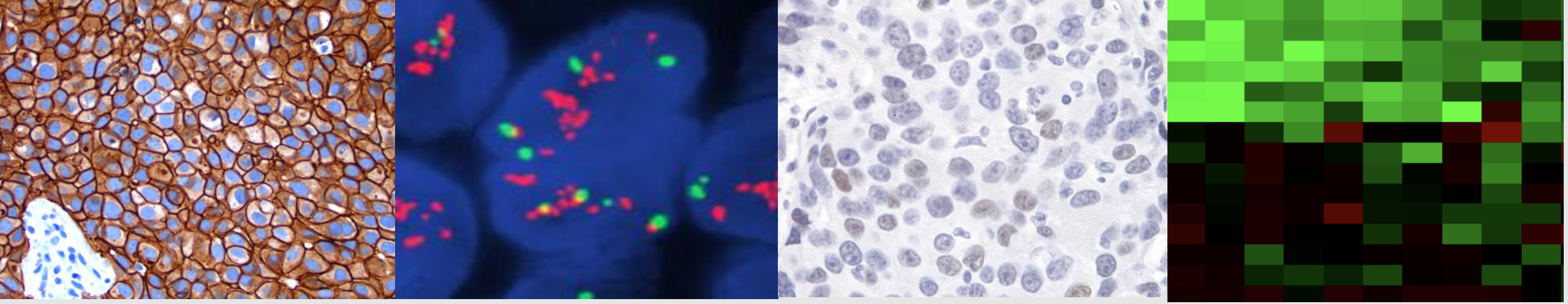

Clinical Game Changers in Breast Pathology: Ensuring best practices in predictive markers and beyond

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Vice Chair of Education, Director of Residency Training
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Bio: Dr Kimberly Allison

- Professor of Pathology at Stanford University School of Medicine
  - Director of Breast Pathology
  - Director of Breast Pathology Fellowship
  - Vice Chair of Education and Director of Pathology Residency Training
- Co-Chair of the ASCO/CAP 2020 Update in Hormone Receptor Testing in Breast Cancer, 2018 HER2 Update Steering Committee
- NCCN Breast Cancer Guidelines Committee
- AJCC Breast Cancer Staging Committee
- WHO 5th edition

Disclosures:

- Scientific Advisory Board of Mammotome, Inc

Today's Learning Objectives

1. Describe the latest biomarkers in breast cancer and how they are used in clinical management.
2. Be familiar and able to apply with the latest guideline ASCO/CAP and NCCN recommendations for prognostic/predictive testing in breast cancer.
3. Review grey zones and unusual results to be aware of and advise on.

Key Skills for Practice:

- ER interpretation and results that need confirmation and correlation
- HER2 interpretation and results require confirmation and correlation
- Correlate histology with ER and HER2 results (recognize unusual results)

Key Topics for Learning:

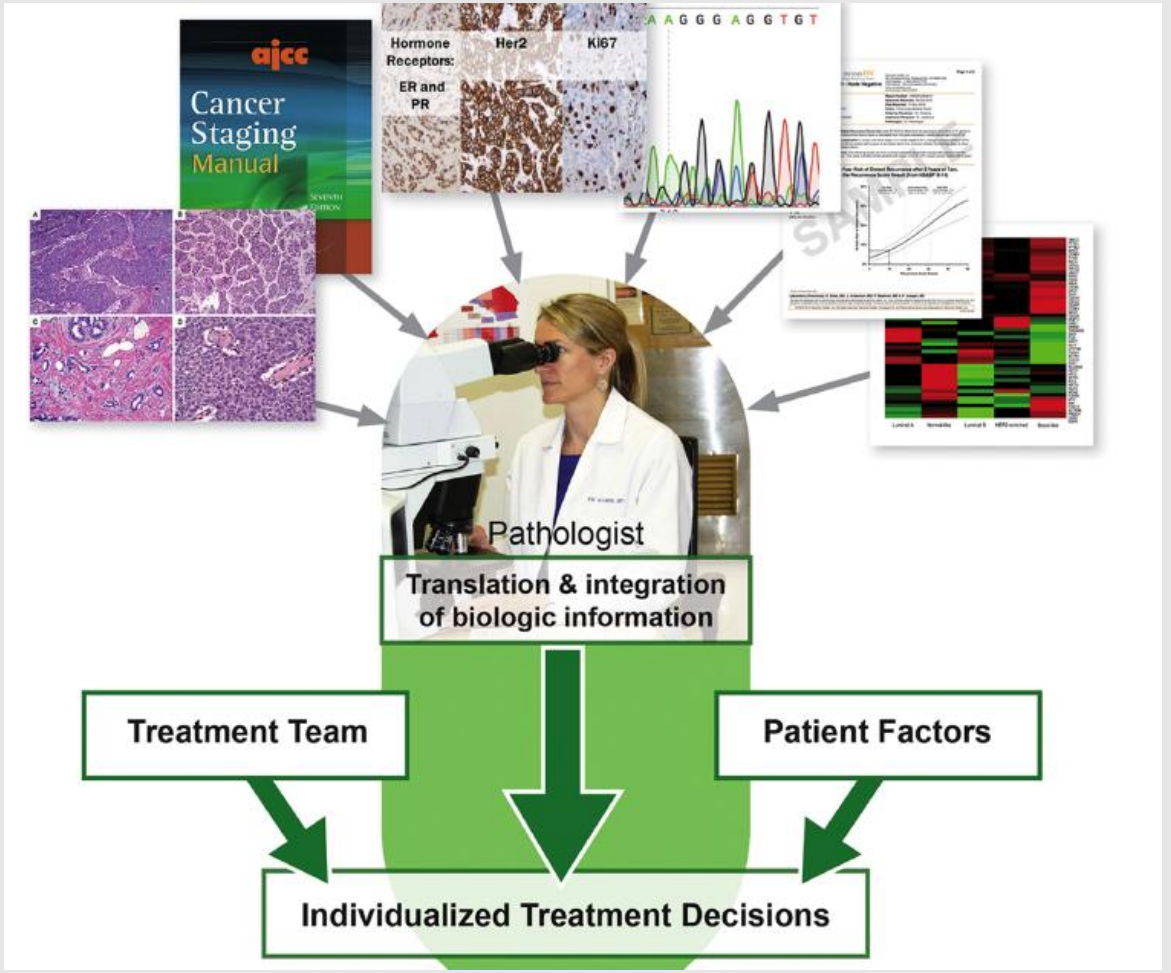
- What are treatment and testing options in metastatic breast cancer?
- How is Ki67 used in breast cancer?
- How is PDL1 testing used in breast cancer?
- What is “HER2 Low” ?

Pathologist as Diagnostic Oncologist

1. Interpretation, reporting and integration
2. Understand clinical relevance
3. Know standards/Guidelines
4. Grey zones/Unusual results
5. Communicate/Consult

YOU guide treatment decisions

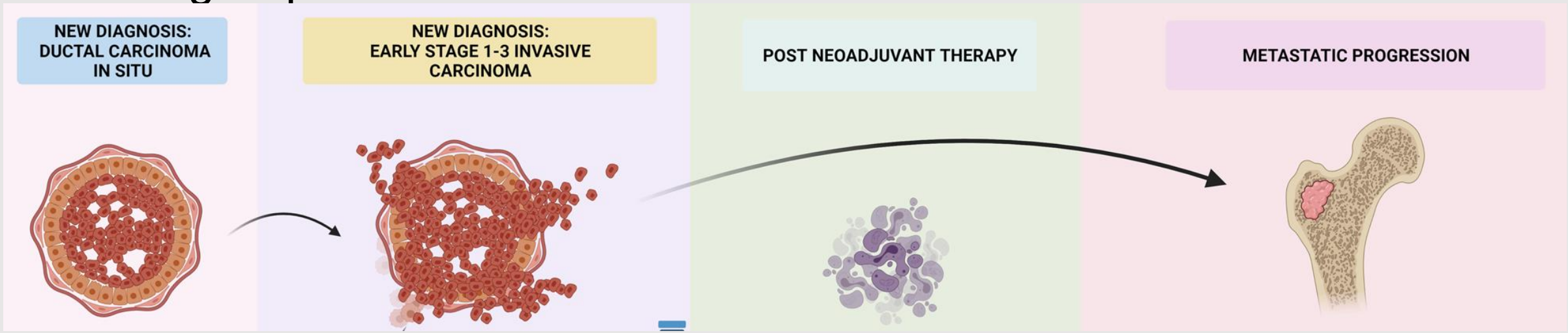
YOU are the expert



Allison KH. Ancillary Prognostic and Predictive Testing in Breast Cancer: Focus on Discordant, Unusual or Borderline Results. Surgical Pathology 11 (2018) 147-176

Breast Cancer Biomarkers Used in Clinical Management

- Different biomarkers relevant at different stages, timepoints and subgroups

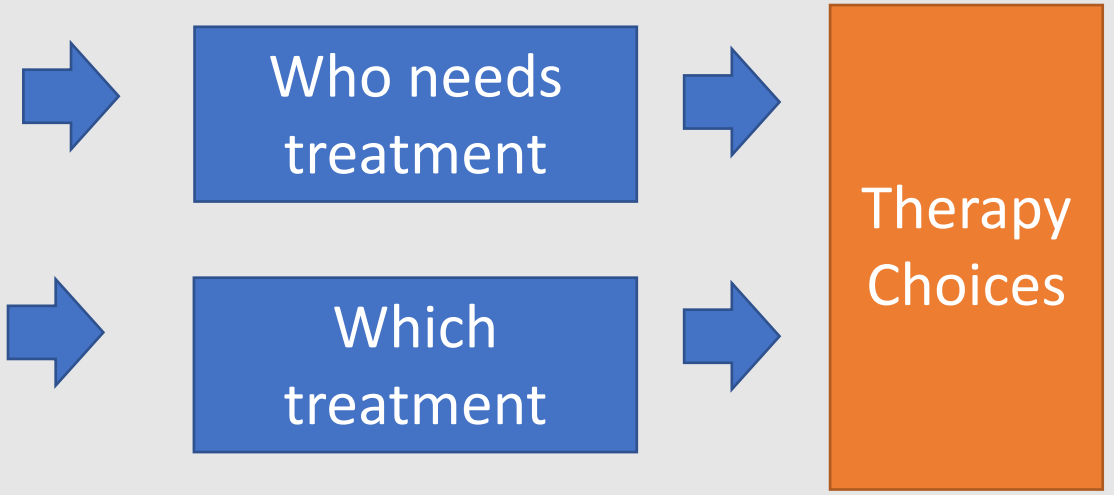


- Not all biomarkers are “molecular”
- Some are prognostic and some are predictive → both used in management decisions

Najjar S, Allison KH. Updates on breast biomarkers. Virchows Arch. 2022 Jan 14. doi: 10.1007/s00428-022-03267-x. Epub ahead of print. PMID: 35029776.

Prognostic vs Predictive Factors

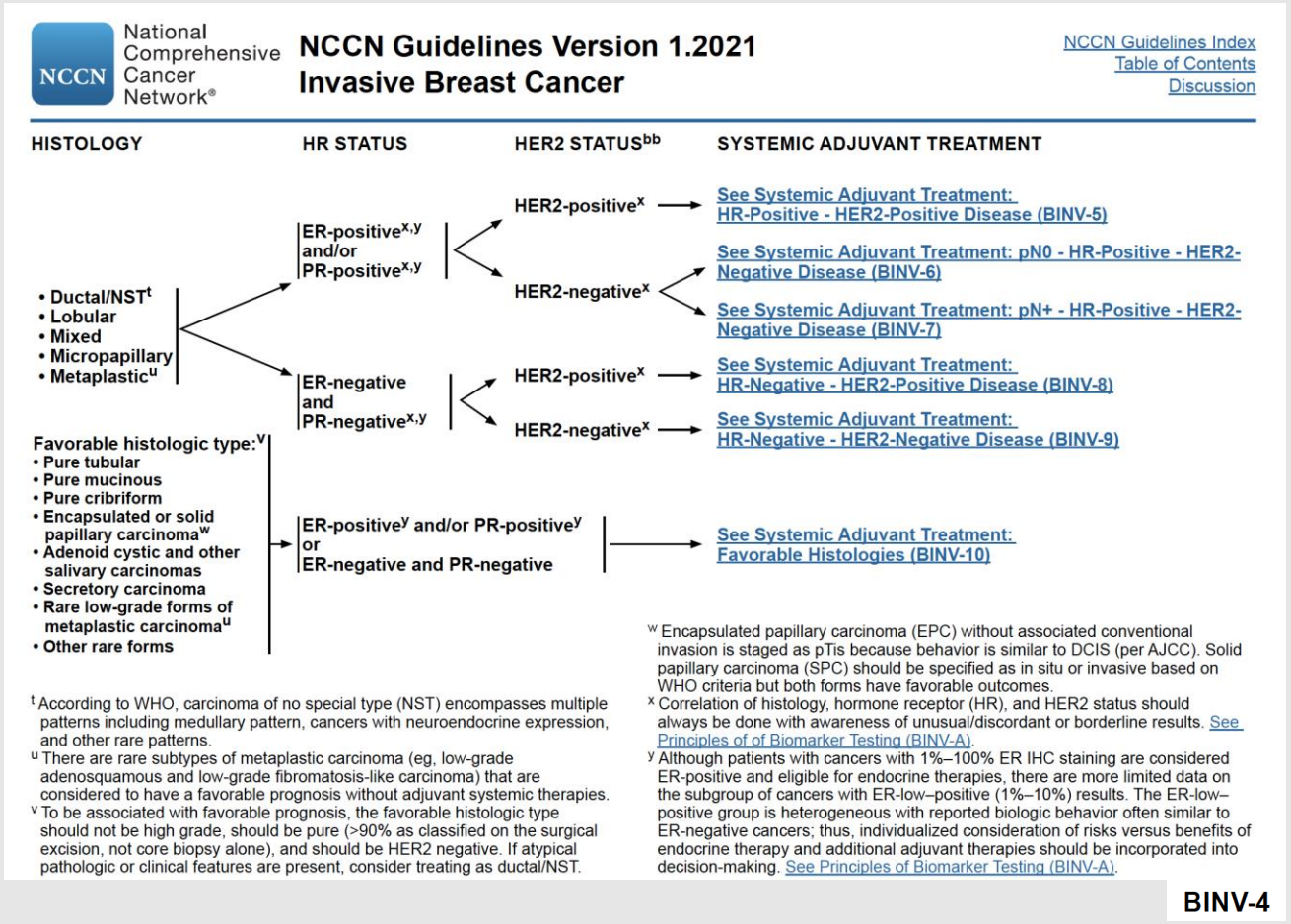
- **Prognostic Factor**: Defines natural history/outcomes (without therapy or with standard therapy)
- **Predictive Factor:** Associated likelihood of benefit from specific treatment



Evidence: Randomized controlled trial showing biomarker linked to response to Rx
Guidelines/FDA Approved methods on how to test and interpret.

Most post powerful prognostic factors: Determine overall treatment pathways

- *Special Histologic Type*
- ER status, HER2 status
- TNM stage:
  - Stages 1-3
  - Stage 4



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NCCN Guidelines Version 1.2021
Invasive Breast Cancer

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HISTOLOGY	HR STATUS	HER2 STATUS[bb]	SYSTEMIC ADJUVANT TREATMENT
• Ductal/NST[t] • Lobular • Mixed • Micropapillary • Metaplastic[u]	ER-positive[x,y] and/or PR-positive[x,y]	HER2-positive[x]	See Systemic Adjuvant Treatment: HR-Positive - HER2-Positive Disease (BINV-5)
		HER2-negative[x]	See Systemic Adjuvant Treatment: pN0 - HR-Positive - HER2-Negative Disease (BINV-6)
			See Systemic Adjuvant Treatment: pN+ - HR-Positive - HER2-Negative Disease (BINV-7)
	ER-negative and PR-negative[x,y]	HER2-positive[x]	See Systemic Adjuvant Treatment: HR-Negative - HER2-Positive Disease (BINV-8)
		HER2-negative[x]	See Systemic Adjuvant Treatment: HR-Negative - HER2-Negative Disease (BINV-9)
Favorable histologic type:[v] • Pure tubular • Pure mucinous • Pure cribriform • Encapsulated or solid papillary carcinoma[w] • Adenoid cystic and other salivary carcinomas • Secretory carcinoma • Rare low-grade forms of metaplastic carcinoma[u] • Other rare forms	ER-positive[y] and/or PR-positive[y] or ER-negative and PR-negative		See Systemic Adjuvant Treatment: Favorable Histologies (BINV-10)

[t] According to WHO, carcinoma of no special type (NST) encompasses multiple patterns including medullary pattern, cancers with neuroendocrine expression, and other rare patterns.

[u] There are rare subtypes of metaplastic carcinoma (eg, low-grade adenosquamous and low-grade fibromatosis-like carcinoma) that are considered to have a favorable prognosis without adjuvant systemic therapies.

[v] To be associated with favorable prognosis, the favorable histologic type should not be high grade, should be pure (>90% as classified on the surgical excision, not core biopsy alone), and should be HER2 negative. If atypical pathologic or clinical features are present, consider treating as ductal/NST.

[w] Encapsulated papillary carcinoma (EPC) without associated conventional invasion is staged as pTis because behavior is similar to DCIS (per AJCC). Solid papillary carcinoma (SPC) should be specified as in situ or invasive based on WHO criteria but both forms have favorable outcomes.

[x] Correlation of histology, hormone receptor (HR), and HER2 status should always be done with awareness of unusual/discordant or borderline results. See Principles of of Biomarker Testing (BINV-A).

[y] Although patients with cancers with 1%–100% ER IHC staining are considered ER-positive and eligible for endocrine therapies, there are more limited data on the subgroup of cancers with ER-low–positive (1%–10%) results. The ER-low–positive group is heterogeneous with reported biologic behavior often similar to ER-negative cancers; thus, individualized consideration of risks versus benefits of endocrine therapy and additional adjuvant therapies should be incorporated into decision-making. See Principles of Biomarker Testing (BINV-A).

BINV-4

BCC 2021
ST. GALLEN INTERNATIONAL BREAST CANCER CONFERENCE
17 – 20 March 2021, Vienna /Austria

St Gallen 2019 Summary: Burstein HJ. *Annals of Oncology* 30:1541-1557, 2019

Is ER Prognostic or Predictive?

A. Prognostic
B. Predictive
C. Both
D. Neither

Is ER Prognostic or Predictive?

A. Prognostic
B. Predictive
C. Both
D. Neither

ER Neg cancers have worse OS than ER Positive cancers

➔ Prognostic

ER Pos cancers may benefit from hormone therapy but ER negative cancers do not

➔ Predictive

Multiple current uses of ER/PR Testing

1. Determining potential benefit from endocrine therapies
2. Overall treatment pathways determined by ER+ vs ER- (ex. NCCN guidelines)
3. Surrogates for intrinsic/molecular subtype determination (along with HER2)
4. Prognostic role (ex. AJCC prognostic subgroups)
5. Metastatic setting: ER+ vs ER- treatments
6. Diagnostic testing (is metastatic cancer breast?)

Test validated for as a predictive biomarker = Guideline's focus

Is the 1% threshold valid for all uses?

Is PR Prognostic or Predictive?

A. Prognostic
B. Predictive
C. Both
D. Neither

Is PR Prognostic or Predictive?

A. Prognostic
B. Predictive
C. Both
D. Neither

Lower PR correlates with worse outcomes in hormone treated ER+ cancers

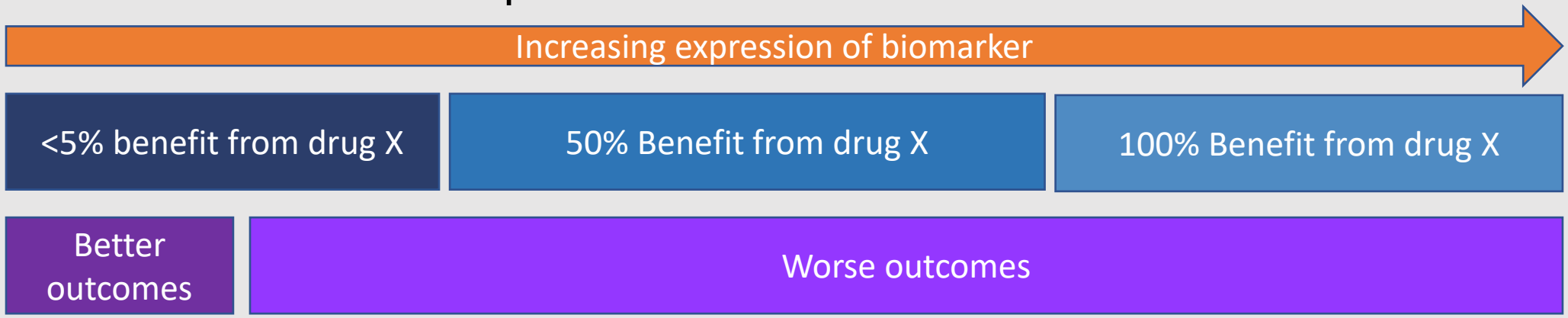
→ Prognostic in specific population of breast cancers

Both PR positive and PR negative cases can respond to endocrine therapy

→ Not predictive of endocrine therapy benefit

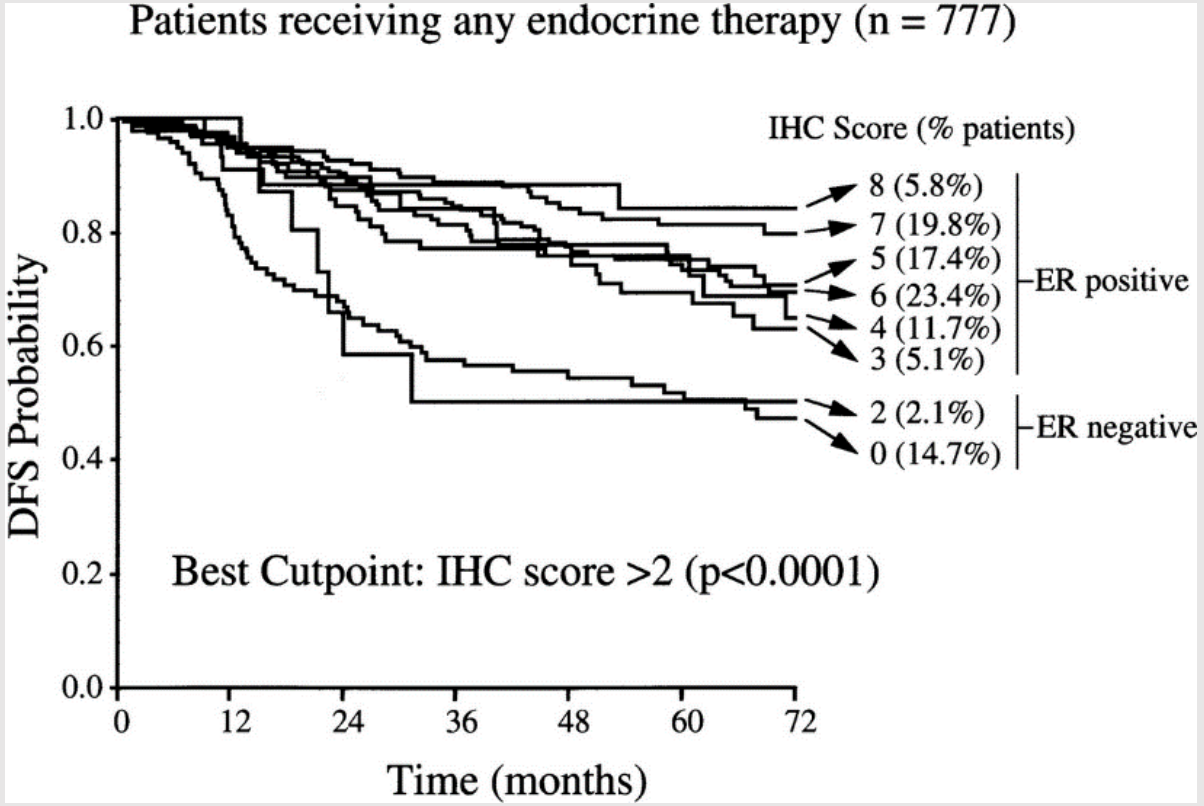
Setting Thresholds for Biomarkers

- Dependent on what trying to prognosticate vs predict:
  - Prognostic and predictive thresholds might not be the same
  - Most ideal predictive threshold will depend on risk/benefit in giving drug
- There will usually be a grey zone near the threshold
  - More variability in test results
  - Less clear clinical implications



Allred study: Showing best predictive ER threshold?

- All patients received endocrine therapy
- Actually only Prognostic…
- Samples were not standard



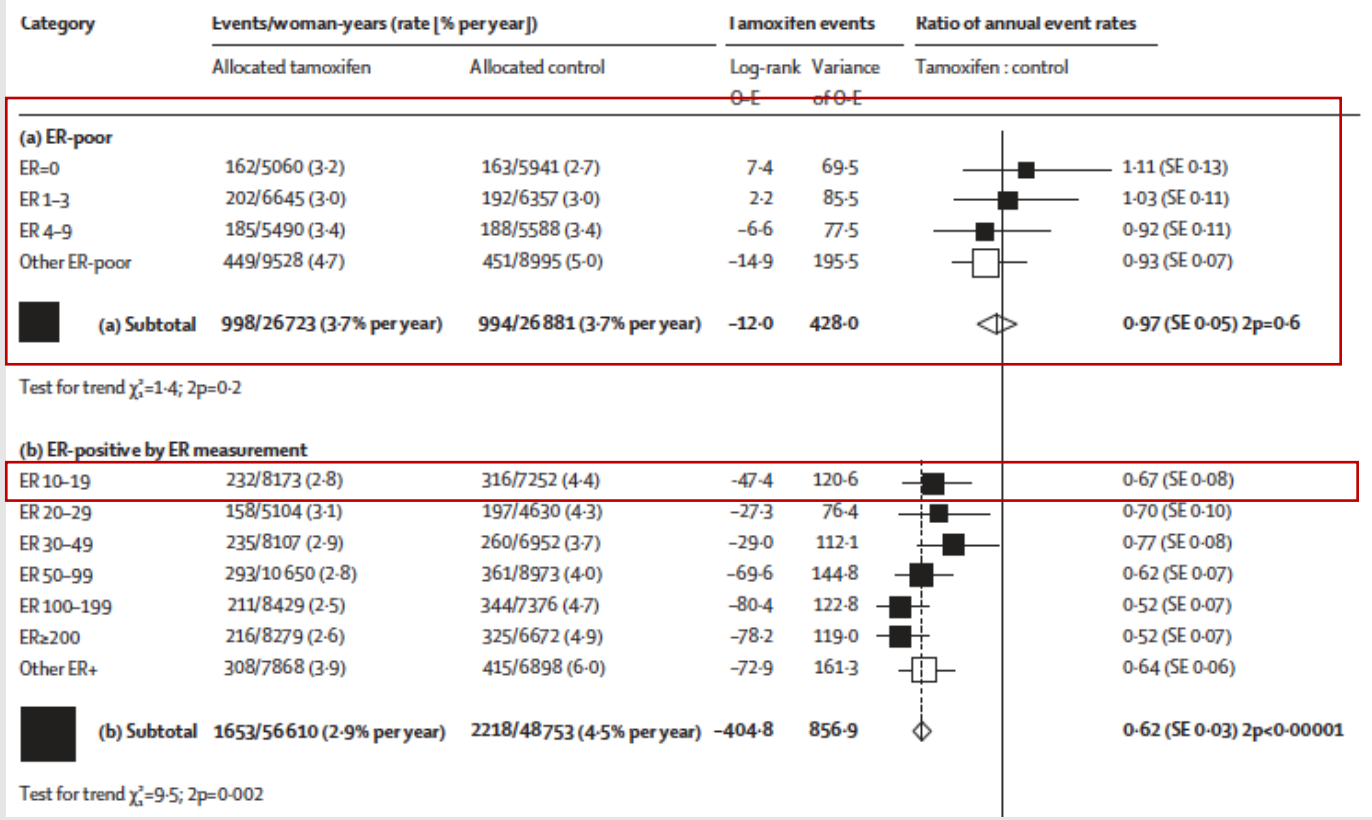
Harvey et al JCO 1999

Clinical Trial data: Best predictive threshold?

Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

*Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**

Lancet 2011; 378 771-84



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
(a) ER-poor					
ER=0	162/5060 (3·2)	163/5941 (2·7)	7·4	69·5	1·11 (SE 0·13)
ER 1–3	202/6645 (3·0)	192/6357 (3·0)	2·2	85·5	1·03 (SE 0·11)
ER 4–9	185/5490 (3·4)	188/5588 (3·4)	−6·6	77·5	0·92 (SE 0·11)
Other ER-poor	449/9528 (4·7)	451/8995 (5·0)	−14·9	195·5	0·93 (SE 0·07)
(a) Subtotal	**998/26723 (3·7% per year)**	**994/26881 (3·7% per year)**	**−12·0**	**428·0**	**0·97 (SE 0·05) 2p=0·6**
Test for trend χ^2_1=1·4; 2p=0·2					
(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<0·00001**
Test for trend χ^2_1=9·5; 2p=0·002					

- Limited clinical data on threshold – mostly based on LBA data
- 20 tamoxifen trials with over 200,000 women-years of follow-up
- Points to 10 fmol ER/mg at best threshold.
  - 10-19 fmol ER/mg had recurrence reduced by 1/3 with 5 yrs Tam

Correlates best with 1% by IHC

Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; and Antonio C. Wolff, MD[21]

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ASCO Guidelines

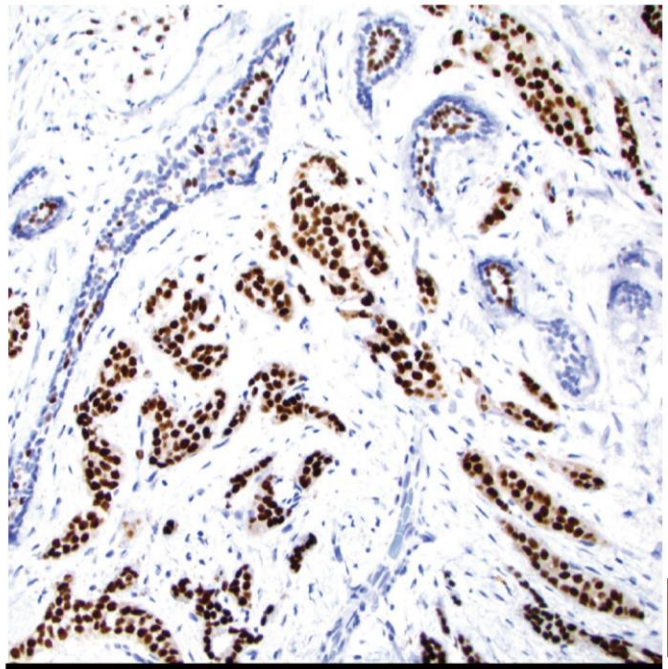
UPDATED JANUARY 2020

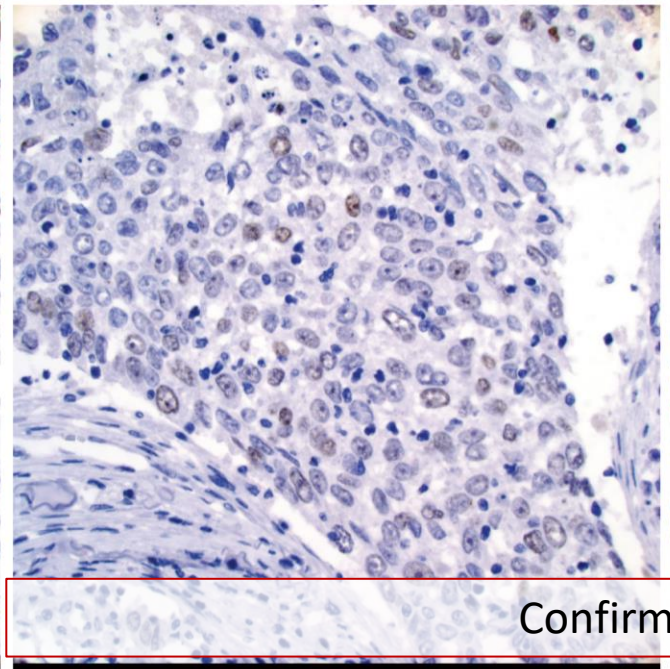
- Samples with 1-100% of tumor nuclei positive for ER or PgR are interpreted as positive.
- For reporting of ER (not PgR), if 1-10% of tumor cell nuclei are immunoreactive, the sample should be reported as ER Low Positive with a recommended comment.
- New recommendation for laboratories to establish a specific standard operating procedure to ensure the validity of low positive (1-10%) or negative (0 or < 1%) interpretations and results. *(See Supplement for Example SOP)*

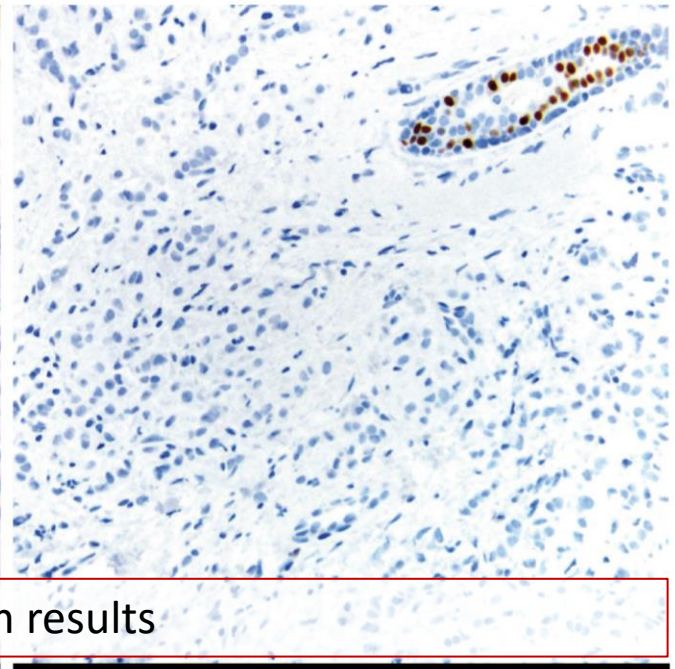
Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

ER STAIN INTERPRETATION

Evaluate overall percentage of cancer in sample with nuclear staining and intensity of stain



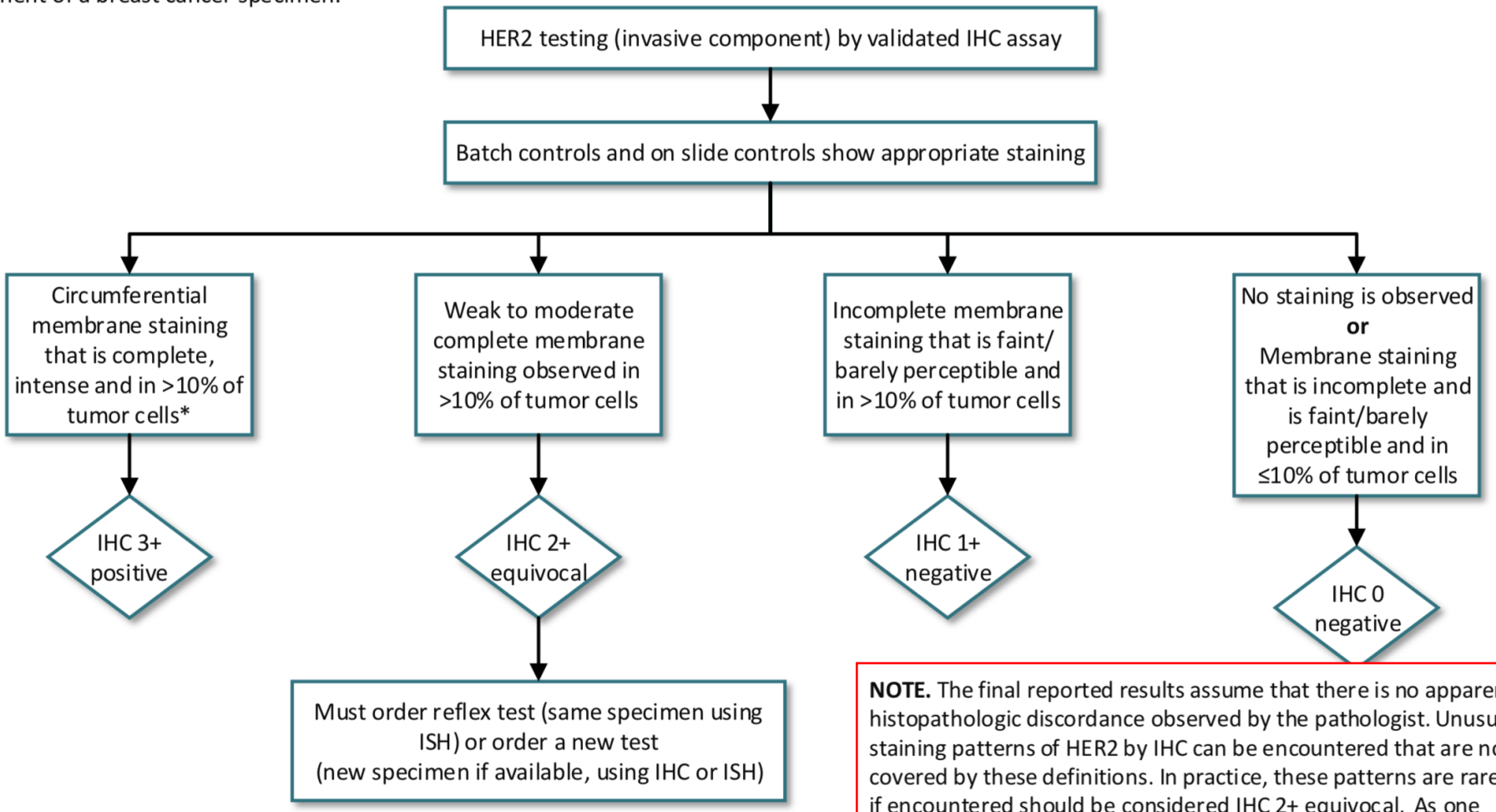


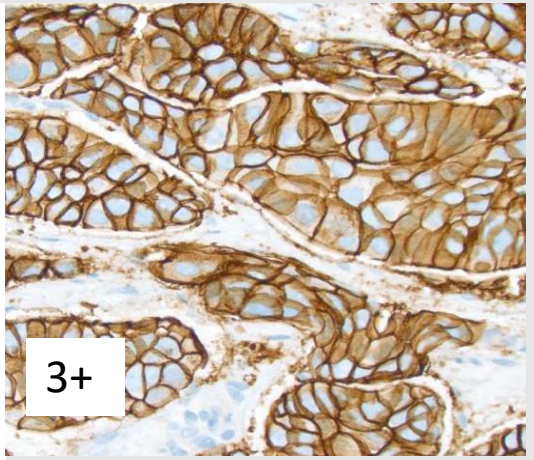


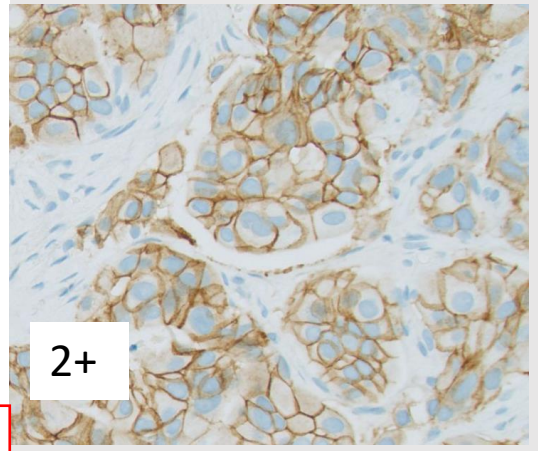
Confirm results

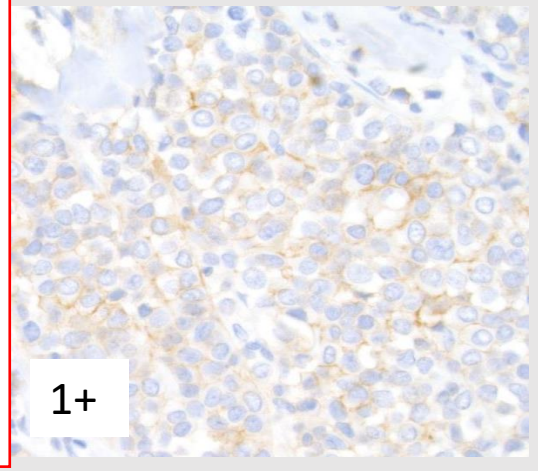
>10% staining	1-10% staining	Example of a cancer with no staining and a positve internal control
If ≥ 1% of cells stain		If < 1% or 0% cells stain*
Interpretation: Positive (include % and intensity)	**Report as ER Low Positive w/comment**	Interpretation: **Negative** (include if result was <1% or 0%)

Figure 1. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) protein expression by immunohistochemistry (IHC) assay of the invasive component of a breast cancer specimen.









NOTE. The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. Unusual staining patterns of HER2 by IHC can be encountered that are not covered by these definitions. In practice, these patterns are rare and if encountered should be considered IHC 2+ equivocal. As one example, some specific subtypes of breast cancers can show IHC staining that is moderate to intense but incomplete (basolateral or lateral) and can be found to be HER2 amplified. Another example is circumferential membrane IHC staining that is intense but within ≤10% of tumor cells (heterogeneous but very limited in extent). Such cases can be considered 2+ equivocal but additional samples may reveal different percentages of HER2 positive staining. (*)Readily appreciated using a low power objective and observed within a homogeneous and contiguous invasive cell population

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.

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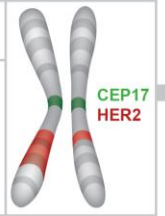
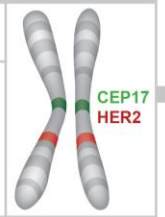
ASCO Guidelines

Grey Zones in Dual Probe HER2 ISH Test Interpretation: 2018 Update Summary

Most cases

Group 1 Result		
Ratio	HER2 sign/cell	
≥ 2.0	≥ 6.0	

→ HER2 Positive

Group 5 Result		
Ratio	HER2 sign/cell	
< 2.0	< 4.0	

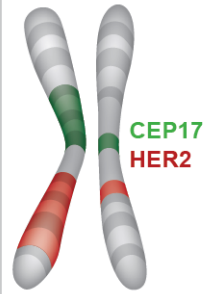
→ HER2 Negative

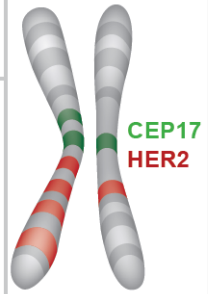
Grey Zones and Borderline Results:
Confirmation, correlation and explanation

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.
WHO 5th edition Tumours of the Breast 2019

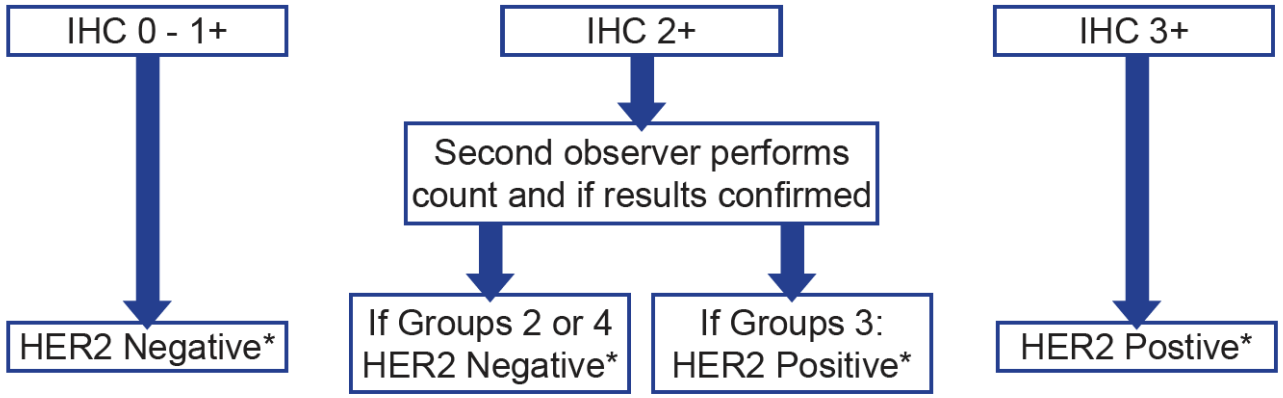
Unusual HER2 ISH Result Categories Requiring Additional Work-Up

Group 2 Result		
Ratio	HER2 sign/cell	
≥ 2.0	< 4.0	

Group 3 Result		
Ratio	HER2 sign/cell	
< 2.0	≥ 6.0	

Group 4 Result		
Ratio	HER2 sign/cell	
< 2.0	4.0 - 6.0	

Review concurrent IHC from the same sample

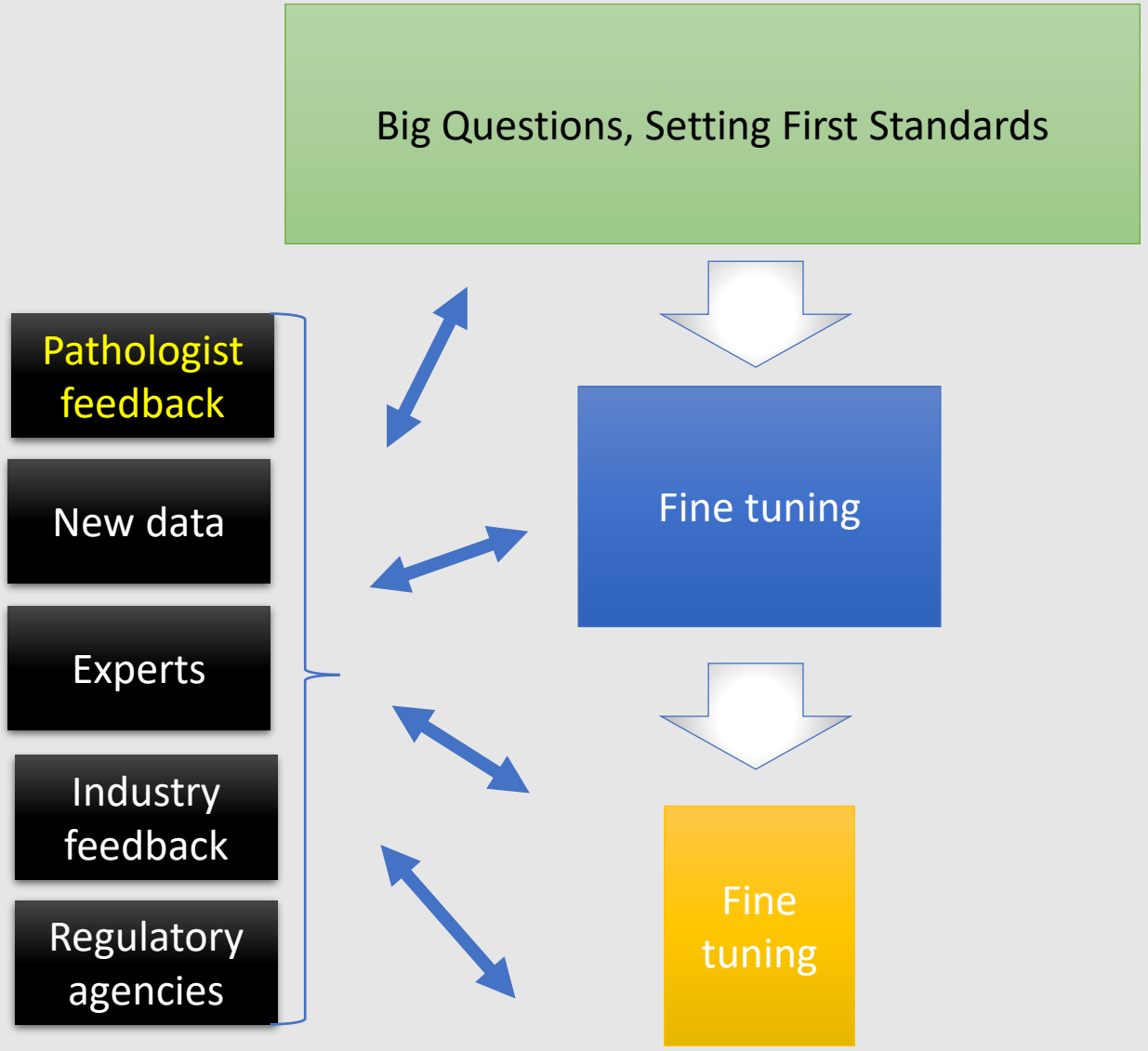


*As determined by concurent IHC and ISH. Report comments recommended (see ASCO/CAP guidelines for details). {29846104}

Report final result based on IHC + ISH, include required comments

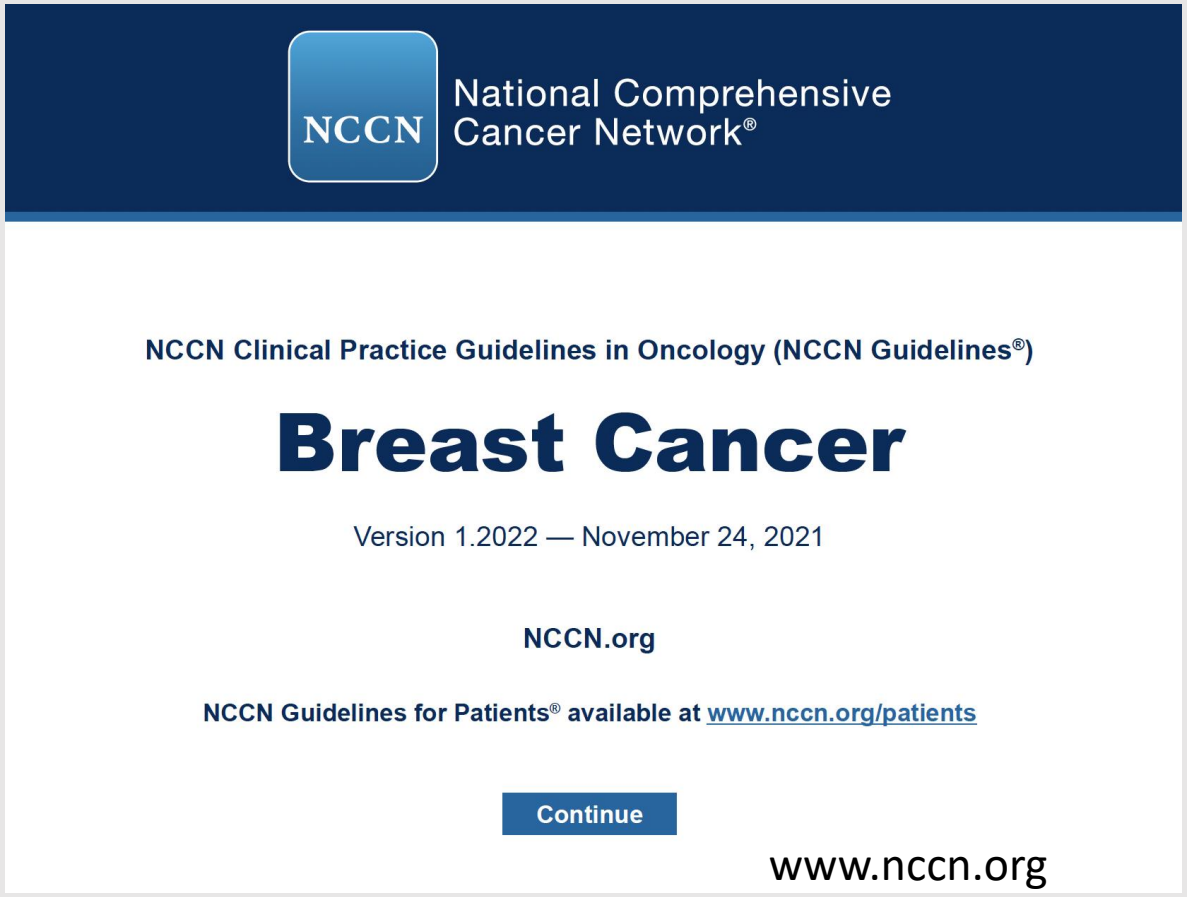
Guidelines in Breast Cancer are Living Documents

- First focused on big questions and standards for all cases
- Subsequent updates based on new data, feedback
- Fine tuning, often focused on less common scenarios



Initial Breast Cancer Diagnosis: What matters most clinically?

