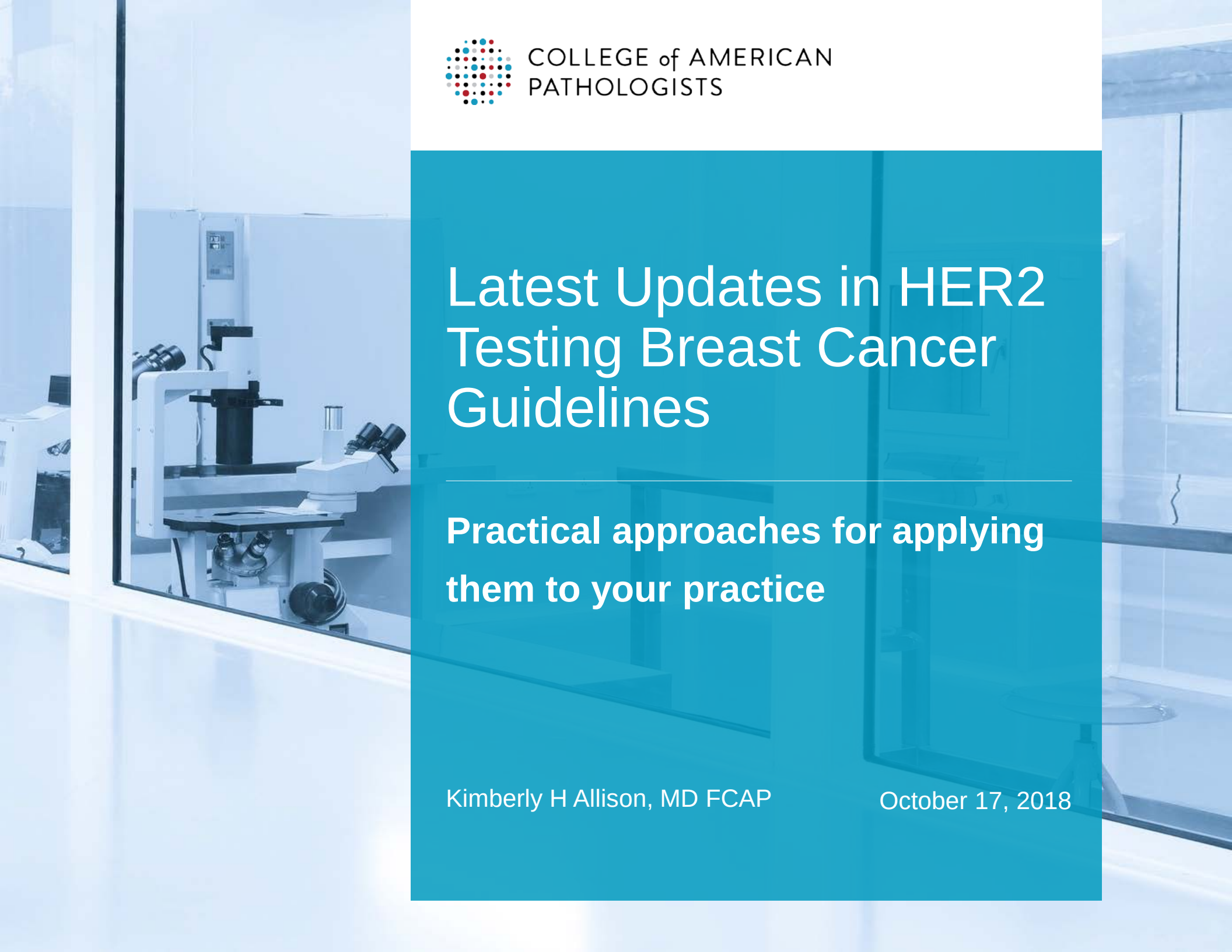

COLLEGE of AMERICAN PATHOLOGISTS

Latest Updates in HER2 Testing Breast Cancer Guidelines

Practical approaches for applying them to your practice

Kimberly H Allison, MD FCAP

October 17, 2018

Webinar Host

- **This series is sponsored by the Personalized Healthcare Committee (PHC)**
- **Today's webinar host is PHC member, Eric Walk, MD**

Housekeeping

- **This presentation will be recorded. The recording and PDF will go out to all registrants in one week**
- **All lines are muted during the presentation**
- **Please send in your questions as you think of them via the "Question Box" in your control panel**

Kimberly H. Allison, MD

- **Professor, Department of Pathology Stanford University Medical Center**
- **Residency Program Director for the Department of Pathology**
- **Specialist in breast cancer diagnosis (breast pathology)**
- **Member of the CAP/ASCO HER2 Testing Guidelines Review committee**

Disclaimer

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Disclaimer, continued

- **Opinions expressed by the speaker are the speaker's own and do not necessarily reflect an endorsement by the CAP of any organizations, equipment, reagents, materials, or services used by participating laboratories.**

Disclosures

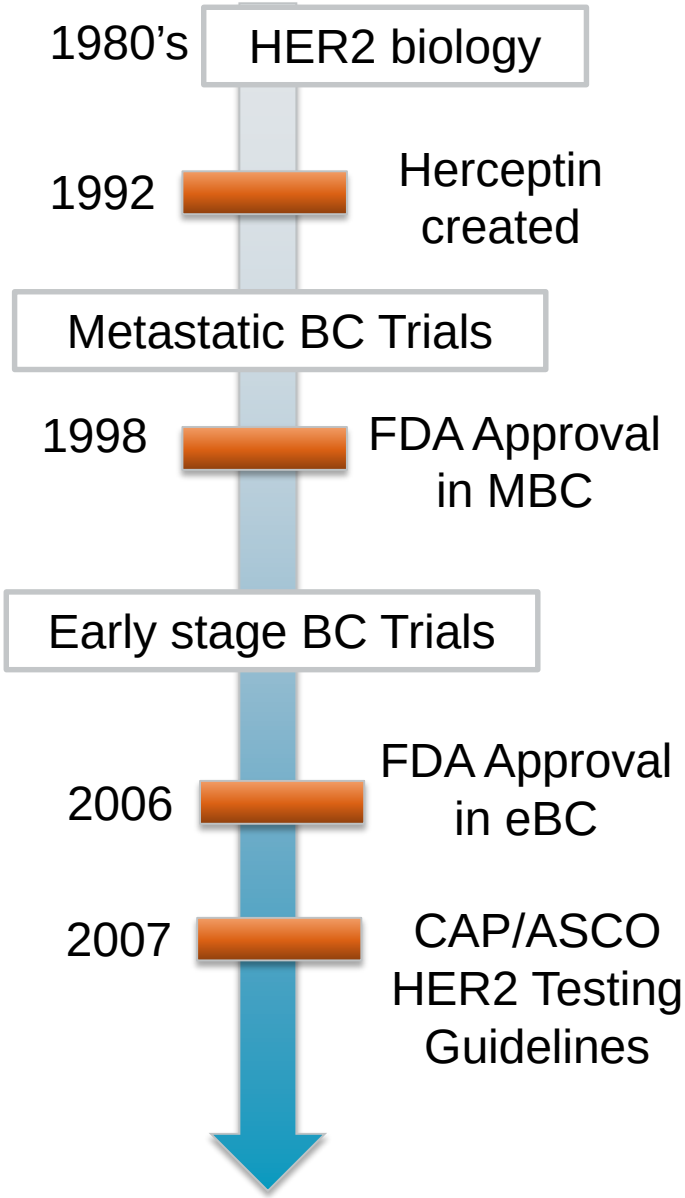
- **I have no relevant financial disclosures**

Summary of Learning Topics

- Review the current state of HER2 Testing in Breast Cancer
- Discuss issues that the 2018 CAP/ASCO Update address and what has remained the same
- Impact of the 2018 Update's changes on laboratory SOPs and reporting
- Learn from case-based examples how to apply the HER2 Guidelines Update to patient samples

A Brief History of HER2 Testing in Breast Cancer

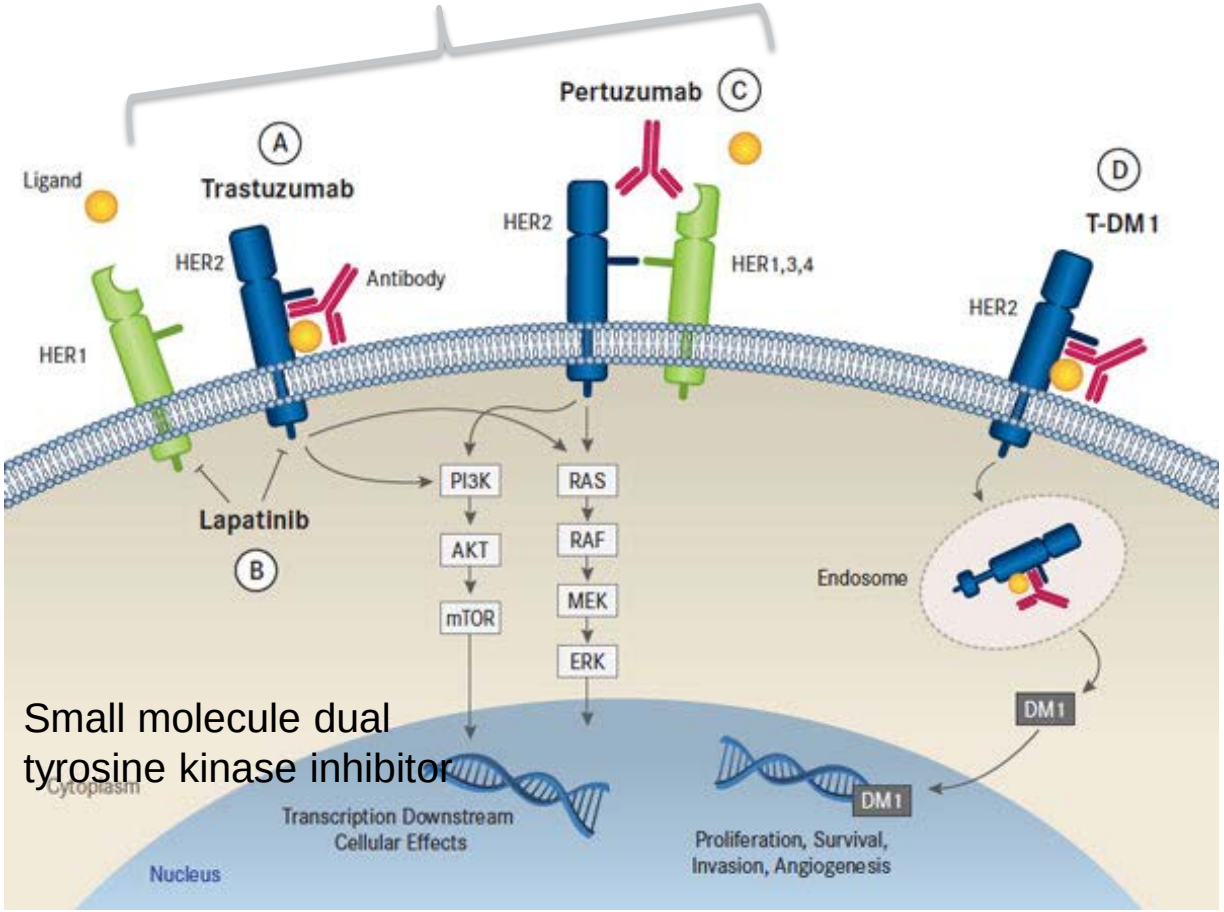
- **First prognostic: HER2 amplification associated with worst outcomes**
- **HER2 targeted therapy developed → Need for accurate testing to PREDICT response to treatment (collaboration between drug + testing industries)**
- **Accurate/standardized HER2 testing needed on ALL cases**
- **2007 First CAP-ASCO HER2 Testing Guideline Published**



HER2 Targeted Therapies Today

- **Combination therapy**
- **Novel agents**
- **Continued need for accurate HER2 testing**

