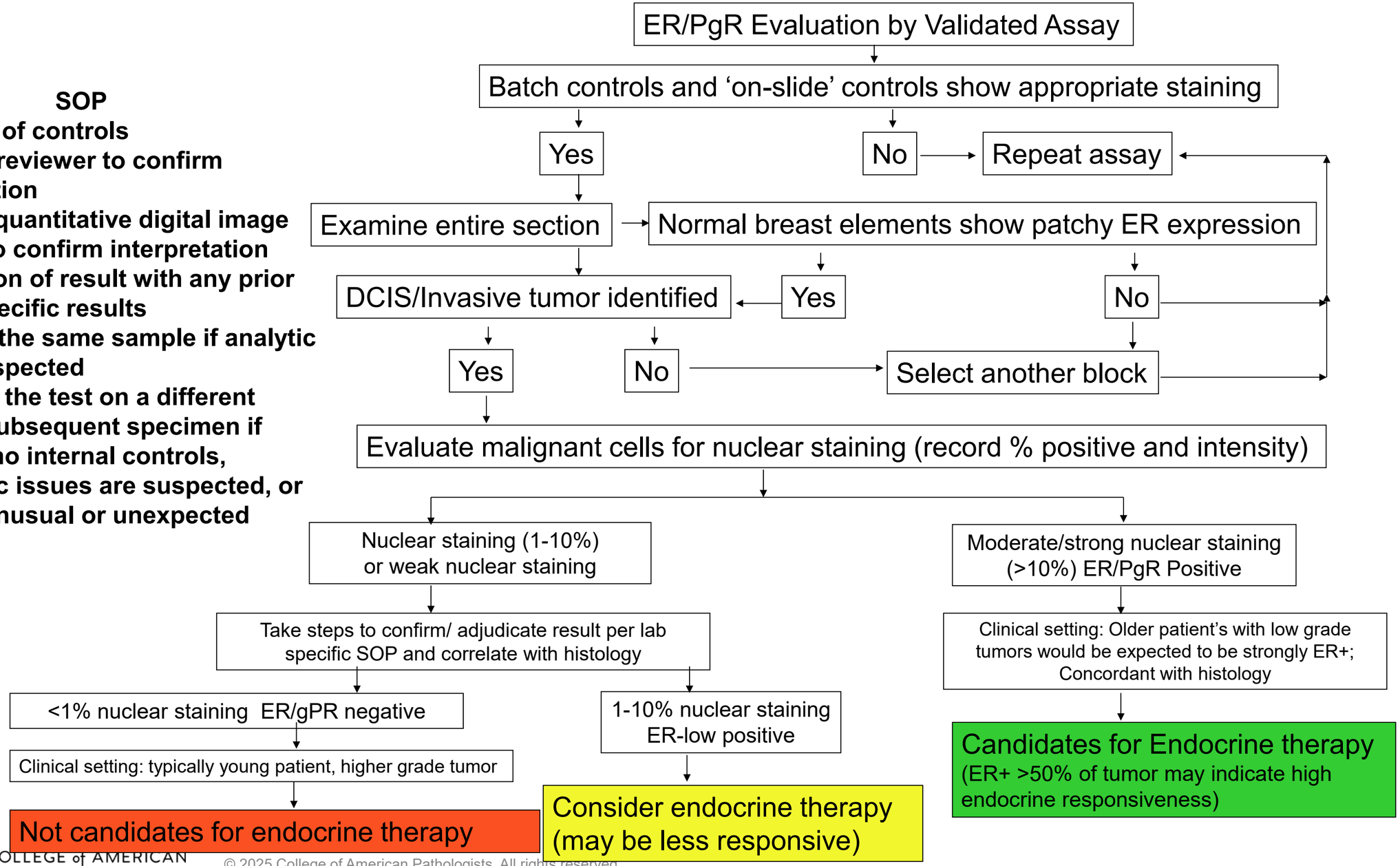
Adapted from Allison K et al., J Clin Oncol 38:1346-1366.

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ER/PgR Interpretation algorithm

SOP

- **Re-review of controls**
- **A second reviewer to confirm interpretation**
- **Validated quantitative digital image analysis to confirm interpretation**
- **Comparison of result with any prior patient-specific results**
- **Retesting the same sample if analytic issues suspected**
- **Repeating the test on a different block or subsequent specimen if there are no internal controls, preanalytic issues are suspected, or result is unusual or unexpected**



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Courtesy of David Hicks MD with modification

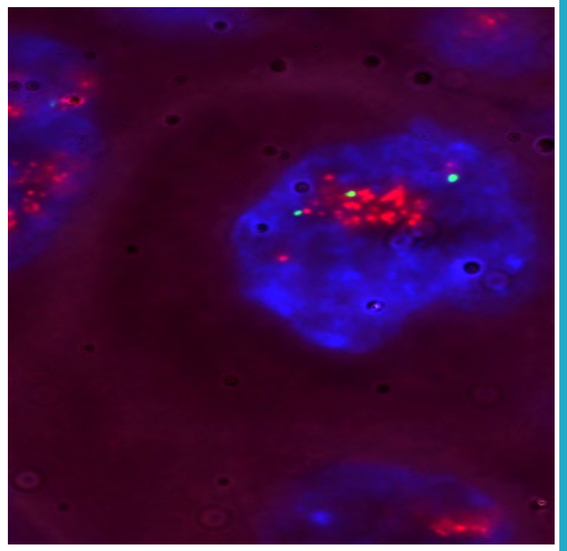
HER2 Testing and Evaluation

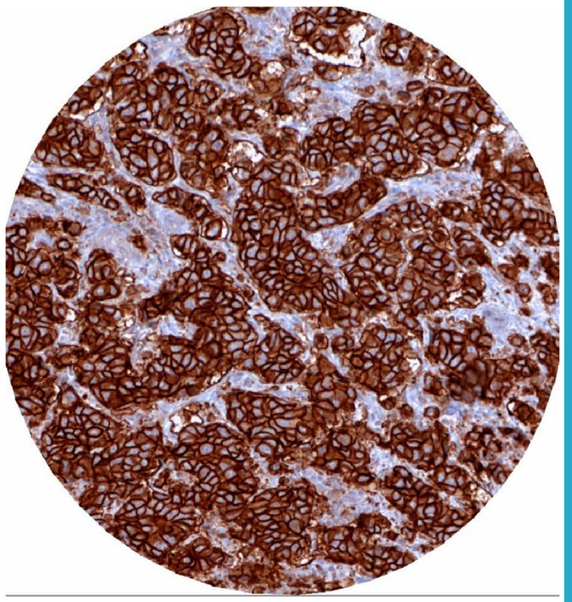
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HER2 Testing Methods

Biological event		Testing Method
✓Gene amplification	→	Southern blot/**ISH***/PCR
↓		
mRNA synthesis	→	Northern blot/RTPCR
↓		
Protein synthesis	→	Western blot
↓		
✓Cell membrane protein	→	**IHC***
↓		
Protein shed from cell surface	→	Serum ELISA





***Both ISH and IHC have been clinically validated and FDA approved for patient selection for HER2-targeted therapy**

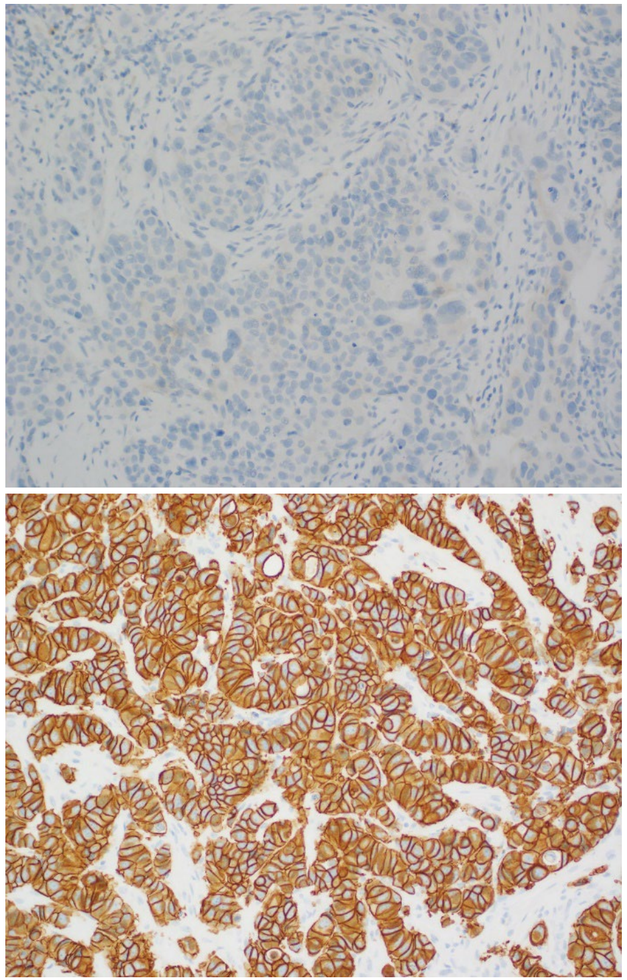
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HER2 Immunohistochemistry (IHC)

- **Evaluates the level of HER2 protein expression**
  - Using a HER2-specific antibody and detection system
  - Staining intensity correlates with level of HER2 expression
  - Semiquantitative method

- **Proper interpretation is critical**
  - Strict criterion for (-): good agreement with absence of *HER2* gene amplification
  - Broad equivocal category: for cases that are not clearly + or -
  - Strict criterion for (+): good agreement with *HER2* gene amplification

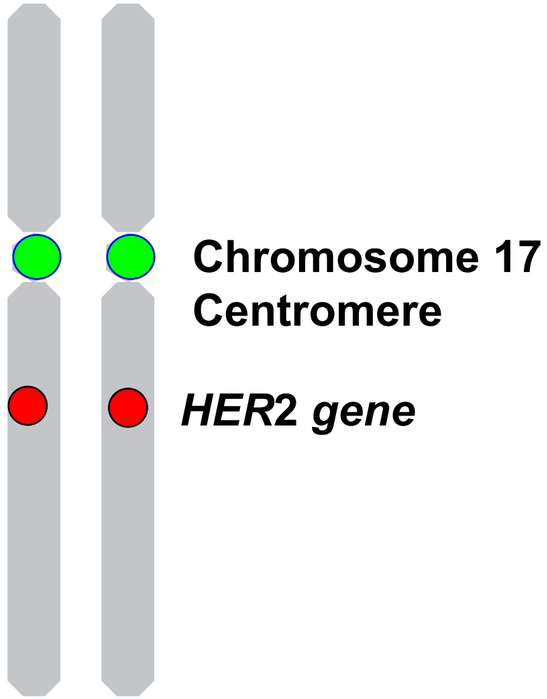


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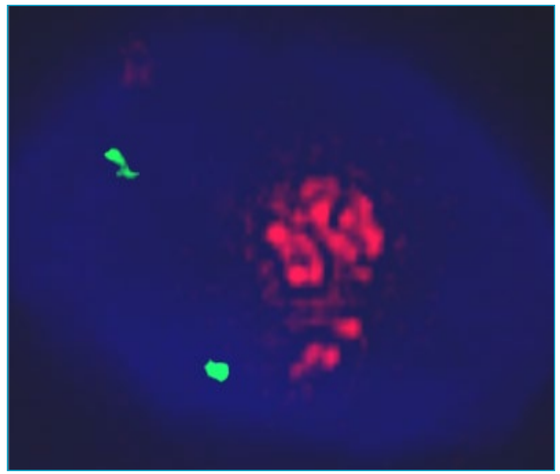
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HER2 ISH Testing

- **ISH: Evaluates the number of *HER2* gene copies in the nucleus**
  - **Uses labeled DNA probes coupled with a microscope that detects the signal**
  - **The signal is proportional to the *HER2* gene copy number**
  - **Morphologically driven, direct visualization of tumor cells**



Probe hybridized with target



Count *HER2* & CEP17

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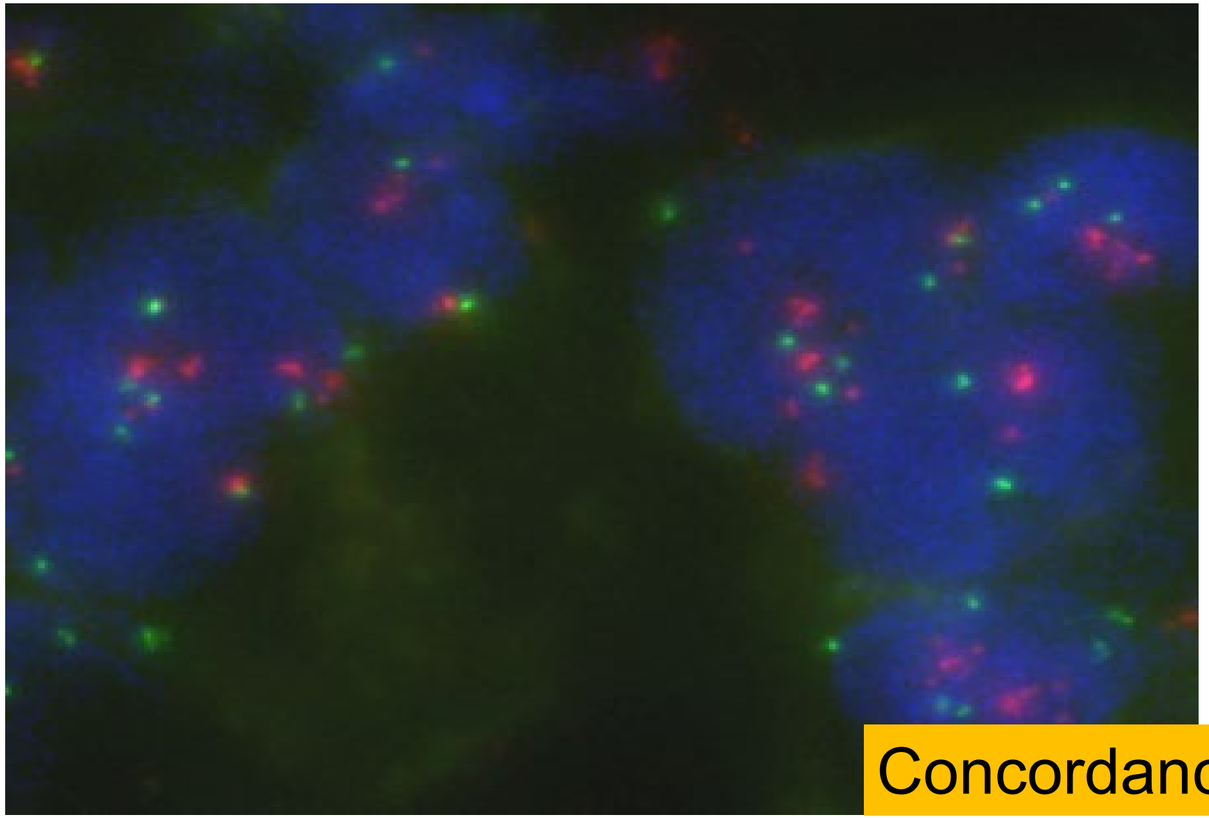
Fluorescence ISH vs Chromogenic ISH

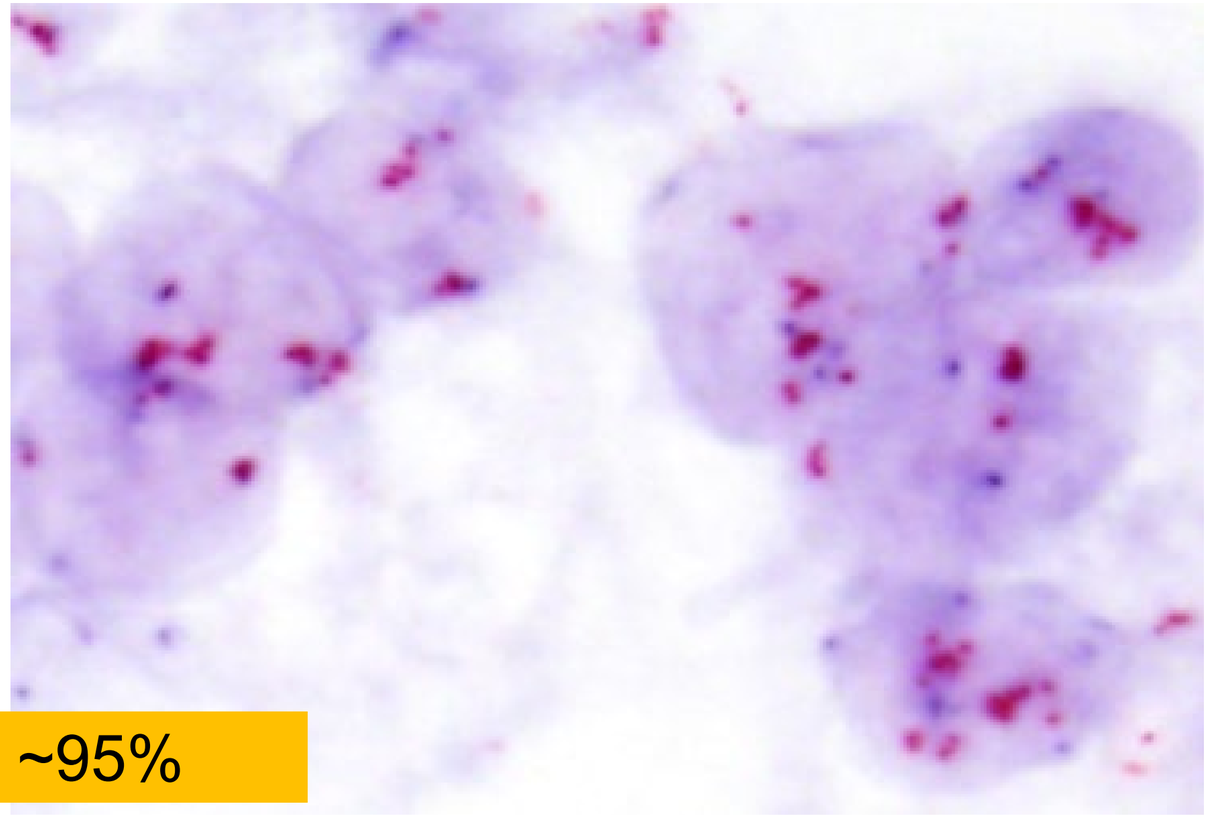
FISH

- Fluorescence detection of hybridized probe, need fluorescence microscope
- Fluorescence faded quickly
- Morphological features difficult to visualize

CISH

- Chromogenic detection of hybridized probe, regular bright field microscope
- Permanent signals
- Morphology features visualized





Concordance: ~95%

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Images provided by David Hicks, MD.

Role of Molecular Assay in HER2 Testing

- High correlation between *HER2* gene expression and mRNA levels
- Modest correlation between intrinsic subtype by gene expression profiling and clinical HER2 receptor status
- **HER2 molecular techniques are not currently recognized as alternative methods to assess HER2 status in clinical practice**

❑ If there is discordance with the clinical gene expression test result

- HER2 test (IHC+/−ISH) should be reviewed
- If the original HER2 status was on biopsy, repeat HER2 testing should be considered on excision

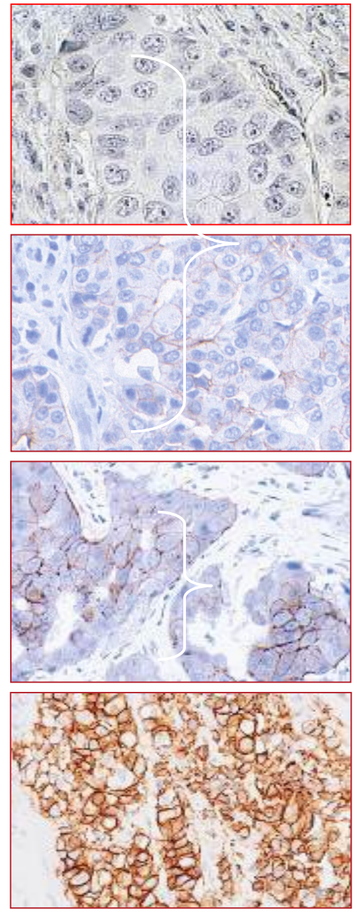
Rakha EA, Tan PH, Quinn C, et al. UK recommendations for HER2 assessment in breast cancer: an update. *J Clin Pathol*. 2023;76(4):217-227. doi:10.1136/jcp-2022-208632

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HER2 Testing: IHC Versus ISH

- IHC & ISH: Complementary methods
  - Examining different aspects of biology
  - Gene amp drives protein overexpression (in majority of cases)
  - Gene amp and protein overexpression highly correlated
- ASCO/CAP
  - **IHC & ISH: clinically validated and equally efficient in identifying patients likely to respond to Herceptin**
  - FFPE tissues used in clinical practice



0

1+

Negative

Validate Assay
95-97% ISH (-)

2+

Equivocal

Reflex ISH

3+

Positive

Validate Assay
95% ISH (+)

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HER2 IHC/ISH Discordance

- **IHC-/ISH+ and IHC+/ISH- discordances present in all antibody clones and ISH methods used**
  - 3-4% of all cases
  - 5.4% of cases with an unequivocal IHC status

	IHC-/ISH+	IHC+/ISH-
2001-2007	3.7%	12.5%
2008-2013	2.0%	18.0%
2014-2018	3.6%	13.8%
2019-	1.2%	5.8%

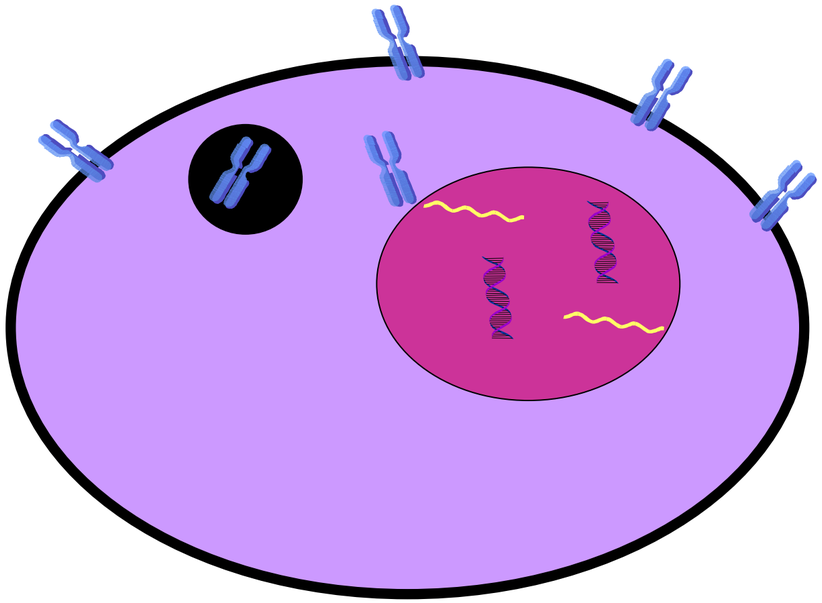
- **No significant difference between FISH and CISH**
- **No significant differences between visual and digital analysis**
- **Possible explanations**
  - Tissue handling (less with guideline implementation)
  - Antibody clones (CB11>HercepTest; IHC-/ISH+:HercepTest ~4B5; IHC+/ISH-: HercepTest>4B5)
  - Interpretation criteria
  - Micropapillary morphology
  - Tumor biology (activation mutation), heterogeneity

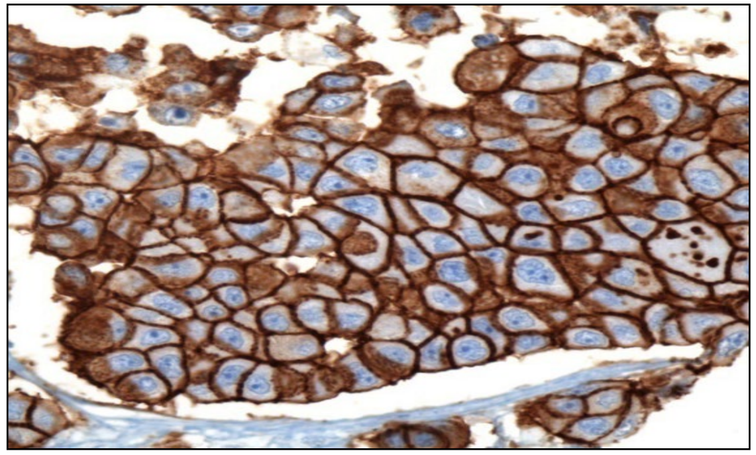
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Memon R, Prieto Granada CN, et al. Discordance Between Immunohistochemistry and In Situ Hybridization to Detect HER2 Overexpression/Gene Amplification in Breast Cancer in the Modern Age: A Single Institution Experience and Pooled Literature Review Study. Clin Breast Cancer. 2022 Jan;22(1):e123-e133. doi: 10.1016/j.clbc.2021.05.004.

Evaluating HER2 IHC Staining



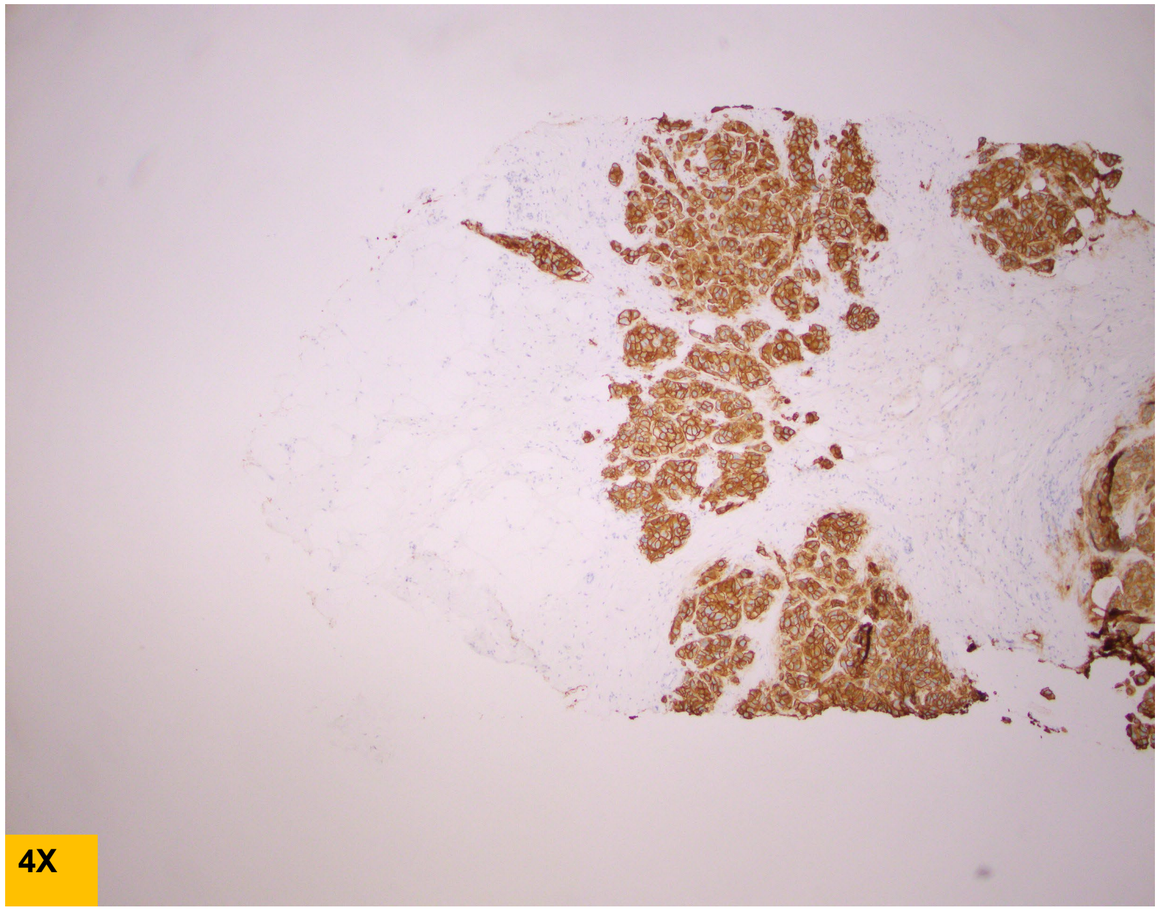


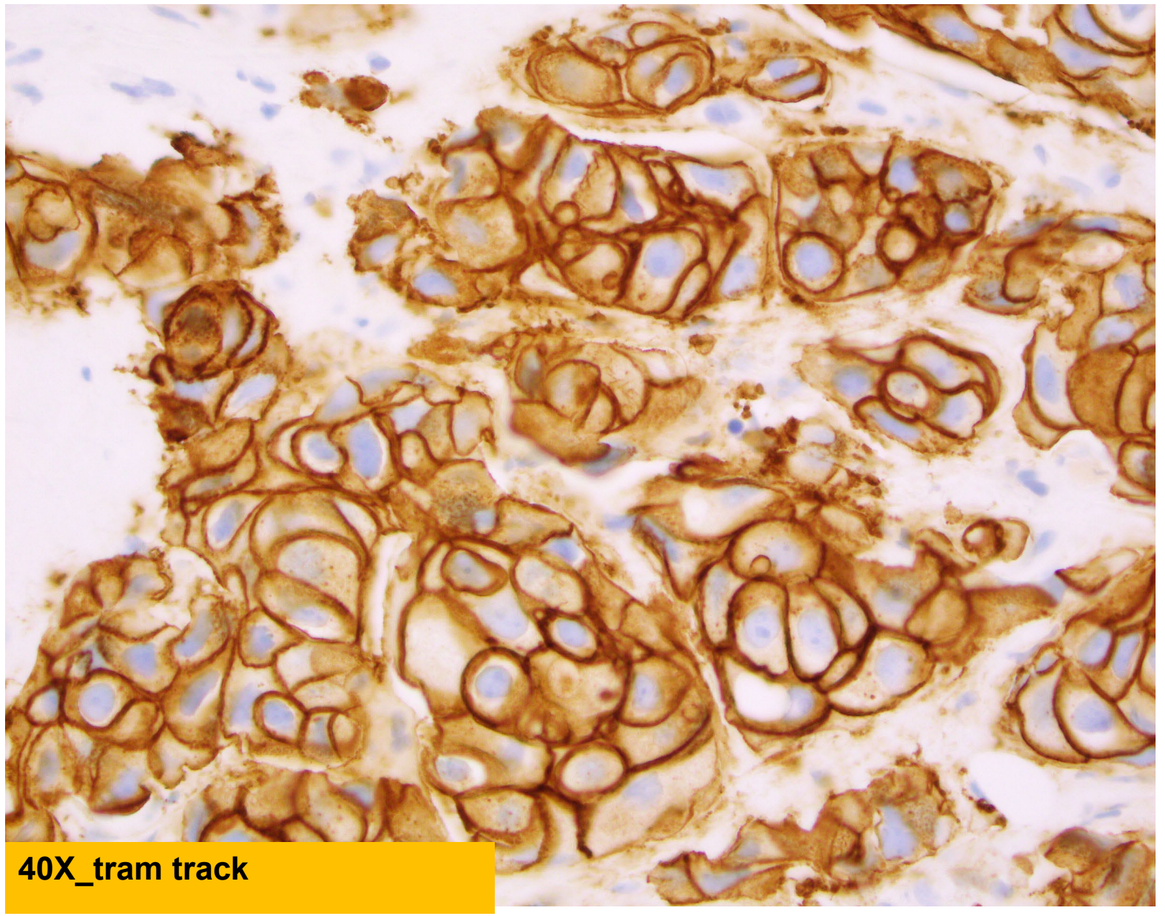
- Membrane staining
  - **Only membrane staining is evaluated (scored) for clinical decision making**
  - Intensity
  - Percentage

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“Magnification Rule” for HER2 Intensity Determination

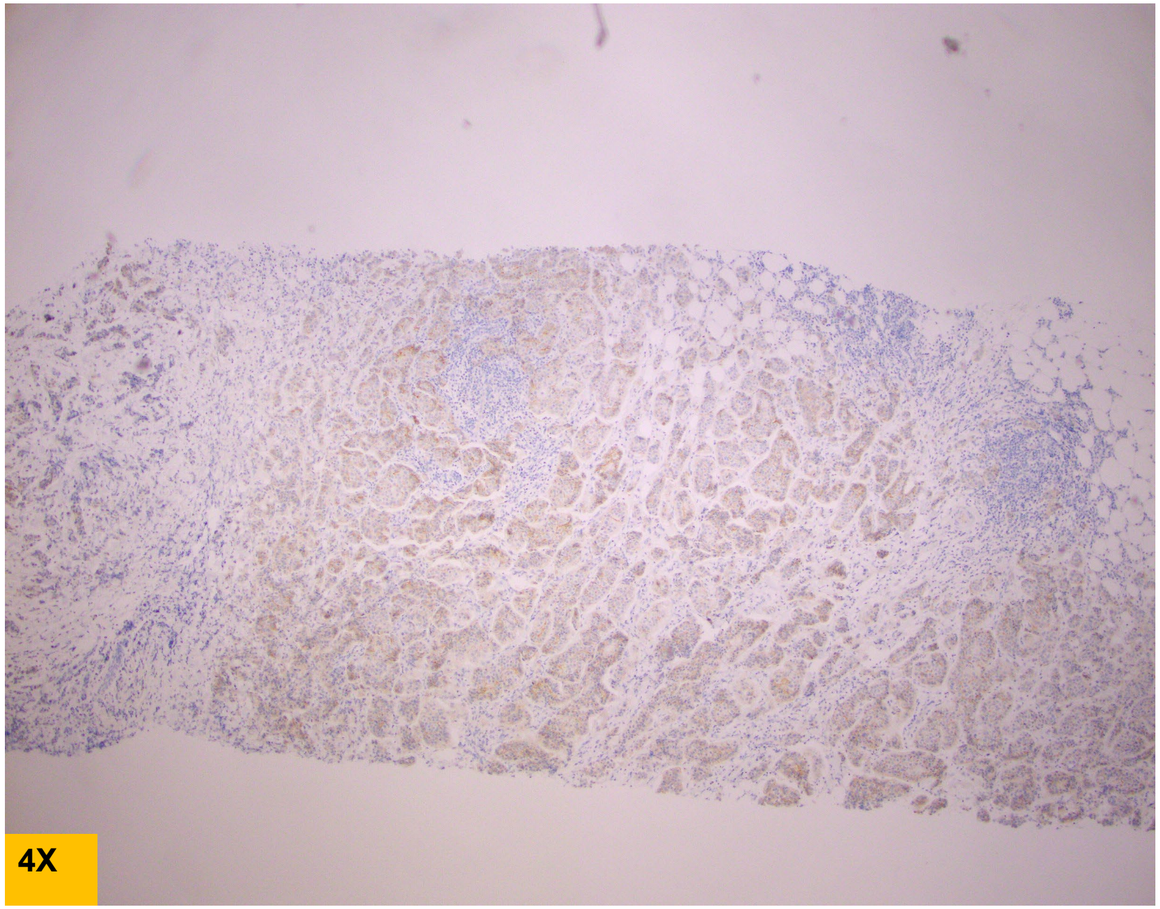


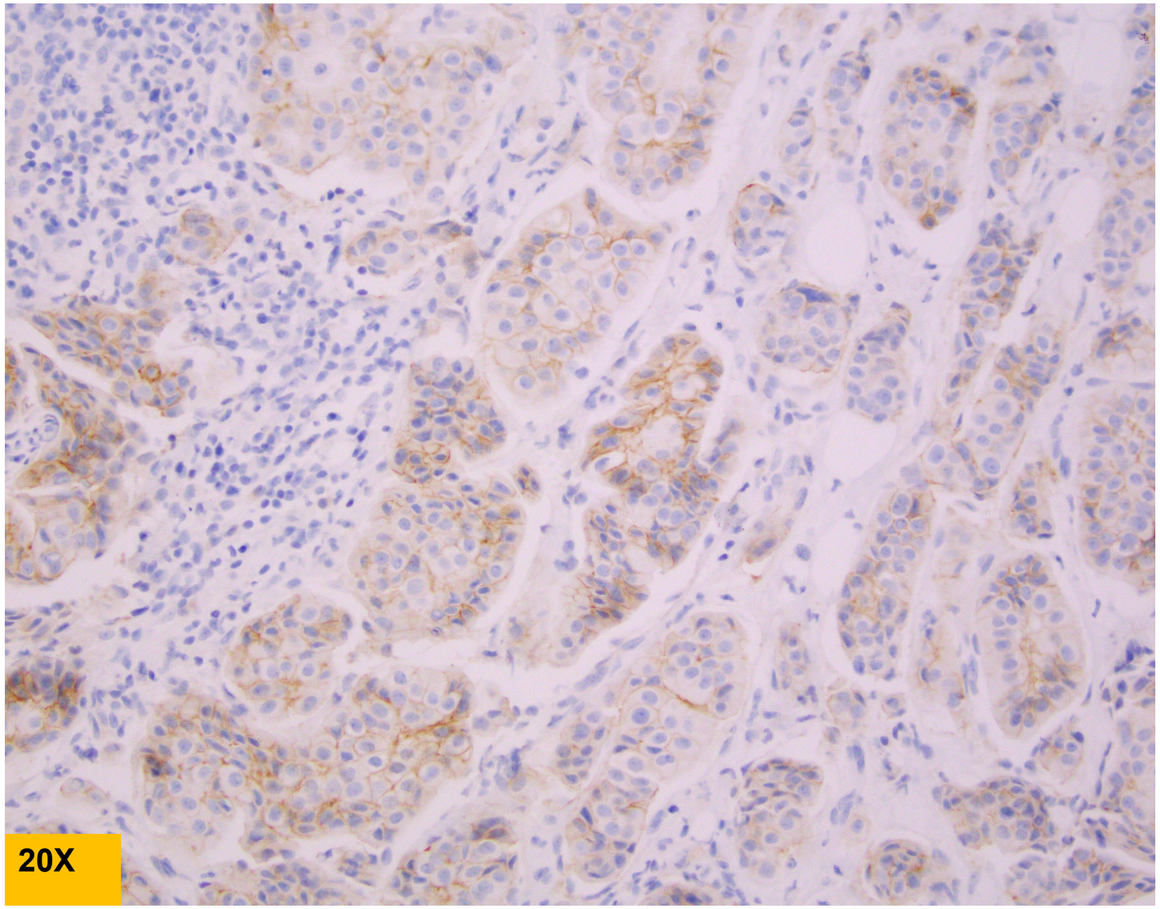


Strong intensity: A clear-cut intense staining perceived at x2-x5 objective lens

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“Magnification Rule” for HER2 Intensity Determination



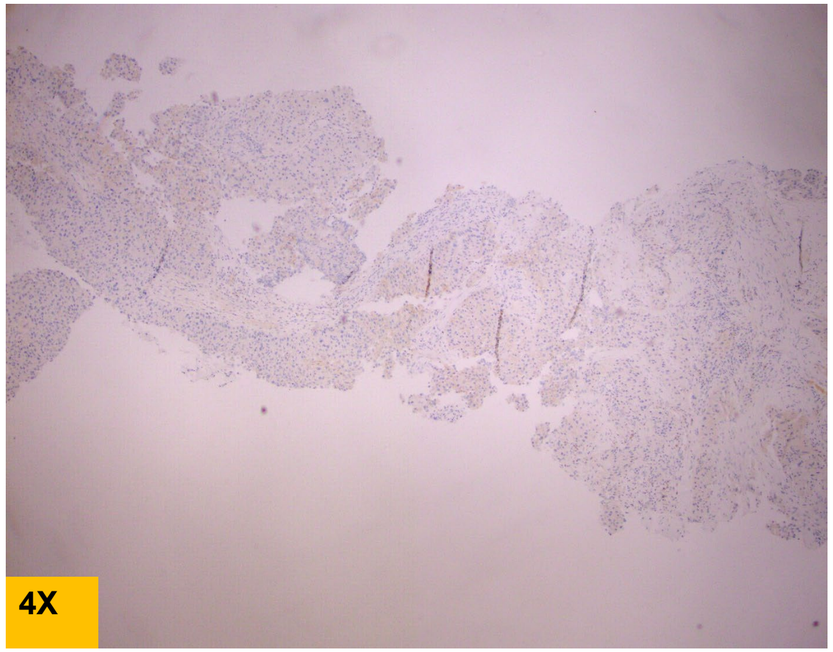


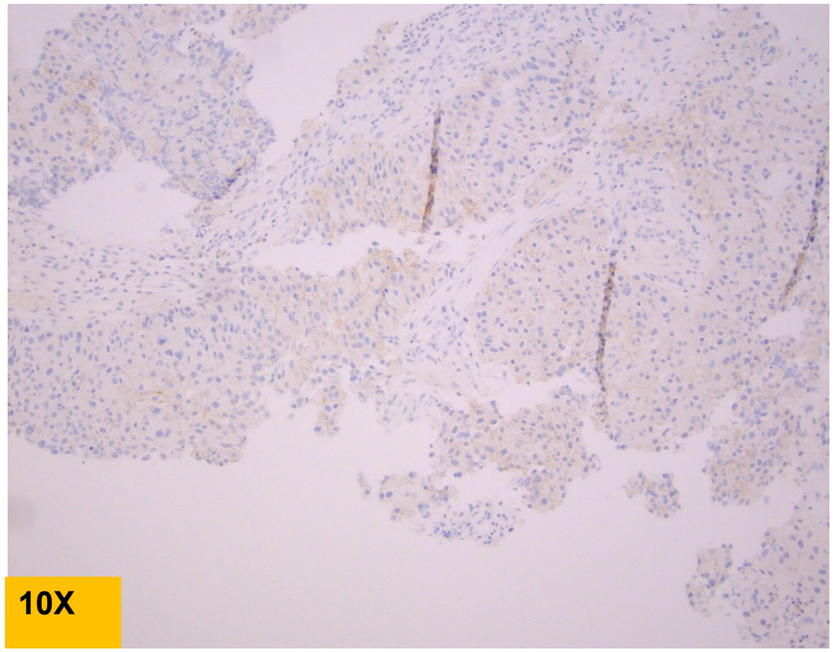
Moderate intensity: clearly appreciated at x10-x20 objective lens

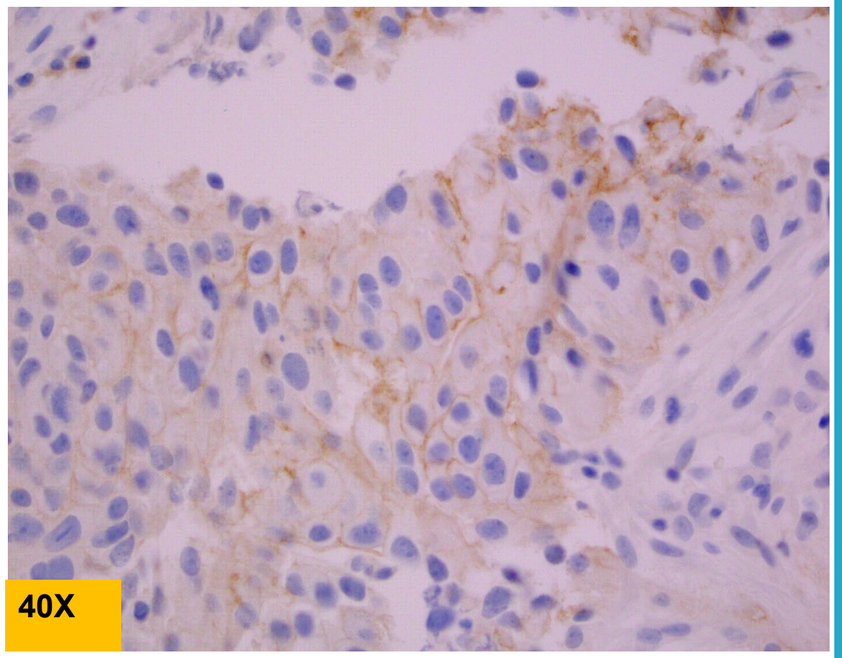
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“Magnification Rule” for HER2 Intensity Determination





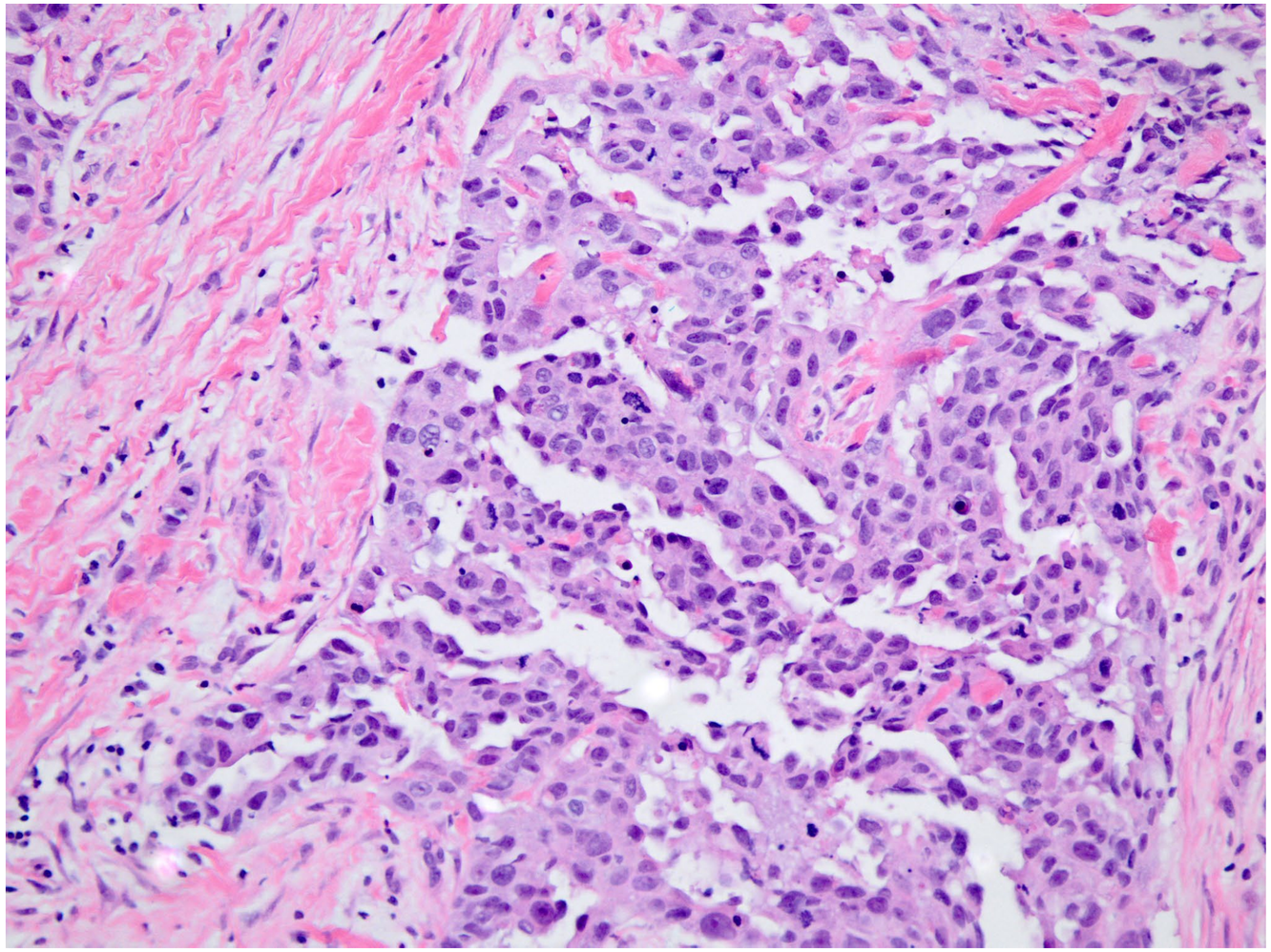


Weak/faint intensity: Detected only on x40 objective lens

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IHC Evaluation Pitfall-Awareness of artifacts