Initial Breast Cancer Diagnosis: What are clinical “game changers”?



NCCN Guidelines are continuously updated and available to download

Allison KH. Prognostic and predictive parameters in breast pathology: a pathologist's primer. Mod Pathol. 2021 Jan;34(Suppl 1):94-106. doi: 10.1038/s41379-020-00704-7. Epub 2020 Nov 5. PMID: 33154551.

ER+, HER2- Invasive Cancer Treatment

Which factors are used to determine if need to add chemotherapy to endocrine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. 21-gene RT-PCR Assay Recurrence Score
6. Nottingham grade
7. Margins

ER+, HER2- Invasive Cancer Treatment

Which factors are used to determine if need to add chemotherapy to endocrine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. 21-gene RT-PCR Assay Recurrence Score
6. Nottingham grade
7. Margins

Treatment of Favorable Histologies

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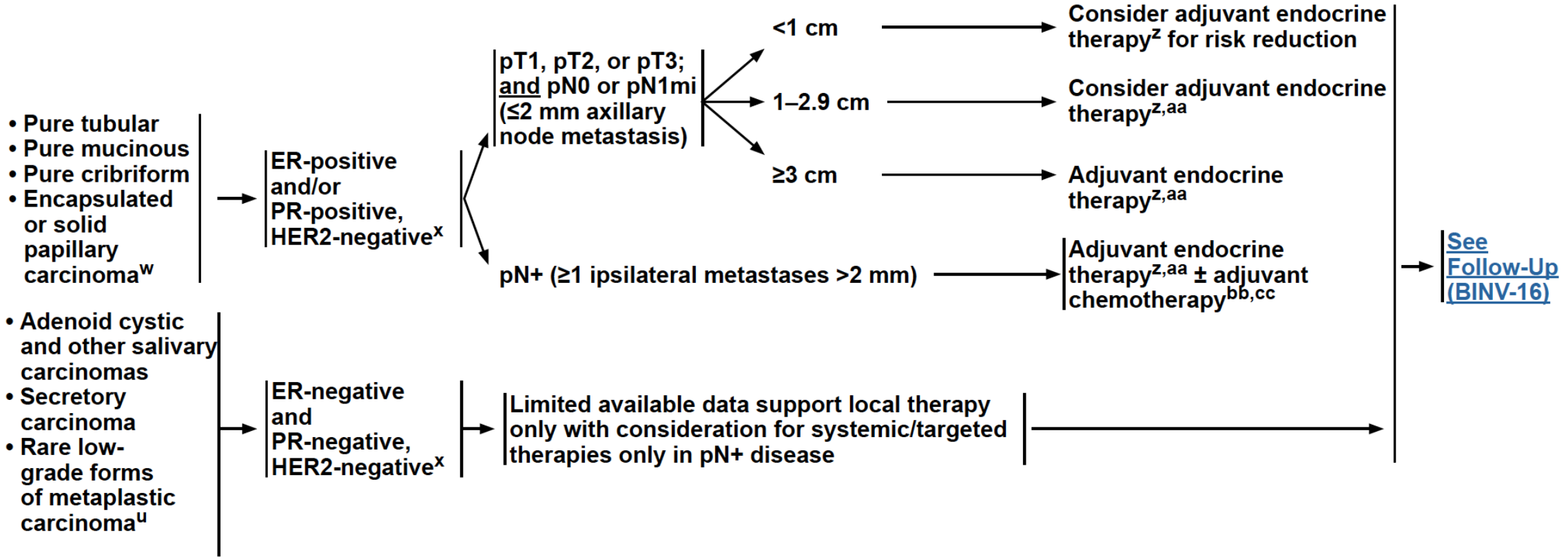
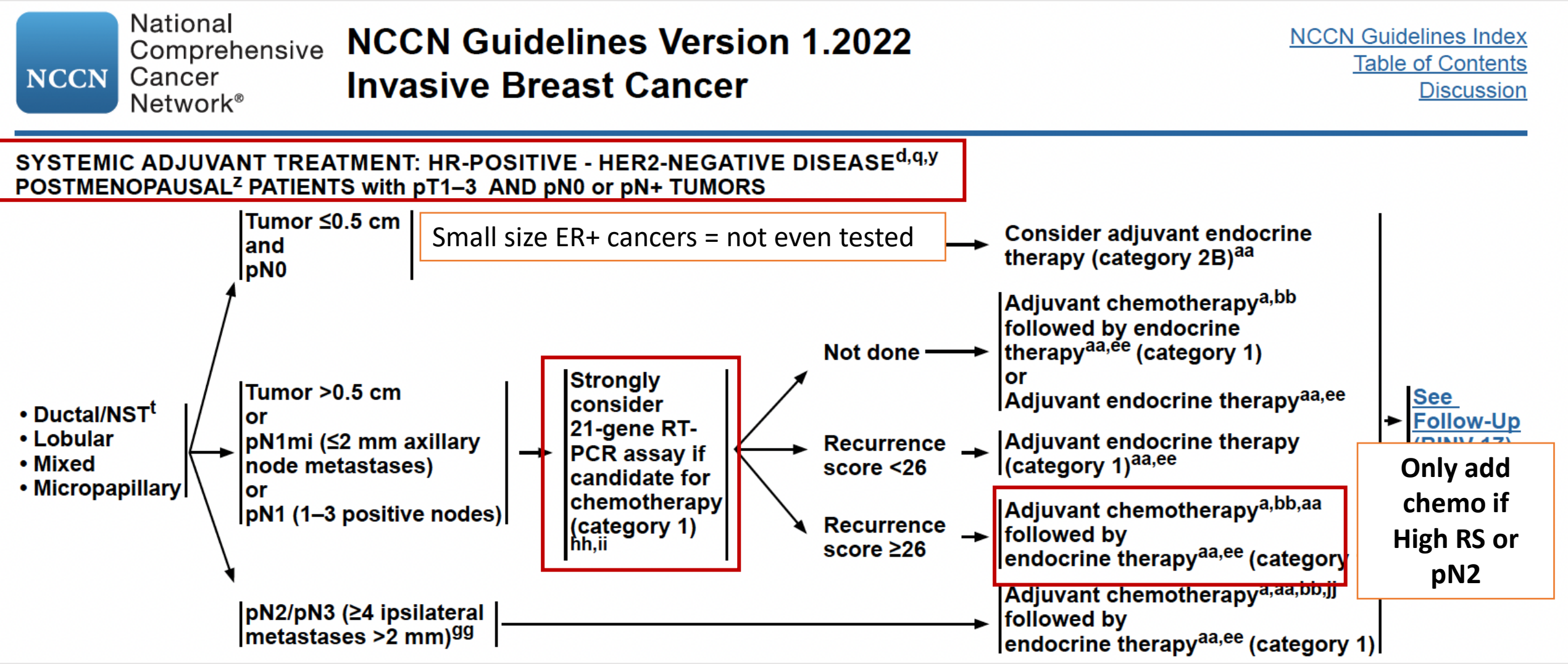
Importance of criteria and correlation

SYSTEMIC ADJUVANT TREATMENT: FAVORABLE HISTOLOGIES[q,v]



ER+, HER2- and Postmenopausal



OncotypeDX: RT-PCR Proliferation-Driven Test

16 Cancer and 5 Reference Genes

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

INVASION
Stromelysin 3
Cathepsin L2

CD68

HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Category	RS (0-100)
Low risk	RS <18
Int risk	RS ≥18 and <31
High risk	RS ≥31

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Gene Expression Assays

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GENE EXPRESSION ASSAYS FOR CONSIDERATION OF ADJUVANT SYSTEMIC THERAPY[a,b]

Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus	Recurrence Risk and Treatment Implications
21-gene (Oncotype Dx) (for pN0)	Yes	Yes	Preferred	1	BINV-N (2 of 5)
21-gene (Oncotype Dx) for pN1 (1–3 positive nodes)[c]	Yes	Yes	Postmenopausal: Preferred	1	BINV-N (2 of 5)
			Premenopausal: Other	2A	
70-gene (MammaPrint) for pN0 and pN1 (1–3 positive nodes)	Not determined	Yes	Other	1	BINV-N (3 of 5)
50-gene (Prosigna) for pN0 and pN1 (1–3 positive nodes)	Not determined	Yes	Other	2A	BINV-N (3 of 5)
12-gene (EndoPredict) for pN0 and pN1 (1–3 positive nodes)	Not determined	Yes	Other	2A	BINV-N (3 of 5)
Breast Cancer Index (BCI)	Predictive of benefit of extended adjuvant endocrine therapy	Yes	Other	2A	BINV-N (4 of 5)

ER+, HER2- and Pre-menopausal with pN0

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2022
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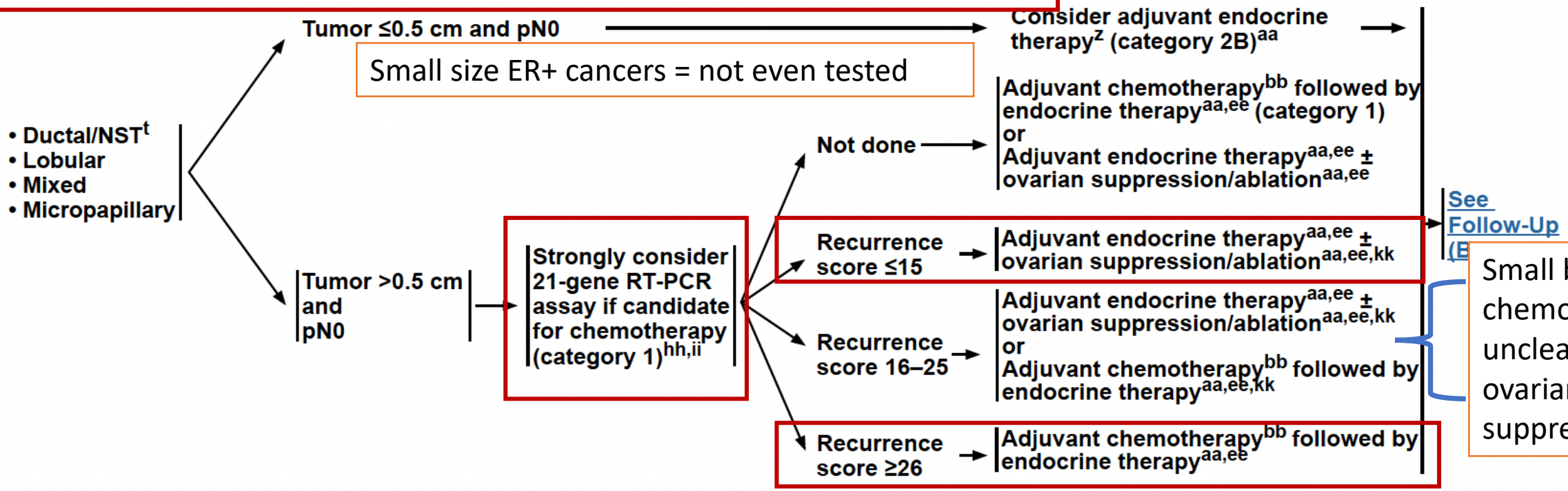
SYSTEMIC ADJUVANT TREATMENT: HR-POSITIVE - HER2-NEGATIVE DISEASE[d,q,y]
PREMENOPAUSAL[z] PATIENTS with pT1–3 AND pN0 TUMORS



[d] See Principles of Biomarker Testing (BINV-A).
[q] See Special Considerations for Breast Cancer in Males (Sex Assigned at Birth) (BINV-I)

ER+, HER2- and Pre-menopausal with pN1+

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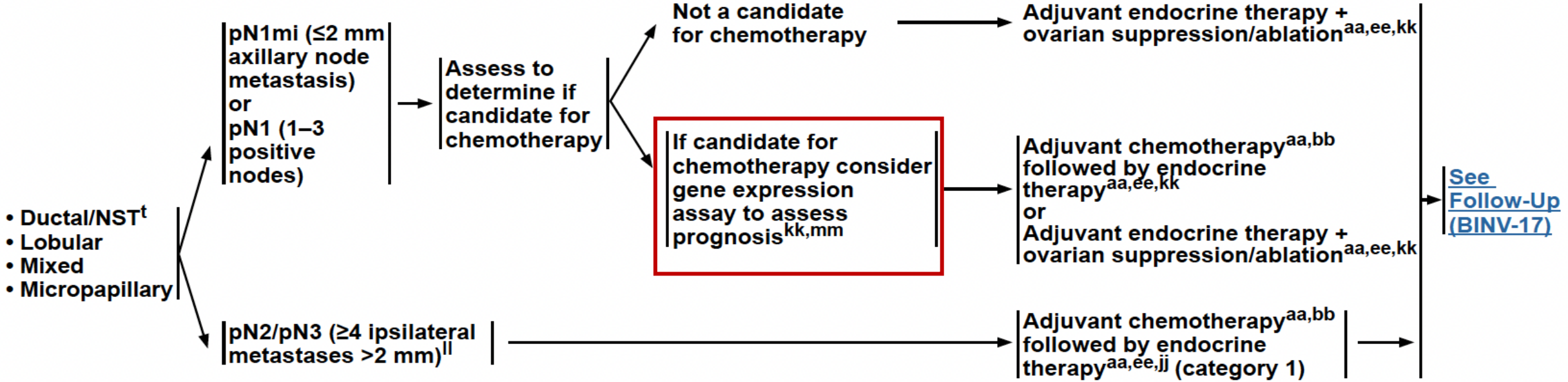
SYSTEMIC ADJUVANT TREATMENT: HR-POSITIVE - HER2-NEGATIVE DISEASE[d,q,y]
PREMENOPAUSAL[z] PATIENTS with pT1–3 AND pN+ TUMORS



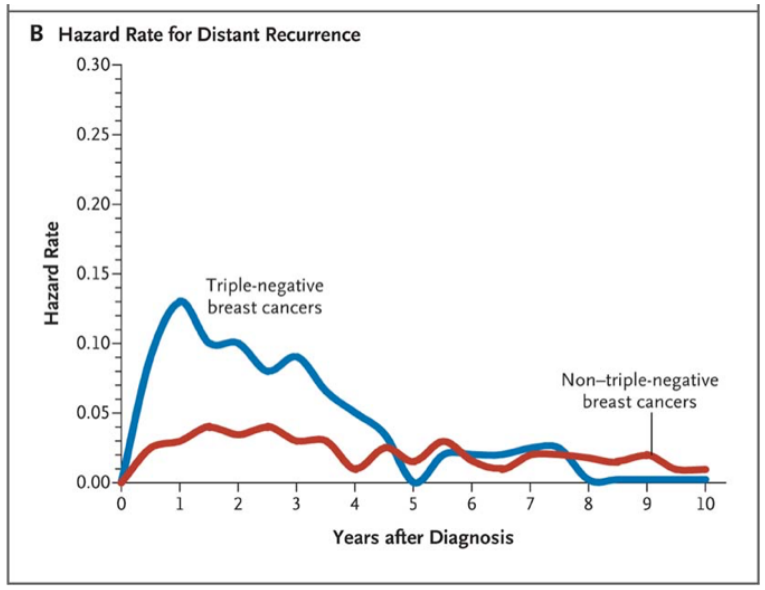
ER+, HER2- Invasive Cancer Treatment

Which factors are used to determine if need to add chemotherapy to endocrine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary (> 0.5 cm)
4. Lymph node status
5. 21-gene RT-PCR Assay Recurrence Score
6. Nottingham grade
7. Margins

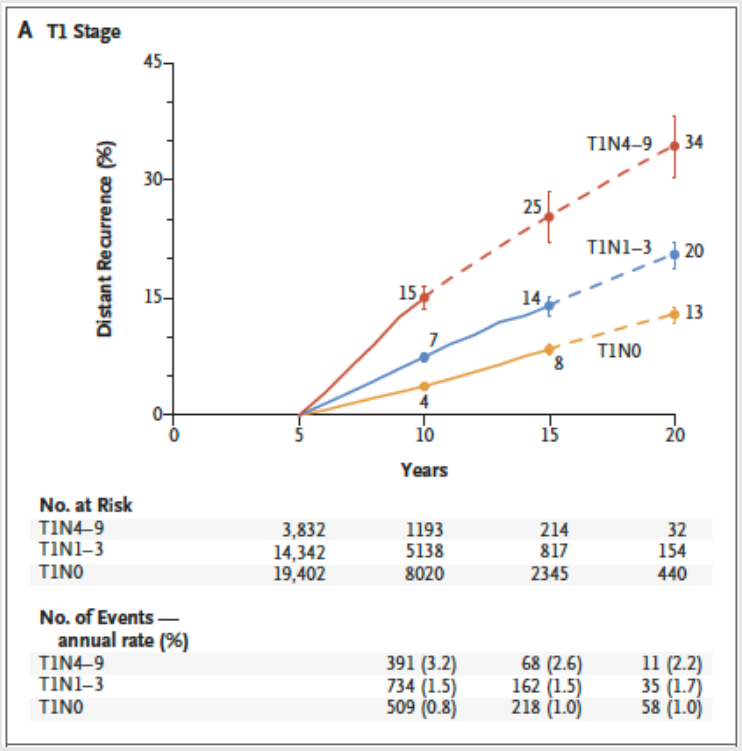
Future Risk in ER+ Breast Cancer

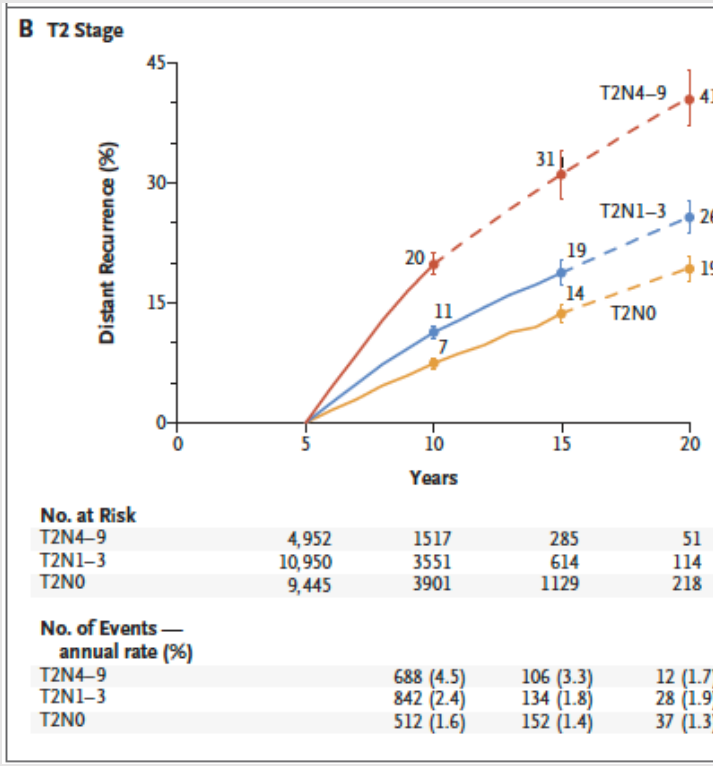
- Within 5 years for ER negative, decades for ER positive



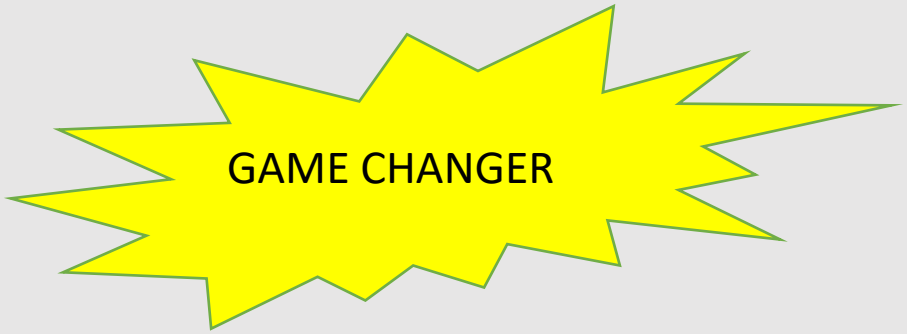
Foulkes WD et al. N Engl J Med 2010;363:1938-1948

- Original T and N stage remain relevant in ER+ cancers long term (high vs low risk)





Pan H et al. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. NEJM (2017) 377 (19) 1836-46



Additional options for risk reduction in highest risk group of ER+, HER2- cancers?

What biomarker is used to determine eligibility?

Adjuvant Abemaciclib Combined With Endocrine Therapy for High-Risk Early Breast Cancer: Updated Efficacy and Ki-67 Analysis From the monarchE Study

O'Shaughnessy J, et al. ESMO 2021. Abstract VP8_2021

HR+, HER2-, node-positive, high-risk EBC

- ≥4 positive axillary lymph nodes (ALN)

OR

- 1-3 ALN and at least 1 of the below:
  - Tumor size ≥5 cm
  - Histologic grade 3
  - Centrally tested Ki-67 ≥20%

Randomized 1:1

- **Abemaciclib + Standard of Care Endocrine Therapy** (N=2808)
- **Standard of Care Endocrine Therapy** (N=2829)

- *Median duration of follow-up: 27 months*
- *Patients off study treatment period: 90%*

Intent to treat population
N=5637

Invasive disease-free survival (IDFS)
Primary objective

Abemaciclib + ET	ET alone

30% reduction in risk of developing an IDFS event
HR=0.70 (nominal p-value <0.0001)

2.7% absolute difference in **2-year IDFS rates**

92.7% vs 90.0%

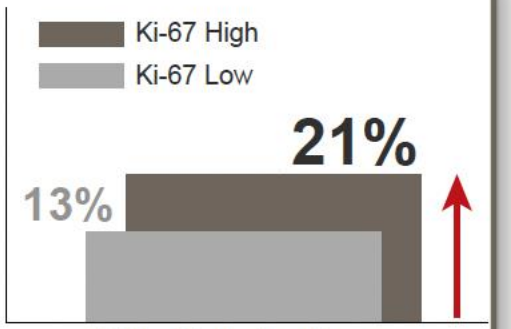
5.4% absolute difference in **3-year IDFS rates**

88.8% vs 83.4%

Cohort 1
N=3917

Inclusion based on clinicopathological risk factors	**Ki-67 High** (≥20%) N=2003	**Ki-67 Low** (<20%) N=1914
Reduction in risk of developing an IDFS event	**37%** *HR=0.63 (p<0.001)*	**30%** *HR=0.70 Exploratory analysis*

Consistent treatment benefit in patients whose tumors had high clinicopathological risk factors, regardless of their Ki-67 index.



Risk of invasive disease at 3 years in control arm

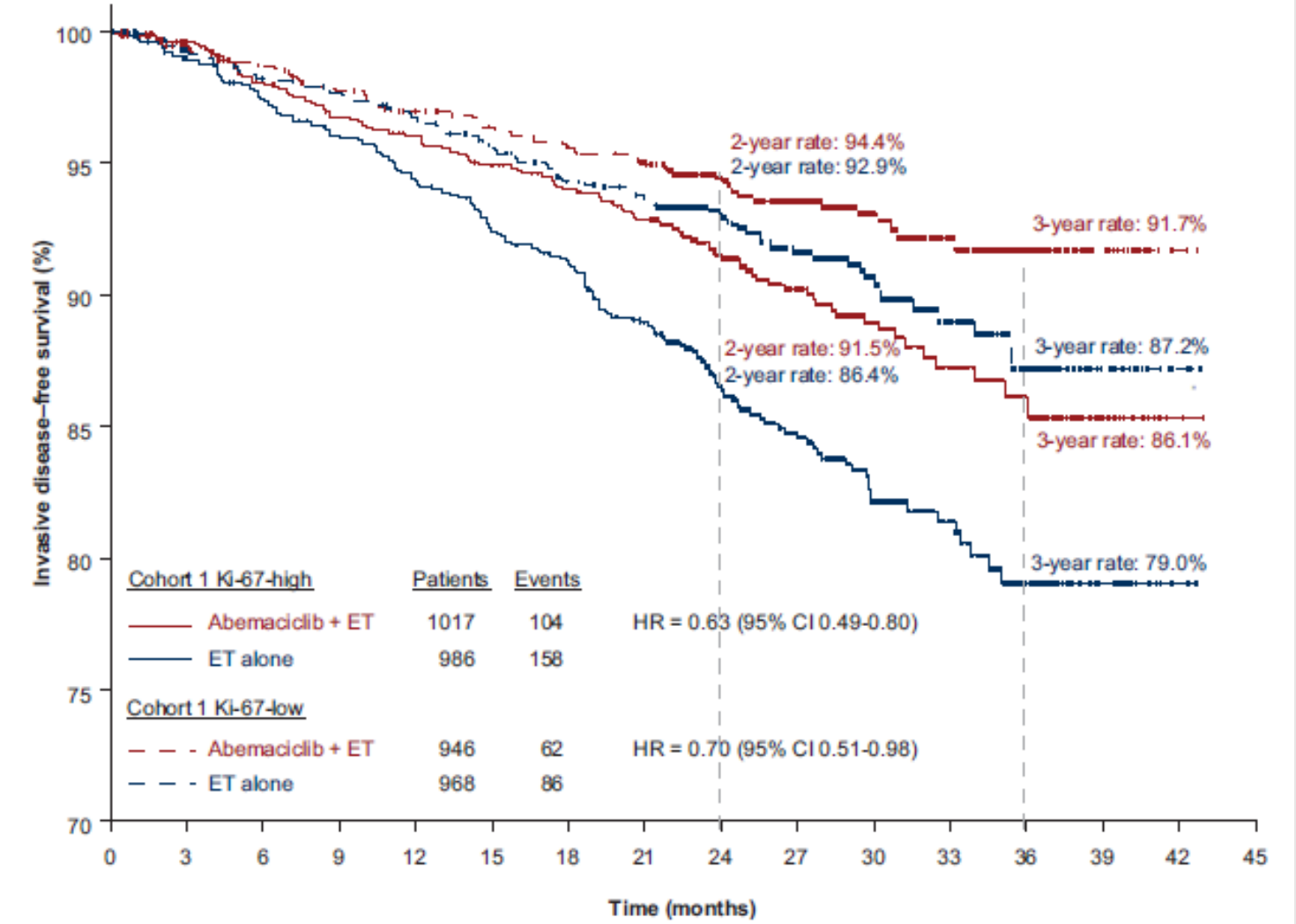
Greater risk of recurrence in patients with high Ki-67 tumors, confirming the prognostic value of Ki-67.

- Abemaciclib + ET significantly reduced the risk of developing an IDFS event in patients with HR+, HER2-, node-positive, high risk EBC.
- The robust treatment benefit was confirmed and maintained beyond the 2-year treatment period with abemaciclib.

monarchE *iDFS*

iDFS in Cohort 1 in Patients With High vs Low Ki-67

- A high Ki-67 index was prognostic of a worsened outcome
- The benefit of abemaciclib + ET vs ET alone was seen regardless of Ki-67 index



eck N, et al. Ann Oncol. 2021;32:1571-1581.

Abemaciclib
FDA Approval and Guideline Recommendations

FDA[a]

In combination with endocrine therapy for the adjuvant treatment of adult patients with HR+, HER2-, node-positive, EBC at high risk of recurrence and Ki-67 ≥ 20% as determined by an FDA approved test

ASCO®[b]

Two years of abemaciclib plus ET can be offered for patients with node-positive HR+/HER2- high-risk breast cancer and:

- Ki-67 ≥ 20%

OR

- ≥ 4 positive ALNs or 1-3 positive ALNs and 1 or more of the following features: histologic Grade 3 disease, tumor size ≥ 5 cm, or Ki-67 ≥ 20%

NCCN[c]

Two years of abemaciclib plus endocrine therapy can be considered in patients with HR+/HER2- high-risk breast cancer:

- ≥ 4 positive lymph nodes

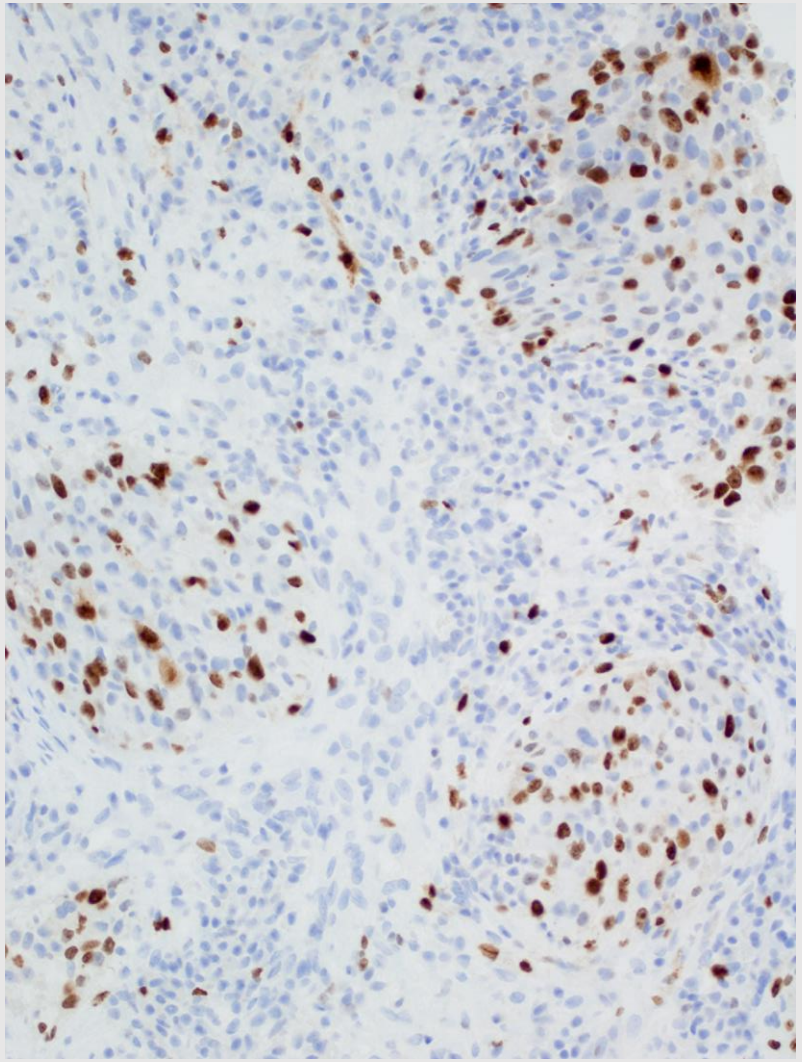
OR

- 1-3 positive lymph nodes and Grade 3 disease or tumor size ≥ 5 cm OR Ki-67 ≥ 20%

- a. Abemaciclib [PI]. Approved 2017. Revised October 2021; b. Giordano SH, et al. J Clin Oncol. 2022;40:307-309; c. NCCN Guidelines®. Breast cancer. V2.2022.

Ki67 assay

- Old assay (MIB-1 most common) many labs already use for proliferation in tumors
- Used in breast cancer as a prognostic factor
  - CAP 2019 Q-probe: 62% of labs report
  - Reproductivity issues (Intern Ki67 WG recommendations)
- Now being used as a “predictive” assay in breast cancer → abemaciclib
- pharmDX = DAKO Omnis platform (few labs have)
- How to validate and score?



Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group

- Ki67 useful only as prognostic indicator in ER+, HER2-, T1-2, N0-1 group
- Only Ki67 ≤ 5% or ≥ 30% valid for decision making (10-20% has Kappa of 0.6)
- Recommend global scoring (not hot spots), low power estimations + counting 100 cells x 4 fields

Box 1: IKWG Scoring Method for Ki67 in Breast Cancer

1) Before first use, access the IKWG website (https://www.ki67inbreastcancerwg.org/) and complete the Ki67 calibration exercise
2) From Tools, link to the Online scoring app (or download and install the Ki67 counting app) and use the global method
3) Using a regular light microscope, review the Ki67-stained breast cancer slide and input estimates of the percent area with negligible, low, medium, or high Ki67 index
4) Score 100 nuclei negative or positive in each field type (as directed by the app)
5) Record "Weighted global score" output as the Ki67 index for that slide

Nielsen TO, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2021 Jul 1;113(7):808-819. doi: 10.1093/jnci/djaa201. PMID: 33369635; PMCID: PMC8487652.

pharmDX Kit

Agilent Dako

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) Interpretation Manual – Breast Carcinoma

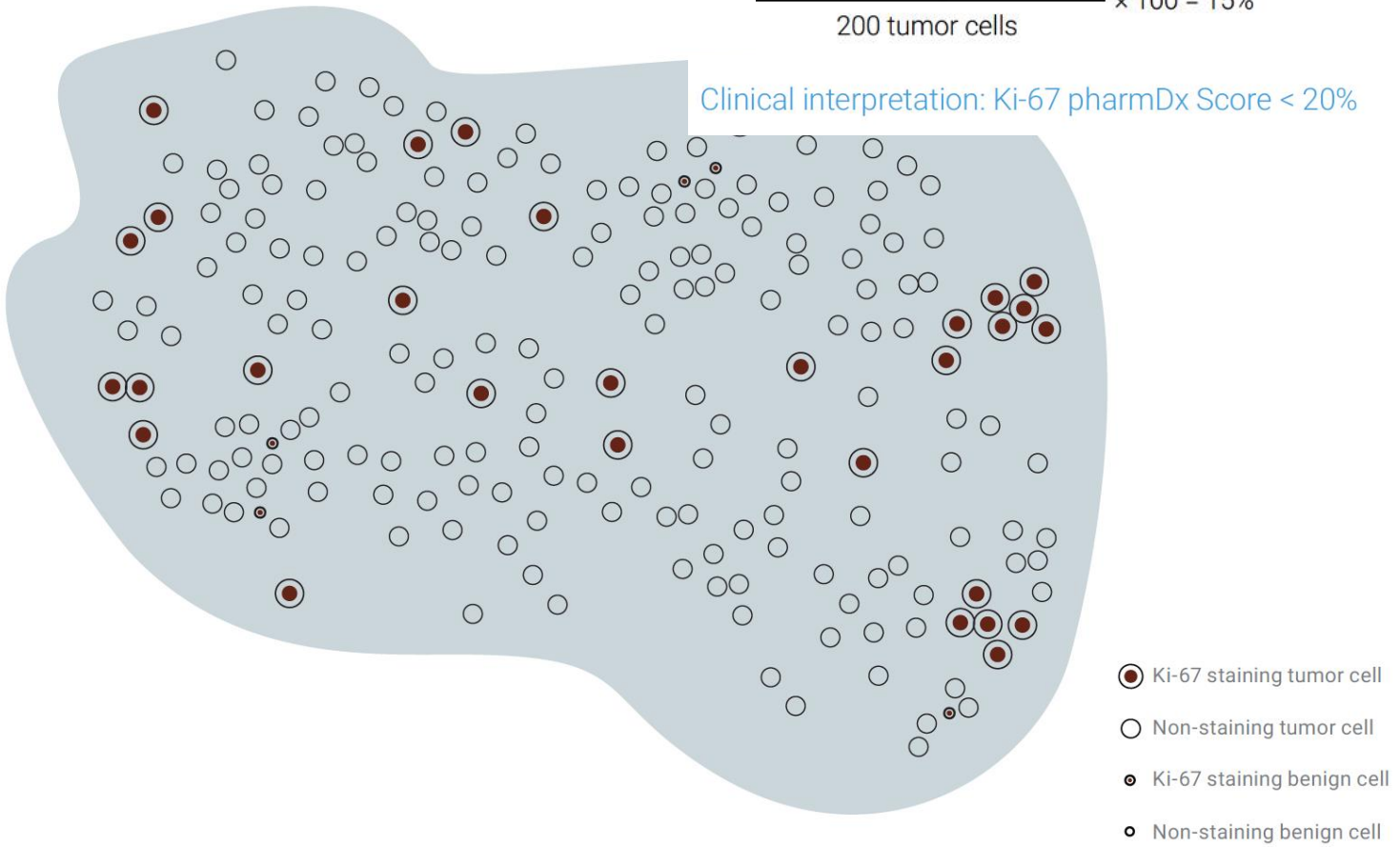
FDA approved for in vitro diagnostic use

$$\text{Ki-67 pharmDx Score (\%)} = \frac{\text{\# Ki-67 staining viable invasive tumor cells}}{\text{Total \# of staining and non-staining viable invasive tumor cells}} \times 100$$

- Calculate % over the entire sample (not just hot spots)
- Include weak staining cells
- “Agilent recommends that scoring be performed within the context of the pathologist’s past experience and best judgment in interpreting IHC stains.”

$$\frac{\text{30 Ki-67 staining tumor cells}}{\text{200 tumor cells}} \times 100 = 15\%$$

Clinical interpretation: Ki-67 pharmDx Score < 20%



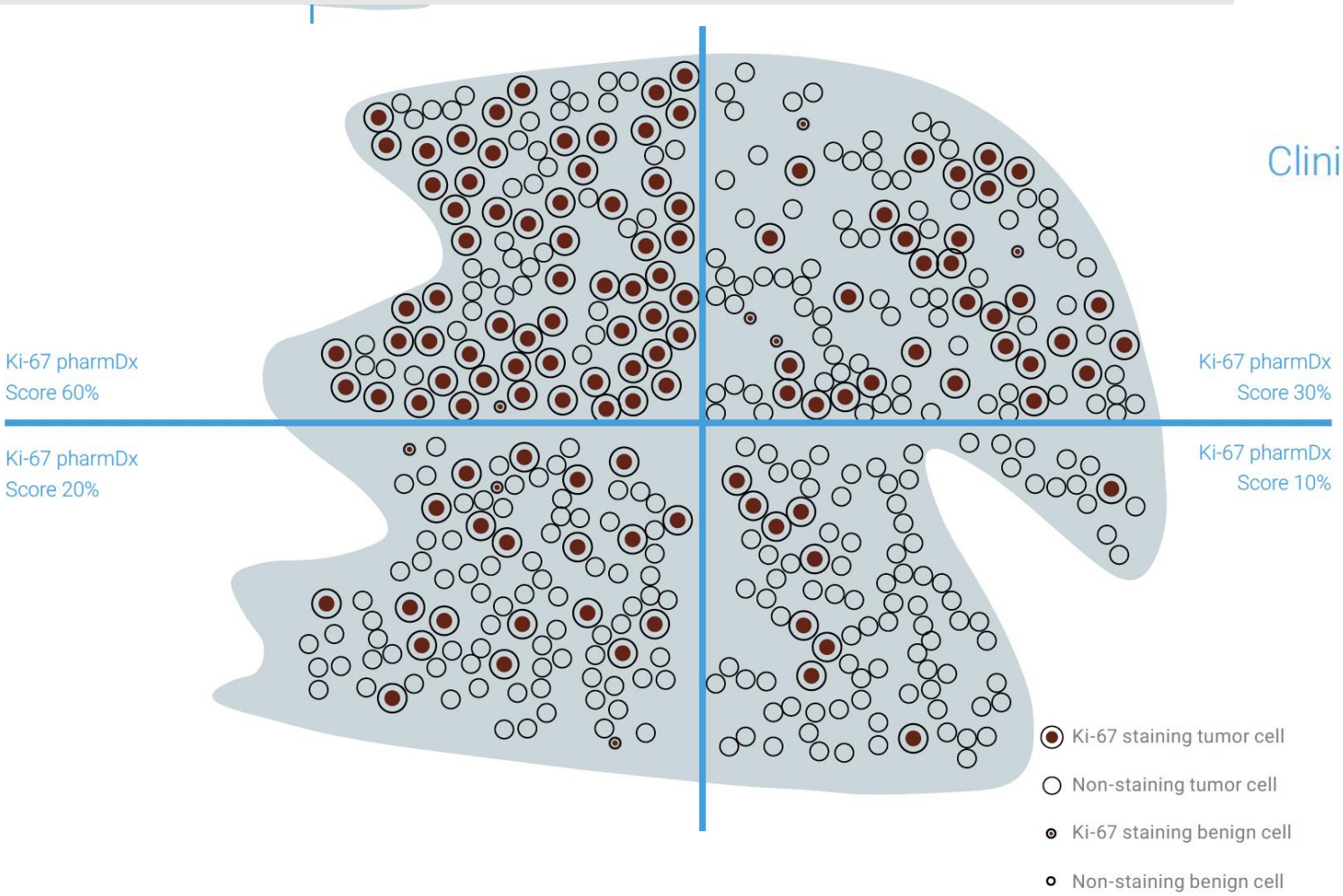
Calculate the Ki-67 pharmDx Score of the entire tumor area:

Estimating Ki67 when Heterogeneous

Assessment:

Ki-67 pharmDx Score = (60% + 30% + 20% + 10%) / 4 = 30%

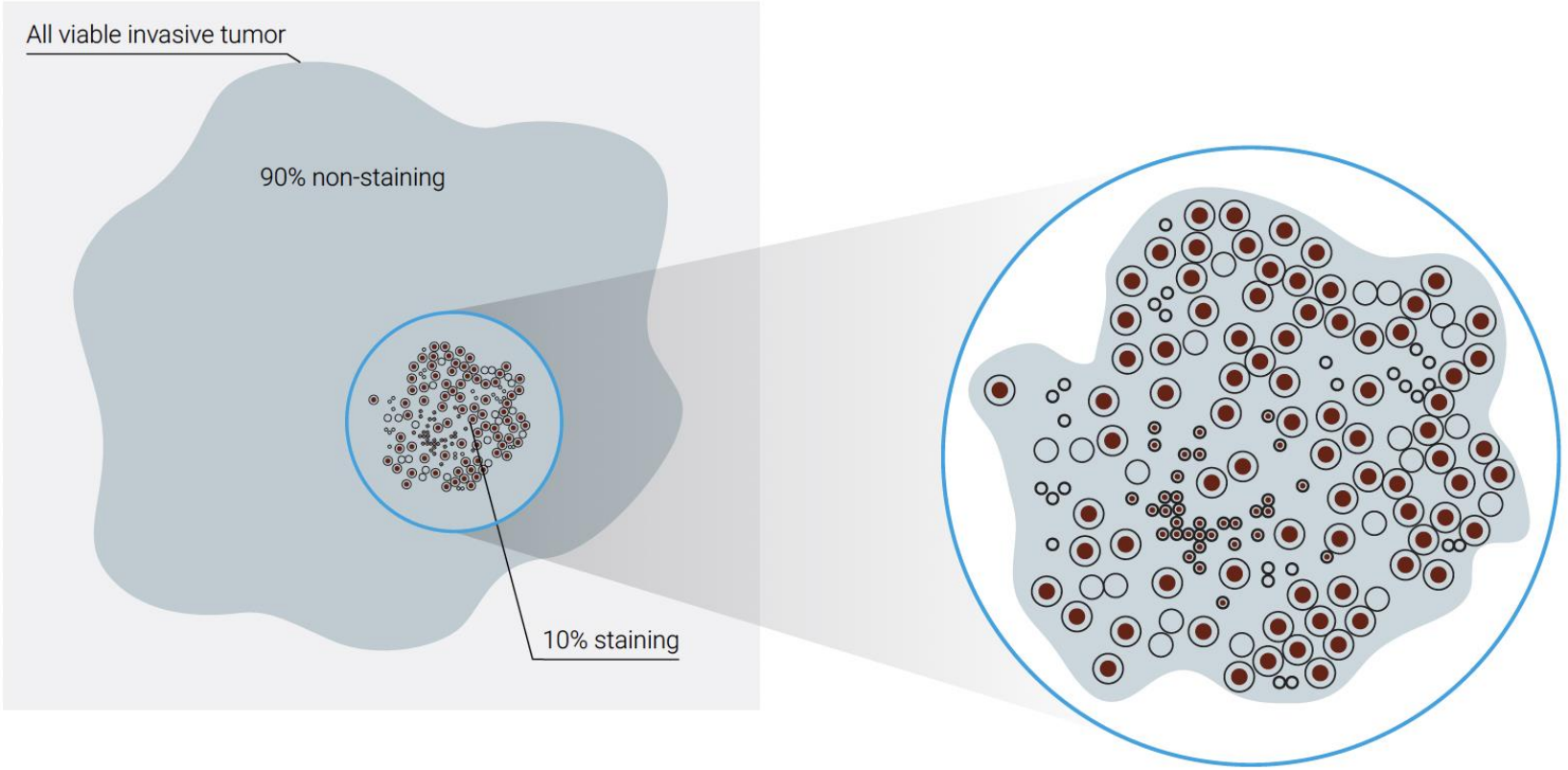
Clinical interpretation: Ki-67 pharmDx Score ≥ 20%



Calculate the Ki-67 pharmDx Score of the entire tumor area:

Ki-67 IHC MIB-1 pharmDx (Dako Omnis) Interpretation Manual

Estimating Ki67 when Focal



Calculate the Ki-67 pharmDx Score of the entire tumor area:

Assessment:

Ki-67 pharmDx Score of area with staining:

$$\text{Ki-67 pharmDx Score (\%)} = \frac{\text{\# Ki-67 staining viable invasive tumor cells}}{\text{Total \# of staining and non-staining viable invasive tumor cells}} \times 100 = \frac{\text{80 Ki-67 staining tumor cells}}{\text{100 tumor cells}} \times 100 = 80\%$$

Ki-67 pharmDx Score of entire tumor area: 10% × 80% = 8%

Clinical interpretation: Ki-67 pharmDx Score < 20%

Considerations for breast Ki-67

What does oncology need? Test volume?