Combination therapy approved neoadjuvantly 2013



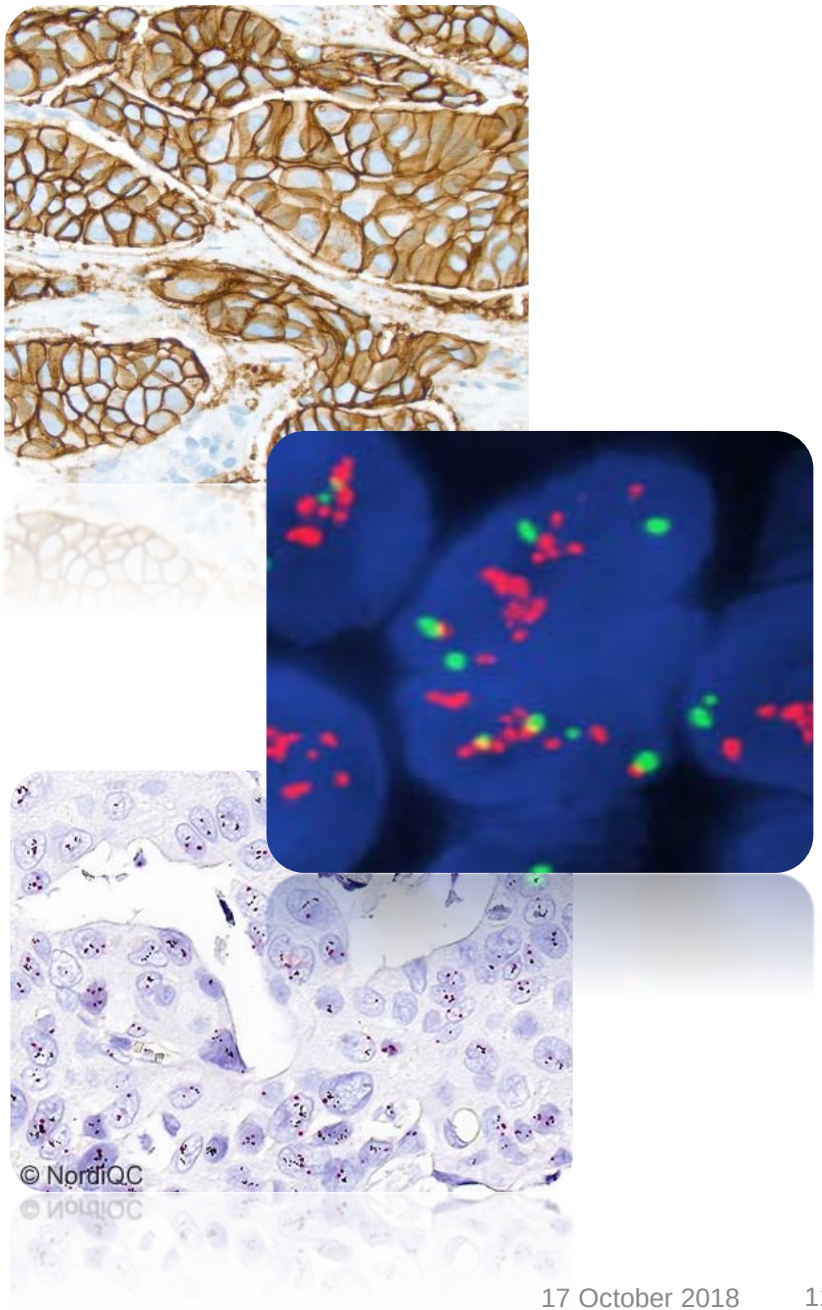
Eleonora Teplinsky, MD and Komal Jhaveri, MD
Published Online: Friday, March 21, 2014
http://www.onclive.com/publications/contemporary-oncology/2014/february-2014/antibody-drug-conjugates-and-t-dm1/1#sthash.IhFlBdjN.dpuf

Methods of HER2 Testing

- **Protein expression:**

  Immunohistochemistry (IHC)
- **Gene amplification status:**

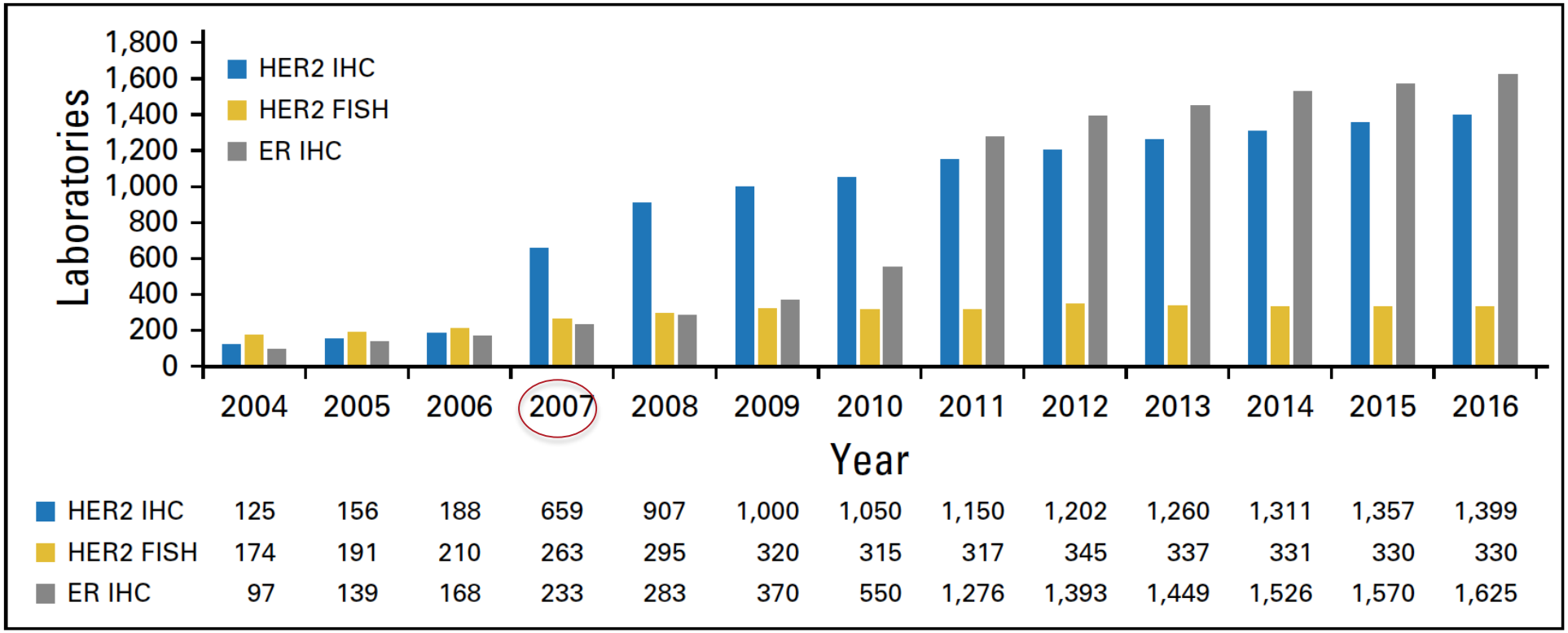
  In situ hybridization (ISH)
  - **FISH, CISH, DISH**
  - **Single probe assays not recommended**
- **Gene expression: mRNA**
  - **Not recommended currently**



HER2 Guidelines: A Brief History of a Living Document

- **2007: First ever joint ASCO+CAP Testing Guideline**
  - **Setting standards for the first time**
  - **Concerns about high false positive rate (local vs central testing for trials) → raised % cells required for IHC 3+ result to 30%**
  - **ISH equivocal only based on ratio 1.8-2.2**
- **2013 Focused Update:**
  - **Concerns about false negatives → returned to FDA IHC standards (10%), created new ISH positive groups (signals/cell and ratio relevant), ISH equivocal group modified, clarified heterogeneity**
  - **Recommendations for retesting and recognizing discordant results**
- **2018 Focused Update:**
  - **Fine tuning, getting rid of ISH equivocal results and addressing workup of uncommon ISH groups**

Current State of CAP PT for HER2 Testing



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
HER2 IHC	125	156	188	659	907	1,000	1,050	1,150	1,202	1,260	1,311	1,357	1,399
HER2 FISH	174	191	210	263	295	320	315	317	345	337	331	330	330
ER IHC	97	139	168	233	283	370	550	1,276	1,393	1,449	1,526	1,570	1,625

- **Dramatic increase in labs participating in CAP PT**
- **Now steady state?**

Fig 7. Number of laboratories participating in predictive marker proficiency testing for human epidermal growth factor receptor 2 (HER2) by immunohistochemistry (IHC), HER2 by fluorescent in situ hybridization (FISH), and estrogen receptor (ER) by IHC through the College of American Pathologists Laboratory Improvement Program.

ASCO/CAP HER2 Testing in Breast Cancer Update
Arch Pathol Lab Med. 2018 May 30. [Epub ahead of print]

VOLUME 36 · NUMBER 20 · JULY 10, 2018
JOURNAL OF CLINICAL ONCOLOGY

Clinical Questions for HER2 2018 Update

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

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

- **Clinical Question 1: What is the most appropriate definition for IHC 2+ (IHC Equivocal)?**
- **Clinical Question 2: Must HER2 testing be repeated on a surgical specimen if initially negative test on core biopsy?**

Unusual Dual Probe ISH Results

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- **Clinical Question 4: Should invasive cancers with an average *HER2* copy number ≥6.0 signals/cell but a *HER2*/CEP17 ratio <2.0 be considered ISH positive?**
- **Clinical Question 5: What is the appropriate diagnostic work-up for invasive cancers with an average *HER2* copy number ≥4.0 but <6.0 signals/cell and a *HER2*/CEP17 ratio <2.0 and initially deemed to have an equivocal *HER2* ISH test result?**

ASCO/CAP HER2 Testing in Breast Cancer Update

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JOURNAL OF CLINICAL ONCOLOGY

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ASCO Guidelines

HER2 Breast on cap.org

Clinical Questions 1 & 2 Previously Addressed

VOLUME 33 · NUMBER 11 · APRIL 10 2015

JOURNAL OF CLINICAL ONCOLOGY

CORRESPONDENCE

National Guidelines and Level of Evidence: Comments on Some of the New Recommendations in the American Society of Clinical Oncology and the College of American Pathologists Human Epidermal Growth Factor Receptor 2 Guidelines for Breast Cancer

Emad A. Rakha and Marian Pigera
Nottingham University Hospitals NHS Trust; University of Nottingham, Nottingham City Hospital, Nottingham, United Kingdom

Abeer Shaaban
St James University Hospital, Leeds, United Kingdom

Sandra J. Shin and Timothy D'Alfonso
New York Presbyterian Hospital, New York, NY

Ian O. Ellis and Andrew H.S. Lee
Nottingham University Hospitals NHS Trust; University of Nottingham, Nottingham City Hospital, Nottingham, United Kingdom

Reply to E.A. Rakha et al

American Society of Clinical Oncology/College of American Pathologists Human Epidermal Growth Factor Receptor 2 Testing Clinical Practice Guideline Upcoming Modifications

Proof That Clinical Practice Guidelines Are Living Documents

M. Elizabeth H. Hammond, MD; David G. Hicks, MD

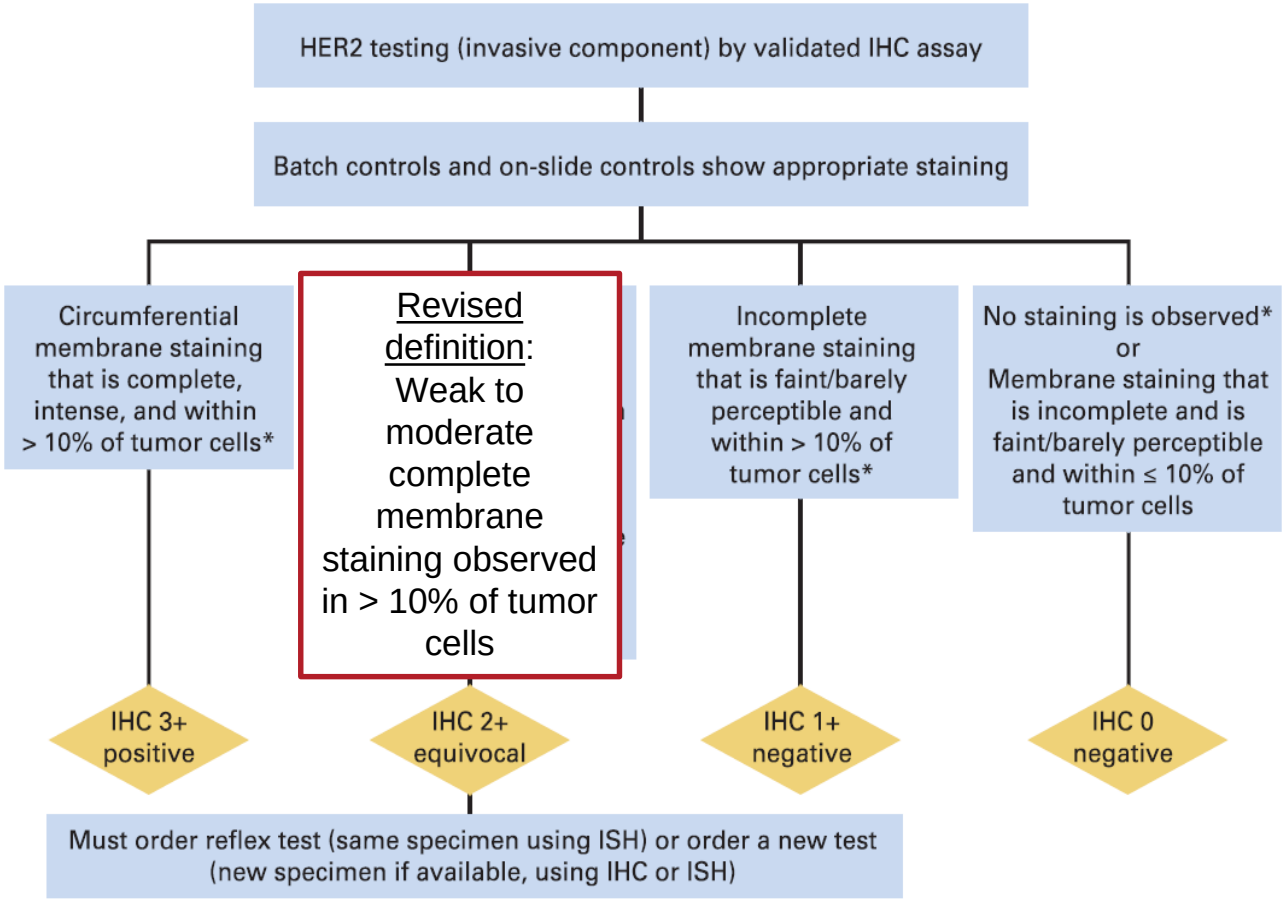
#1: Revised Definition of IHC 2+

CLINICAL QUESTION 1
What is the most appropriate definition for IHC 2+ (IHC Equivocal)?