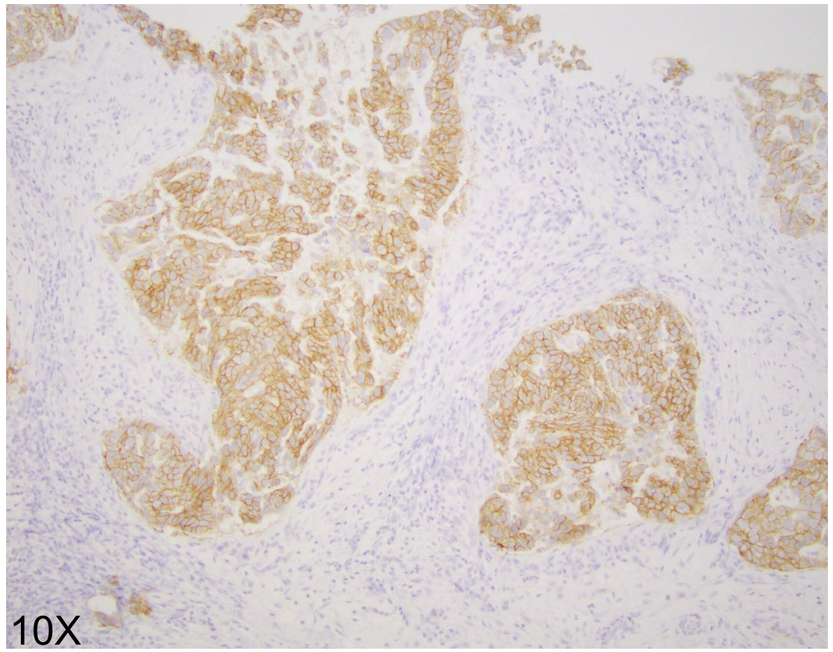
- A 59-year-old woman was found to have a mass on screening mammogram.
- Core biopsy: Invasive ductal carcinoma, histologic grade 3

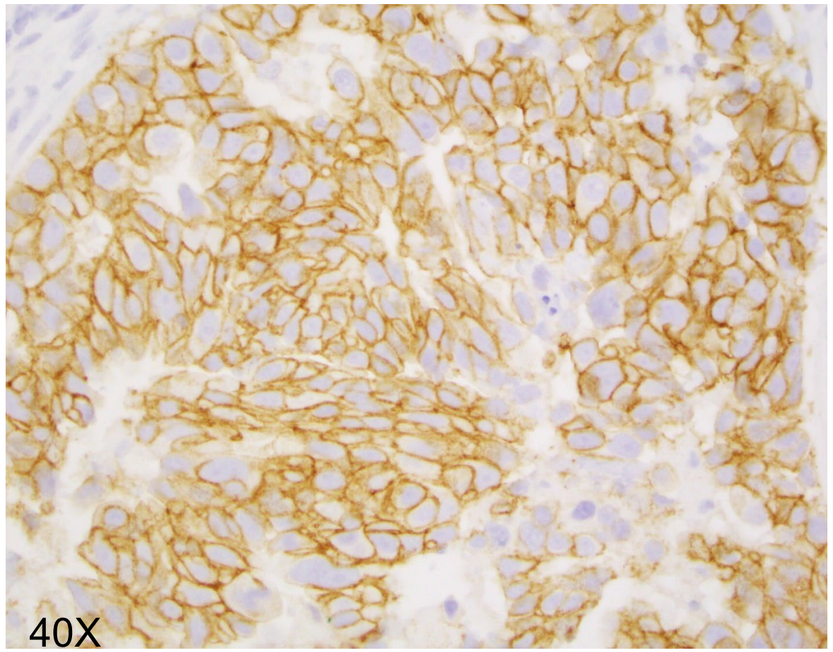


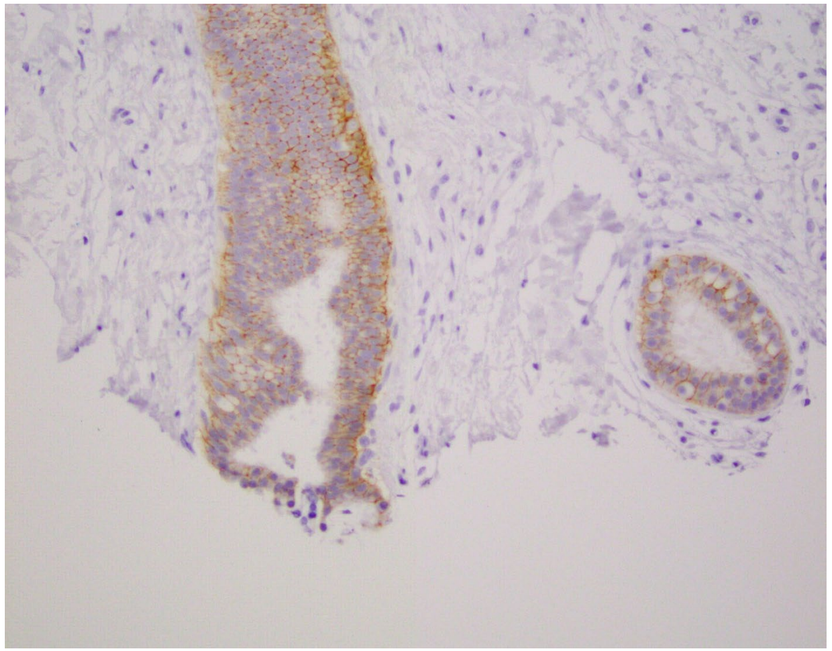
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HER2 IHC







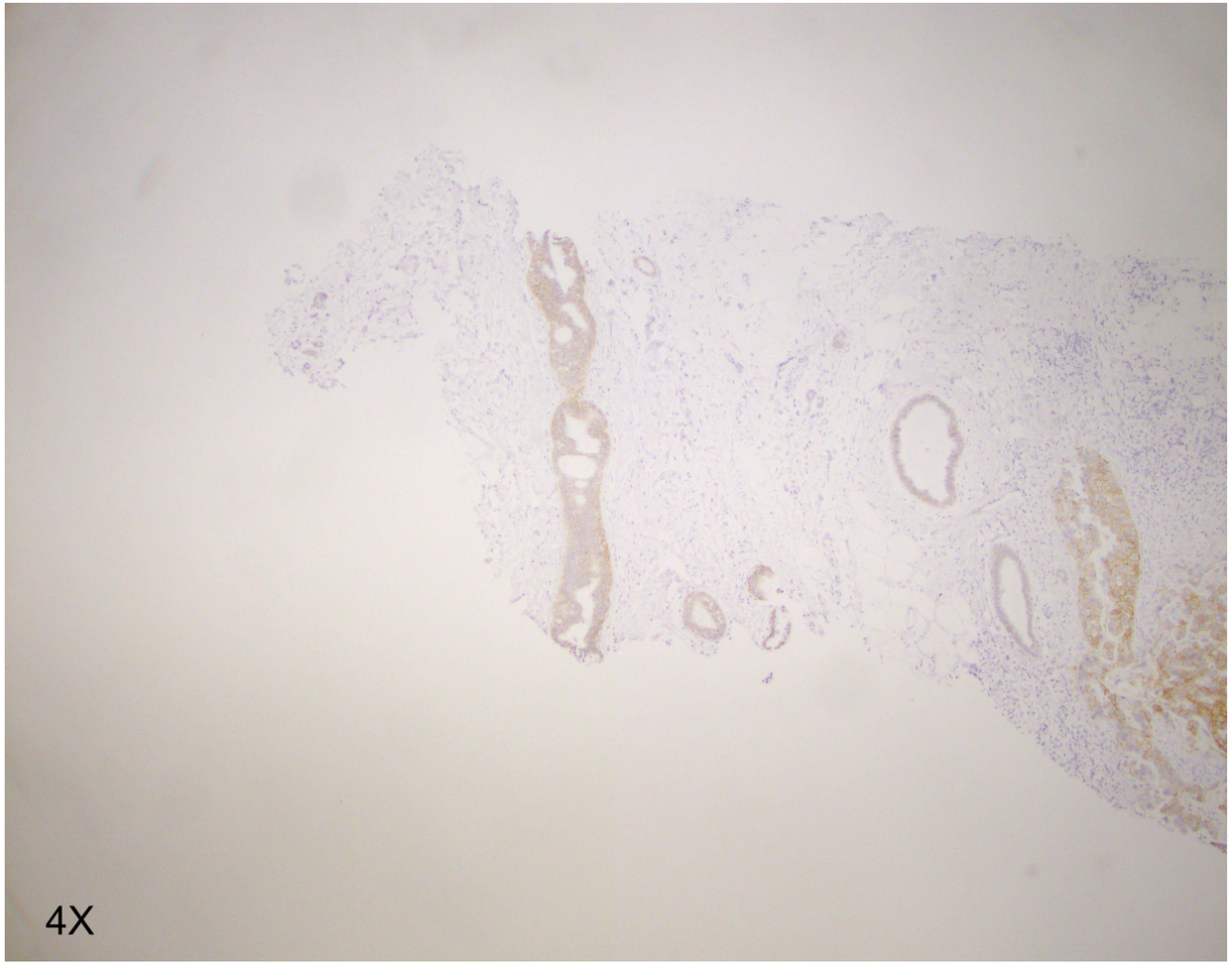
Tumor area

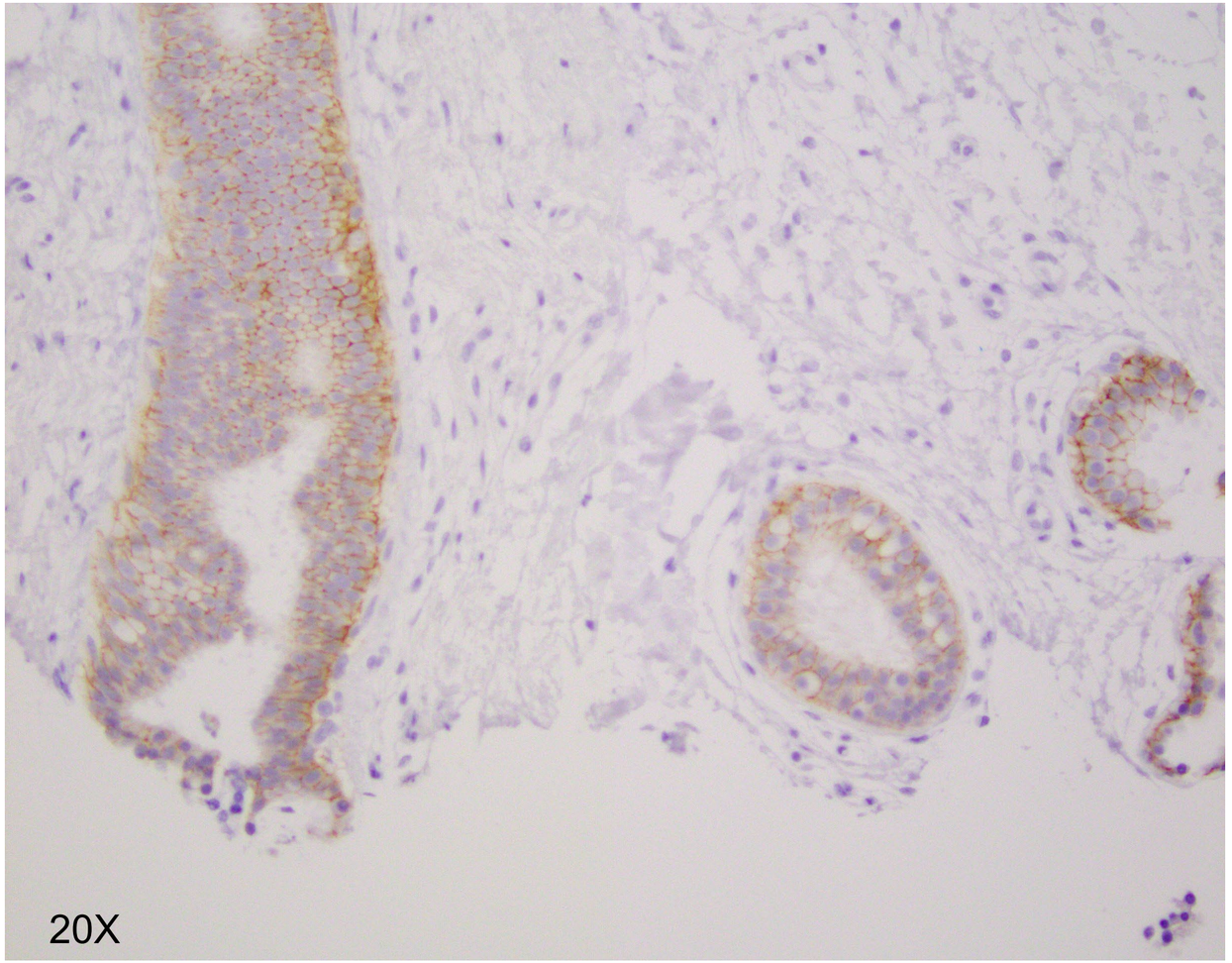
Benign breast glands

How Would You Report the HER2 Status for This Case?

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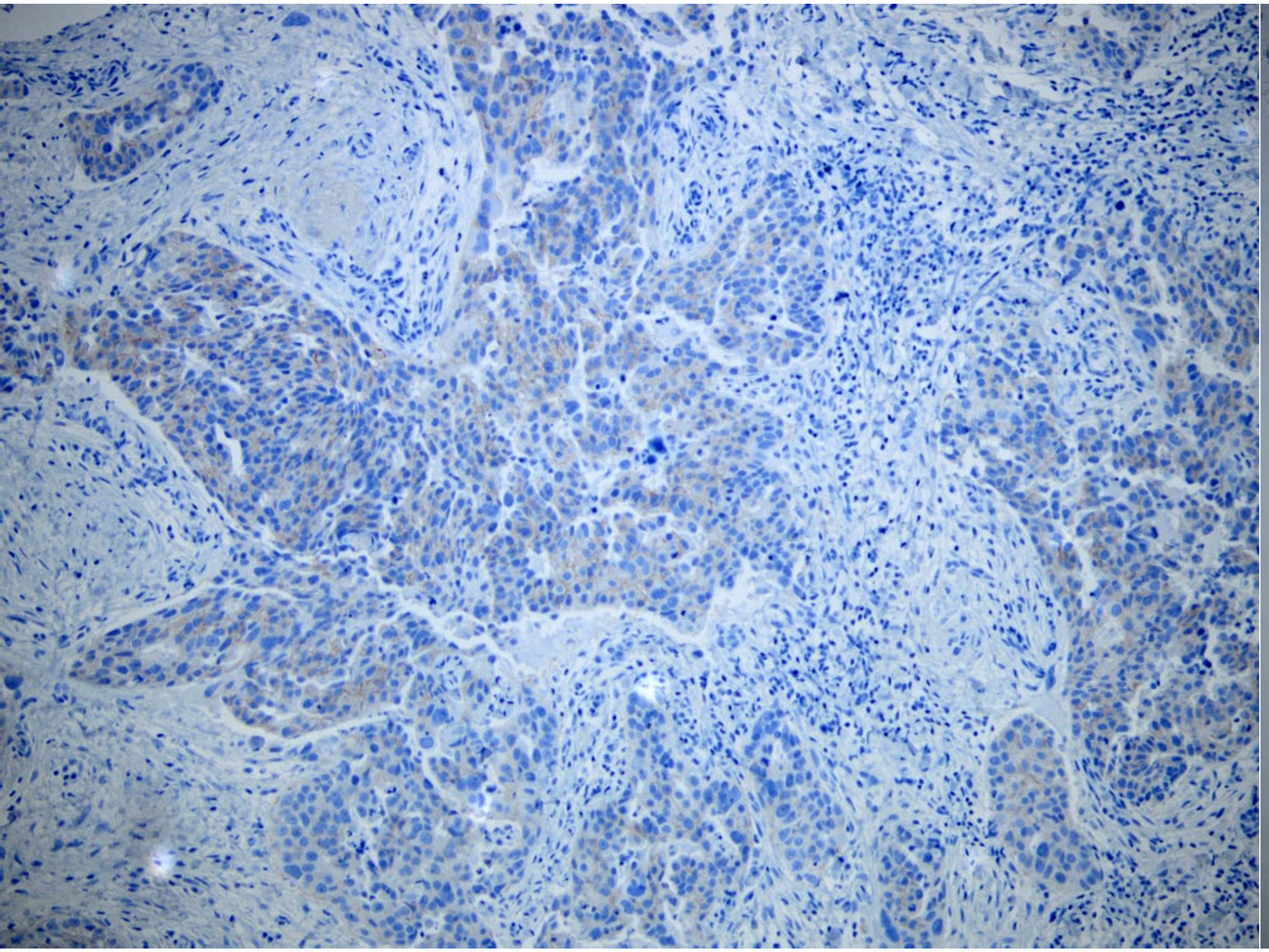


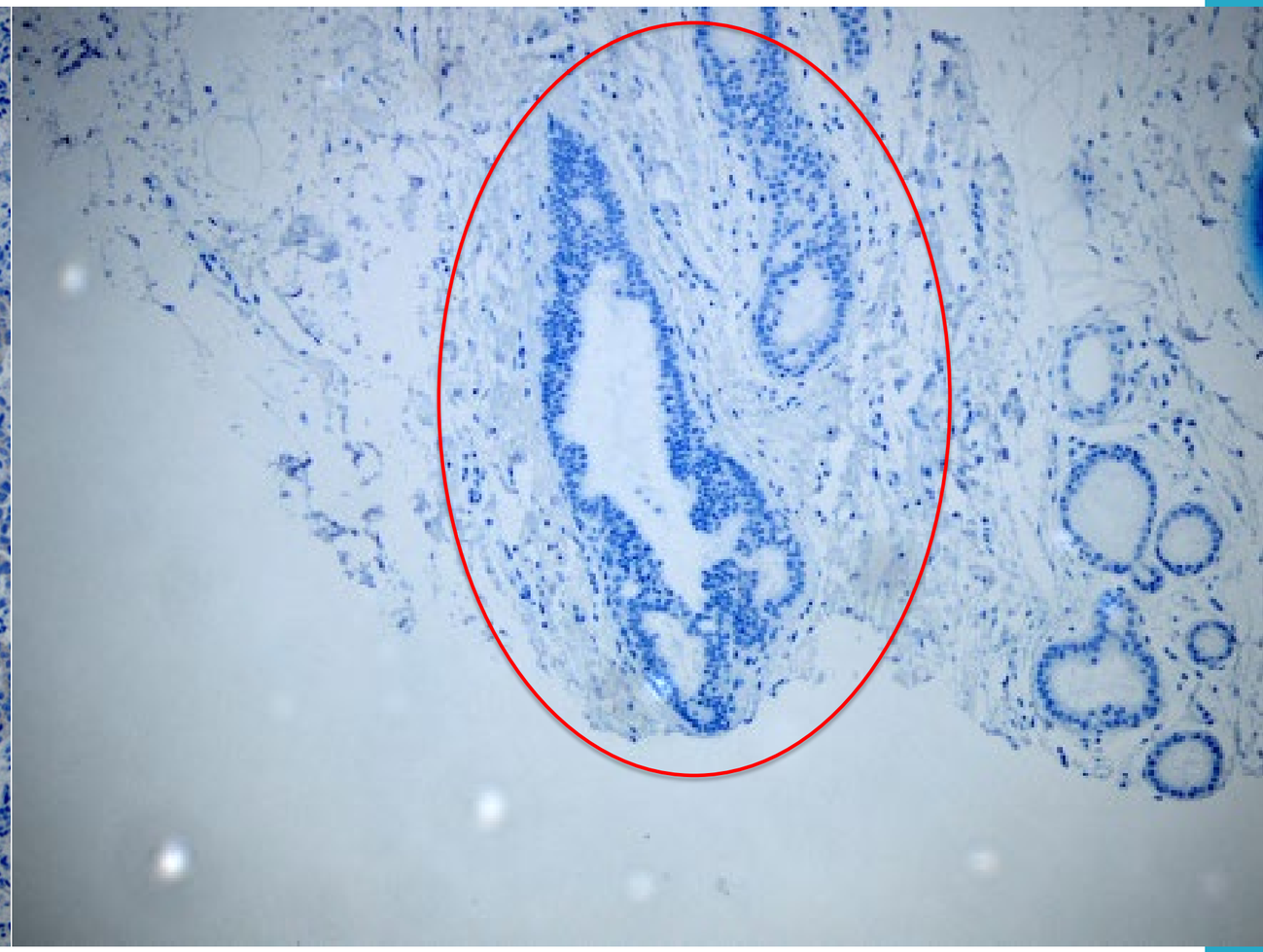
Internal control showing moderate staining

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Repeated HER2 IHC

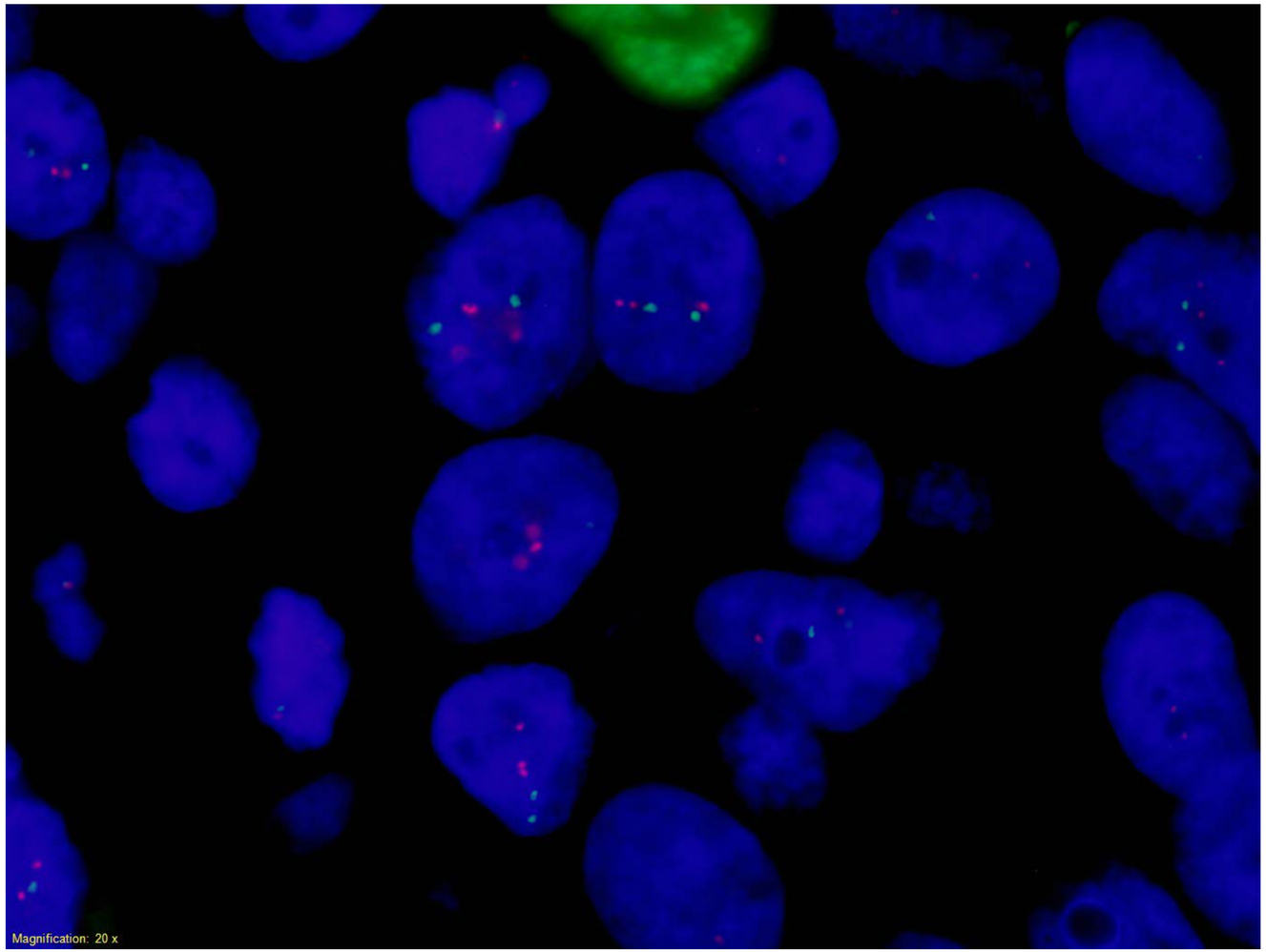




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HER2 FISH: Non-amplified



HER2/CEP17 ratio	1.5
Average # HER2 signals/nucleus	3.7
Average # CEP17 signals/nucleus	2.5

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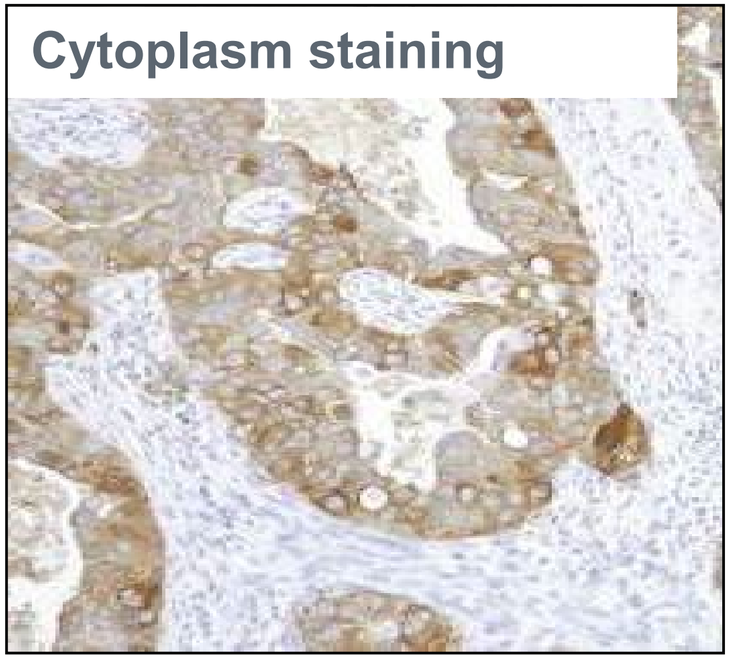
HER2 Control Staining

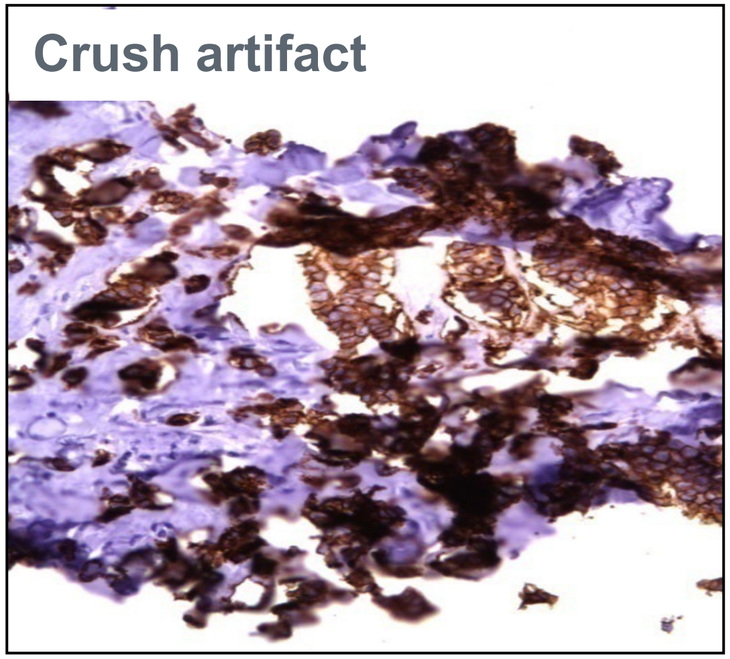
- HER2 weak staining of normal epithelial cells can be seen in multiple organs
- The levels of HER2 protein expression in normal breast epithelium are similar to those observed in non-amplified, non-overexpressing breast cancers.
- Intense staining of the cell membrane is found only in the tumor cells of invasive breast carcinoma.
- If moderate to intense staining of normal epithelium is observed, the slide should be considered inappropriate for interpretation and restaining is required.

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ER/PgR/HER2 Testing Rejection Criteria

- Controls are not as expected
- Artifact involves most of the sample
- Sample has strong membrane staining of normal breast ducts for HER2 (internal controls)
- If the sample meets the IHC rejection criteria, repeating IHC or sending for HER2 FISH analysis can be performed

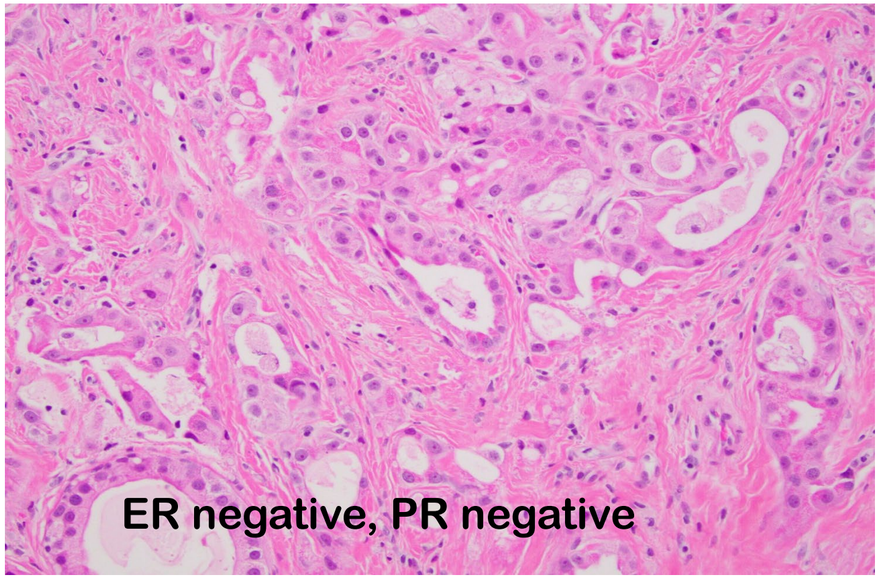




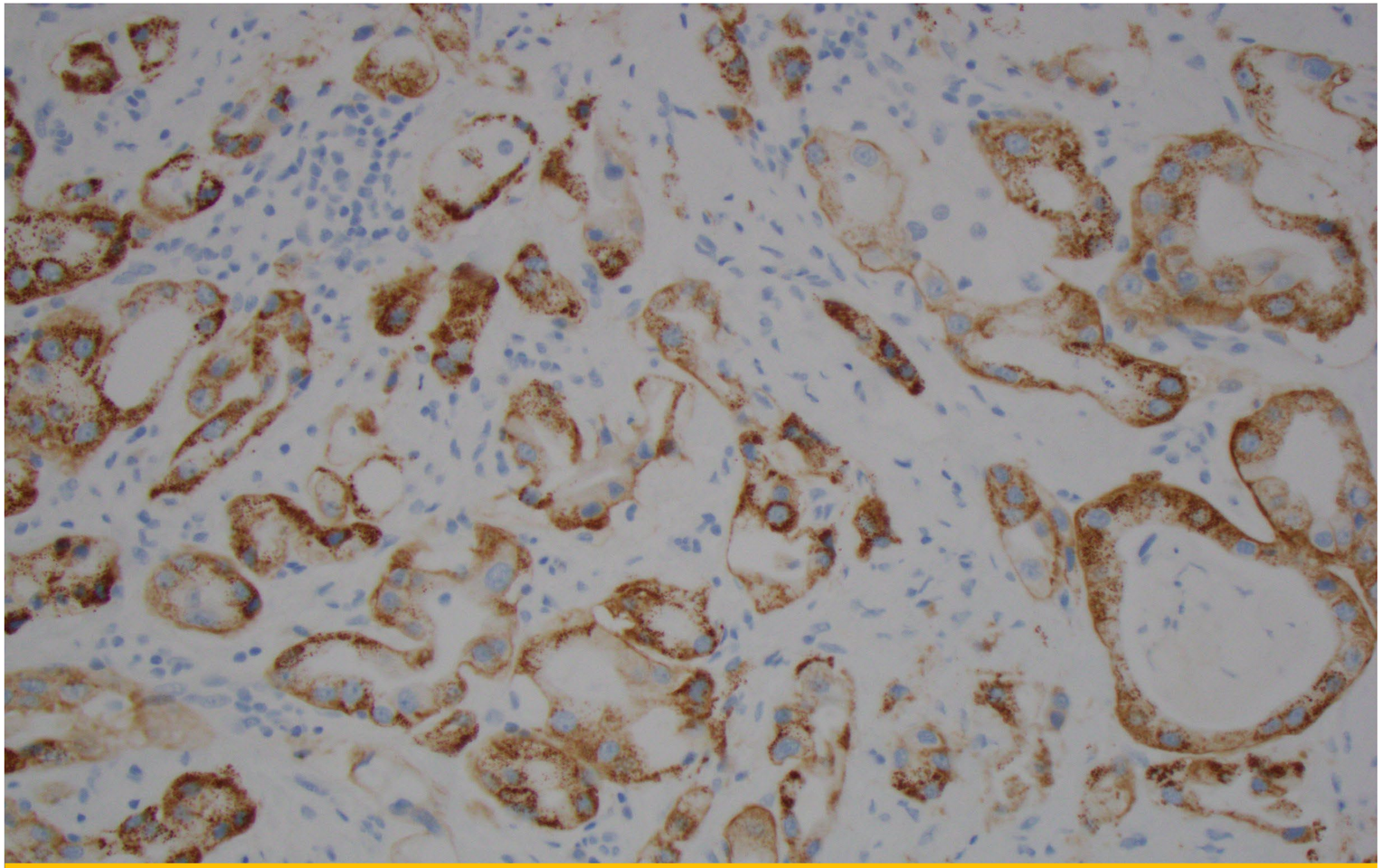
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IHC Evaluation Pitfalls—Cytoplasmic staining



Collection time	12/5/22 at 1502
Fixation start	12/5/22 at 1532
Fixation length	16 hours

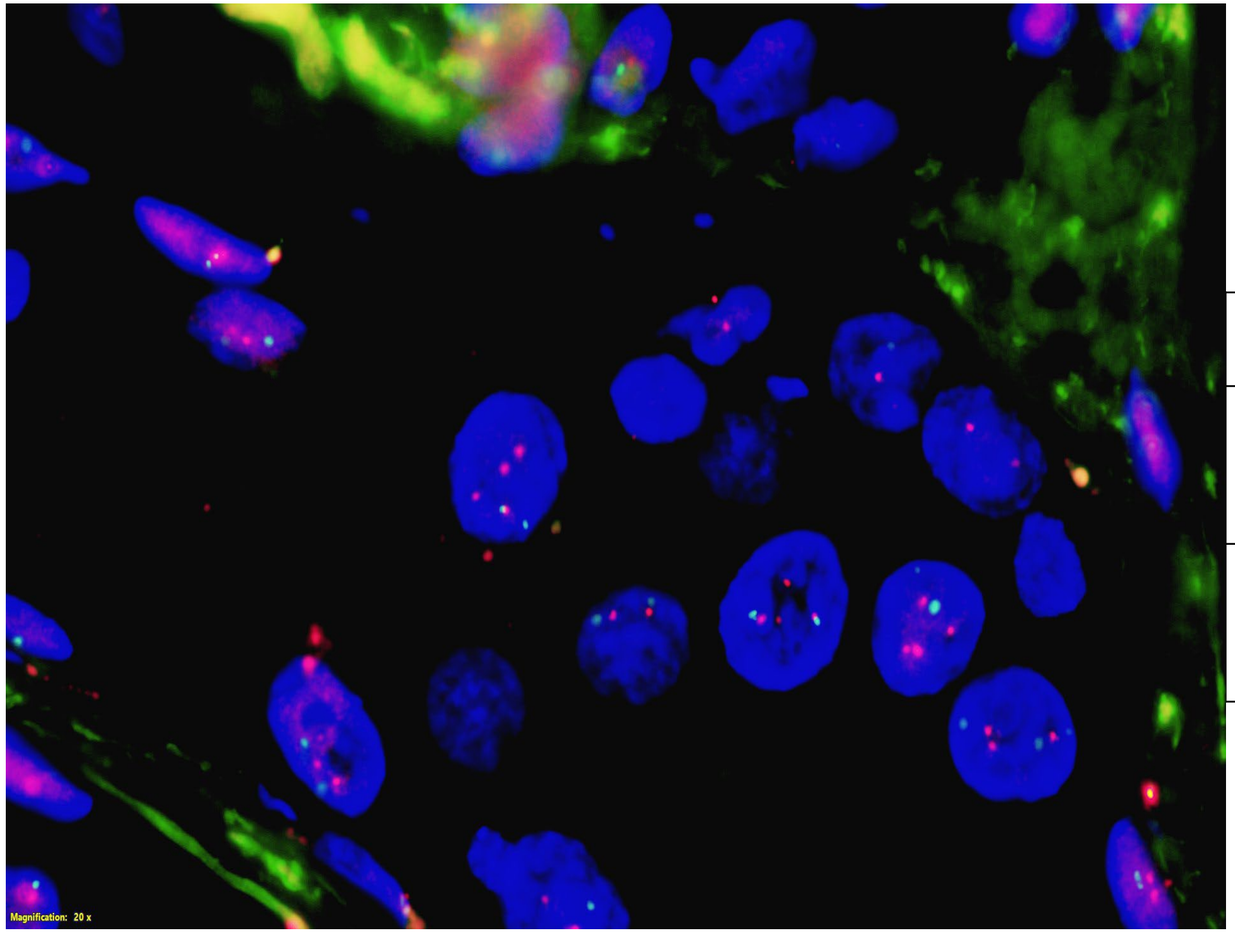


How Would You Report the HER2 Status, any work-up?

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HER2 FISH: Non-amplified



HER2/CEP17 ratio	1.3
Average # HER2 signals/nucleus	3.6
Average # CEP17 signals/nucleus	2.7

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HER2 Cytoplasmic Staining in Breast Cancer

- In the absence of membrane staining in breast cancer is rare
  - May be real or artifact
  - Receptor is transport from cytoplasm to membrane
  - Receptor internalization and degradation
- Not associated with the presence of detectable HER2 mRNA in breast cancer
- Seen in breast carcinoma with neuroendocrine differentiation or apocrine differentiation
- When encountered, better to consider equivocal and send for ISH for confirmation

Horiguchi S, Hishima T, Hayashi Y, et al. HER-2/neu cytoplasmic staining is correlated with neuroendocrine differentiation in breast carcinoma. *J Med Dent Sci*. 2010;57(2):155-163.

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IHC Evaluation Pitfall--Awareness of possible HER2 test discordance

Histopathologic findings with positive HER2 results:

- **Histologic grade 1 carcinoma of the following types:**
  - **Invasive ductal or lobular carcinoma, ER and PR positive**
  - **Tubular (at least 90% pure)**
  - **Mucinous (at least 90% pure)**
  - **Cribriform (at least 90% pure)**
  - **Adenoid cystic carcinoma (90% pure) and often triple-negative**
- **Histopathologic findings with negative HER2 results:**
  - **Histologic grade 3**
  - **Amount of invasive tumor in the core biopsy is small**
  - **Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core**
  - **Core biopsy result is equivocal for HER2 after testing by both ISH and IHC**
  - **Any doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error**

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Analyzing Slides for HER2 IHC
(If all Control Slides Show Appropriate Staining)

EASY	**Obviously 0**
	Obviously 3+
Challenging	**Somewhere between 0 and 1+**
	Somewhere between 1+ and 2+
	Somewhere between 2+ and 3+

- **Distinction between 0 and 1+, refer to 2023 ASCO/CAP recommendation**
- **Distinction between 1+ and 2+, if have any doubt, consider sending case for ISH**
- **Distinction between 2+ and 3+, consider sending case for ISH**

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Recent Updates on HER2 Testing

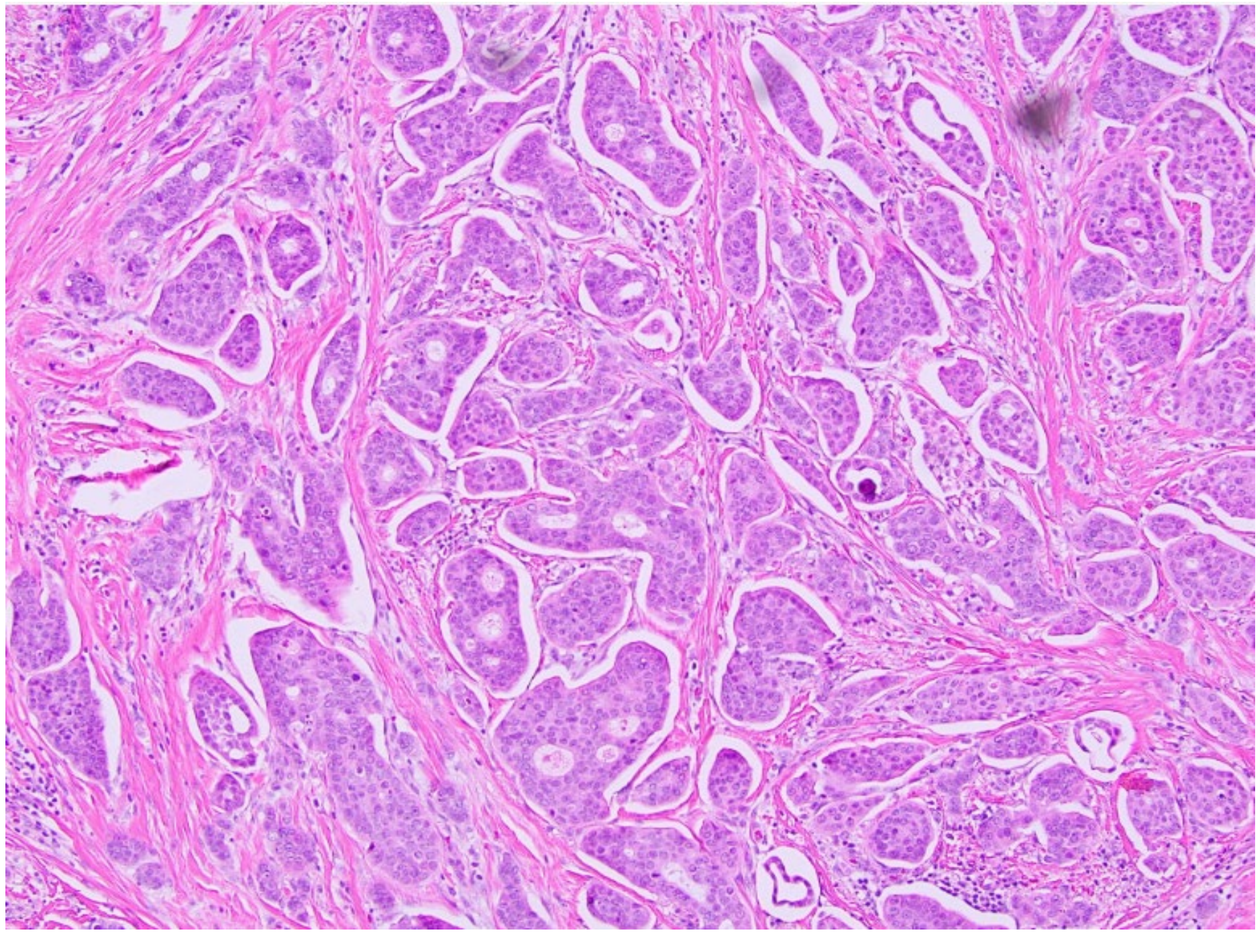
2018 ASCO/CAP

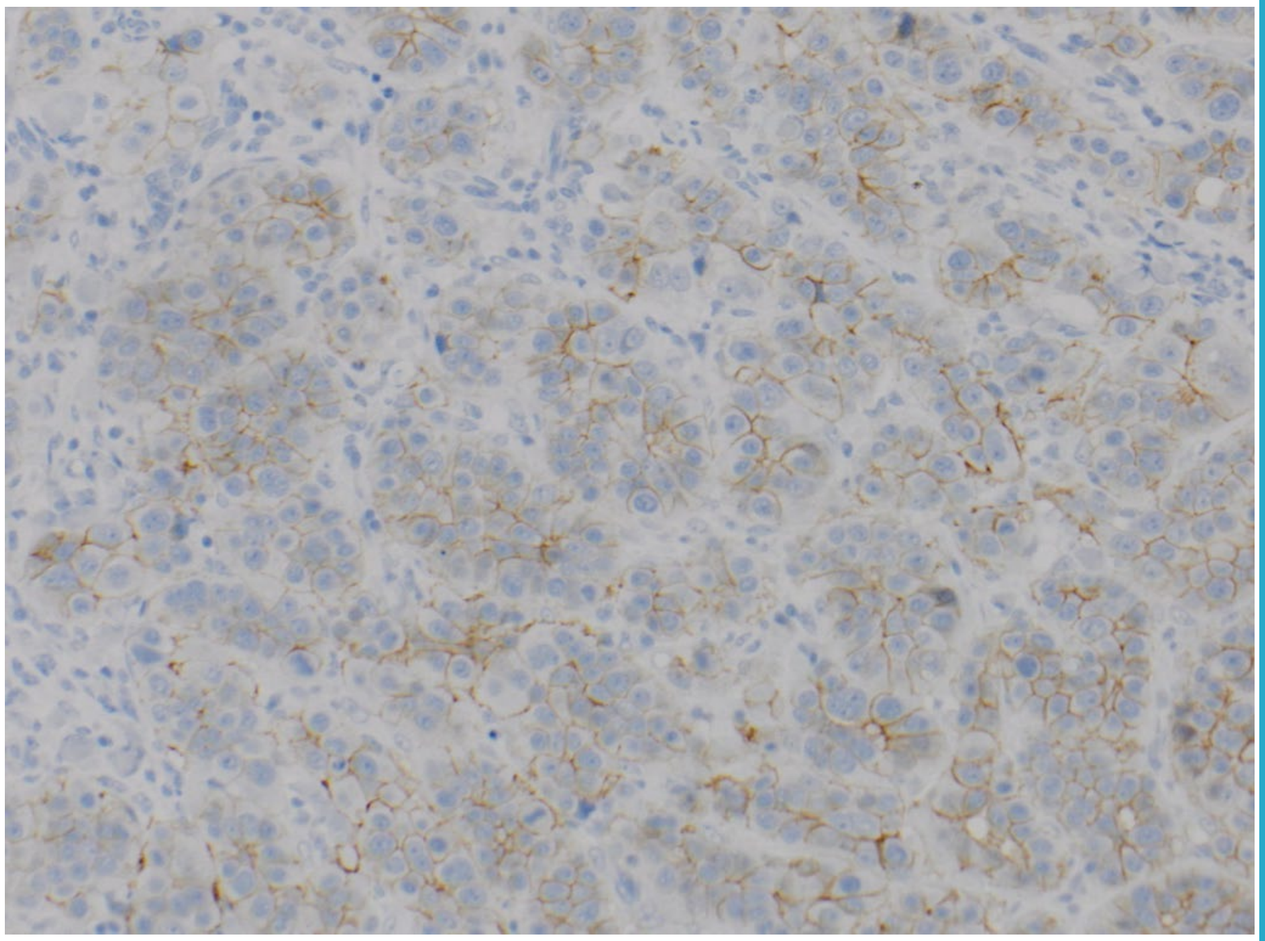
- Clarifying controversial issues in the 2013 guideline
- HER2 ISH unusual groups

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Moderate, incomplete (basolateral) membrane staining

56-year-old woman with a 3.3-cm mass in the right breast, partial mastectomy





IDC with micropapillary features, grade 3, ER: positive, PgR: positive (10% moderate), Ki67: ~60%

How would you report HER2 IHC result? Any additional work-up?