Ki-67 on EVERY breast cancer?

Ki-67 on ER+ breast cancer?

Ki-67 on ER+ LN+?

If low volume,
Consider send out

Ki-67 in house

Current Ki-67 assay

- Compare to FDA assay
  - Cross validate as LDT
  - Scoring, report template
- Changes needed?
  - May need separate assay for breast/drug
  - No change for other purposes

No existing Ki-67 assay

- Consider FDA approved assay
- Validate as predictive marker

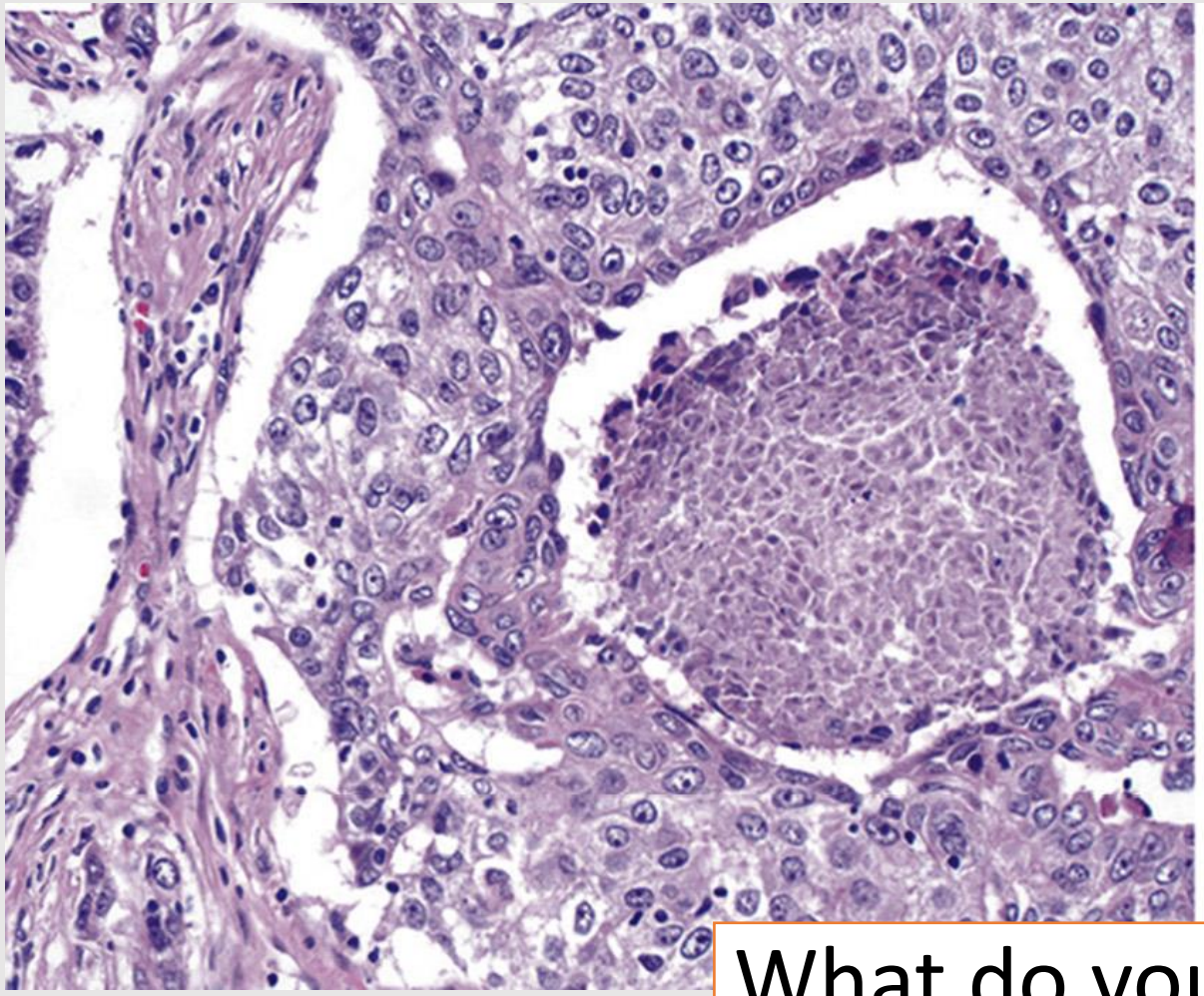
Slide courtesy of Dr Megan Troxell

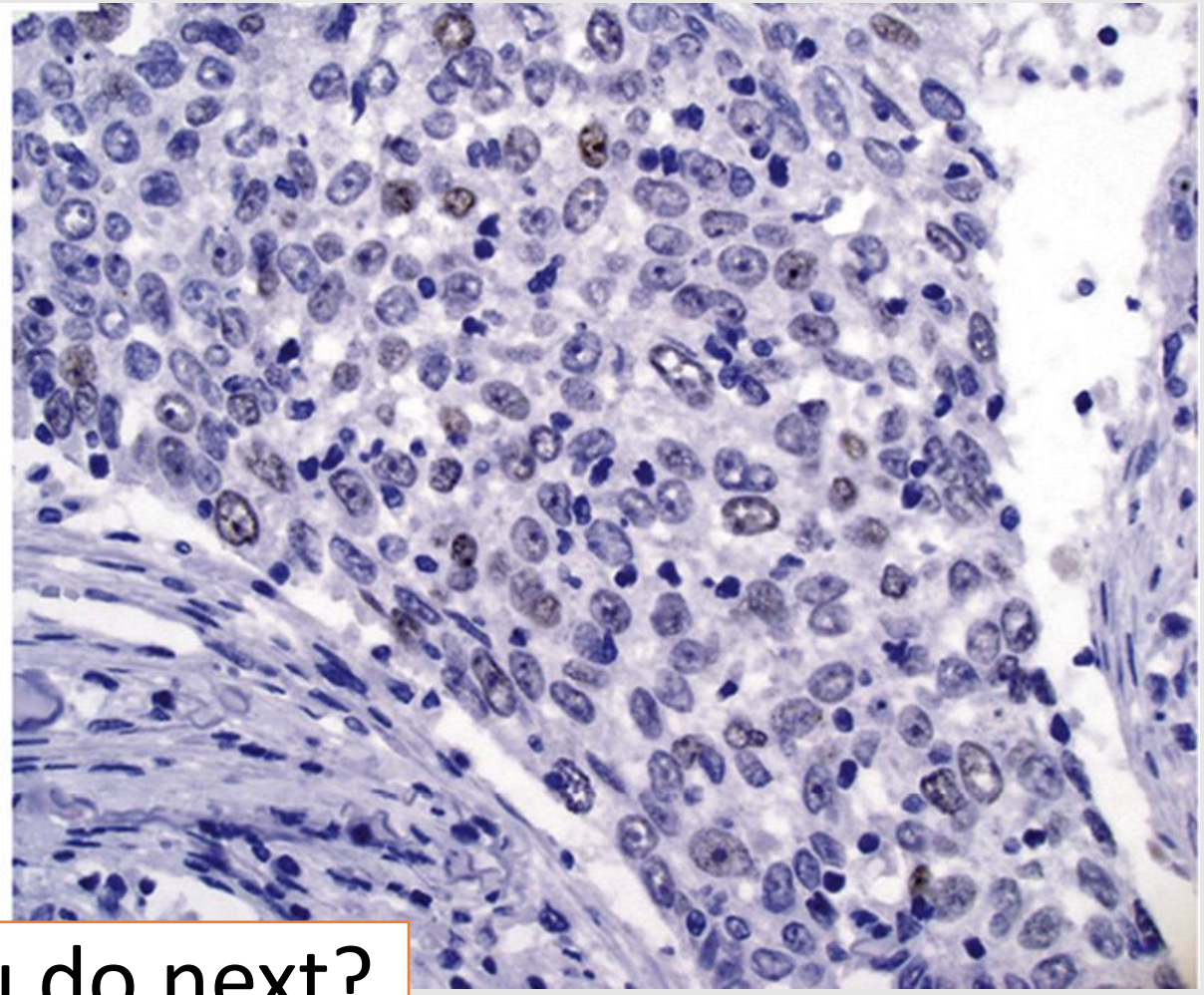
ER+, HER2- Invasive Cancer Treatment

Which factors are used to determine if need to add ~~chemo~~therapy to endocrine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary (> 0.5 cm)
4. Lymph node status
5. Proliferation:
    1. 21-gene RT-PCR Assay Recurrence Score (chemotherapy decision)
    2. Ki67 (abemaciclib in high risk if > 20%)
6. Nottingham grade
7. Margins

Example Case: 35 y/o female with Grade 3 IDC and the following ER stain you estimate to be 1-10% positive (1+)





What do you do next?

Recommendation 2.3 (NEW)

Laboratories should establish and follow an SOP stating the steps the laboratory takes to confirm or adjudicate ER results for cases with weak stain intensity or ≤10% of cells staining (see Supplemental Digital Content Data Supplement 2, Figure 1 for an example SOP).

Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; and Antonio C. Wolff, MD[21]

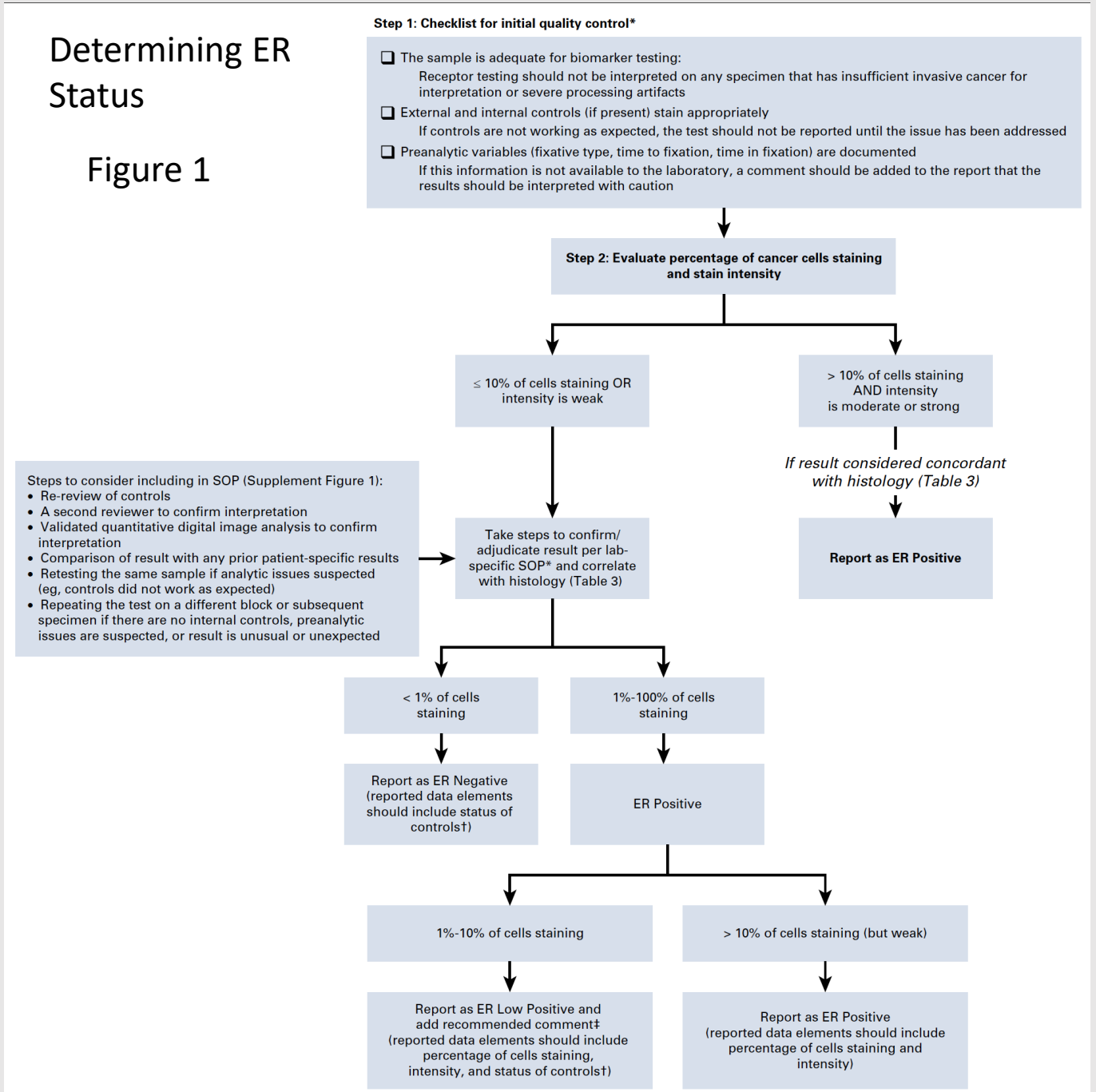
ER staining between 1-10% of invasive cancer cells is considered ER Low Positive (after additional steps taken to confirm the result)

Possible SOP:

- Re-review of controls
- Second reviewer to confirm interpretation
- Validated quantitative digital image analysis to confirm interpretation
- Comparison or result with any prior patient results
- Retesting the same specimen if analytic issues suspected (eg controls did not work as expected)
- Repeat on a different block or subsequent specimen
  - Esp if no internal controls, preanalytic issues suspected, or unusual or unexpected result

Determining ER Status

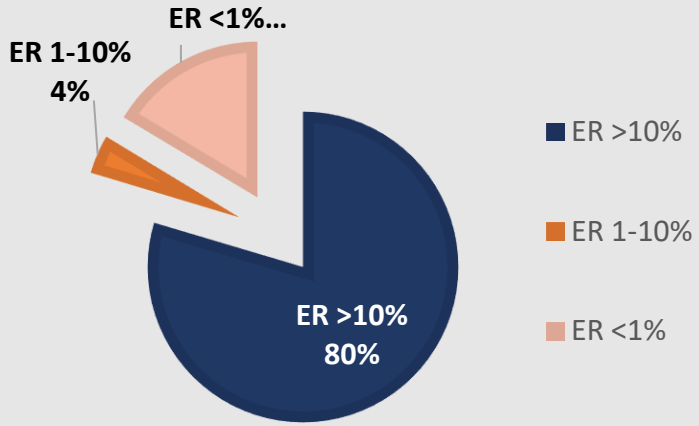
Figure 1



Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309 Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Stanford Practice: Data used to establish an SOP

Interpretation Category (Based on Majority)	Cases in Category	Cases with 100% (6 of 6) agreement	Cases with >80% (5 of 6) agreement
Negative (<1%)	16	67%	87%
Low Positive (1-10%)	6	0%	17%
Positive (>10%)	8	75%	100%

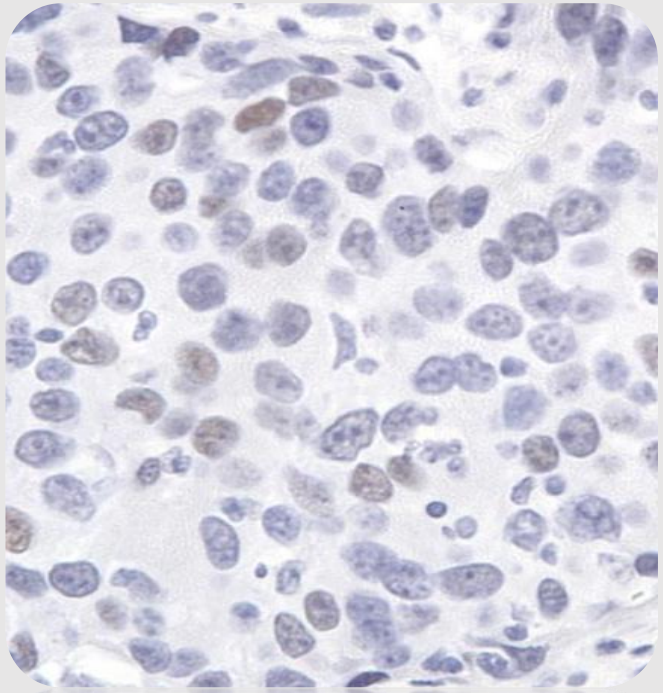


- Test set of 30 cases reported as ER Negative (0 or <1%), Low Positive (1-10%) or Positive (>10%) were identified.
- 5 breast pathologists who perform ER interpretations scored/interpreted each case
- Agreement was very high for > 10%
- Agreement was high for < 1% (best for 0%)
- Agreement was very low for Low Positive (1-10%)
- Decided our SOP should include second pathologist review for cases with 1-10% staining or close to the 1% threshold for positive
  - Would result in second review of approximately 4% of our cases

What do we know about ER Low (1-10%) Positive Cancers?

- Rare (2-3%) & heterogenous
- Often “basal-like” features (histology, response to neoadjuvant chemotherapy and molecular profiles), worse prognosis (even with endocrine RX)
  - Don’t want to exclude these patients from “triple negative” trials…?
- Potential benefit from endocrine therapy (although less than stronger positive):

  May still need to be considered positive for at least at trial of endocrine therapy but intent not to be used to treat similar to other strong ER+ cancers…..



Raghav KP, et al. Cancer 118:1498-1506, 2012
Honma N, et al. Breast 23:754-762, 2014
Chen T, et al. Clin Breast Cancer 18:1-8, 2018
Balduzzi A, et al. Clin Breast Cancer 14:258-264, 2014
Gloyeske NC, et al. Am J Clin Pathol 141:697-701, 2014
Deyarmin B, et al. Ann Surg Oncol 20:87-93, 2013
Yi M, et al. Ann Oncol 25:1004-1011, 2014

Recommended Comment for ER Low Positive

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

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Recommendations on Internal Control Reporting (Recommendation 2.4):

- The status of internal controls should also be reported for cases with 0-10% staining (with a special comment for those lacking internal controls). See Table 2.

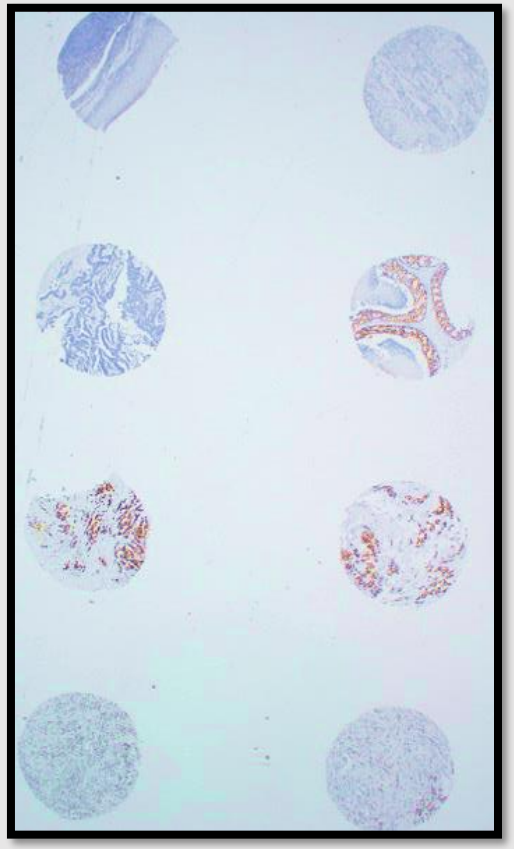
Result:	Additional recommended comment:
No internal controls and ER is 0-10%:	No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

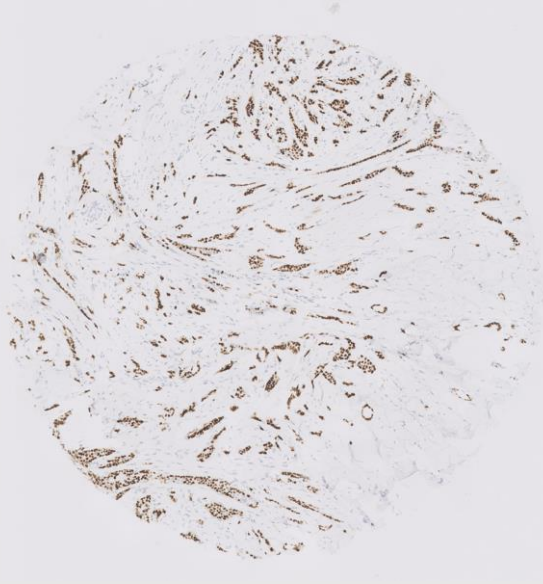
Recommendation 1.5. Optimal internal QA procedures

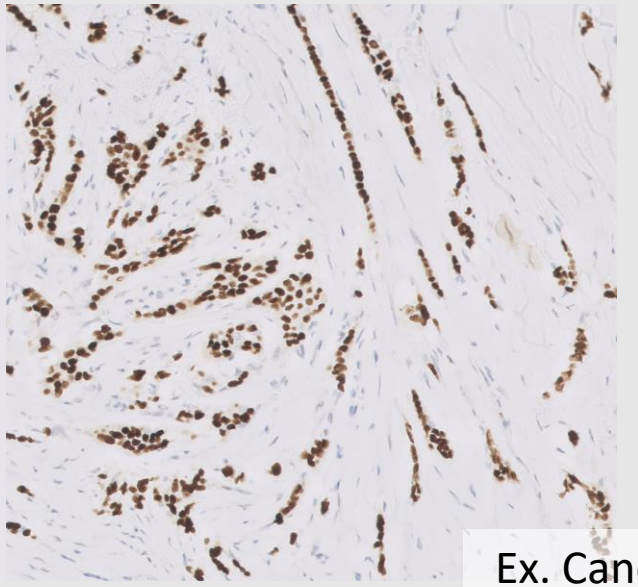
Updated

Standardized operating procedures (SOPs) should be used that include routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections (or other appropriate control) on each tested slide, wherever possible**. External controls should include negative and positive samples as well as samples with lower percentages of ER expression... On-slide controls are recommended.**



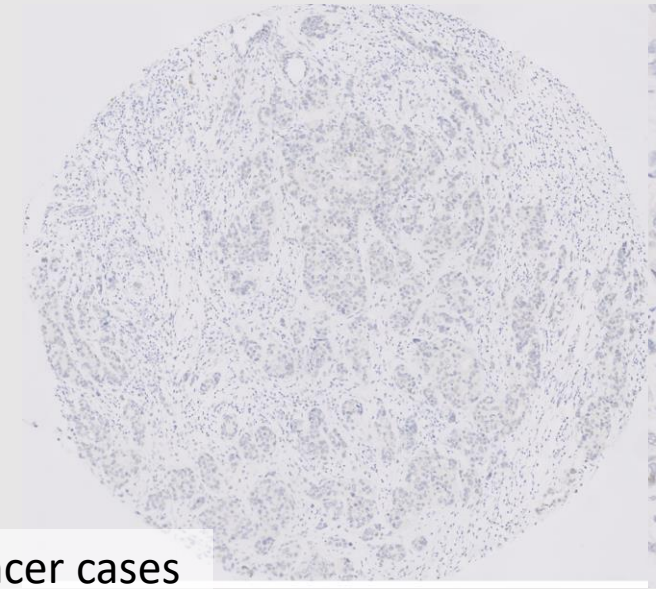
External Controls: Include a spectrum of ER expression, on-slide TMAs or similar preferred

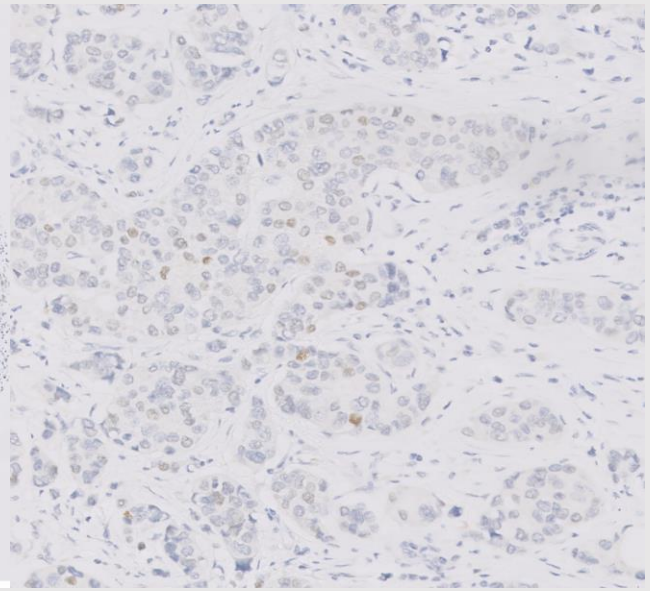




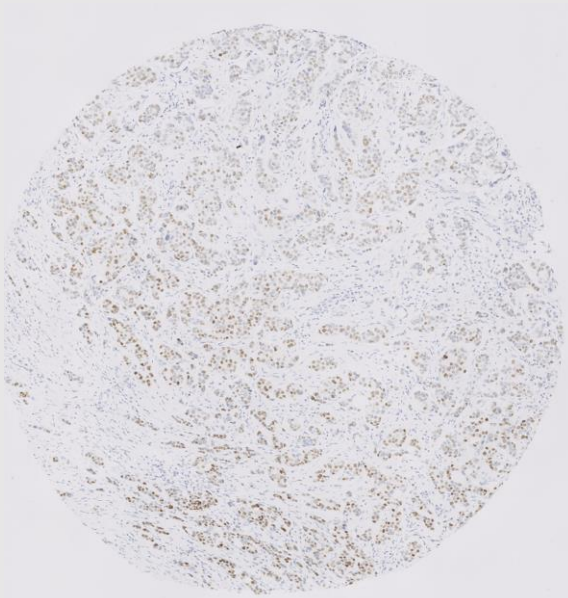
Strong positive (>95%, 3+) control.

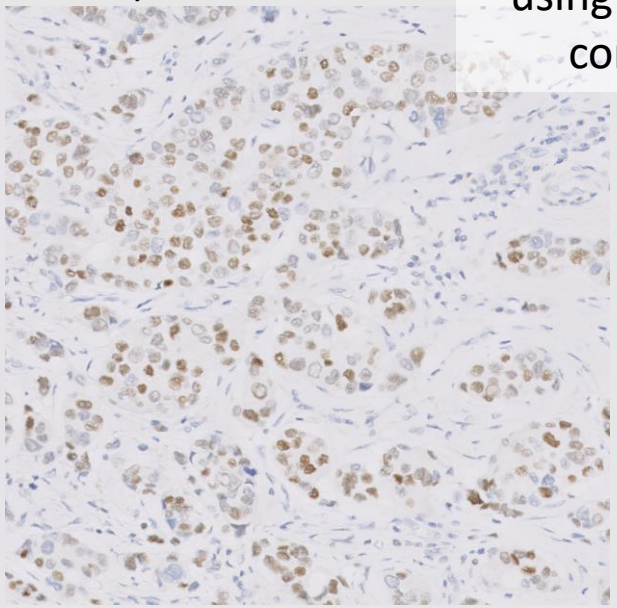
Ex. Cancer cases using external controls



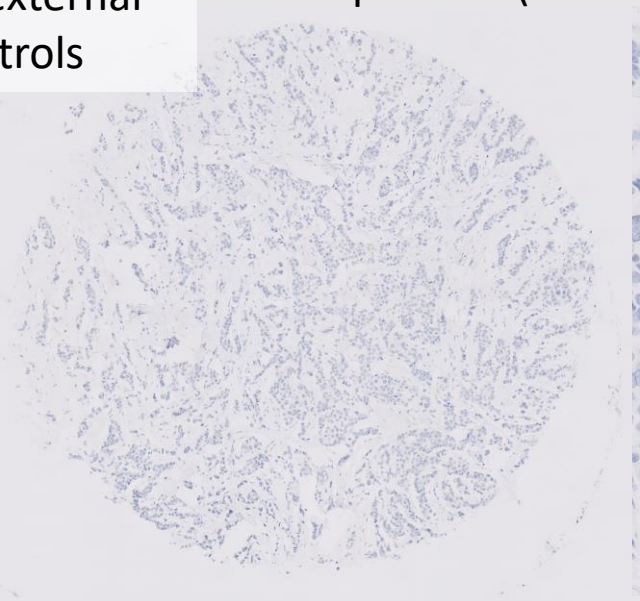


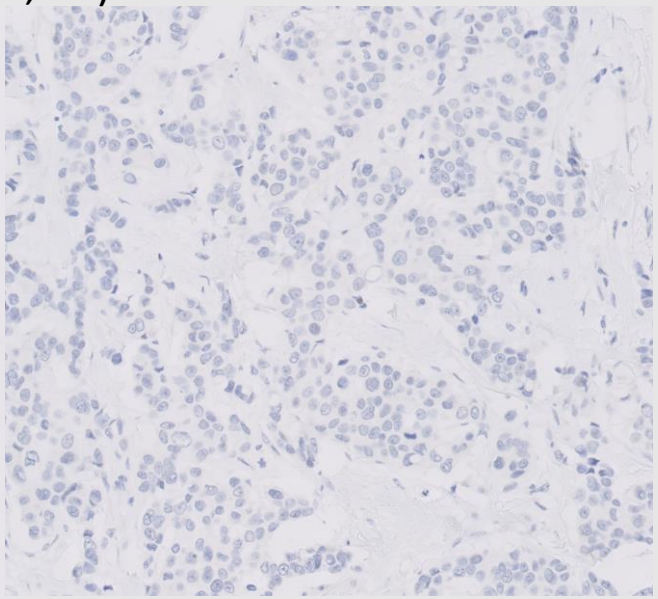
Low positive (1-10%, 1+) control.





Moderate intensity positive (80%, 2+) control.

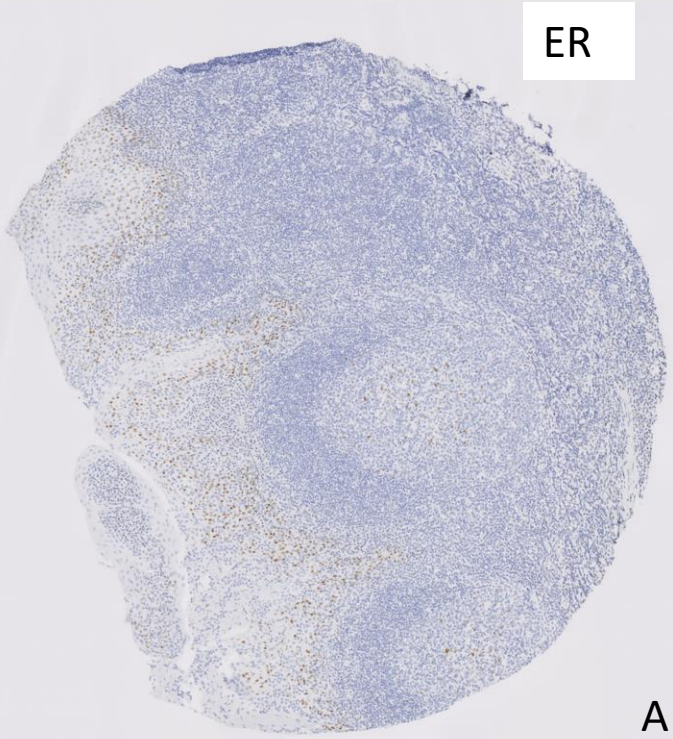


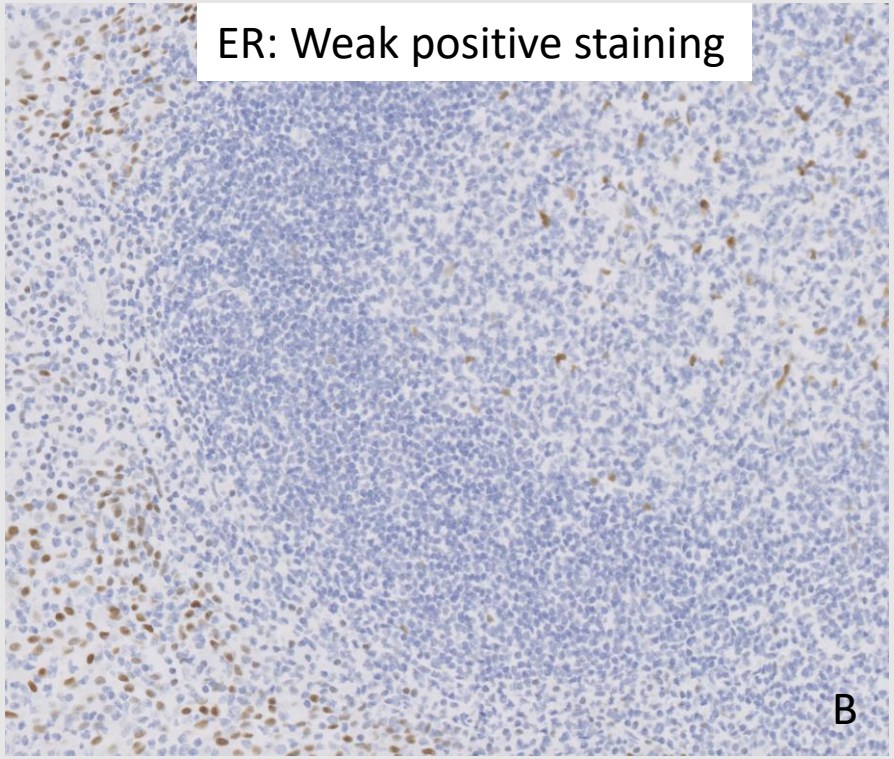


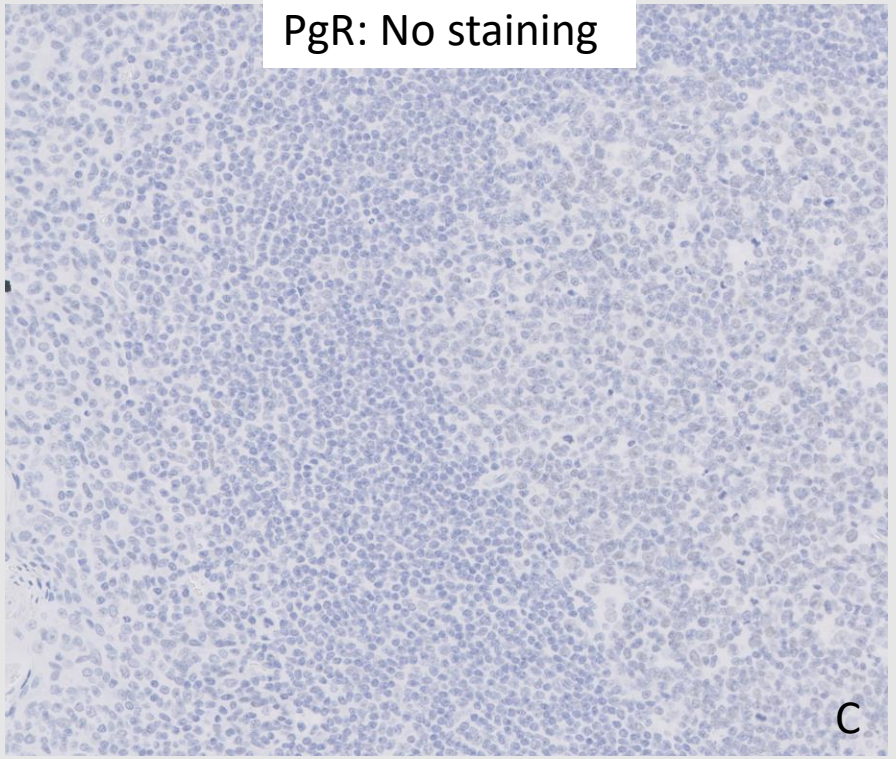
Negative (0%) control.

What tissue is a good low-ER positive control and also serves as a negative control for PR?

TONSIL: An Excellent External Control For Low ER Positive and PgR Negative



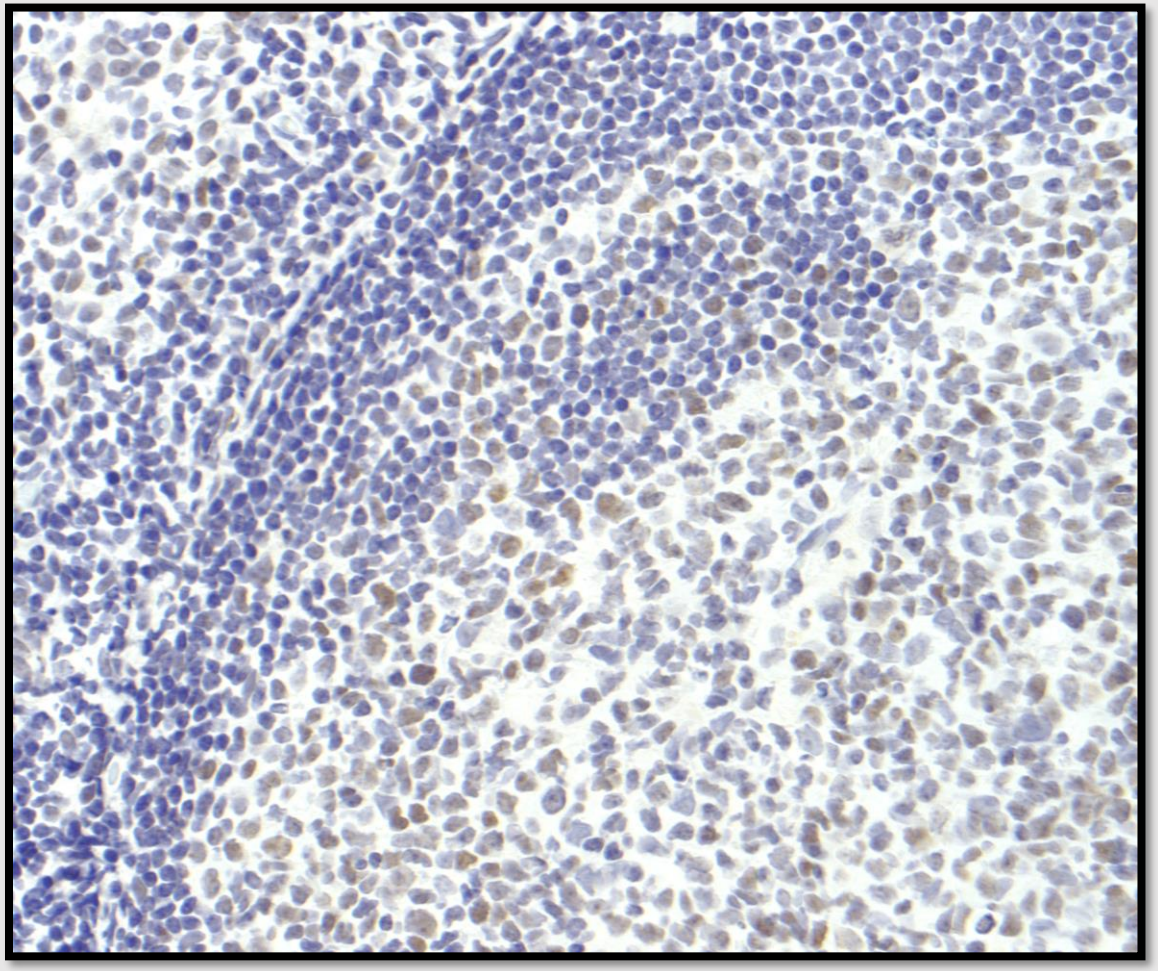




Tonsil is an excellent external control to monitor the analytical sensitivity for ER. Dispersed germinal center cells and the squamous epithelium should be ER positive but the B-cells in the mantle zones should be ER negative (as shown in panels A at 5x and panel B at 20x). Tonsil is an appropriate negative control for PgR. In contrast to ER, no nuclear PgR staining should be seen. Weak positive PgR staining in tonsil should result in work-up to determine if assay drift has occurred.

Example case: External tonsil control for PgR stain reviewed

Tonsil staining for PgR when should be negative….
Need to re-titer assay?
Drift occurring?

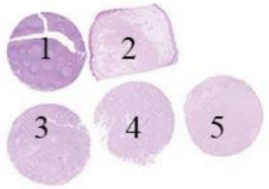


False Positive PR

- More common with SP2 and 1E2 antibodies in CAP PT data and external QA data

www.nordicqc.org

No.	Tissue	PR-positivity*	PR-intensity*
1.	Tonsil	0%	Negative
2.	Uterine cervix	80-90%	Moderate to strong
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	30-70%**	Weak to strong
5.	Breast carcinoma	90-100%**	Moderate to strong



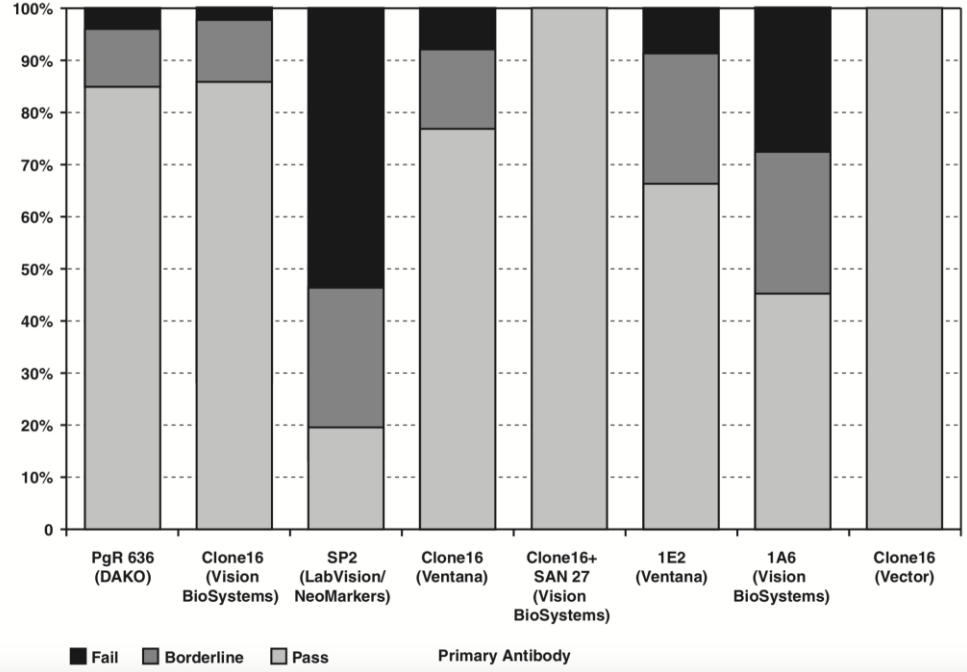
* PR-status and staining pattern as characterized by NordiQC reference laboratories using the mAb clones 16 and PgR 1294.
** PR expression heterogenous.

Troxell ML, Long T, Hornick JL, Ambaye AB, Jensen KC. **Comparison of Estrogen and Progesterone Receptor Antibody Reagents Using Proficiency Testing Data.** Arch Pathol Lab Med. 2017 Oct;141(10):1402-1412.

Potential for False-Positive Staining With a Rabbit Monoclonal Antibody to Progesterone Receptor (SP2)

Findings of the UK National External Quality Assessment Scheme for Immunocytochemistry and FISH Highlight the Need for Correct Validation of Antibodies on Introduction to the Laboratory

Merdol Ibrahim, PhD,[1] Andrew Dodson, MSc,[2] Sarah Barnett, MSc,[1] David Fish, MSc,[3] Bharat Jasani, PhD,[4] and Keith Miller, MSc[1]



Ibrahim M, Am J Clin Pathol. 2008 Mar;129(3):398-409.

ER negative, PR positive breast cancers

- Controversial if real or artifact (PR downstream of ER)
- Rare (<1%), should be worked up
  - Rule out false negative ER
  - Rule out false positive PR
  - Examine controls, Repeat test
- Unclear if benefit from endocrine therapy (PR only considered prognostic in ER+) and poor prognosis

Example Case

- 65 year old with Grade 1 IDC on core biopsy
- Interpret ER stain

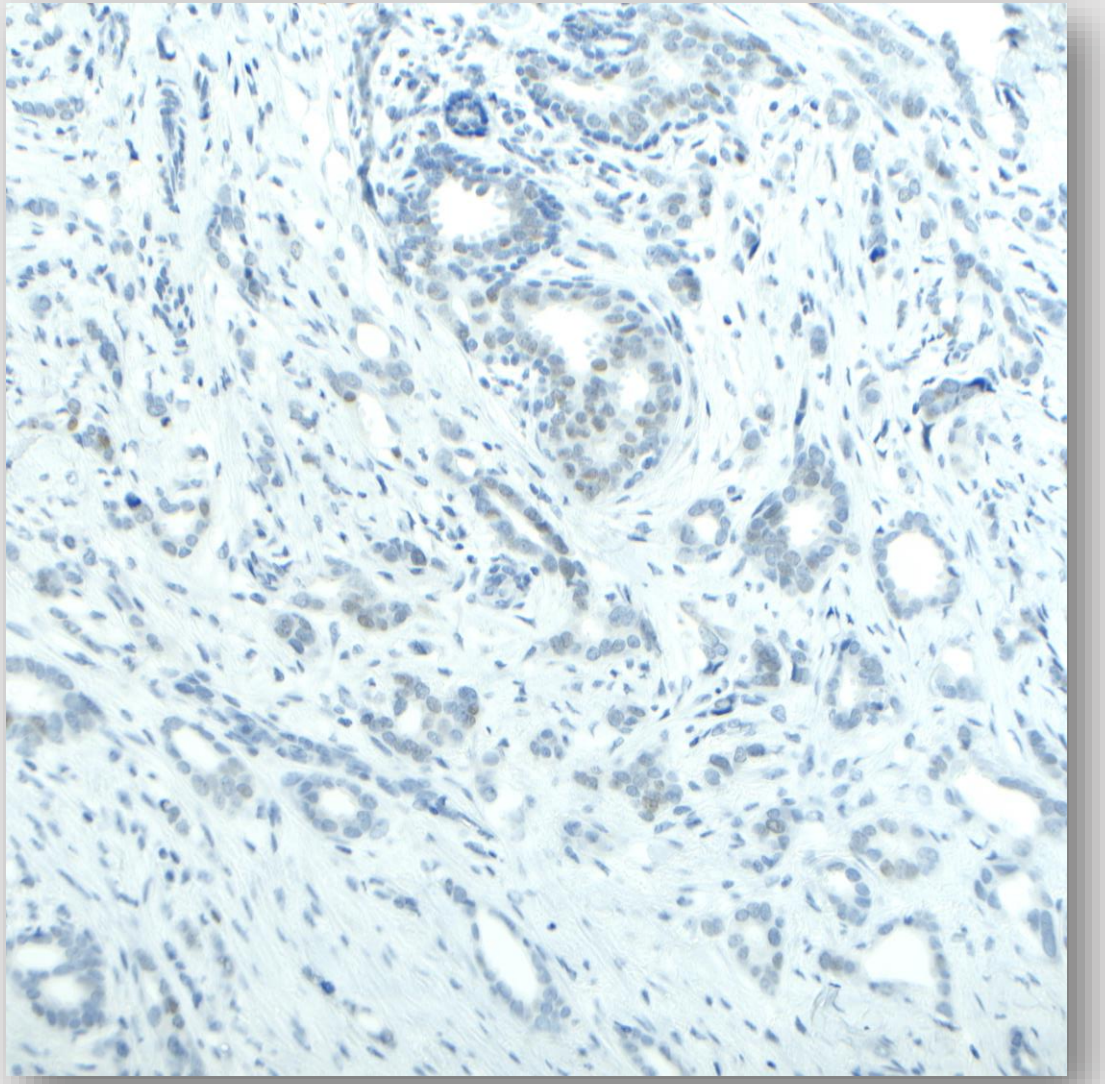
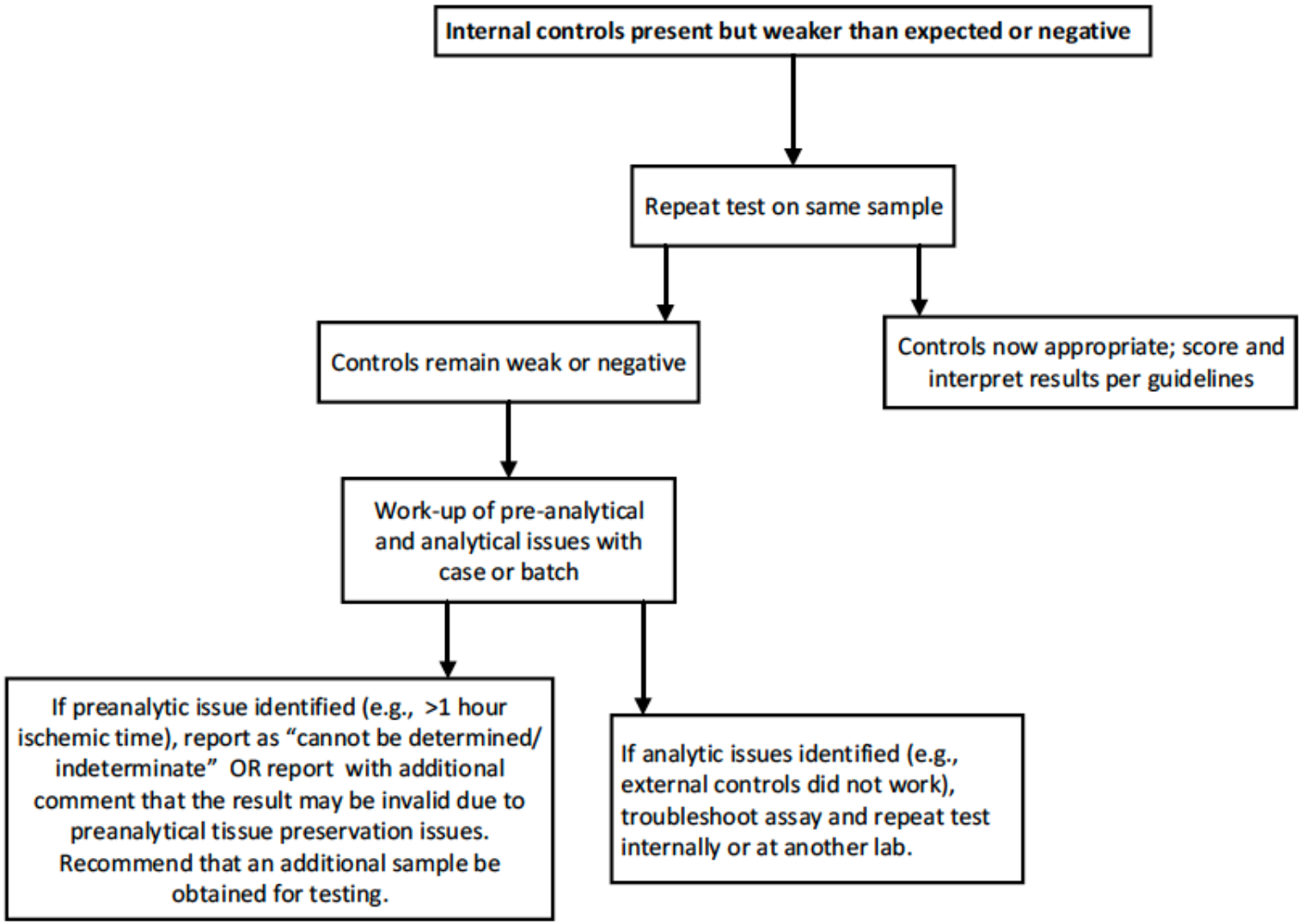
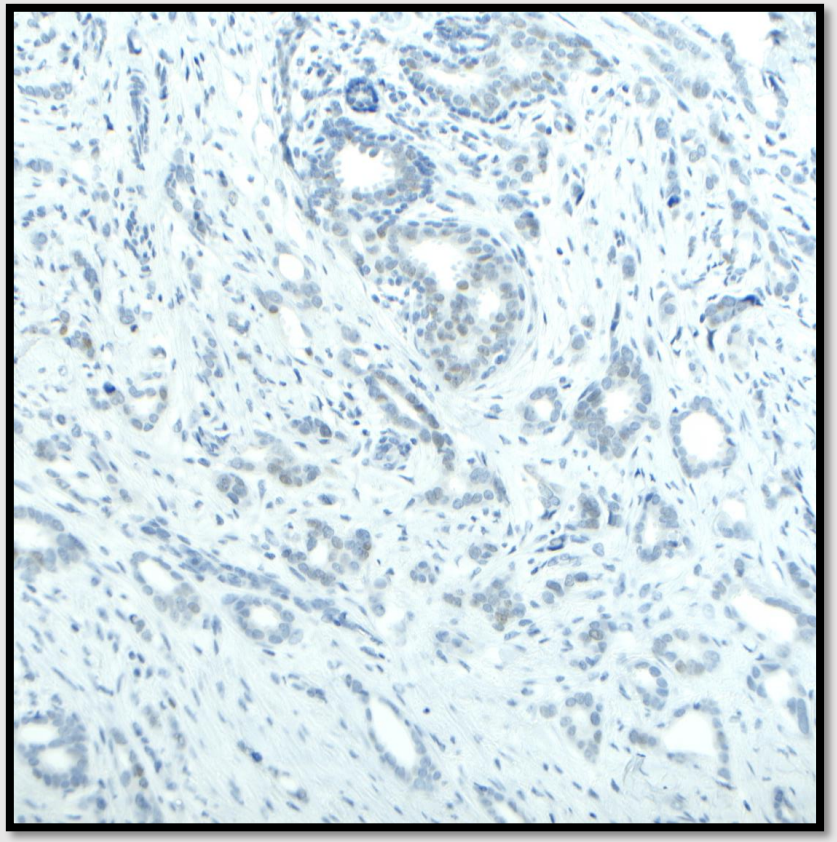


Figure 1c. Internal controls present but weaker than expected or negative





- ✓ Double check stain worked (repeat test)
- ✓ Check pre-analytic variables
- ✓ May need to report as "indeterminate" with recommendations for additional samples if pre-analytic issues identified

Recommendation 2.2.