Recommendation 1

The revised definition of IHC 2+ (equivocal) is invasive breast cancer with "Weak to moderate complete membrane staining observed in >10% of tumor cells." (see Figure 1 in full text)

(Type: Evidence based; Evidence quality: High; Strength of recommendation: Strong)



HER2 Breast on cap.org

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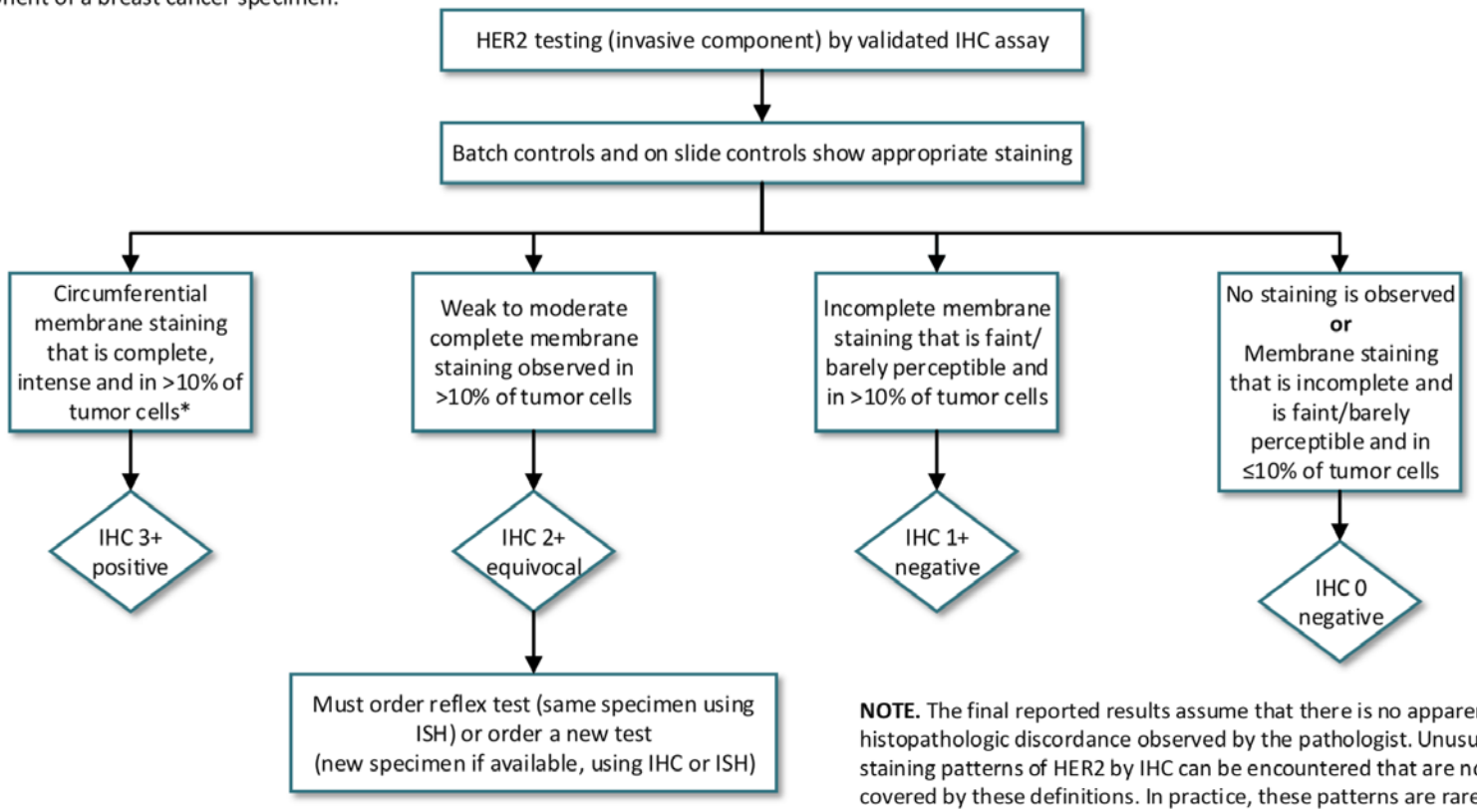
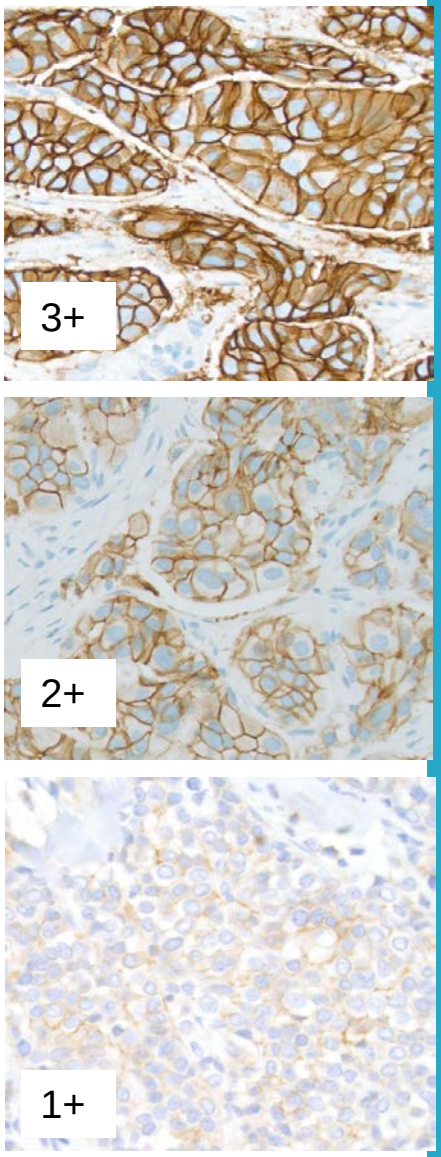
ASCO Guidelines

HER2 IHC Testing Interpretation

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



Unusual staining patterns now in Note

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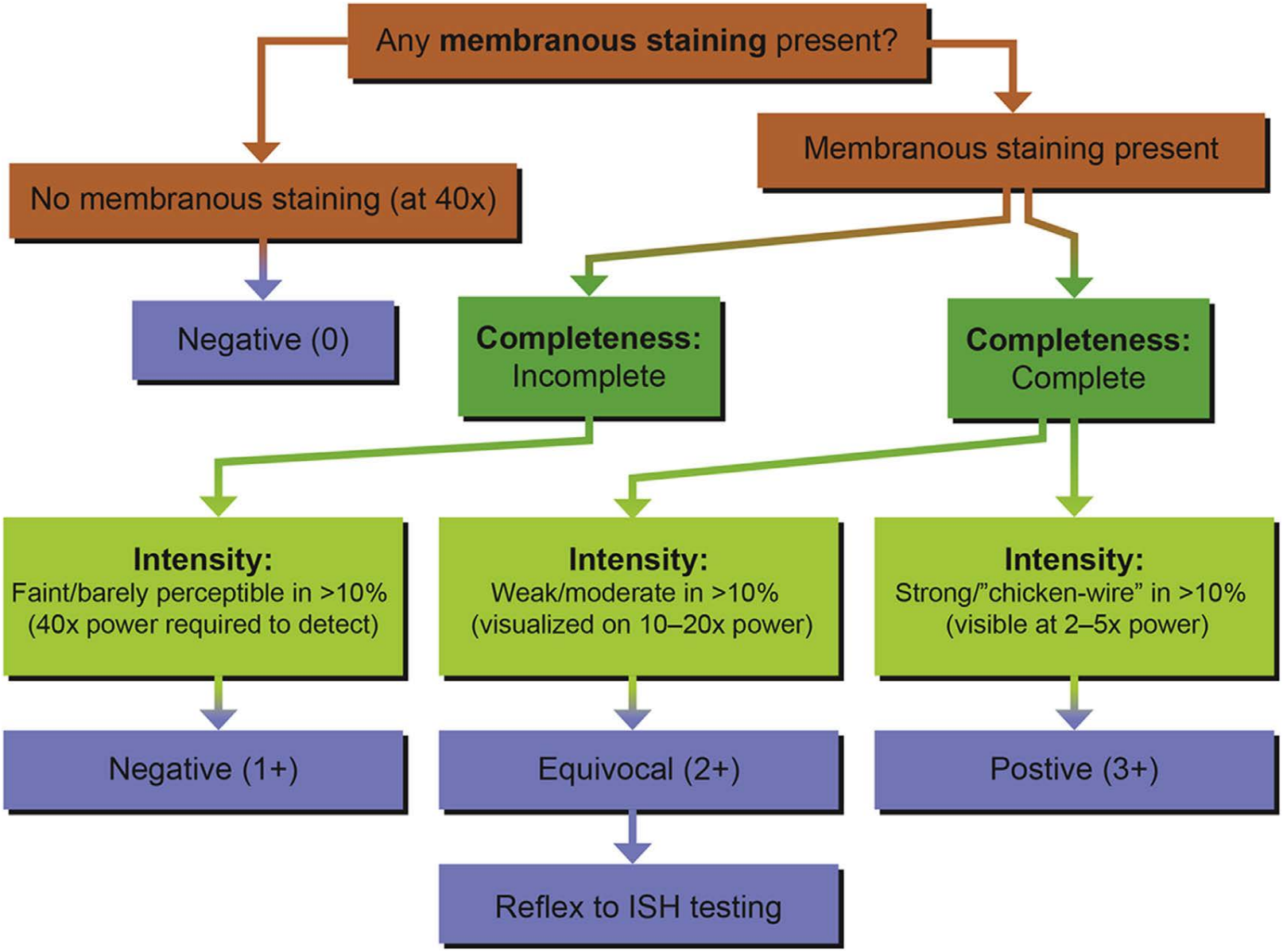
ASCO Guidelines

Work-Aid for HER2 IHC Interpretation



Membranous?