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Clinical Data and Morphology

- 56-year-old with 3.3-cm mass
- Grade 3 cancer, ER+ (strong), PgR + (weak), micropapillary morphology
- High Ki67 (fits for a HER2+ carcinoma)

Preanalytic Data and Technical Issues

- Preanalytical variables and controls appropriate

Interpretation

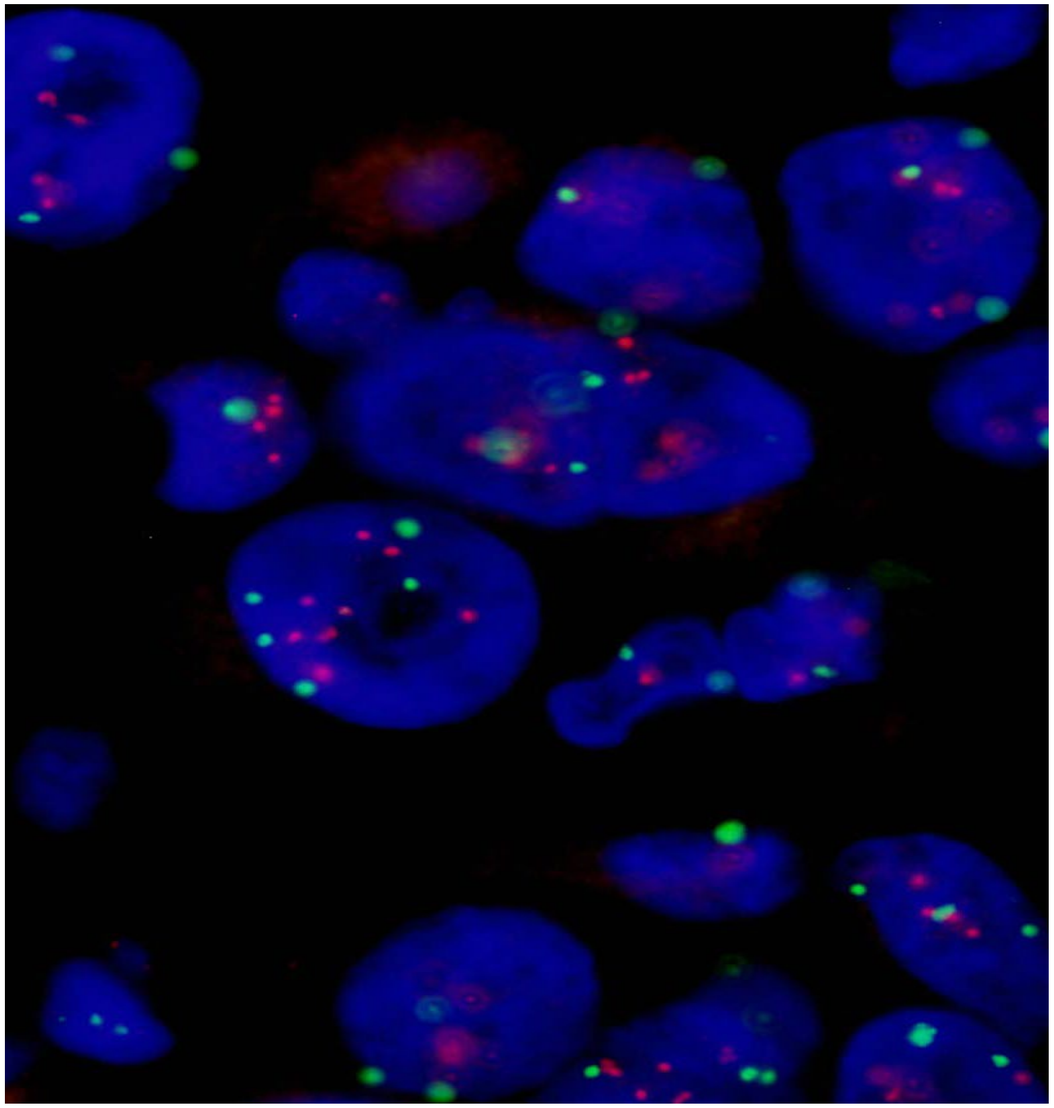
- ER+/PgR+ (weak)/HER2-/Ki-67 high
- HER2 IHC results discordance with other clinical/pathologic finding

Resolution

- Unfavorable pathologic features and micropapillary morphology
- Consider sending for reflex HER2 FISH analysis

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HER2 FISH Analysis



Total number of tumor cells counted: 40 + 40

Interpretation: Amplified

- HER2/CEP17 ratio: 2.6
- Average number of HER2 signals/nucleus: 8.1
- Average number of CEP17 signals/nucleus: 3.1

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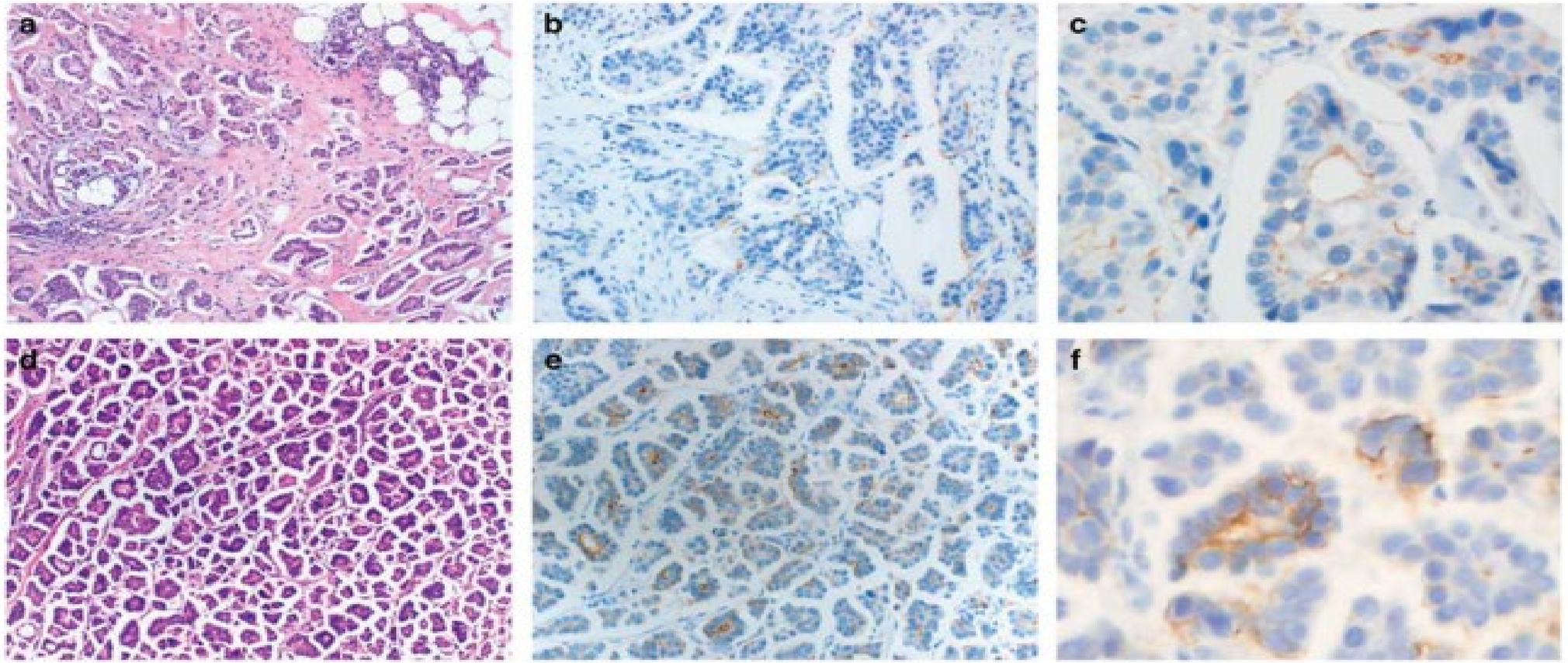


Figure 1 Examples of invasive carcinomas with micropapillary features (**a** and **d**) and corresponding HER2 immunohistochemistry stains at ×20 (**b** and **e**) and ×40 (**c** and **f**) that were scored as 1+, yet were HER2-amplified by fluorescence *in situ* hybridization. Note weak staining in the apical lumens and focal weak lateral immunoreactivity.

HER2 amplification was identified in 21 (47%) of invasive breast carcinoma with micropapillary features, with the corresponding IHC being 1+ (n = 9), 2+ (n = 11), and 3+ (n = 1).

Stewart RL, Caron JE, Gulbahce EH, Factor RE, Geiersbach KB, Downs-Kelly E. HER2 immunohistochemical and fluorescence in situ hybridization discordances in invasive breast carcinoma with micropapillary features. *Mod Pathol*. 2017;30(11):1561-1566. doi:10.1038/modpathol.2017.65

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Updated ASCO/CAP Recommendations

- Unusual HER2 IHC staining pattern can be encountered that are not covered by the definitions in the package insert.
- In practice these patterns are rare and if encountered should be considered HER2 equivocal.

Moderate, incomplete (basolateral) membrane staining

May be seen in cases with unusual breast cancer morphologies

- **Invasive micropapillary carcinoma**
- **Carcinoma with prominent glandular morphology**



Recommend interpreting as equivocal (2+) and sending for ISH testing

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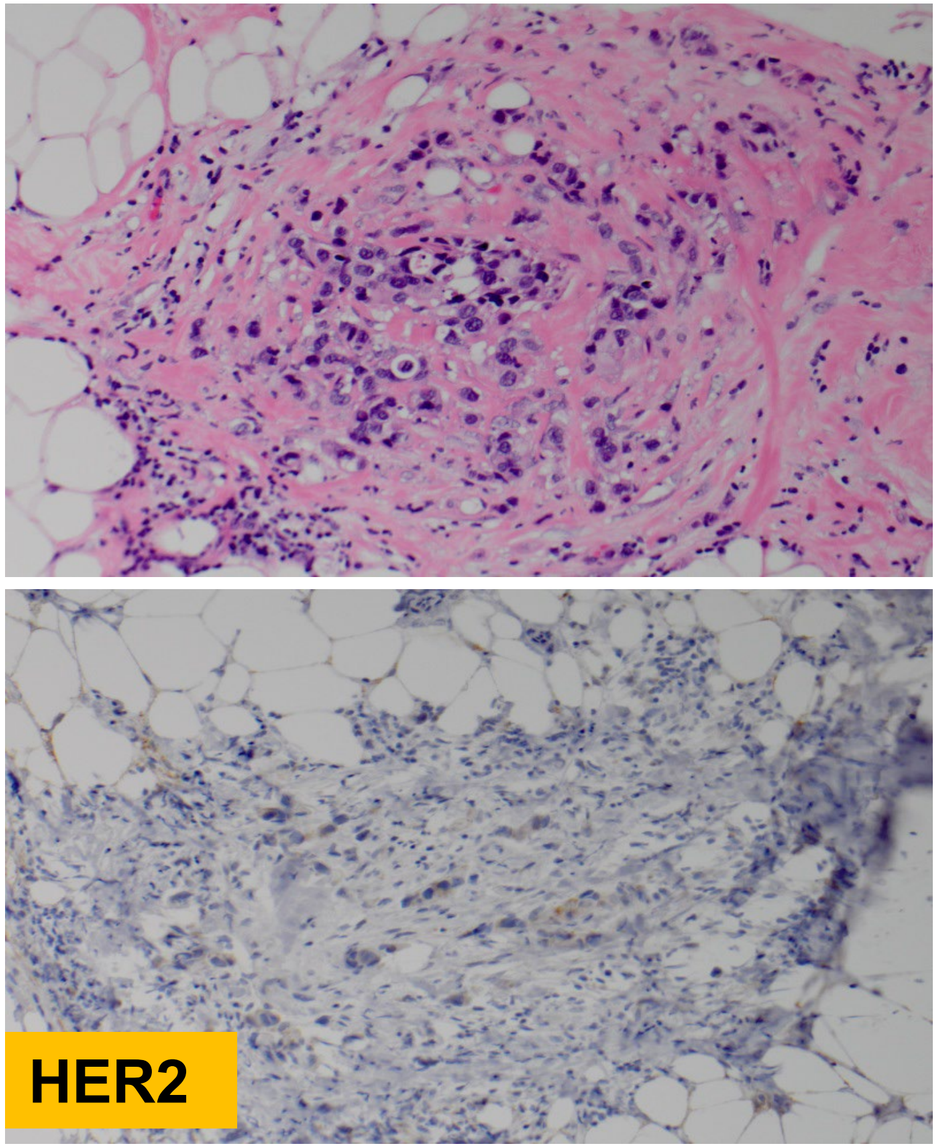
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HER2 Heterogeneity

When to repeat HER2 testing on a Surgical Specimen if Initially Negative Test on Core Biopsy?

60-year-old woman with extensive calcification in the left breast seen on screening mammography

- 1:00 core biopsy:
  - Small focus of invasive ductal carcinoma, nuclear grade 3
  - High-grade DCIS with central necrosis and calcifications
  - ER, PgR, HER2 negative

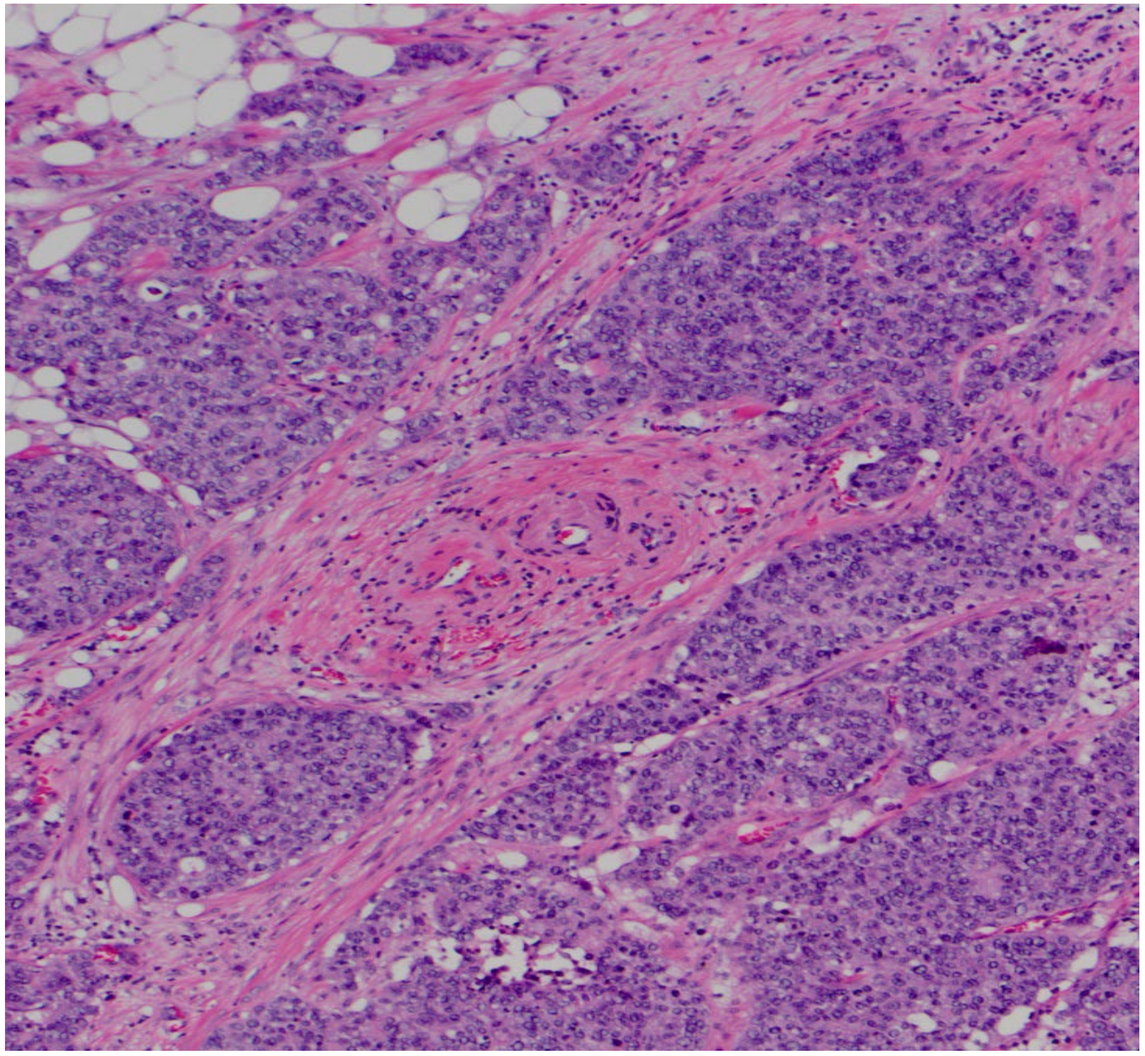


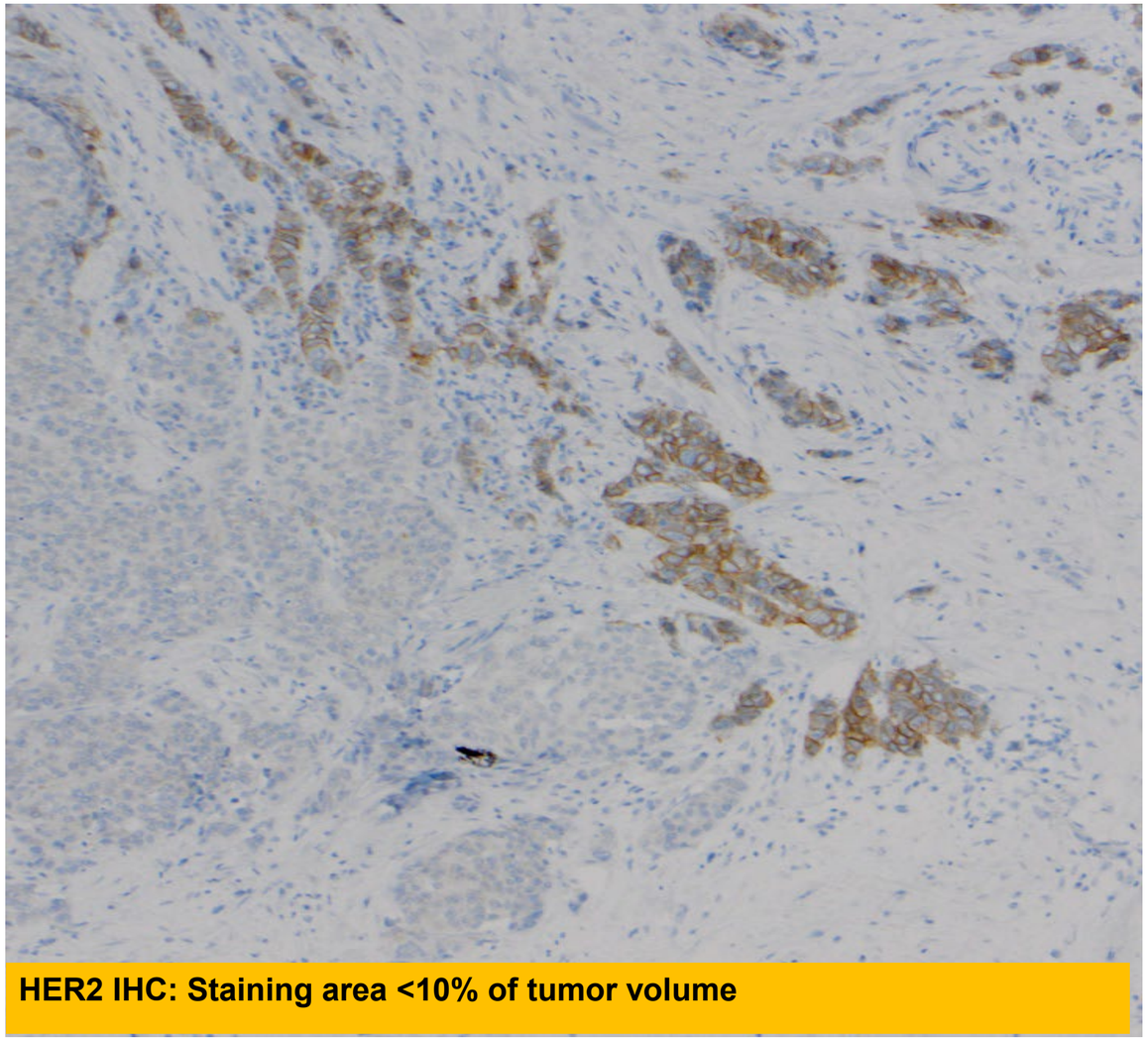
Case provided by David Hicks, MD.

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Mastectomy: Invasive ductal carcinoma, 1.7 cm, histologic grade 3



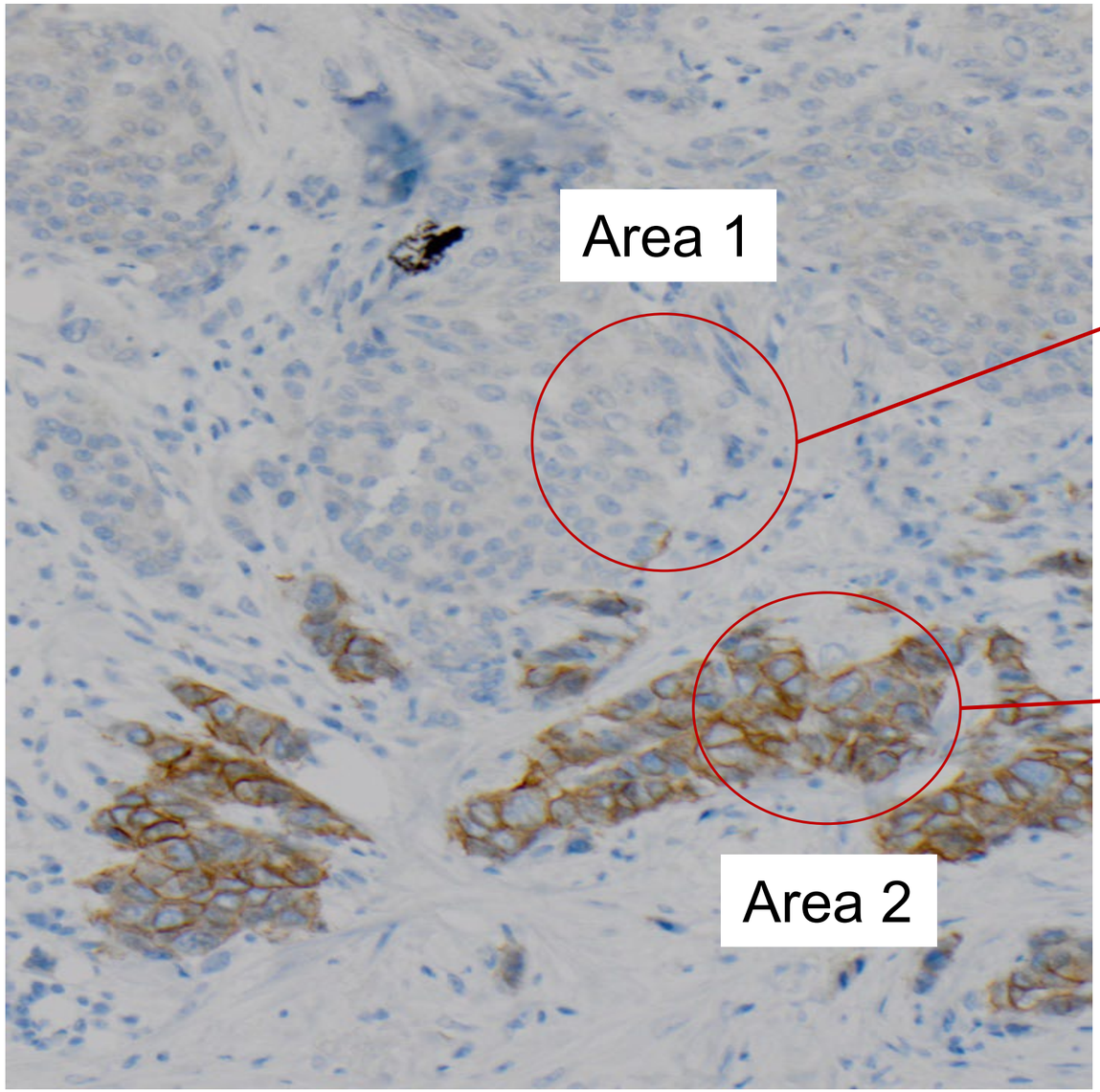


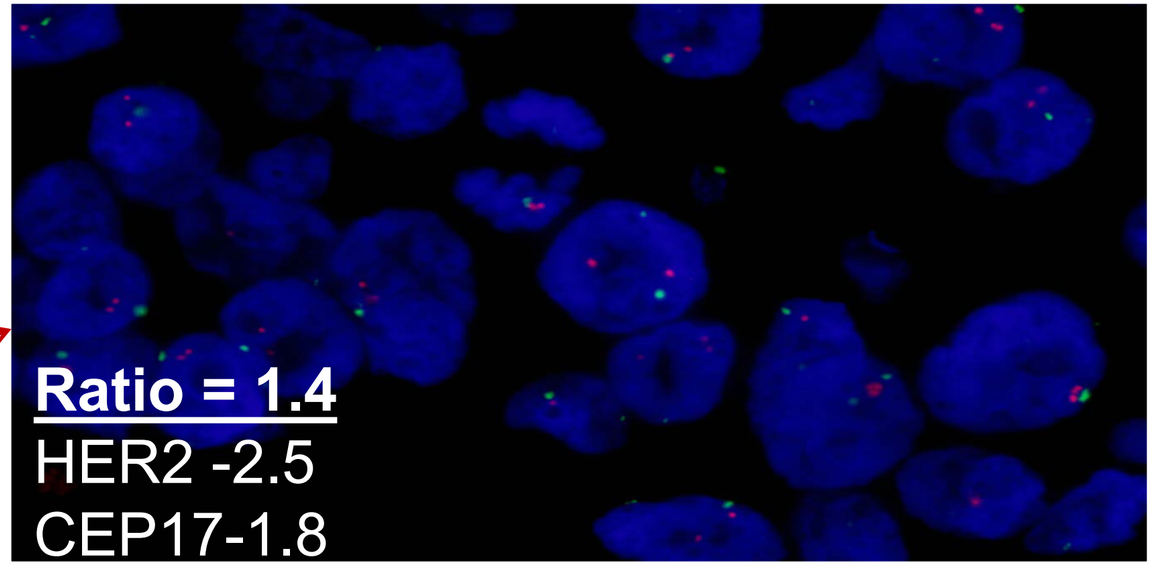
HER2 IHC: Staining area <10% of tumor volume

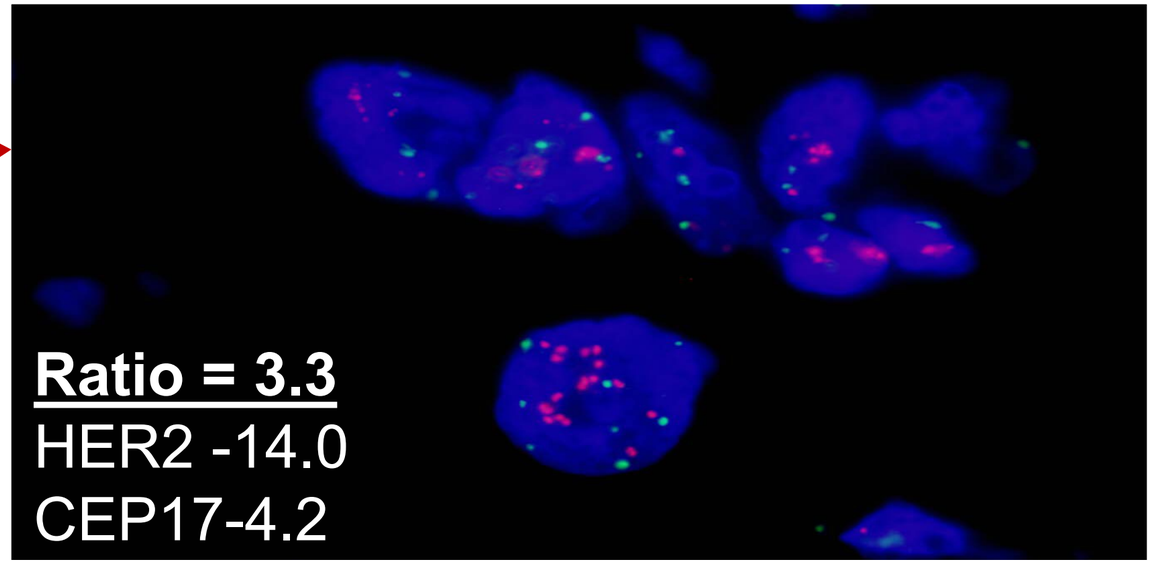
- How Would You Report the HER2 IHC Status in This Case Based on This IHC Slide?
- What is the Most Likely Explanation for the HER2 Discrepancy Between the Core and the Repeat Test on the Excision?

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HER2 FISH Performed on Excision







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ASCO/CAP Recommendations: Must HER2 Testing be Repeated on a Surgical Specimen if Initially Negative Test on Core Biopsy?

2013 recommendation	2018 recommendation
If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen	If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen

- "Recent studies show that rates of concordance for HER2 between core biopsy and excision specimens of 98% to 99% are achievable…"
- "In these cases, the reason for discordance was often either HER2 amplification around the cutoff for positivity or tumor heterogeneity"

Rakha EA, Pigera M, Shaaban A, et al. National guidelines and level of evidence: comments on some of the new recommendations in the American Society of Clinical Oncology and the College of American Pathologists human epidermal growth factor receptor 2 guidelines for breast cancer. *J Clin Oncol*. 2015;33(11):1301-1302. doi:10.1200/JCO.2014.59.7211

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HER2 Intratumoral Heterogeneity (IHC and FISH)

- **Definition:**
  - Coexistence of subpopulation of tumors cells with different HER2 gene or protein expression within a tumor
  - HER2 IHC >10% and <50% of tumor cells with strong circumferential membrane staining
  - HER2/CEP17 ratio of >2, observed in a homogenous contiguous population of >10% and <50% of tumor cells
  - If less than 10% of the total area of the invasive cancer tested, the case should be considered IHC equivocal
- **Heterogeneity can lead to discordant IHC/ISH results that must be reconciled for treatment planning**
  - Use IHC to target areas for HER2 FISH analysis if available
- **The higher grade component of a mixed histology tumor should always be selected for HER2 analysis**
- **Clinical significance of heterogeneity remains unclear, and may affect the response to the anti-HER2 treatment**

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ASCO/CAP Guideline Recommendation

Intra-tumoral Heterogeneity

- **Scan entire FISH slide on low magnification prior to counting**
  - **Look for clusters of amplified cells and determine the % of the tumor that these cells represent.**
- **If there is a distinct population of cells, with increased HER2 signals/cell consisting of more than 10% of tumor cells (observed in homogenous and contiguous population):**
  - **Count and report this population separately.**
  - **Report the HER2 test result as:**
    - **<u>Results</u>: HER2 positive.**
    - **Report HER2 signals (and ratio, if available) for both the minority amplified and the majority non-amplified portions of the tumor.**

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Which Specimen Should be Tested?

Core needle biopsy

Considerations

Fixation consistency
More likely to have timely and consistent tissue fixation with CNB samples.

Artifacts
Prone to edge effects and crush artefacts resulting in misinterpretation of staining.

Heterogeneity
Poorly reflects histologic features and biologic profile of the entire tumor.

Excisional biopsy

Considerations

Repeat testing the excision is based on clinic-pathological factors.

Repeat testing on excision

May repeat test on excision if initial HER2 is (-)

- Tumor is grade 3
- Amount of invasive tumor in the core biopsy specimen is small
- Resected specimen contains high-grade carcinoma that is morphologically distinct from that in the core
- Core biopsy result is borderline for HER2 after testing by both IHC and ISH
- There is doubt about the handling of the core biopsy specimen (long ischemic time, short time in fixative, different fixative), or the test is suspected by the pathologist to be negative on the basis of testing error

Metastatic disease

Sample source
Perform test at metastatic site, if tissue sample is available; Myriad of specimen types from liver, bone, lung, and cytology specimen

Bony specimen
Strong acidic decalcifying agent impact HER2 but EDTA have shown to have very little effect on HER2

Cytology specimen
Fixative variability between use of (formalin vs alcohol-based fixatives)

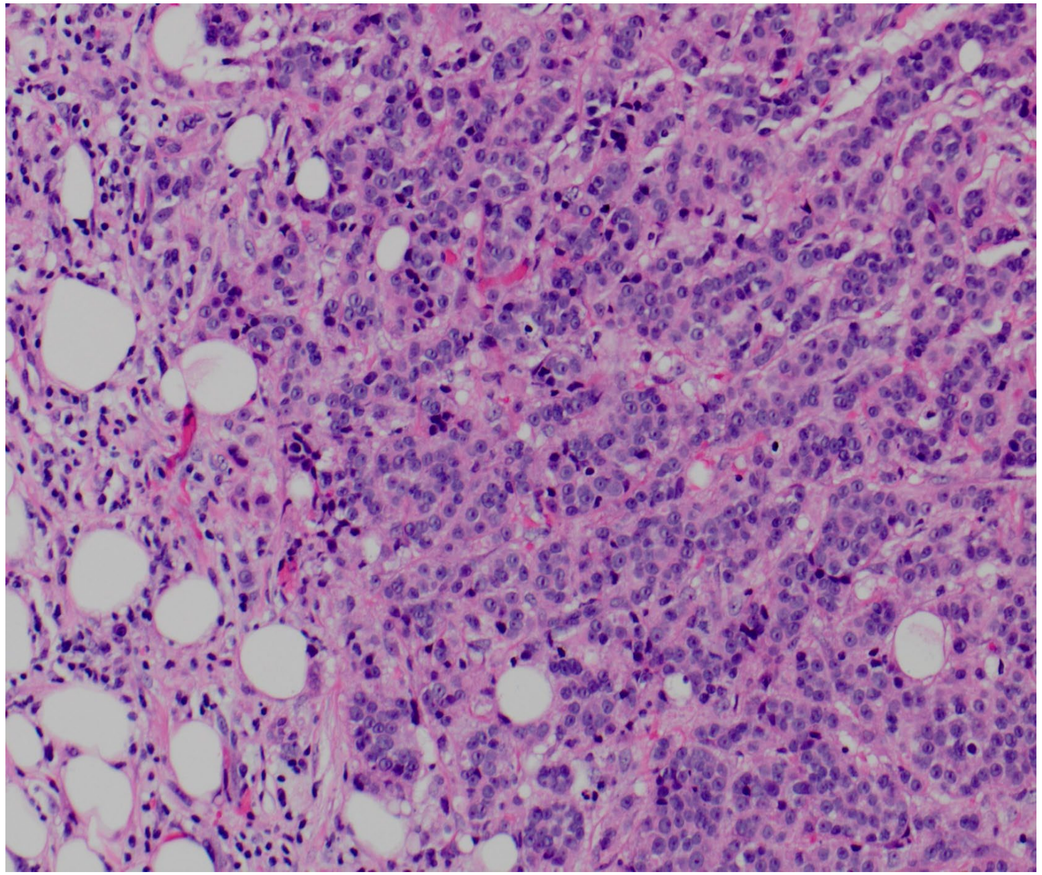
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HER2 Unusual ISH Result (Group 2)
HER2 IHC 2+ with HER2/CEP17 Ratio ≥2.0 but an Average *HER2* Copy Number <4.0 Signals/Cell

71-year-old woman with a mass in right breast

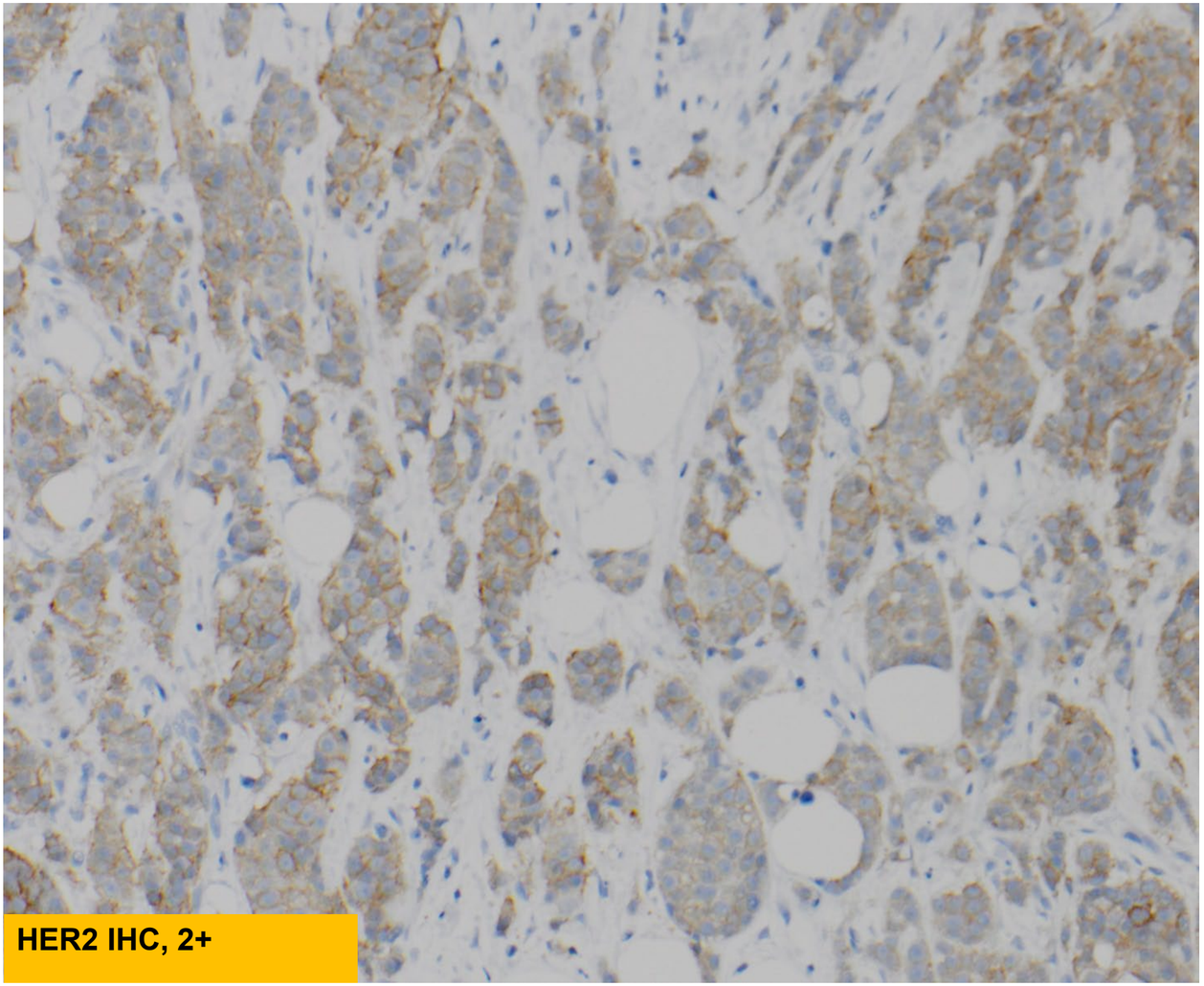
Breast, right, core needle biopsy:

- Invasive ductal carcinoma, nuclear grade 1
  - ER: Positive (Allred score 8)
  - PgR: Positive (Allred score 8)
  - Her2: Read as equivocal (image on next slide)
  - Ki67: ~15%

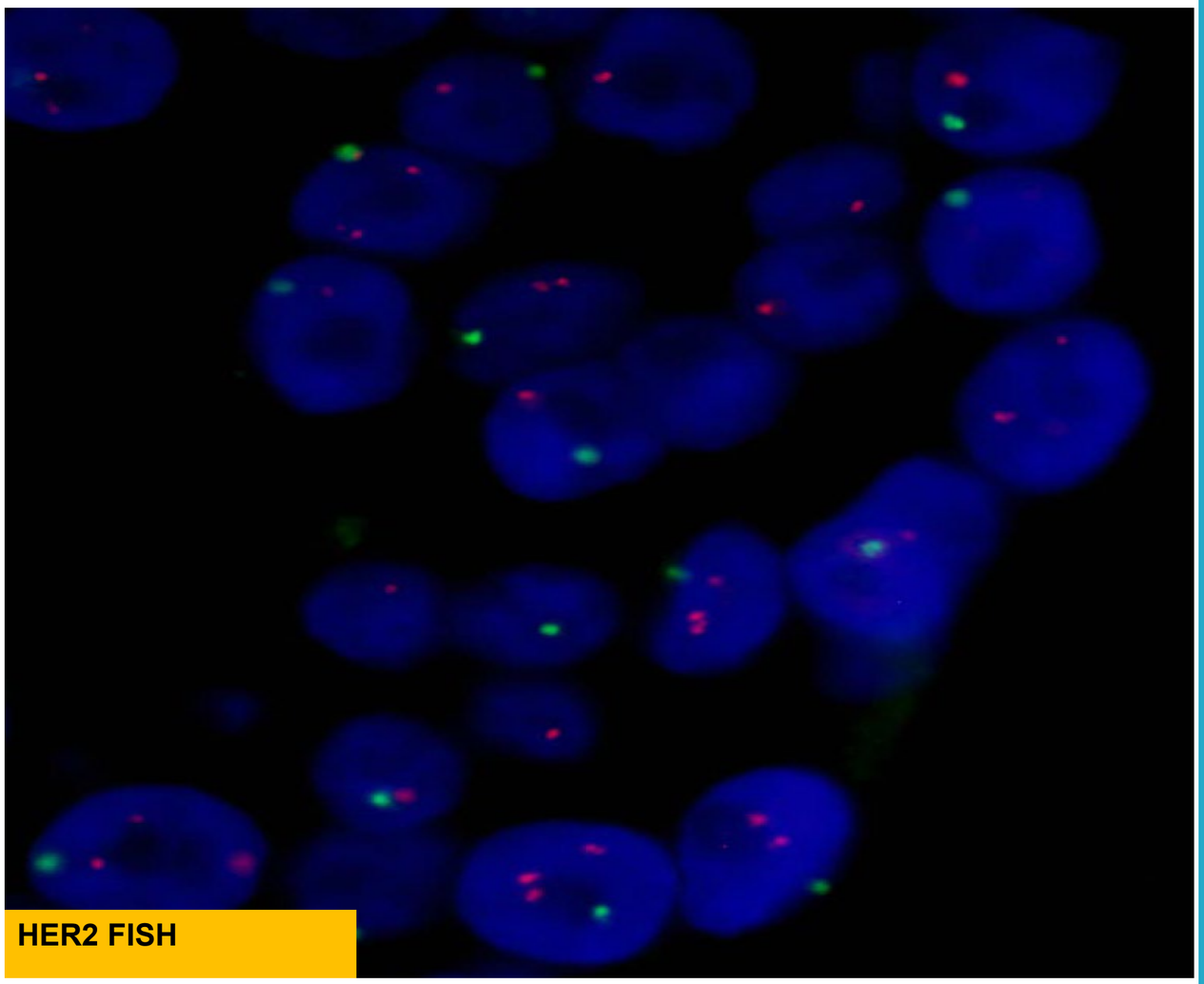


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HER2 IHC, 2+



HER2 FISH

HER2/CEP17 ratio: 3.1

Total number of tumor cells counted: 20 + 20

Average number of HER2 signals/nucleus: 3.8

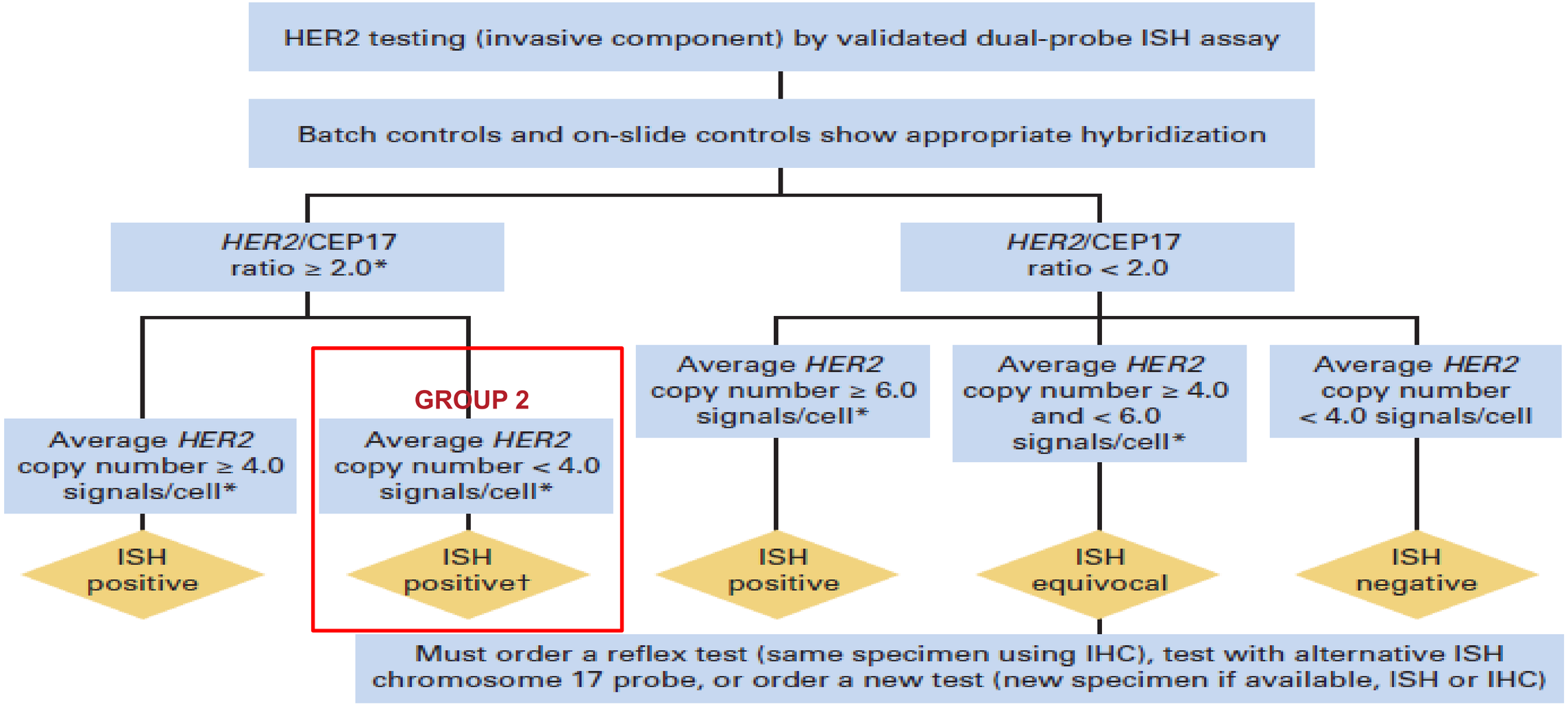
Average number of CEP17 signals/nucleus: 1.2

How Would You Report the HER2 Status for This Case?