Updated

Interpretation of any ER result should include evaluation of the concordance with the histologic findings of each case. Clinicians should also be aware of when results are highly unusual/discordant and work with pathologists to attempt to resolve or explain atypical reported findings (see manuscript Table 3 as an aid in this process).

Strong Recommendation

Invasive Breast Cancer Histopathologic Concordance with Estrogen Receptor Staining

HIGHLY UNUSUAL ER NEGATIVE RESULTS	HIGHLY UNUSUAL ER POSITIVE RESULTS
Low grade invasive carcinomas of no special type (also known as invasive ductal carcinoma)	Metaplastic carcinomas of all subtypes
Lobular carcinomas (classic type)	Adenoid cystic carcinomas and other salivary gland-like carcinomas of the breast
Pure tubular, cribriform, or mucinous carcinomas	Secretory carcinoma
Encapsulated papillary and solid papillary carcinomas	Carcinomas with apocrine differentiation

Note: If a result is considered highly unusual/discordant additional steps should be taken to check the accuracy of the histologic type or grade as well as the pre-analytic and analytic testing factors. This work-up may include 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.

Also these should be HER2 Negative

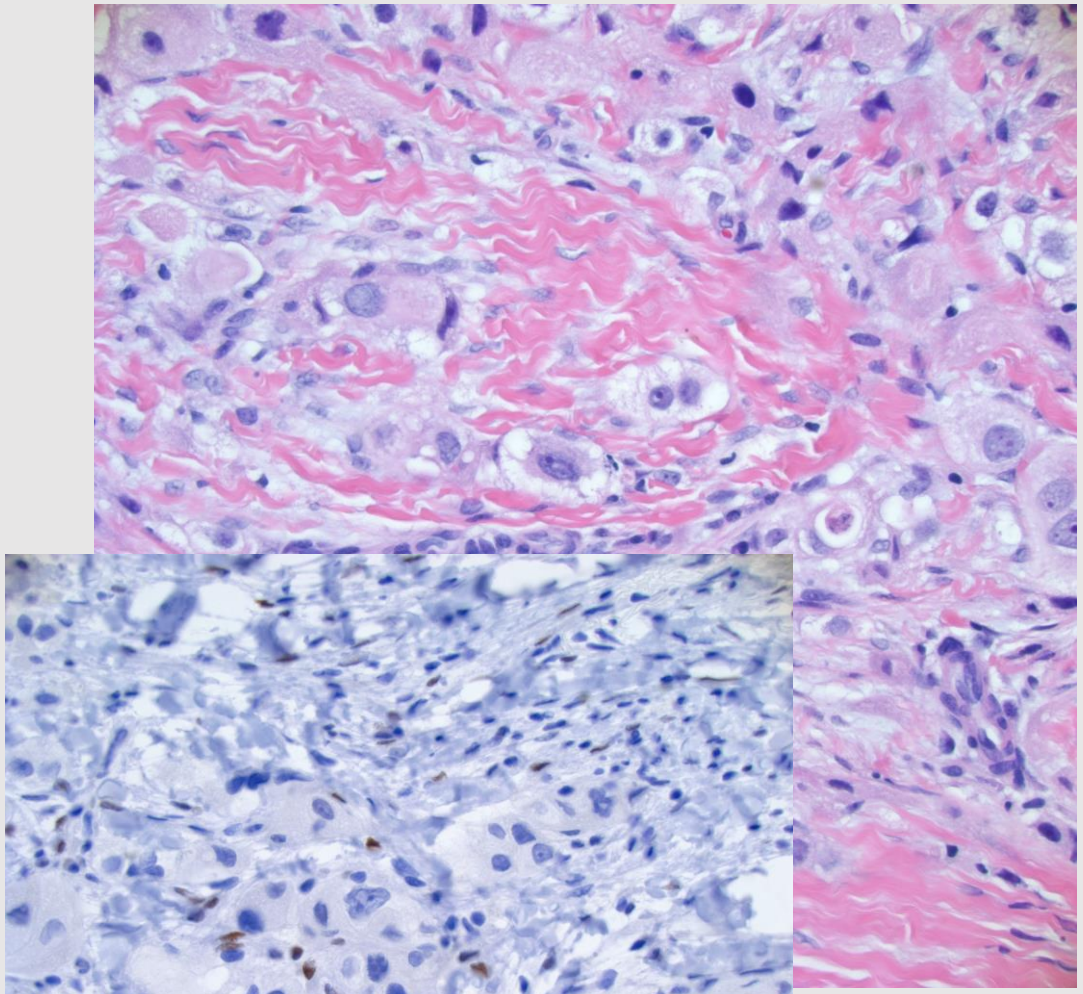
Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Example case:

You are reviewing as a second opinion a case with the following diagnosis from the original lab:

DIAGNOSIS: INVASIVE LOBULAR CARCINOMA

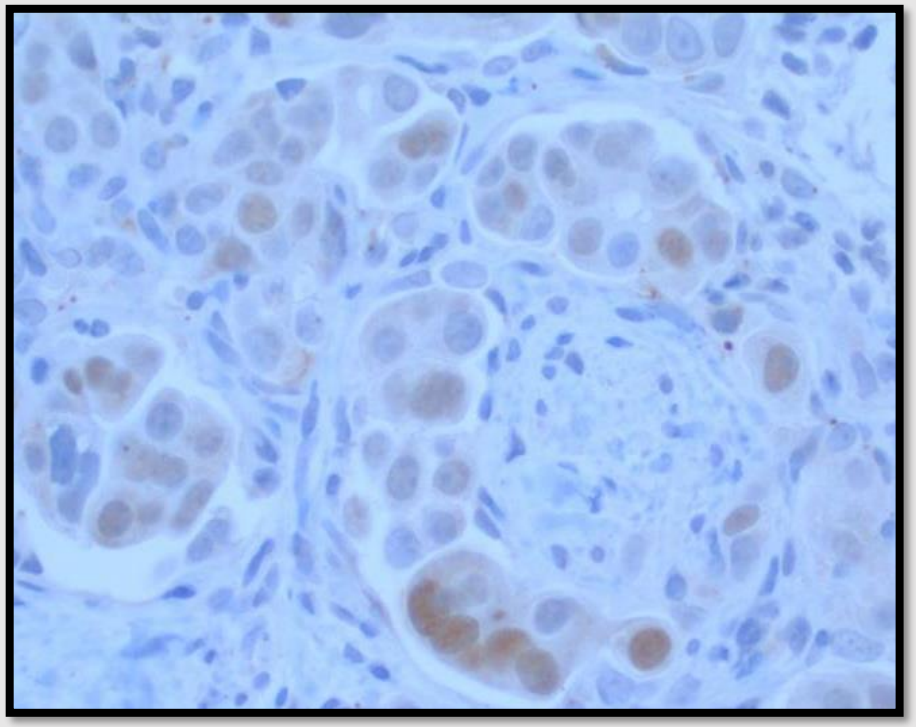
- ER negative (0%) with positive internal controls
- PR negative (0%) with positive internal controls
- HER2 negative (0) by IHC



Revised Diagnosis: Invasive Pleomorphic Lobular Carcinoma

Example Case:

- Grade 3 invasive ductal carcinoma, LN neg
- Core Biopsy outside read by image analysis : ER 2%
- Core biopsy by our review: ER 10%, 1+
- Excision at Stanford: ER 20%, 1-2+
- Sent for Oncotype DX:
  - High RS (54; 34% recur)



Quantitative Single Gene Report

The *Oncotype* DX assay uses RT-PCR to determine the RNA expression of the genes below. These results may differ from ER, PR, or HER2 results reported using other methods or reported by other laboratories.[1]

The ER, PR, and HER2 Scores are also included in the calculation of the Recurrence Score.

ER Score = **6.2** Negative

The ER Score positive/negative cut-off of 6.5 units was validated from a study of 761 samples using the 1D5 antibody (immunohistochemistry) and 607 samples using the SP1 antibody (immunohistochemistry). The standard deviation for the ER Score is less than 0.5 units.[2]

Clinical Experience:

For ER positive breast cancer, the magnitude of tamoxifen benefit increases as the ER Score increases from 6.5 to ≥12.5.[3]

Please note: The Average Rate of Distant Recurrence reported on Page 1 based on the Recurrence Score was determined in patients who received 5 years of tamoxifen treatment and takes into account the magnitude of tamoxifen benefit indicated by the ER Score.

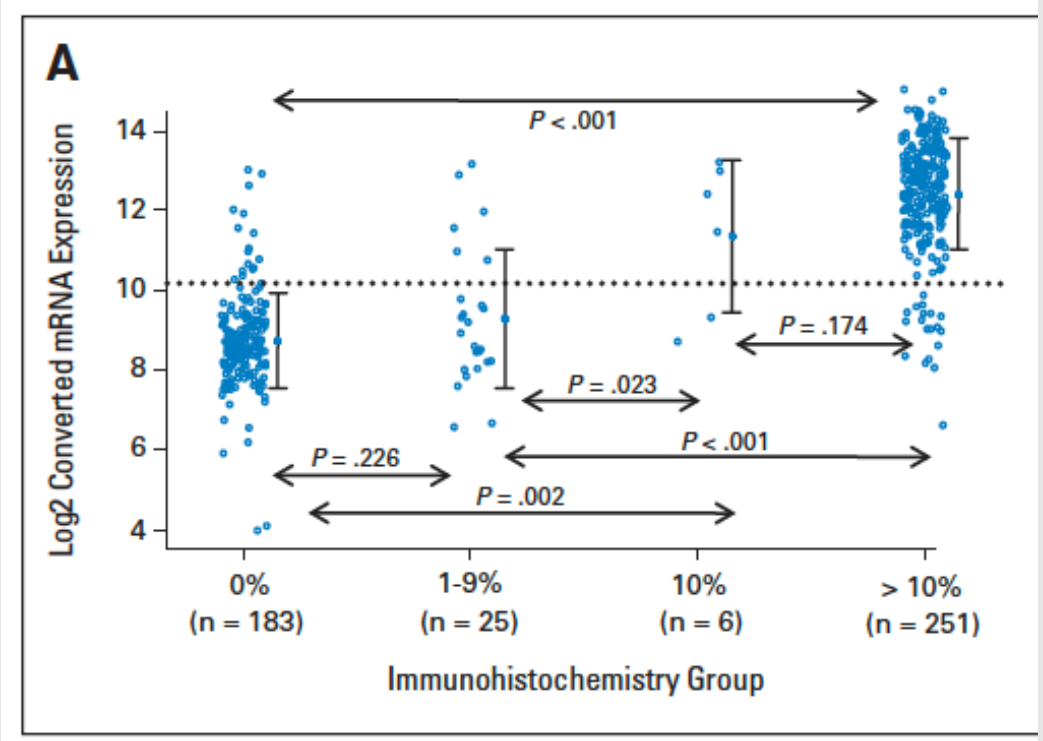
Cases close to threshold for positive are more likely to have different results by different assays, methods or samples. Any positive result is treatable but need to acknowledge data limited.

mRNA methods may be be less sensitive than IHC in detecting low level ER expression

Estrogen Receptor (ER) mRNA and ER-Related Gene Expression in Breast Cancers That Are 1% to 10% ER-Positive by Immunohistochemistry

Takayuki Iwamoto, Daniel Booser, Vicente Valero, James L. Murray, Kimberly Koenig, Francisco J. Esteva, Naoto T. Ueno, Jie Zhang, Weiwei Shi, Yuan Qi, Junji Matsuoka, Elliana J. Yang, Gabriel N. Hortobagyi, Christos Hatzis, W. Fraser Symmans, and Lajos Pusztai

- Cancers with 1-9% ER staining by IHC had features overlapping with ER <1% cases (basal-like PAM-50, worse survival)
- Were often below threshold of positive for mRNA assay….



J Clin Oncol 30:729-734. © 2012 by American Society of Clinical Oncology

Park City History

- Prior mining town
- In 1946 Bob Burns and Otto Carpenter used parts from mines, car engines + lodgepole pines to build lifts
- Deer Valley Chairlifts named after them
- Miners could pay $1.50 to ride lifts + lesson

Scavenged abandoned mines and built mechanized lift towers from discarded mining equipment, hewn aspen wood and nearby lodgepole pines. 1947
https://www.skiutah.com/blog/authors/lexi/ski-utah-resort-histories-deer

Early Stage ER Negative Invasive Cancer Treatment

Which factors are used to determine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. Proliferation
6. Nottingham grade
7. Margins

Early Stage ER Negative Invasive Cancer Treatment

Which factors are used to determine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. Proliferation → uniformly high
6. Nottingham grade → uniformly high
7. Margins

NCCN on favorable histologic types

National Comprehensive Cancer Network®

NCCN Guidelines Version 8.2021
Invasive Breast Cancer

NCCN Guidelines Index
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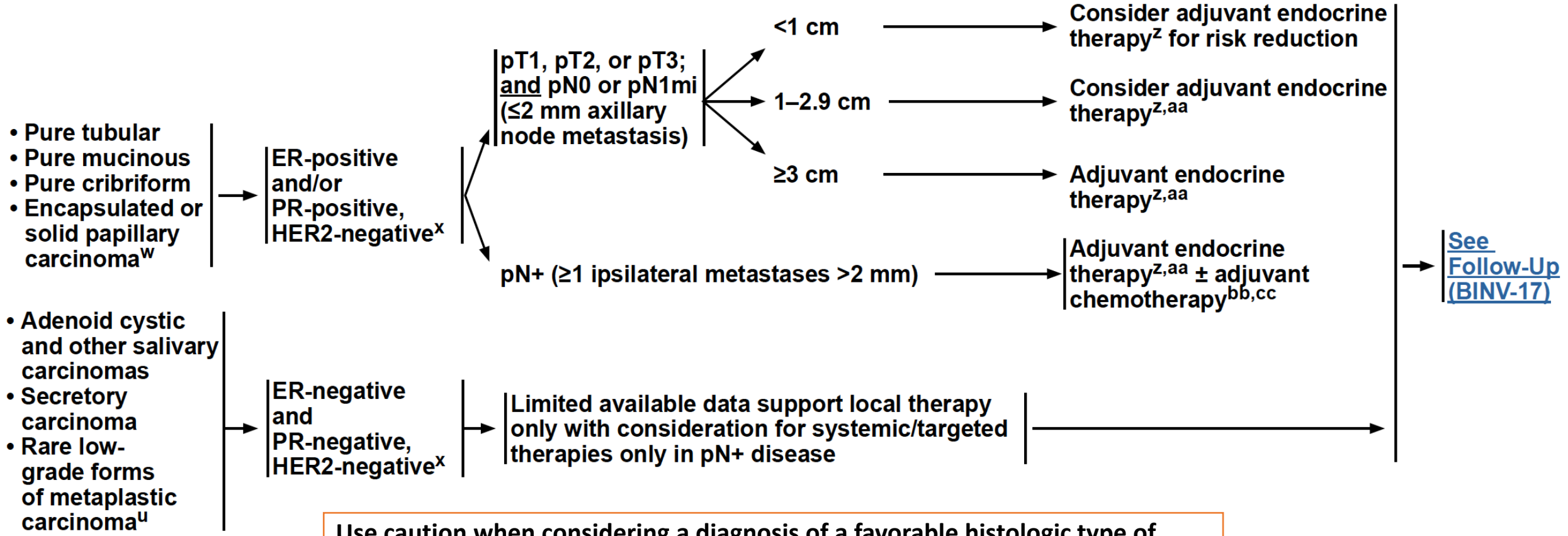
Discussion

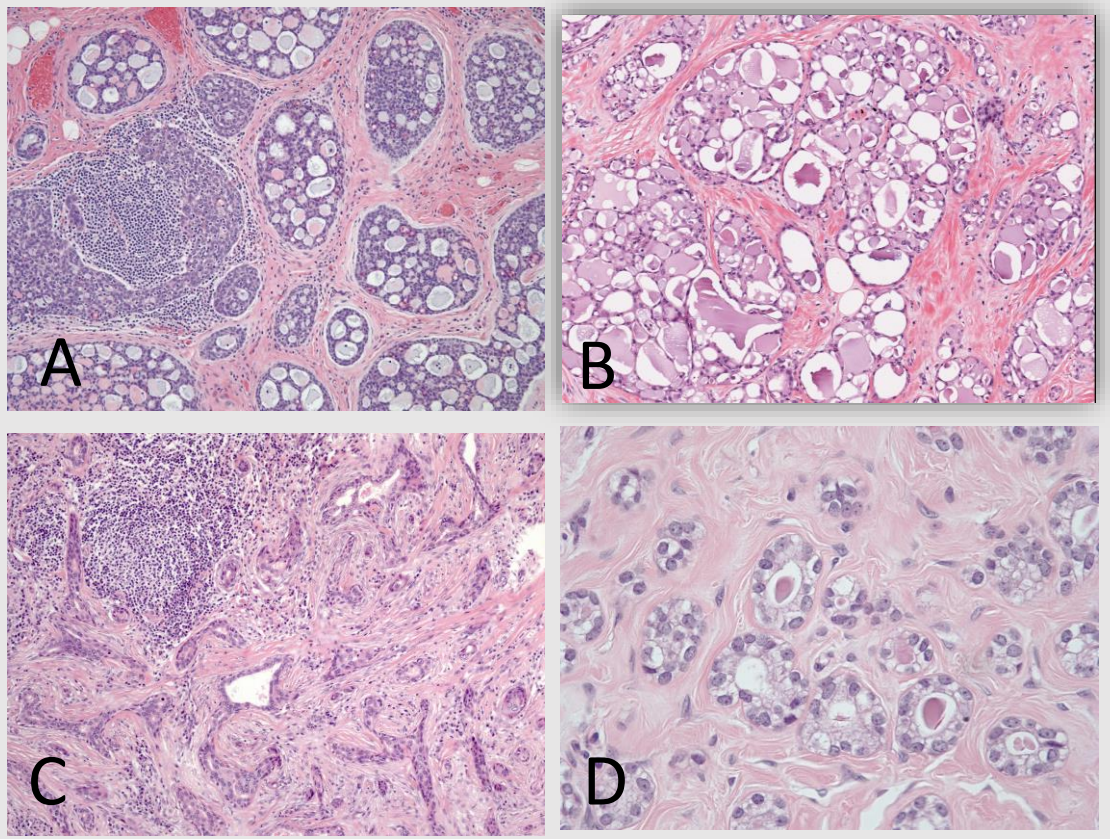
SYSTEMIC ADJUVANT TREATMENT: FAVORABLE HISTOLOGIES[q,v]



Use caution when considering a diagnosis of a favorable histologic type of breast cancer on core biopsy and correlate with ER and HER2 results.

Triple Negative/Basal Low Grade Processes:

- **Adenoid cystic carcinoma (classic type, not basaloid variant)**
- Low grade metaplastic carcinomas (adenosquamous carcinomas, fibromatosis-like, etc)
- Secretory carcinoma: t(12;15) *ETV6-NTRK3* translocation
- Well differentiated apocrine carcinomas (less well defined)
- Microglandular adenosis (not “invasion”?)



These may NOT behave like the typical high grade triple negative cancer!

If neoadjuvant chemotherapy being considered → discussion at tumor board appropriate

Early Stage ER Negative Invasive Cancer Treatment

Which factors are used to determine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. Proliferation
6. Nottingham grade
7. Margins

What other Biomarker?

Early Stage ER Negative Invasive Cancer Treatment

Which factors are used to determine therapy?

1. Histologic type
2. Menopausal status/age
3. Size of primary
4. Lymph node status
5. Proliferation
6. Nottingham grade
7. Margins

What other Biomarker?

HER2!

Size and LN influence on treating ER- Cancer:

- Per NCCN, if ER-/HER2- (triple negative):
  - LN+ or > 1.0 cm → treat with chemotherapy
  - 0.6 -1.0 cm or pN1mi ***consider*** chemotherapy
  - pN0 and < 0.5 cm → no adjuvant therapy

What size or LN would chemo be standard vs considered vs avoided?

- Per NCCN, if ER-/HER2+:
  - LN+ *OR* > 1 cm → Chemo + Herceptin = clear benefit
  - < 1 cm and LN negative ***consider*** chemotherapy
  - Add Pertuzumab if high risk LN+ or large (KATHERINE trial)

NEED TO FIND even small foci of HER2+ invasion
SIZE ALL ACCURATELY

Often neoadjuvant treatment so need to get ER and HER2 status correct up front

See www.NCCN.org

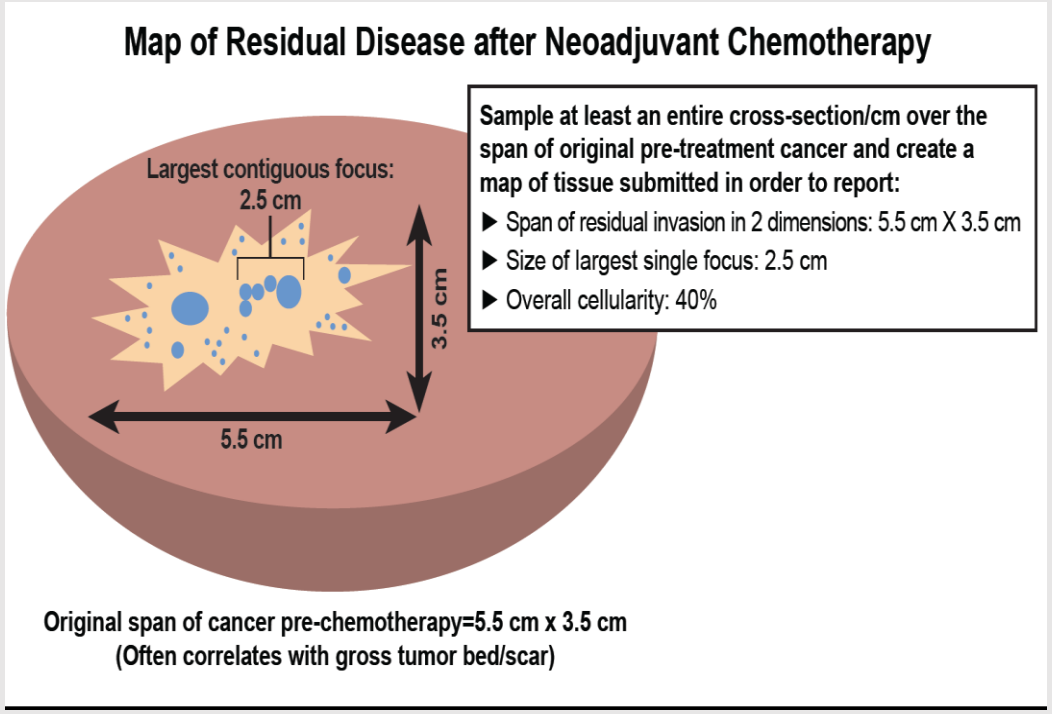
What is one of the most powerful prognostic indicators of residual risk after initial treatment of ER Negative cancers?

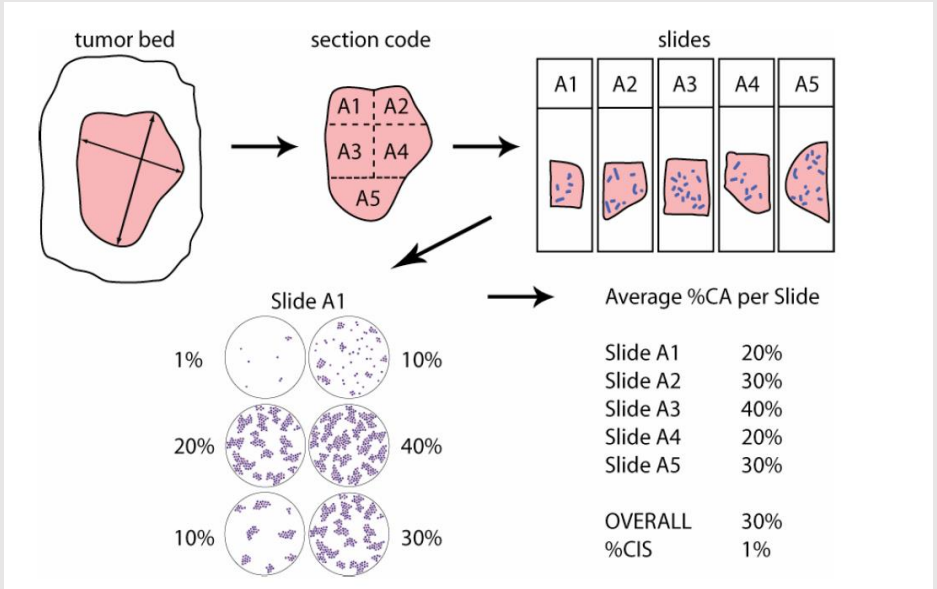
Residual disease post neoadjuvant treatment

- Need to standardize post-neoadjuvant sampling and residual cancer pathology measurements post-neoadjuvant therapy:

  Ex. **Residual Cancer Burden**

  http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

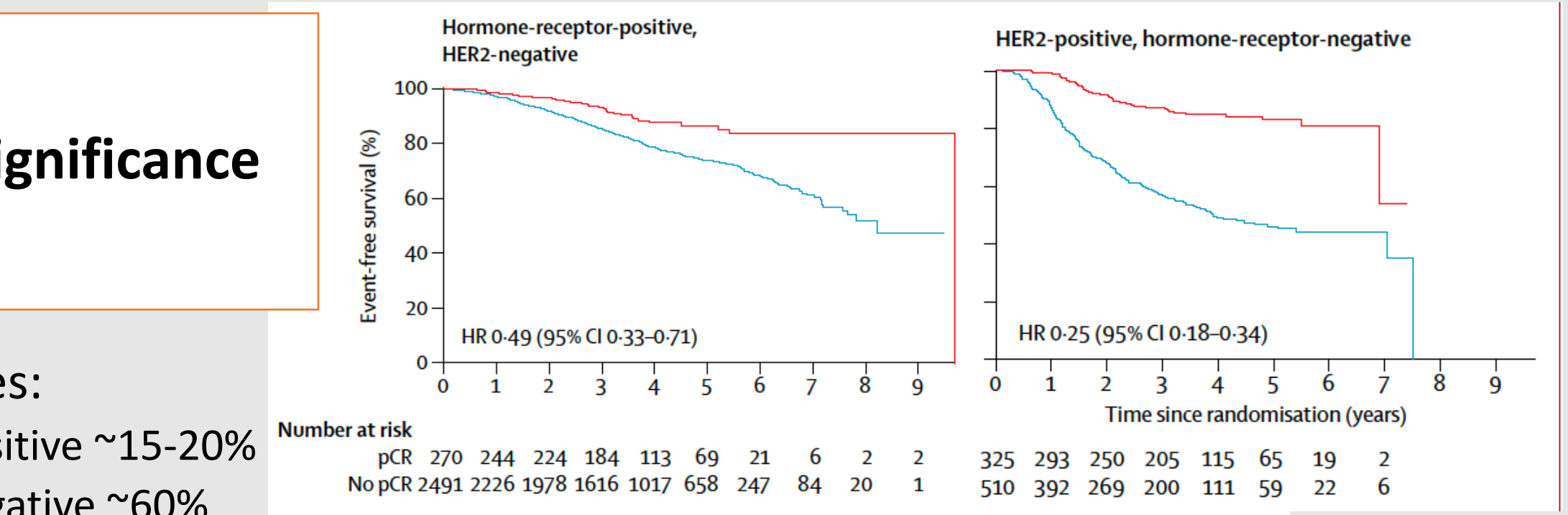


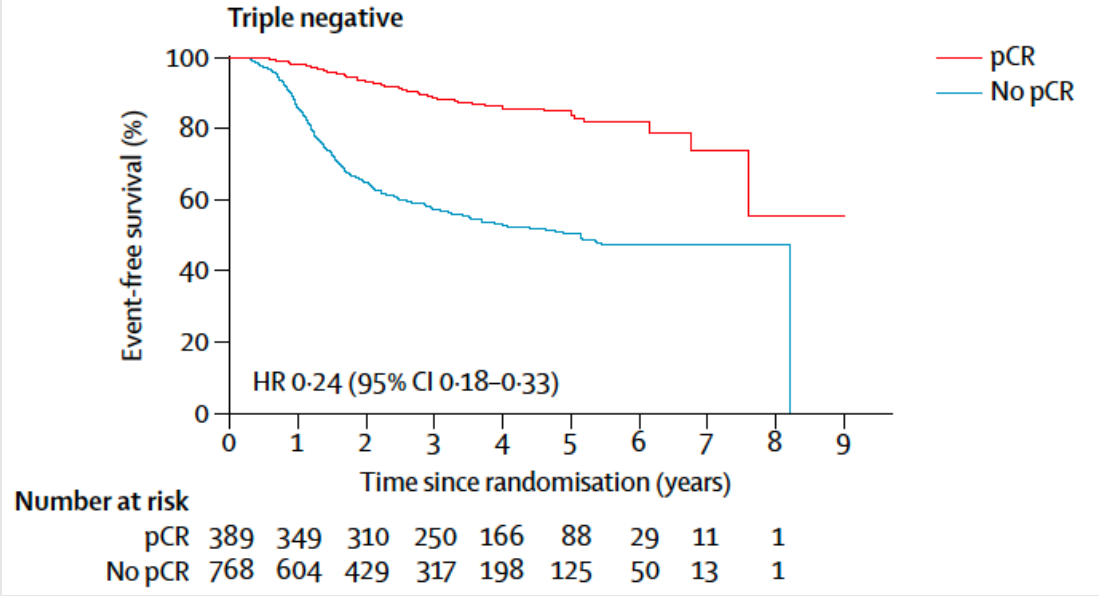


Annals of Oncology 30: 1541–1557, 2019
doi:10.1093/annonc/mdz235

pCR Significance

- pCR Rates:
  - ER positive ~15-20%
  - ER negative ~60%
- Biggest differences in survival with pCR in:
  - HER2+/ER-
  - Triple Neg
  - Also in Grade 3 ER+





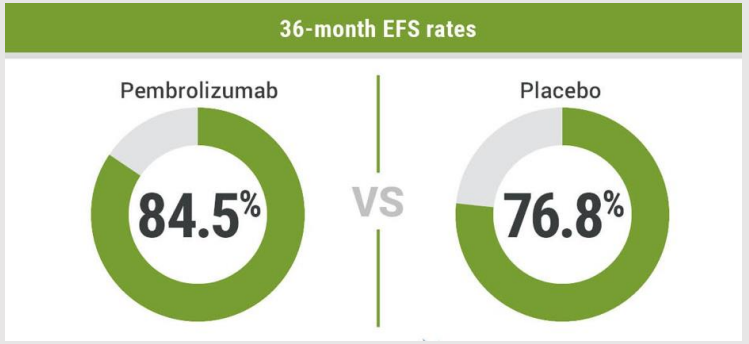
Cortazar P, et al. ..CTNeoBC pooled analysis. Lancet. 2014 Jul 12;384(9938):164-72. PMID: 24529560.

Post-treatment Triple Negative: Options if not a pCR

- Consider oral capecitabine (Xeloda)
- Pembrolizumab FDA approved in early stage 2-3 triple negative breast cancer (neoadj + adjv 27 weeks)

→ Is PDL-1 testing needed?

NO! (only in the metastatic setting)



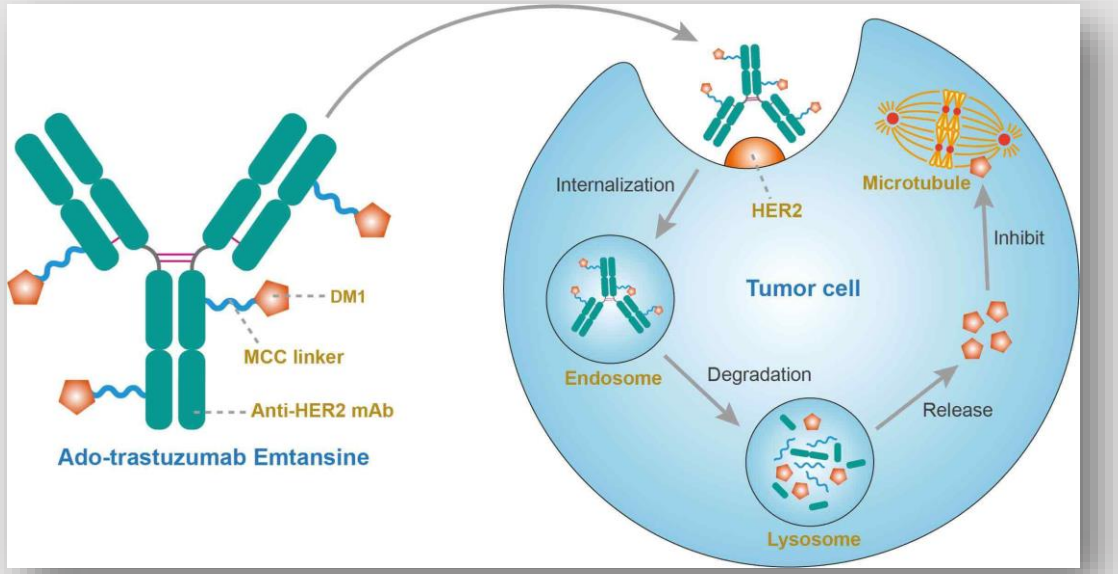
Masuda N, et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. N Engl J Med. 2017 Jun 1;376(22):2147-2159. PMID: 28564564.

Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med 2020; 382:810. and Schmid P, et al. Abstract 179. Presented at: ESMO Virtual Plenary; July 15, 2021

Post-treatment HER2 positive with residual disease

- Antibody-drug conjugate Ado-trastuzumab emtansine (TDM1)

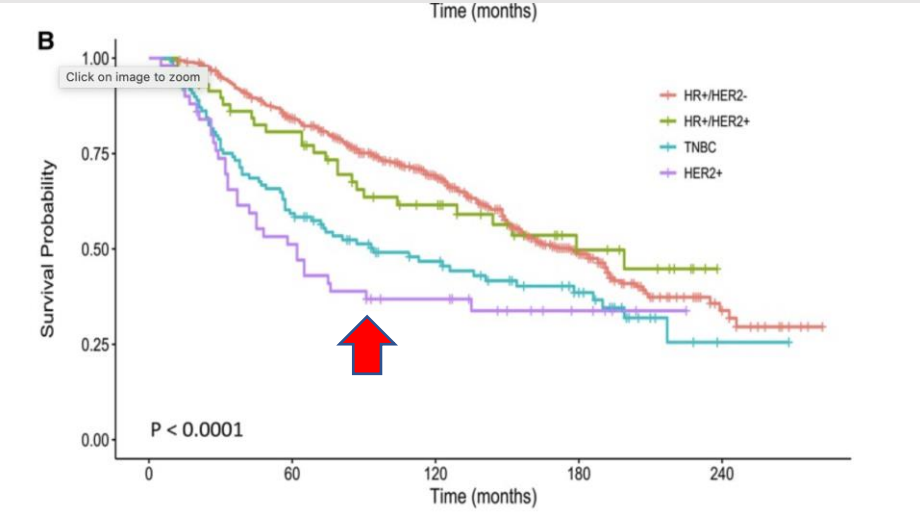
(vs continue with HER2 targeted alone if pCR)

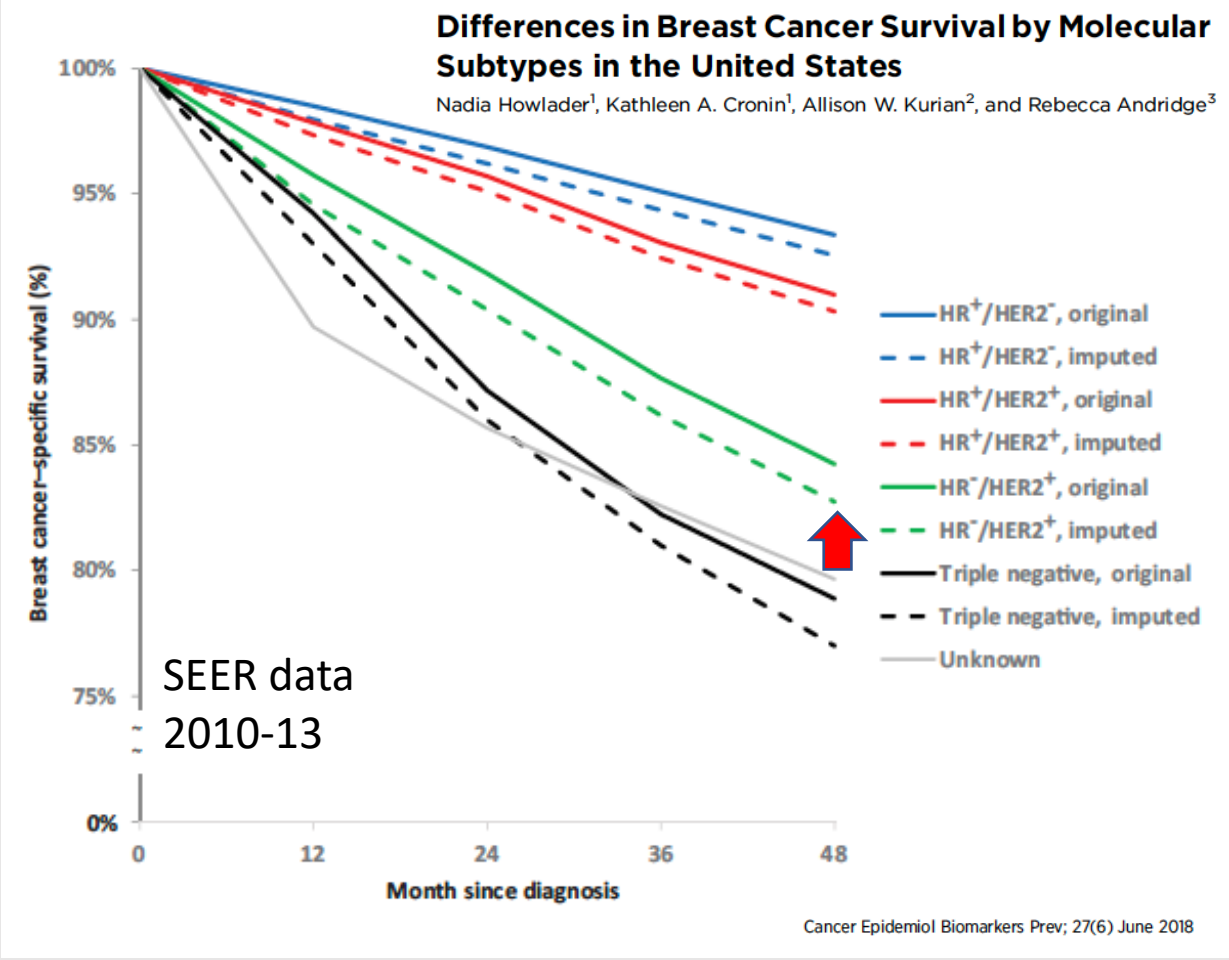


Denduluri N, et al. Selection of Optimal Adjuvant Chemotherapy and Targeted Therapy for Early Breast Cancer: ASCO Guideline Update. J Clin Oncol. 2021 : 33079579.

Survival in HER2+ cancers

- Now better 5-year survival than triple negatives
- If survive past 5 years curve flattens….likely long term survivor/”cure”



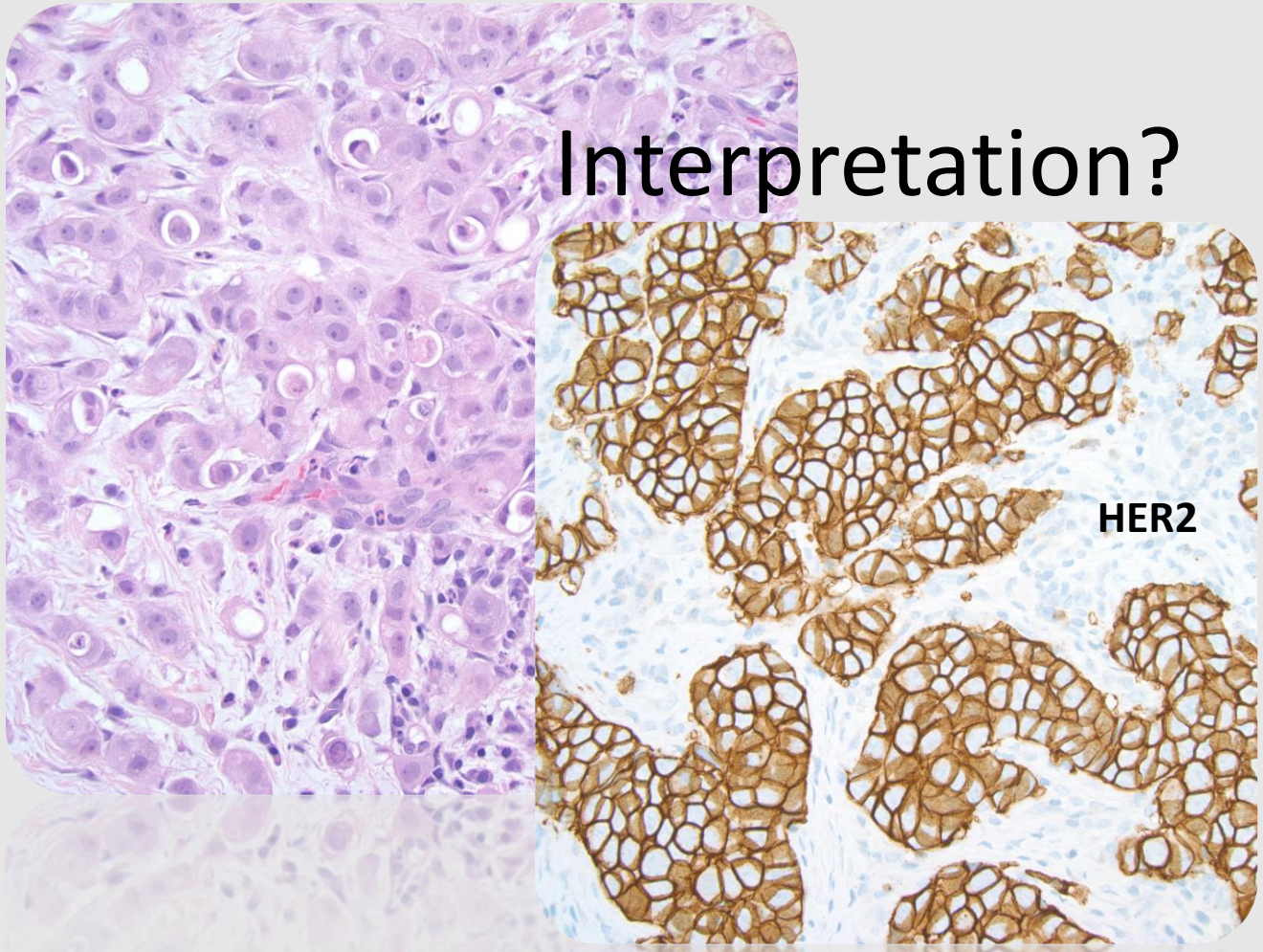


Yang SX, Polley EC. Breast Cancer Res Treat. 2019; 175(2): 287–295.

Test Case:

33 y/o postpartum female with 8 cm mass with following H&E and HER2 IHC stain. What is your interpretation of the HER2 IHC test?

A. HER2 Positive (3+)
B. HER2 Equivocal (2+)
C. HER2 Negative (1+)



Algorithm for interpreting HER2 IHC staining in invasive breast cancer

Any membranous staining present?