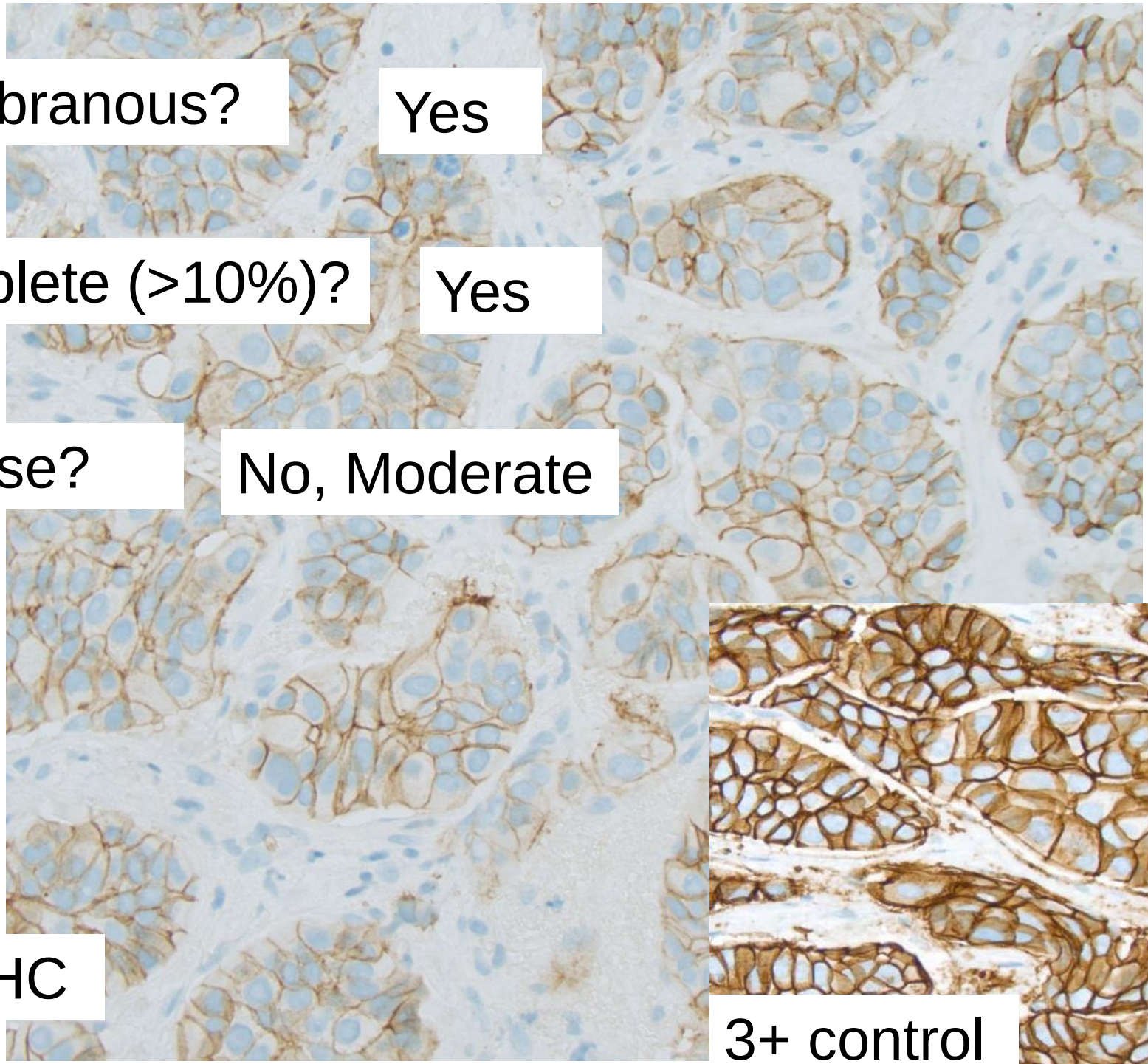
Yes

Complete (>10%)?

Yes

Intense?

No, Moderate



2+ IHC

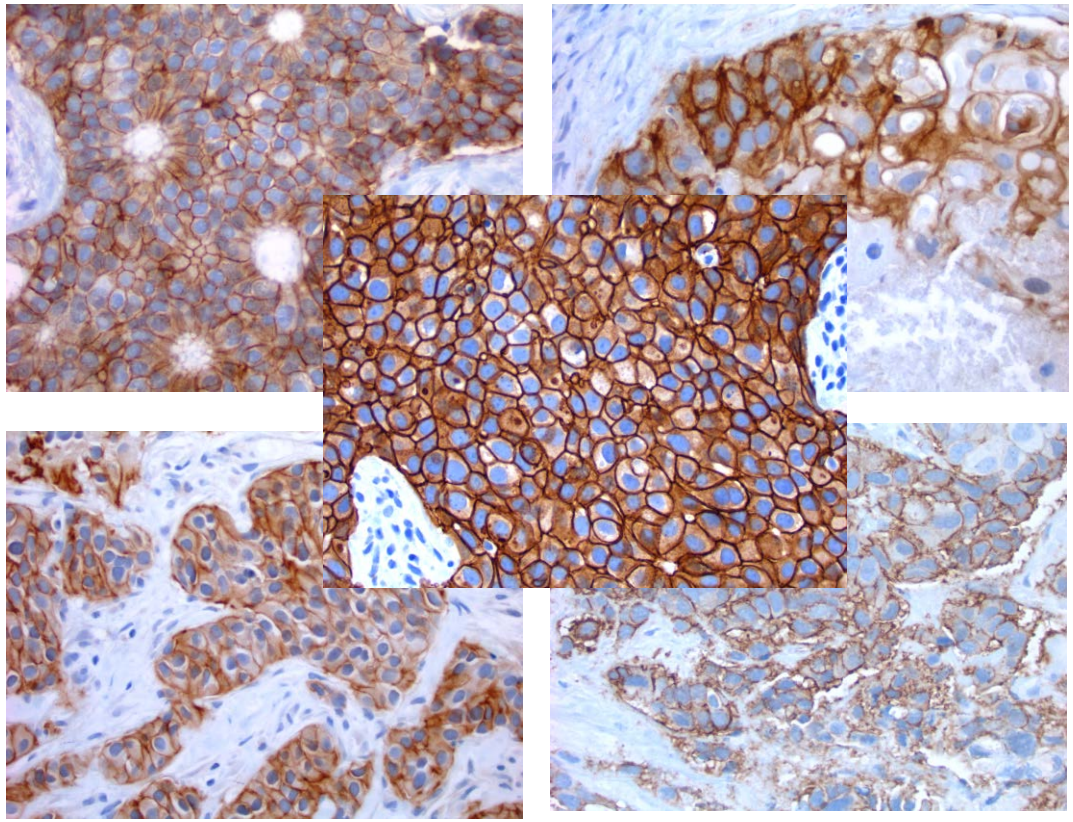
3+ control

Achieving 95% Cross-Methodological Concordance in HER2 Testing

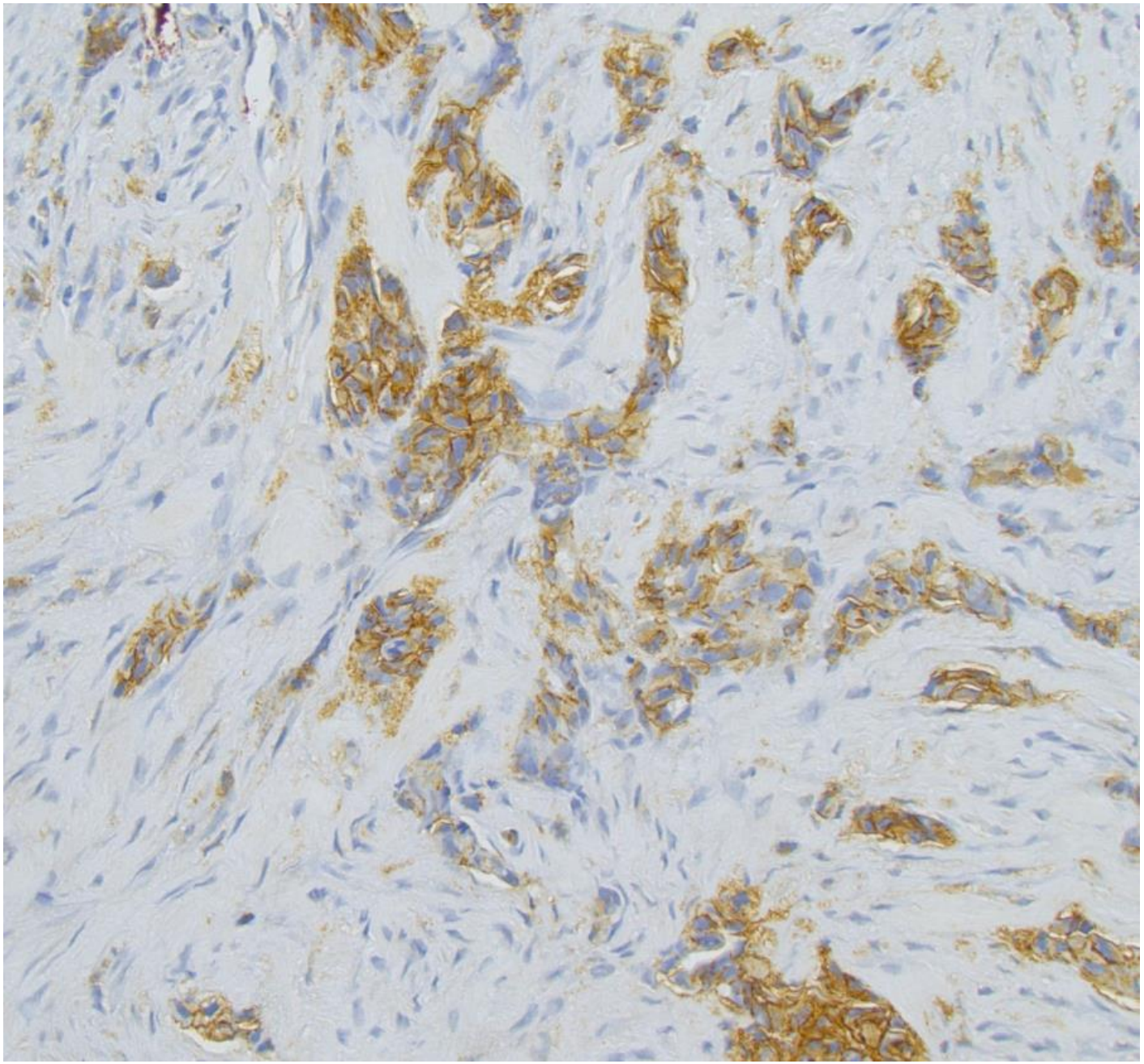
Causes and Implications of Discordant Cases

Erin E. Grimm, MD, Rodney A. Schmidt, MD, PhD, Paul E. Swanson, MD, Suzanne M. Dintzis, MD, PhD, and Kimberly H. Allison, MD

- 697 cases with both IHC and FISH results
- 96% overall concordance
- Most common reason for discordance on review: Over-interpretation of IHC stain intensity



Am J Clin Pathol 2010;134:284-292
DOI: 10.1309/AJCPUQB18XZOHHBJ



A. 0

B. 1+

C. 2+

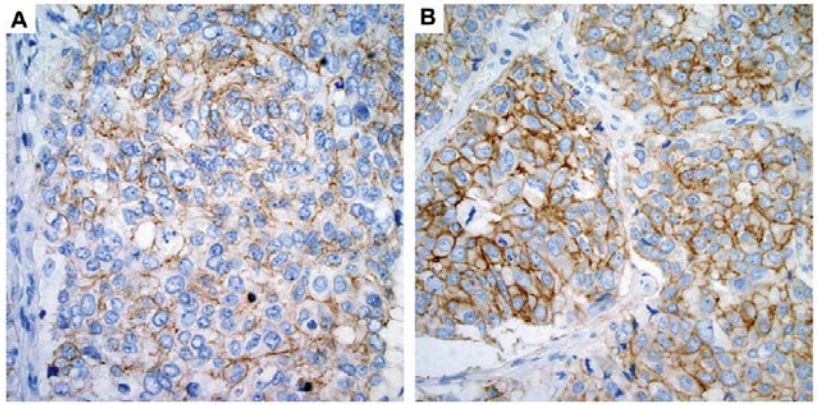
D. 3+

E. Other

2013+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**
- **Reason for indeterminate result should be reported**
- **Another method of testing can be attempted or another sample requested**