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Algorithm for Evaluation of HER2 by Dual-Probe ISH 2013 Guideline



Wolff AC, Hammond ME, Hicks DG, et al. 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. *J Clin Oncol*. 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984

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Table 3. Distribution by Dual FISH and IHC Testing Results in Reported Data Sets

Initial Test Results	Laboratory: HERA Central Laboratory[15]	BCIRG Central Laboratory[10]	USC Breast Cancer Analysis Laboratory[12]	Mayo Clinic Cytogenetics Laboratory[11]	UK NEQAS 2009-2016*	Stanford/ UCSF/ UWMC[16]
FISH distribution						
n	6,018	10,468	7,526	2,851	11,116	8,068
Group 1 ratio ≥ 2.0; *HER2* ≥ 4.0	55.0 (≥ 6.0, 48.7; ≥ 4.0-6.0, 6.3)	40.8	17.7	11.8	14.2	13.8
Group 2 ratio ≥ 2.0; *HER2* < 4.0	0.8	0.7	0.4	1.3	3.7	1.4
Group 3 ratio < 2.0; *HER2* ≥ 6.0	0.4	0.5	0.6	3.0	1.1	0.8
Group 4 ratio < 2.0; *HER2* ≥ 4.0 < 6.0 (after alternative probe: pos, equivocal, neg)	1.9	4.1	4.6	14.2 (7.5, 5.5, 1.3)	7.6	5.2
Group 5 ratio < 2.0; *HER2* < 4.0	41.9	53.9	76.7	69.6	73.4	78.8
IHC distribution						
n	3,089	4,331	7,526	1,922	11,116	3,027
0	IHC 0-1+, 2.0	54.5	51.7	2.4	0.5	IHC 0-1+, 38.1
1+ (including 0 or 1+)	—	9.4	31.0	8.0	1.8	—
2+ (including (1+/2+ or 2+3+)†	61.8	13.7	9.0	87.1†	96.5†	2+, 46.6
3+	36.2	22.4	8.4	2.5	1.3	3+, 15.3

NOTE. Data are presented as % unless otherwise indicated.

Abbreviations: BCIRG, Breast Cancer International Research Group; FISH, fluorescent in situ hybridization; HERA, Herceptin Adjuvant trial; neg, negative; pos, positive; UCSF, University of California, San Francisco; UK NEQAS, United Kingdom National External Quality Assessment Service; USC, University of Southern California; UWMC, University of Washington Medical Center.

*Andrew Dodson, personal communication October 2016.

†IHC 1+ or 2+ and 2+ or 3+ were grouped together with IHC 2+. In each column for a specific laboratory or study, the top set of percentages describes the distribution of group 1 to 5 results when tested using a dual-probe FISH assay, while the bottom set of percentages describes the distribution of IHC tests results of the samples submitted to that laboratory or study for dual-probe ISH testing and as described in each publication.

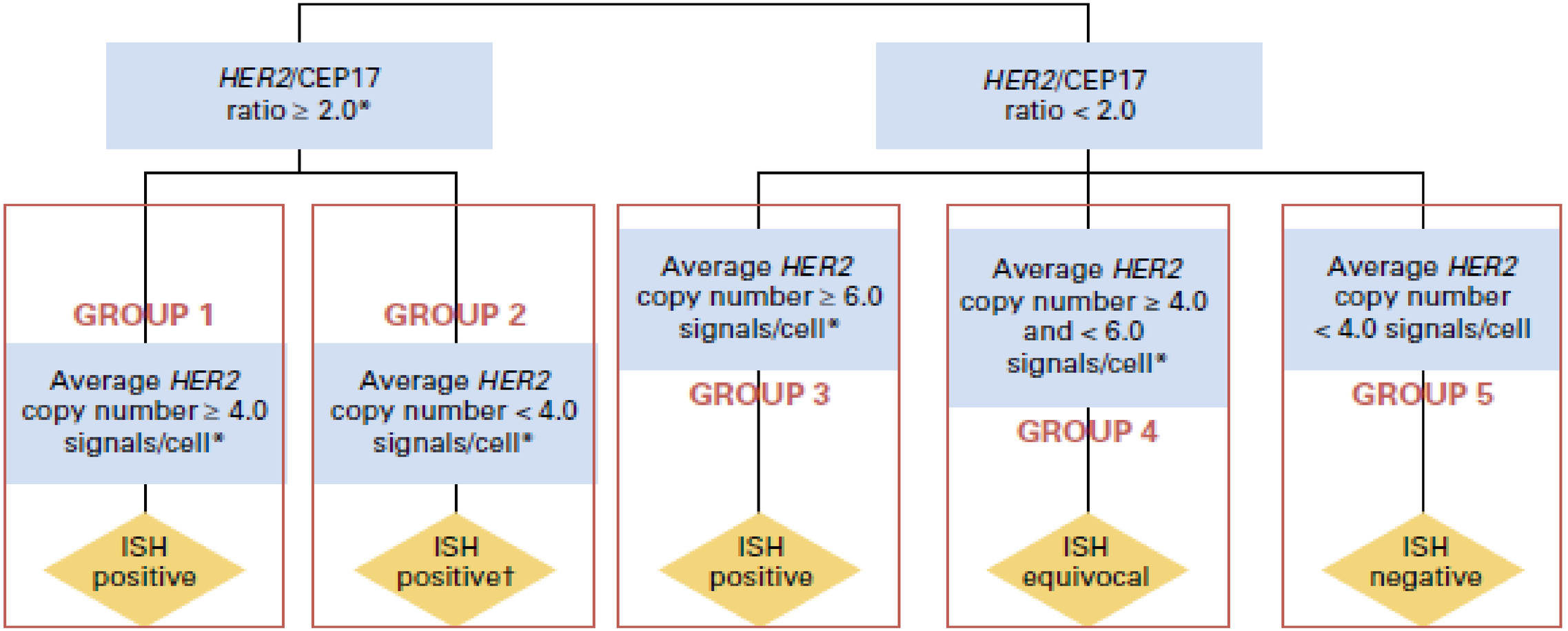
ISH group 2:

Incidence:0.4%-3.7%

Causes: Amplification of the *HER2* gene and an associated increased HER2 copy number, with a *loss* of chromosome 17 copy number, resulting in HER2 amplified monosomy of chromosome 17

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Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738



Incidence: 0.7%
IHC review: > 90% (0/1+)

Group 2: No apparent benefit from trastuzumab therapy

Press MF, Sauter G, Buyse M, et al. HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials. *J Clin Oncol*. 2016;34(29):3518-3528. doi:10.1200/JCO.2016.66.6693

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Revised 2018 Recommendation: If a Case has a HER2/CEP17 Ratio of ≥2.0 but the Average HER2 Signals/Cell is <4.0, a Definitive Diagnosis Will be Rendered Based on Further Workup

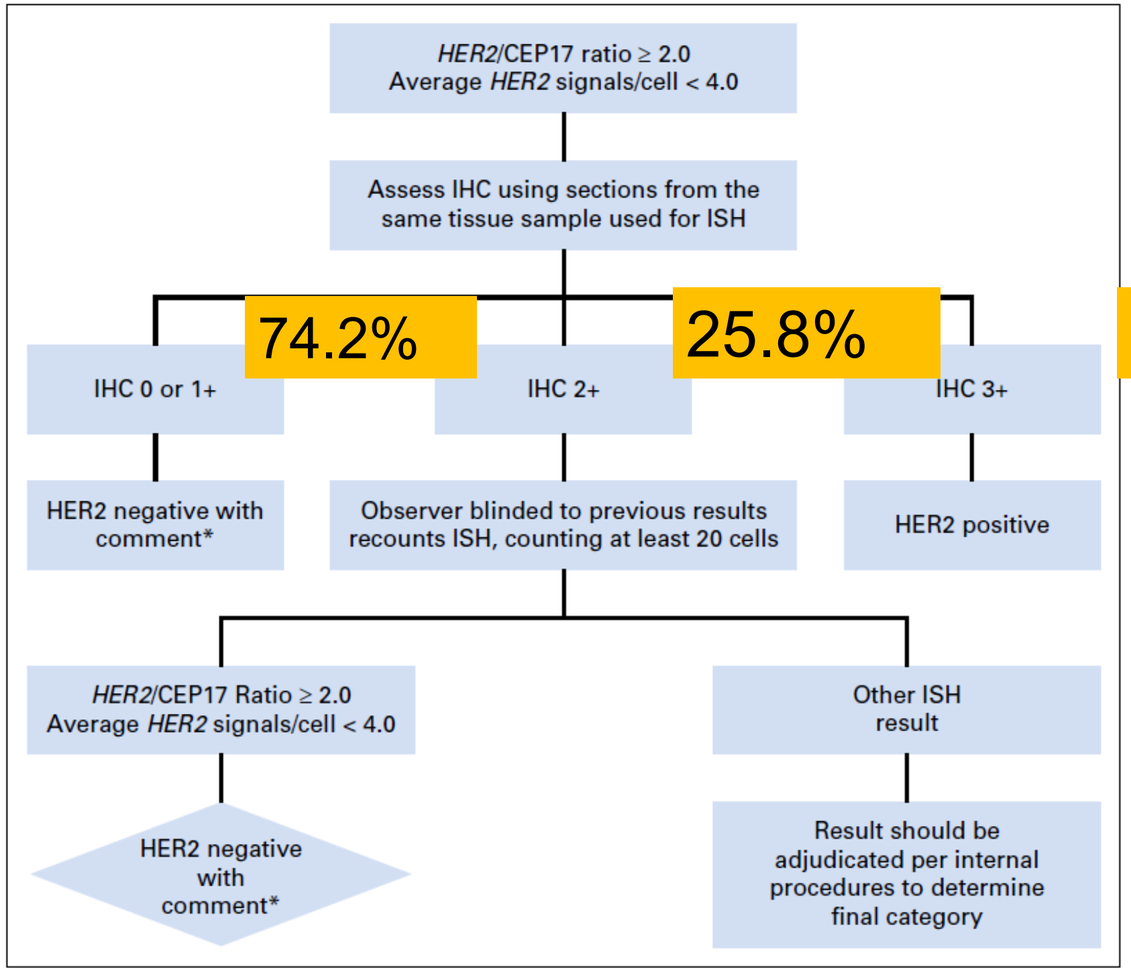


Fig 4. Clinical Question 3, group 2. (*) Evidence is limited on the efficacy of HER2-targeted therapy in the [o]f cases with a *HER2*/CEP17 ratio ≥ 2.0 [and] *HER2* copy number of < 4.0 per cell. In [an] [e]ation of adjuvant trastuzumab trials, patients in this subgroup who were randomly assigned to the trastuzumab arm did not seem to derive an improvement in disease-free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement ISH and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low *HER2* copy number by ISH and the lack of protein overexpression. CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

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Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738

Index Case Final HER2 Status (FISH Group 2 and IHC 2+)

Breast, right, core needle biopsy

Integrated final HER2 status based on IHC and FISH results:

- **Interpretation - Negative (please see comment)**

Comment: The integrated final HER2 results for this case are based on a consideration of both the IHC and FISH results per the 2018 ASCO/CAP HER2 focused update. The HER2 immunohistochemistry (IHC) was equivocal (2+) and fluorescence in situ hybridization (FISH) for *HER2* was performed on the same tissue block which identified the following signal pattern:

HER2/CEP17 ratio > = 2 & average HER2 copy # < 4

Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a *HER2*/CEP17 ratio >=2.0 and an average *HER2* copy number <4.0/cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomized to the trastuzumab arm did not appear to derive an improvement in disease free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement FISH and define HER2 status. If IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low HER2 copy number by FISH and lack of protein overexpression. In this scenario, clinical correlation with other patient factors, pathologic features of the patient's cancer (such as ER expression and tumor grade) and available test results from other specimens may be used when selecting appropriate treatments, including chemotherapy and HER2 targeted therapies.

Patient did not receive HER2-targeted therapy

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Table 2 Comparison of *HER2* IHC score in 2 groups of tumors with amplified *HER2* FISH results

HER2 IHC results	*HER2* FISH results	
	Group A (*HER2/CEP17* ≥2; *HER2* copy <4)	Group B (*HER2* amplified; *HER2* copy ≥4)
HER2 negative (0, 1+)	11 (61.1%)	14 (9.3%)
HER2 equivocal (2+)	7 (38.9%)	36 (23.8%)
HER2 positive (3+)	0	101 (66.9%)
Total	18	151

1.5% of all breast cancers, are associated with low or absent HER2 protein expression

Zare SY et al., Breast cancers with a HER2/CEP17 ratio of 2.0 or greater and an average HER2 copy number of less than 4.0 per cell. Human Pathology (2019) 83, 7–13

HER2 ISH group 2

- **0.6% of breast cancer.**
- **Repeat testing on different block may change the initial results (3/13, 23% changed to HER2 positive).**
- **pCR is seen with anti-HER2 therapy when repeat testing converts to HER2 positive.**
- **All three cases with pCR are ER ≤10% and Ki67 ≥40%.**
- **Repeat HER2 tests on alternative samples may be considered to guide anti-HER2 therapy.**

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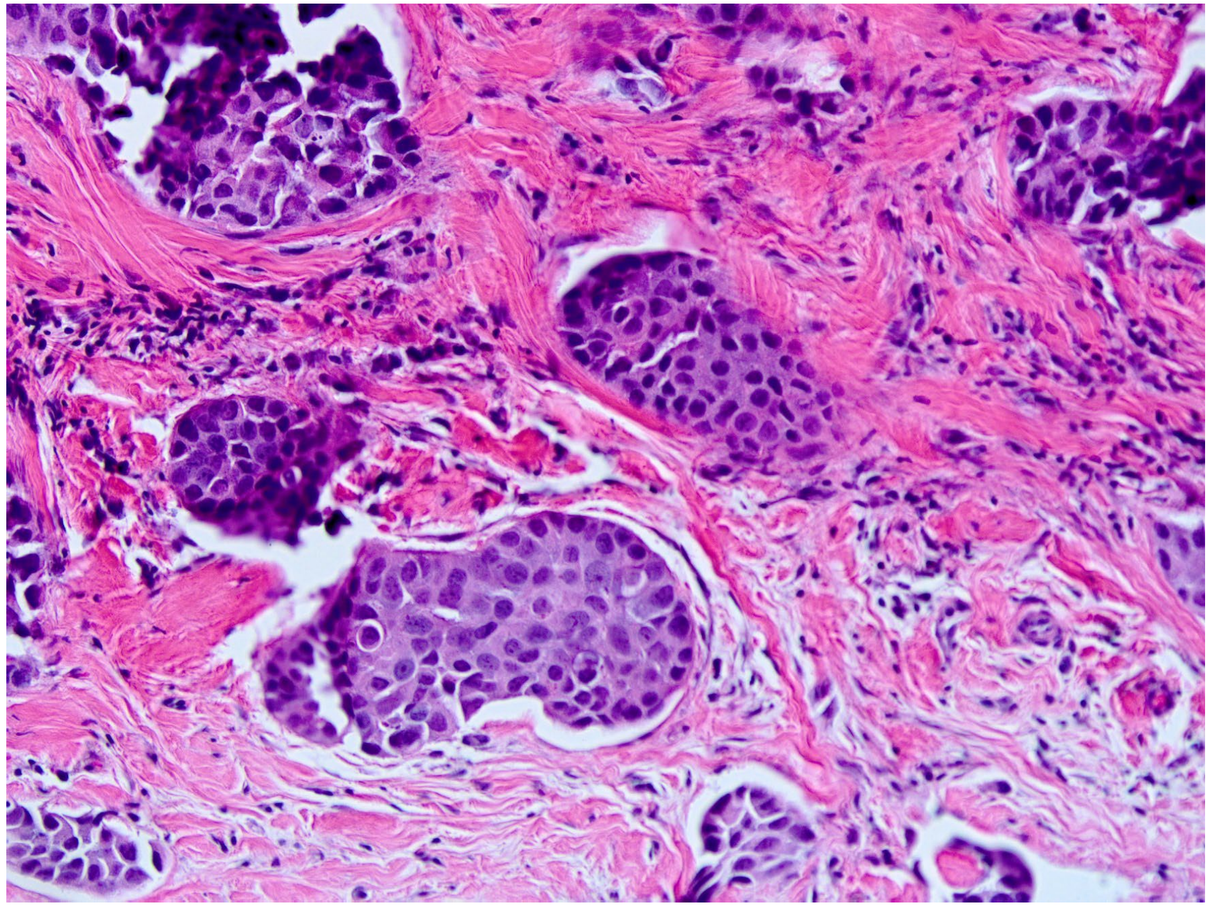
Wang M, Ding Q, Gu J, et al. Breast Cancer With a HER2 FISH Group 2 Result: Should HER2 Tests be Repeated?. *Clin Breast Cancer*. 2023;23(4):415-422. doi:10.1016/j.clbc.2023.02.006

HER2 Unusual ISH result (Group 3)
HER2 IHC 2+ with an Average *HER2* Copy Number ≥6.0 Signals/Cell but a HER2/CEP17 Ratio <2.0

77-year-old female with irregular, mildly hyperdense nodule in left breast

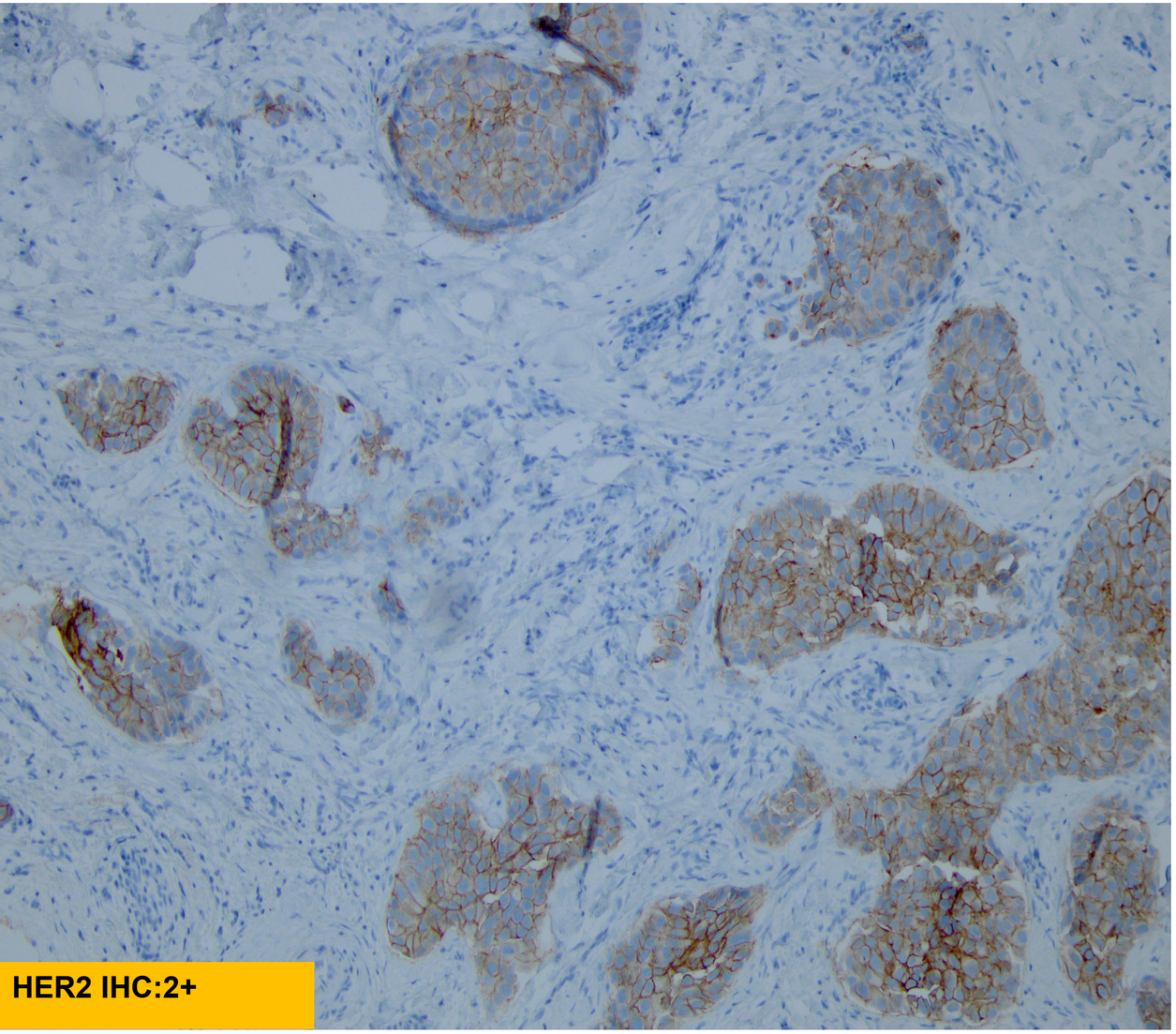
Breast, left at 2:30 (mass), core needle biopsy:

- Invasive ductal carcinoma, nuclear grade 2
  - ER: Positive
  - PgR: Positive
  - Her2: Equivocal (image on next slide)

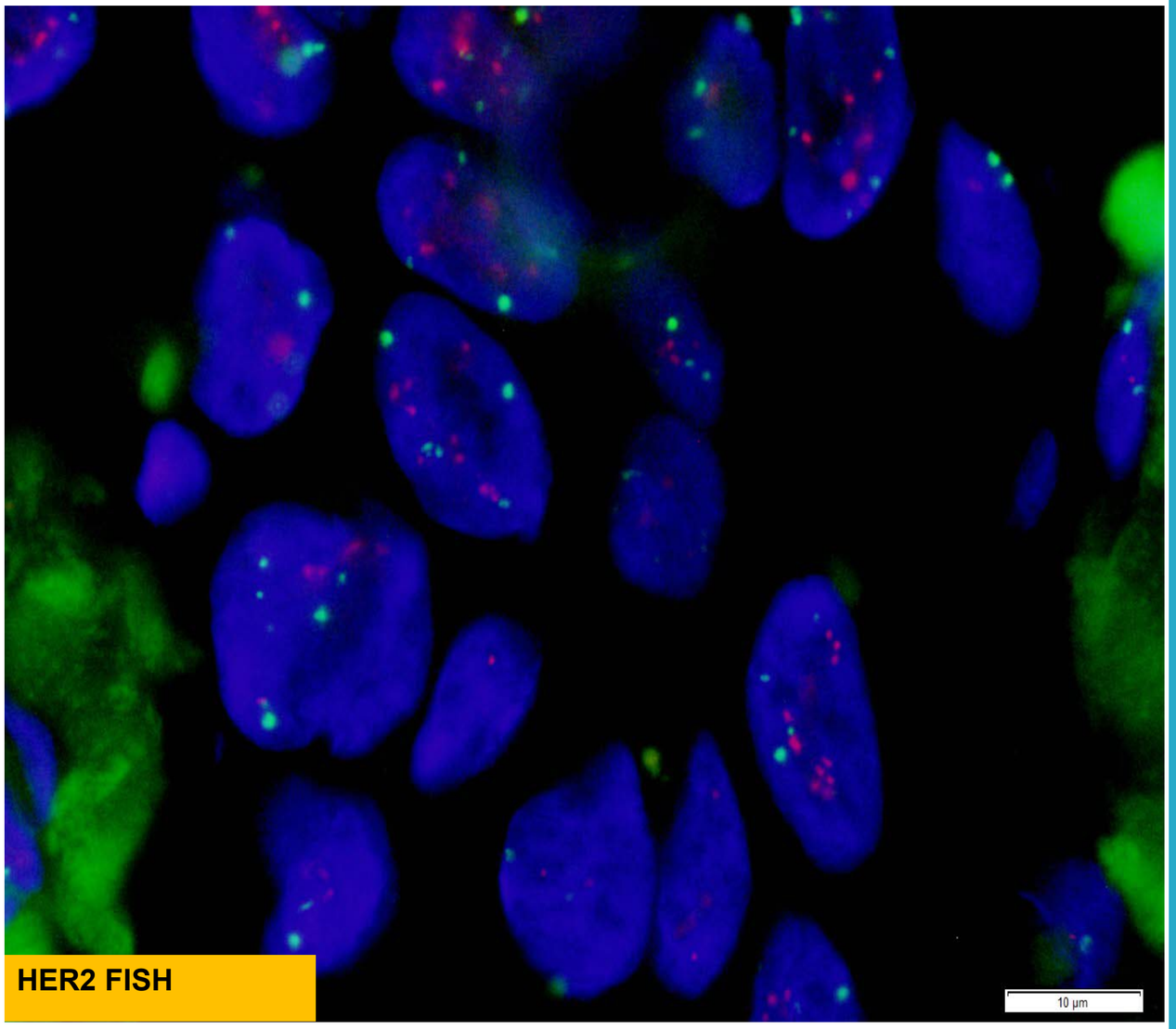


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HER2 IHC:2+



HER2 FISH

HER2/CEP17 ratio: 1.6
Total number of tumor cells counted: 20 + 20
The average HER2 signal copy number: 9.4
The average CEP17 signal copy number: 6.0

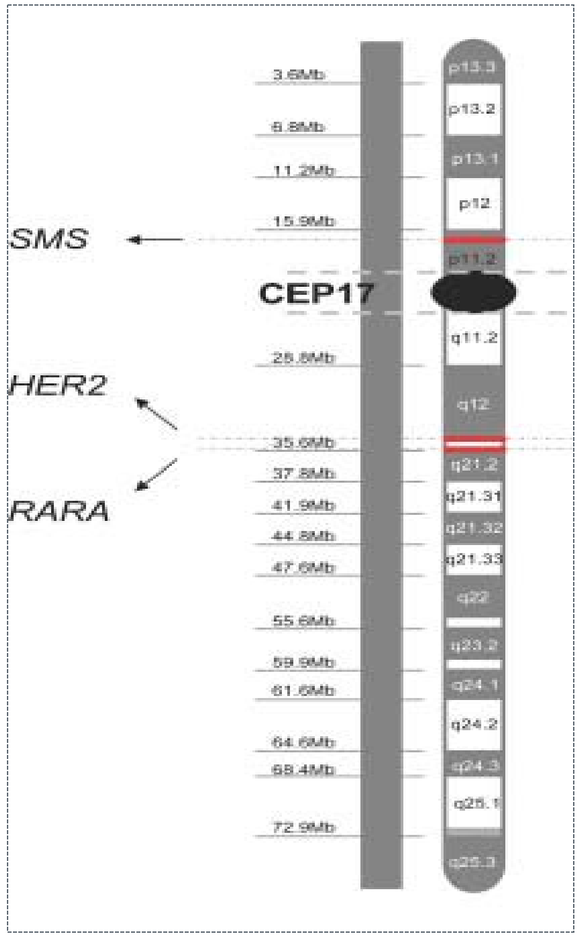
How Would You Report the HER2 Status for This Case?

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Does the Average CEP17 # in FISH Predict Polysomy in Breast Cancer?

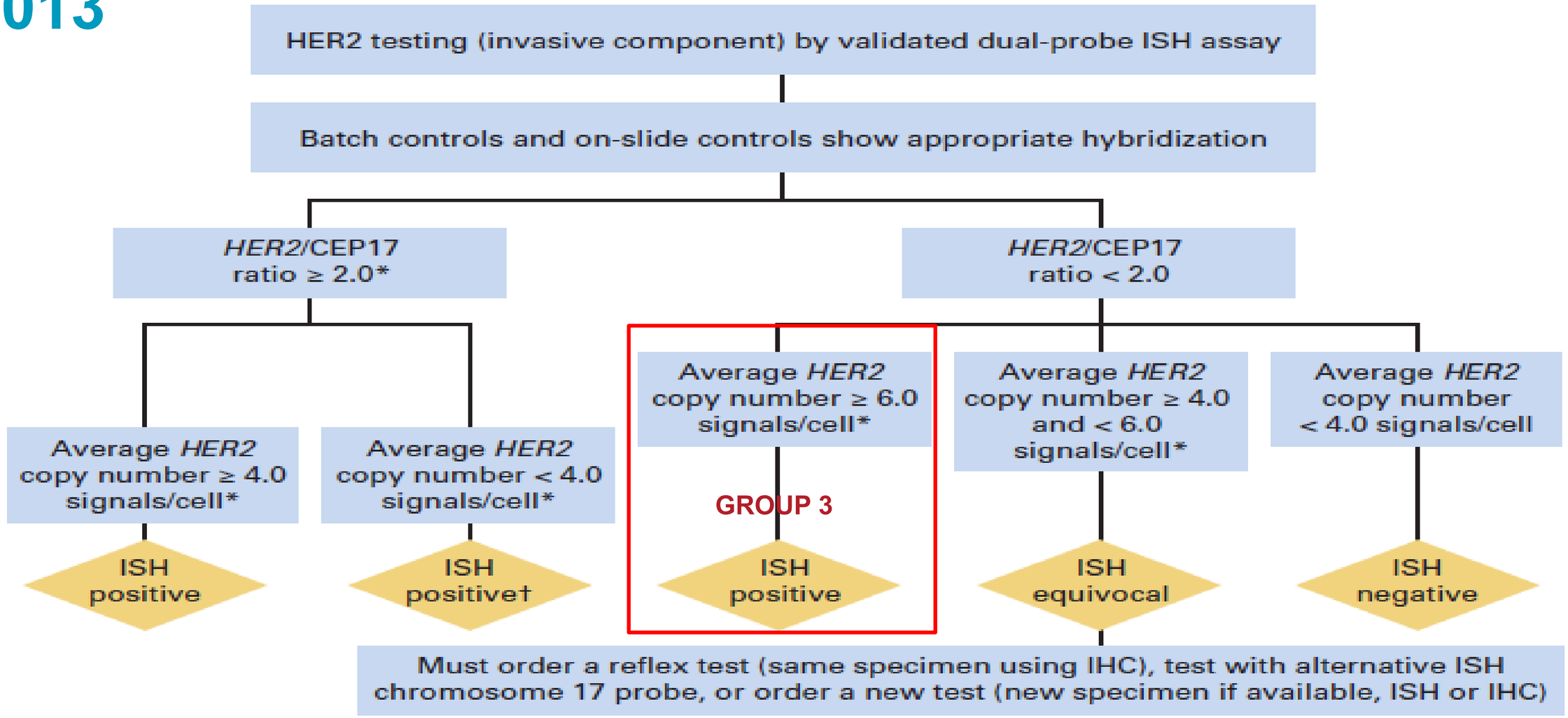
- Aberrant CEP17 copy # in FISH assays may not represent true chromosome 17 polysomy.
- Array CGH studies suggest true chromosome 17 polysomy is likely a rare event in breast cancer.
- CEP17 copy number >3.0 in FISH is frequently related to gain or amplification of the centromeric region.
  - Correction with CEP17 probes may provide misleading *HER2* gene status assessment results.
  - Cases with HER2/CEP17 <2 showing polysomy 17 should be further evaluated for HER2 by IHC.
  - Increased *HER2* gene copies (≥6 *HER2* genes or HER2/CEP17 ratio ≥2.2), regardless of the evaluation method, is positively correlated with HER2 protein expression.



Marchiò C, Lambros MB, Gugliotta P, et al. Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. *J Pathol*. 2009;219(1):16-24. doi:10.1002/path.2574.

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Algorithm for Evaluation of HER2 by Dual-Probe ISH-2013



Wolff AC, Hammond ME, Hicks DG, et al. 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. *J Clin Oncol*. 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984

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Table 3. Distribution by Dual FISH and IHC Testing Results in Reported Data Sets

Initial Test Results	Laboratory					
	HERA Central Laboratory[15]	BCIRG Central Laboratory[10]	USC Breast Cancer Analysis Laboratory[12]	Mayo Clinic Cytogenetics Laboratory[11]	UK NEQAS 2009-2016*	Stanford/ UCSF/ UWMC[16]
FISH distribution						
n	6,018	10,468	7,526	2,851	11,116	8,068
Group 1 ratio ≥ 2.0; *HER2* ≥ 4.0	55.0 (≥ 6.0, 48.7; ≥ 4.0-6.0, 6.3)	40.8	17.7	11.8	14.2	13.8
Group 2 ratio ≥ 2.0; *HER2* < 4.0	0.8	0.7	0.4	1.3	3.7	1.4
Group 3 ratio < 2.0; *HER2* ≥ 6.0	0.4	0.5	0.6	3.0	1.1	0.8
Group 4 ratio < 2.0; *HER2* ≥ 4.0 < 6.0 (after alternative probe: pos, equivocal, neg)	1.9	4.1	4.6	14.2 (7.5, 5.5, 1.3)	7.6	5.2
Group 5 ratio < 2.0; *HER2* < 4.0	41.9	53.9	76.7	69.6	73.4	78.8
IHC distribution						
n	3,089	4,331	7,526	1,922	11,116	3,027
0	IHC 0-1+, 2.0	54.5	51.7	2.4	0.5	IHC 0-1+, 38.1
1+ (including 0 or 1+)	—	9.4	31.0	8.0	1.8	—
2+ (including (1+/2+ or 2+3+)†	61.8	13.7	9.0	87.1†	96.5†	2+, 46.6
3+	36.2	22.4	8.4	2.5	1.3	3+, 15.3

NOTE. Data are presented as % unless otherwise indicated.

Abbreviations: BCIRG, Breast Cancer International Research Group; FISH, fluorescent in situ hybridization; HERA, Herceptin Adjuvant trial; neg, negative; pos, positive; UCSF, University of California, San Francisco; UK NEQAS, United Kingdom National External Quality Assessment Service; USC, University of Southern California; UWMC, University of Washington Medical Center.

*Andrew Dodson, personal communication October 2016.

†IHC 1+ or 2+ and 2+ or 3+ were grouped together with IHC 2+. In each column for a specific laboratory or study, the top set of percentages describes the distribution of group 1 to 5 results when tested using a dual-probe FISH assay, while the bottom set of percentages describes the distribution of IHC tests results of the samples submitted to that laboratory or study for dual-probe ISH testing and as described in each publication.

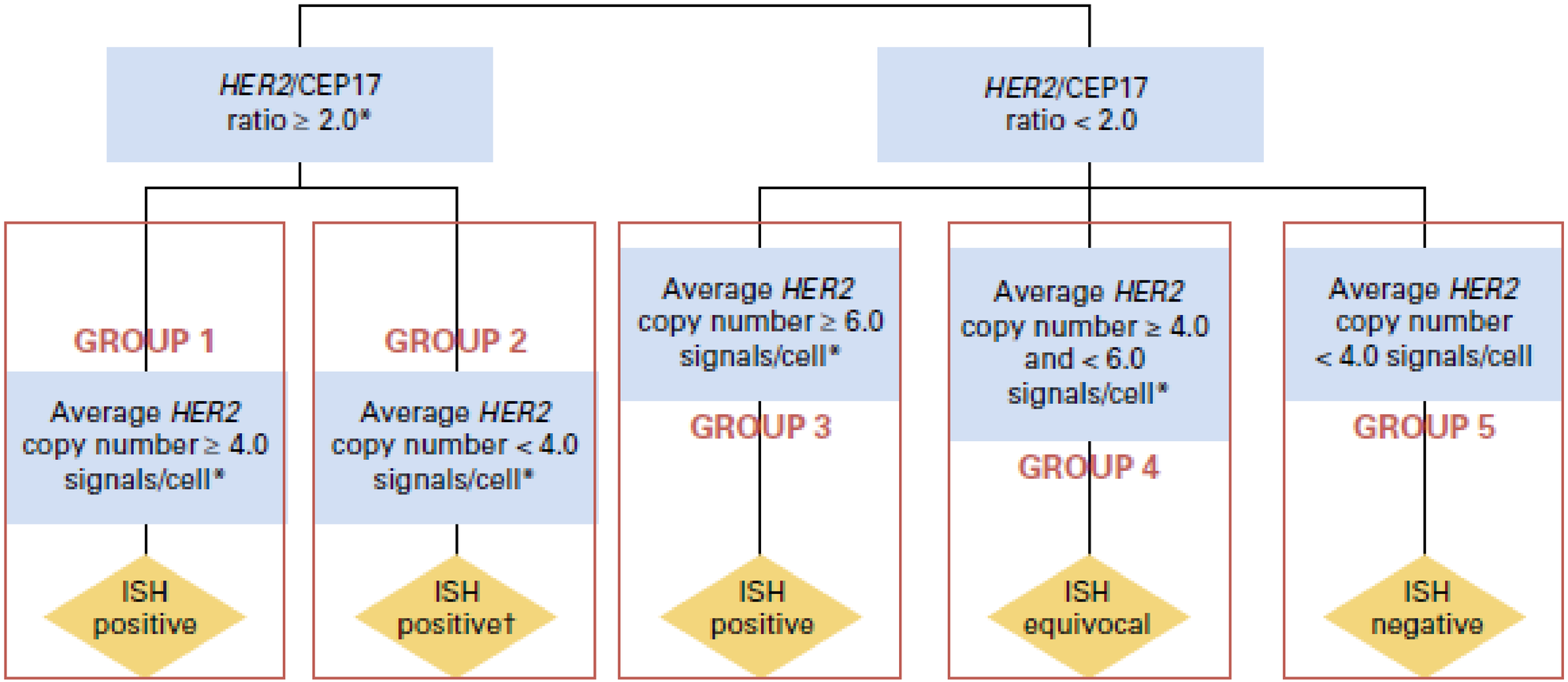
ISH Group 3:

Incidence:0.4%-3.0%

Causes: Polysomy of ch17, or more commonly co-amplification of both the HER2 and CEP17

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Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738



Incidence: 0.5%

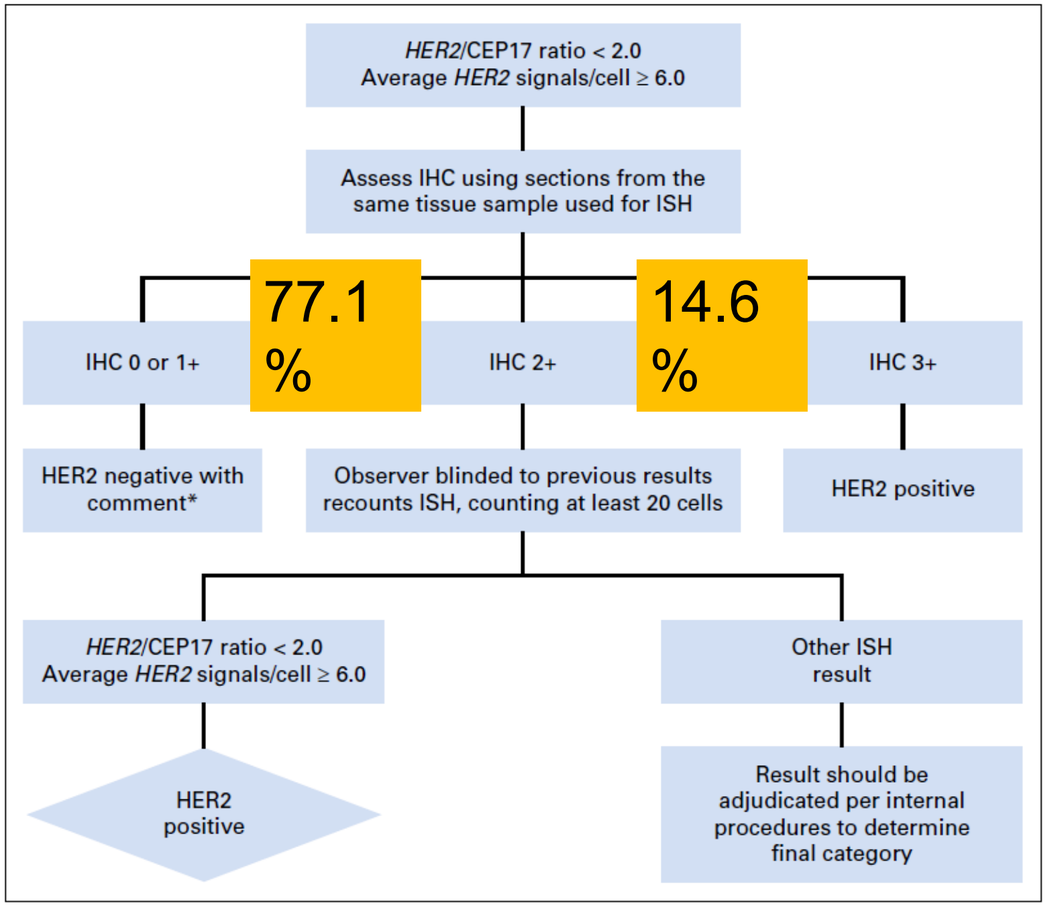
IHC review: ~76% low expression

Insufficient data for trastuzumab benefit

Press MF, Sauter G, Buyse M, et al. HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials. *J Clin Oncol*. 2016;34(29):3518-3528. doi:10.1200/JCO.2016.66.6693

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Revised 2018 Recommendation: If a Case has an Average of ≥6.0 HER2 Signals/Cell With a HER2/CEP17 Ratio of <2.0, Formerly Diagnosed as ISH Positive for HER2, a Definitive Diagnosis Will be Rendered Based on Further Workup



al Question 4, group 3. (*) There are in-sufficient data on the efficacy of HER2-targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative. CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738

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Index Case Final HER2 Status (FISH Group 3 and IHC 2+)

Breast, left at 2:30 (mass), core needle biopsy

Integrated final HER2 status based on IHC and FISH results:

- **Interpretation - positive (please see comment)**

Comment: The integrated final HER2 results for this case is based on a consideration of both the IHC and FISH results per the 2018 ASCO/CAP HER2 focused update. The HER2 immunohistochemistry (IHC) was equivocal (2+) and fluorescence in situ hybridization (FISH) for HER2 was performed on the same tissue block which identified the following signal pattern:

HER2/CEP17 ratio < 2 & average HER2 copy # > = 6

This patient is eligible for HER2-targeted therapy based on the 2013 ASCO/CAP HER2 Testing Guideline Update. This invasive cancer has >= 6.0 mean HER2 signals/cell but also has increased centromere (CEP17) control signals, resulting in a HER2:CEP17 ratio < 2.0. Because array-based comparative genomic hybridization (aCGH) studies have shown that true polysomy (duplication of the entire chromosome) is actually rare, while gain of the pericentromeric region of chromosome 17 is more commonly observed, the ASCO/CAP Guideline recommends considering these cases HER2 positive. However, there is limited data to indicate if patients receive benefit from HER2 targeted therapy in this setting without over-expression of the HER2 protein by IHC. This sample was scored as (2+) by IHC. Clinical correlation with other patient factors and the pathologic features of the patient's cancer should be used in this setting when considering treatment with HER2-targeted therapies.