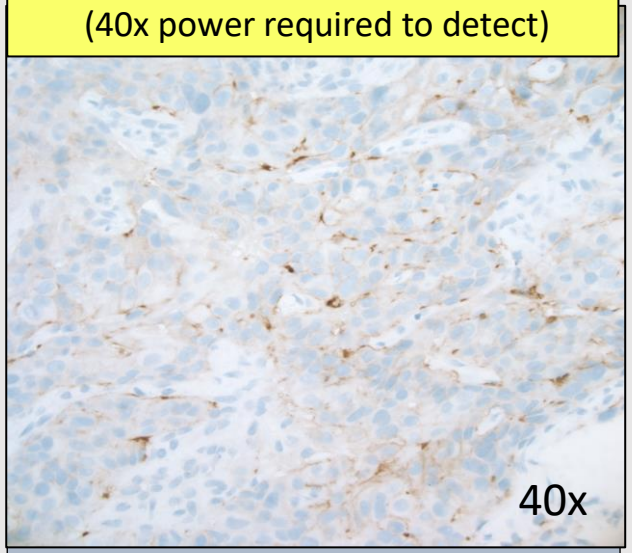
No membranous staining (at 40x)

Membranous staining present

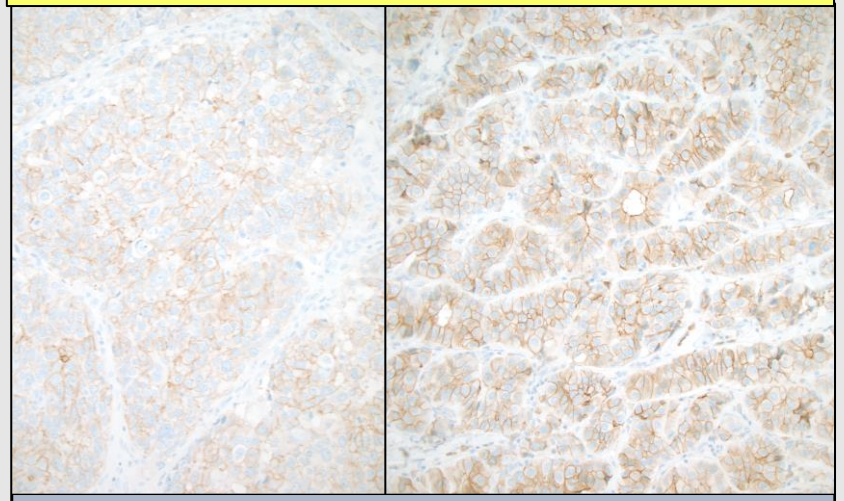
Completeness: Incomplete

Completeness: Complete

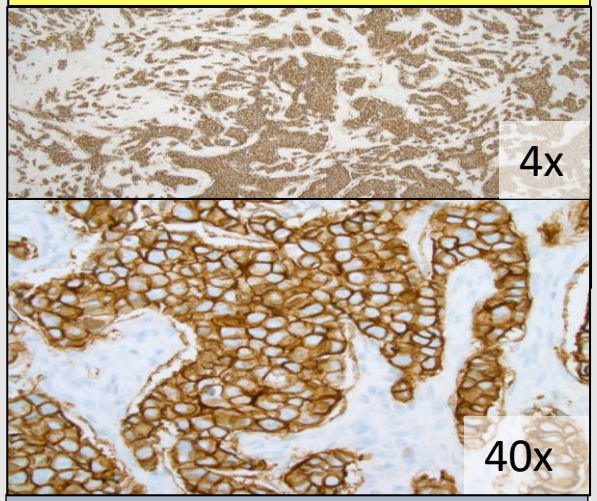
Intensity:
Faint/Barely perceptible in > 10%
(40x power required to detect)



Intensity:
Weak/Moderate in > 10%
(visualized on 10-20x power)



Intensity:
Strong / "chicken-wire" in > 10%
(visible at 2-5x power)



Negative (0)

Negative (1+)

Equivocal (2+)

Positive (3+)

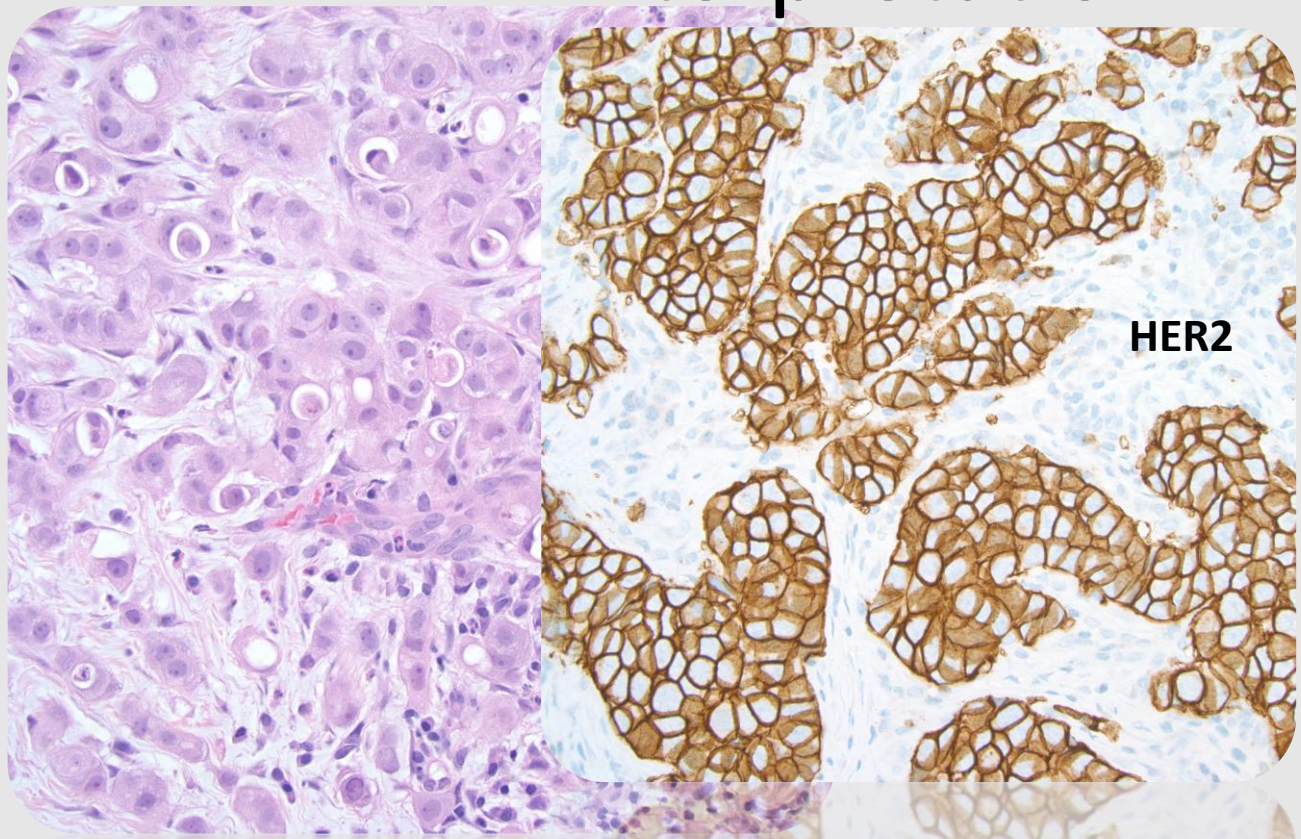
Reflex to ISH testing

Test Case:

Interpretation?

33 y/o postpartum with 8 cm mass with following H&E and HER2 IHC stain. What is your interpretation of the HER2 IHC test?

A. **HER2 Positive (3+)**
B. HER2 Equivocal (2+)
C. HER2 Negative (1+)



REFERENCE:
Wolff AC, Hammond ME, 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: 2105–22.

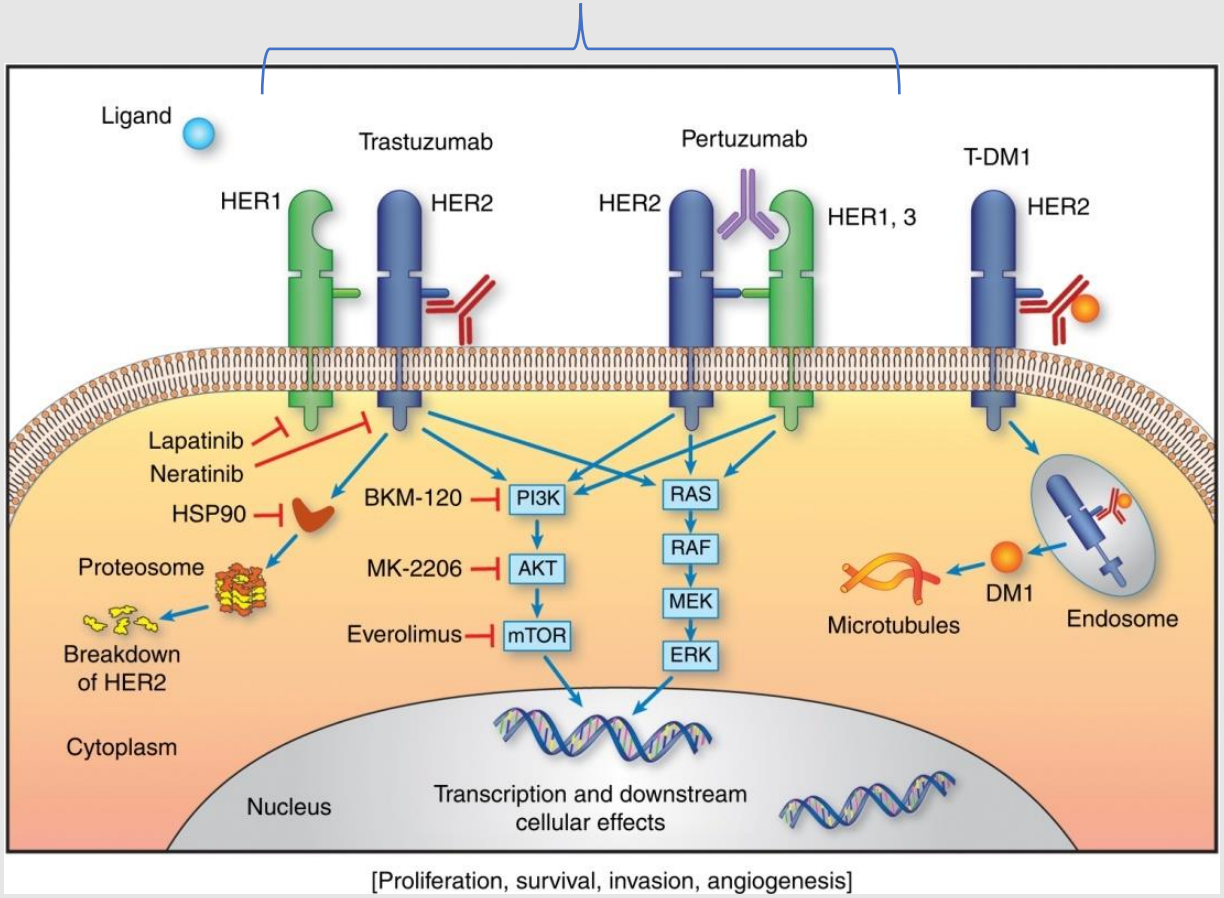
What's next for the patient?

- 6 months neoadjuvant chemotherapy (AC/T) plus Herceptin x 1 year
- Treated to a complete pathologic response in both breast and axilla
  - 5 year survival difference w/ CPR: 42% → 80-95%

Latest in Targeted treatments for HER2 Positive Breast Cancer

Trastuzumab (Herceptin) + Pertuzumab (Perjeta) + chemo combination therapy approved neoadjuvantly in 2013, now standard in higher risk cases either adjuvant or neoadj (APHINITY trial)

Trastuzumab (Herceptin) + chemo:
First approved in 2006 in non-metastatic setting



Ado-trastuzumab (TDM1)

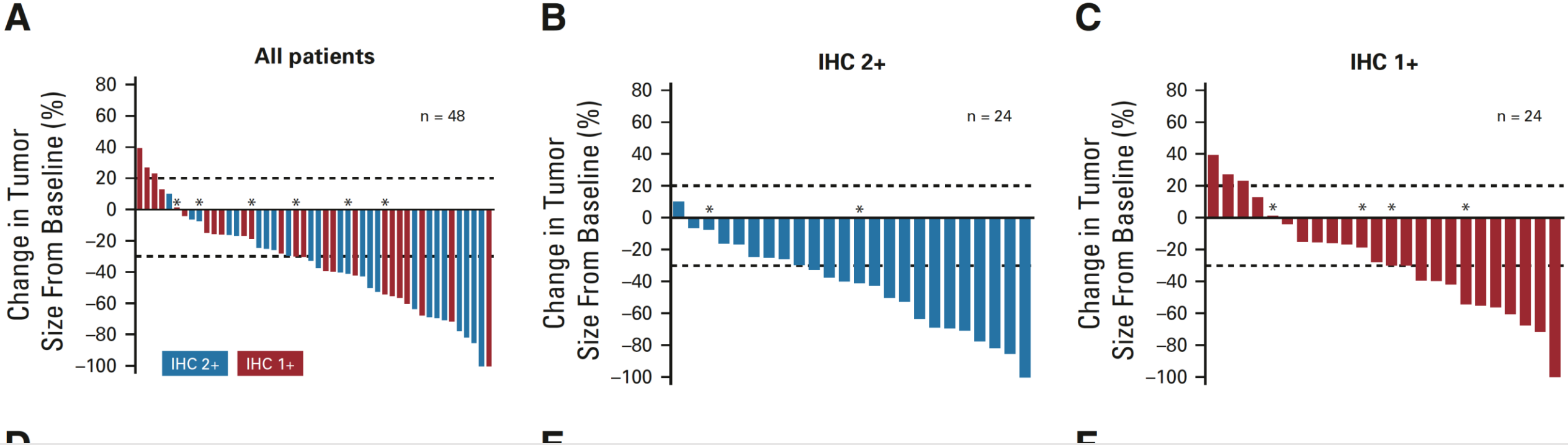
- Trastuzmab linked to chemotherapy for intra-cellular release (ADC).
- FDA approved to add on post-neoadjuvant treatment if pCR not achieved (KATHERINE trial).
- Used in metastatic setting.

Now also **T-DXd** as second line in mets

Tucatinib
oral TKI effective in brain mets (HER2CLIMB trial)

BJC British Journal of Cancer
Multidisciplinary Journal of Cancer Research

T-DXD in “HER2-Low”



- Exciting results in “HER2-low”= 1+ to 2+ by IHC, negative for gene amplification
- Metastatic setting only

Trastuzumab Deruxtecan Is Effective in HER2-Low Breast Cancer. Cancer Discov. 2020 Apr;10(4):488. doi: 10.1158/2159-8290.CD-RW2020-030. Epub 2020 Feb 28. PMID: 32111601.

HER2 Low?

- 0 vs 1+ threshold largely untested to determine if clinical validity…..
- Some evidence if include HER2 0 may also benefit = irrelevant if “HER2 low”
- Mostly ER+ cancers but HER2 Low is not a biologically defining biomarker
- Would need to validate antibodies around new threshold…. Lots of issues here (no gold standard, heterogeneity, pre-analytics, variability)
- PREMATURE TO USE HER2 0 vs 1+ as a clinically relevant threshold OUTSIDE OF A CLINCIAL TRIAL

<u>Trials testing HER2 Low:</u>

DESTINY → only including HER2-low

DAISY → including HER2+, HER2-low and HER2 0 (SABCS poster PD8-02, Abstract #617)

HER2-Low Breast Cancers

New Opportunities and Challenges

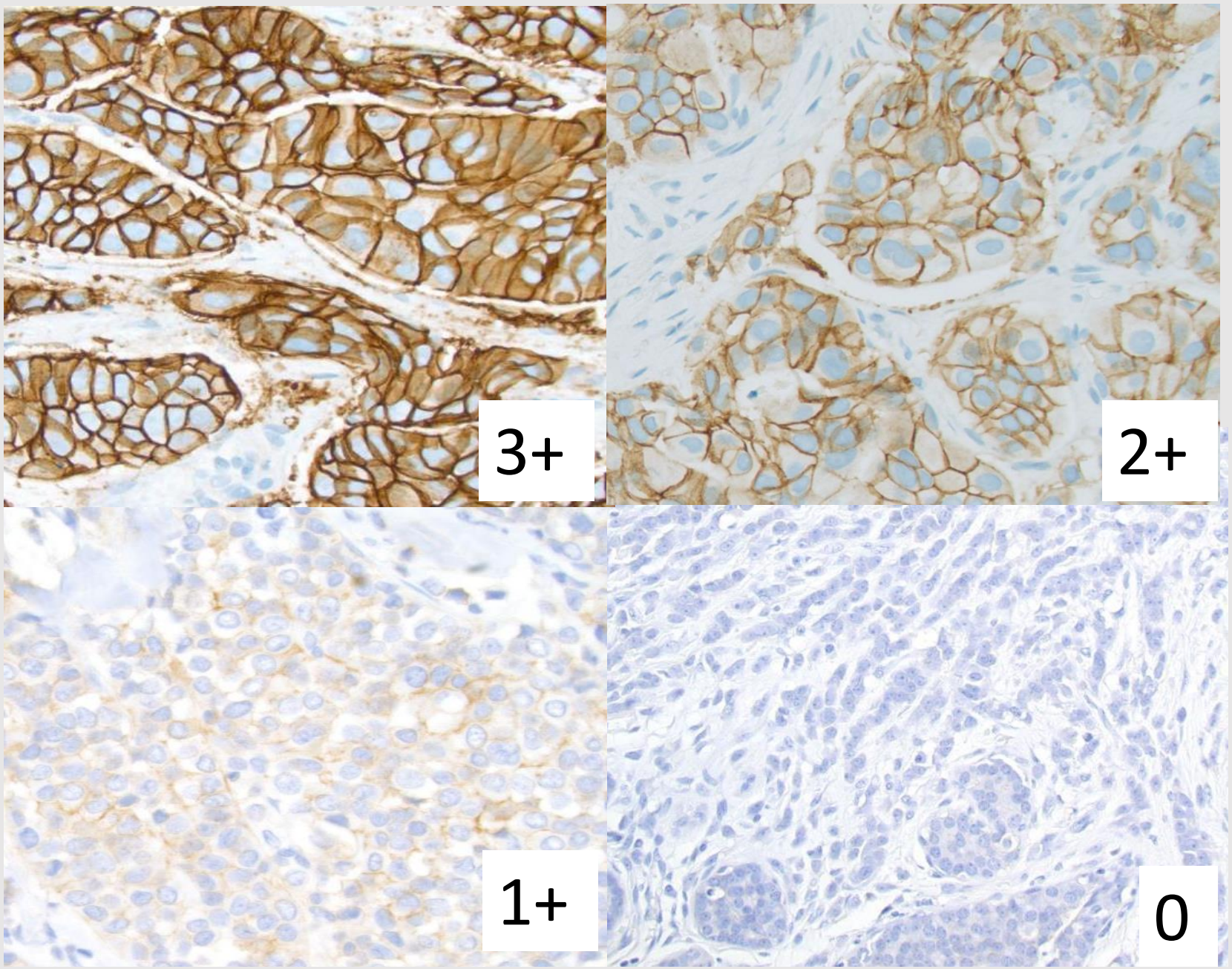
Huina Zhang, MD, PhD, Hani Katerji, MD, Bradley M. Turner, MD, MPH, MHA, and David G. Hicks, MD

From the Department of Pathology, University of Rochester Medical Center, Rochester, NY, USA.

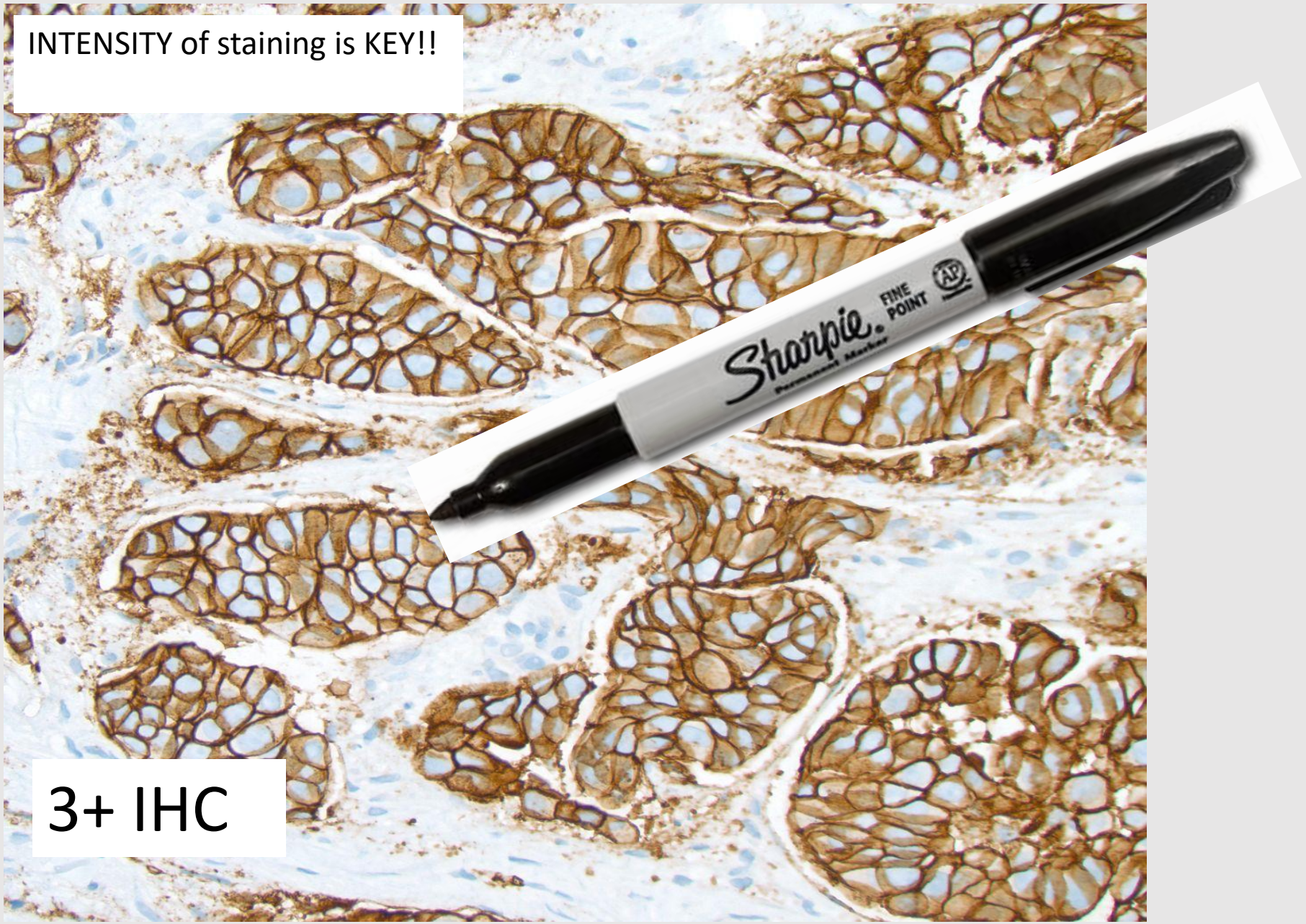
Am J Clin Pathol. 2021 Sep 14:aqab117. doi: 10.1093/ajcp/aqab117. Epub ahead of print. PMID: 34519765.

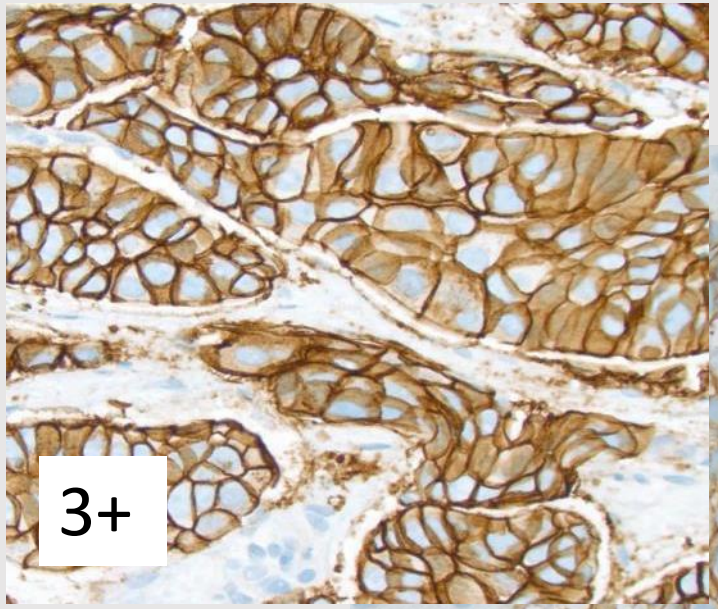
HER2 IHC pitfalls and challenges

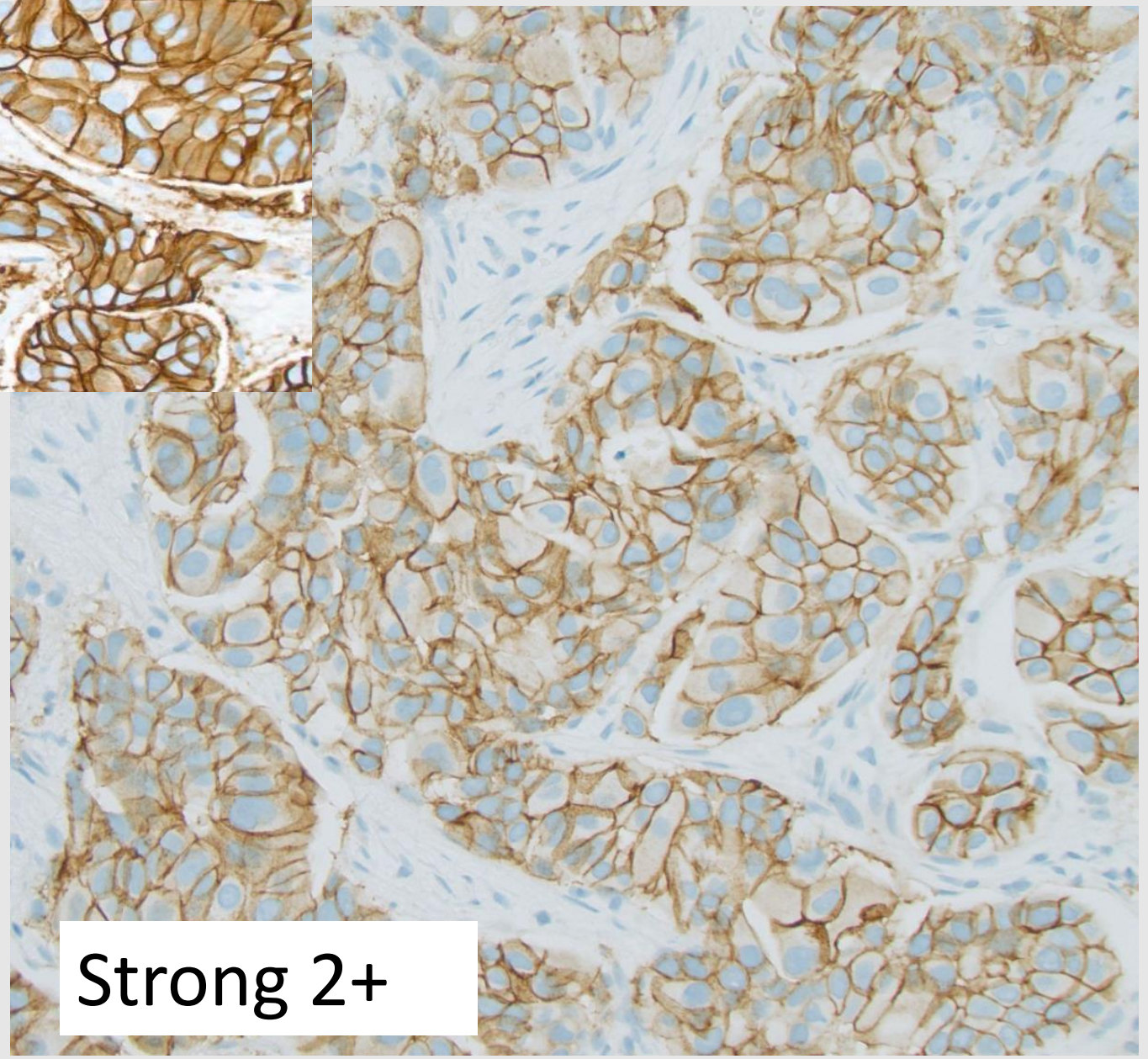
- Overinterpretation of stain intensity
- Artifacts
- Faded slide/cut too long prior to testing
- Unusual staining patterns
- Discordant with histology
- Heterogeneity
- Discordant with FISH


3+
2+
1+
0

INTENSITY of staining is KEY!!

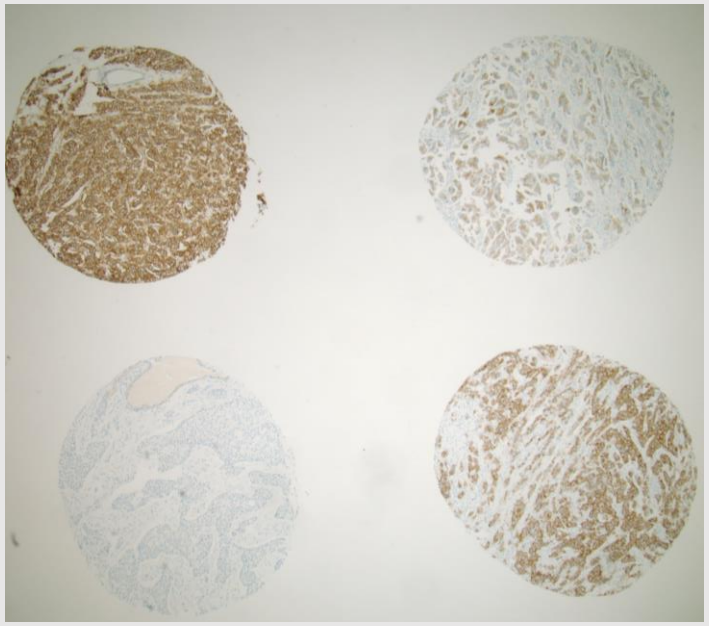






Importance of good controls:

- Preferably on slide
- Range of stain intensities



2018 Guidelines: What is HER2 Indeterminate?

- Inadequate specimen handling
- Artifacts (crush or edge)
- Analytical testing failure
- Controls not as expected
- Unstained slide cut > 6 weeks prior
- For ISH:
  - Not at least 2 areas to count, >25% of signals unscorable/weak, > 10% of signals occur over cytoplasm, nuclear resolution poor, auto-fluorescence strong

Cold ischemic time < 1 hour
Formalin fixation 6-72 hours

- Reason for indeterminate result should be reported
- Another method of testing can be attempted or another sample requested

ASCO/CAP HER2 Testing Guideline Update—Wolff et al

Beware of the old unstained section

ASCO/CAP Guidelines:

Do not use unstained sections cut > 6 weeks from testing

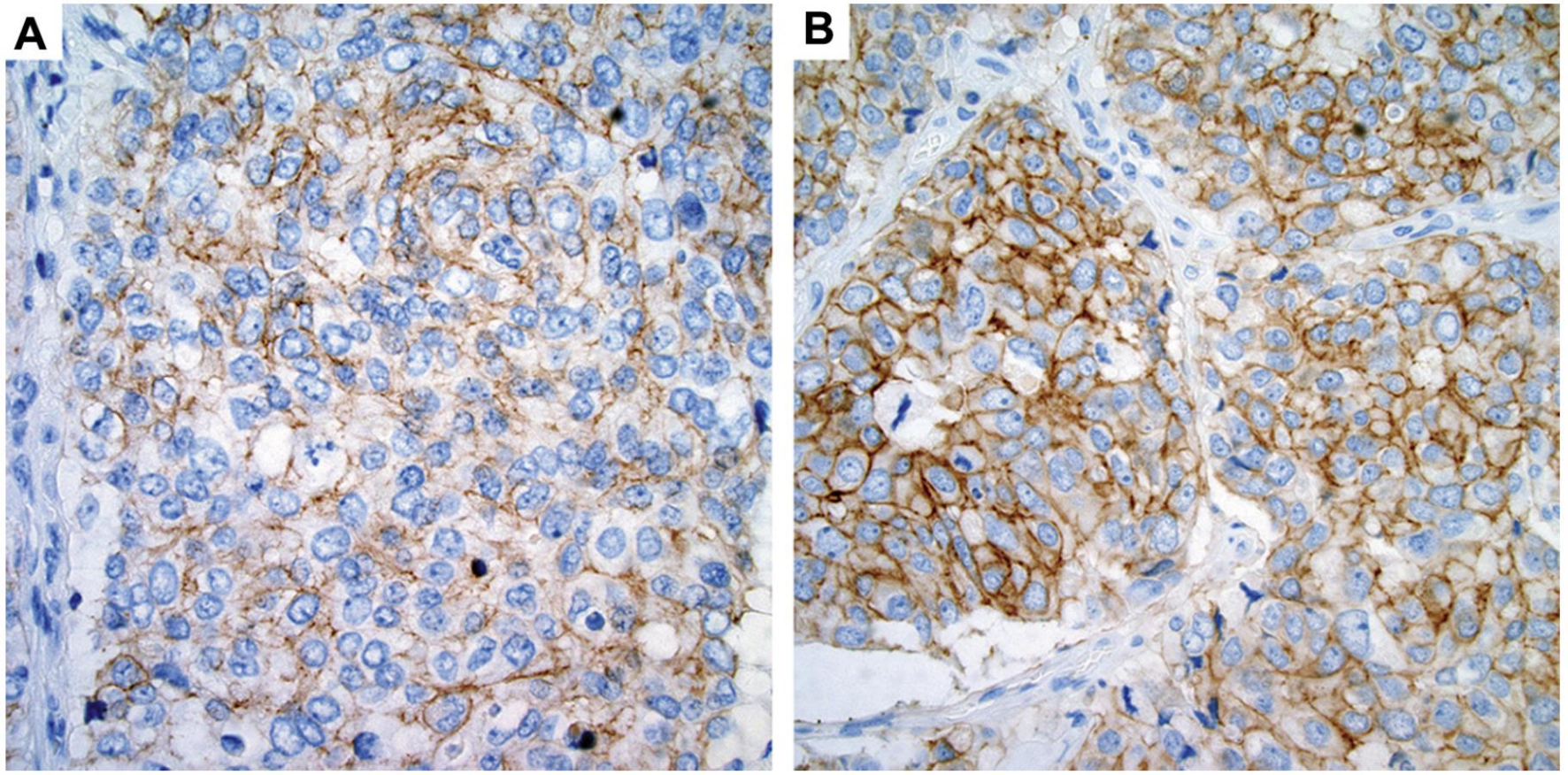
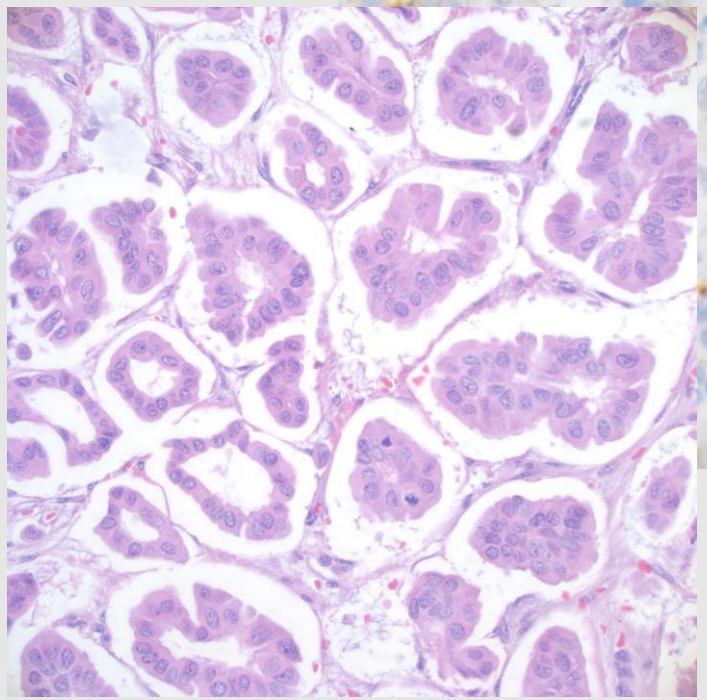


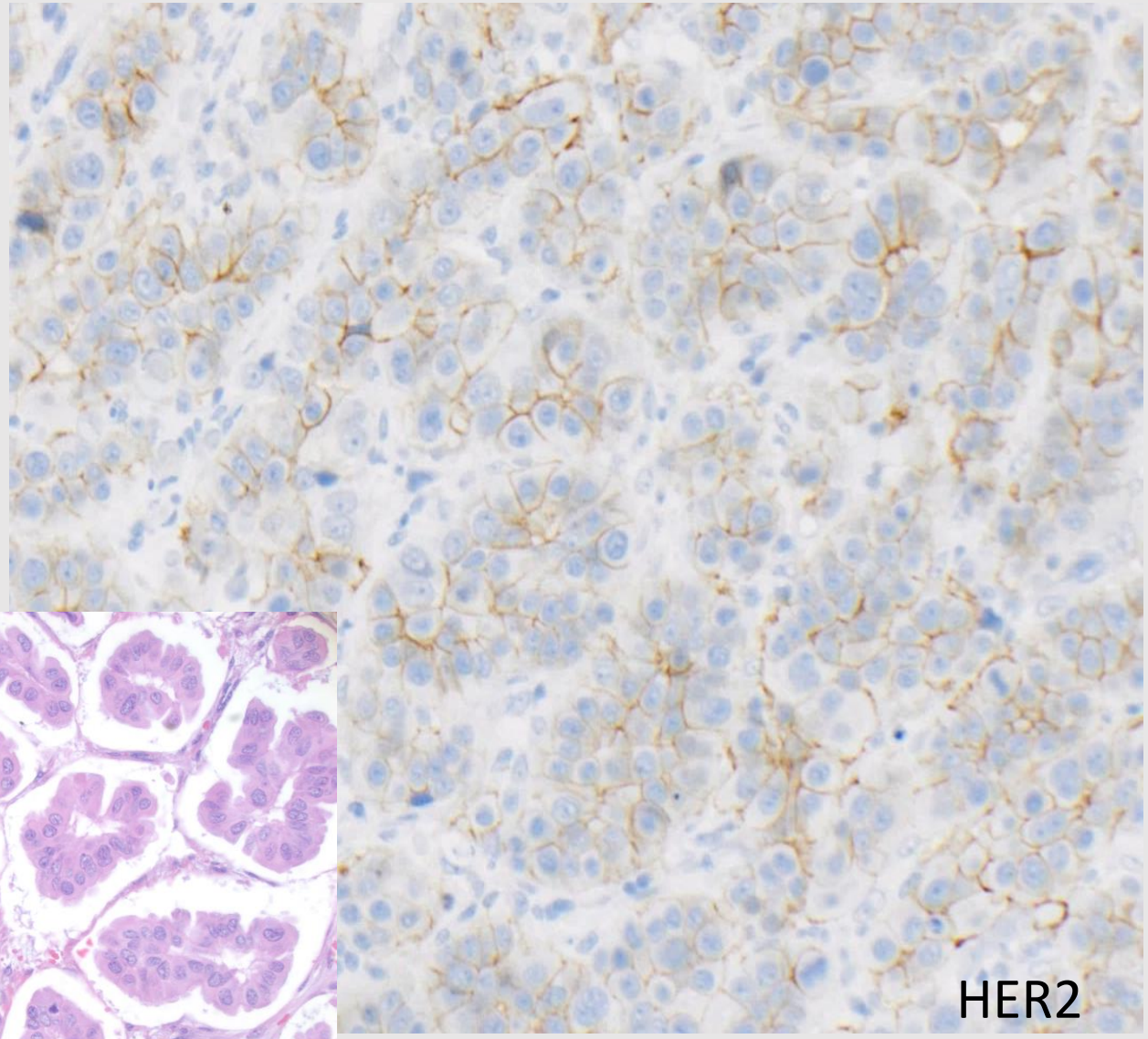
Fig. 7. An example of the effect of time since unstained sections were cut from the tissue block on HER2 expression. Sections from the same tissue block were stained for HER2. Only 1+ staining was noted after more than 6 weeks elapsed since cutting the unstained sections from the tissue block (as shown in *A*). However, 2+ staining is present when the test is run on freshly cut unstained sections (*B*) [H&E, original magnification *A*, *B* ×400].

Surgical Pathology 11 (2018) 147–176
https://doi.org/10.1016/j.path.2017.09.006

HER2 Test Case

- 51 year old
- Grade 2 invasive cancer
- ER > 95% Positive
- You are interpreting the HER2 IHC stain





Algorithm for interpreting HER2 IHC staining in invasive breast cancer

Any membranous staining present?

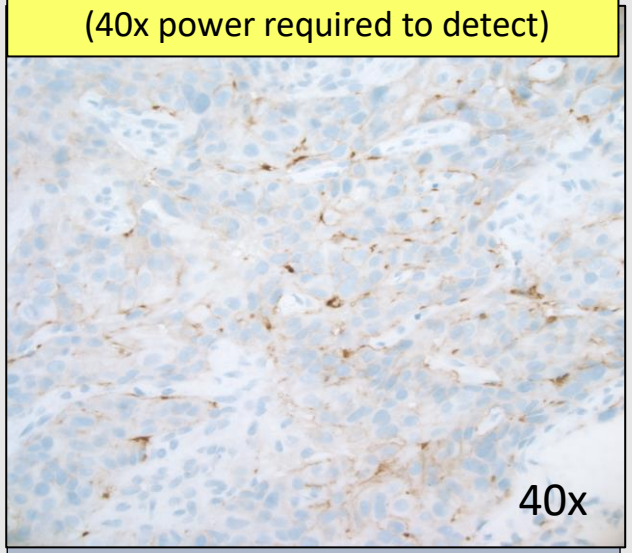
No membranous staining (at 40x)

Membranous staining present

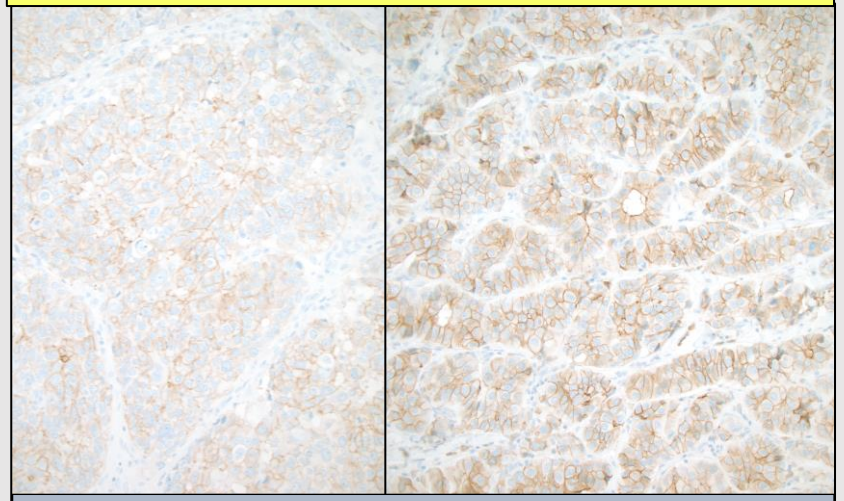
Completeness: Incomplete

Completeness: Complete

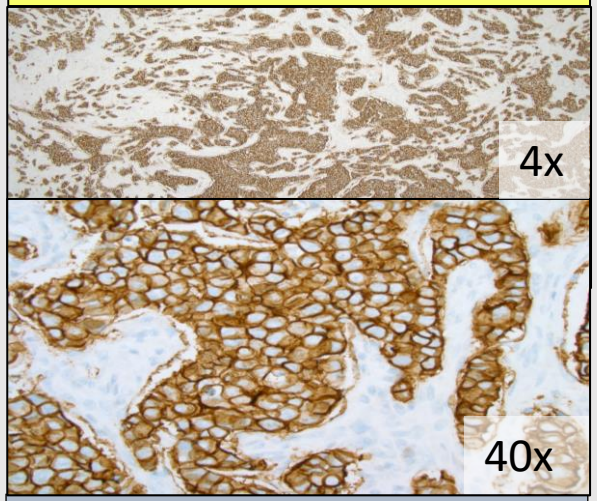
Intensity:
Faint/Barely perceptible in > 10%
(40x power required to detect)



Intensity:
Weak/Moderate in > 10%
(visualized on 10-20x power)



Intensity:
Strong / "chicken-wire" in > 10%
(visible at 2-5x power)



Negative (0)

Negative (1+)

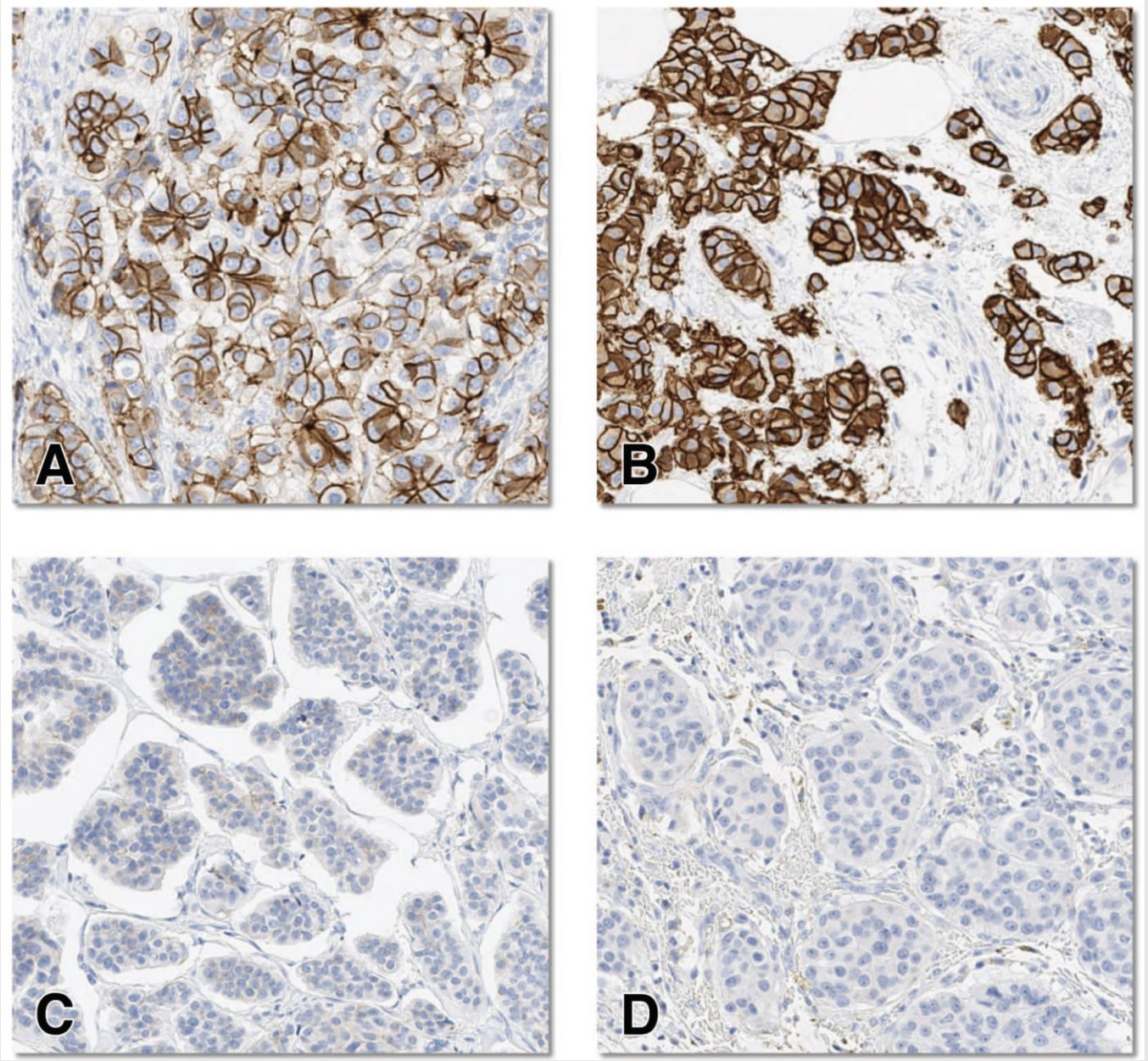
Equivocal (2+)

Positive (3+)

Reflex to ISH testing

HER2 staining in micropapillary carcinoma

- Basolateral ”U-shaped” staining common (~50%)
  - When intensity strong typically amplified
  - When intensity weak to moderate can also be amplified (30%) → should call 2+ and reflex to ISH

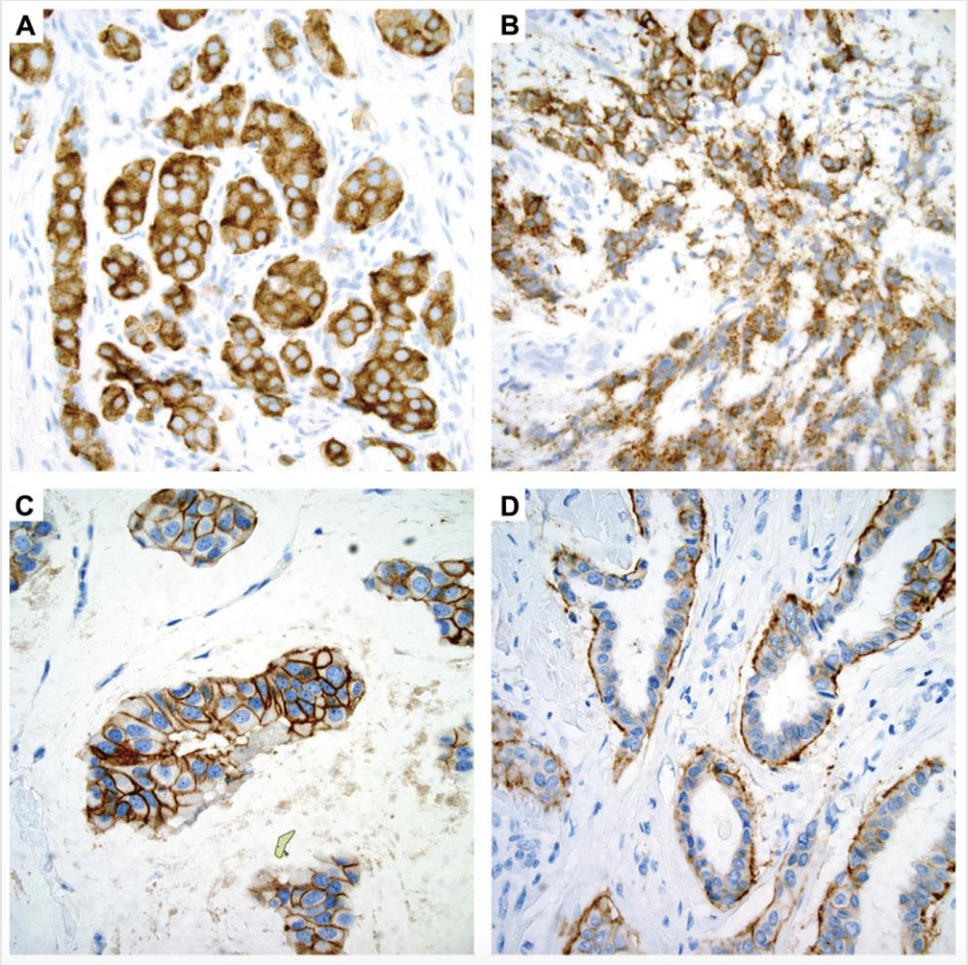


Perron M, Wen HY, Hanna MG, Brogi E, Ross DS. HER2 Immunohistochemistry in Invasive Micropapillary Breast Carcinoma: Complete Assessment of an Incomplete Pattern. Arch Pathol Lab Med. 2021 Aug 1;145(8):979-987.