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



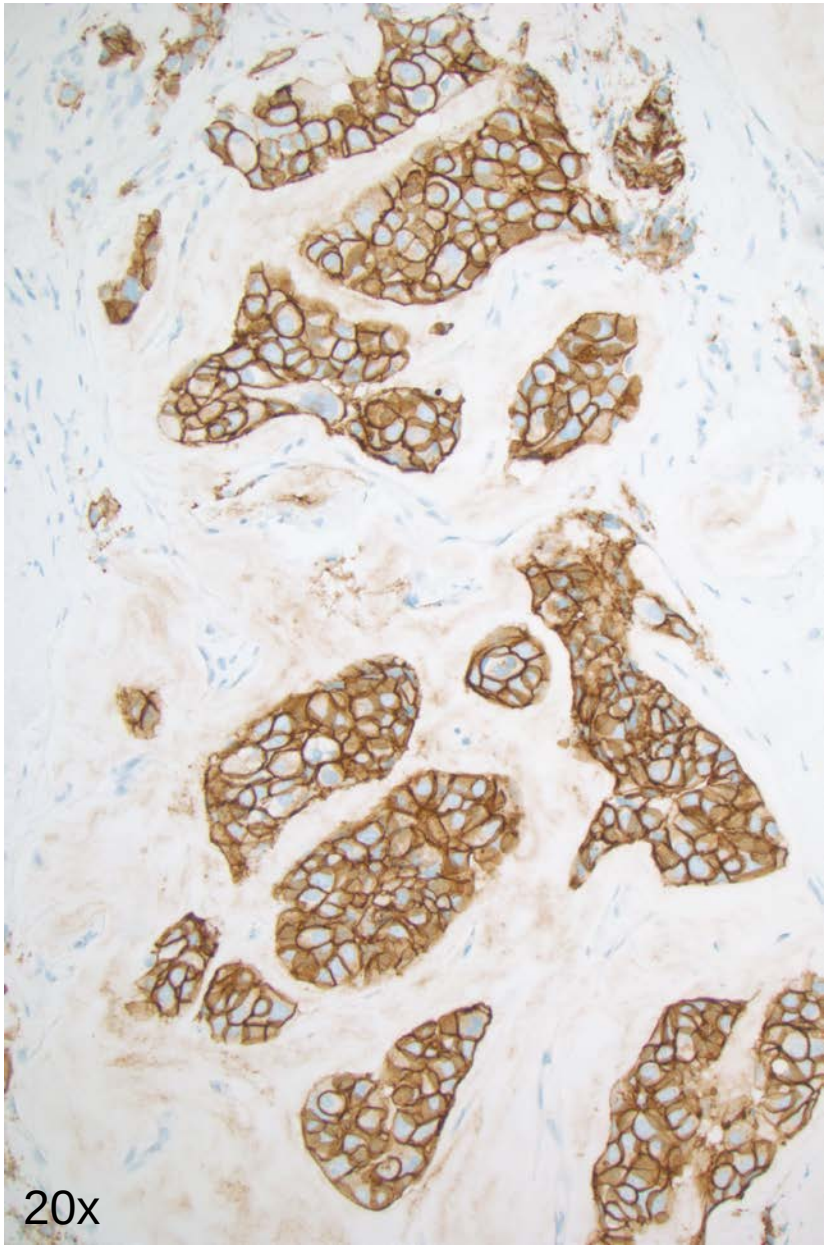
Cut > 6 weeks prior Re-cut and stained

ASCO/CAP HER2 Testing Guideline Update—Wolff et al

Test Case

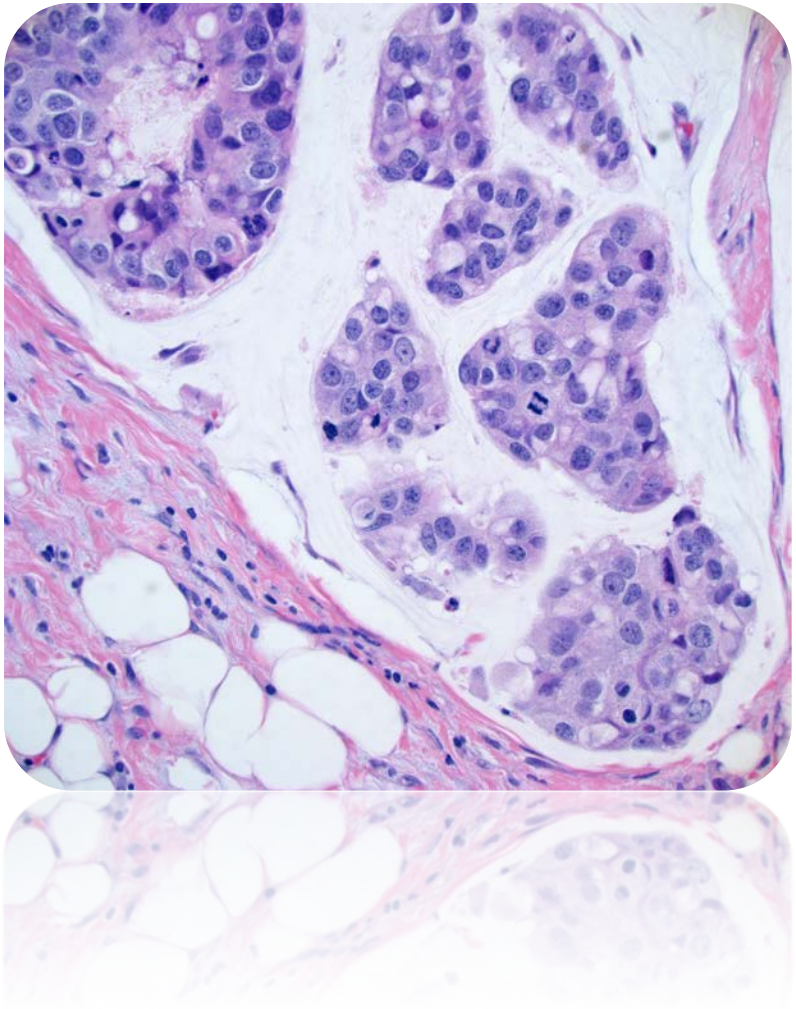
42 year old with a diagnosis of invasive mucinous carcinoma. You receive the HER2 IHC for interpretation. How do you report the case?

A. **IHC 3+ (positive)**
B. **IHC 2+ (equivocal)**
C. **IHC 1+ (negative)**
D. **Other**



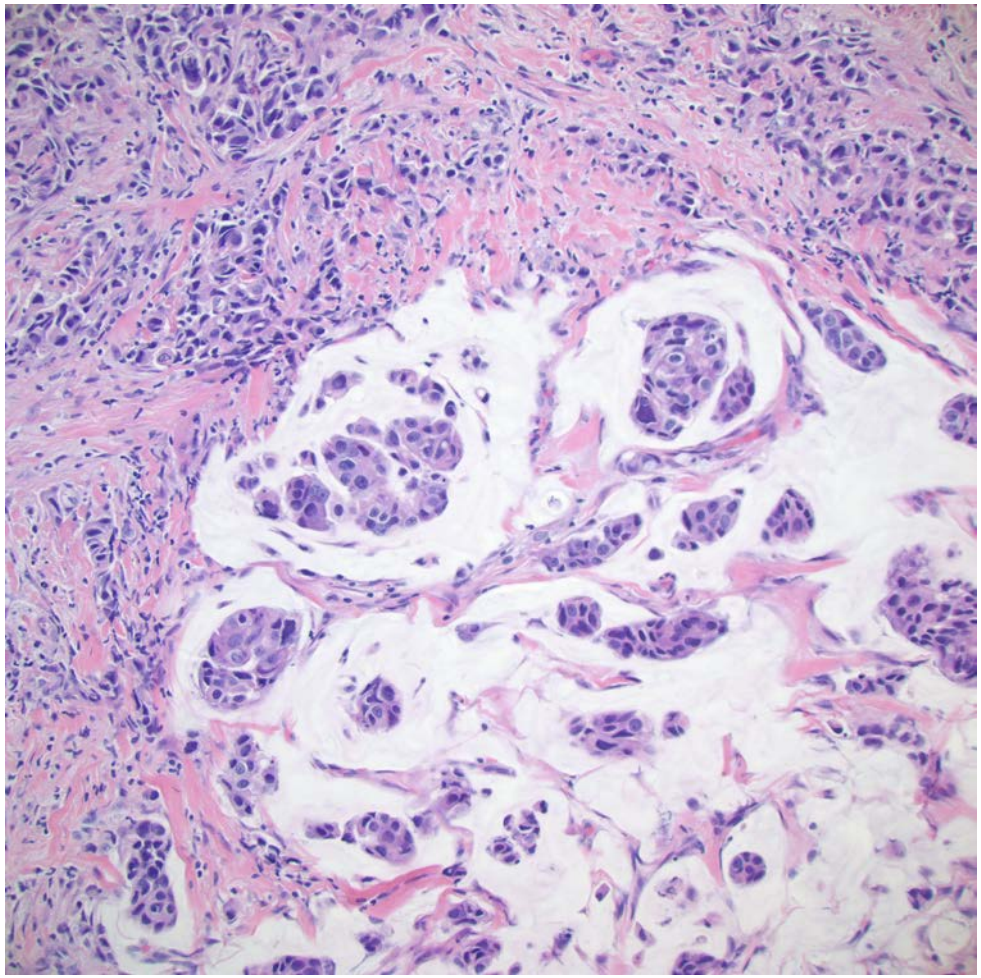
Beware of the “mucinous” carcinoma!

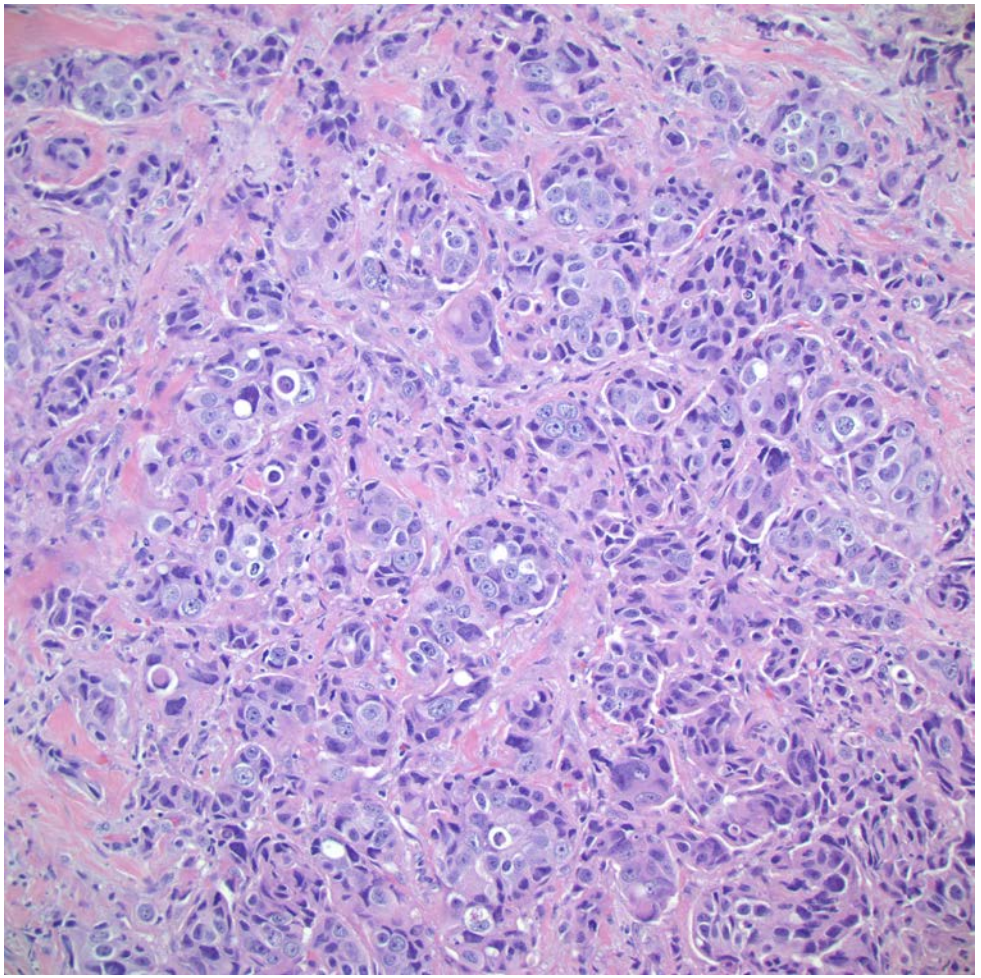
- **To qualify as “Good Prognosis Subtype: Pure mucinous carcinoma”**
  - **Should be pure, ER+ and not high grade**
- **Should NEVER be:**
  - **HER2 positive**
  - **ER negative**
  - **Classified on core biopsy**