Patient completed Trastuzumab, in addition to Taxol

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A recent study on HER2 IHC 2+ and ISH group 3 (62 cases)

- Histologic grade 2 or 3, ER positive, with an average HER2 signals/cell of 7.3.
- Luminal molecular type: 80%; HER2 molecular subtype:10%.
- 10% achieved pCR.
- No statistically significant difference in disease outcomes between patients who completed anti-HER2 treatment and those who did not.
- Advanced clinical stage, and ER/PgR negativity (univariate analysis) was associated with unfavorable disease outcomes
- Only clinical stage (multivariate analysis) was the most significant factor associated with disease outcomes.
- Role of perioperative HER2-targeted therapy needs further investigation

Ajabnoor R et al. Breast Cancer With HER2 Immunohistochemical Score 2 and Average HER2 Signals/Cell 6 or More and HER2/CEP17 Ratio Less Than 2 ('ISH Group 3'): A Multiinstitutional Cohort Analysis Emphasizing Outcome and Molecular Subtype Mod Pathol 2024;37(8):100530

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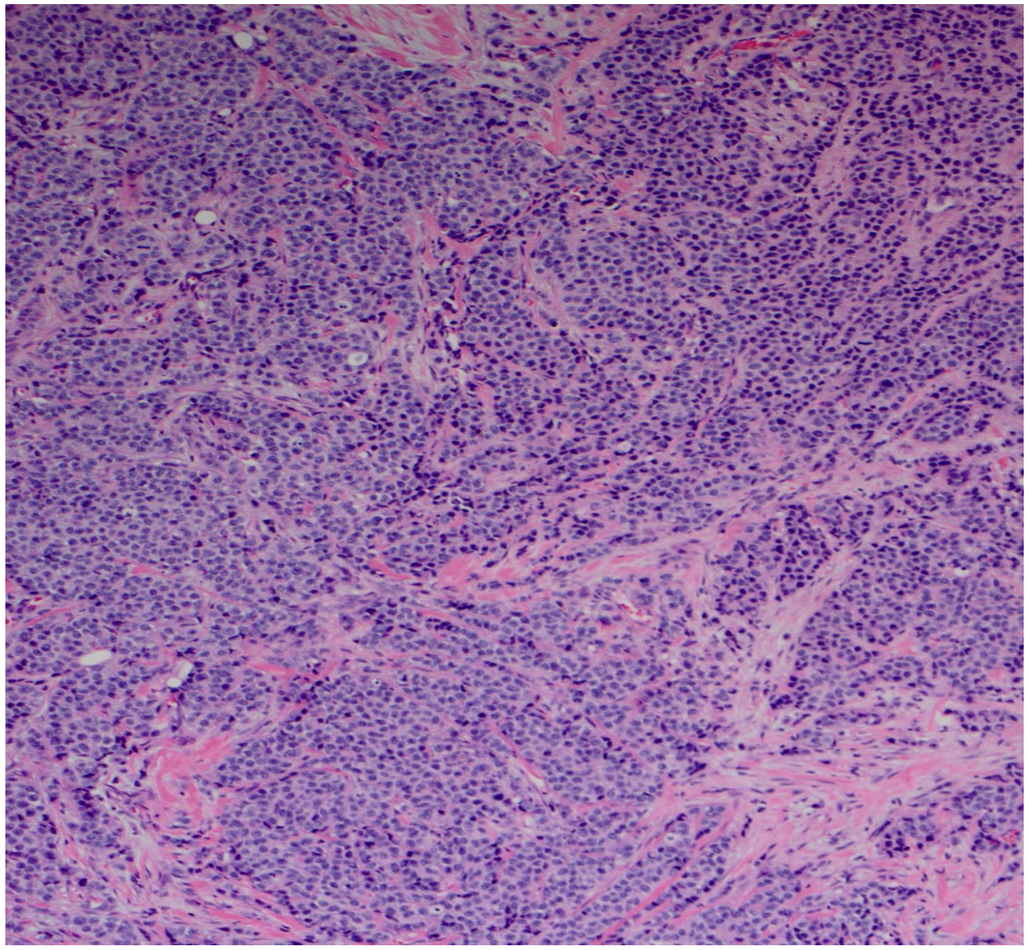
HER2 Unusual ISH Result (Group 4)

HER2 IHC 2+ with an average *HER2* copy number ≥4.0 but <6.0 signals/cell, but a HER2/CEP17 ratio <2.0

51-year-old woman, right breast mass discover by mammographic screening

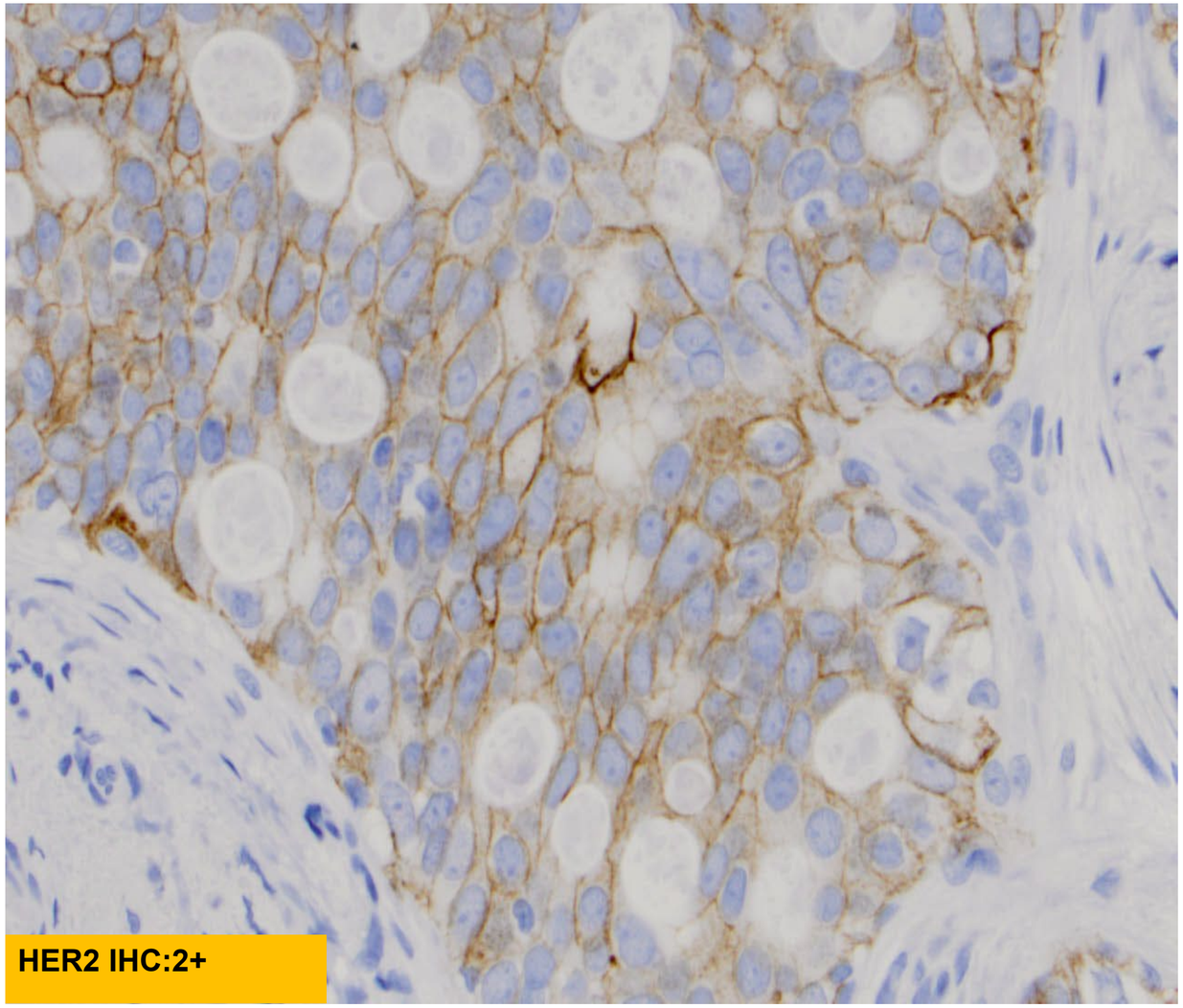
Breast, right, core needle biopsy:

- Invasive ductal carcinoma, nuclear grade 2
  - ER: positive (Allred score 8)
  - PgR: positive (Allred score 4)
  - HER2 IHC: equivocal (2+)
  - Ki67: 10%
  - FISH analysis ordered

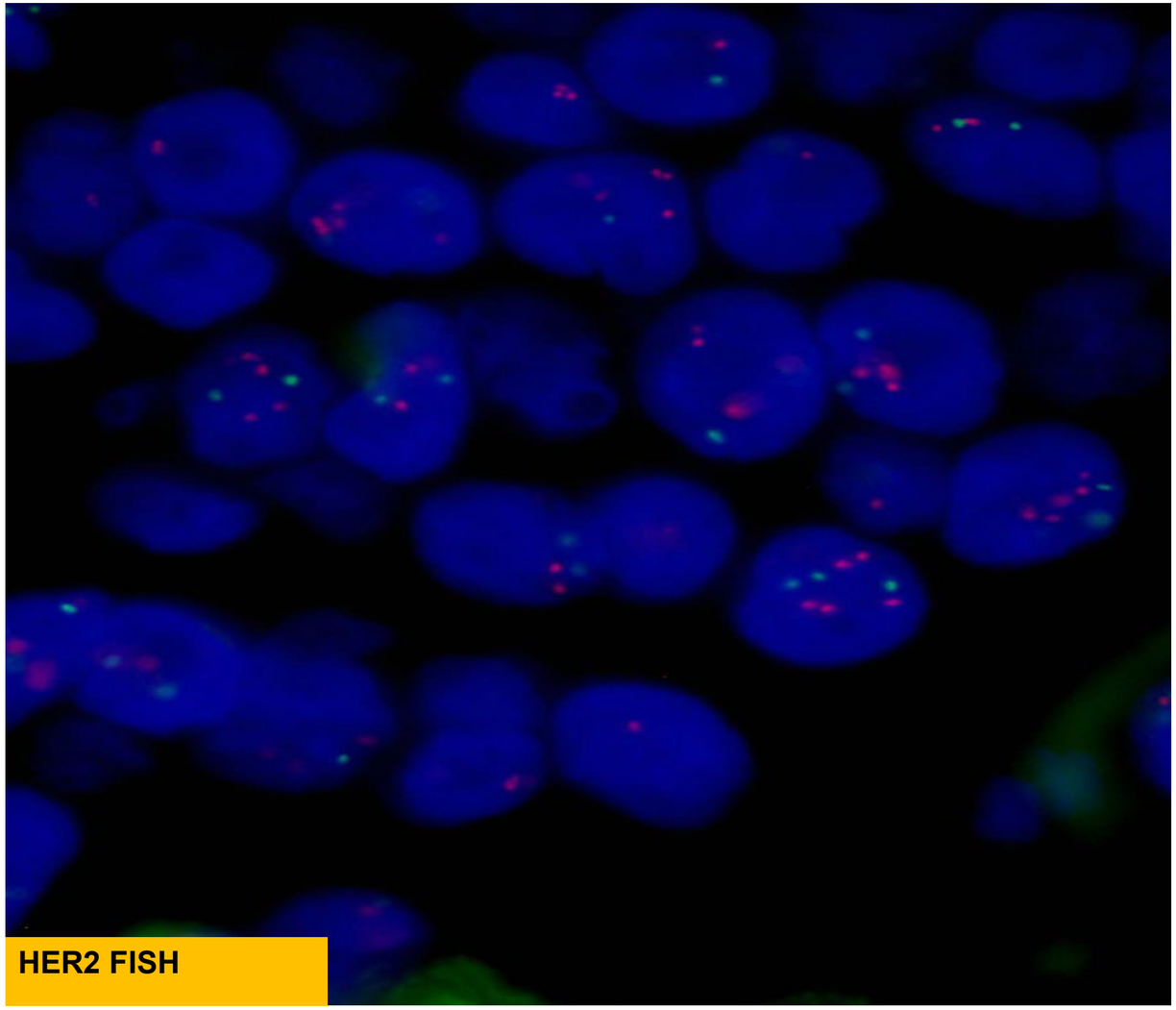


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HER2 IHC:2+



HER2 FISH

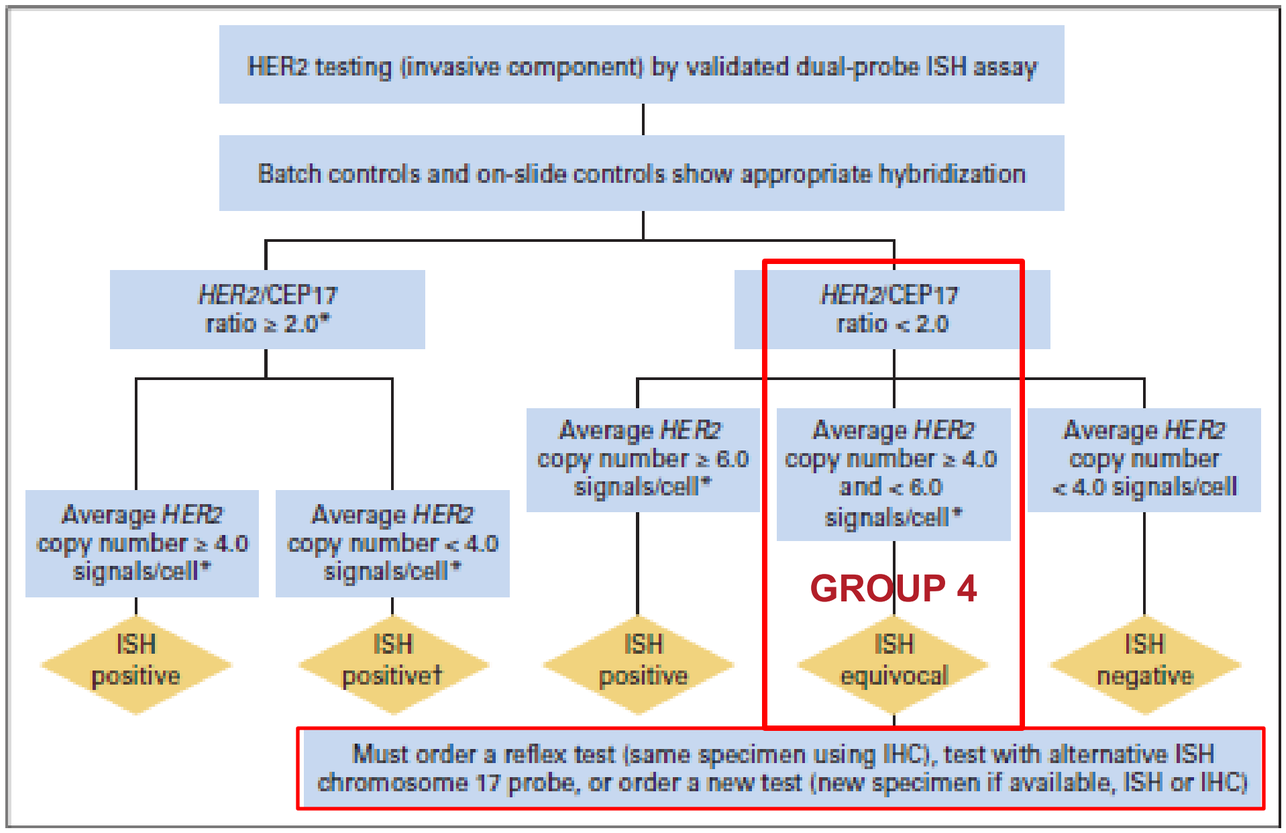
HER2/CEP17 ratio: 1.7
Total number of tumor cells counted: 20 + 20
The average HER2 signal copy number: 5.4
The average CEP 17 signal copy number: 3.1

How Would You Report the HER2 Status for This Case?

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Algorithm for Evaluation of HER2 by Dual-Probe ISH-2013



Wolff AC, Hammond ME, Hicks DG, et al. 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. *J Clin Oncol*. 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984

Fig 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (*HER2*) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (*HER2* gene) assay (dual-probe ISH). Amplification in a dual-probe ISH assay is defined by examining first the *HER2*/CEP17 ratio followed by the average *HER2* copy number (see Data Supplement 2E for more details). If there is a second contiguous population of cells with increased HER2 signals per cell, and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or immunohistochemistry [IHC] slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and also reported. Although categories of HER2 status by ISH can be created that are not covered by these definitions, in practice they are rare and if encountered should be considered ISH equivocal (see Data Supplement 2E). NOTE. The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. (*) Observed in a homogeneous and contiguous population. (†) See Data Supplement 2E for more information on these rare scenarios.

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Table 3. Distribution by Dual FISH and IHC Testing Results in Reported Data Sets

Initial Test Results	Laboratory: HERA Central Laboratory[15]	BCIRG Central Laboratory[10]	USC Breast Cancer Analysis Laboratory[12]	Mayo Clinic Cytogenetics Laboratory[11]	UK NEQAS 2009-2016*	Stanford/ UCSF/ UWMC[16]
FISH distribution						
n	6,018	10,468	7,526	2,851	11,116	8,068
Group 1 ratio ≥ 2.0; *HER2* ≥ 4.0	55.0 (≥ 6.0, 48.7; ≥ 4.0-6.0, 6.3)	40.8	17.7	11.8	14.2	13.8
Group 2 ratio ≥ 2.0; *HER2* < 4.0	0.8	0.7	0.4	1.3	3.7	1.4
Group 3 ratio < 2.0; *HER2* ≥ 6.0	0.4	0.5	0.6	3.0	1.1	0.8
Group 4 ratio < 2.0; *HER2* ≥ 4.0 < 6.0 (after alternative probe: pos, equivocal, neg)	1.9	4.1	4.6	14.2 (7.5, 5.5, 1.3)	7.6	5.2
Group 5 ratio < 2.0; *HER2* < 4.0	41.9	53.9	76.7	69.6	73.4	78.8
IHC distribution						
n	3,089	4,331	7,526	1,922	11,116	3,027
0	IHC 0-1+, 2.0	54.5	51.7	2.4	0.5	IHC 0-1+, 38.1
1+ (including 0 or 1+)	—	9.4	31.0	8.0	1.8	—
2+ (including (1+/2+ or 2+3+)†	61.8	13.7	9.0	87.1†	96.5†	2+, 46.6
3+	36.2	22.4	8.4	2.5	1.3	3+, 15.3

NOTE. Data are presented as % unless otherwise indicated.

Abbreviations: BCIRG, Breast Cancer International Research Group; FISH, fluorescent in situ hybridization; HERA, Herceptin Adjuvant trial; neg, negative; pos, positive; UCSF, University of California, San Francisco; UK NEQAS, United Kingdom National External Quality Assessment Service; USC, University of Southern California; UWMC, University of Washington Medical Center.

*Andrew Dodson, personal communication October 2016.

†IHC 1+ or 2+ and 2+ or 3+ were grouped together with IHC 2+. In each column for a specific laboratory or study, the top set of percentages describes the distribution of group 1 to 5 results when tested using a dual-probe FISH assay, while the bottom set of percentages describes the distribution of IHC tests results of the samples submitted to that laboratory or study for dual-probe ISH testing and as described in each publication.

ISH Group 4:

Incidence:1.9%-14.2%

Causes: Mainly heterozygous deletions

Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738

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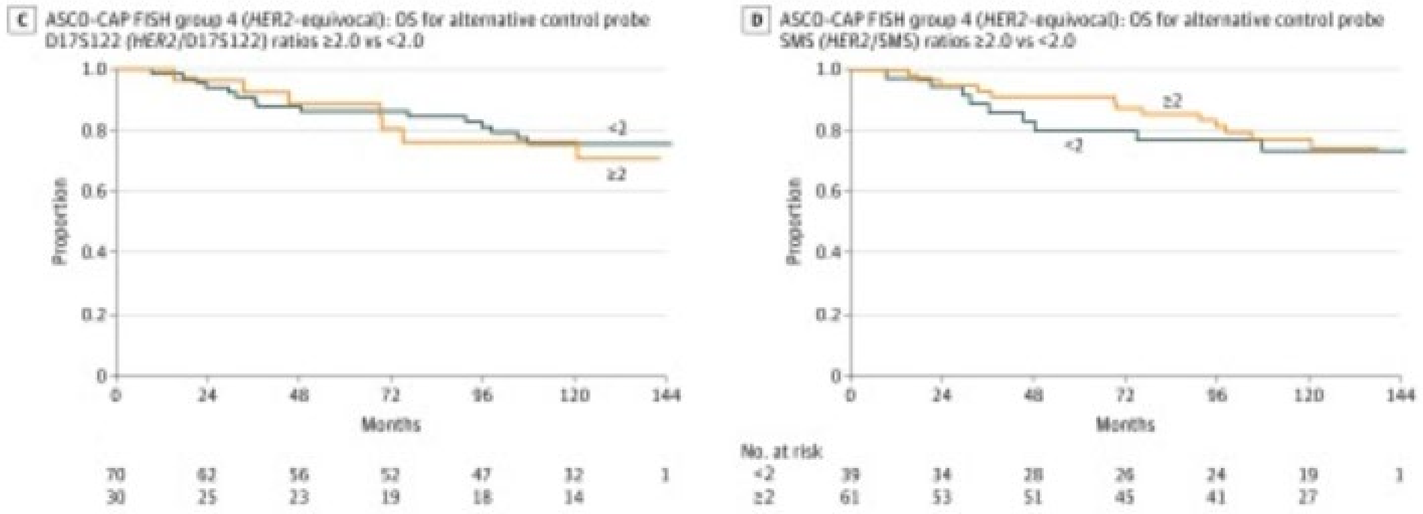


Table 1. Chromosome 17 Regional Gene Copy Gains and Losses Based on GISTIC Among Alternative Control Genomic Sites Compared With *ERBB2/HER2* Gene Copy Gains and Losses in the METABRIC Cohort Including 1915 Participants[a]

	ERBB2/HER2 Gene Copy No. Status, No. (%)				
Alternative Control (region)	*HER2* Loss	*HER2* Normal	*HER2* Gain	*HER2* Amp	Total
LIS1					
Gain	9 (2.5)	25 (2.7)	61 (18.7)	13 (4.5)	108 (5.6)
Normal	46 (12.7)	622 (66.5)	64 (19.6)	87 (29.9)	819 (42.8)
Loss	308 (84.8)	288 (30.8)	201 (61.7)	191 (65.6)	988 (51.6)
Total	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)
TP53					
Gain	3 (0.8)	15 (1.6)	60 (18.4)	4 (1.4)	82 (4.3)
Normal	41 (11.3)	624 (66.7)	59 (18.1)	81 (27.8)	805 (42.0)
Loss	319 (87.9)	296 (31.7)	207 (63.5)	206 (70.8)	1028 (53.7)
Total	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)
D17S122 (*TEKT3*)					
Gain	10 (2.7)	11 (1.2)	55 (16.9)	8 (2.7)	84 (4.4)
Normal	50 (13.8)	643 (68.8)	67 (20.5)	84 (28.9)	844 (44.1)
Loss	303 (83.5)	281 (30.0)	204 (62.6)	199 (68.4)	987 (51.5)
Total	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)
RAI1					
Gain	7 (1.9)	39 (4.2)	78 (23.9)	35 (12.0)	159 (8.3)
Normal	57 (15.7)	640 (68.4)	64 (19.9)	89 (30.6)	851 (44.4)
Loss	299 (82.4)	256 (27.4)	183 (56.1)	167 (57.4)	905 (47.3)
Total	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)
SMS (*TOP3A*)					
Gain	12 (3.3)	42 (4.5)	79 (24.2)	41 (14.1)	174 (9.1)
Normal	54 (14.9)	647 (69.2)	71 (21.8)	94 (32.3)	866 (45.2)
Loss	297 (81.8)	246 (26.3)	176 (54.0)	156 (53.6)	875 (45.7)
Total:	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)
TOP2A/RARA (*TOP2A*)					
Gain	2 (0.5)	19 (2.0)	284 (87.1)	117 (40.2)	422 (22.0)
Normal	22 (6.1)	899 (96.1)	36 (11.0)	77 (26.5)	1034 (54.0)
Loss	339 (93.4)	17 (1.8)	6 (1.8)	97 (33.3)	459 (24.0)
Total	363 (100)	935 (100)	326 (100)	291 (100)	1915 (100)

Abbreviations: D17S122, the 17p-arm genomic locus which is duplicated in Charcot-Marie-Tooth disease; GISTIC, genomic identification of significant targets in cancer; *HER2*, human epidermal growth factor receptor 2 gene; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; *RARA*, retinoic acid receptor-alpha gene; SMS, Smith-Magenis Syndrome locus; *TOP2A*, topoisomerase-II-alpha gene.

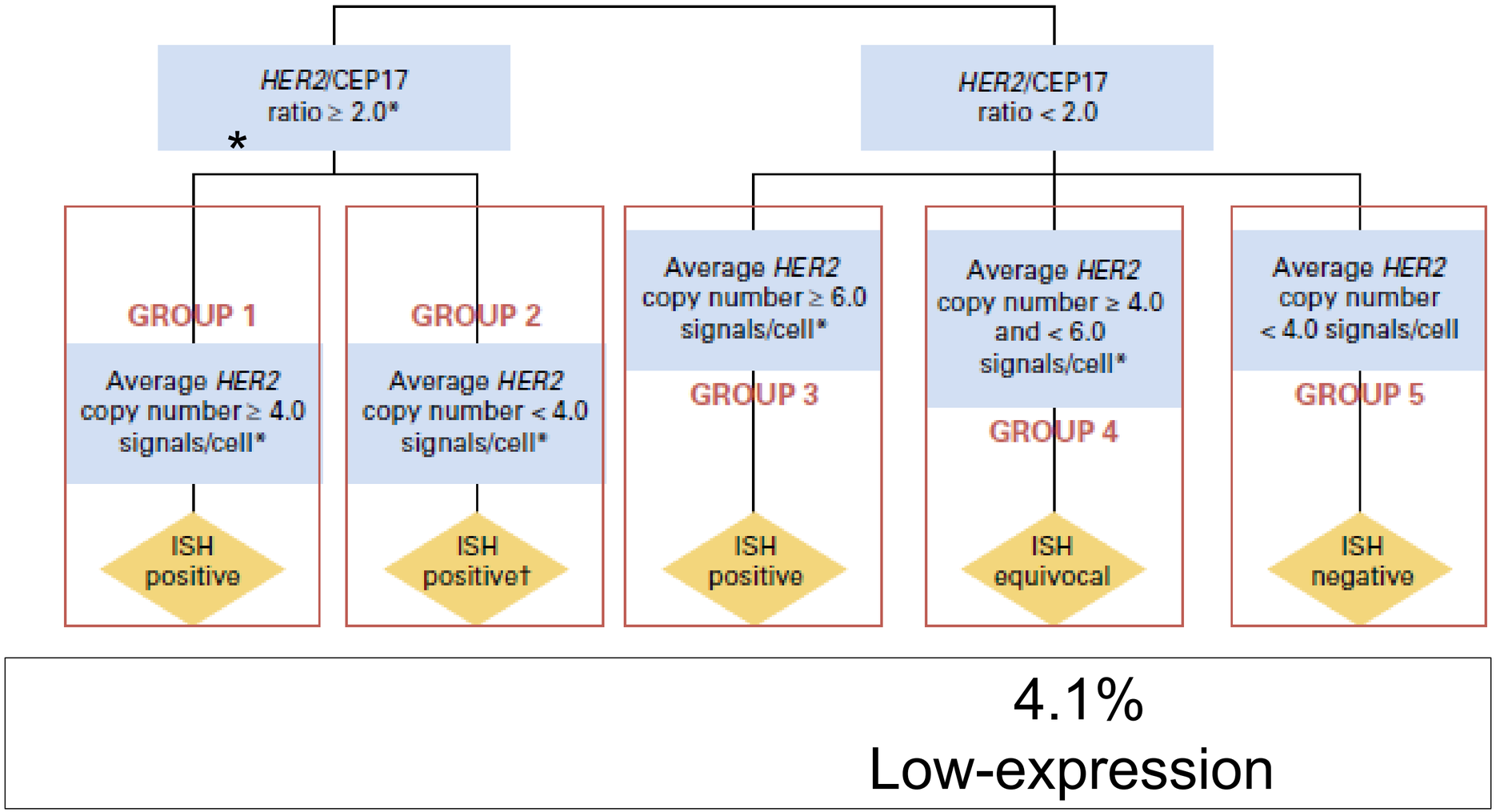
[a] Genomic Identification of Significant Targets in Cancer, a tool to identify genes targeted by somatic copy-number alterations; note that a subset of cases was not evaluable by GISTIC, thus 1915 of 1980 cases are reported here.

- **1915 METABRIC cases and 100 BCIRG 005 cases**
- **Chromosome 17 p-arm genomic sites had a high rate of heterozygous deletions (26%-32%)**
- **ISH positive using alternative control probes: No significantly worse prognosis vs either the overall ISH-equivocal or ISH-negative patients or *HER2*-to-alternative control probe ratio of less than 2.0**
- **The indiscriminate use of alternative controls: may lead to false-positive determinations and should be avoided**

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Press MF, Seoane JA, Curtis C, et al. Assessment of ERBB2/HER2 Status in HER2-Equivocal Breast Cancers by FISH and 2013/2014 ASCO-CAP Guidelines. *JAMA Oncol*. 2019;5(3):366-375. doi:10.1001/jamaoncol.2018.6012



Not enrolled, outcomes with chemo did not differ significantly from group 5

Press MF, Sauter G, Buyse M, et al. HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials. *J Clin Oncol*. 2016;34(29):3518-3528. doi:10.1200/JCO.2016.66.6693

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Revised 2018 Recommendation: If a Case has an Average HER2 Copy Number ≥4.0 but <6.0 Signals/Cell and a HER2/CEP17 Ratio <2.0

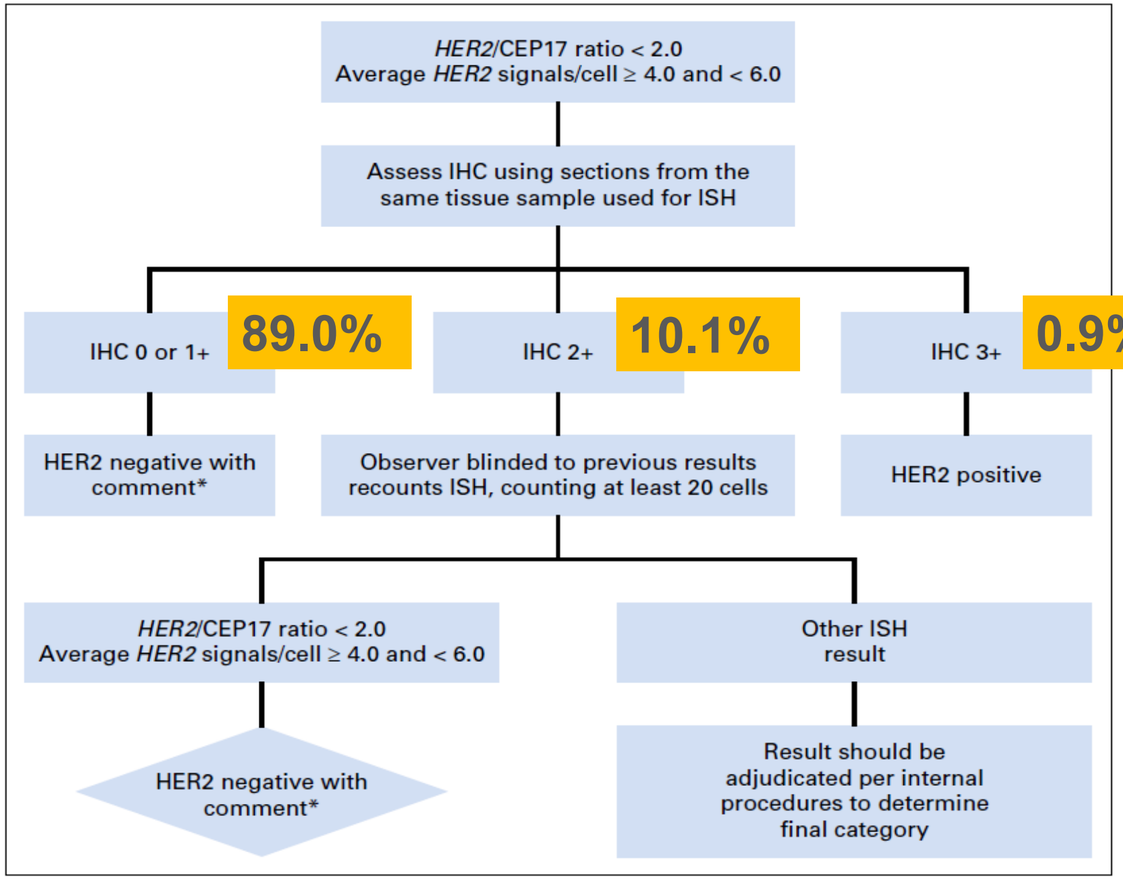


Fig 6. Clinical Question 5, group 4. (*) It is uncertain whether patients with an average of ≥ 4.0 and < 6.0 *HER2* signals per cell and a *HER2*/CEP17 ratio of < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH ratio threshold for positive, there is a higher likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen. CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

- ❑ No equivocal case
- ❑ Alternative probes are not recommended

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Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738

Index Case Final HER2 Status (FISH Group 4 and IHC 2+)

Breast, right, core needle biopsy

Integrated final HER2 status based on IHC and FISH results:

- **Interpretation - Negative (please see comment)**

Comment: The integrated final HER2 results for this case is based on a consideration of both the IHC and FISH results per the 2018 ASCO/CAP HER2 focused update. Immunohistochemistry (IHC) was equivocal (2+) and fluorescence in situ hybridization (FISH) for HER2 was performed on the same tissue block which identified the following signal pattern:

HER2/CEP 17 ratio < 2.0 with an average HER2 signal/tumor cell of >= 4.0 and < 6.0

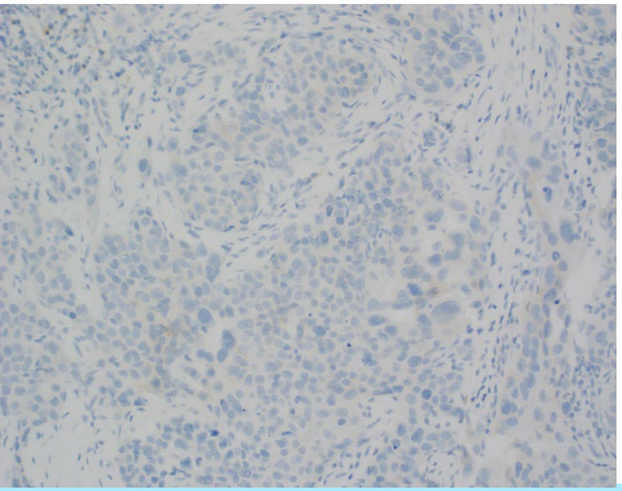
It is uncertain whether patients with > =4.0 and < 6.0 average HER2 signals/cell and HER2/CEP17 ratio < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the threshold for positive, there is a higher likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen. In this scenario, clinical correlation with other patient factors, pathologic features of the patient's cancer (such as ER expression and tumor grade) and test results from other specimens may be used when selecting appropriate treatments, including chemotherapy and HER2-targeted therapies.

Patient did not receive HER2-targeted therapy

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HER2 testing by validated IHC assay-2018 Guideline

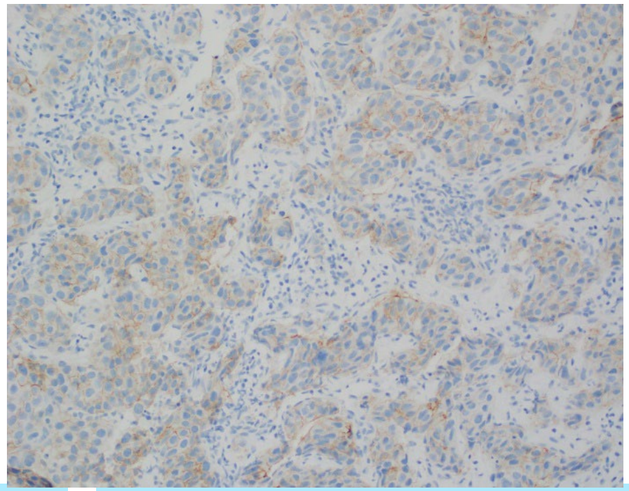
Batch controls and on-site controls show appropriate staining



No staining or incomplete, faint/barely perceptible membrane staining in ≤10% of tumor cells

IHC 0

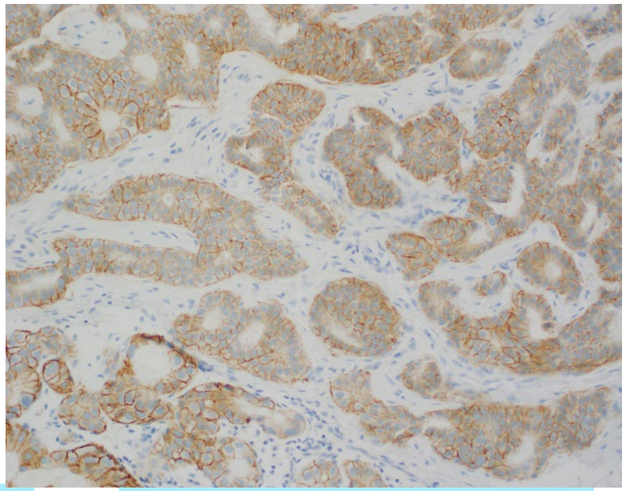
HER2 Negative



Incomplete, faint/barely perceptible membrane staining in >10% of tumor cells

IHC 1+

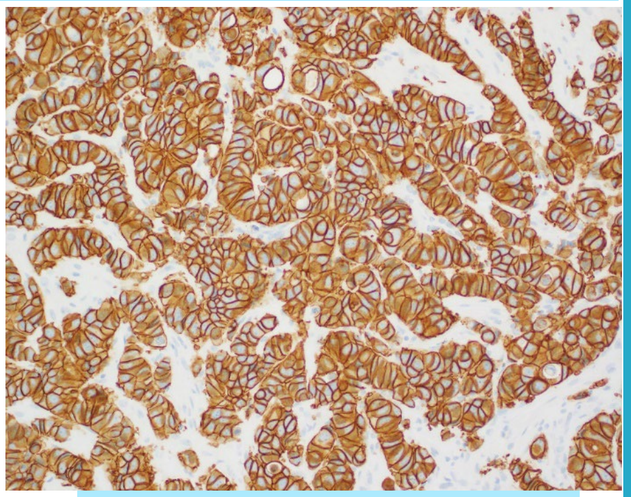
HER2 Negative



Weak to moderate complete membrane staining in >10% of tumor cells

IHC 2+

Reflex ISH testing



Circumferential, complete, intense membrane staining in >10% of tumor cells

IHC 3+

HER2 Positive

Group 1
Positive
HER2/CEP17 ratio of ≥2.0, with an average HER2 copy number ≥4.0 signals/cell

Group 2
Negative
HER2/CEP17 ratio of ≥2.0, with an average HER2 copy number <4.0 signals/cell

Group 3
Positive
HER2/CEP17 ratio of <2.0, with an average HER2 copy number ≥6.0 signals/cell

Group 4
Negative
HER2/CEP17 ratio of <2.0, with an average HER2 copy number ≥4.0 and < 6 signals/cell

Group 5
Negative
HER2/CEP17 ratio of <2.0, with an average HER2 copy number <4.0 signals/cell

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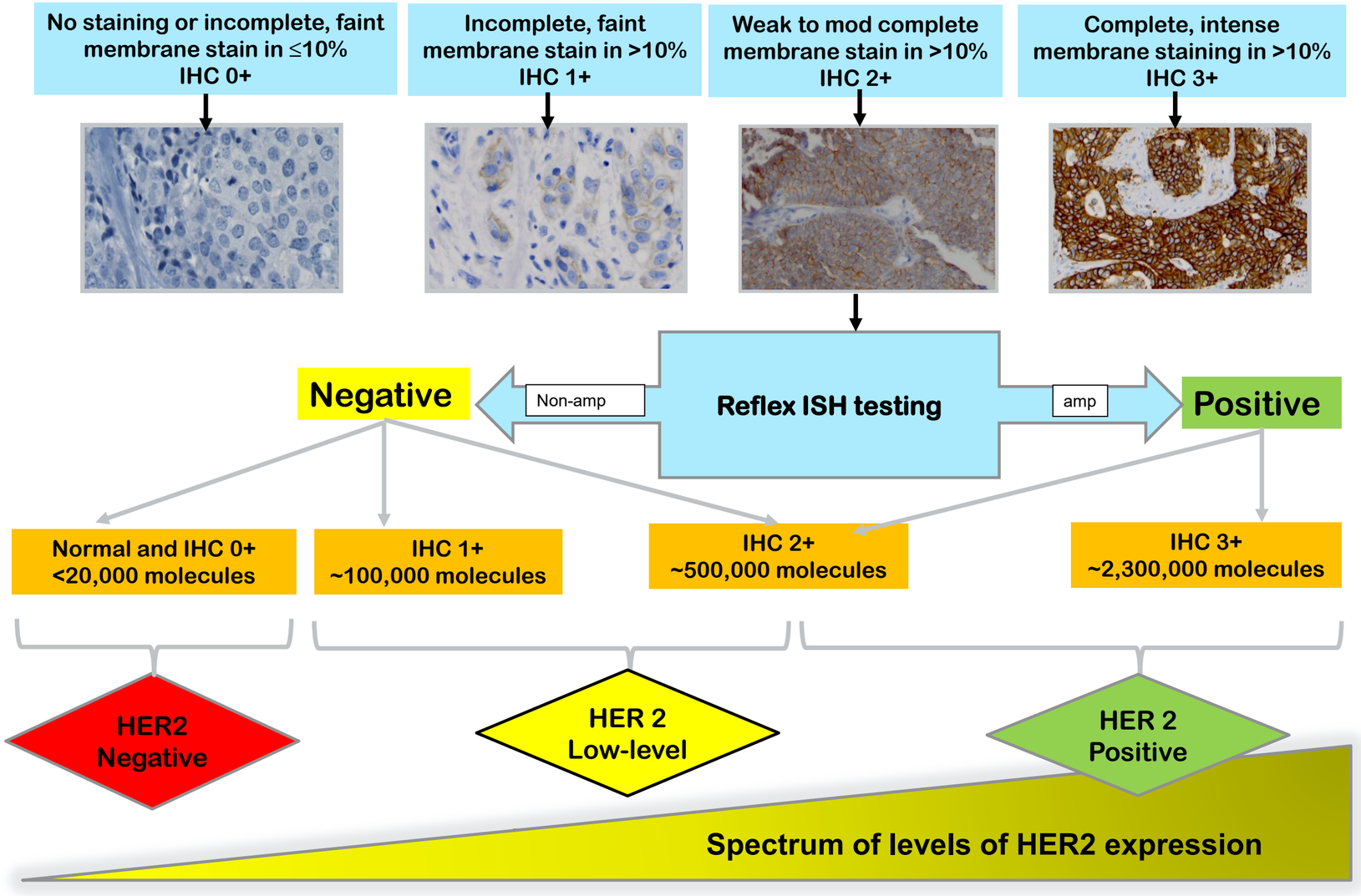
Zhang H, Moisini I, Ajabnoor RM, Turner BM, Hicks DG. Applying the New Guidelines of HER2 Testing in Breast Cancer. *Curr Oncol Rep*. 2020;22(5):51. Published 2020 Apr 29. doi:10.1007/s11912-020-0901-4

Recent Updates on HER2 Testing

2023 ASCO/CAP: HER2-Low Breast Cancer

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HER2 Levels as an Expression Spectrum: Paradigm Shift in Breast Cancer

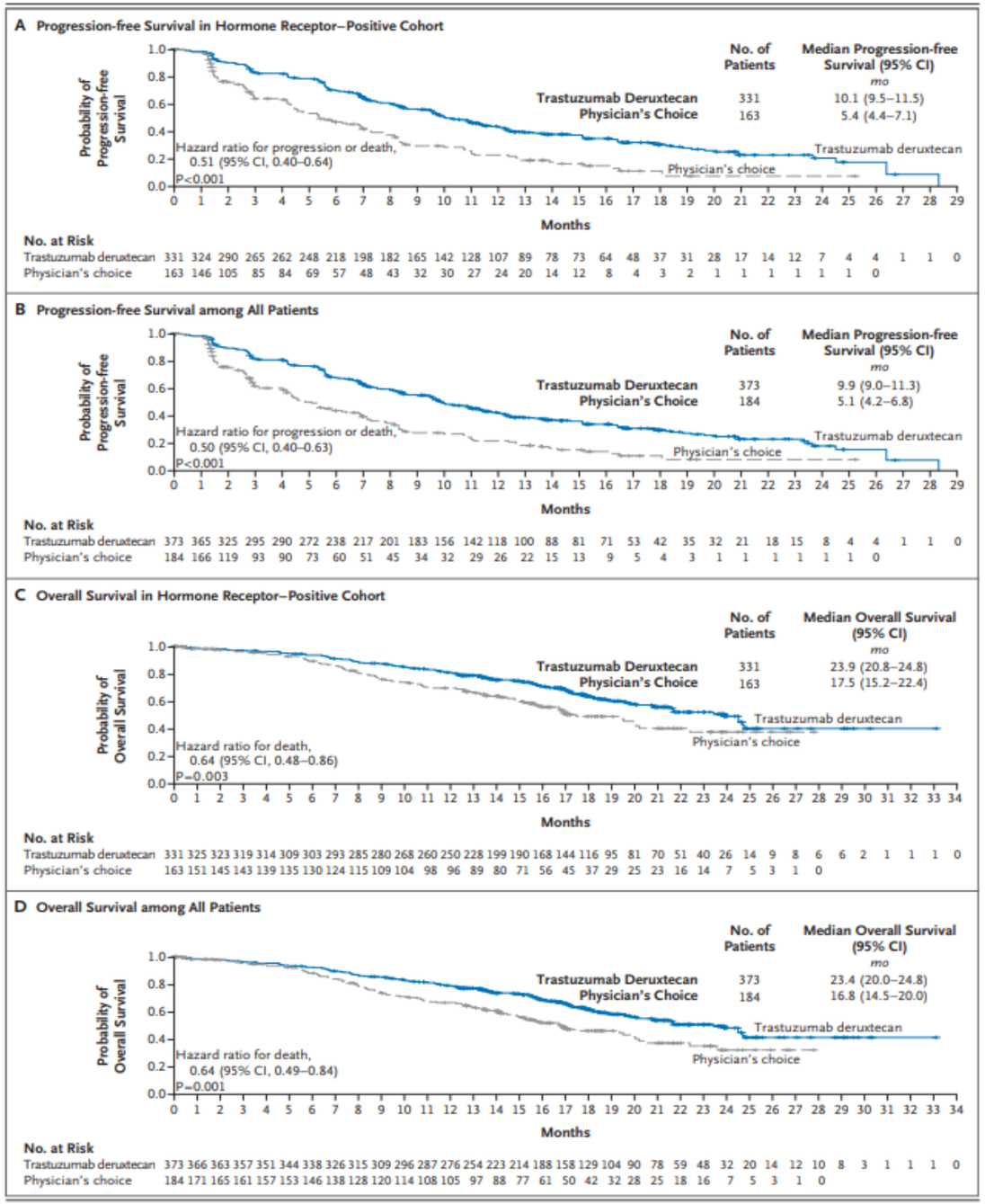


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Courtesy of David Hicks MD

Trastuzumab Deruxtecan (T-Dxd) in Previously Treated HER2-Low Advanced Breast Cancer



- 557 pt enrolled: T-Dxd: 373; TPC: 184 (2:1)
  - 494 HR+, 88.7%; 63 HR- 11.3%
- T-DXd significantly improved PFS and OS
  - ORR: 52.3% vs 16.3% (overall); 52.6% vs 16.3% (HR+)
- Similar benefit across all subgroups, including HER2 IHC status
- Supports the use of T-DXd as a treatment option for HER2-low (IHC 1+, IHC 2+/ISH-) mBC

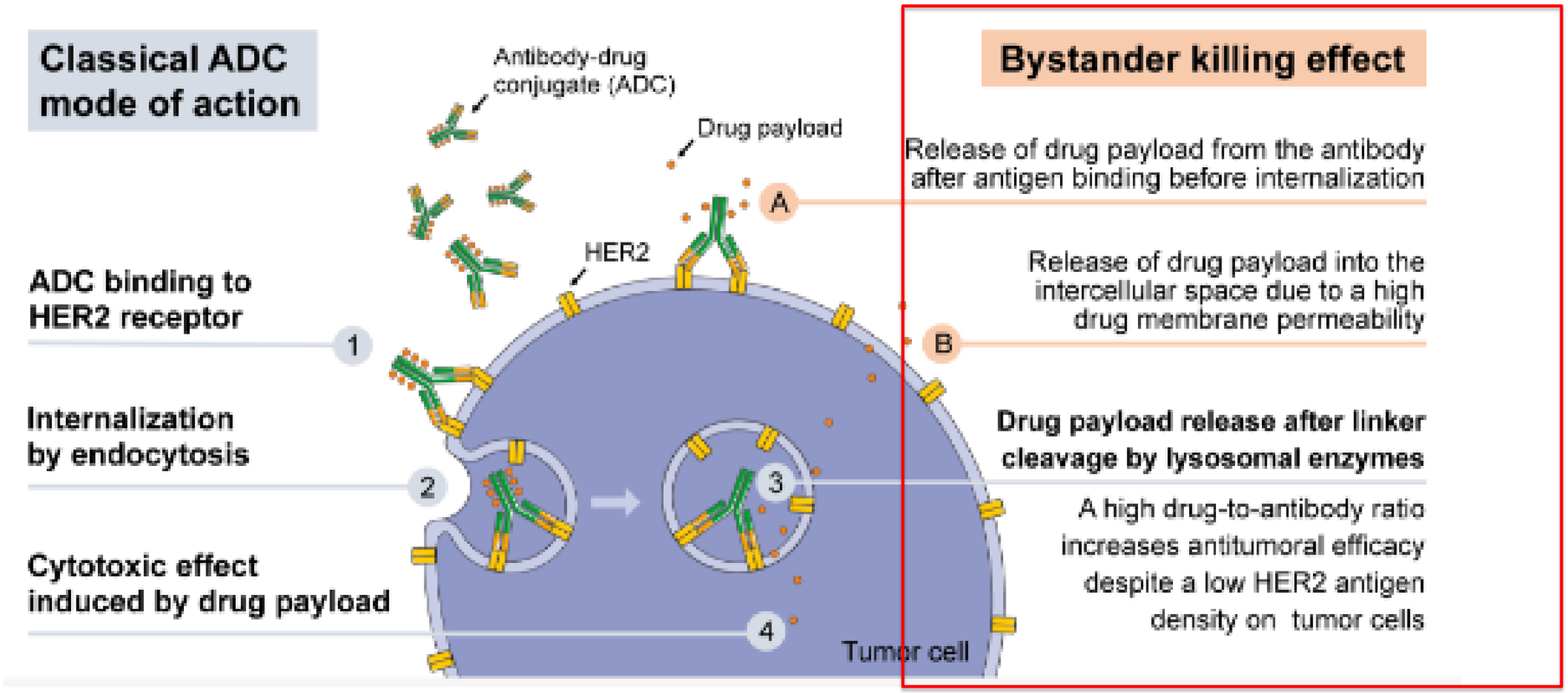
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Modi S, Jacot W, Yamashita T, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. *N Engl J Med*. 2022;387(1):9-20. doi:10.1056/NEJMoa2203690