Unusual HER2 Staining Patterns

Unusual IHC Patterns (can call 2+):

- Granular staining
- Basolateral staining only (more frequent in micropapillary carcinomas and may be amplified)
- Only basal staining



See review:
Allison KH, Ancillary Prognostic and Predictive Testing in Breast Cancer Focus on Discordant, Unusual, and Borderline Results Surgical Pathology 11 (2018) 147–176 https://doi.org/10.1016/j.path.2017.09.006

HER2 Test Case

- 51 year old
- Grade 2 invasive cancer
- ER > 95% Positive
- Your interpretation of the HER2 IHC stain:
  - **Equivocal 2+ (unusual staining pattern)**
  - **Refer for ISH testing**

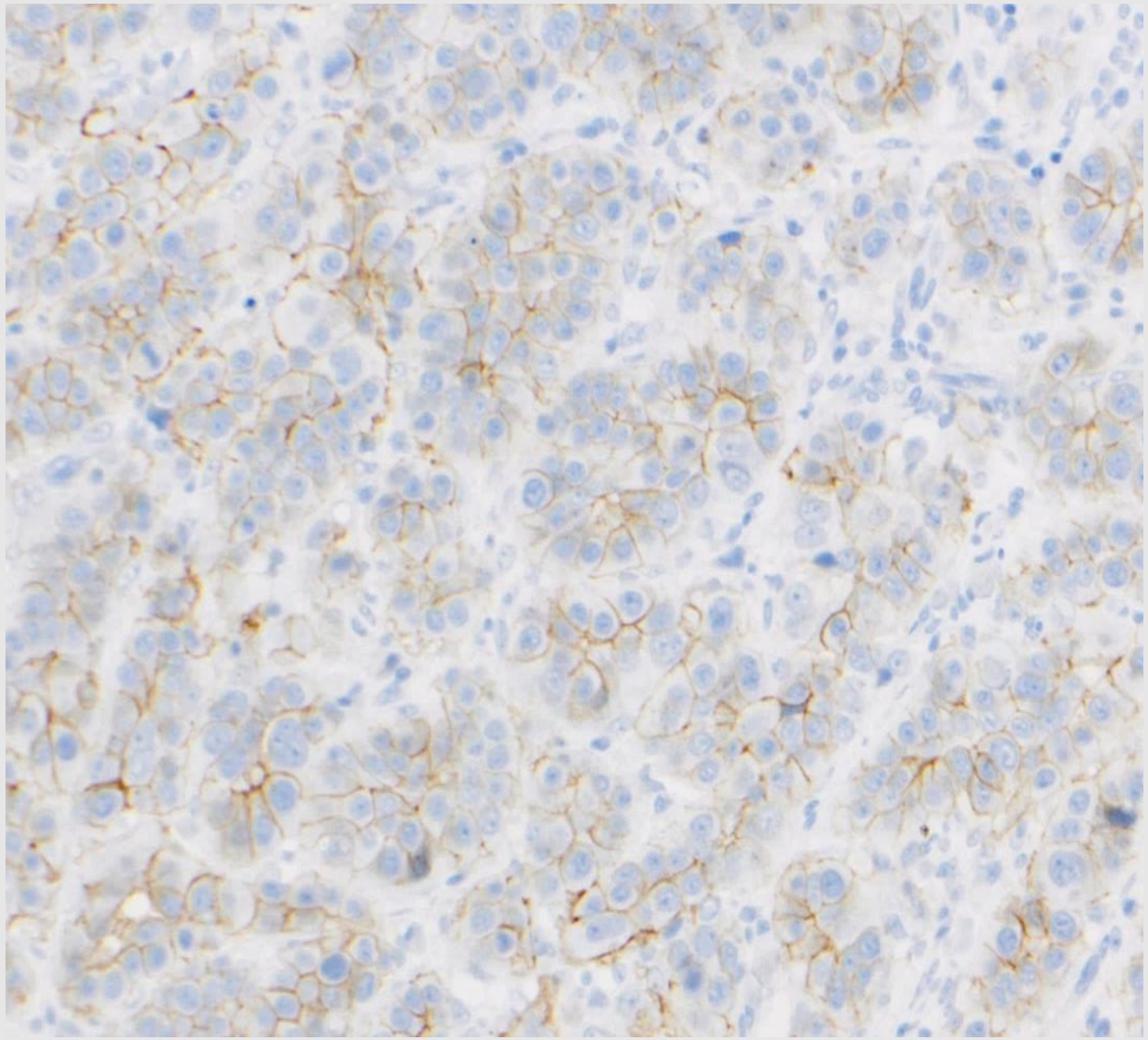
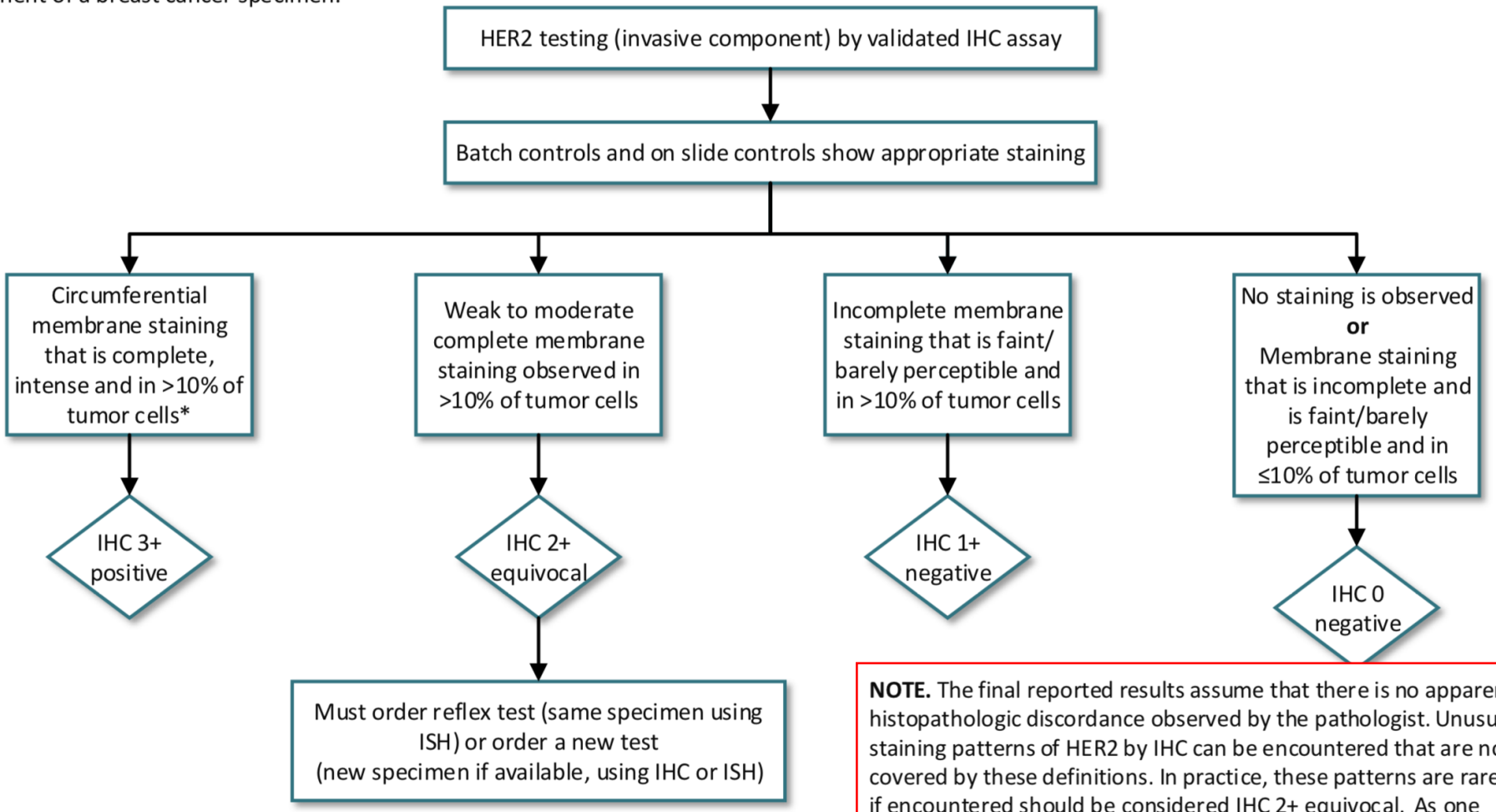
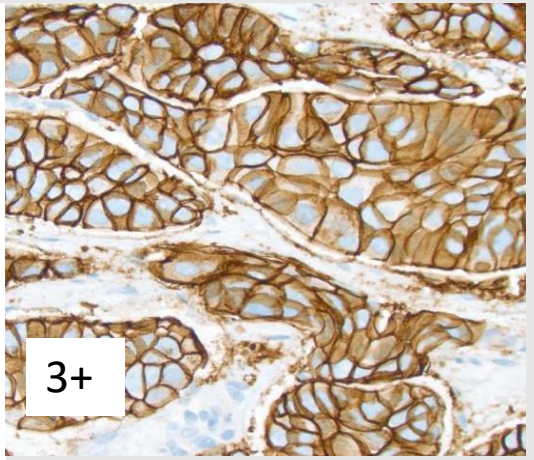
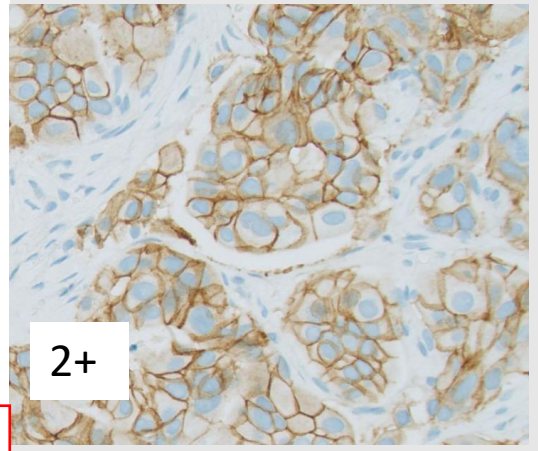
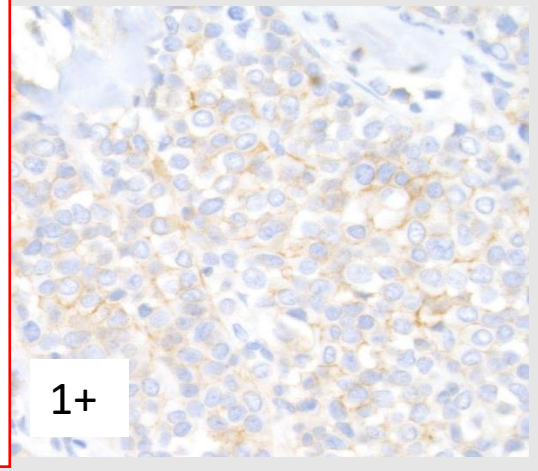


Figure 1. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) protein expression by immunohistochemistry (IHC) assay of the invasive component of a breast cancer specimen.









NOTE. The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. Unusual staining patterns of HER2 by IHC can be encountered that are not covered by these definitions. In practice, these patterns are rare and if encountered should be considered IHC 2+ equivocal. As one example, some specific subtypes of breast cancers can show IHC staining that is moderate to intense but incomplete (basolateral or lateral) and can be found to be HER2 amplified. Another example is circumferential membrane IHC staining that is intense but within ≤10% of tumor cells (heterogeneous but very limited in extent). Such cases can be considered 2+ equivocal but additional samples may reveal different percentages of HER2 positive staining. (*)Readily appreciated using a low power objective and observed within a homogeneous and contiguous invasive cell population

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.

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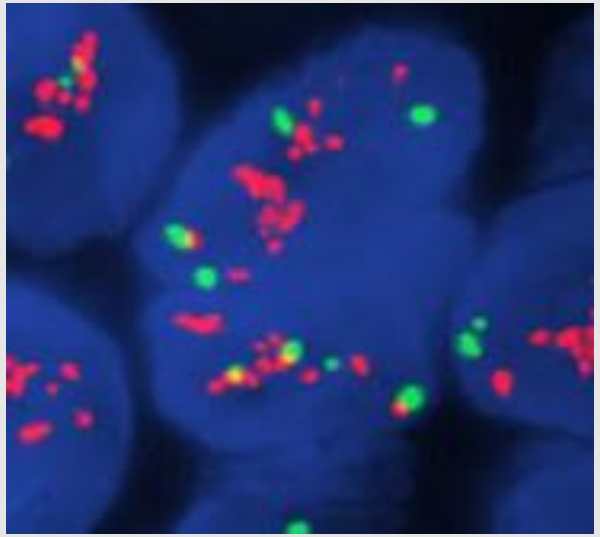
ASCO Guidelines

HER2 In Situ Hybridization (ISH) Testing

Cell	HER2	CEP17
1	15	2
2	9	2
3	7	1
4	12	2
5	10	2
6	10	1
7	8	3
8	2	2
9	2	2
10	8	2
11	15	1
12	12	3
13	8	2
14	2	2
15	7	2
16	9	2
17	12	1
18	12	2
19	15	2
20..	10	3
Mean	9.25	1.95
Ratio	4.74	

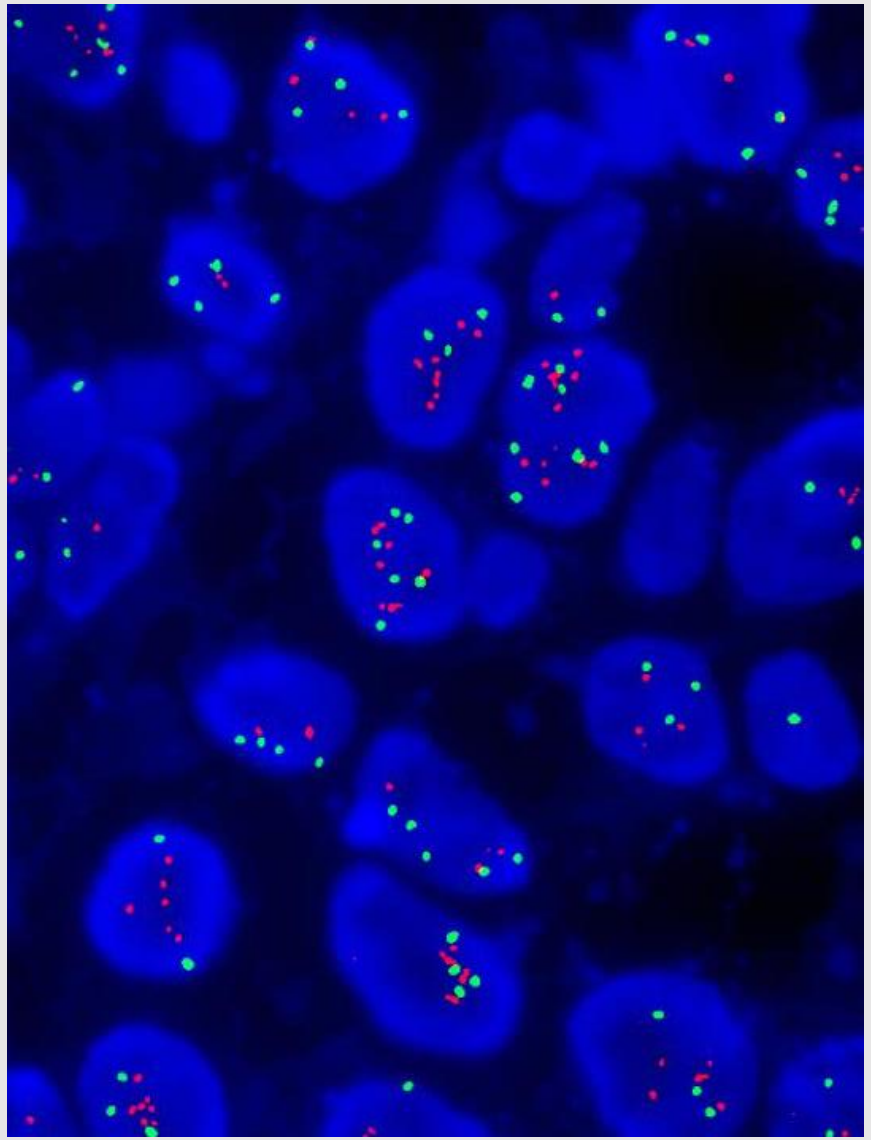
- Example of FISH case Positive for HER2 gene amplification
  - (Dual Probe)



Must include both mean signals/cell and ratio on report

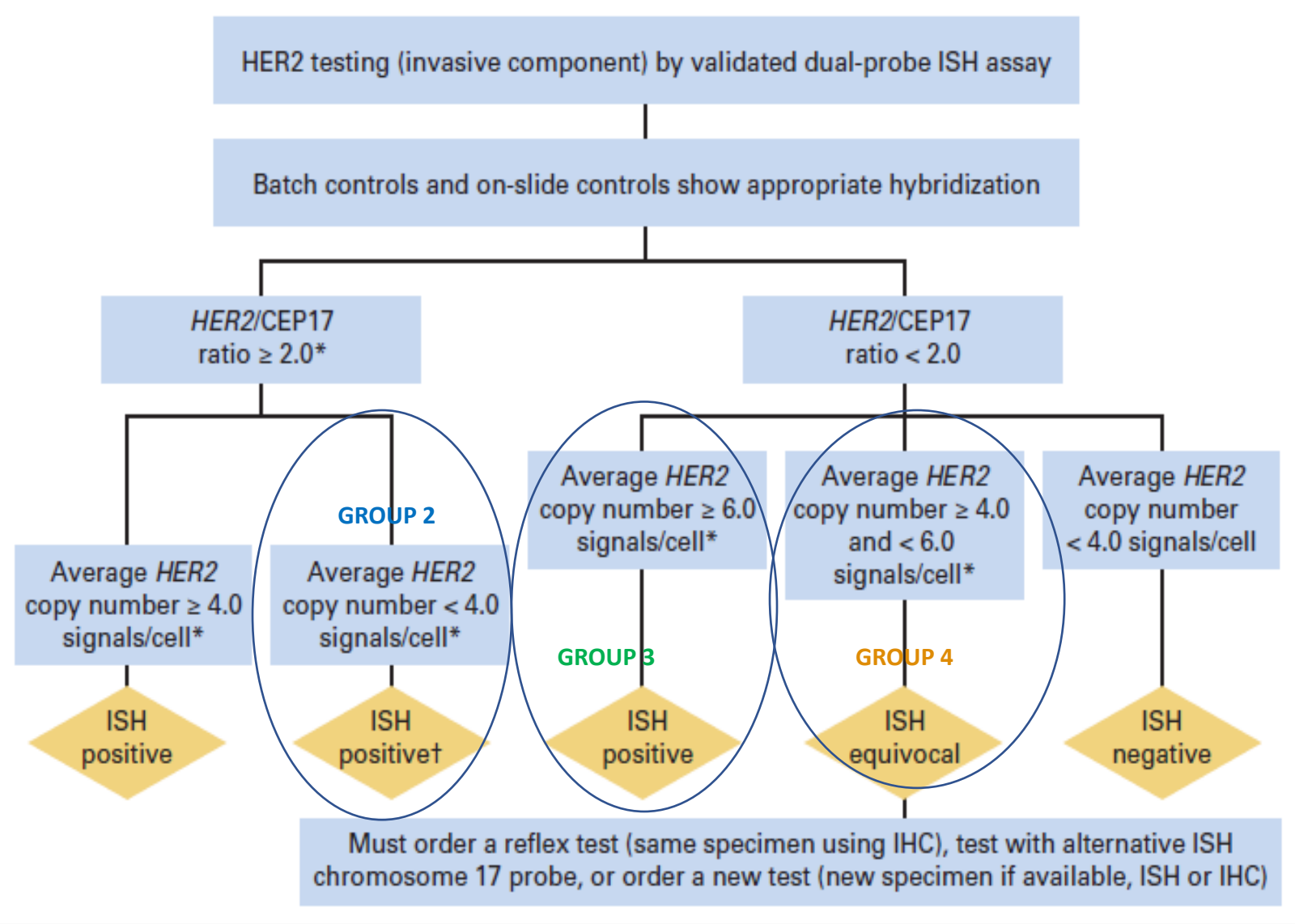
Our Test Case Results:

Cell	HER2	CEP17
1	7	3
2	1	2
3	5	4
4	8	4
5	4	3
6	6	5
7	3	3
8	1	4
9	5	3
10	4	3
11	6	4
12	8	3
13	5	3
14	6	2
15	3	1
16	2	2
17	5	5
18	6	3
19…	4	3
…..(50 total)		
Mean	**4.6**	2.4
Ratio	**1.9**	



- Ratio < 2.0
- Mean HER2 signal/cell between 4-6

2013 HER2 Testing by Dual-Probe ISH



Group nomenclature from Press, JCO and APLM 2016

HER2 Focused Update Clinical Questions About Unusual ISH Groups 2-4

- **Group 2**: HER2/CEP17 ratio ≥2.0, HER2 copies <4, **HER2 Positive? (Monosomy for CEP17)**
- **Group 3**: HER2/CEP17 ratio <2.0, HER2 copies ≥6, **HER2 Positive? (Co amplified/ polysomy of CEP17)**
- **Group 4**:HER2/CEP17 ratio <2.0. HER2 copies ≥4 but <6, **HER2 Equivocal?**
- **Are alternative probes recommended?**

Group 4 Cases: What do we know?

Labs	HERA central lab *(Mod Pathol 2015)*	BCIRG central lab *(JCO 2016)*	Press reference lab *(APLM 2016)*	Jenkins reference lab *(JCO 2016)*	UK NEQAS 2009-2016 partial *(unpublished)*	*Stanford/UCSF/ UWMC (Mod Pathol 2017)*
FISH distribution	n=6,018	n=10,468	n=7,526	n=2,851	n=11,116	n=8,068
Group 4 *ratio <2.0; HER2 ≥4.0 <6.0 (after alternative probe: pos, equivocal, neg)*	1.9%	4.1%	4.6%	14.2% *(7.4%, 5.5%, 1.3%)*	7.6%	5.2%

- Frequency depends on population testing
- Mostly ER+ (80-85%), Rarely HER2 3+,frequently IHC neg (0-1+), often 2+
- Alternative probes previously used frequently with variable results not clinically validated → no longer recommended
- Limited clinical trial data (not in original HER2 trials) but BCIRG-005 Data support ***they do no worse that non-Group 4 cases w/o HER2 Rx***

Press MF et al. J Clin Oncol. 2016 Oct 10;34(29):3518-3528
Ballard M et al. Mod Pathol. 2017 Feb;30(2):227-235.

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

COLLEGE of AMERICAN PATHOLOGISTS

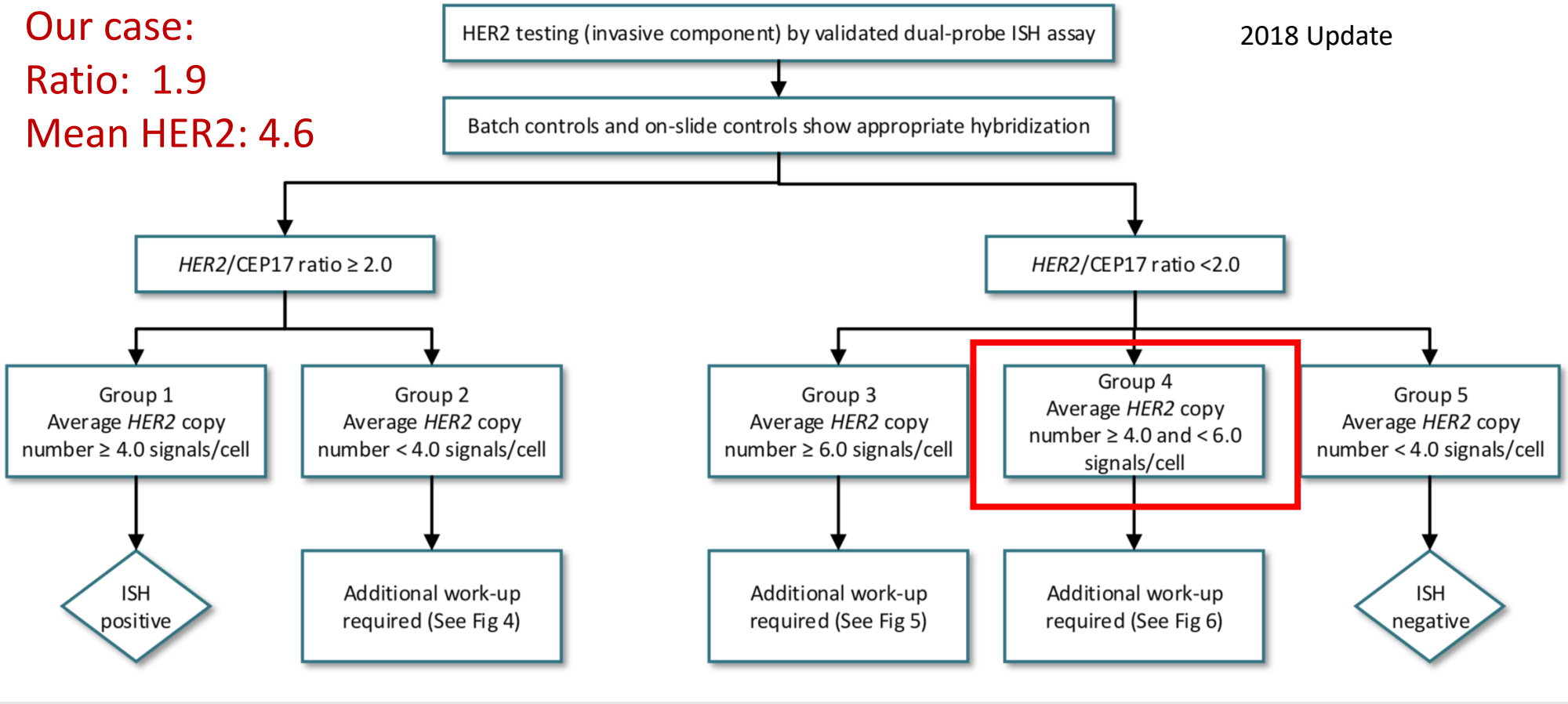
ASCO Guidelines

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

Figure 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).

Our case:
Ratio: 1.9
Mean HER2: 4.6

2018 Update



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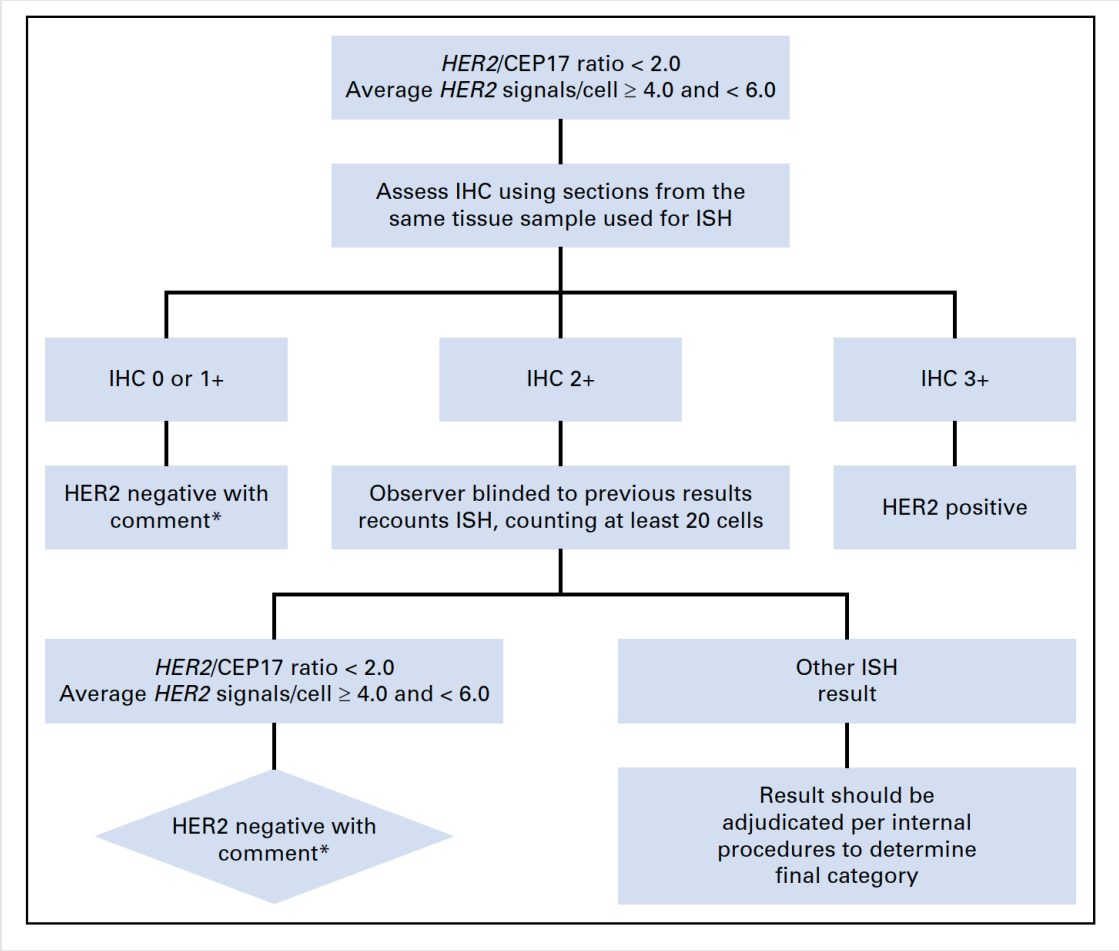
Group 4 ISH Cases: Additional Workup

- Review or perform concurrent IHC:
- If Neg IHC (0-1+) = HER2 Negative
- If Positive IHC (3+) = HER2 Positive
- If 2+ → second observer counts (at least 20 cells) and if still Group 4 = HER2 Negative



Group 4: Only positive if IHC is 3+

HER2 Case 1 FISH Results:

RESULTS: HER2:CEP17 Ratio 1.9
Mean HER2 signals/cell: 4.6
Result category: Ratio <2.0 and 4-5.9 HER signals/cell (Group 4 result)

INTERPRETATION: HER2 NEGATIVE (**BASED ON IHC AND FISH, SEE COMMENT**)
Concurrent IHC result: 2+ Equivocal

REQUIRED

COMMENT: This case has an uncommon FISH result ("Group 4," previously considered equivocal). Per the 2018 HER2 Testing Update, a concurrent IHC result has been used in the interpretation of the final result (and the FISH result recounted by a second observer). 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 high likelihood that repeat testing will result in different results by chance alone. Therefore, per guideline recommendations, when IHC results are not 3+ positive, the sample is considered HER2 negative without additional testing on the same specimen.

HER2 Case 1 Scenario 2

- IHC 2+
- Initial Group 4 ISH result close to threshold for Group 1 result
  - Ratio 1.9
  - Mean HER2 signals/cell 4.6
- Recounts/adjudicated results:
  - Ratio 2.1
  - Mean HER2 signals/cell 4.8

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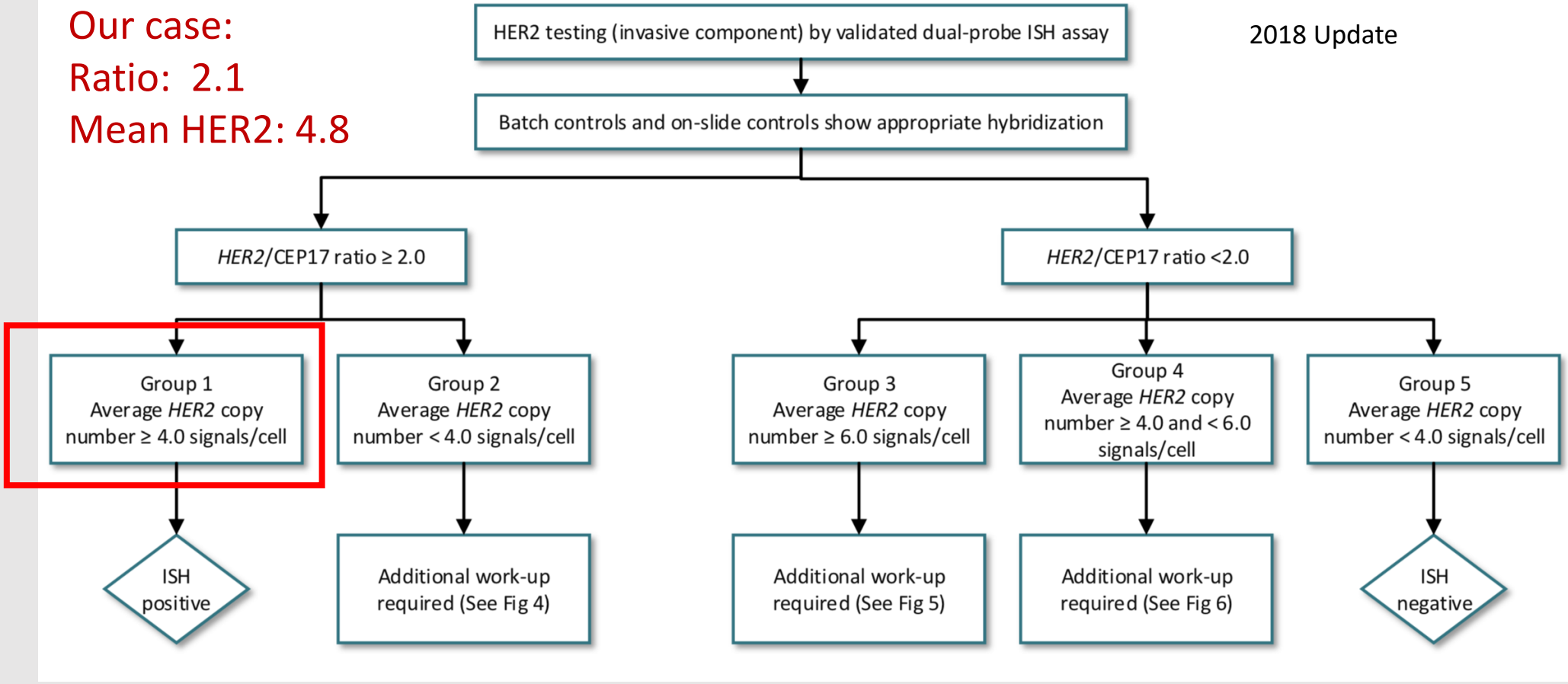
Figure 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (HER2 gene) assay (dual-probe ISH).

Our case:
Ratio: 2.1
Mean HER2: 4.8

2018 Update



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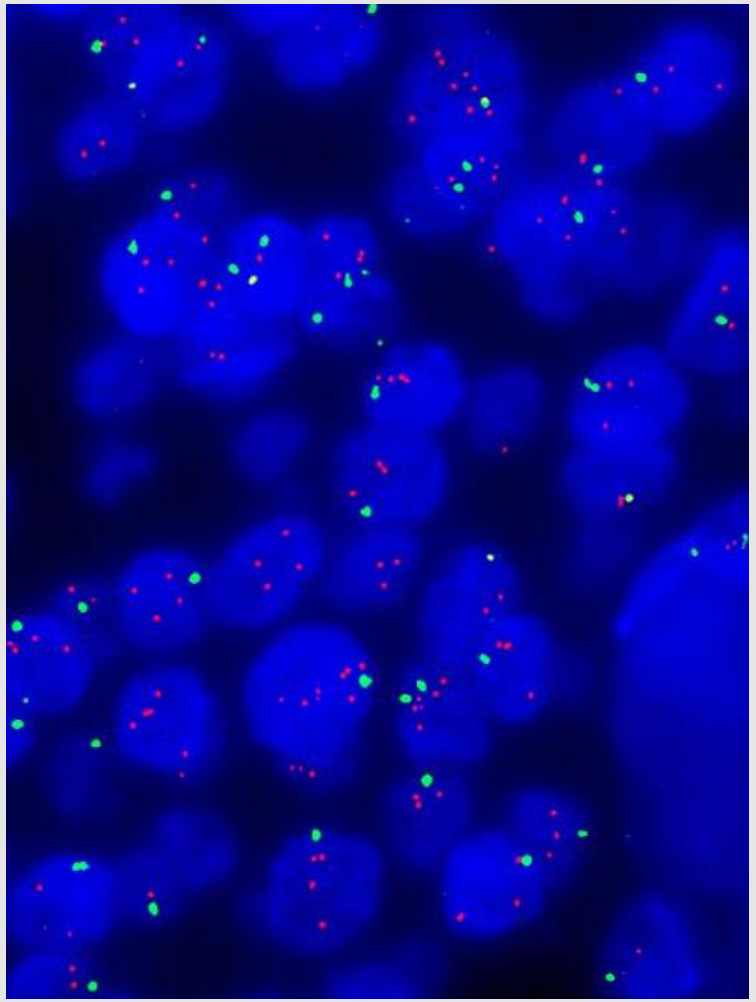
Variability close to a threshold

- Is expected
- Double check results
- Additional counts with new observers
- Check with prior/other results
- Consider histologic features
- Can send for consultation
- Borderline positives likely to be negative by RT-PCR (Oncotype) (not recommended as an alterative test)
- Acknowledge the results are in a borderline zone!

Low Amplified Results

- Ratio ≥ 2.0 but 4.0-5.9 mean HER2/cell
- Considered a positive result by ASCO/CAP (not addressed separately)
- In original trials because ratio ≥ 2.0
- Can be discordant with IHC result (0-1+)
- Identified most often in labs that dual test
- Features overlap with Group 4 (ER+, etc.)
- Likely a heterogeneous group
- What to do?



Grimm EV, et al. HER2 Testing: Insights From Pathologists' Perspective on Technically Challenging HER2 FISH Cases. Appl Immunohistochem Mol Morphol. 2021 Oct 1;29(9):635-642. PMID: 34282066.

Stanford's Approach to Reporting Low Amplified Cases

RESULTS: HER2:CEP17 Ratio 2.1
Mean HER2 signals/cell: 4.8
Result category: Ratio ≥2.0 and 4-5.9 HER signals/cell (Group 1, low amplified)

INTERPRETATION: HER2 LOW AMPLIFIED with concurrent equivocal IHC result (2+) (See Comment)

- **COMMENT**: This patient is eligible for HER2 targeted therapy based on the 2018 ASCO/CAP HER2 Testing Guideline Update. This invasive cancer has a low level of increased HER2 signals (4-6) and a HER2:CEP17 ratio > 2.0. Because this case was close to the threshold for HER2 positive, additional cells were counted by a second independent observer and the results above are an average of the two counts. Although there is limited data to suggest benefit of HER2 targeted therapy in this setting, these patients were considered eligible for the first generation of trastuzumab trials. 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.

Case 1 Take Homes:

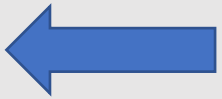
- IHC interpretation
  - 2+ definition revised/updated
  - Be aware of unusual staining patterns
- ISH interpretation:
  - Unusual ISH Group results require additional workup (concurrent IHC, additional counts)
  - Group 4 cases (formerly ISH equivocal) are most often considered negative when concurrent IHC reviewed
    - Alternative probes no longer recommended
  - Issue of cases close to thresholds (ex. Low Amplified results and consultation around)

HER2 Case 2:

- 65 year old women
- You are the pathologist reviewing her lumpectomy specimen
- Prior core biopsy:
  - Invasive mucinous carcinoma, grade 1
  - ER Positive (>95%,3+)
  - PR Positive (40%, 2+)
  - HER2 Positive by FISH
  - Ki67 5-10%

HER2 Case 2:

- 65 year old women
- You are the pathologist reviewing her lumpectomy specimen
- Prior core biopsy report:
  - Invasive mucinous carcinoma, grade 1
  - ER Positive (>95%,3+)
  - PR Positive (40%, 2+)
  - HER2 Positive by FISH
  - Ki67 5-10%



Unusual result!

Discordant results and repeat testing

DISCORDANT/UNUSUAL RESULTS:

A new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:

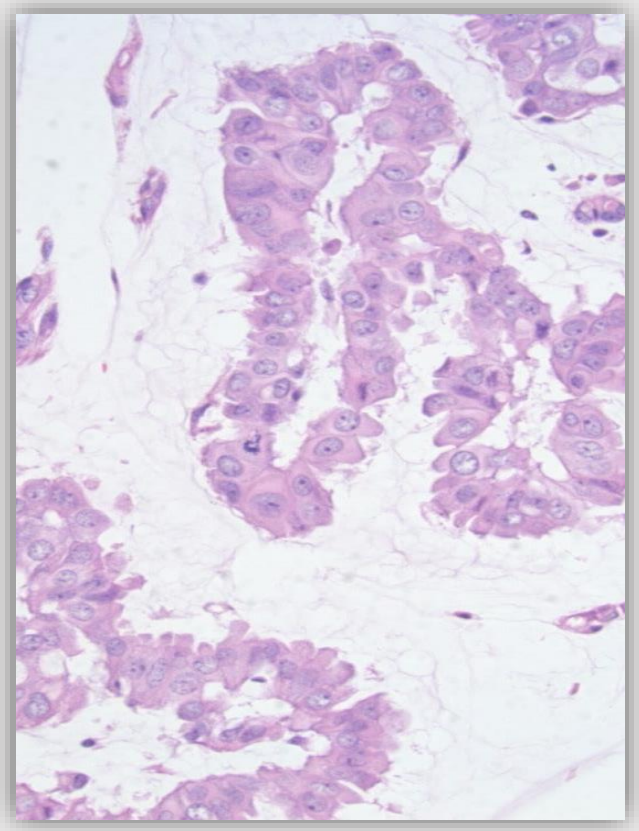
Histologic **grade 1 carcinoma** of the following types:

- Infiltrating ductal or lobular carcinoma, ER and PgR+
- Tubular (at least 90% pure)
- **Mucinous** (at least 90% pure)
- Cribriform (at least 90% pure)
- Adenoid cystic carcinoma (90% pure)

Beware of the “mucinous” carcinoma!