Mucinous features/Mucin Production ≠ Mucinous carcinoma

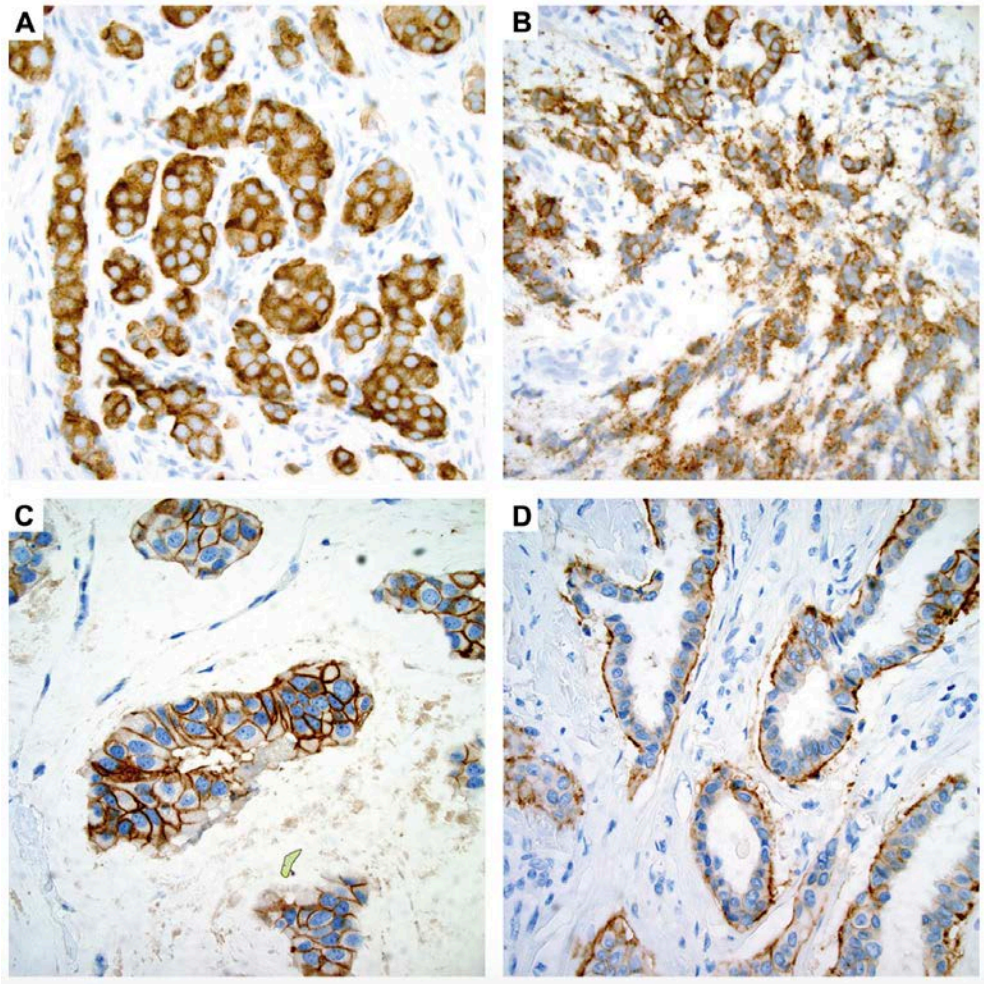
Re-review of histology:
Not pure mucinous carcinoma





Unusual Staining Patterns and Discordant Results

Unusual IHC Patterns (either 2+ or insufficient)



DISCORDANT 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:

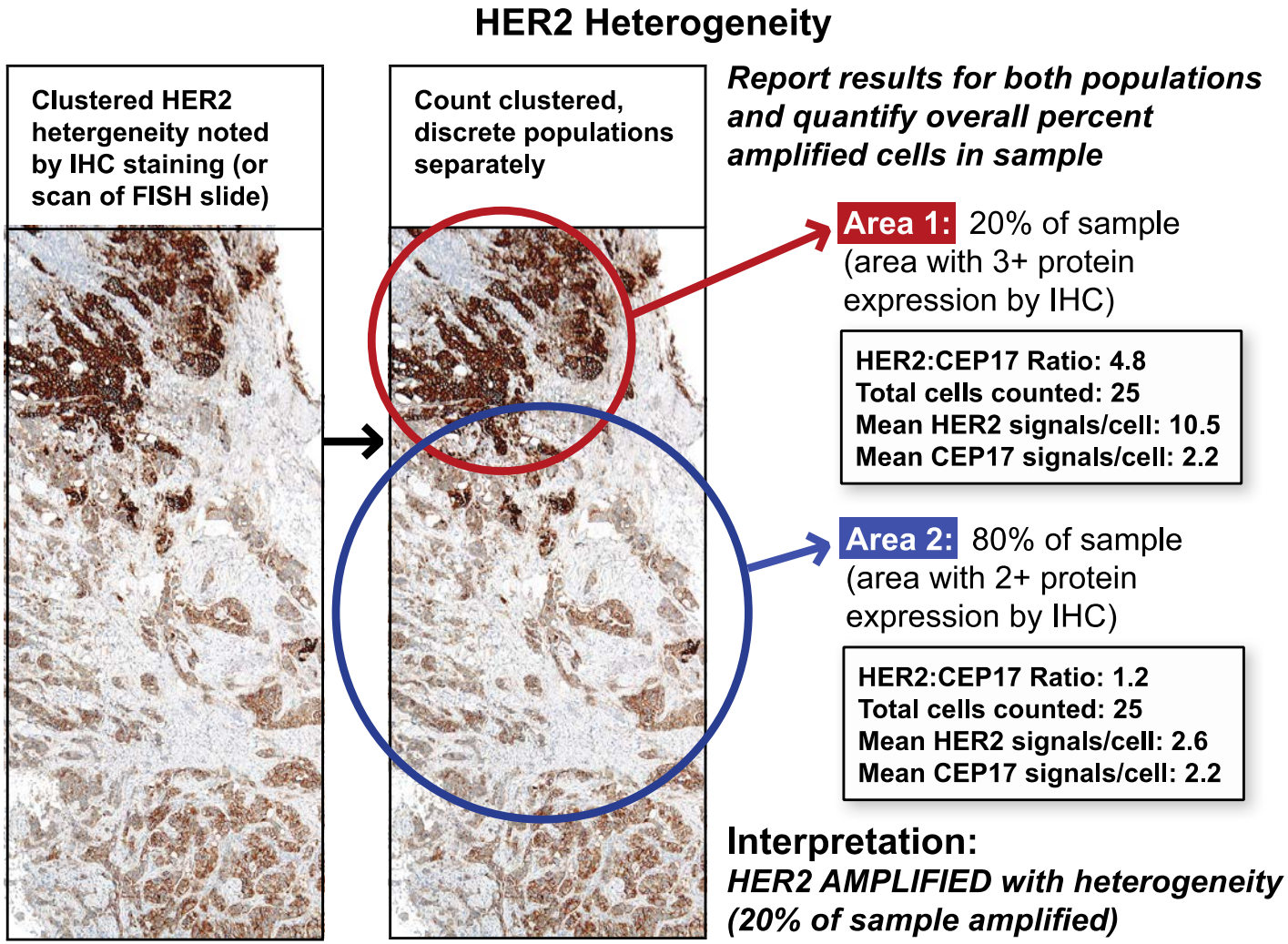
- Infiltrating ductal or lobular carcinoma, ER and PgR+
- Tubular (at least 90% pure)
- Mucinous (at least 90% pure)
- Cribriform (at least 90% pure)
- Adenoid cystic carcinoma (90% pure)

See Table 2 in Guidelines Update

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

Unusual IHC Staining Patterns:



- If 3+ staining in > 10% = Positive Test (but note heterogeneity present in report)
- If 3+ staining in < 10% = Equivocal result by IHC

Summary of Recommendations

CLINICAL QUESTION 2

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

Recommendation 2

On the basis of some criteria (including a tumor grade 3), "If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test *may* be ordered on the excision specimen…" (see Table 2 in full text)

(Type: Evidence based; Evidence quality: High; Strength of recommendation: Strong)

COLLEGE of AMERICAN PATHOLOGISTS

HER2 Breast on cap.org

ASCO Guidelines

HER2 Negative on Core Biopsy; When to Consider Retesting in the Excision?

- **Tumor is Grade 3**

See Table 2 in Guidelines Update

- **Amount of invasion in core was small**
- **Resection has high grade carcinoma that is morphologically distinct from that in core**
- **Unusual or discordant HER2 results on core* (Table 2 being updated – currently states if equivocal by IHC+ISH)**
- **Doubt about specimen handling of core**
- **Pathologist suspects testing error**

Can make POLICY or USE PATHOLOGIST JUDGEMENT

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ASCO Guidelines

HER2 Breast on cap.org

HER2 Testing by ISH/FISH: Typical results

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	

CEP17

HER2

ean CEP17 signals/cell: 2.0

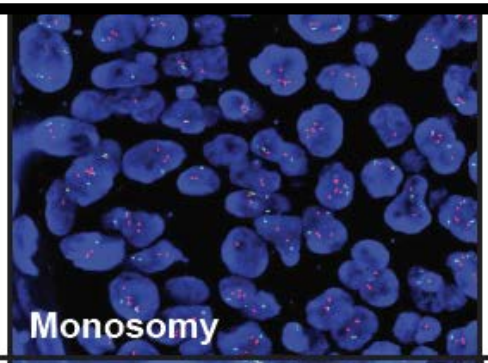
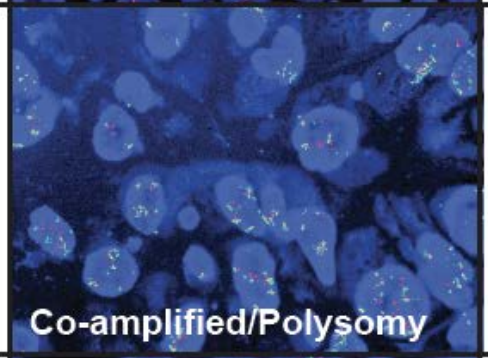
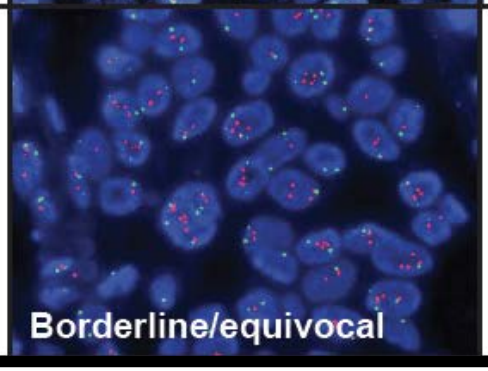
"Group 1"
Ratio ≥ 2.0. +
Mean HER2 ≥ 4.0

"Group 5"
Ratio < 2.0
Mean HER2 < 4.0

Must include both mean signals/cell and ratio on report

Unusual FISH Categories: Groups 2-4

2013 Guideline Interpretation:

Group 2		≥ 2.0	< 4.0	
Group 3		< 2.0	≥ 6.0	
Group 4		< 2.0	4.0 - 6.0	

Positive

Controversial...

Positive

Equivocal

Retest:
New sample or lab, new technique (alt probes)

How Common are Group 2-4 Cases?

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

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

- **Group 2: 0.4 - 3.7% (most ~1%)**
- **Group 3: 0.4- 3.0% (most ~1%)**
- **Group 4: 1.9 - 14.2% - most ~ 4-5% (highest in 2+ cases using refr lab)**

Groups 2-4 overall: ~ 3 - 8%

ASCO/CAP HER2 Testing in Breast Cancer Update

Arch Pathol Lab Med. 2018 May 30. [Epub ahead of print]

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JOURNAL OF CLINICAL ONCOLOGY

Groups 2-4 and Discordance with IHC and Grade

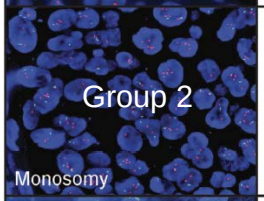
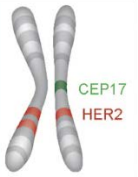
'Non-classical' HER2 FISH results in breast cancer: a multi-institutional study

Morgan Ballard[1], Florencia Jalikis[2], Gregor Krings[3], Rodney A Schmidt[2], Yunn-Yi Chen[3], Mara H Rendi[2], Suzanne M Dintzis[2], Kristin C Jensen[1,4], Robert B West[1], Richard K Sibley[1], Megan L Troxell[1] and Kimberly H Allison[1]