Proposed Mechanism for T-DXd in HER2-Low Breast Cancer

T-Dxd: Bystander killing effect
- Cleavable linker
- High DAR ratio (8:1)
- Membrane-permeable payload



FDA approves first HER2-targeted therapy (T-Dxd) for unresectable or metastatic HER2-low breast cancer on 08/05/2022

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Rinnerthaler G, Gampenrieder SP, Greil R. HER2 Directed Antibody-Drug-Conjugates beyond T-DM1 in Breast Cancer. *Int J Mol Sci.* 2019;20(5):1115. Published 2019 Mar 5. doi:10.3390/ijms20051115

~50% of Breast Cancer Cases Could be Considered HER2-Low

- Enriched in HR+ BCs, ductal phenotype
- Heterogeneous group
  - Luminal A: 50.8%-65.5%
  - Luminal B: 22.8%-28.8%
  - HER2:1.1%-3.6%
  - Basal:4.6%-17.7%
- Less responsive to NAC with low pCR rates (0%-29.2%)
- More mutations involved in *PI3K-Akt* signaling and reduced *TP53* mutations

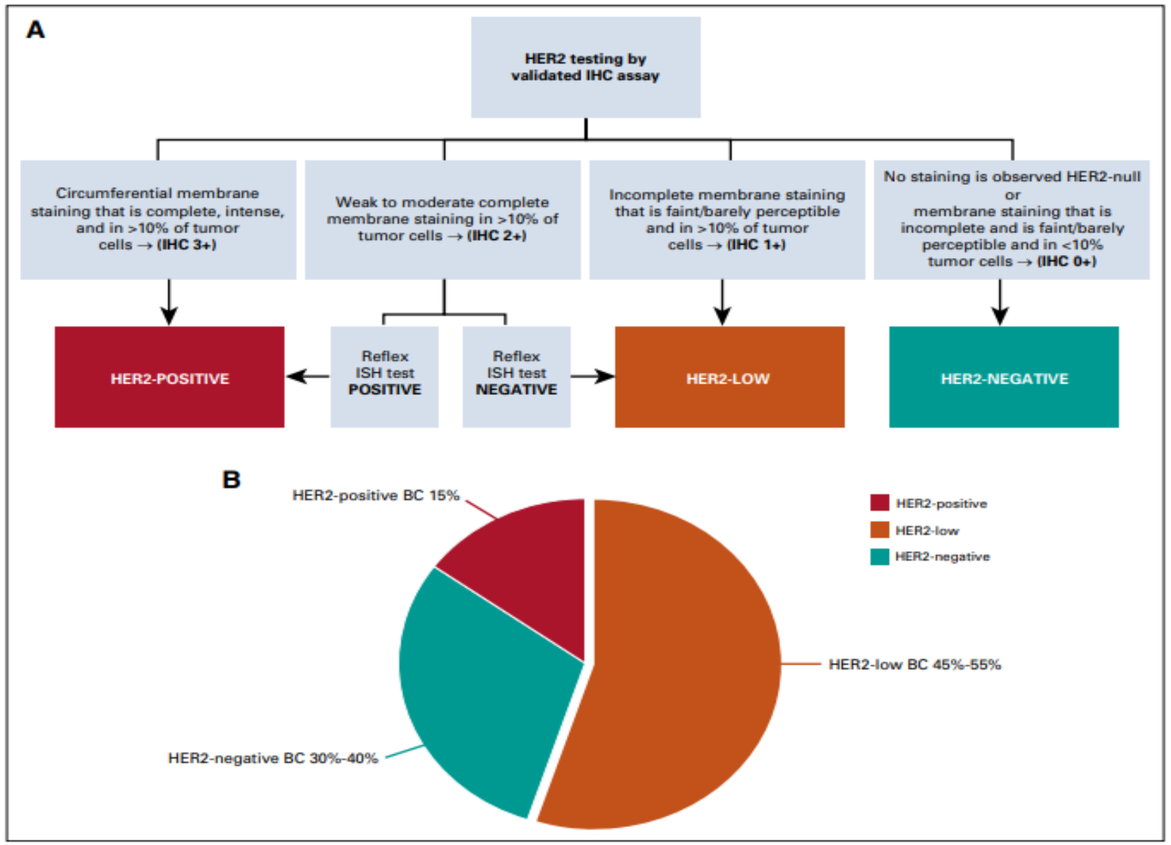


FIG 1. Proposal of an algorithm for defining HER2-low BC. (A) The majority of clinical trials testing anti-HER2 agents in HER2-low BC enroll patients affected by cancers with an HER2 IHC score of 1+ or 2+ with a negative reflex ISH assay, regardless of the molecular subtype (eg, luminal, triple negative). Accordingly, IHC and ISH currently represent the most appropriate techniques to define HER2-low BC. Nevertheless, novel quantitative testing methods (more extensively discussed in the text) might allow a better dissection of HER2 expressions, particularly for HER2-negative samples, dissecting HER2 absence (HER2 null) from faint or barely perceptible staining in < 10% of tumor cells. (B) If activity of these drugs was confirmed, the percentage of patients deriving benefit from anti-HER2 treatments could reach 60% to 70%.[9] BC, breast cancer; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Tarantino P, Hamilton E, Tolaney SM, et al. HER2-Low Breast Cancer: Pathological and Clinical Landscape. *J Clin Oncol*. 2020;38(17):1951-1962. doi:10.1200/JCO.19.02488

Zhang H, Karakas C, Tyburski H, et al. HER2-low breast cancers: Current insights and future directions. *Semin Diagn Pathol*. 2022;39(5):305-312. doi:10.1053/j.semdp.2022.07.003

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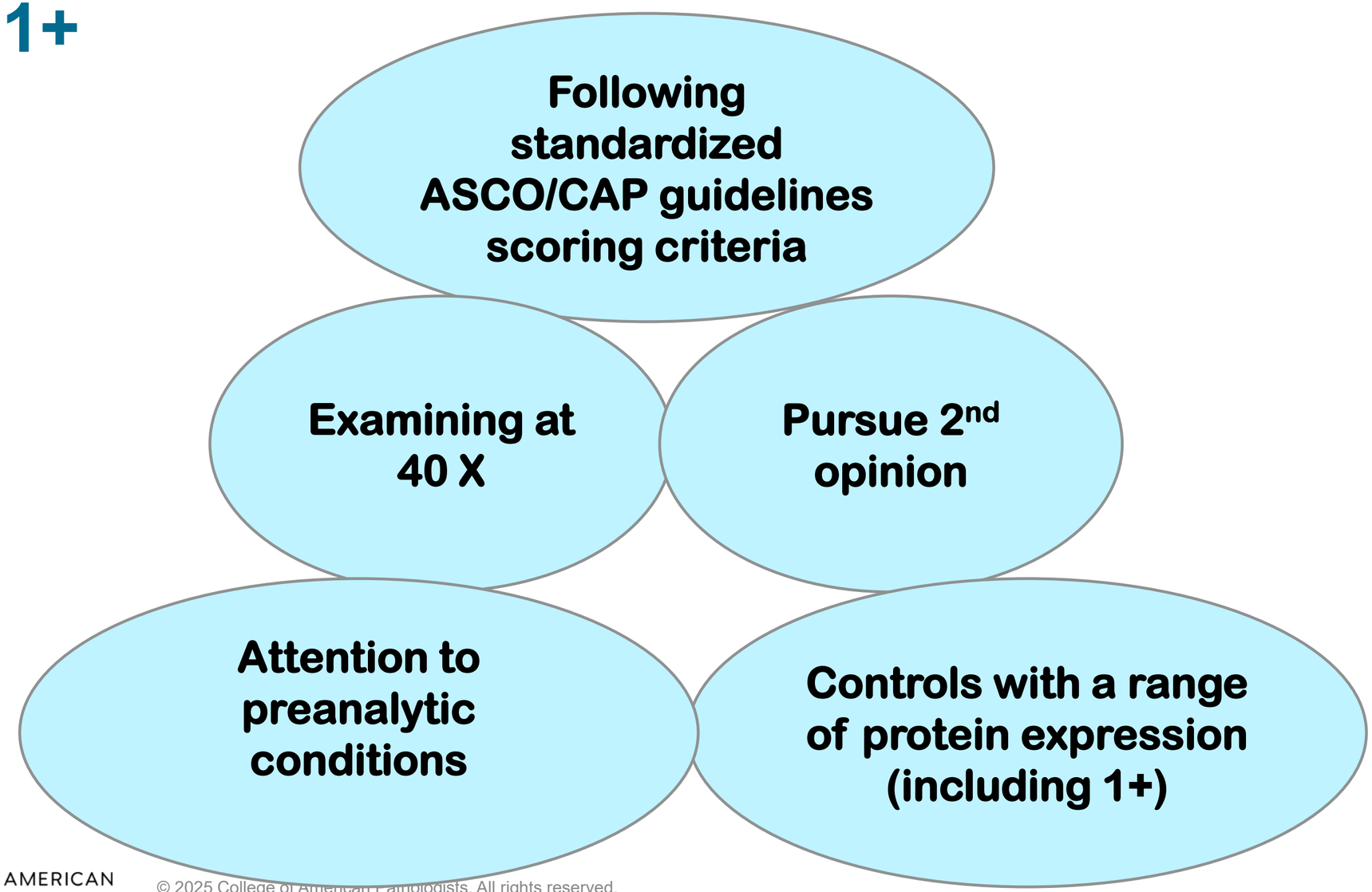
2023 ASCO/CAP HER2 Testing Guideline Update

- Affirms the 2018 ASCO/CAP recommendations
- Acknowledges a new indication for T-DXd when HER2 is IHC 1+ or 2+ without amplification by ISH
- Best practices to distinguish IHC 0 from 1+ are clinically relevant
- Offers a new HER2 testing reporting comment to highlight the current relevance of IHC 0 versus 1+ results, and best practice recommendations to distinguish these often subtle differences
- **Premature to create new categories of HER2 low-expression**

Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-College of American Pathologists Guideline Update. *J Clin Oncol*. 2023;41(22):3867-3872. doi:10.1200/JCO.22.02864.

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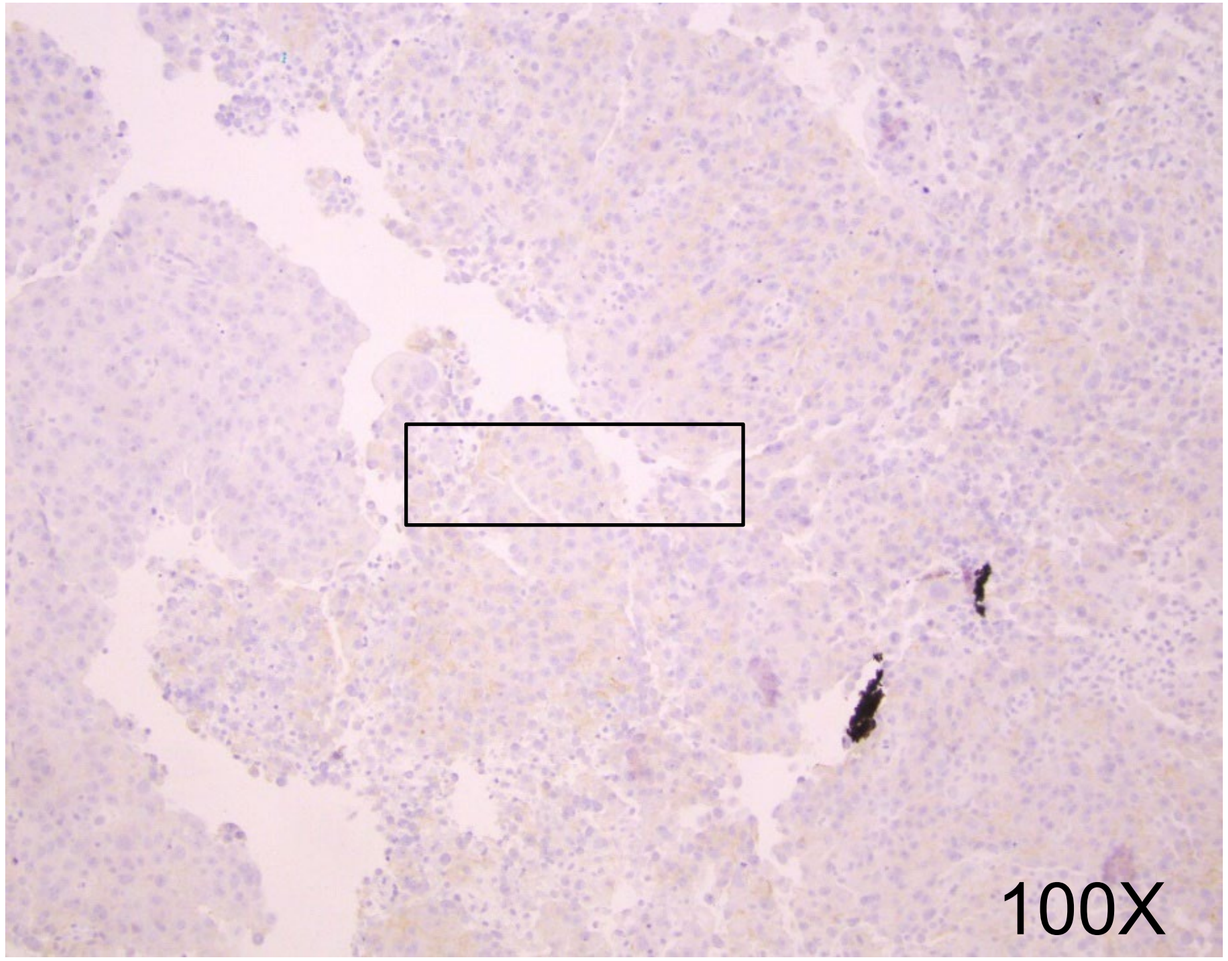
2023 ASCO/CAP HER2 Testing Guideline Update on Best Practice Recommendations to Distinguish HER2 IHC 0 Versus 1+

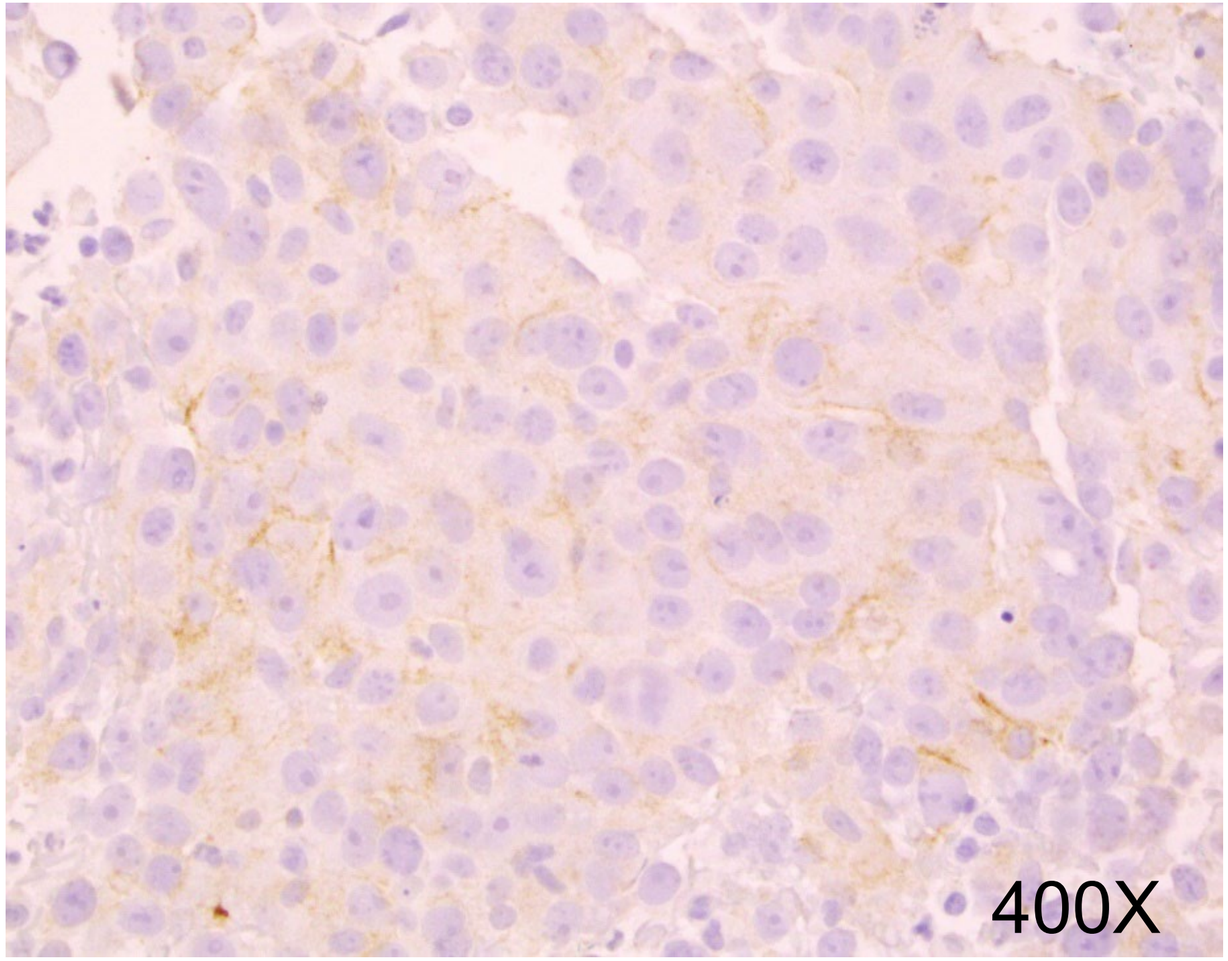


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Wolff AC, Somerfield MR, Dowsett M, et all. J Clin Oncol. 2023;41(22):3867-3872

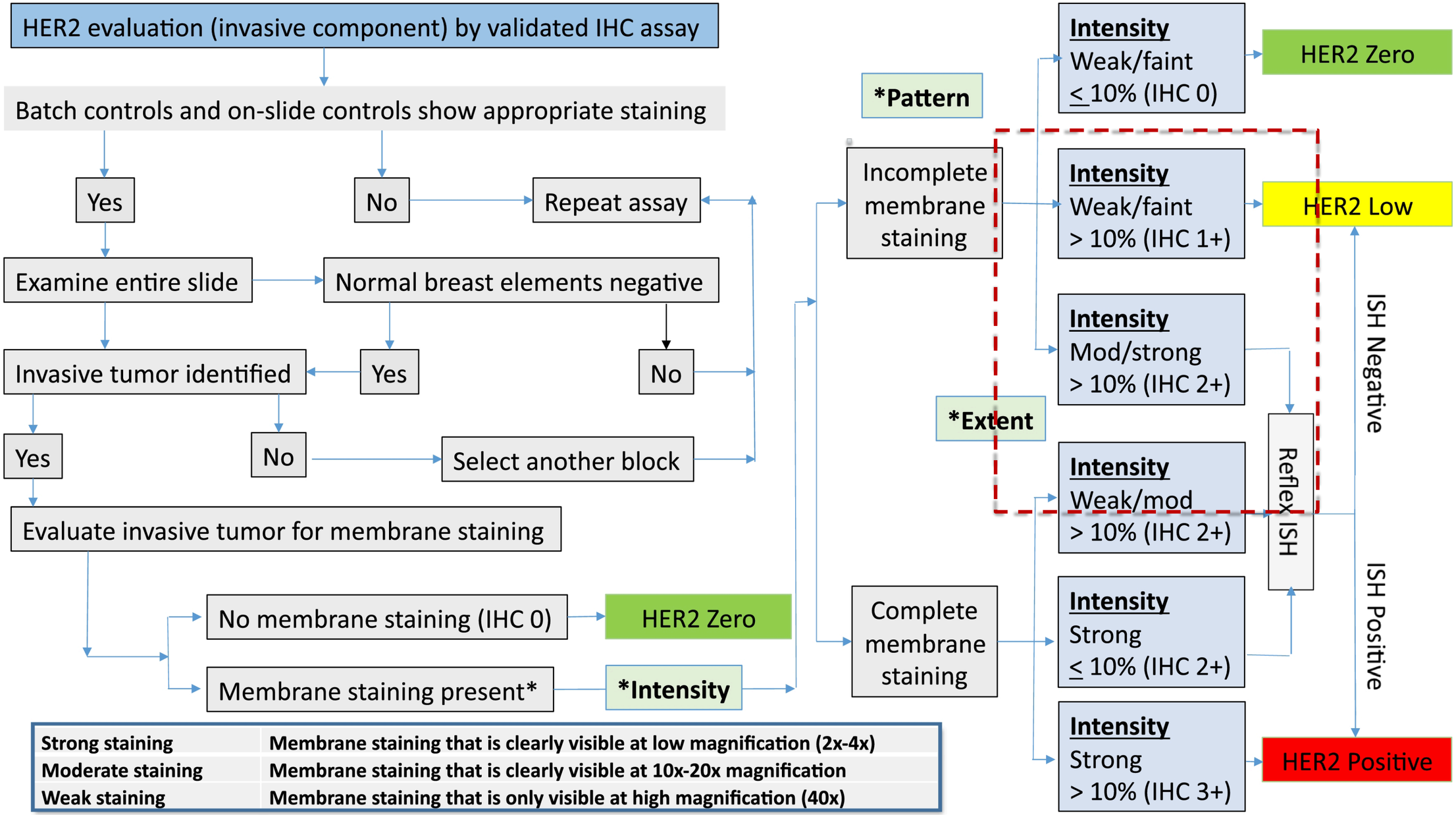




Examining HER2 IHC at high magnification (40x) when discriminating 0 from 1+ staining

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Strong staining	**Membrane staining that is clearly visible at low magnification (2x-4x)**
Moderate staining	**Membrane staining that is clearly visible at 10x-20x magnification**
Weak staining	**Membrane staining that is only visible at high magnification (40x)**

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Zhang H, Karakas C, Tyburski H, et al. HER2-low breast cancers: Current insights and future directions. *Semin Diagn Pathol*. 2022;39(5):305-312. doi:10.1053/j.semdp.2022.07.003

Challenges and Unanswered Questions on HER2-Low Breast Cancer-Interobserver Reproducibility

Cohort 1:
Reviewed 2 years of CAP HER2 IHC proficiency test results from ~1400 labs

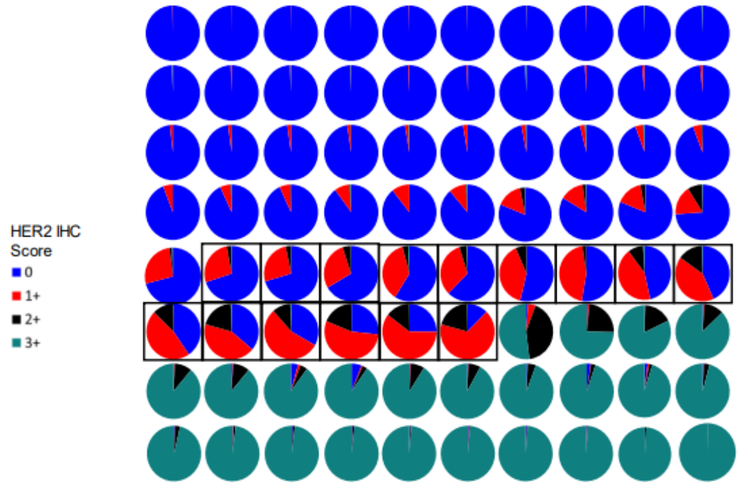
- Analyzed the frequency and distribution of each score in 80 cases

- 52/80 (65%) had ≥90% concordance, limited to 0 or 3+ scores
- 56/80 were considered 0 or 1+
  - **25% of these cores had <70% inter-observer concordance**

Cohort 2:
18 pathologists, 170 cases

- Acceptable value: 17/18 raters agreement (>90%)
  - **17 of 18 raters agreed on 24/92 cases (26%): 0 /1+**
  - 17 of 18 raters agreed on 26/45 cases (58%): 2/3+

eFigure. A, Distribution of HER2 IHC scores in 80 cores in CAP surveys.
Percentage of raters who gave HER2 IHC scores of 0, 1+,2+ or 3+ per core. Black boxes indicate 15/80 cores with < 70% concordance.



Fernandez AI, Liu M, Bellizzi A, et al. Examination of Low ERBB2 Protein Expression in Breast Cancer Tissue. *JAMA Oncol*. 2022;8(4):1-4. doi:10.1001/jamaoncol.2021.7239

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Challenges and Unanswered Questions on HER2-Low Breast Cancer-Interantibody reproducibility

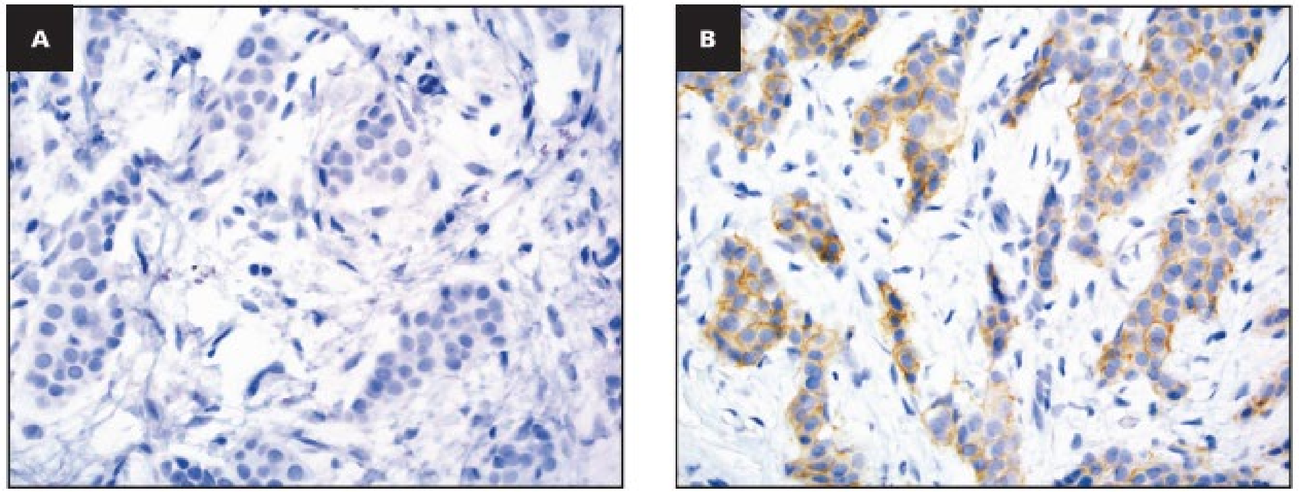


FIGURE 4 Example of a breast cancer case with HER2 HercepTest (**A**, ×400) and PATHWAY anti-HER2 (4B5) (Ventana) (**B**, ×400) stains. It was scored as 0 by 6 observers on HercepTest and as 1+ by 6 observers on 4B5.

TABLE 2 Antibody Agreement (HercepTest vs 4B5) by 6 Observers

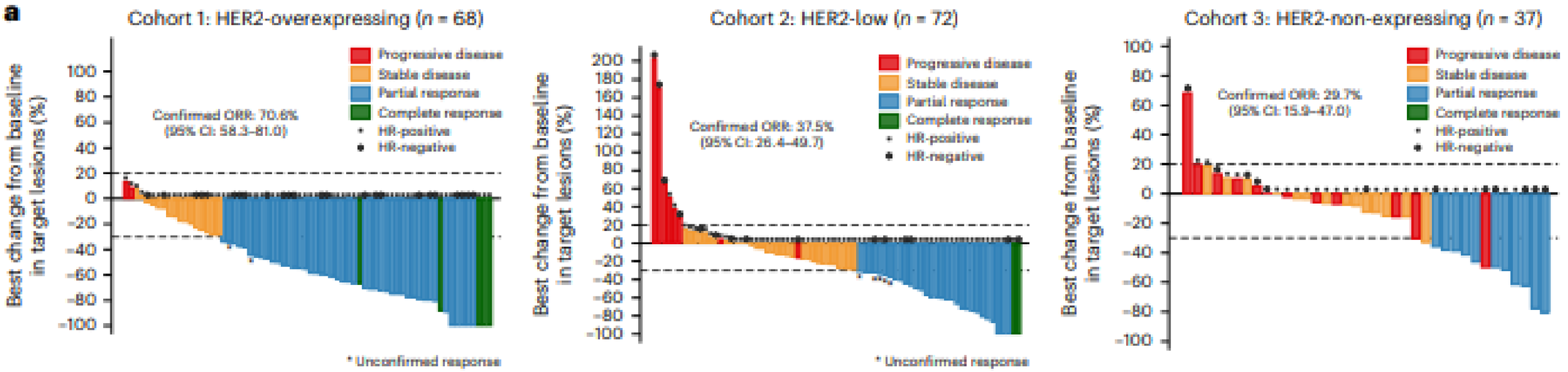
Observer	Observed Agreement, No. (%)	κ	Weighted κ	95% CI
1	68.4	0.54	0.66	0.415-0.668
2	54.4	0.35	0.54	0.222-0.480
3	54.4	0.36	0.56	0.227-0.492
4	58.8	0.44	0.60	0.317-0.566
5	57.0	0.40	0.55	0.266-0.527
6	53.5	0.36	0.55	0.232-0.494
Average	57.8	0.41	0.58	

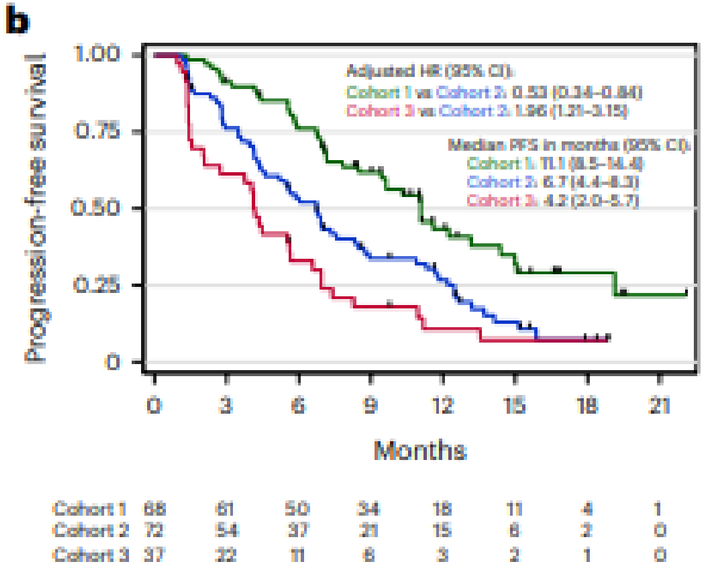
CI, confidence interval.

Karakas C, Tyburski H, Turner BM, et al. Interobserver and Interantibody Reproducibility of HER2 Immunohistochemical Scoring in an Enriched HER2-Low-Expressing Breast Cancer Cohort. *Am J Clin Pathol*. 2023;159(5):484-491. doi:10.1093/ajcp/aqac184

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Challenges and Unanswered Questions on HER2-Low Breast Cancer-The definition is evolving (DAISY Trial)





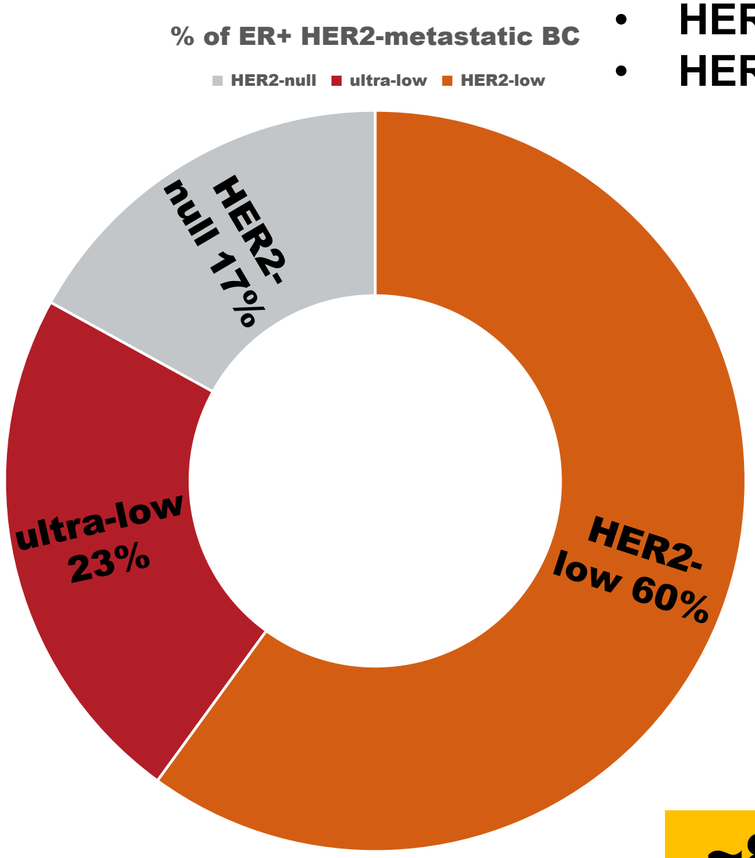
	ORR	Median duration of response	Median PFS
Cohort 1	70.6%	9.7 months	11.1 months
Cohort 2	37.5%	7.6 months	6.7 months
Cohort 3	29.7%	6.8 months	4.2 months

Mosele F, Deluche E, Lusque A, et al. Trastuzumab deruxtecan in metastatic breast cancer with variable HER2 expression: the phase 2 DAISY trial. *Nat Med*. 2023;29(8):2110-2120. doi:10.1038/s41591-023-02478-2

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Challenges and Unanswered Questions on HER2-Low Breast Cancer-The definition is evolving (DESTINY BREAST-06 trial)



- HER2 null: HER2 IHC 0+ without any staining
- HER2-ultra-low: HER2 IHC 0+ with any staining
- HER2-low: HER2 IHC 1+ and 2+/ISH –

- 866 pts (HER2-low, n=713; HER2-ultralow, n=153) were randomized
- T-DXd showed a statistically significant and clinically meaningful PFS benefit vs TPC in ER+/HER2-low mBC after ≥1 endocrine-based therapy (HR, 0.62 [95% CI 0.51, 0.74], P<0.0001; median, 13.2 vs 8.1 mo).
- HER2-ultralow results were consistent with HER2-low.

~85% of ER+, HER2- mBC may benefit from T-DXd

Curigliano G et al. Trastuzumab deruxtecan (T-DXd) vs physician's choice of chemotherapy (TPC) in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-low or HER2-ultralow metastatic breast cancer (mBC) with prior endocrine therapy (ET): Primary results from DESTINY-Breast06 (DB-06).Journal of Clinical Oncology Volume 42, Number 17_suppl (2024 ASCO meeting abstract)

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HER2-Low Breast Cancer

- Targetable by new generation of HER2-targeting ADCs
- Currently defined as breast cancers with HER2 IHC score of 1+, or 2+ with negative ISH results, and the definition is continue to evolve
- Represents a heterogeneous group of breast cancer and are enriched in HR+ tumors; Whether it represents a distinct biologic/clinical entity is controversial
- **Premature to create new result categories of HER2 low-expression**
- More accurate and reliable methods are needed to better define the most appropriate patient population for treatment
- Adhere to 2023 ASCO/CAP guideline recommendations on HER2 IHC scoring

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Issues not specified in the current guidelines

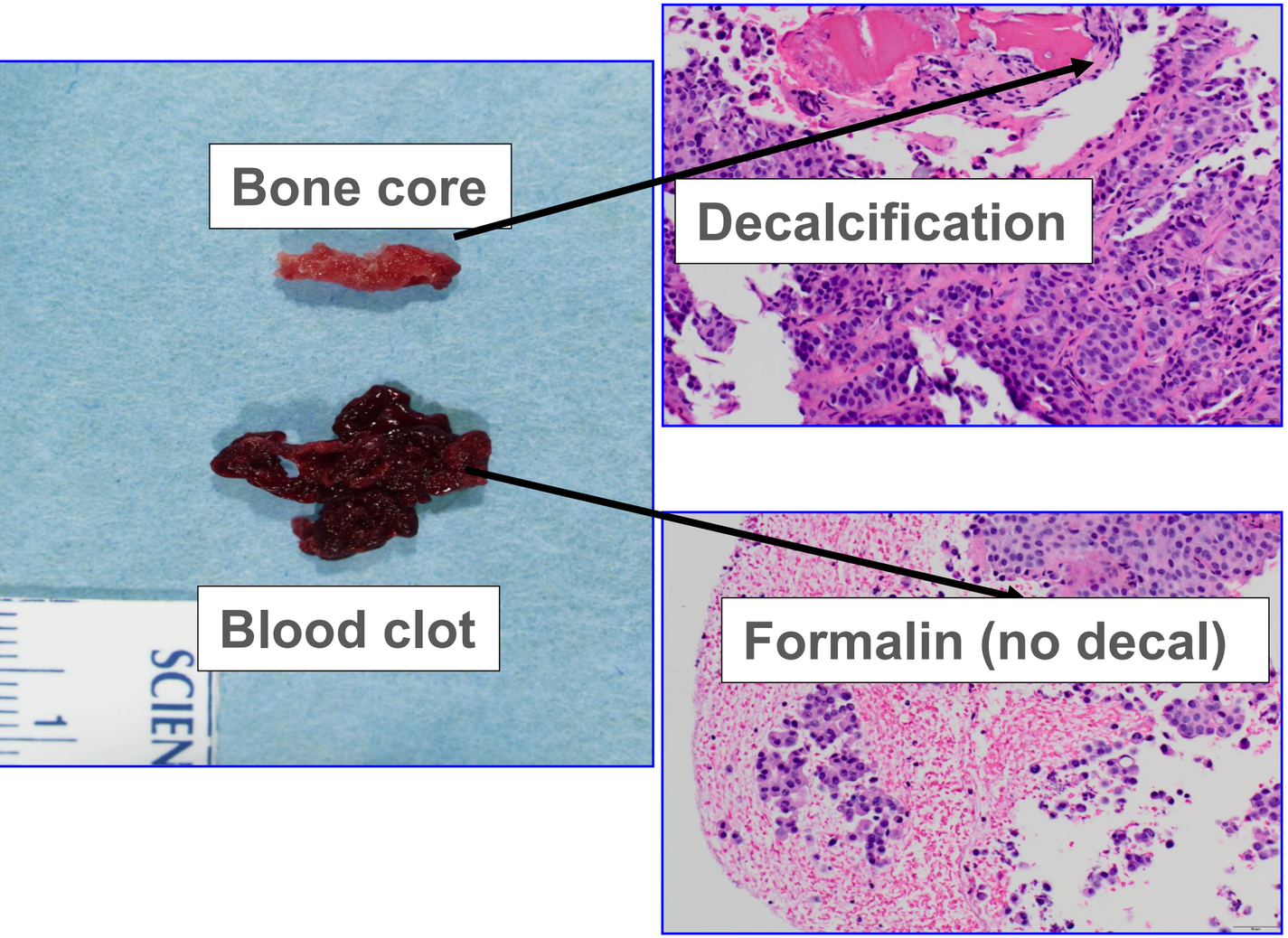
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ER/PgR/HER2 Testing in Bone Specimen

- Bone is the most common site of breast cancer involvement
- Decalcification affects antigenicity of the tumor cells and DNA quality
- No specific recommendation
  - Decalcification artifacts should be rejected
  - Decalcified in a strong acid solution may be rejected
- Studies suggested EDTA decalcification is the preferred method

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Core Biopsy of Bone for Suspected Breast Cancer



Strategies for cases requiring decalcification

- Separating the blood clot from fragments of bone and processing separately
- Use of molecular friendly decalcifying agents (EDTA)
- ISH is more sensitive than IHC, low threshold for FISH testing

- *Comment:* The bone biopsy contained a core tissue block that underwent decalcification and a blood clot that contained clumps of tumor cells that was not decalcified. The biomarker analyses (ER, PR, and HER2) were performed on the blood clot section because this would be a more suitable sample for testing.

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Repeating ER/PgR/HER2 After Neoadjuvant Chemotherapy

- **Biomarker status conversion in about 15-25% of cases (HER2 using binary scoring system)**
  - Loss of expression: Unclear whether alter the treatment; poor prognosis in some studies
  - Gain of expression: Favorable or targetable treatment
- **No unanimous consensus or uniform practice**
- **Routine reassessment is not recommended**
  - Positive ER/PgR/HER2 on pretreatment biopsy
- **Reassessment must be performed**
  - Part of clinical trial protocol
  - Unknown biomarker status
- **Reassessment should be considered**
  - Results were negative/equivocal prior to treatment
  - No treatment response
  - Heterogeneous tumor or multiple residual tumors with different morphology

Provenzano E, Bossuyt V, Viale G, et al. Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group. *Mod Pathol*. 2015;28(9):1185-1201. doi:10.1038/modpathol.2015.74

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Part III

Approaches to ER/PgR and HER2 Interpretation in Breast Cancer

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- **Adhere to ASCO/CAP guidelines**
- **Correlate the ER/PgR/HER2 results with tumor morphology and clinical information**



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Clinicopathologic Characteristics of Common Molecular Subtypes of Breast Cancer

Molecular subtypes	Luminal A	Luminal B	HER2 enriched	Basal-like
% of breast cancer	~30%-40%	~20%-30%	10%-15%	15%-20%
Age	Older age	Younger	Younger	Younger
Histologic grade	1-2	2-3	2-3	Typically 3
Common histologic types	IC-NST(well-differentiated), classic lobular, tubular, cribriform, mucinous, neuroendocrine	IC-NST, micropapillary	IC-NST, apocrine, pleomorphic lobular	IC-NST, medullary features, metaplastic, adenoid cystic, secretory
ER status by IHC	Positive (high)	Positive (maybe low)	Negative	Mostly negative
PgR status by IHC	Usually positive	Negative or low positive	Negative	Mostly negative
HER2 by IHC or FISH	Negative	Positive in ~40% (luminal HER2)	Positive (Classic HER2)	Negative
Ki-67	Low (<10%)	Typically high (>14%)	Typically high (>20%)	Typically very high (>50%)
Natural history	Indolent, possible late recurrence	More aggressive than luminal A	Worse natural history; Sensitive to HER2-targeted therapy	Worse natural history except for some special types, early recurrence more likely
Local recurrence	0.8%-8%	1.5%-8.7%	4%-15% (without HER2-targeted therapy)	3%-17%
Neoadjuvant response (pCR rate)	~2%	~6%	≥ 57%	~34%

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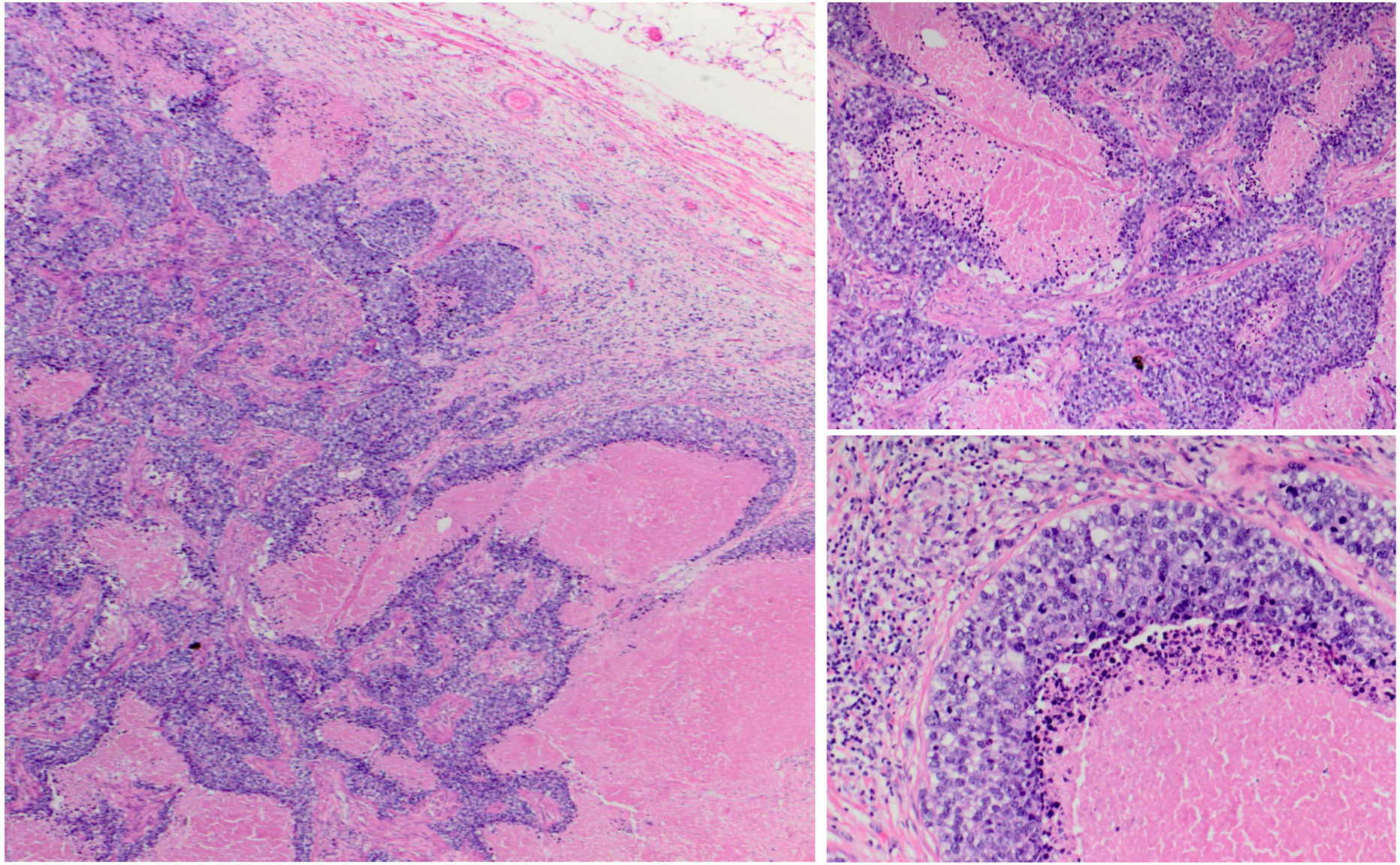
Zhang, H., Hicks, D.G. (2021). Breast and Gynecologic Tumors. In: Ding, Y., Zhang, L. (eds) Practical Oncologic Molecular Pathology. Practical Anatomic Pathology. Springer, Cham. https://doi.org/10.1007/978-3-030-73227-1_5.