- Must be pure, ER+ and not high grade to correlate with good prognosis subtype

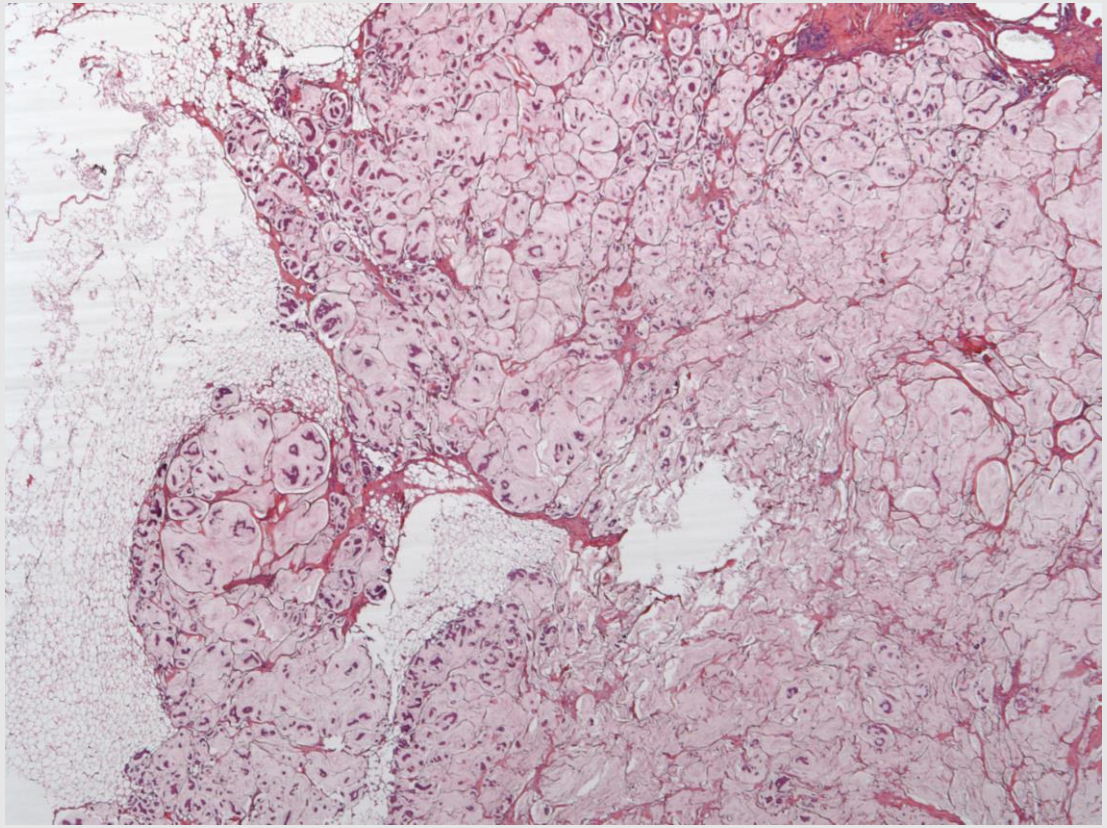
- Should NOT be:
  - HER2 positive
  - ER negative
  - High grade
  - Classified on core biopsy

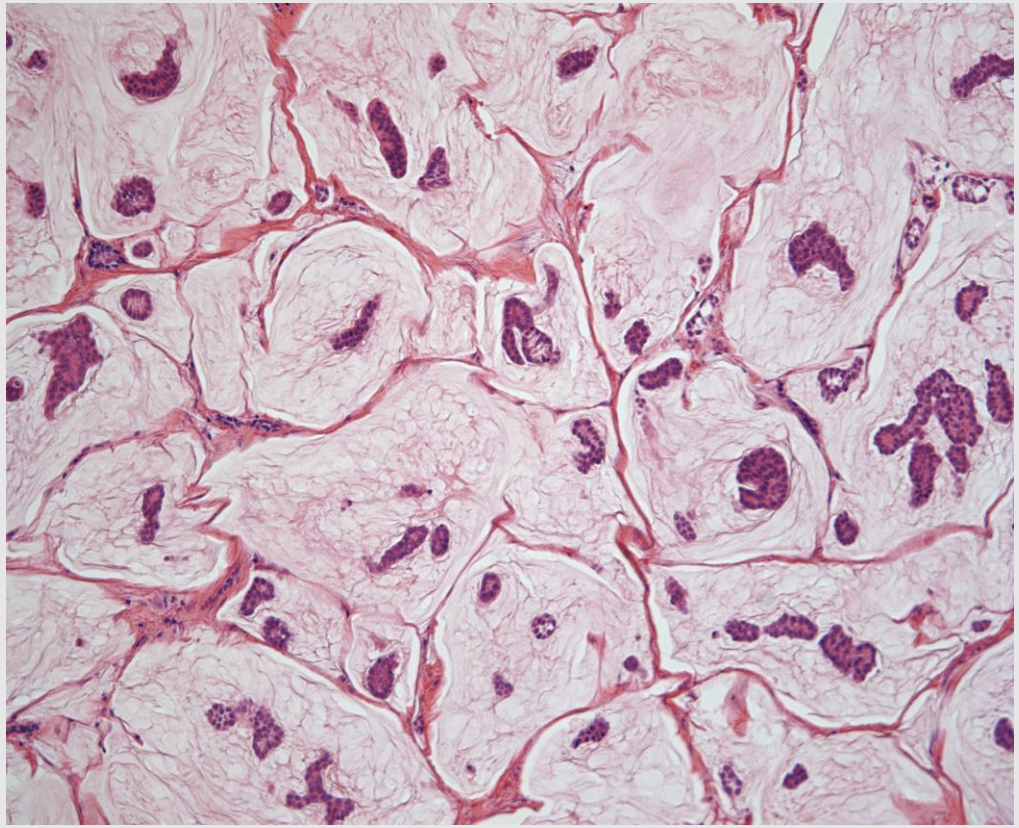


Mucinous features/Mucin Production ≠ Mucinous carcinoma

Per WHO 5th Edition: “Best classified as invasive breast cancer with mucin production”

HER2 Case 2: Lumpectomy findings



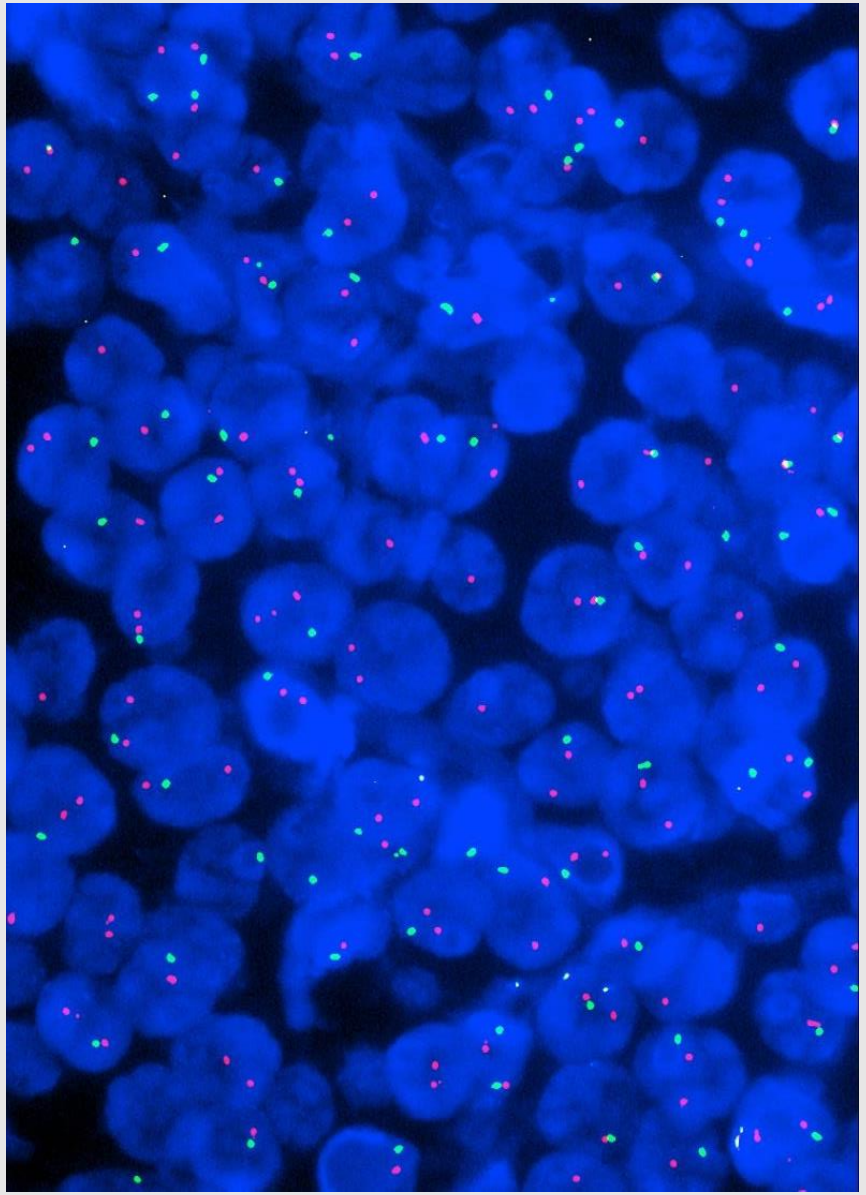


Pure mucinous carcinoma, low grade

HER2 Status?

FISH Results Case 2:

Cell	HER2	CEP17
1	3	1
2	2	2
3	3	1
4	2	1
5	2	1
6	2	1
7	3	1
8	3	1
9	2	2
10	3	1
11	2	1
12	2	1
13	2	1
14	3	1
15	3	1
16	2	2
17	3	1
18	2	2
19…	3	1
…..(50 total)		
Mean	**3.7**	1.2
Ratio	**3.1**	



- Ratio $\geq$ 2.0
- Mean HER2 < 4

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

COLLEGE of AMERICAN PATHOLOGISTS

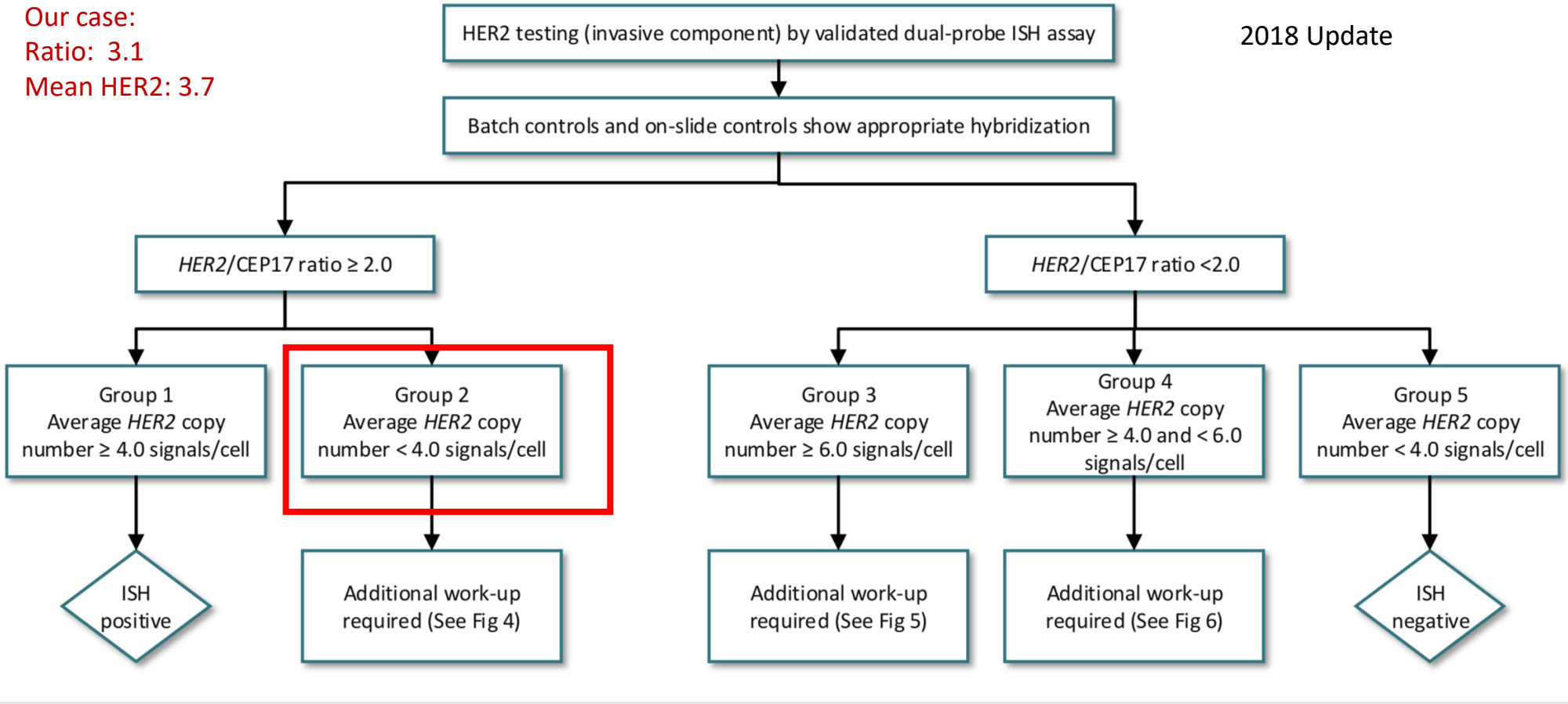
ASCO Guidelines

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

Figure 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).

Our case:
Ratio: 3.1
Mean HER2: 3.7

2018 Update



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Group 2 Cases:

- Frequency is very low:

Labs	HERA central lab *(Mod Pathol 2015)*	BCIRG central lab *(JCO 2016)*	Press reference lab *(APLM 2016)*	Jenkins reference lab *(JCO 2016)*	UK NEQAS 2009-2016 partial *(unpublished)*	*Stanford/UCSF/ UWMC (Mod Pathol 2017)*
FISH distribution	n=6,018	n=10,468	n=7,526	n=2,851	n=11,116	n=8,068
Group 2 *ratio ≥2.0; HER2 <4.0*	0.8%	0.7%	0.4%	1.3%	3.7%	1.4%

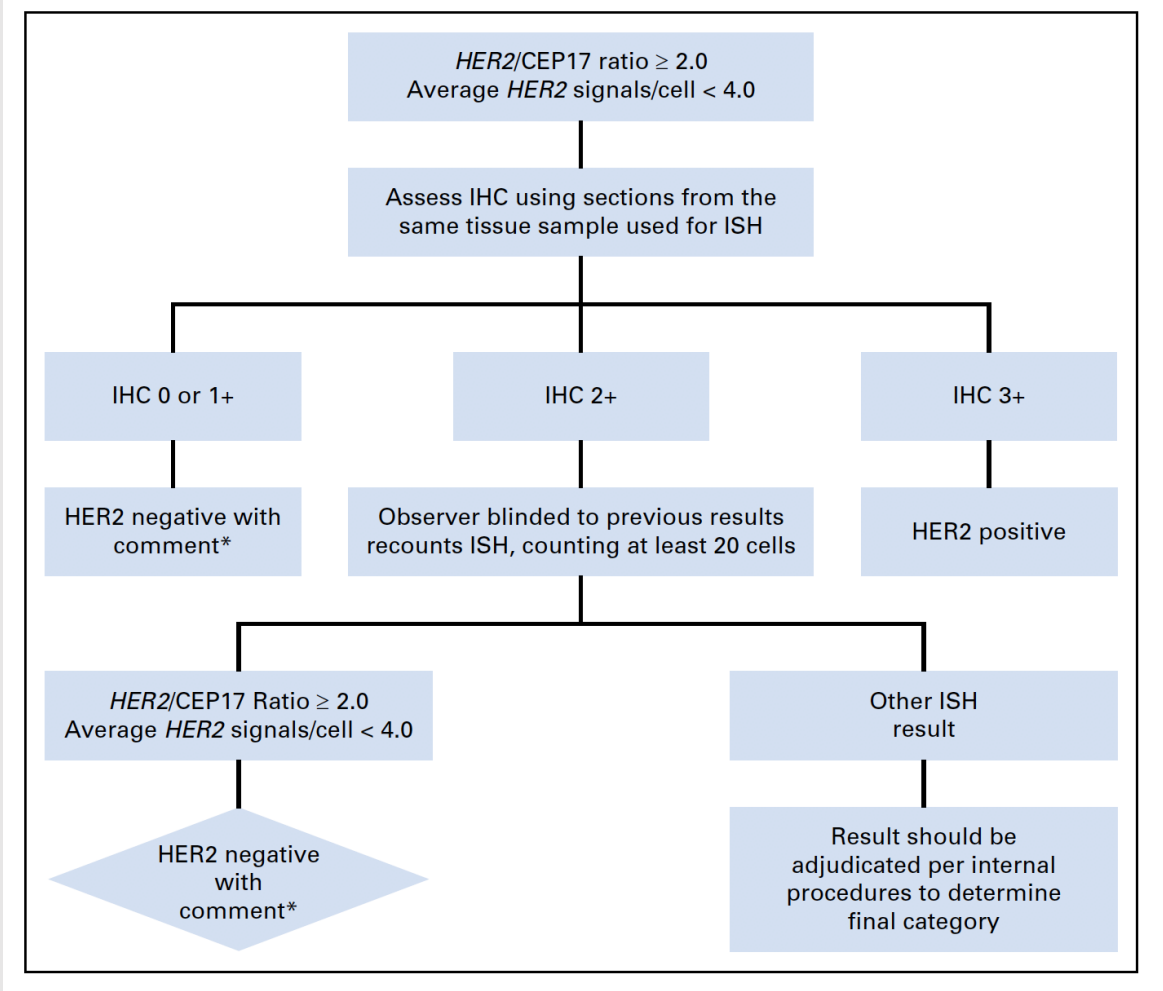
- Mostly ER+, Very rare to be HER2 3+ , predominantly IHC neg (0-1+)
- Previously considered amplified because ratio positive and would have been included in original trials → OFTEN DISCORDANT WITH IHC RESULTS
- Limited clinical trial data from BCIRG-006 data support they ***do not derive significant benefit from HER2 targeted therapy***

Press MF et al. J Clin Oncol. 2016 Oct 10;34(29):3518-3528.
Ballard M et al. Mod Pathol. 2017 Feb;30(2):227-235.

Group 2 ISH Cases: Additional Workup

Review or perform concurrent IHC:

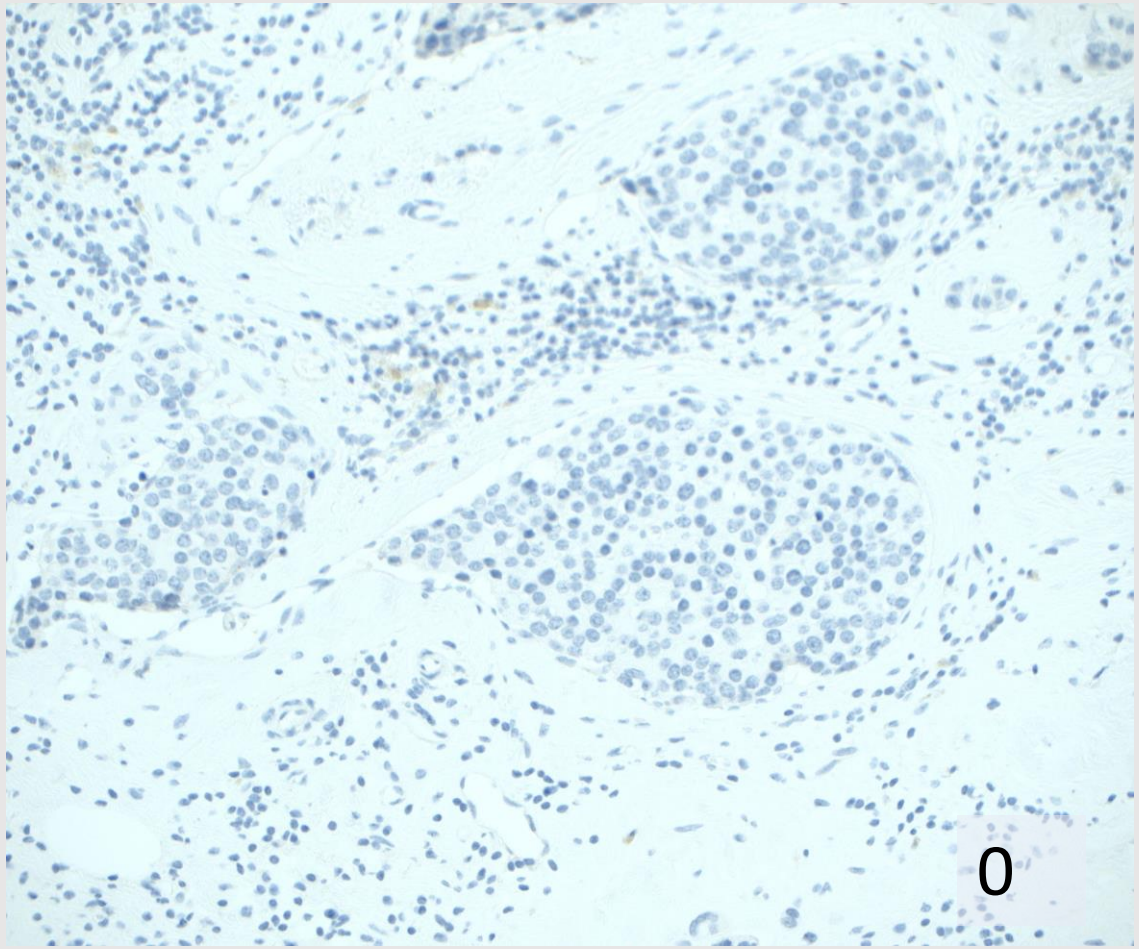
- If Neg IHC (0-1+) = HER2 Negative
- If Positive IHC (3+) = HER2 Positive
- If 2+ → second observer counts (at least 20 cells) and if still Group 2 = HER2 Negative



Group 2: Only positive if IHC is 3+

Concurrent IHC Result:

- How to report results?



RESULTS: HER2:CEP17 Ratio 3.1

Mean HER2 signals/cell: 3.7

Result category: Ratio ≥2.0 and < 4.0 HER signals/cell (Group 2 result)

INTERPRETATION: HER2 NEGATIVE (BASED ON IHC AND FISH, SEE COMMENT)

Concurrent IHC result: 0

REQUIRED

- **COMMENT**: This case has an uncommon HER2 FISH result ("Group 2" or "Monosomy-like"). Per the 2018 HER2 Testing Update, a concurrent IHC result has been used in the interpretation of the final result (and the FISH result recounted by a second observer). Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a HER2/CEP17 ratio of > 2.0 and an average HER2 copy number of < 4.0 per cell. In the first generation 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. Per guideline recommendations, when the IHC result is not 3+ positive, the specimen is considered HER2 negative because of the low HER2 copy number by ISH and the lack of protein overexpression.

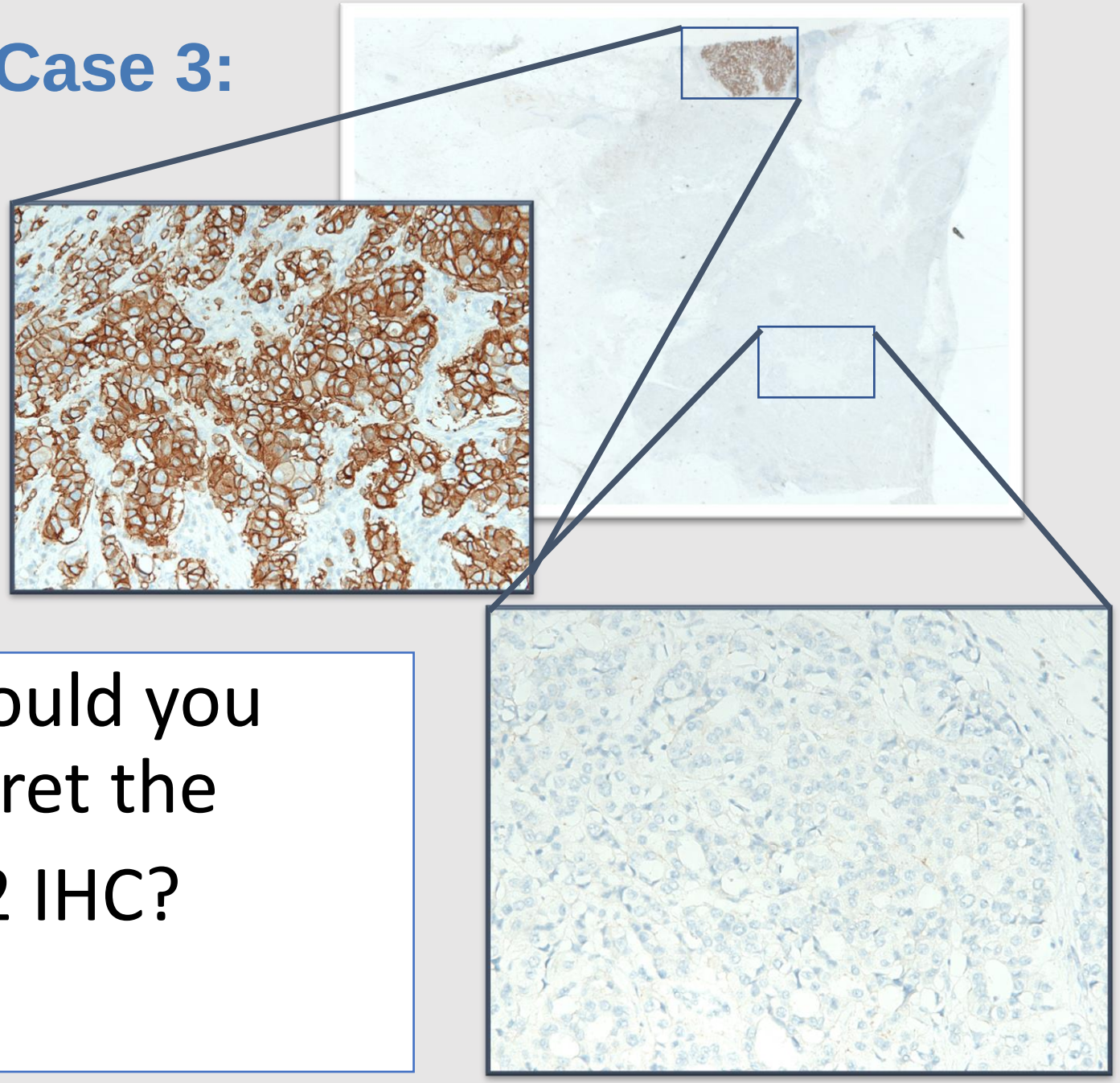
HER2 Case 2 Take Homes

- Be aware of unusual/discordant results
  - Grade 1 and/or favorable special histologic types not HER2+

- Group 2 ISH results (ratio ≥ 2.0 but < 4.0 mean HER2)
  - Unusual ISH Group results require additional workup (concurrent IHC, additional counts)
  - Group 2 cases are most often considered negative when concurrent IHC reviewed

HER2 Case 3:

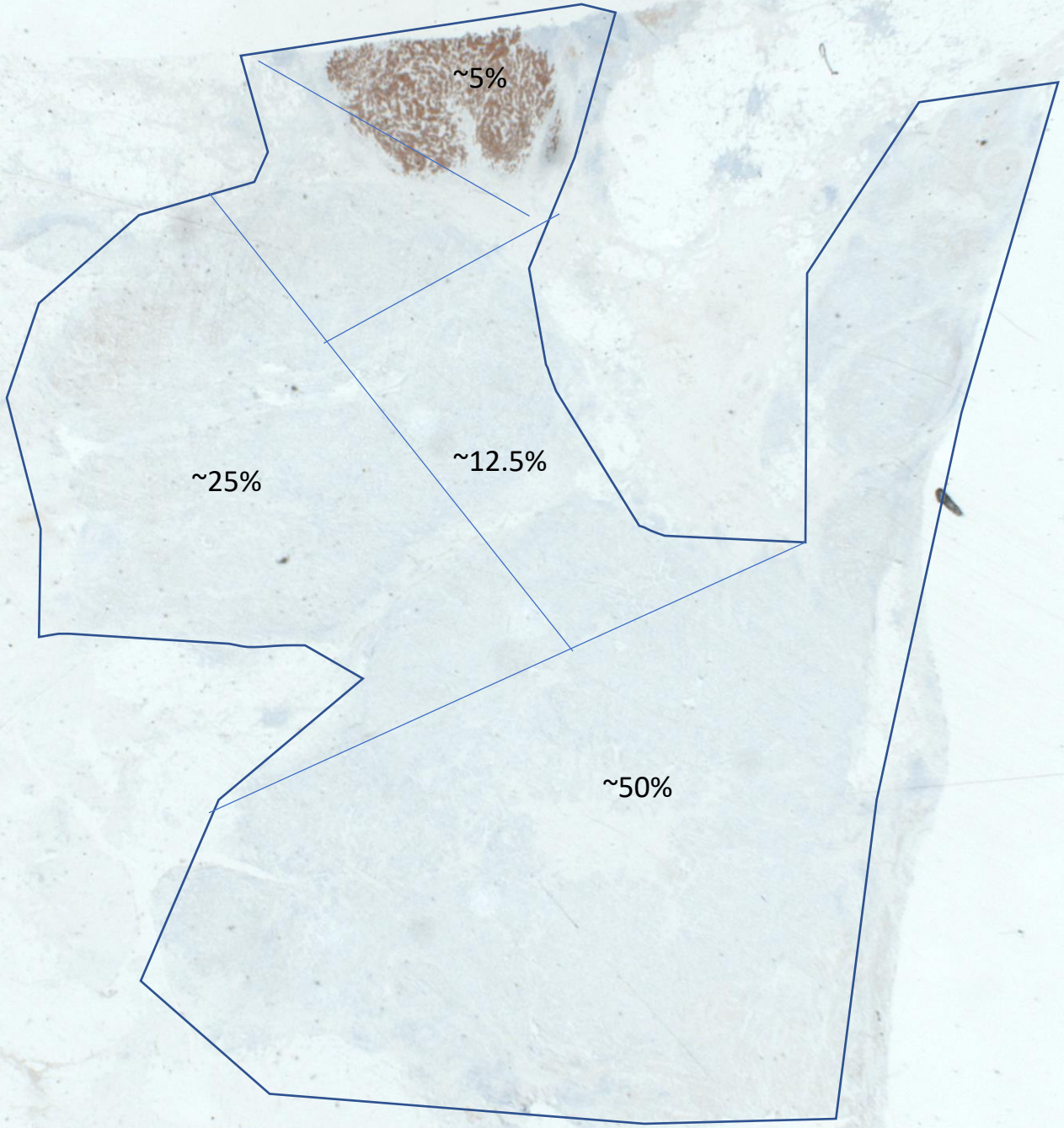
Invasive ductal carcinoma:
Grade 3 of 3



- How would you interpret the
- HER2 IHC?

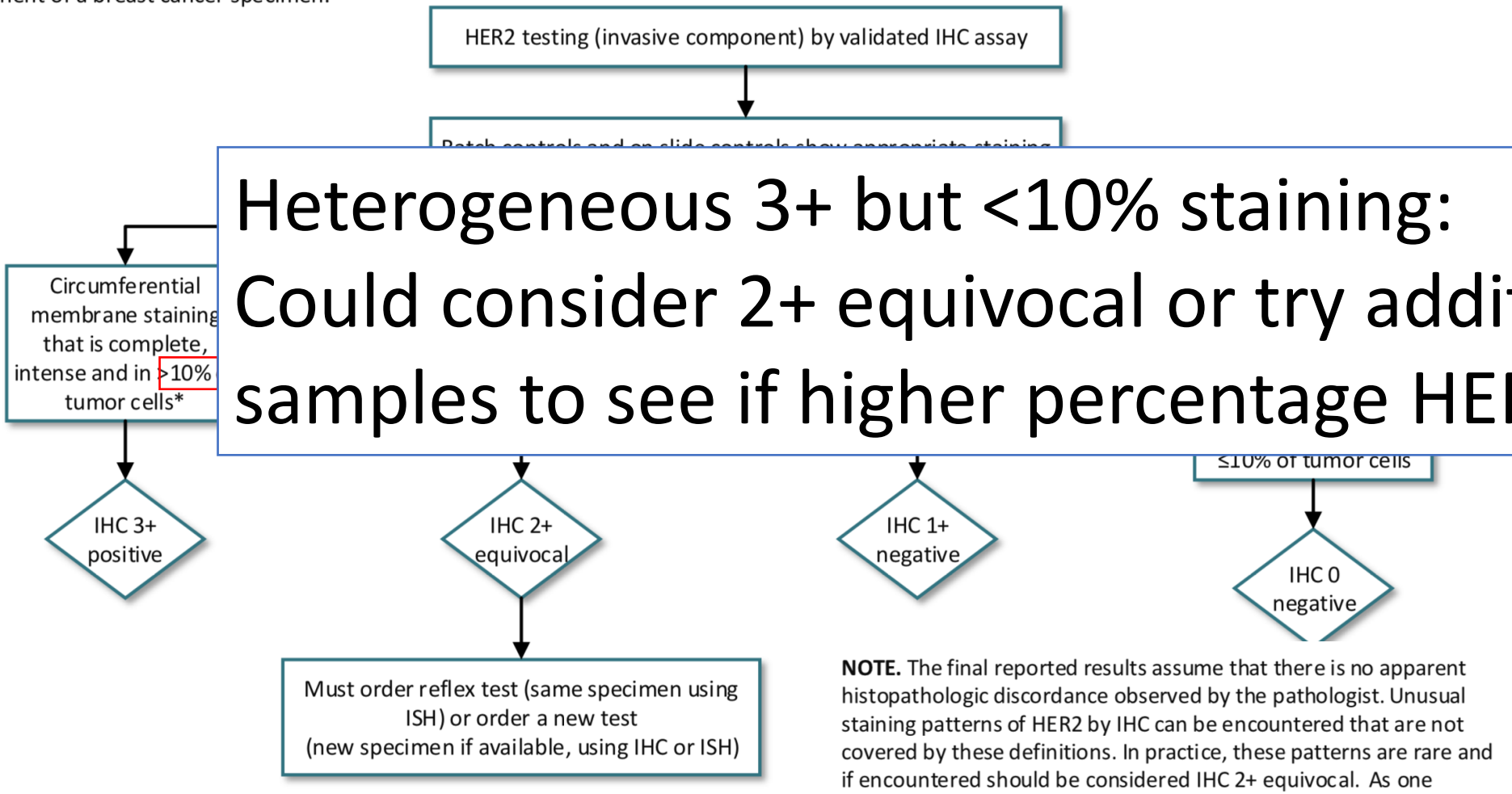
What percent is staining 3+?

Only 5% of sample is 3+ = Below the 10% threshold for positive……

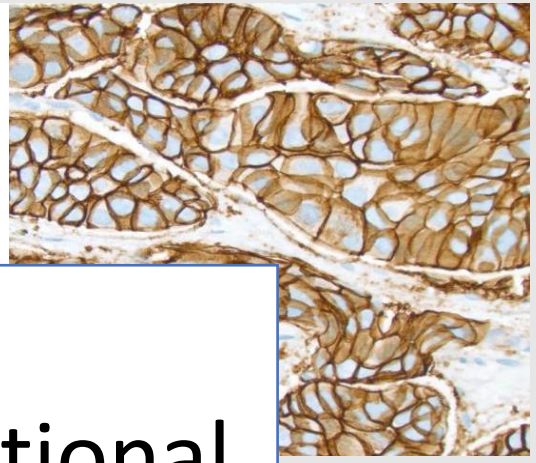


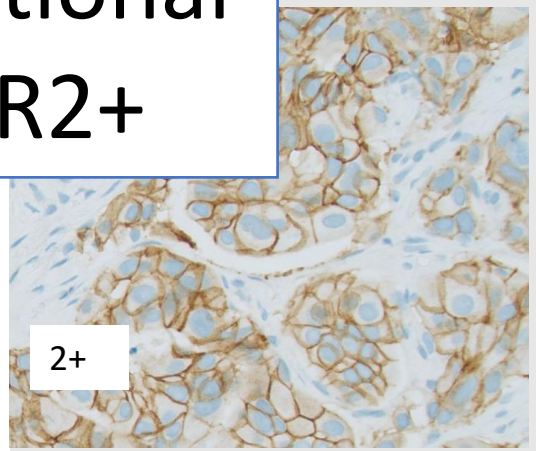
HER2 IHC stain

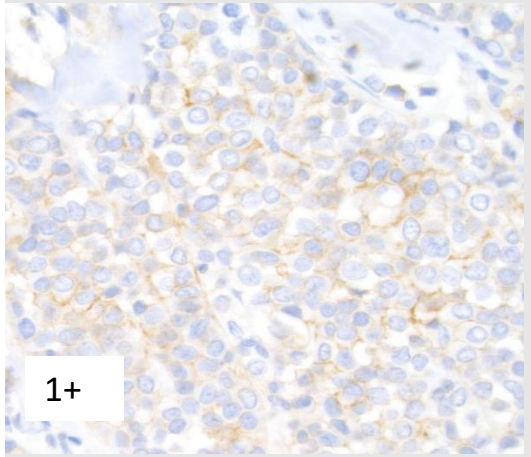
Figure 1. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) protein expression by immunohistochemistry (IHC) assay of the invasive component of a breast cancer specimen.



Heterogeneous 3+ but <10% staining:
Could consider 2+ equivocal or try additional samples to see if higher percentage HER2+







NOTE. The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. Unusual staining patterns of HER2 by IHC can be encountered that are not covered by these definitions. In practice, these patterns are rare and if encountered should be considered IHC 2+ equivocal. As one example, some specific subtypes of breast cancers can show IHC staining that is moderate to intense but incomplete (basolateral or lateral) and can be found to be HER2 amplified. Another example is circumferential membrane IHC staining that is intense but within ≤10% of tumor cells (heterogeneous but very limited in extent). Such cases can be considered 2+ equivocal but additional samples may reveal different percentages of HER2 positive staining. (*)Readily appreciated using a low power objective and observed within a homogeneous and contiguous invasive cell population

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.

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ASCO Guidelines

HER2 Heterogeneity by FISH (unchanged since 2013)

- Must score separately an aggregated positive population that is > 10% of total tumor population
- Report must include:
  - HER2 status as positive with the percentage of the total tumor that is amplified
  - Ratio and signals/cell of both populations

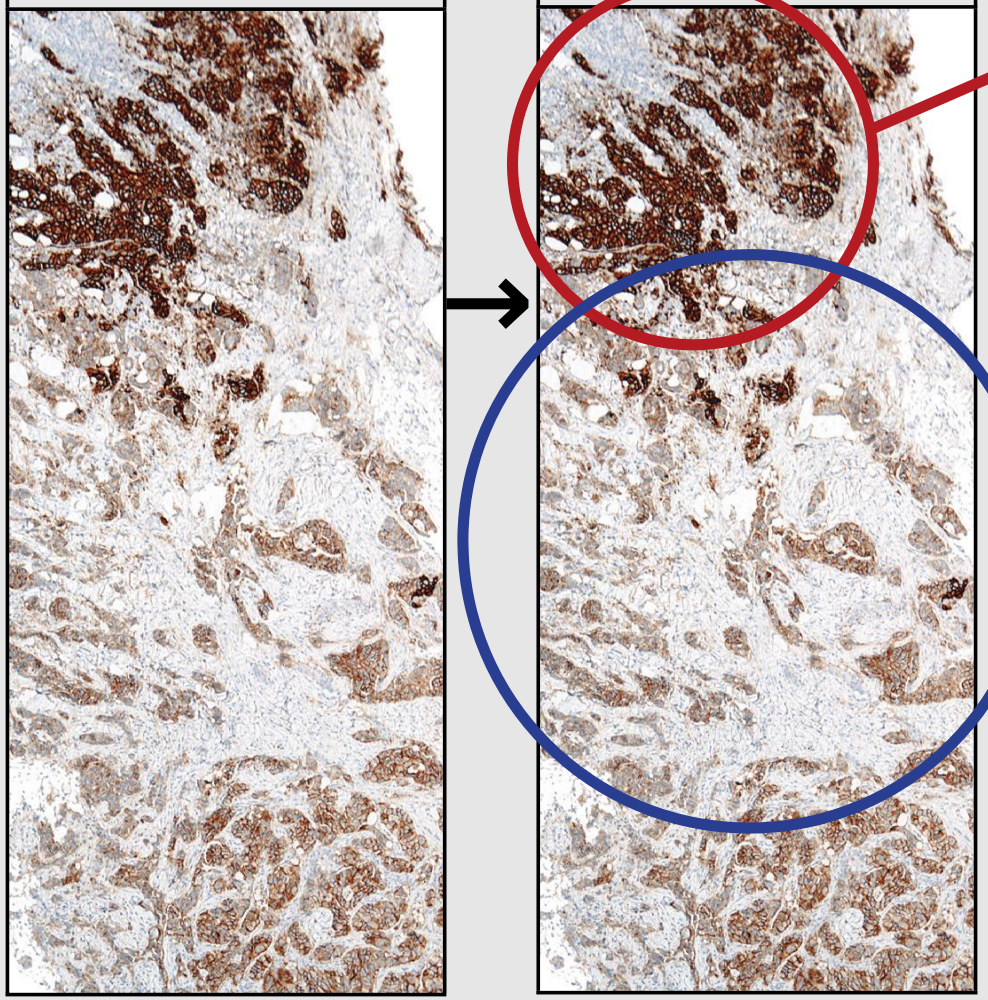
See Table 1 “ISH Interpretation” and Data Supplement 8: ISH Interpretation Criteria

ASCO/CAP HER2 Testing Guideline Update—Wolff et al

HER2 Heterogeneity

Clustered HER2 hetergeneity noted by IHC staining (or scan of FISH slide)

Count clustered, discrete populations separately



Report results for both populations and quantify overall percent amplified cells in sample

Area 1: 20% of sample (area with 3+ protein expression by IHC)

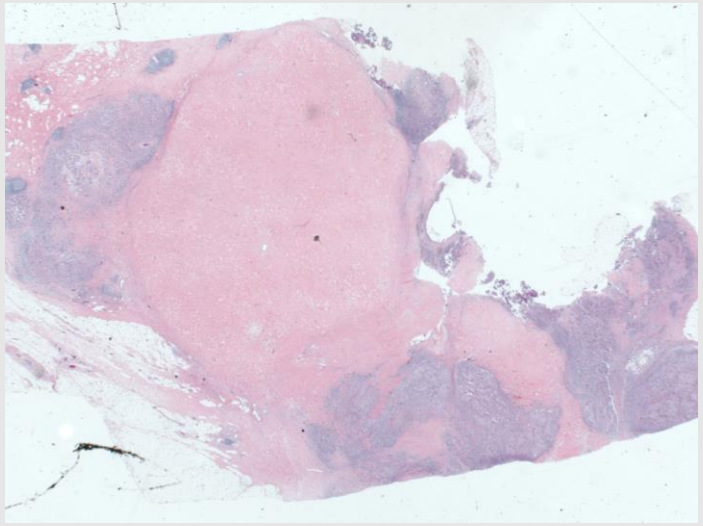
HER2:CEP17 Ratio: 4.8
Total cells counted: 25
Mean HER2 signals/cell: 10.5
Mean CEP17 signals/cell: 2.2

Area 2: 80% of sample (area with 2+ protein expression by IHC)

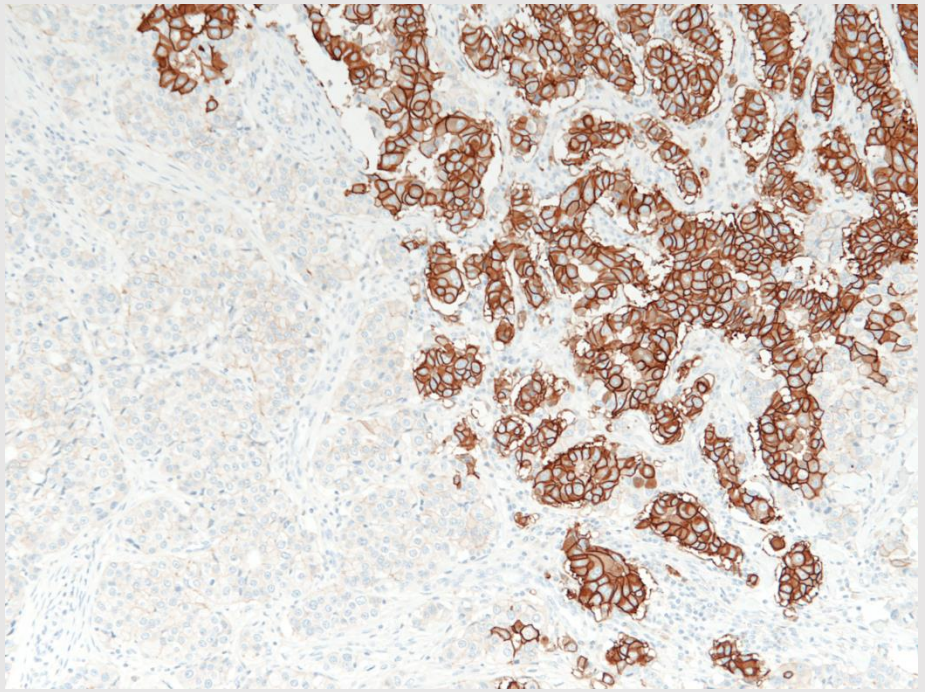
HER2:CEP17 Ratio: 1.2
Total cells counted: 25
Mean HER2 signals/cell: 2.6
Mean CEP17 signals/cell: 2.2

Interpretation:
HER2 AMPLIFIED with heterogeneity (20% of sample amplified)

HER2 Case 3:

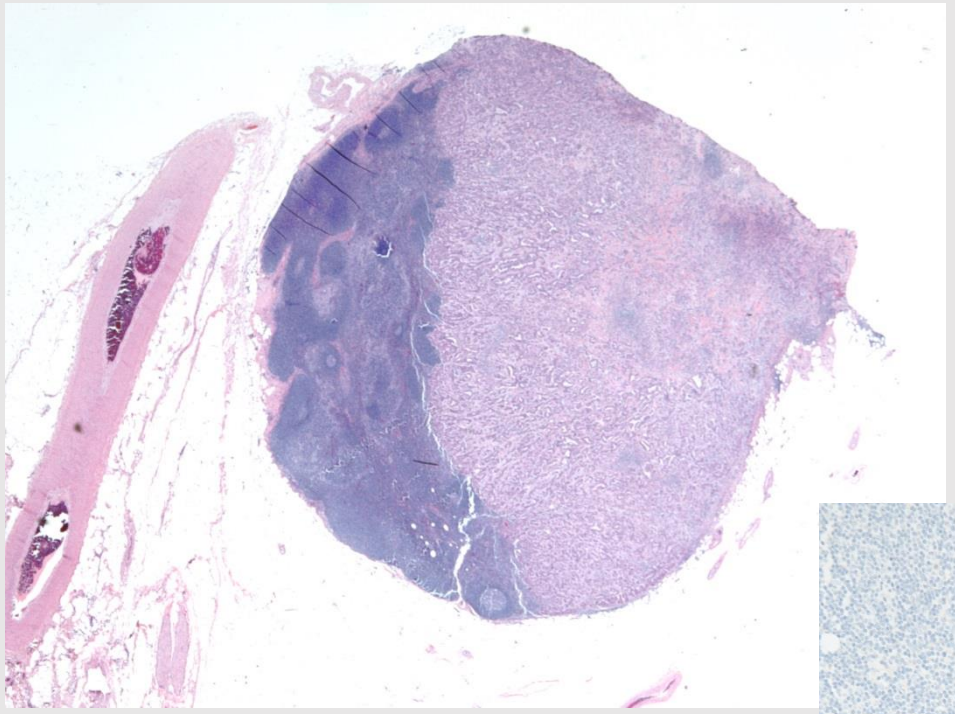


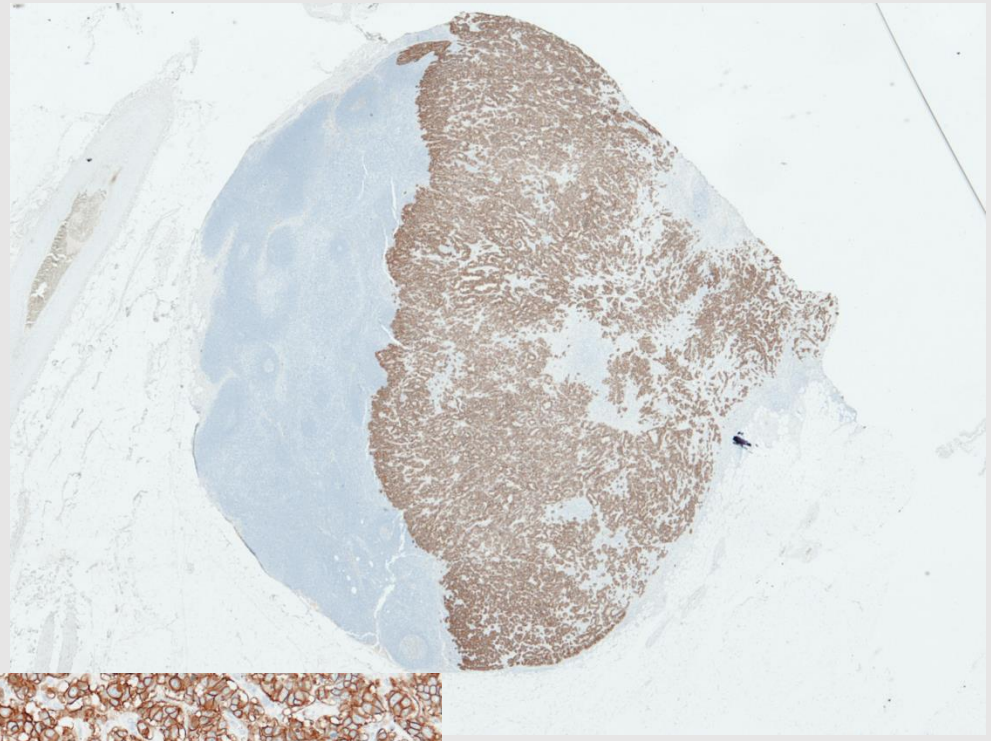
Testing an additional block:

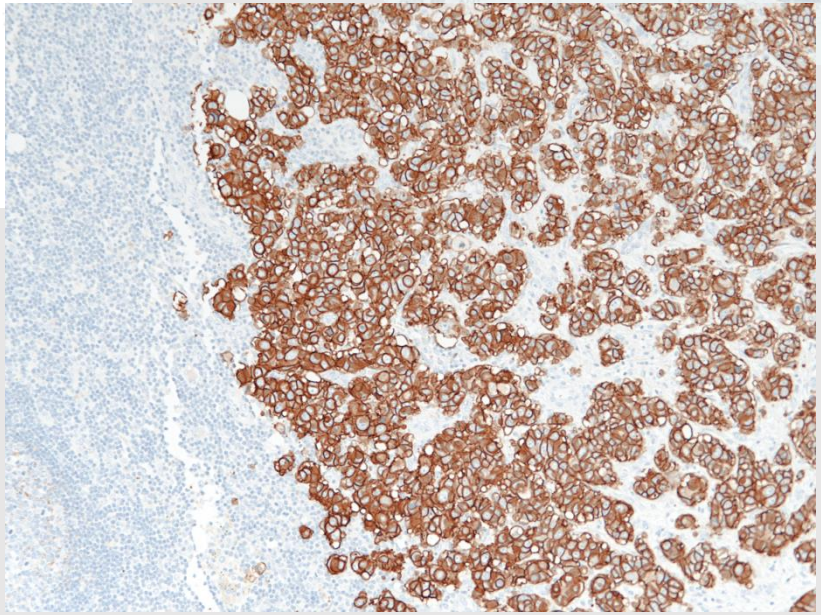


50% HER2 3+

HER2 Case 3:







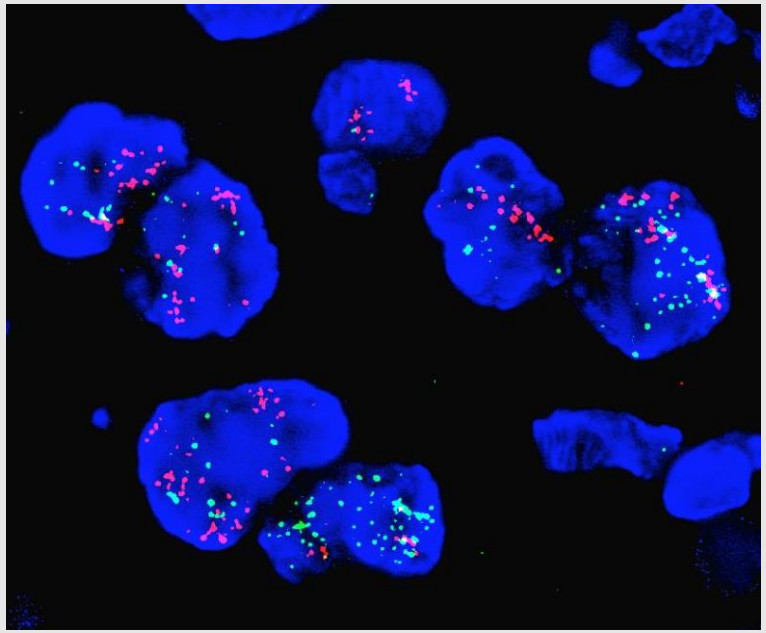
Testing the positive lymph node:

100% HER2 3+

Heterogeneous primary → Uniformly positive metastasis

HER2 Case 3: FISH on LN met

Cell	HER2	CEP17
1	10+	10+
2	10+	10+
3	8	6
4	10+	10+
5	10+	10+
6	10+	10+
7	10+	10+
8	10+	10+
9	10+	10+
10	10+	10+
11	10+	10+
12	10+	10+
13	10+	10+
14	10+	10+
15	10+	10+
16	5	10+
17	10+	10+
18	10+	10+
19…	10+	10+
…..(50 total)		
Mean	**9.2**	9.2
Ratio	**1.0**	



- Ratio < 2.0
- Mean HER2 > 6

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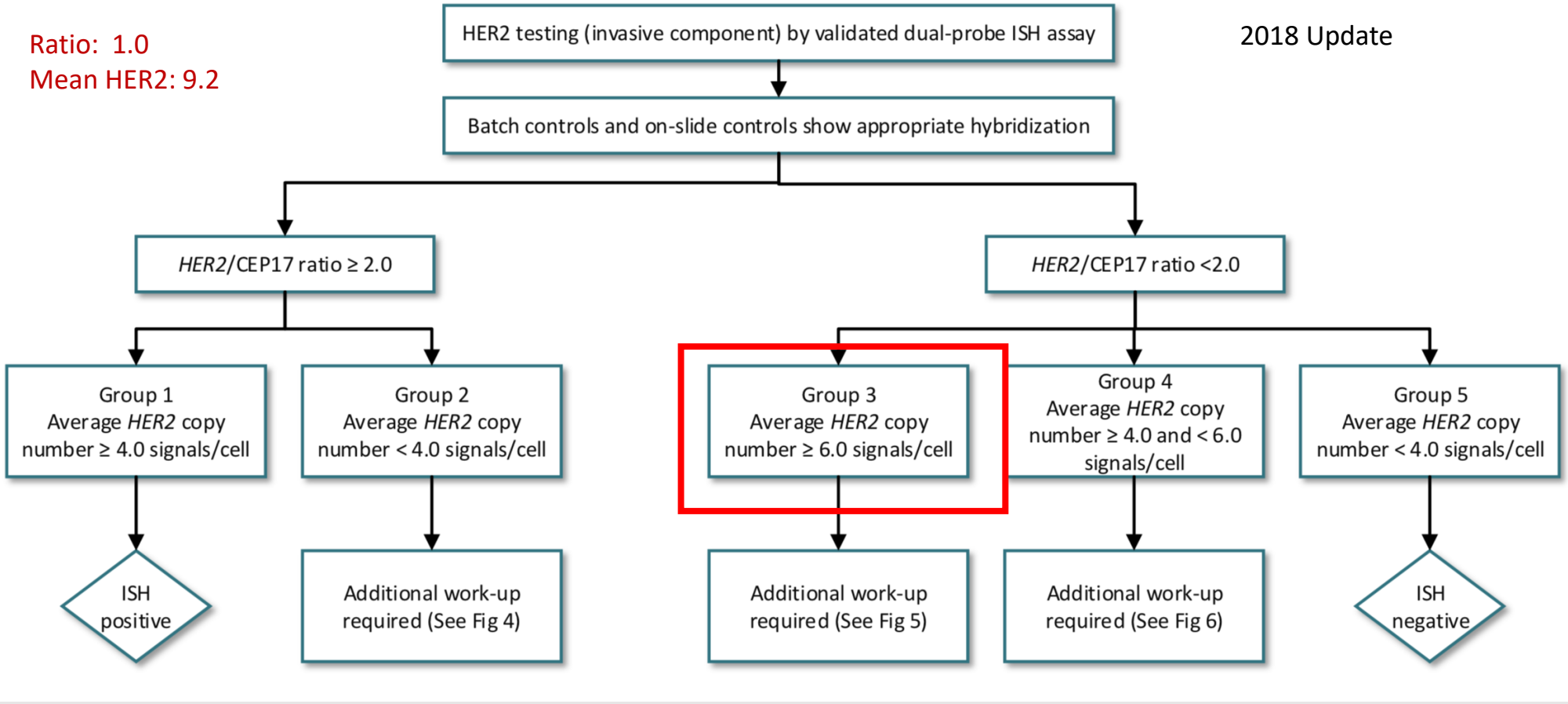
Figure 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (HER2 gene) assay (dual-probe ISH).