MODERN PATHOLOGY (2016), 1–9

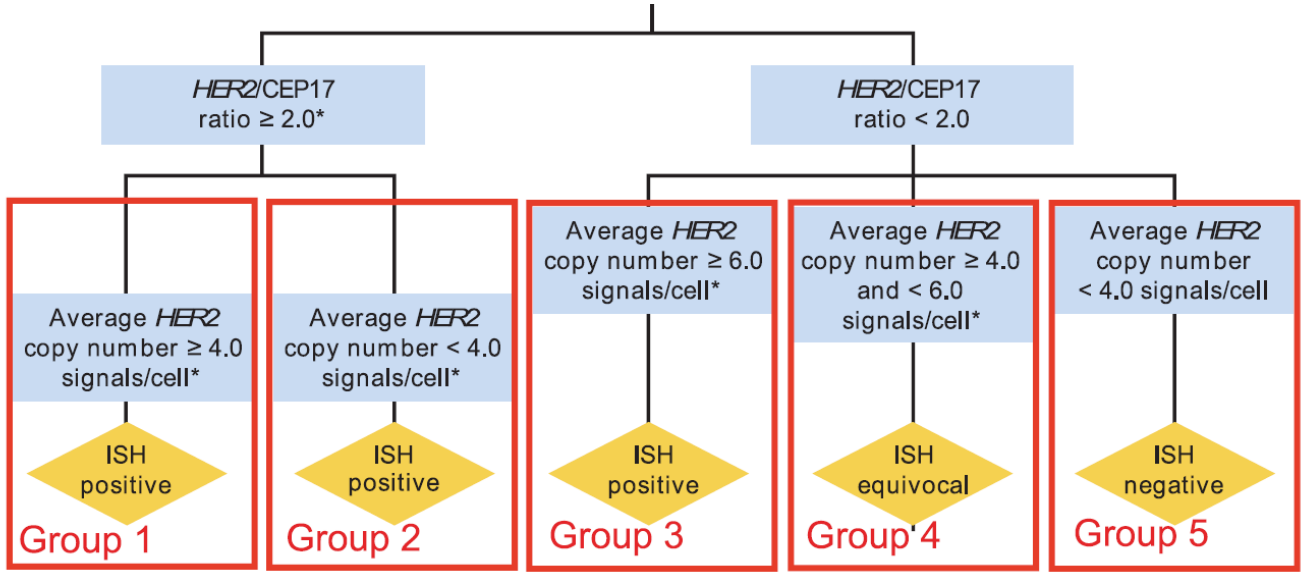
More often discordant with IHC results or other features

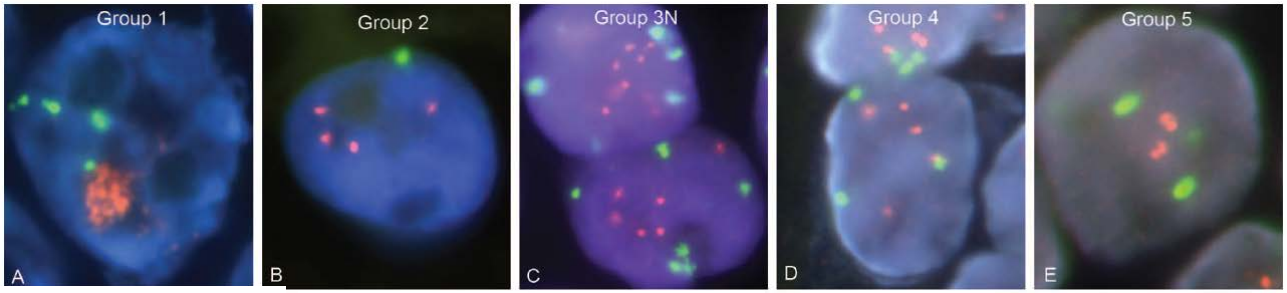
> 8,000 cases with IHC and FISH results from Stanford, UCSF, UWMC

				IHC 0-1+	IHC 3+	ER+	Grade 1
	< 2.0	4.0 - 6.0		25.4%	7.3%	82.2%	9.1%
	≥ 2.0	< 4.0		30.1%	12.4%	78.8%	13.3%
	< 2.0	≥ 6.0		13.2%	31.7%	75.0%	5.6%
	≥ 2.0	4.0 - 6.0		21.6%	10.0%	81.0%	9.6%
Classic Amplified (Group 1):				< 3%	68.5%	69.2%	3.5%
Classic Non-amplified (Group 5):				53.5%	0.9%	81.3%	25.1%

Assessing the New American Society of Clinical Oncology/College of American Pathologists Guidelines for *HER2* Testing by Fluorescence In Situ Hybridization

Central HER2 testing lab for BCIRG trials (N= 10,468)





	Classic amplified (≥6)	"Monosomy"	Co-amplified	Equivocal	Negative
% HER2 3+ by IHC:	49.6%	0%	11.1%	0.7%	0.05%
Low amplified (4-6)	2%				

Press MF et al.

Arch Pathol Lab Med. doi: 10.5858/arpa.2016-0009

Clinical Evidence in Group 2-4 Cases

- **Limited by their rarity**
- **Group 2 (ratio >2.0) no significant benefit from HER2 RX in HER2+ trial (BGIRG-006)**
- **Groups 3 and 4 were not typically included in HER2 targeted trials because ratio negative**
  - **Group 4: Do not have worse outcomes in ER+/HER2- trial analysis (BCIRG-005)**
  - **Group 3: Heterogeneous group, "co-amplified," benefit of HER2 RX indeterminate/mixed**

VOLUME 34 · NUMBER 29 · OCTOBER 10, 2016

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

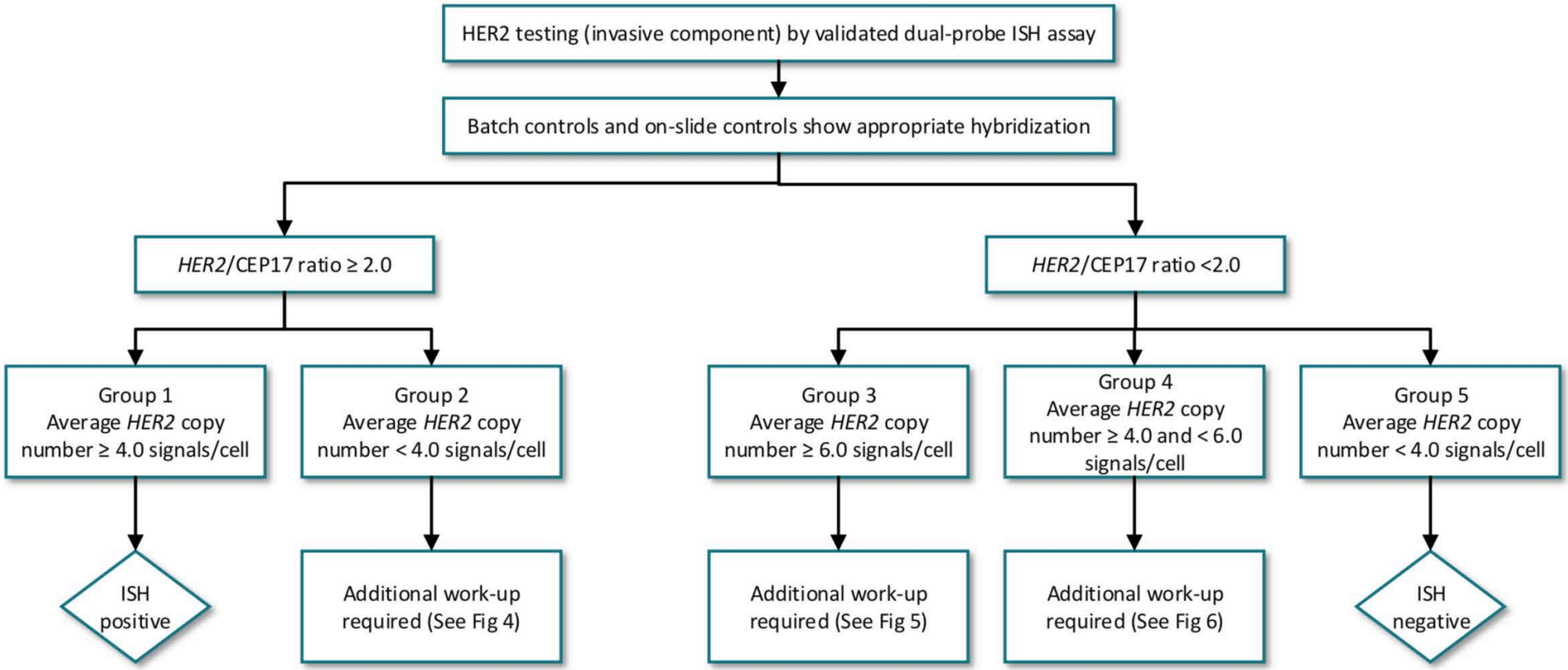
HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials

Michael F. Press, Guido Sauter, Marc Buyse, Hélène Fourmanoir, Emmanuel Quinaux, Denice D. Tsao-Wei, Wolfgang Eiermann, Nicholas Robert, Tadeusz Pienkowski, John Crown, Miguel Martin, Vicente Valero, John R. Mackey, Valerie Bee, Yanling Ma, Ivonne Villalobos, Anaamika Campeau, Martina Mirlacher, Mary-Ann Lindsay, and Dennis J. Slamon

Other testing methods besides IHC are not clinically validated (such as alternative probes) and can give variable results

HER2 FISH Testing: 2013→ 2018 Update

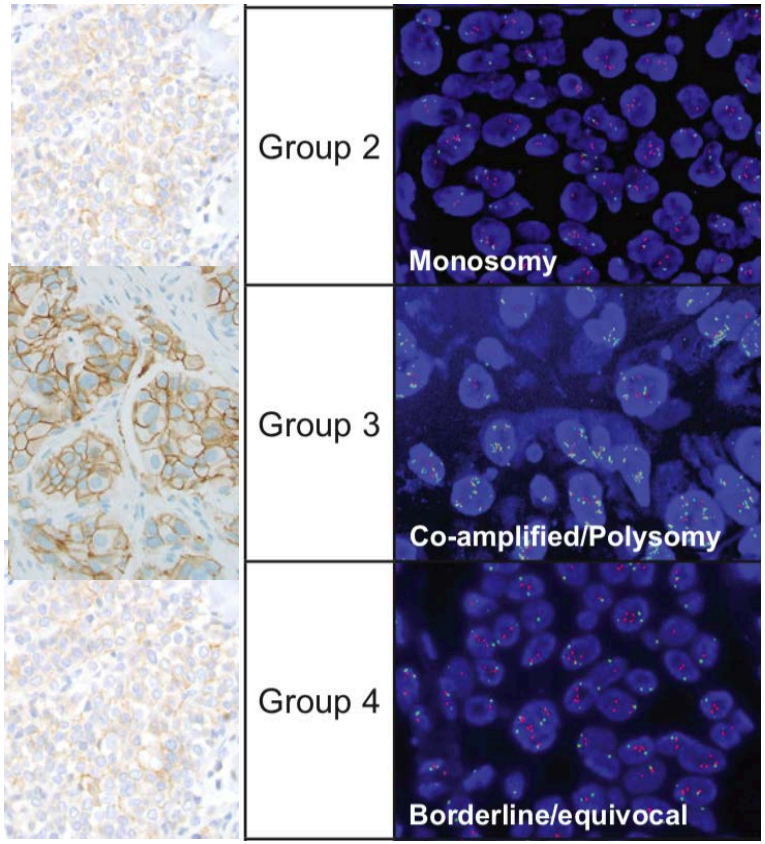
Figure 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (HER2 gene) assay (dual-probe ISH).



Additional Work-up for Group 2-4 Cases

- **Concurrent IHC review by FISH Lab:**
  - **If NEGATIVE (0-1+) → Result as HER2 NEGATIVE***
  - **If POSITIVE (3+) → Result as HER2 POSITIVE**
  - **If Equivocal (2+)→ Additional counting of FISH result by second observer, if stays in same group then result as:**
    - **NEGATIVE* if Groups 2 and 4**
    - **POSITIVE if Group 3**

      ***Comments required for these results**
- **No more FISH equivocal results!**
- **Alternative probes are not recommended as standard practice (allowed in consultation on challenging cases)**



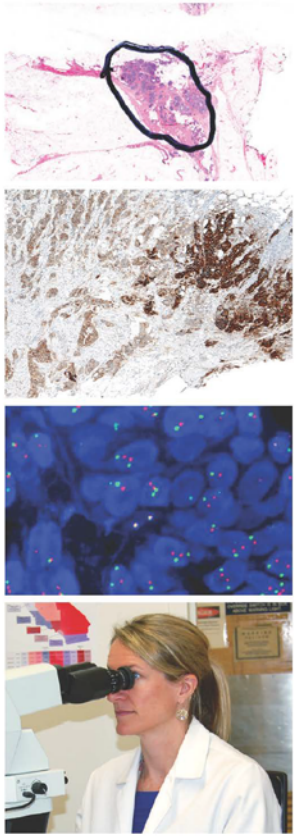
Implementation: Concurrent IHC and FISH Review

Since 2013:

The pathologist should scan the entire ISH slide before counting at least 20 cells or use IHC to define the areas of potential *HER2* amplification.