Case Presentation #1

- 47-year-old woman with prior history of breast cancer 3 years ago
  - Poorly differentiated invasive ductal carcinoma
  - Nuclear grade 3
  - ER, PR, and HER2 negative
- Sentinel lymph node biopsy (x3) negative
- Treatment: Mastectomy followed by chemotherapy and XRT
- Patient now presents with new ipsilateral axillary mass
- FNA shows poorly differentiated carcinoma
- Patient underwent axillary dissection

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Case provided by David Hicks, MD

H&E Axillary Lymph Node Metastasis



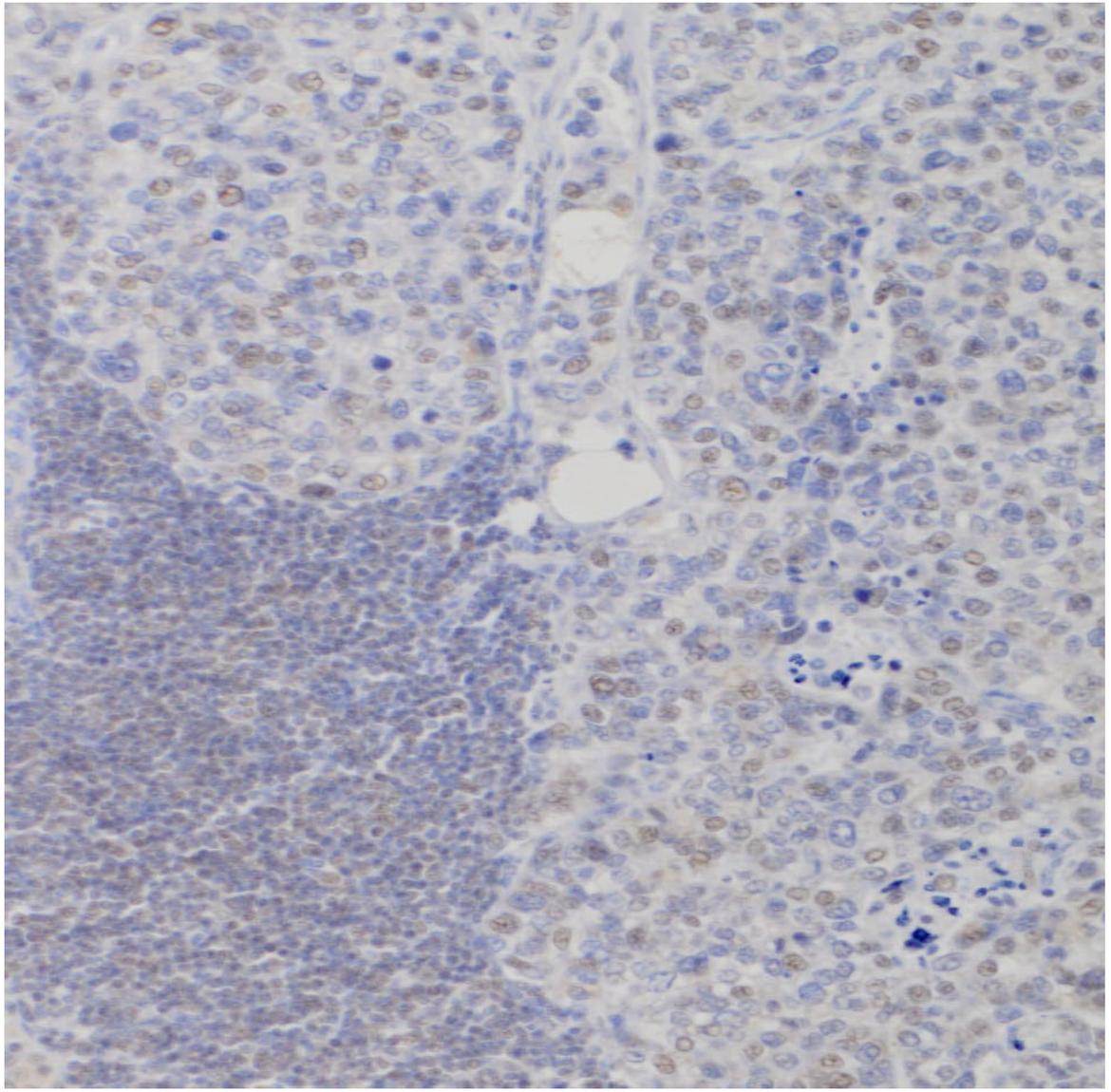
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ER IHC

Control: endometrium

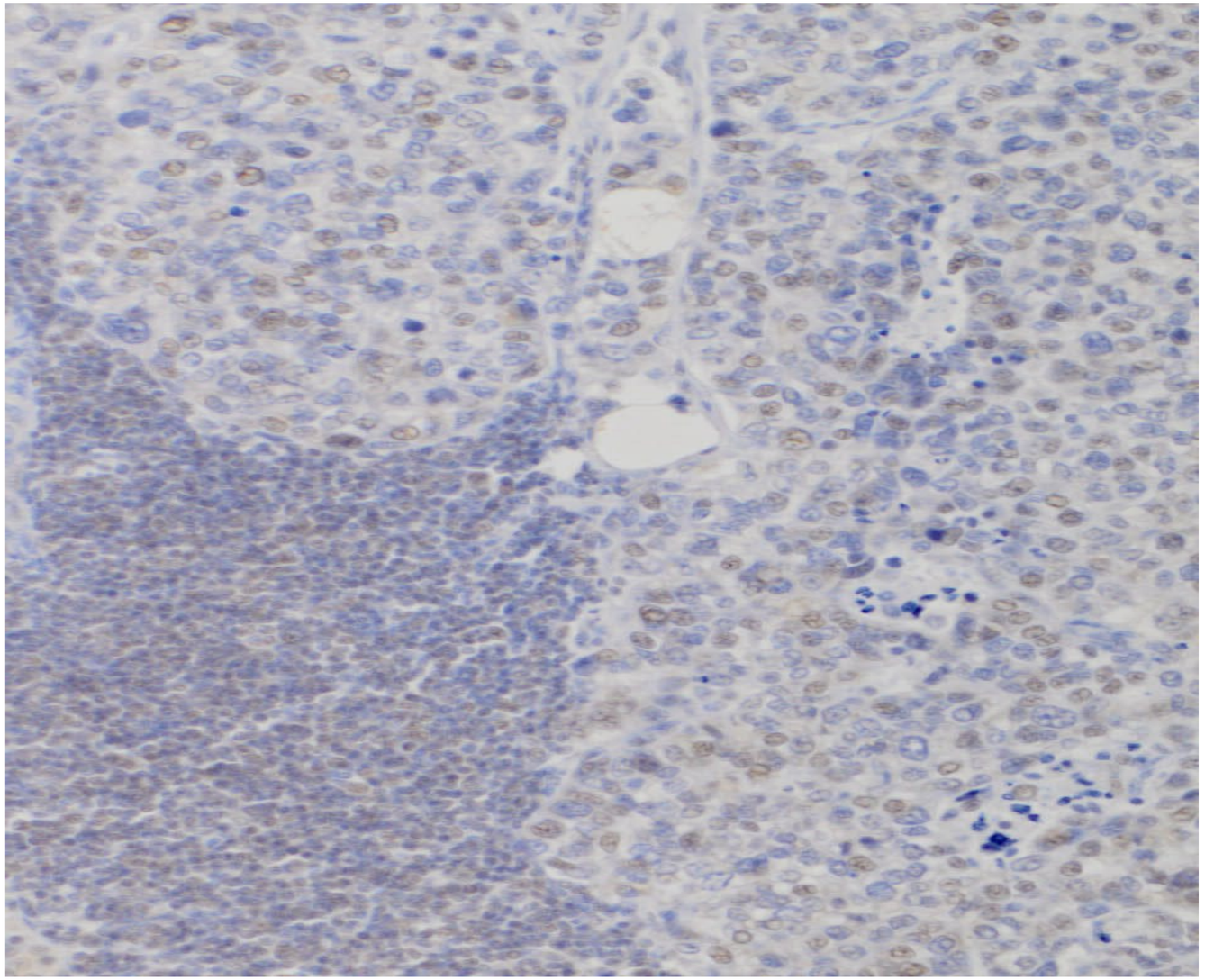




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What is the Most Appropriate Interpretation of the ER Results for This Case?



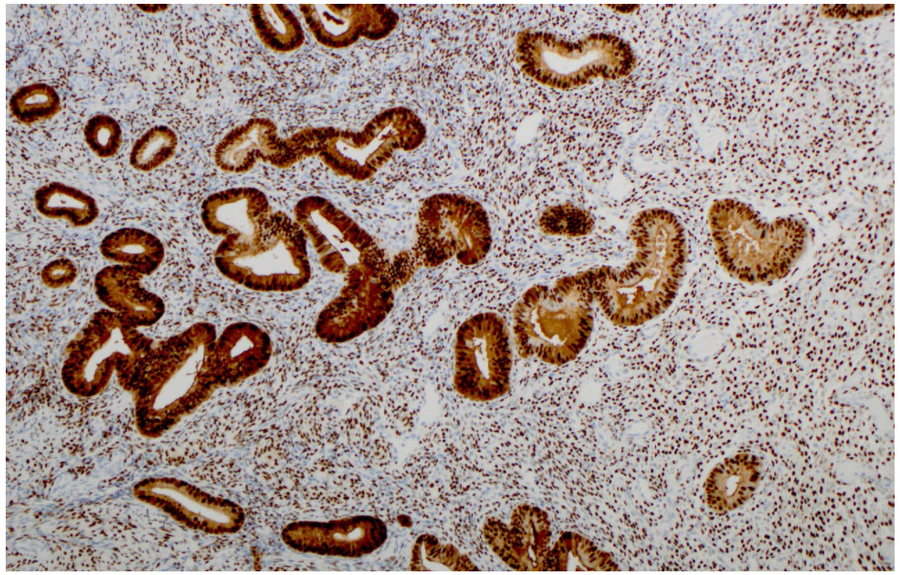


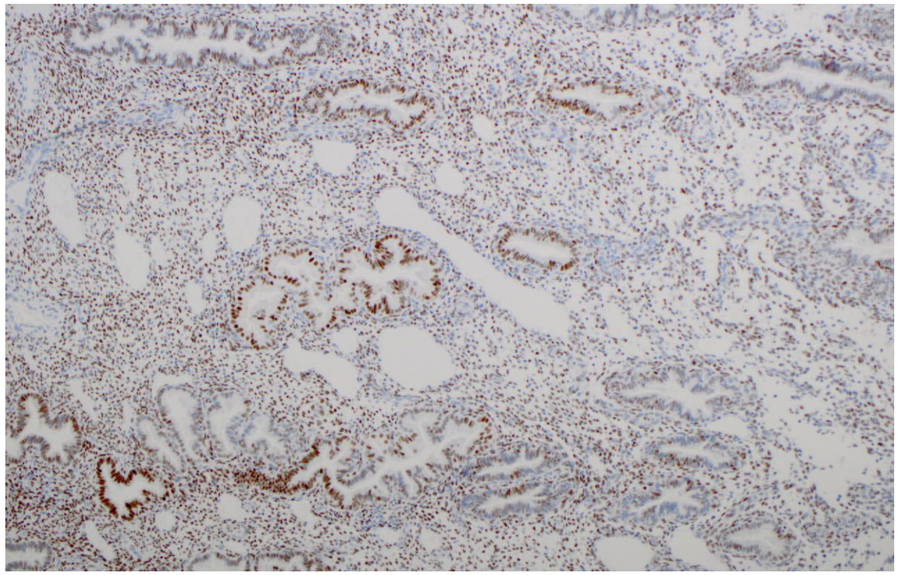
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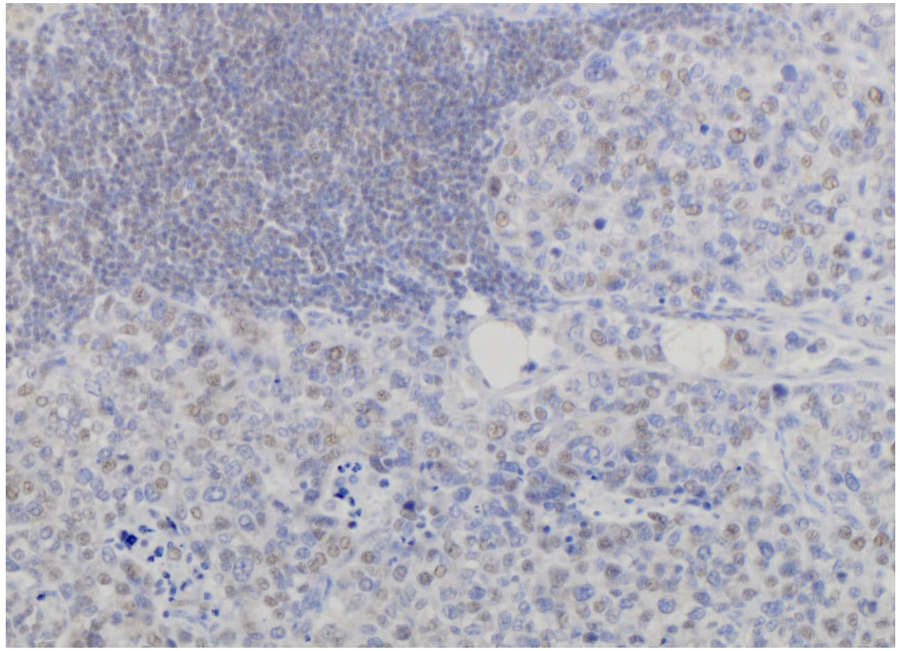
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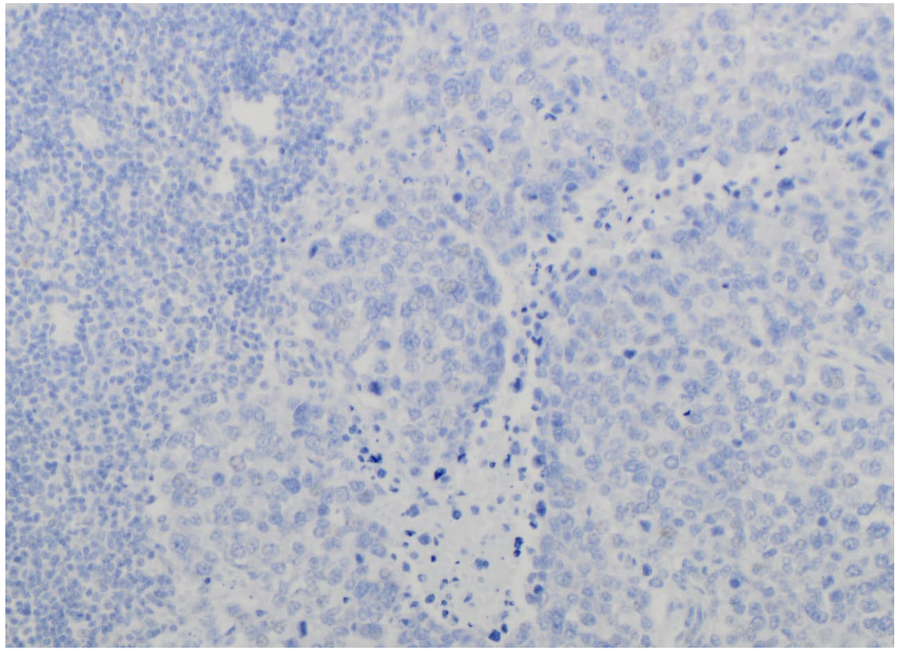
Original ER IHC

Repeat ER IHC





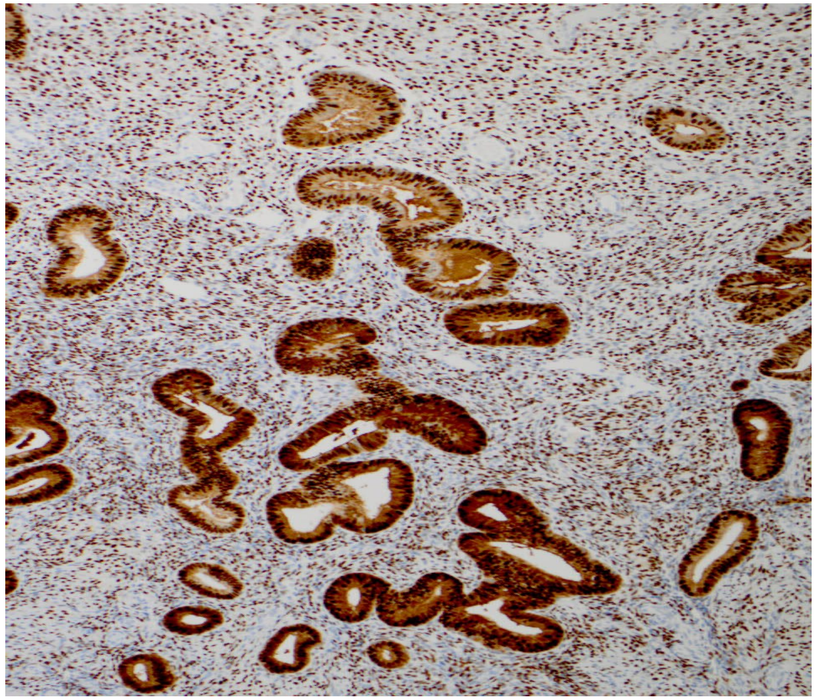




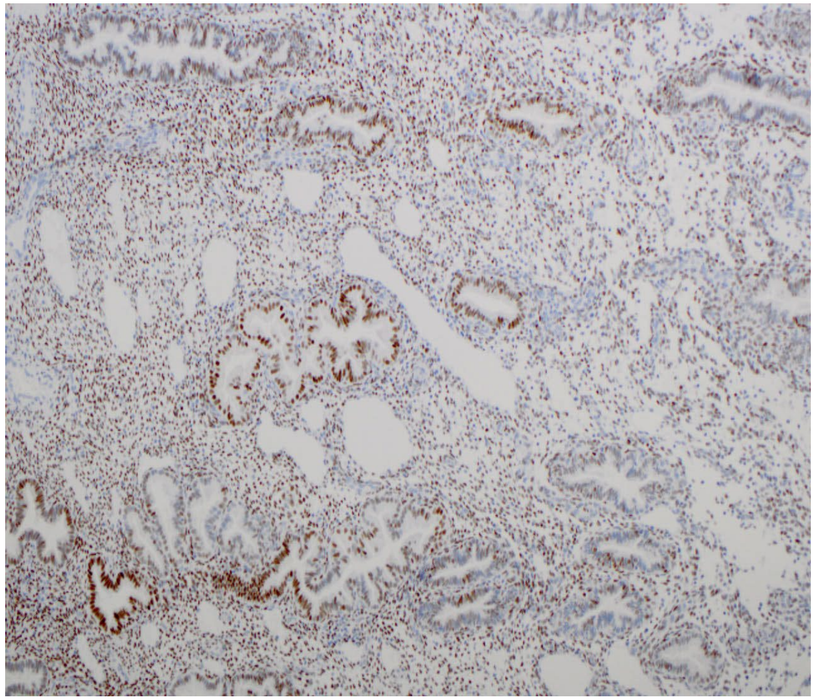
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- **Question on the initial ER IHC (appears to have positive staining)**
  - The high-grade morphology and geographic necrosis is more likely to be seen in ER negative tumors
  - Loss of ER expression in recurrent disease is rare, while a gain of ER expression is more common (unless the initial result was a false negative)
  - The positive control is over-stained with significant cytoplasmic staining
  - The lymphocytes adjacent to the tumor cells also appear to be positive
- **Repeat ER IHC stain showed an expected result for the positive controls and the tumor now appears to be ER negative**



Control staining is too strong



Control staining is appropriate

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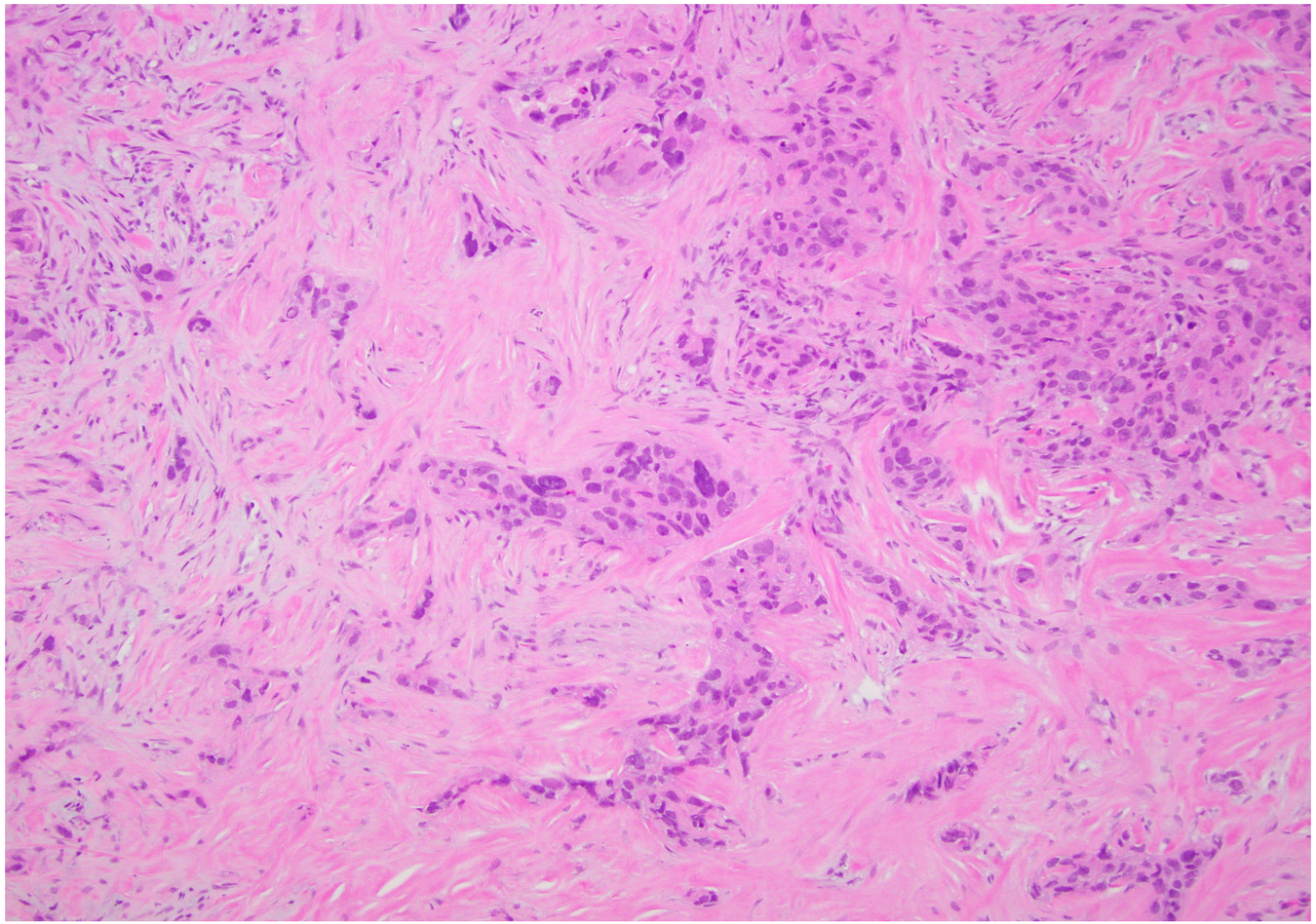
Case #1 Take Home Messages

- ER, PgR, and HER2 results should always be correlated with the morphologic and clinical feature of the patient's breast cancer.
- Both positive and negative controls should always be carefully evaluated to make sure the assay is performing appropriately.
- Aberrant staining of cells not normally expected to expressing a protein antigen and overstaining of the control should be a clue that there is a problem with the test, and it should be repeated.
- External controls should include negative and positive samples as well as samples with lower percentages of ER expression (such as tonsil). On-slide controls are recommended.

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Case Presentation #2

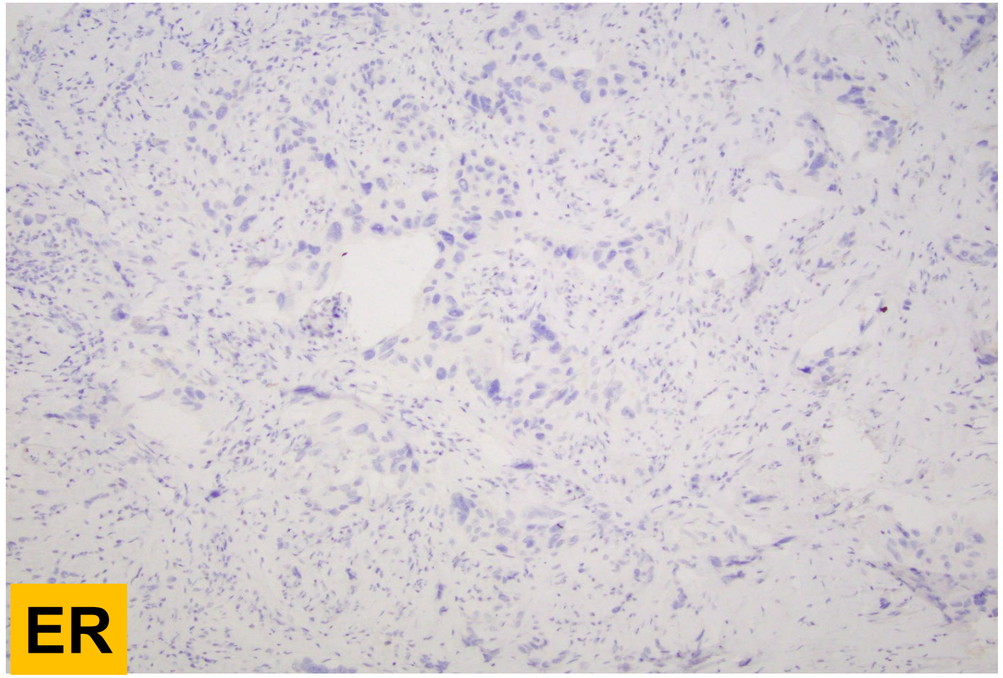
- A 78-year-old woman with 4-mm breast mass on ultrasound
- Core biopsy was performed

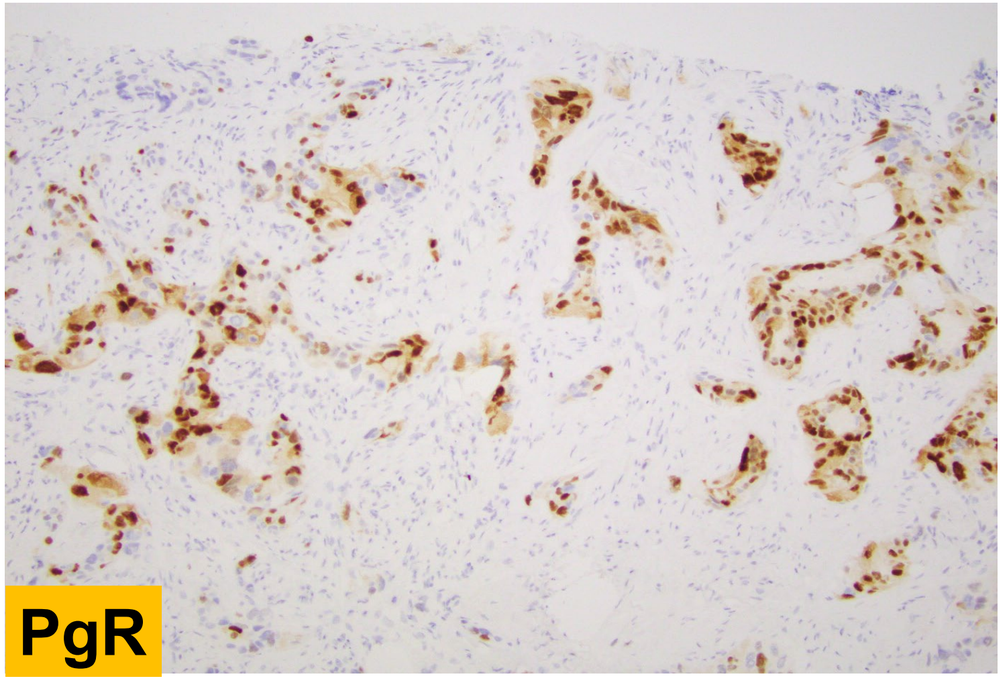


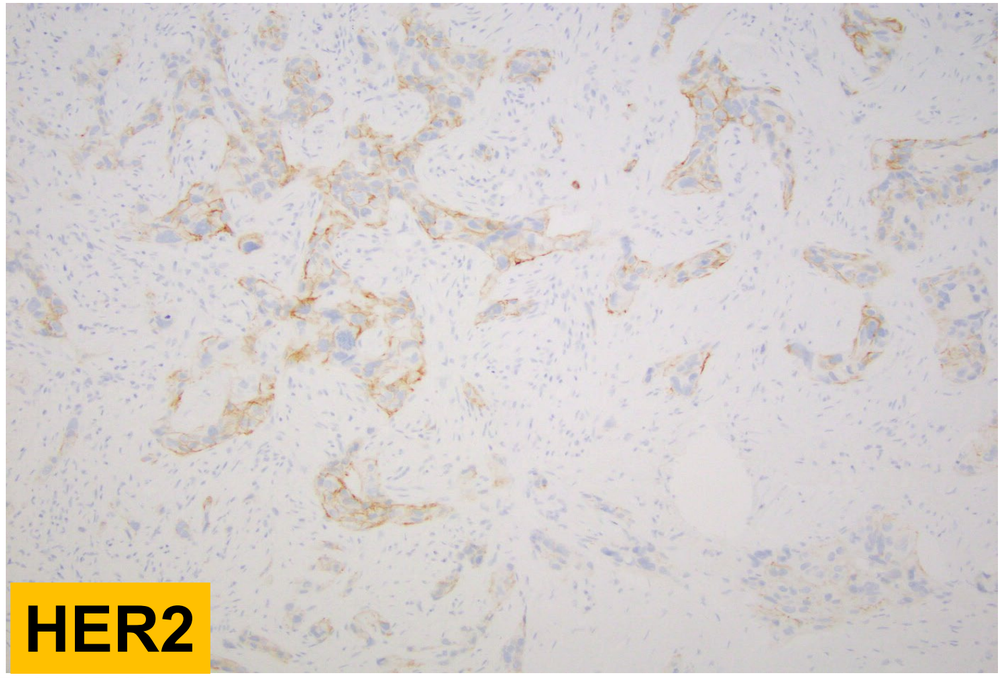
Invasive ductal carcinoma, histologic grade 3
Breast cancer biomarker was ordered

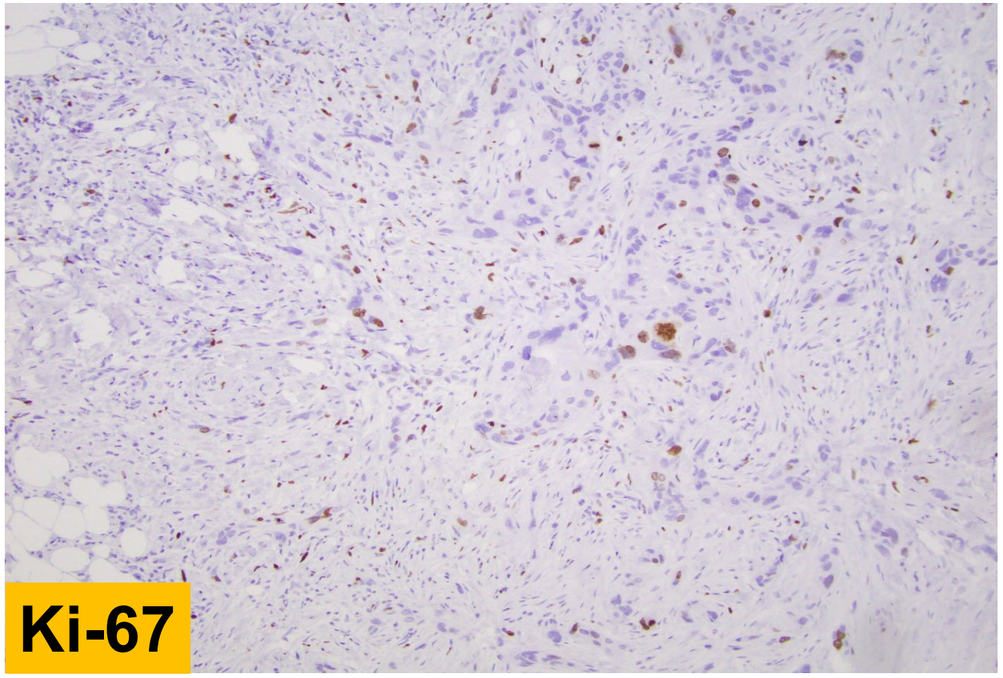
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How Would You Report the ER/PR Status in This Case?

A. ER negative/PR positive

B. ER negative/PR negative

C. Indeterminate

D. Seek colleague's opinion

E. Repeat ER/PR IHCs



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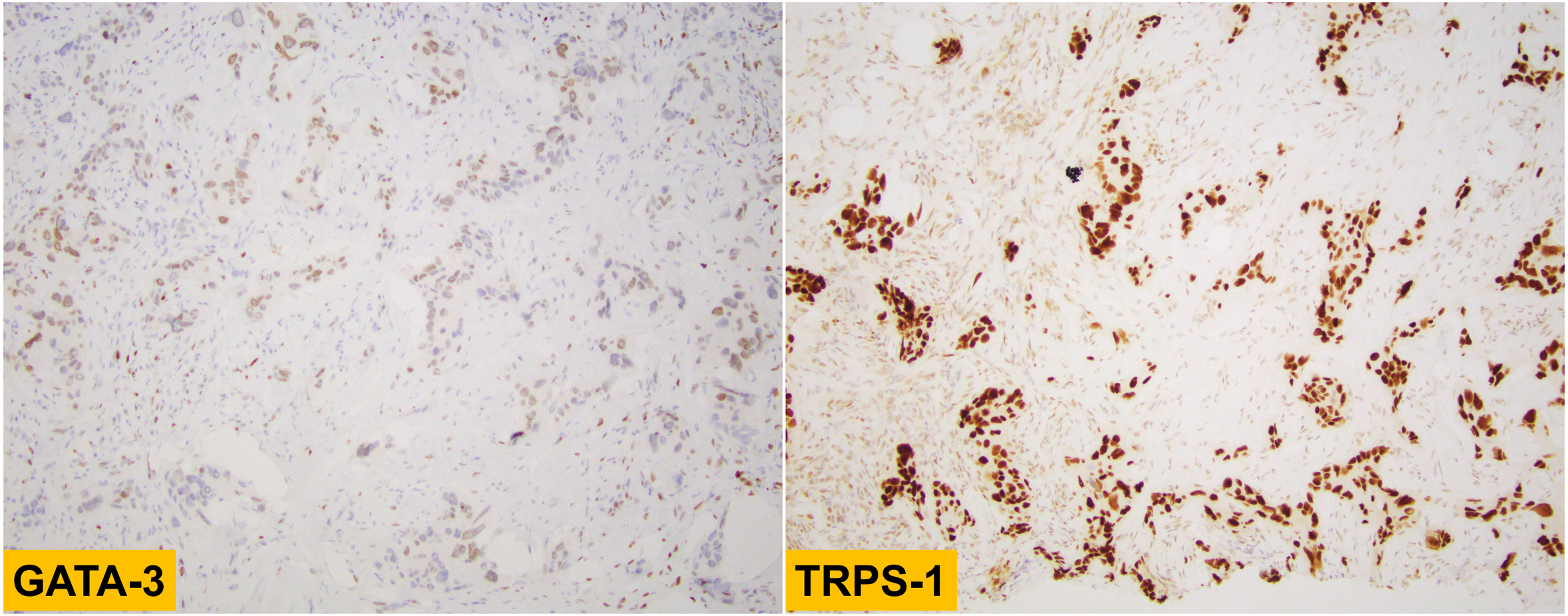
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Preanalytic Information

Collection time	12/7/22 at 15:50
Fixation start	12/7/22 at 15:50
Fixation length	12 hours

External control works appropriately
No internal control present on this biopsy material
What to do next?

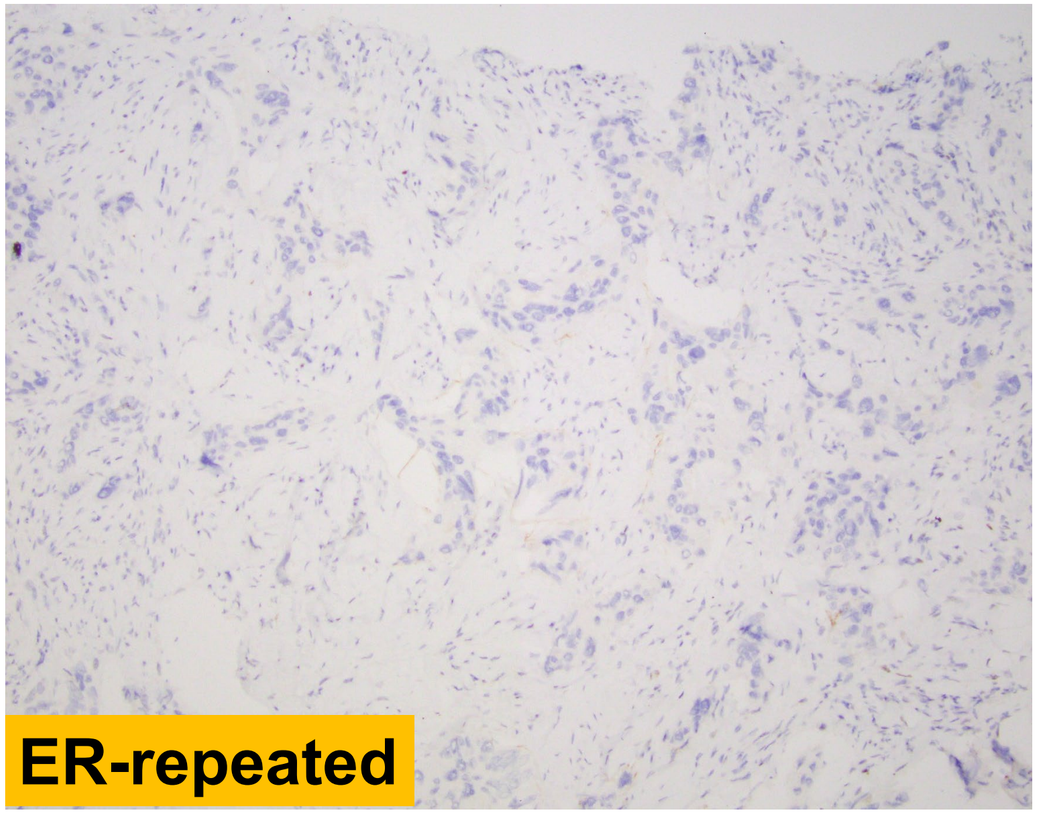
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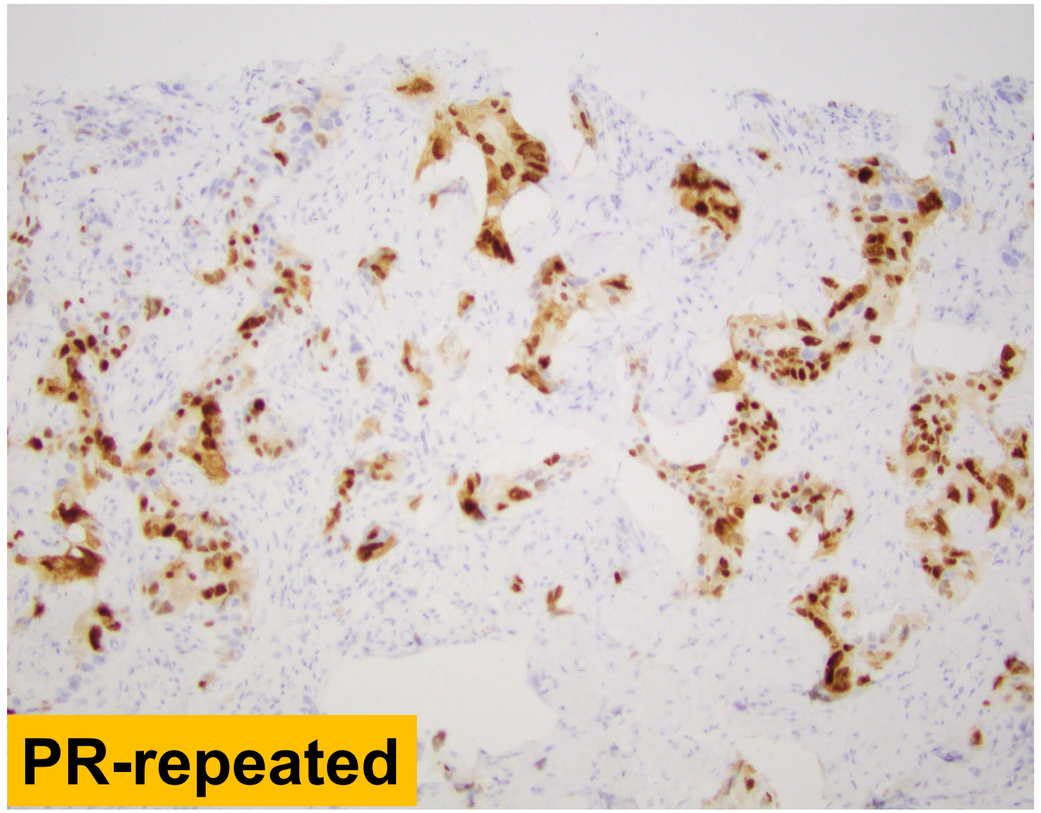
Confirmed breast primary of tumor

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ER-repeated



PR-repeated

Breast, right, 3:00 (mass), core needle biopsy:

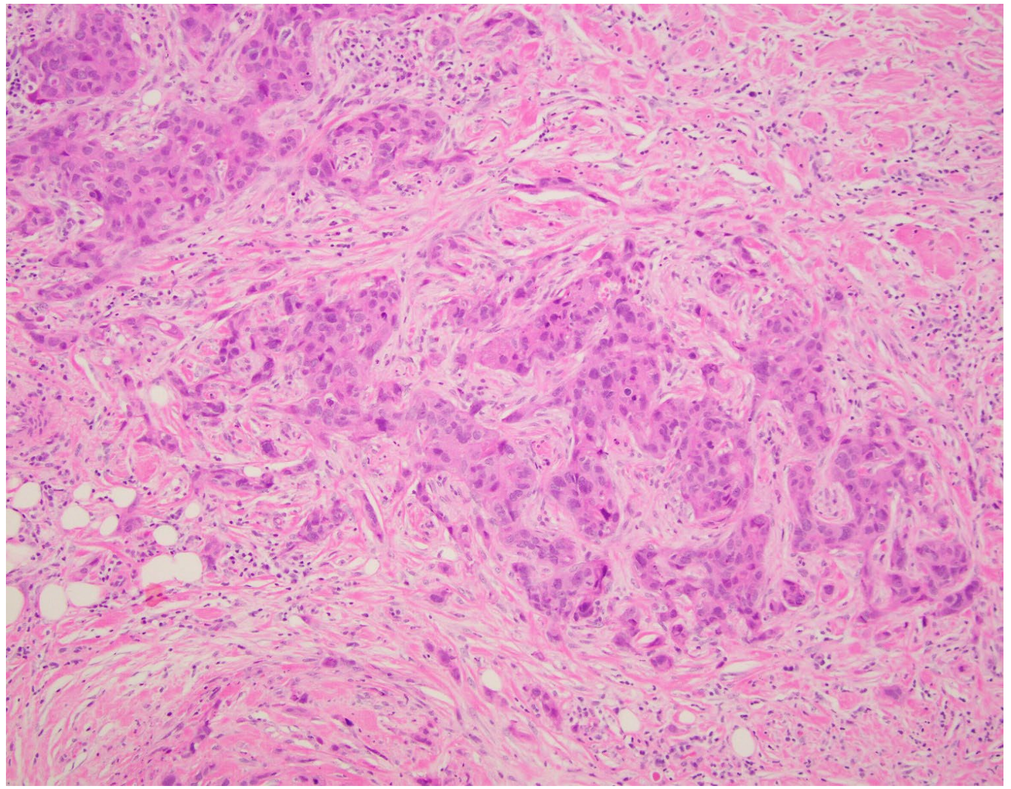
- Invasive ductal carcinoma (see comment)
  - Estrogen receptor: Negative (Allred score 0)
  - Progesterone receptor: Positive (Allred score 6)
  - Her2: Negative (score 1+)
  - Ki67: ~12%

Comment: ***Given lack of benign epithelial cells on the section for biomarker studies and the unusual ER-/PR+ phenotype in the tumor cells, a repeated biomarker study on the excisional material may be considered.***

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Partial Mastectomy and Sentinel Lymph Node Biopsy

- Invasive ductal carcinoma, size 9 mm, grade 3
  - **Repeated: ER-/PR+**
  - HER2-/Ki-6712%
  - No associated DCIS, angiolymphatic invasion
  - Margins negative
  - One negative lymph node (0/1)
  - Pathologic (anatomic) stage: pT1b pN0(sn)



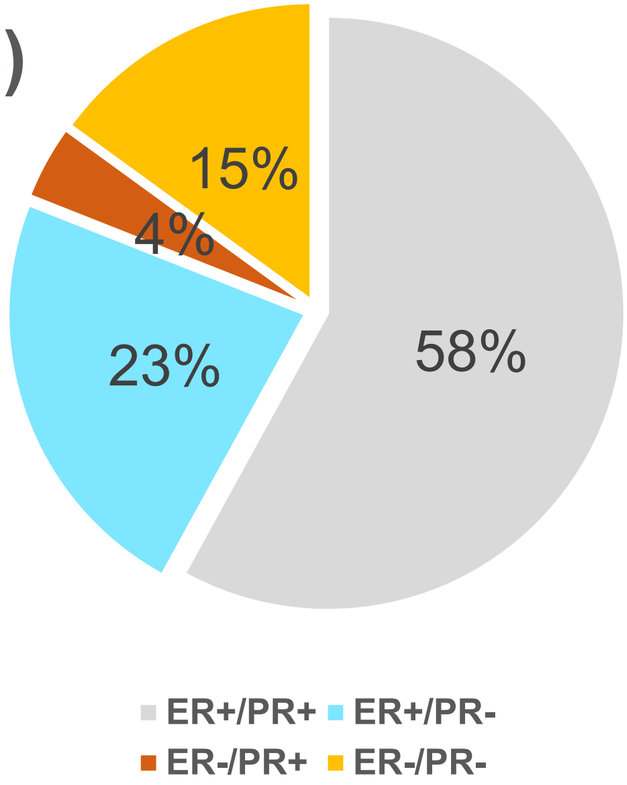
Tumor board recommendation: Adjuvant anastrozole and radiation therapy