Ratio: 1.0
Mean HER2: 9.2

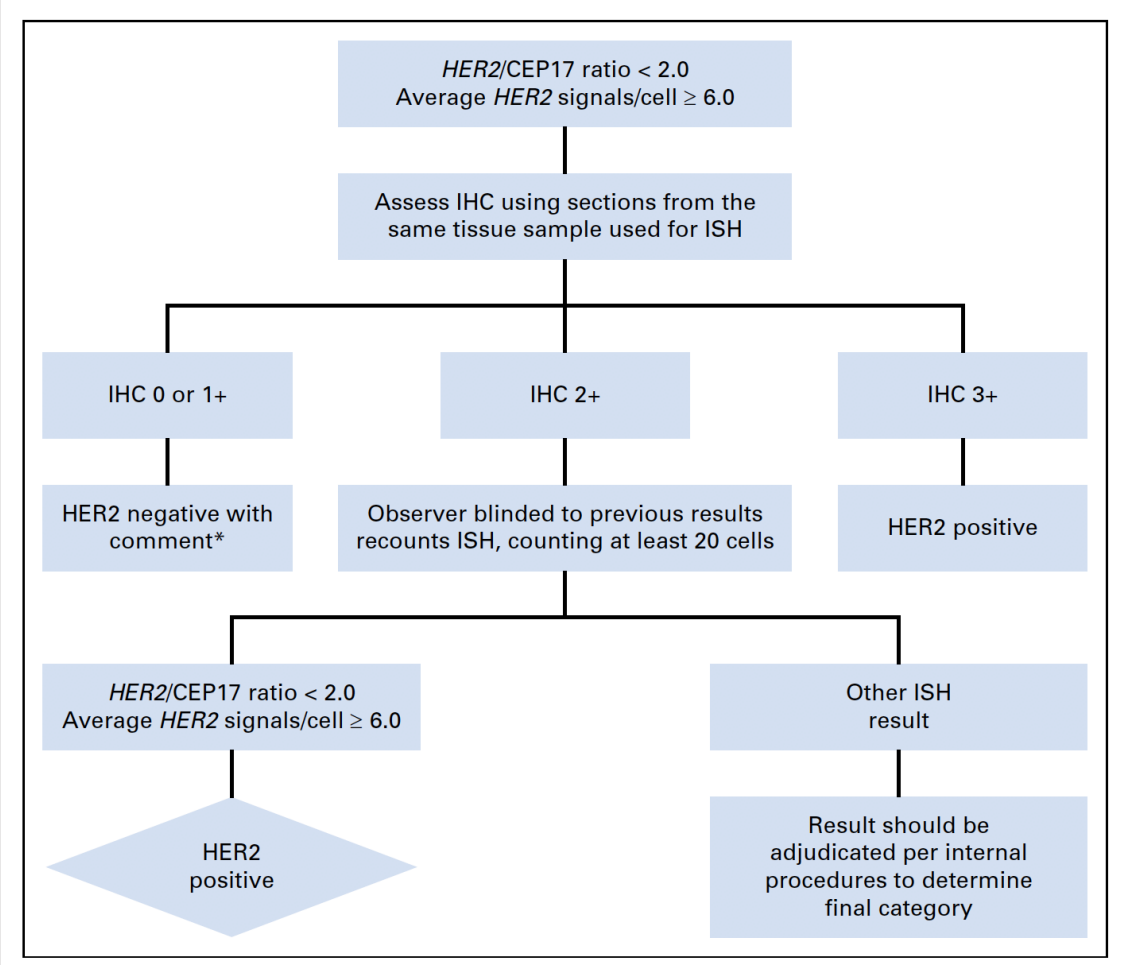
2018 Update



JOURNAL OF CLINICAL ONCOLOGY

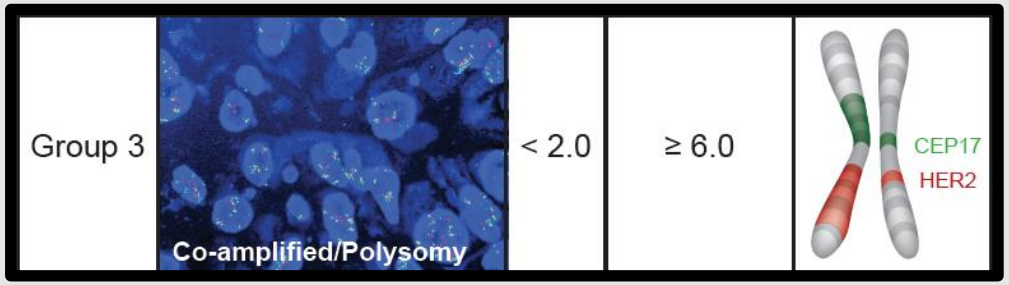
Group 3 ISH Cases: Additional Workup

- Review or perform concurrent IHC:
- If Neg IHC = HER2 Negative
- If IHC (3+) = HER2 POSITIVE
- If IHC 2+ → second observer counts (at least 20 cells) and if still Group 3 = HER2 POSITIVE



Different than Groups 2 and 4!!
Group 3: Positive if IHC is 2+ or 3+

Additional Data on Group 3 ISH Cases



- Often TRUE HER2 AMPLIFICATION: Molecular data supporting "co-amplified" rather than polysomy

- Often HER2 3+: HERA trial re-analysis: Of 21 cases (originally considered FISH negative) 15 of 20 (75%) were positive by IHC (3+)

- May be a Heterogenous Group/Category: Press/USC: (N= 48)
  - Group 3A: > 12.3 HER2 signals/cell, 75% 2-3+ by IHC (N= 8)
  - Group 3B: average of 6.8 HER2 signals/cell, 87.5% IHC 0-1+ (N= 40)

Stoss OC, et al. Mod Pathol 28:1528-34, 2015

Press MF et al. *Arch Pathol Lab Med.* doi: 10.5858/arpa.2016-0009

RESULTS: HER2:CEP17 Ratio 1.0

Mean HER2 signals/cell: 9.2

Result category: Ratio < 2.0 and > 6.0 HER signals/cell (Group 3 result)

INTERPRETATION: HER2 POSITIVE (BASED ON IHC AND FISH, SEE COMMENT)

Concurrent IHC result: 3+

REQUIRED

- **COMMENT**: This case has an uncommon FISH result ("Group 3" or "Co-amplified"). Per the 2018 HER2 Testing Update, a concurrent IHC result has been used in the interpretation of the final result (and the FISH result recounted by a second observer). There are insufficient 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. Per guideline recommendations, when concurrent IHC results are negative (0 or 1+), the specimen be considered HER2 negative. However, in the setting of equivocal or positive IHC results (2-3+) the case is considered HER2 positive.

Reporting heterogeneous cases

FINAL DIAGNOSIS: Invasive ductal carcinoma,

Heterogeneous for HER2 over-expression:

-- 30% positive for HER2 over-expression (3+) by IHC and positive for gene amplification by FISH

-- 70% negative for HER2 over-expression (1+) and negative for gene amplification by FISH

COMMENT:

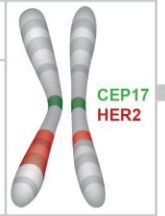
This invasive cancer has two distinct, clustered subpopulations (heterogeneous) with different HER2 status. A distinct, clustered subpopulation, representing 30% of the tested invasive carcinoma is positive for HER2 by both protein over-expression and gene amplification (blocks A3, A4 and A5 tested). The remainder of the invasive cancer in this sample is HER2 negative. The 2013 and 2018 CAP/ASCO HER2 testing guidelines consider this a HER2 positive result and the patient should be considered eligible for HER2 targeted therapy.

HER2 Case 3 Take Homes

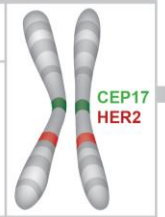
- Heterogeneity interpretation and reporting
  - Clustered populations with different results (give percent)
  - > 10% 3+ by IHC is positive (if < 10% consider additional testing)
  - Report separate populations by FISH –if >10% is amplified considered positive
- Group 3 ISH results (ratio < 2.0 but > 6.0 mean HER2)
  - Unusual ISH Group results require additional workup (concurrent IHC, additional counts)
  - Group 3 cases are considered HER2 positive if IHC is 2+ or 3+ (majority will be positive but not all)

Grey Zones in Dual Probe HER2 ISH Test Interpretation: 2018 Update Summary

Most cases

Group 1 Result		
Ratio	HER2 sign/cell	
≥ 2.0	≥ 6.0	

→ HER2 Positive

Group 5 Result		
Ratio	HER2 sign/cell	
< 2.0	< 4.0	

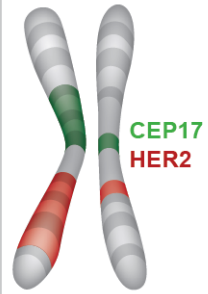
→ HER2 Negative

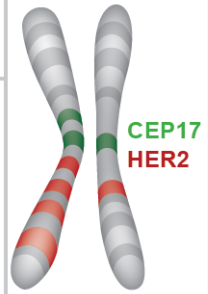
Grey Zones and Borderline Results:
Confirmation, correlation and explanation

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.
WHO 5th edition Tumours of the Breast 2019

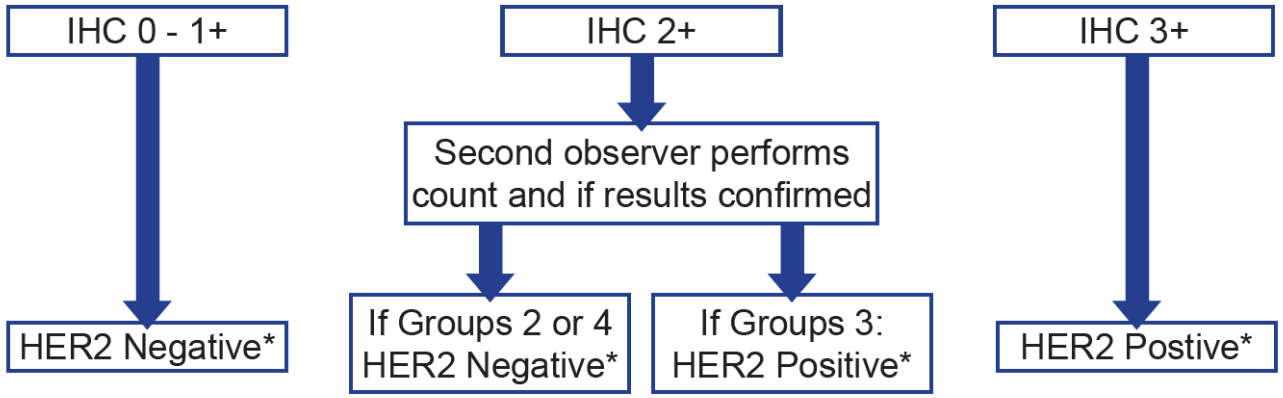
Unusual HER2 ISH Result Categories Requiring Additional Work-Up

Group 2 Result		
Ratio	HER2 sign/cell	
≥ 2.0	< 4.0	

Group 3 Result		
Ratio	HER2 sign/cell	
< 2.0	≥ 6.0	

Group 4 Result		
Ratio	HER2 sign/cell	
< 2.0	4.0 - 6.0	

Review concurrent IHC from the same sample



*As determined by concurent IHC and ISH. Report comments recommended (see ASCO/CAP guidelines for details). {29846104}

Report final result based on IHC + ISH, include required comments

Result Categories in HER2 FISH Reports

- HER2 NEGATIVE
- HER2 NEGATIVE (BASED ON IHC AND FISH, SEE COMMENT)
  - Concurrent IHC result: _____

- HER2 POSITIVE
- HER2 POSITIVE (BASED ON IHC AND FISH, SEE COMMENT)
  - Concurrent IHC result: _____

- HER2 POSITIVE WITH HETEROGENEITY
  - ___ % of sample with gene amplification (clustered)
    - Correlating with areas of ___ protein expression by IHC
  - Free text option (can use both)

Impact of 2018 ASCO/CAP HER2 Guidelines Update

- New SOPs and reporting needed for Group 2-4 cases
    - Need for labs to evaluate IHC and ISH concurrently for a minority of cases (complex to implement for some labs, need for implementation timeline from CAP)
- Reduced need for repeat testing or alterative probe testing
- Final HER2 status will not be Equivocal
- Reduction in IHC – ISH discordant results
- Small overall decrease in HER2 positive rate (<1- 8% overall)
    - Most of Group 2 and Group 4 (prior Equivocal) cases now considered HER2 Negative

Liu ZH, et al. Breast Cancer Res Treat. 2019 Feb 2.
Curado M, et al. Virchows Arch. 2019 Apr 5.
Gordian-Arroyo AM, et al. Am J Clin Pathol. 2019 Apr 8.
Hoda RS et al. Arch Pathol Lab Med. 2019 Oct 24.

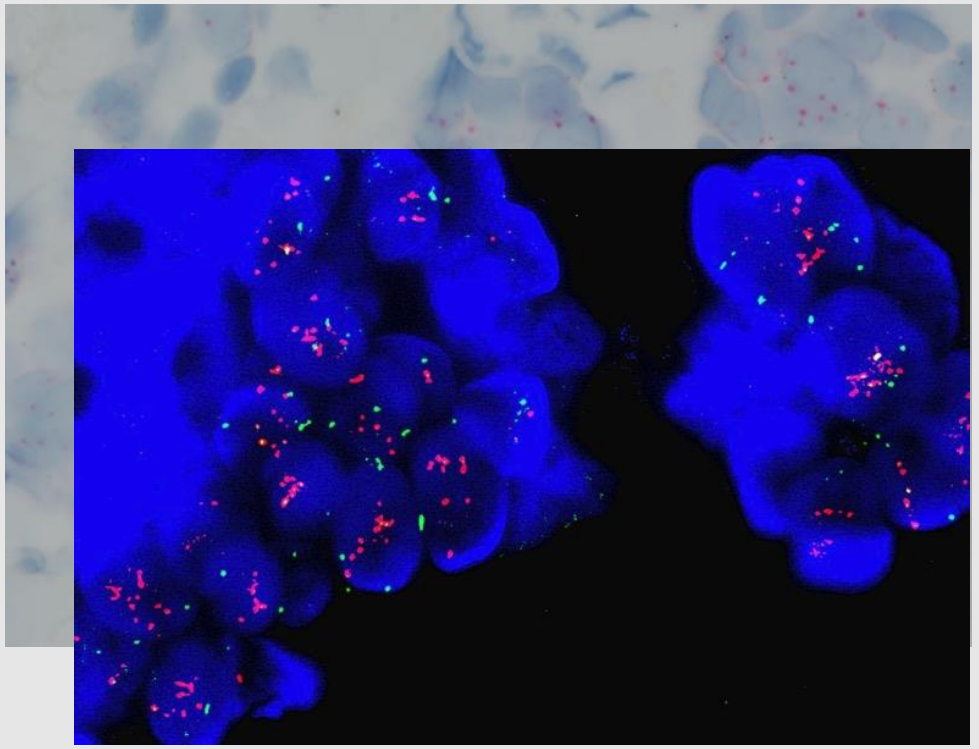
DISH Testing Pitfalls

HER2 Dual In Situ Hybridization

Correlations and Cautions

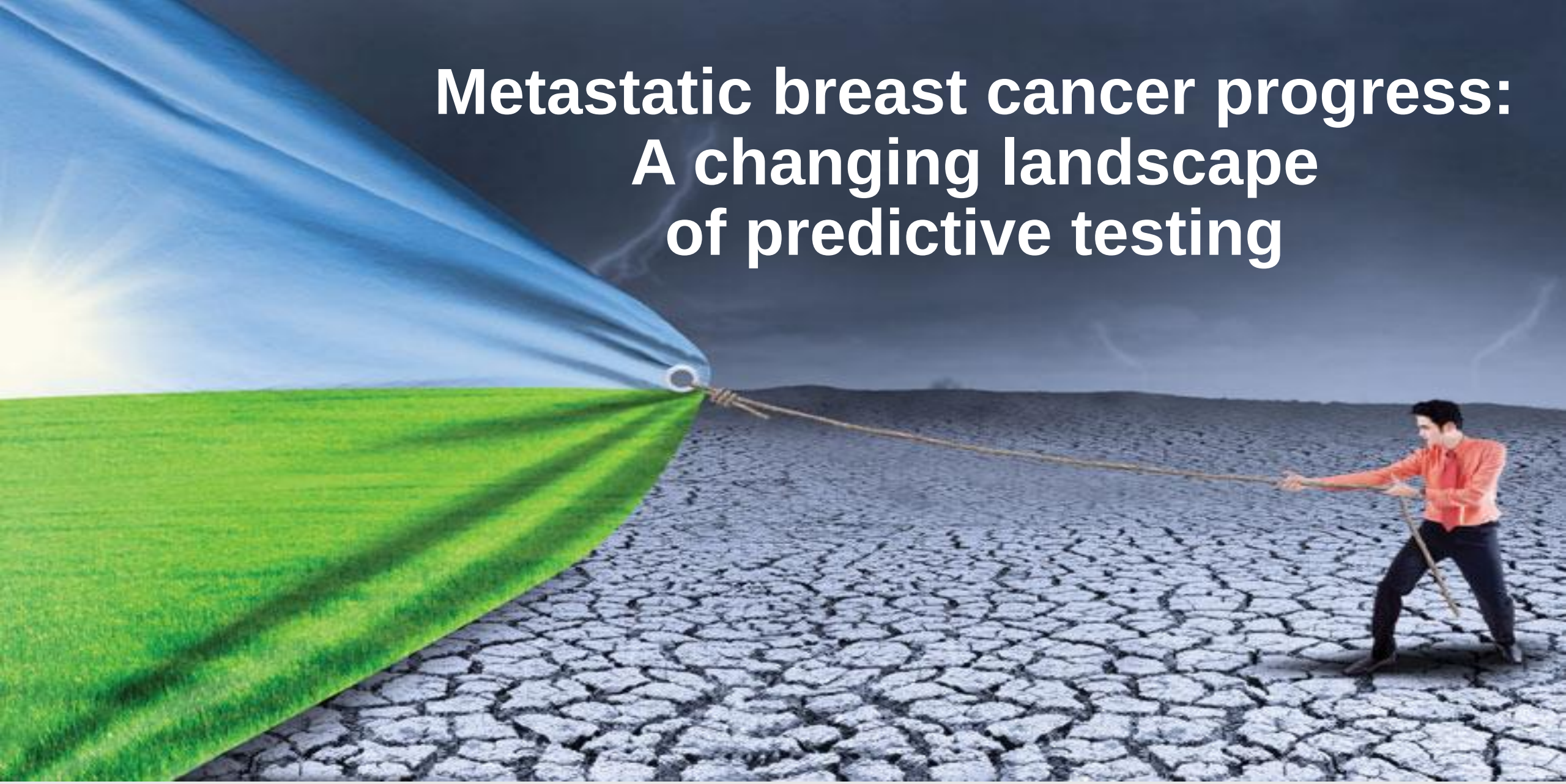
Megan Troxell, MD, PhD; Richard K. Sibley, MD; Robert B. West, MD, PhD; Gregory R. Bean, MD, PhD; Kimberly H. Allison, MD

- Poor hybridization and/or staining (lack of ISH signals)
- Excess or clumped stain (punctate or clumped black material outside of and overlaying nuclei).
- Evaluated with a 60x + objective focusing up and down to carefully assess for small or weak signals, and for appropriate signal localization as noted above.



(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2019-0510-OA)

Metastatic breast cancer progress: A changing landscape of predictive testing



Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

- Any new metastasis or recurrence should be biopsied for ER/PR and HER2 status
  - However, treatment path can be based on status of primary

Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

#2. Test for germline BRCA1/2 status
RX: PARP inhibitors

- OlympiAD Trial (Olaparib monotherapy was superior to standard in germline BRCA+ metastatic breast cancer) – (2021 UPDATE: Now also in some high risk early stage!)
- EMBRACA Trial (Talazoparib monotherapy superior was superior to standard in germline BRCA+ metastatic breast cancer)

*FDA approved companion diagnostic for olaparib and talazoparib = BRCAnalysis CDx (*PCR and Sanger sequencing with large deletions/duplications by multiplex PCR)

Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 2017;377:523-533.
Litton J, Rugo H, Ettl J, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 2018;379:753-763.

Testing Considerations in Recurrent or Newly Metastatic Disease

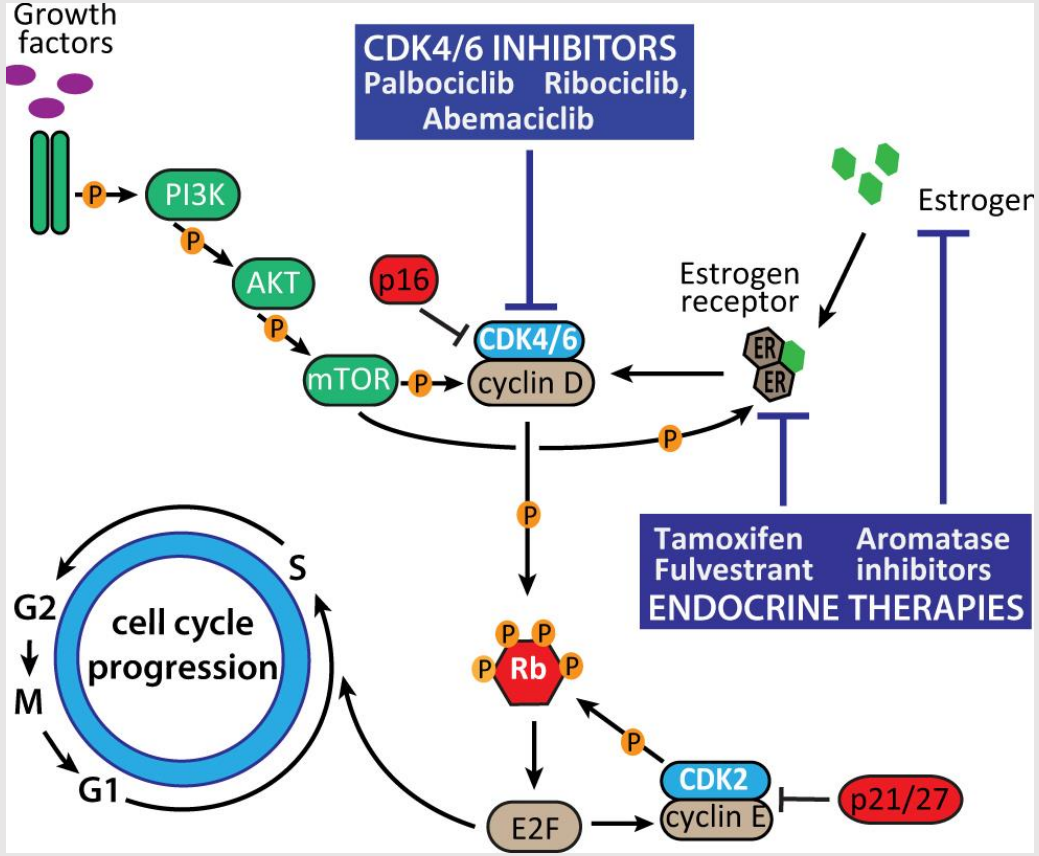
Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

ER Positive, HER2 Negative
RX: AI/SERM + CDK4/6 inhibitor (abemaciclib, palbociclib, ribociclib), mTOR inhibitor (everolimus)

No new biomarker (Ki67 not required here)

What else?



Endocrine-Related Cancer 26, 1; 10.1530/ERC-18-0317

Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

ER Positive, HER2 Negative
RX: AI/SERM + CDK4/5 inhibitor

Test for PIK3CA mutation
RX: Alpelisib + fulvestrant to inhibit PI3K + ER pathways

SOLAR1 Trial

Companion diagnostic test concurrently approved by FDA = *therascreen* PIK3CA RGQ PCR Kit (11 mutations) in FFPE tissue (or ctDNA..)

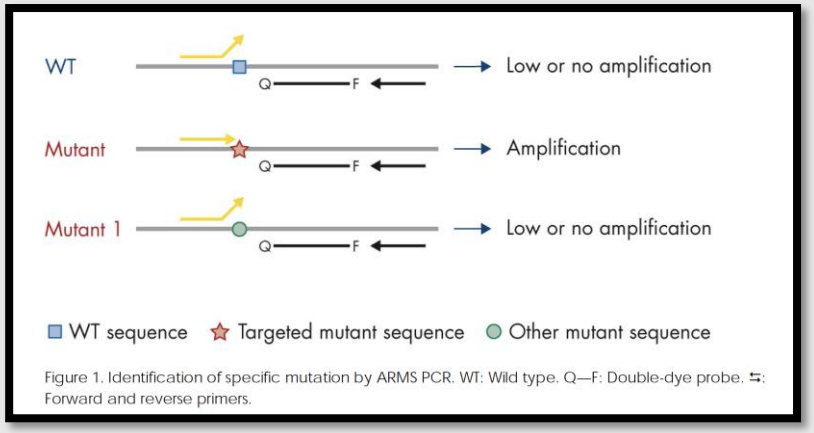
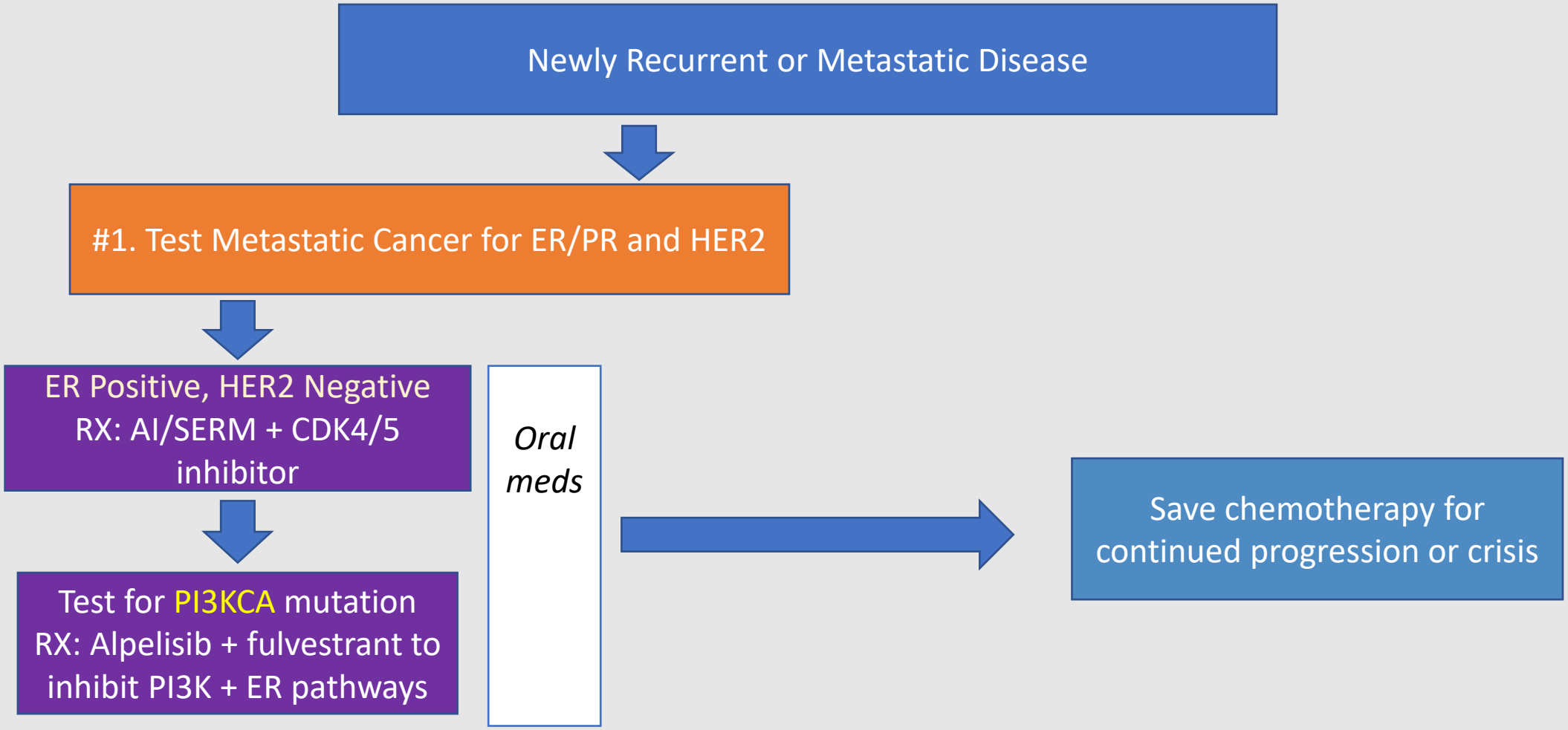


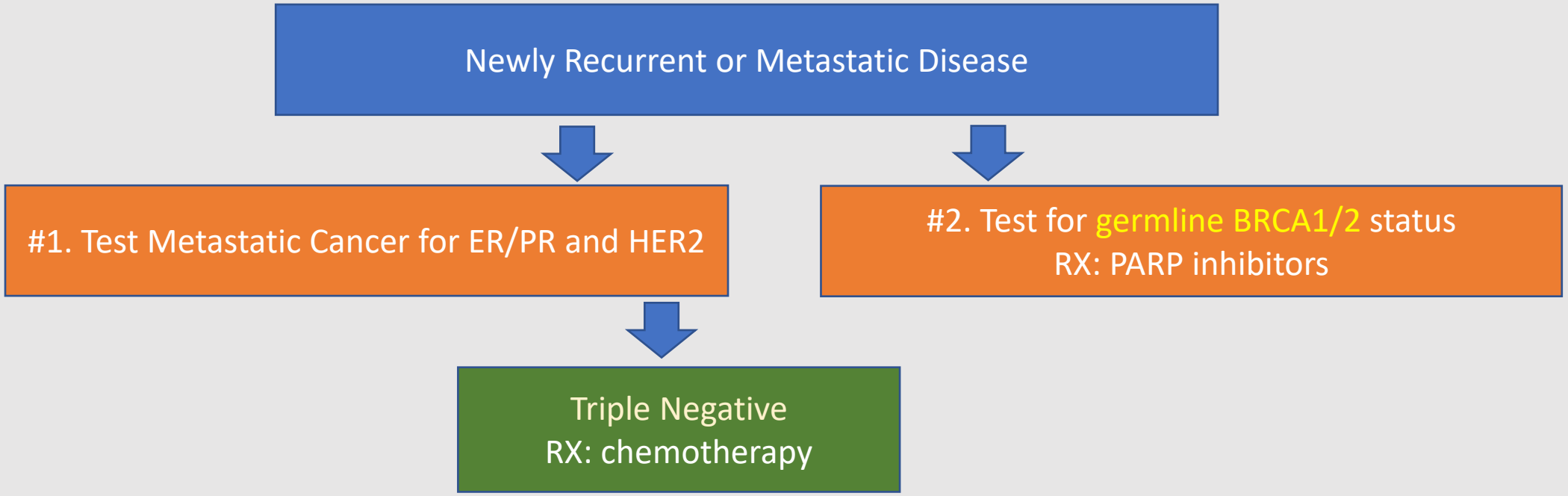
Figure 1. Identification of specific mutation by ARMS PCR. WT: Wild type. Q—F: Double-dye probe. ⇆: Forward and reverse primers.

Andre F, Ciruelos E, Rubovszky G, et al. Alpelisib for *PIK3CA*-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019;380(20):1929-1940.

Testing Considerations in Recurrent or Newly Metastatic Disease



Testing Considerations in Recurrent or Newly Metastatic Disease



Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

#2. Test for germline BRCA1/2 status
RX: PARP inhibitors

Triple Negative
RX: chemotherapy

Test for PD-L1 expression

IMpassion130 Trial	**SP142 IHC antibody using IC area scoring system ≥1%**	RX: Atezolizumab immunotherapy + alb-paclitaxel
KEYNOTE-355 Trial	**22C3 IHC antibody using CPS scoring system ≥10**	RX: Pembrolizumab + chemo

Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018 Nov 29;379:2108-2121.

Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

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RX: PARP inhibitors

Triple Negative
RX: chemotherapy

Test for PD-L1 expression

KEYNOTE-355 Trial

22C3 IHC antibody using CPS scoring system ≥10

RX: Pembrolizumab + chemo

Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018 Nov 29;379:2108-2121.

Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

#1. Test Metastatic Cancer for ER/PR and HER2

HER2+
RX: TDM-1 , T-DXd plus others

Possibly T-DXd for HER2 Low or 0?

#2. Test for germline BRCA1/2 status
RX: PARP inhibitors

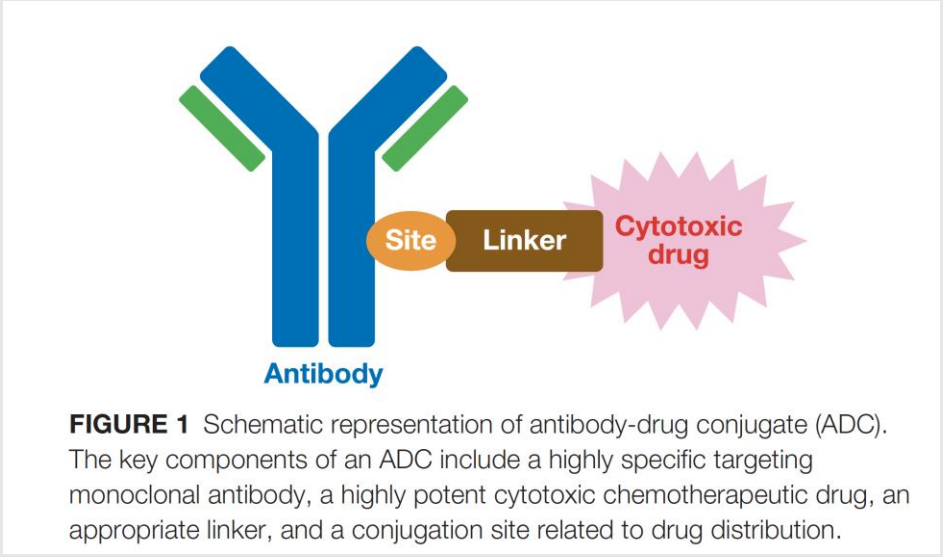


FIGURE 1 Schematic representation of antibody-drug conjugate (ADC). The key components of an ADC include a highly specific targeting monoclonal antibody, a highly potent cytotoxic chemotherapeutic drug, an appropriate linker, and a conjugation site related to drug distribution.

Testing Considerations in Recurrent or Newly Metastatic Disease

Newly Recurrent or Metastatic Disease

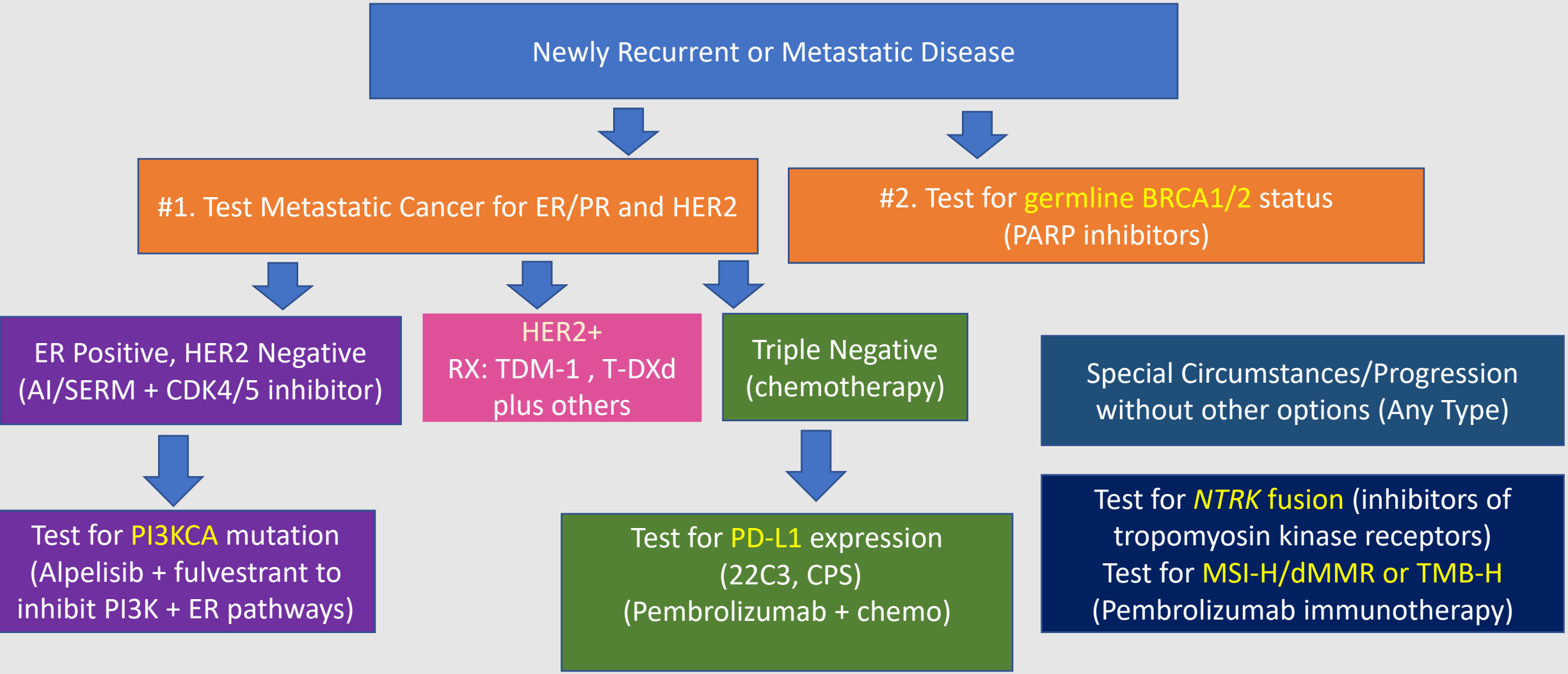
Special Circumstances/Progression without other options (Any Type)

Test for *NTRK* fusion RX: inhibitors of tropomyosin kinase receptors
(Secretory carcinoma of the breast >90%, other breast <5%)
Test for MSI-H/dMMR or TMB-H RX:Pembrolizumab immunotherapy

No specific companion diagnostic test

Drilon A, et al. N Engl J Med 2018;378(8):731-739.
Andre F, et al. . N Engl J Med . 2019;380(20):1929-40.
Le DT, et al. Science. 2017;357(6349):409-13.

REVIEW: Testing Considerations in Recurrent or Newly Metastatic Disease



ADDITIONAL TARGETED THERAPIES AND ASSOCIATED BIOMARKER TESTING FOR RECURRENT UNRESECTABLE (LOCAL OR REGIONAL) OR STAGE IV (M1) DISEASE

Biomarkers Associated with FDA-Approved Therapies

Breast Cancer Subtype	Biomarker	Detection	FDA-Approved Agents	NCCN Category of Evidence	NCCN Category of Preference
Any[a]	*BRCA1* mutation *BRCA2* mutation	Germline sequencing	Olaparib Talazoparib	Category 1 Category 1	Preferred
HR-positive/ HER2-negative[b]	*PIK3CA* activating mutation	PCR (blood or tissue block if blood negative), molecular panel testing	Alpelisib + fulvestrant[c]	Category 1	Preferred second- or subsequent-line therapy
TNBC	PD-L1 expression (using 22C3 antibody) Threshold for positivity combined positive score ≥10	IHC	Pembrolizumab + chemotherapy (albumin-bound paclitaxel, paclitaxel, or gemcitabine and carboplatin)[d]	Category 1	Preferred first-line therapy[h]
Any	*NTRK* fusion	FISH, NGS, PCR (tissue block)	Larotrectinib[e] Entrectinib[e]	Category 2A	Useful in certain circumstances
Any	MSI-H/dMMR	IHC, PCR (tissue block)	Pembrolizumab[d,f] Dostarlimab-gxly[g]	Category 2A	
Any	TMB-H (≥10 mut/mb)	NGS	Pembrolizumab[d,f]	Category 2A	

[a] Assess for germline *BRCA1/2* mutations in all patients with recurrent or metastatic

RECURRENT/STAGE IV (M1) DISEASE

CLINICAL STAGE

WORKUP[a]

- History and physical exam
- Discuss goals of therapy, adopt shared decision-making, and document course of care
- CBC
- Comprehensive metabolic panel, including liver function tests and alkaline phosphatase
- Imaging for systemic staging:
  - Chest diagnostic CT ± contrast
  - Abdominal ± pelvic diagnostic CT with contrast or MRI with contrast
  - Brain MRI with contrast if suspicious CNS symptoms
  - Spine MRI with contrast if back pain or symptoms of cord compression
  - Bone scan or sodium fluoride PET/CT[ss] (category 2B)
  - FDG PET/CT[tt] (optional)
  - X-rays of symptomatic bones and long and weight-bearing bones abnormal on bone scan
- Biomarker testing:
  - Biopsy of at least first recurrence of disease (consider re-biopsy if progression)
  - Evaluation of ER/PR and HER2 status[d,ddd,eee]
  - Comprehensive germline and somatic profiling to identify candidates for additional targeted therapies, see Additional Targeted Therapies and Associated Biomarker Testing for Recurrent or Stage IV (M1) Disease (BINV-R)
- Genetic counseling if patient is at risk[c] for hereditary breast cancer
- Assess for distress[g]

Stage IV (M1) or Recurrent →

→ See Treatment of Local and Regional Recurrence (BINV-19) and Supportive care[fff]

→ See Systemic Treatment of Recurrent Unresectable (local or regional) or Stage IV (M1) (BINV-20)[ggg] and Supportive care[fff]

STANDARD BIOMARKERS IN BREAST CANCER AT DIFFERENT TIME POINTS IN DISEASE PROGRESSION

NEW DIAGNOSIS: EARLY STAGE 1-3

- **ER (By IHC)**
  - Overall treatment pathway determined
  - Treatment with endocrine therapy
- **HER2 (By IHC or ISH)**
  - Overall treatment pathway determined
  - Treatment with HER2 targeted therapy (plus chemotherapy)
- **FOR ER+, HER2- SUBSET: Multi-Gene Panel***
  - Chemotherapy benefit estimated (plus endocrine therapy)

POST –NEOADJUVANT TREATMENT

- **RESIDUAL DISEASE POST NEOADJUVANT TREATMENT**
  - ADDITIONAL THERAPIES OFFERED
- **NO RESIDUAL DISEASE POST NEOADJUVANT TREATMENT**
  - CONTINUE WITH STANDARD THERAPY

METASTATIC PROGRESSION

- **REPEAT ER AND HER2 TESTING**
  - ER POSITIVE:
    Endocrine therapy + CDK4/6 inhibitors (as first line)
  - **PIK3CA MUTATION**
    - Second line option of alpelisib + fulvestrant
  - HER POSITIVE:
    Various HER2 antibody + chemotherapy combinations
  - ER AND HER NEGATIVE:
  - **PDL-1 (By IHC, 22C3 and CPS score)**
    - PDL-1 inhibitor (Pembrolizumab) + chemo
- **TEST ALL FOR BRCA1/2 (Germline)**
  - PARP inhibitor therapy (olaparib or talazoparib)
- **COMPREHENSIVE GENOMIC PROFILING (to identify uncommon targets)**
- **Option to test MSI/MMR and TMB**
  - PDL-1 inhibitor (Pembrolizumab)
- **Option to test for NTRK fusion**
  - Larotrectinib or Entrectinib

Questions?
allisonk@stanford.edu