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Case #2 Take home message: ER-/PgR+ Breast Cancer

- PgR expression is regulated by ERa
- ER-/PgR+ breast cancer is exceedingly rare (~1%-4%)
- Early studies considered it as technical artifact, especially in the absence of normal internal controls
- Increasing evidence has suggested it is a unique phenotype tends to be high-grade and larger tumor and occurs in younger women
- Respond to endocrine therapy and considered as hormone receptor tumors

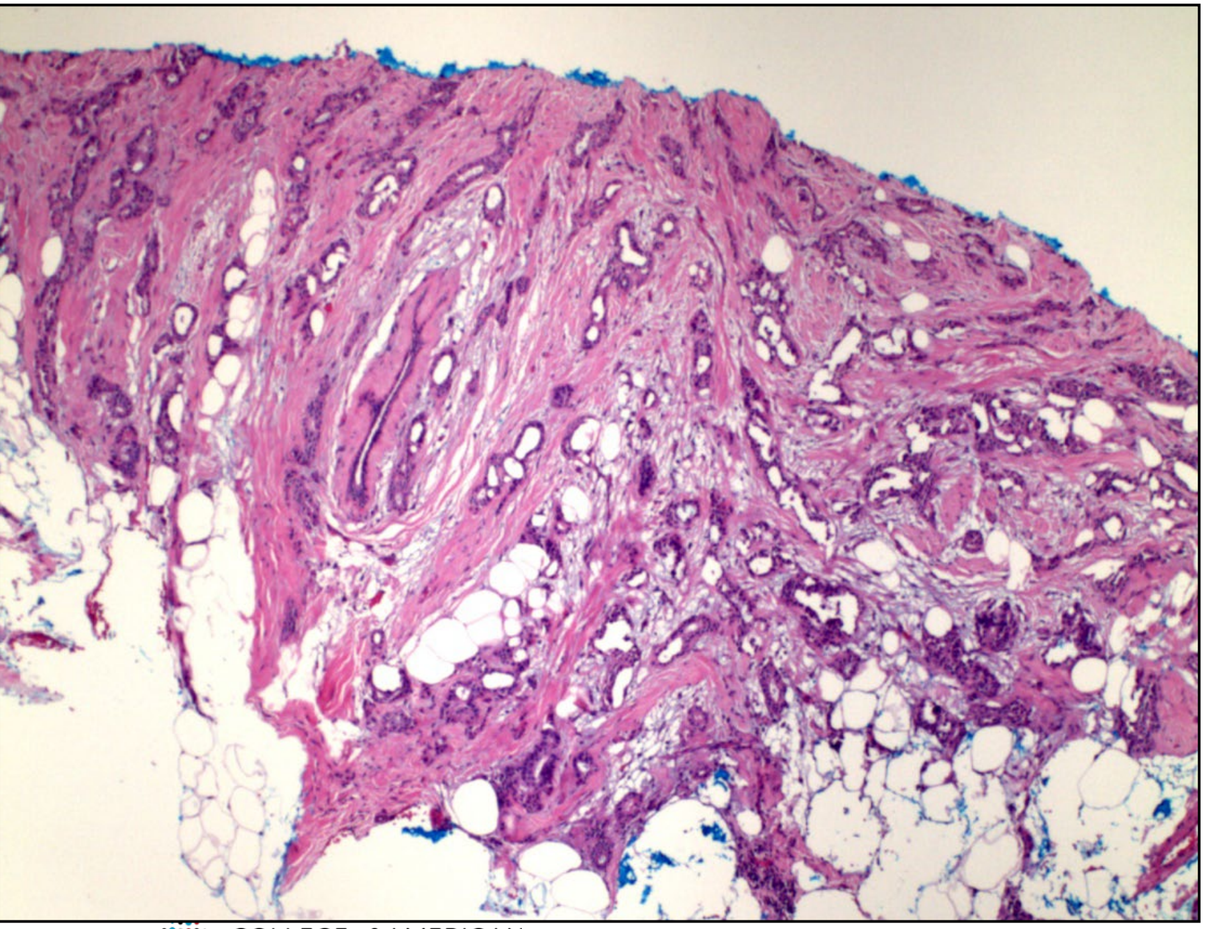


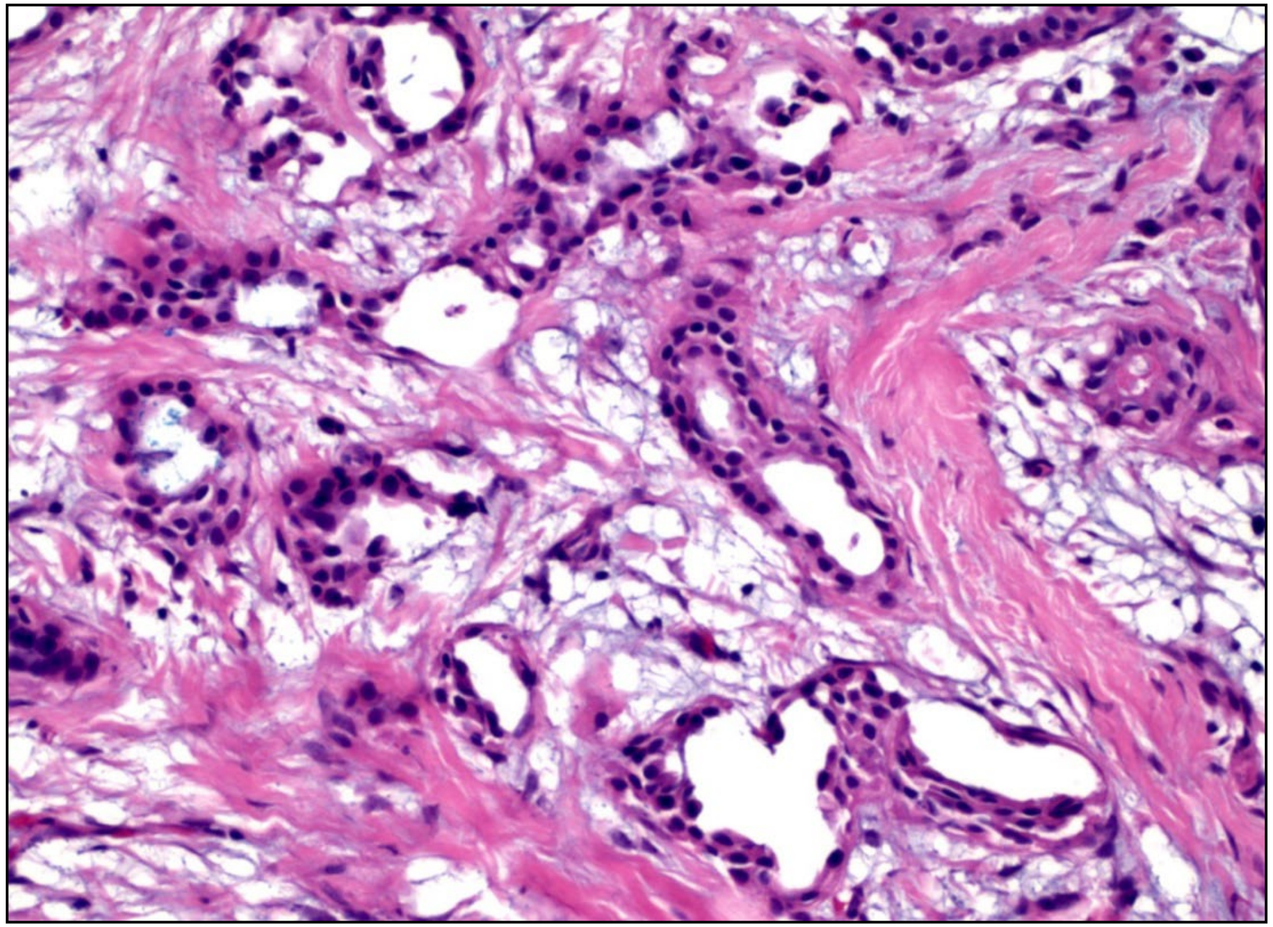
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Case Presentation #3

- 66-year-old woman, irregular mass on ultrasound
- Invasive ductal carcinoma, histologic grade I on core biopsy
- Tumor size: 0.9 cm

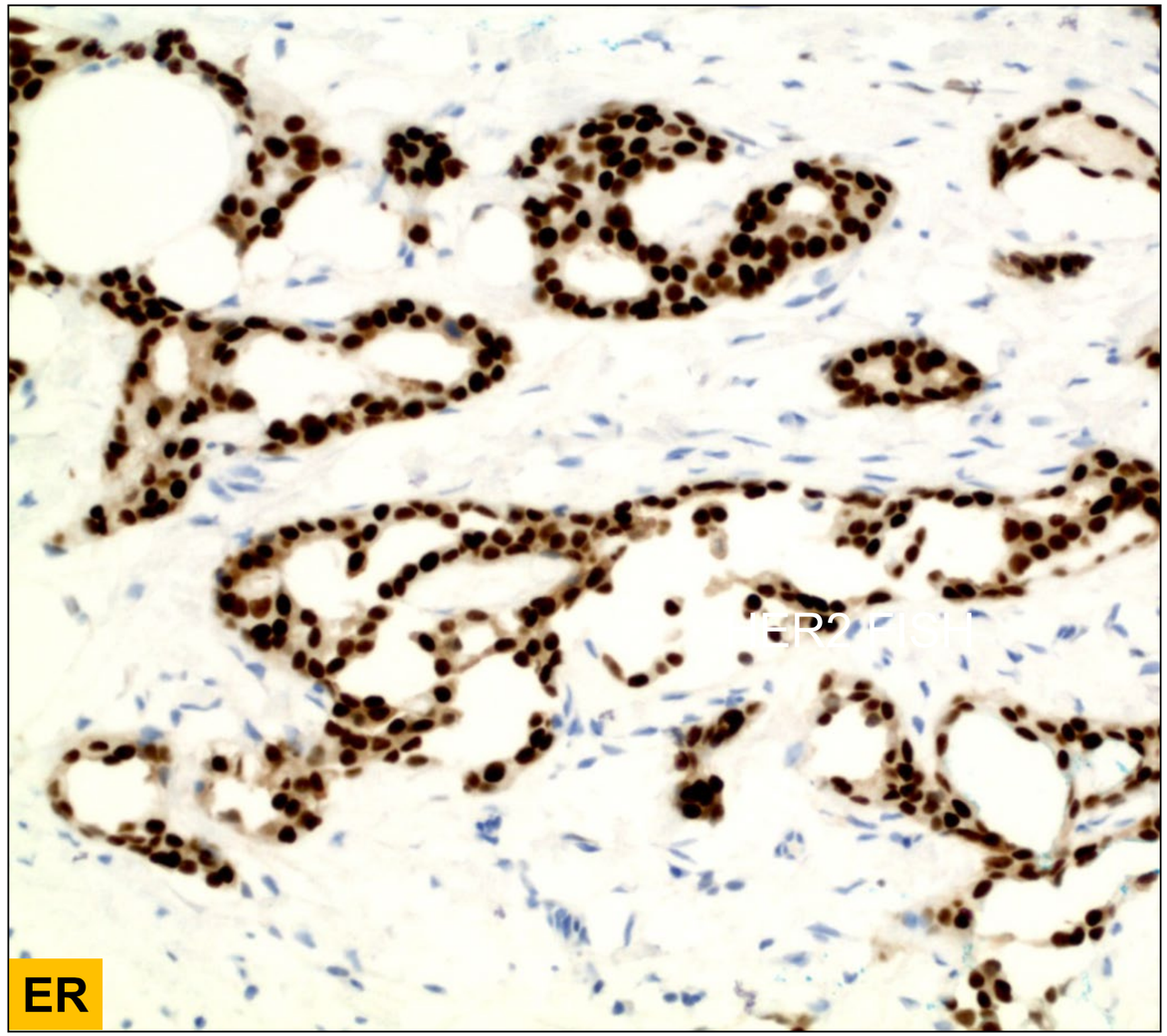


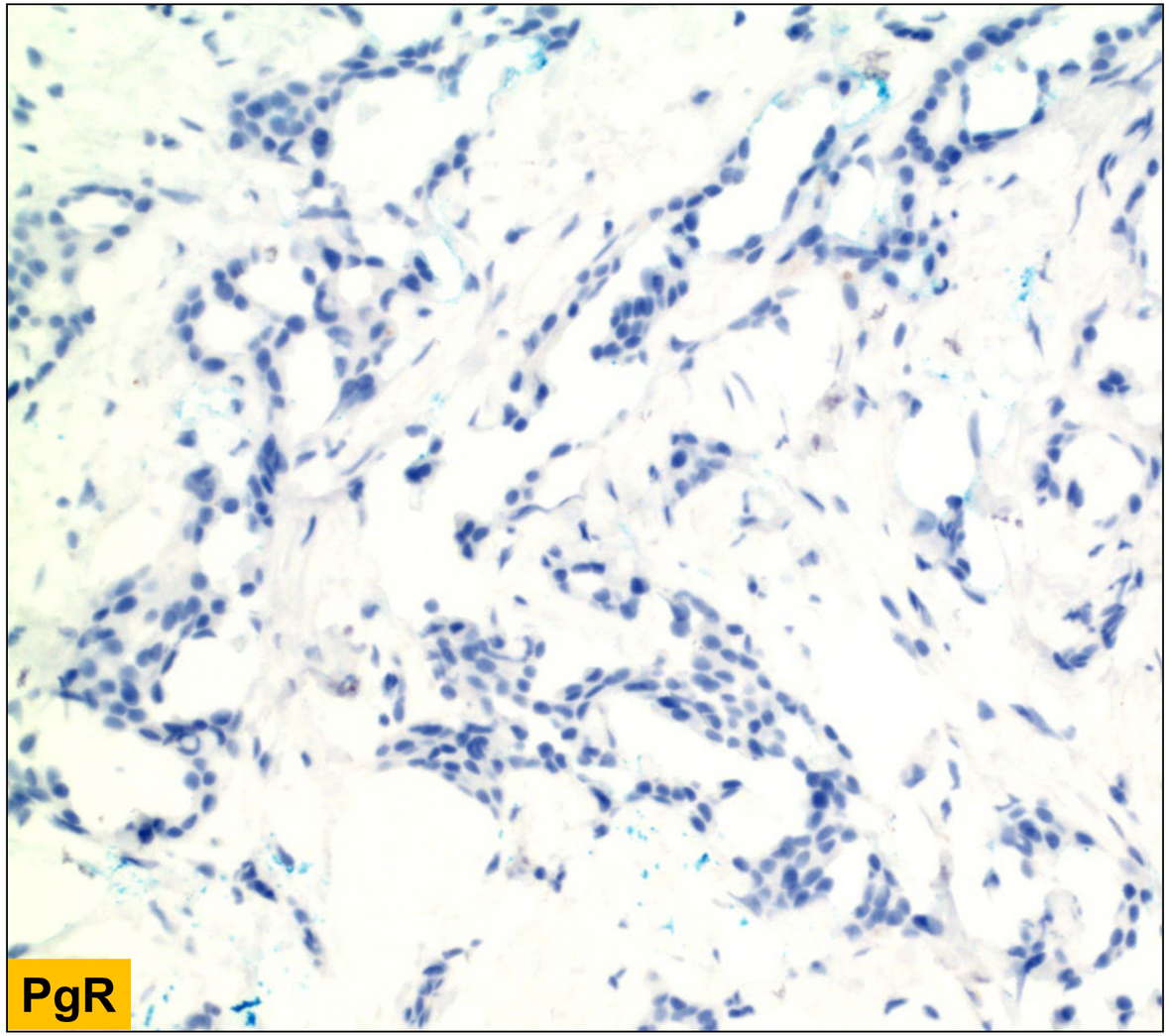


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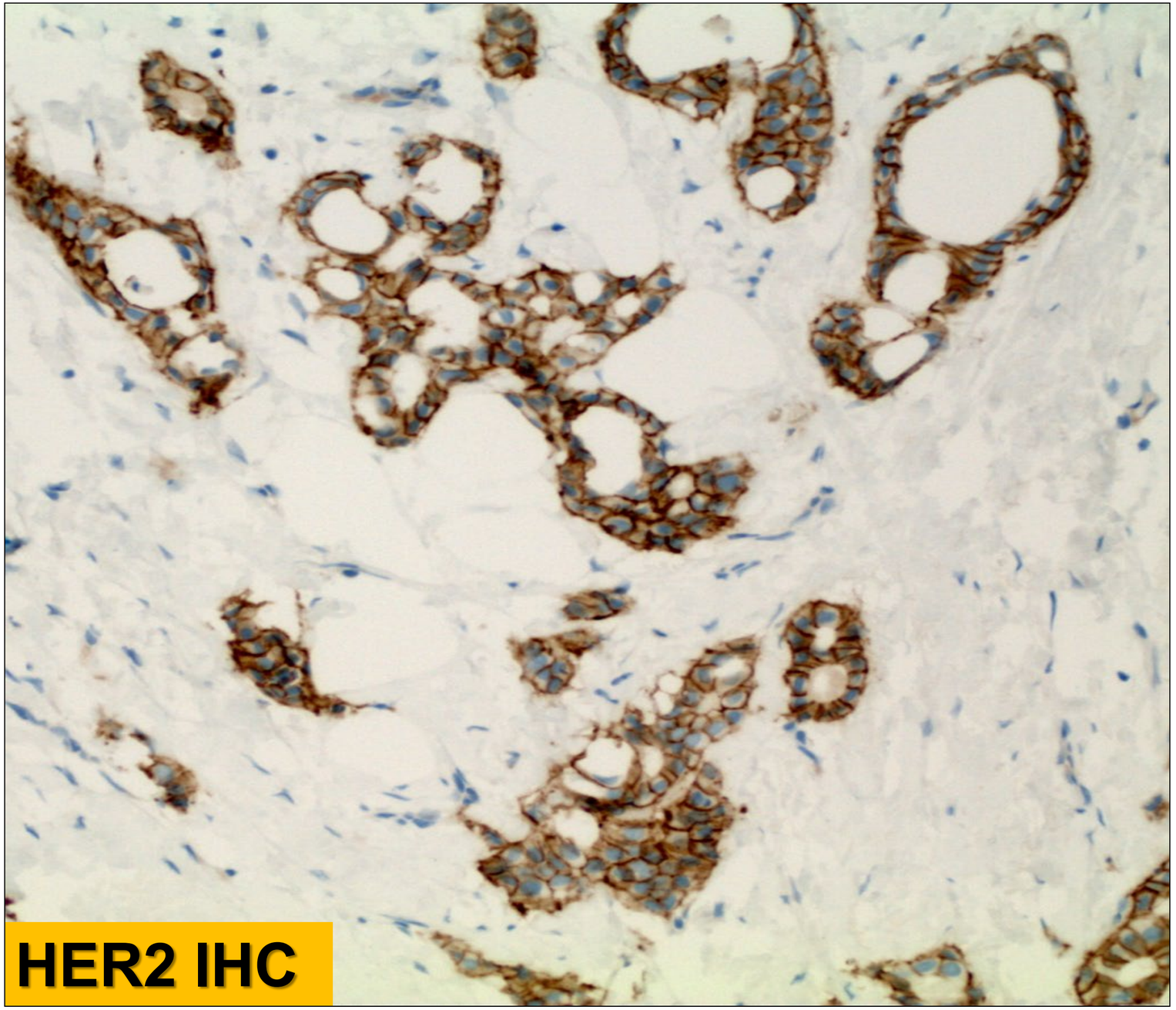
Case provided by David Hicks, MD.





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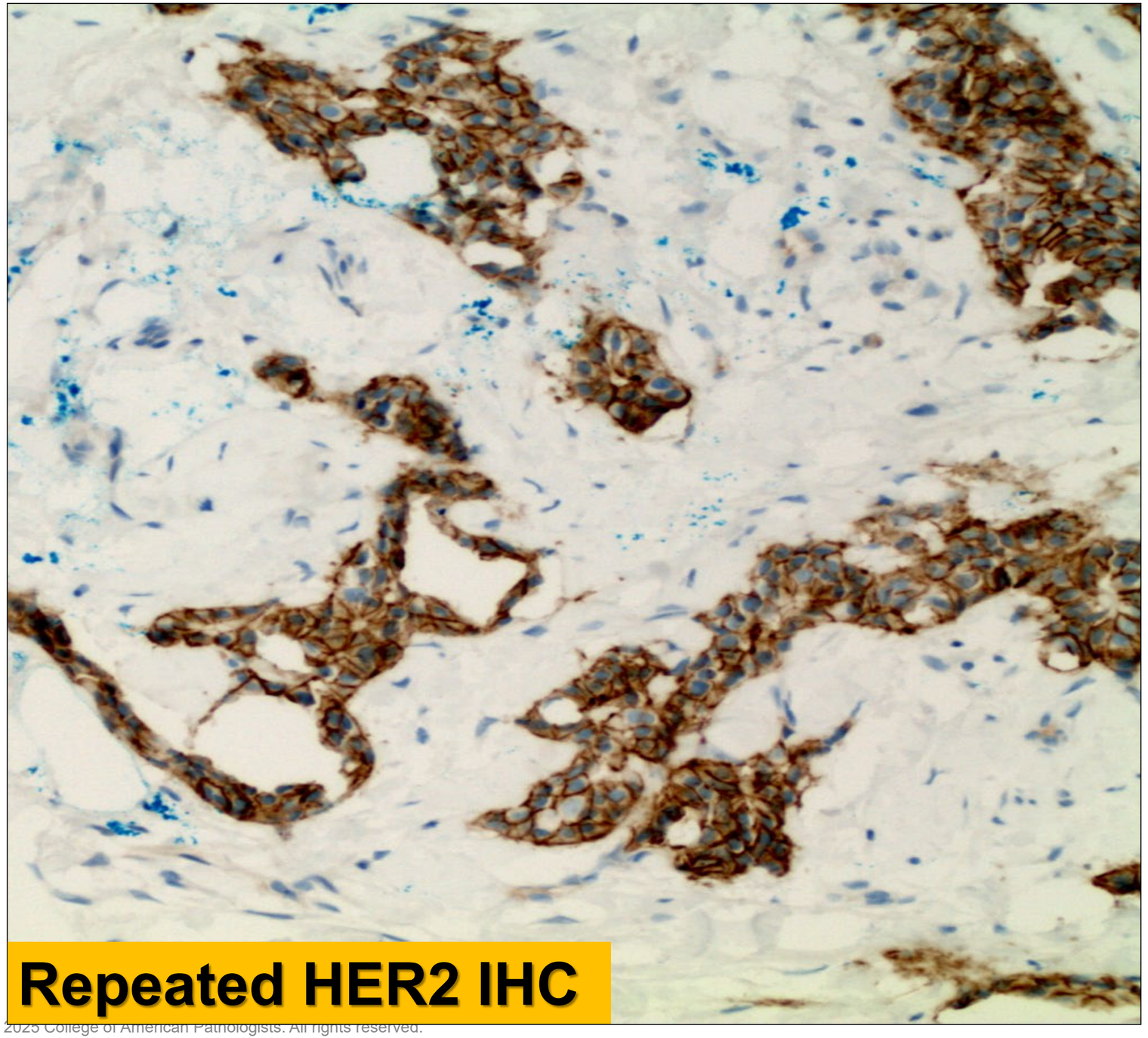
Which of the Following Would You Least Consider on the Interpretation of HER2 Status in This Case?

A. Report case as HER2 positive
B. Recheck tumor grade
C. Check internal and external controls
D. Repeat HER2 IHC
E. Confirm with FISH
F. Seek colleague's/consensus opinion



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Repeated HER2 IHC

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Clinical Data and Morphology

- 66-year-old, 9 mm on imaging
- Invasive ductal carcinoma, histologic grade 1
- Expected: ER positive/PR positive/HER2 negative

Preanalytic Data and Technical Issues

✓ Preanalytical variables and controls appropriate

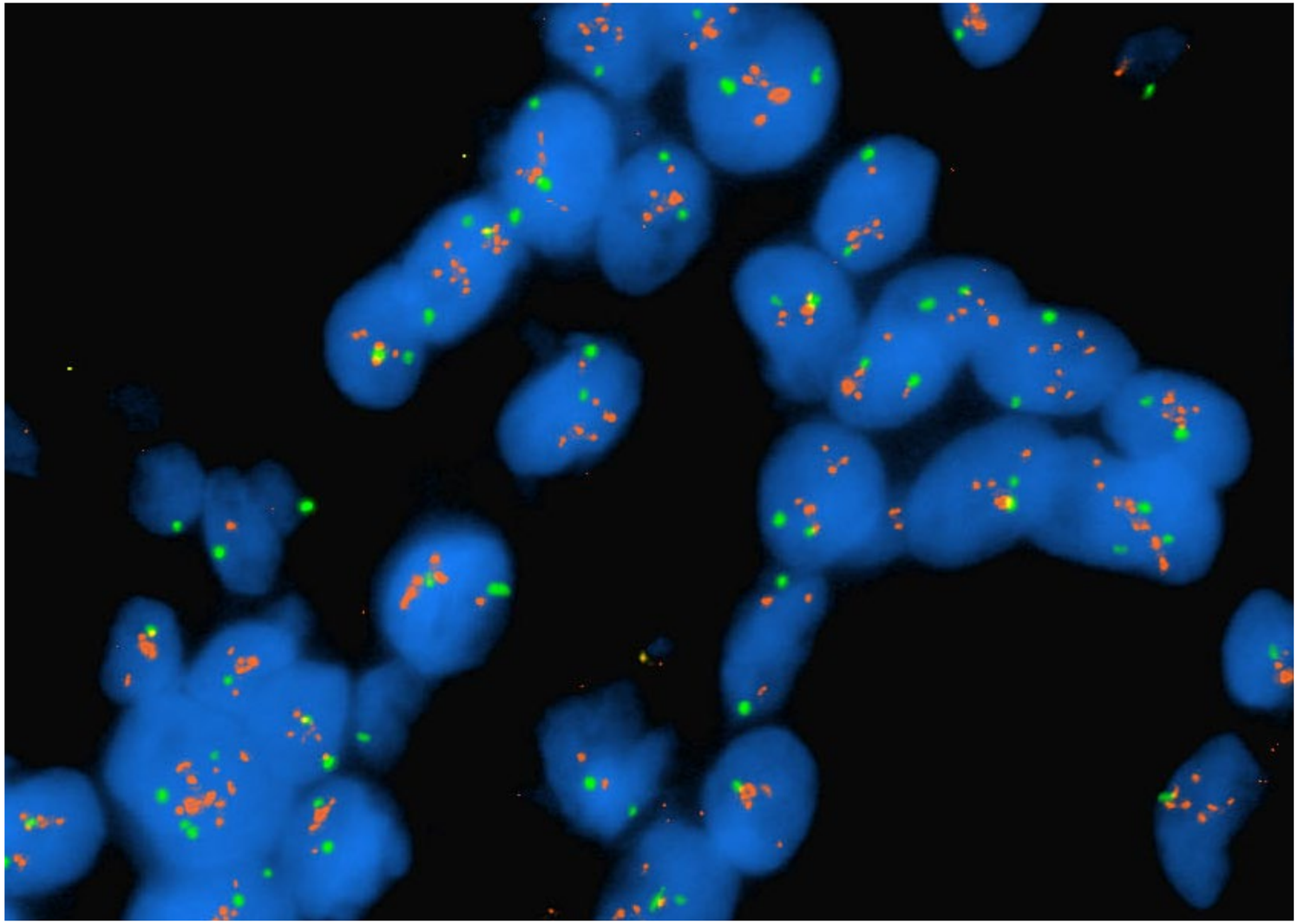
Does HER2 status fit with clinical and morphological profile?

- No (Grade 1 IDC, very unusual to be HER2 positive)

Solution

- Send to ISH testing

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HER2 FISH results: Amplified
HER2/CEP17 = 3
Ave. HER2 # = 9
Ave. CEP17 # = 3

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Case #3 Take home message:

Approach for Troubleshooting-- Discordant, Difficult to Interpret, or do not fit the Clinical or Morphological Profile of the Patient

Gather more information

- Clinical information
- Specimen handling and fixation information
- Processing information
- Interpretation information

Determine the source of the issue

Technical problem

- Preanalytical tissue requirements not met
- Improper assay procedure
- Problem with controls

Interpretation problem

- Artifacts present
- Improper use of criterion for interpretation
- Lack of training

Unusual tumor biology

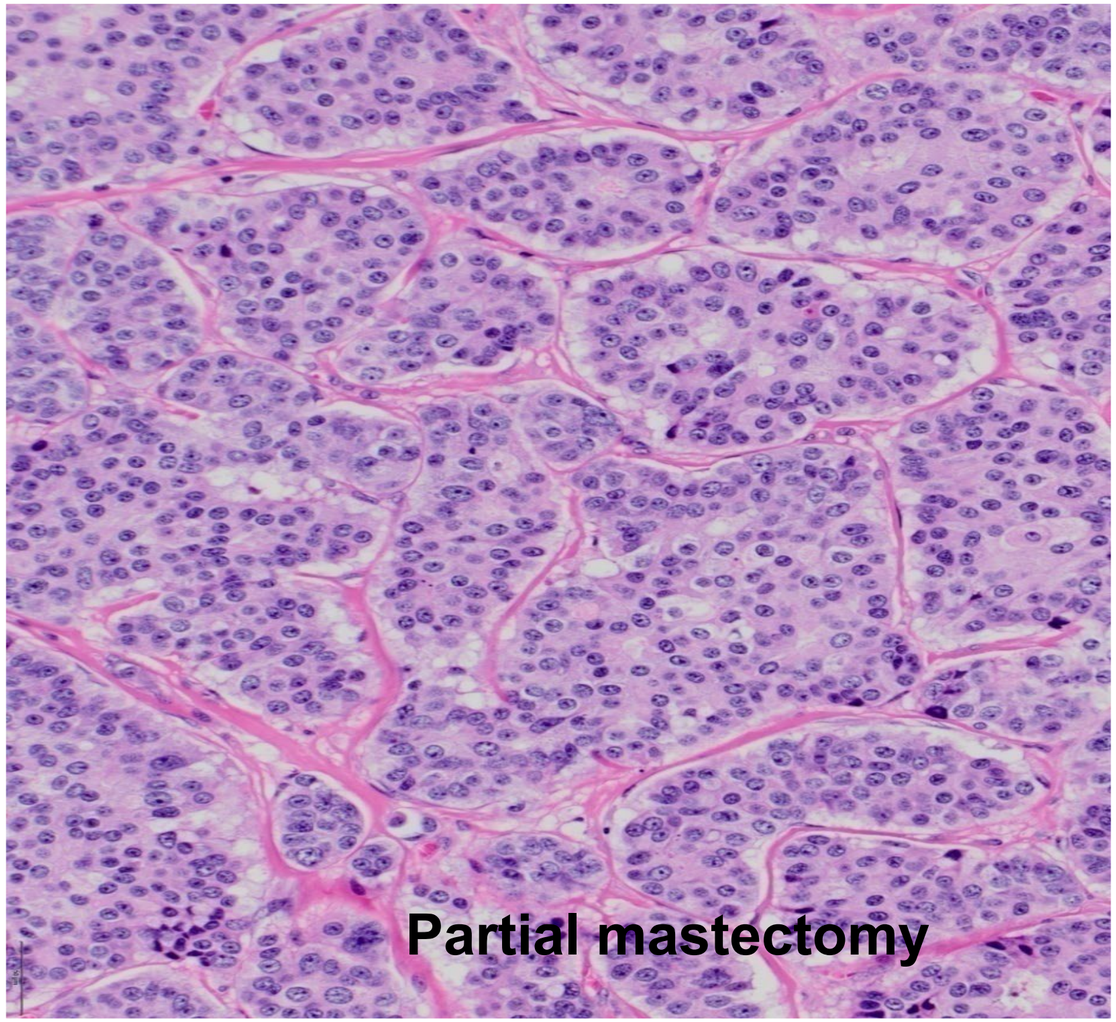
- Correlate with morphology
- pursue additional testing

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Case Presentation #4

- 51-year-old woman with right breast mass
- Core biopsy done at outside facility
  - IDC, grade 3
  - Per outside report: ER/PgR positive, HER2 equivocal, no record of follow-up ISH analysis
- Patient referred for surgical treatment, partial mastectomy performed

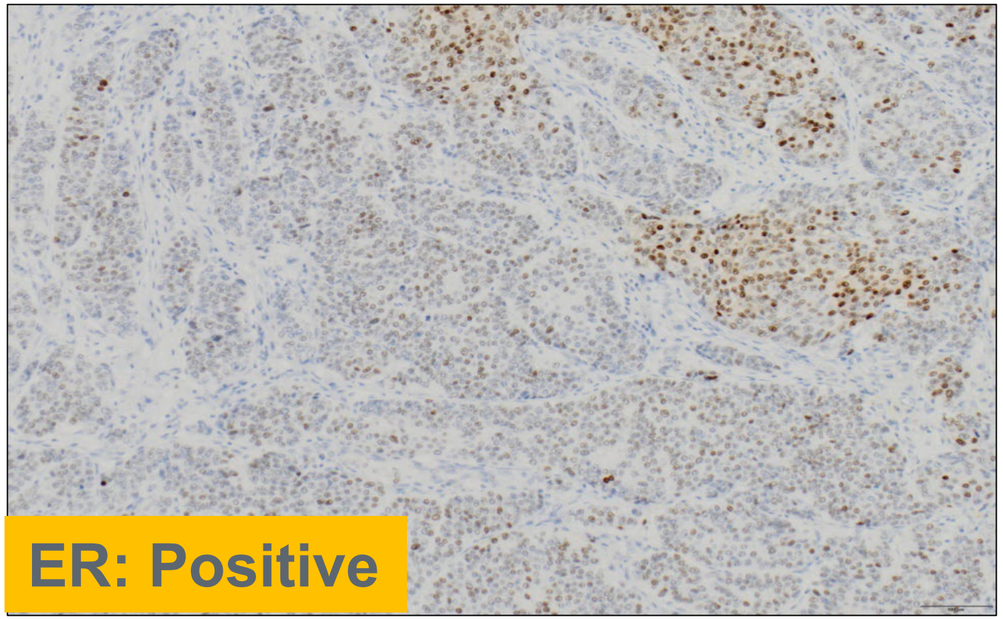
Collection time	7/11/13 4:00 pm
Fixation start	7/11/13 4:20 pm
Fixation length	16 hrs
Tumor size	3.5 cm
Lymph node	2/31



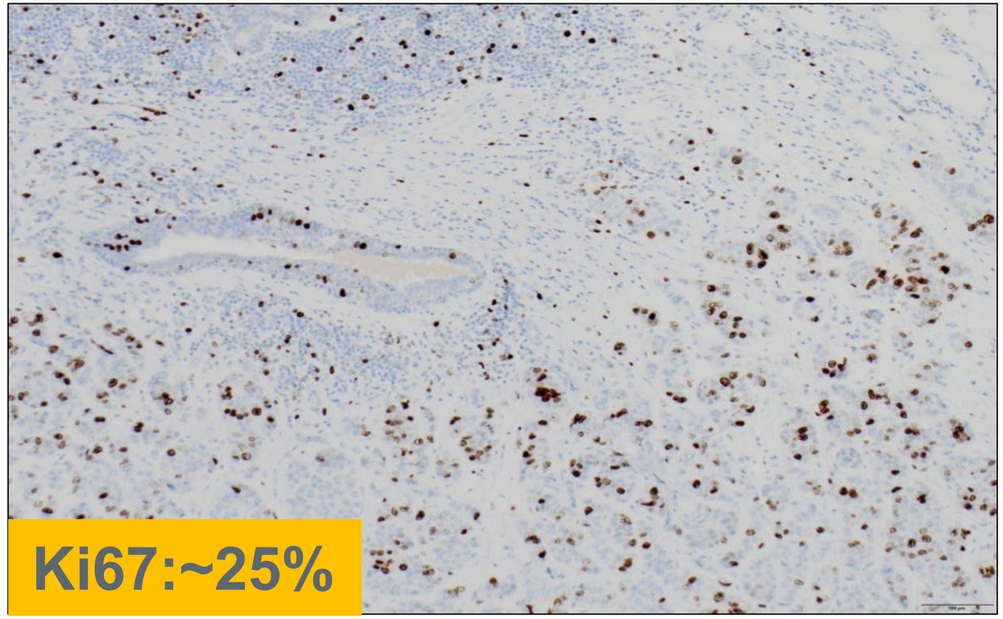
Case provided by David Hicks, MD.

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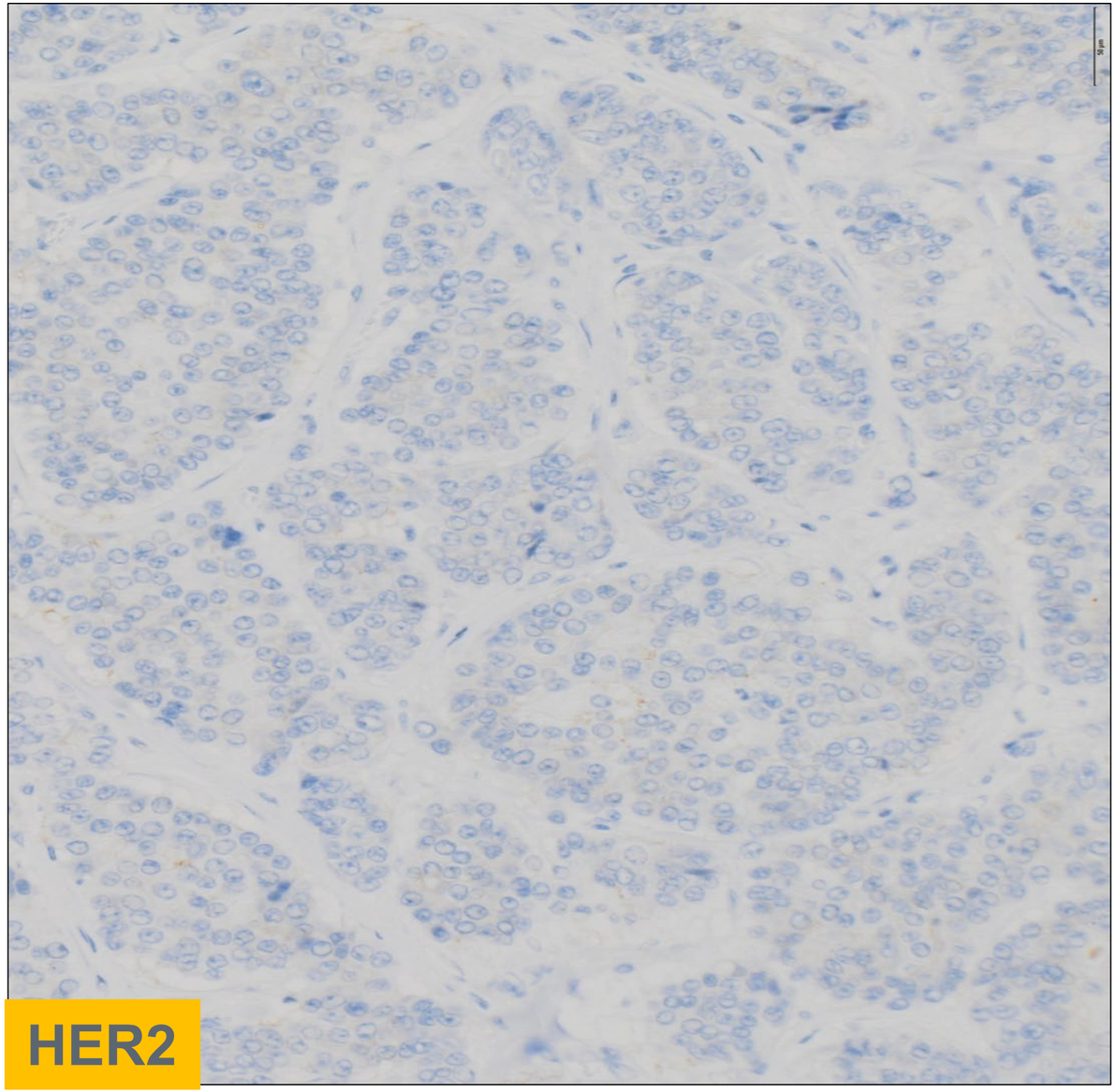
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ER: Positive



Ki67:~25%



HER2

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Clinical Data and Morphology

- 51-year-old
- Grade 2 invasive ductal carcinoma, ER/PgR positive
- Ki-67 ~25%
- Lymph node (2/31)
- HER2 equivocal at outside institution, unknown FISH result

Preanalytic Data and Technical Issues

- Preanalytical variables and controls appropriate

Interpretation Issues

- Is the interpretation of IHC 0?
- Outside called equivocal

Solution

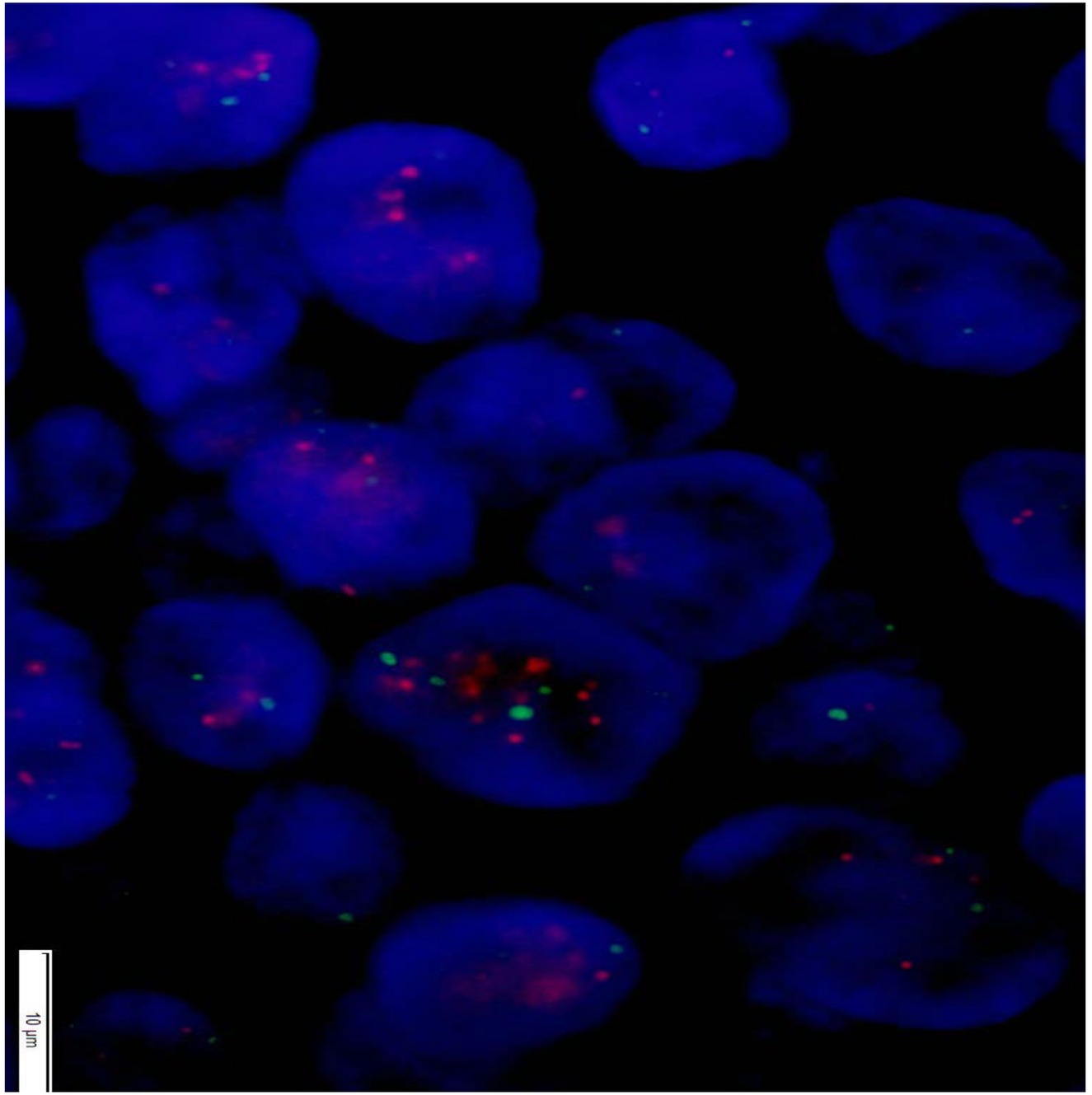
- Consider “FISHing”



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- **HER2/CEP17 = 2.6**
  - **Cells counted + 20+20**
  - **Ave # HER2 = 7.1**
  - **Ave # CEP17 = 2.7**
- **Interpretation: *amplified***



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Case #4 Take home message:

Indications for HER2 Reflex or Repeat Testing

❶ Equivocal

- **IHC 2+ and/or ISH dual probe *HER2*/CEP17 ratio <2.0 with an average *HER2* copy number ≥4.0 and <6.0 signals/cell).**
- **Would prefer to repeat on the excision despite the 2018 focused update recommendations for FISH equivocal results.**

❷ Discordant (when to consider repeating)

- **HER2-negative result** in a case with one or more the following unfavorable histologic features: **high-grade tumor*, small amount of invasive tumor on core biopsy (especially if high-grade),*** peritumoral lymphatic invasion, ER negative, ER positive/PR negative, high proliferative index (Ki67 >20%). (*Specifically noted in the ASCO/CAP HER2 Guideline.)

❸ Indeterminate

- **Technical issues** preventing the test from being reported as positive, negative, or equivocal (eg, inadequate specimen handling, artifacts such as crush or edge, or analytic testing failure).

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ER/PgR/HER2 Testing Summary: Key Takeaways

Standardization of all testing components is critical

- **Preanalytic variables, including controlling cold ischemic time, prompt tissue fixation, adequate tissue fixation, and processing**
- **Analytic variables, including the use of FDA-approved testing kits and proper controls**
- **Postanalytic variables, including training for interpretation and awareness of artifacts, following guideline reporting, and ongoing proficiency and competency testing**
- **Correlate the ER/PgR/HER2 results with tumor morphology and clinical information**



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How Can I Make a Difference for Breast Cancer Patients and Clinicians?

1. Stay informed on treatment information related to molecular analysis and breast cancer care.
2. Help ensure the biologic quality of tissue samples for biomarker studies by standardized preanalytic variables.
3. Provide clear, concise, and comprehensive reports.
4. Become an active participant and consultant to the multidisciplinary breast care team.
5. Check that the biomarker results correlate with the clinical profile for each patient.
6. Question cases that do not correlate and consider additional testing.



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Acknowledgements

University of Rochester Medical Center

- Dr. David Hicks
- Dr. Bradley Turner
- Breast Pathology Team
- Department of Pathology

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CAP Learning Team

Thank you all for your attention!



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