Initial Scoring of HER2 FISH Test

1	Pathologist review of H&E and/or IHC stained slide to localize invasive cancer to evaluate (exclude DCIS).
2	Review controls (repeat if not as expected).
3	Review entire slide to examine for heterogeneity or use IHC stain to guide where to count FISH. If more than one distinctly clustered population has different levels of protein expression or gene amplification, they should be scored separately.
4	Count a minimum of 20 non-overlapping cells in at least 2 separate areas (at least 10 cells/area).
5	If close to the threshold for positive (ratio 1.8-2.2 or between 4-6 HER2 signals/cell) have an additional observer count at least an additional 20 cells.
6	Pathologist review of cell counts and confirmation that the appropriate area was scored. Correlation with histology and additional findings before case interpreted and reported.



Implementation: Concurrent IHC and FISH Review

New in 2018:

For Single ISH Probe = required concurrent IHC review on *all* ISH cases

For Dual Probe only required on Group 2-4 cases to ensure counting in area of strongest staining for recount

Many labs already do concurrent IHC + FISH review

For institutions/labs that do not currently: Need to have process for dual review (local practice considerations to dictate best method)

Implementation: When to Recount ISH Results

- **Group 2-4 initial results with IHC 2+:**
  - **Labs doing FISH only on IHC 2+ cases would recount *all* Group 2-4 results (likely <10% of cases; 3% of Stanford/UCSF/UWMC cohort)**
  - **At Stanford (Dual test) we also opted to recount *all* Group 2-4 results**
    Recounts when near threshold for positive still beneficial (ratio 1.8-2.2)
- **Need resources for a blinded second count (enough techs)**
- **Final count to report: Usually average of the two counts unless different results → "result adjudicated per internal procedures to define the final result category"**

Implementation: Reporting Categories

- **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)**

Cell	HER2	CEP17
1	4	1
2	3	2
3	4	2
4	3	2
5	4	1
6	2	1
7	2	1
8	4	1
9	3	1
10	3	1
11	5	2
12	2	2
13	4	1
14	3	2
15	3	1
16	3	2
17	2	2
18	4	1
19	4	1
20	4	1
Mean	3.3	1.4
Ratio	2.4	

Test Case

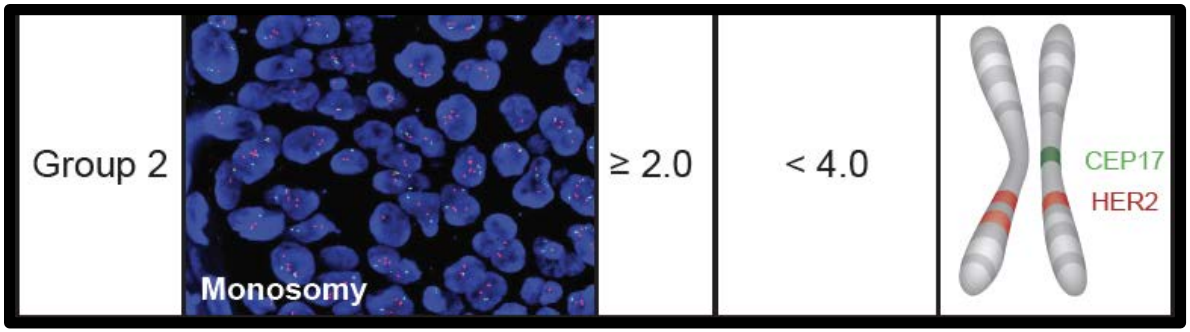
By the 2018 Update these HER2 FISH results are considered:

A. Positive

B. Negative

C. Equivocal

D. Additional work-up required



Cell	HER2	CEP17
1	4	1
2	3	2
3	4	2
4	3	2
5	4	1
6	2	1
7	2	1
8	4	1
9	3	1
10	3	1
11	5	2
12	2	2
13	4	1
14	3	2
15	3	1
16	3	2
17	2	2
18	4	1
19	4	1
20	4	1
Mean	3.3	1.4
Ratio	2.4	

Test Case

By the 2018 Update these HER2 FISH results are considered:

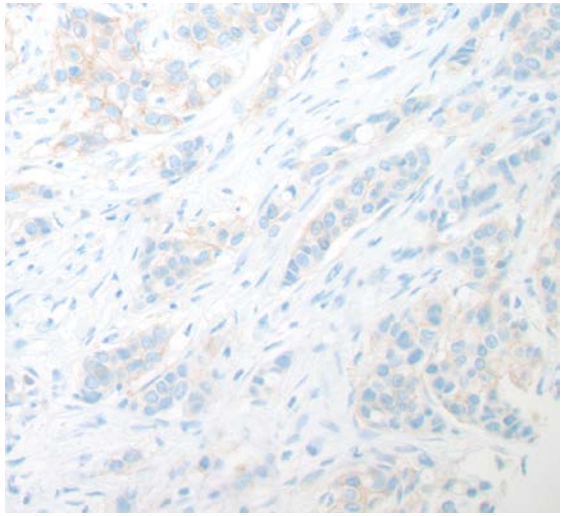
A. Positive

B. Negative

C. Equivocal

D. <u>Additional work-up required</u>

Concurrent IHC is 1+



Group 2 FISH Results

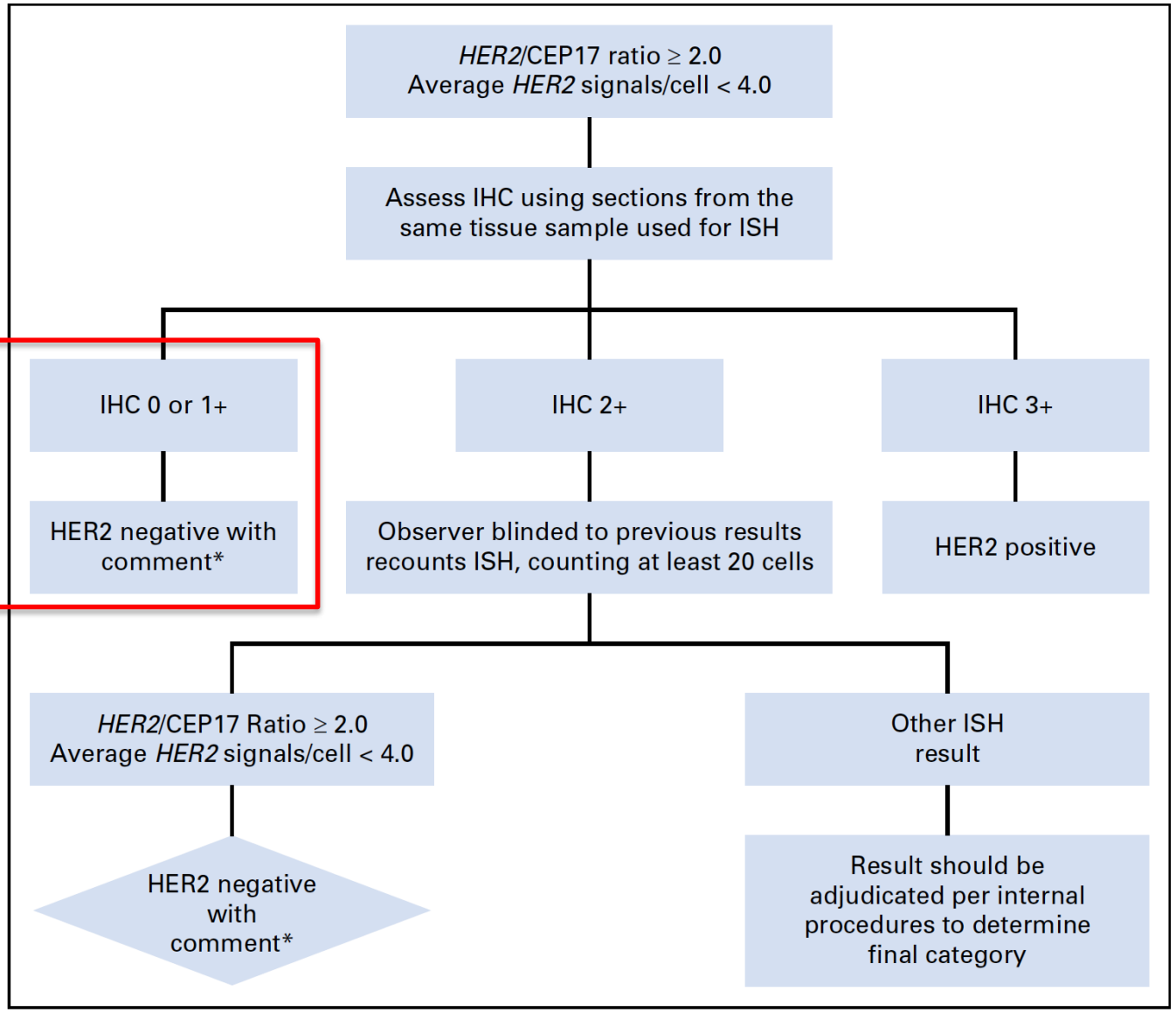


Fig 4. Clinical Question 3, group 2. (*) Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a *HER2*/CEP17 ratio ≥ 2.0 and an average *HER2* copy number of < 4.0 per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who 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. IHC expression for HER2 should be used to complement ISH and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low *HER2* copy number by ISH and the lack of protein overexpression. CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Example Report for Group 2 Result

INTERPRETATION:

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

Concurrent IHC result: 1+

COMMENT (*only required if negative*):

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.

Cell	HER2	CEP17
1	9	8
2	9	7
3	7	8
4	6	6
5	10	7
6	2	2
7	8	7
8	9	8
9	2	2
10	8	7
11	9	7
12	12	8
13	8	8
14	2	2
15	7	7
16	8	9
17	12	10
18	9	9
19	10	8
20	10	8
Mean	7.85	6.9
Ratio	1.14	

Test Case

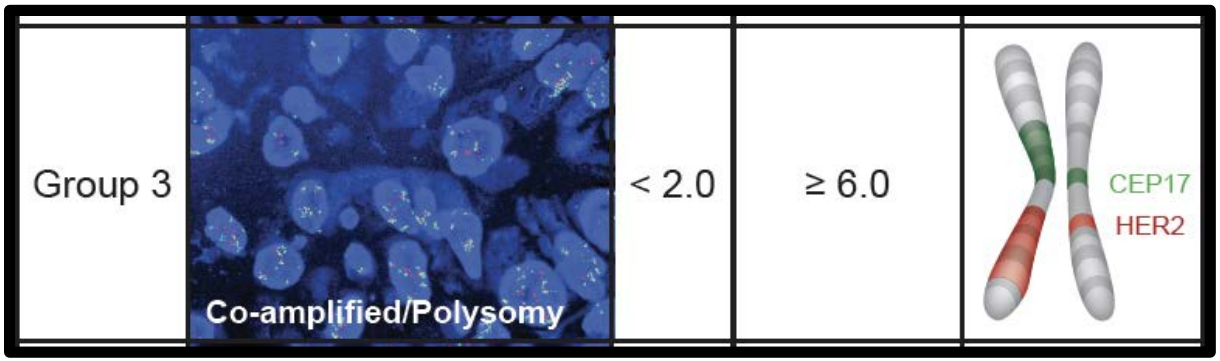
By the 2018 Update these HER2 FISH results are considered:

A. Positive

B. Negative

C. Equivocal

D. Additional work-up required



Cell	HER2	CEP17
1	9	8
2	9	7
3	7	8
4	6	6
5	10	7
6	2	2
7	8	7
8	9	8
9	2	2
10	8	7
11	9	7
12	12	8
13	8	8
14	2	2
15	7	7
16	8	9
17	12	10
18	9	9
19	10	8
20	10	8
Mean	7.85	6.9
Ratio	1.14	

Test Case

By the 2018 Update these HER2 FISH results are considered:

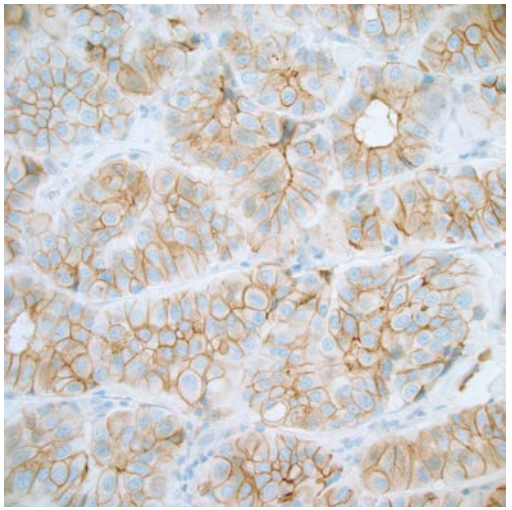
A. Positive

B. Negative

C. Equivocal

D. <u>Additional work-up required</u>

Concurrent IHC is 2+



Group 3 FISH Results

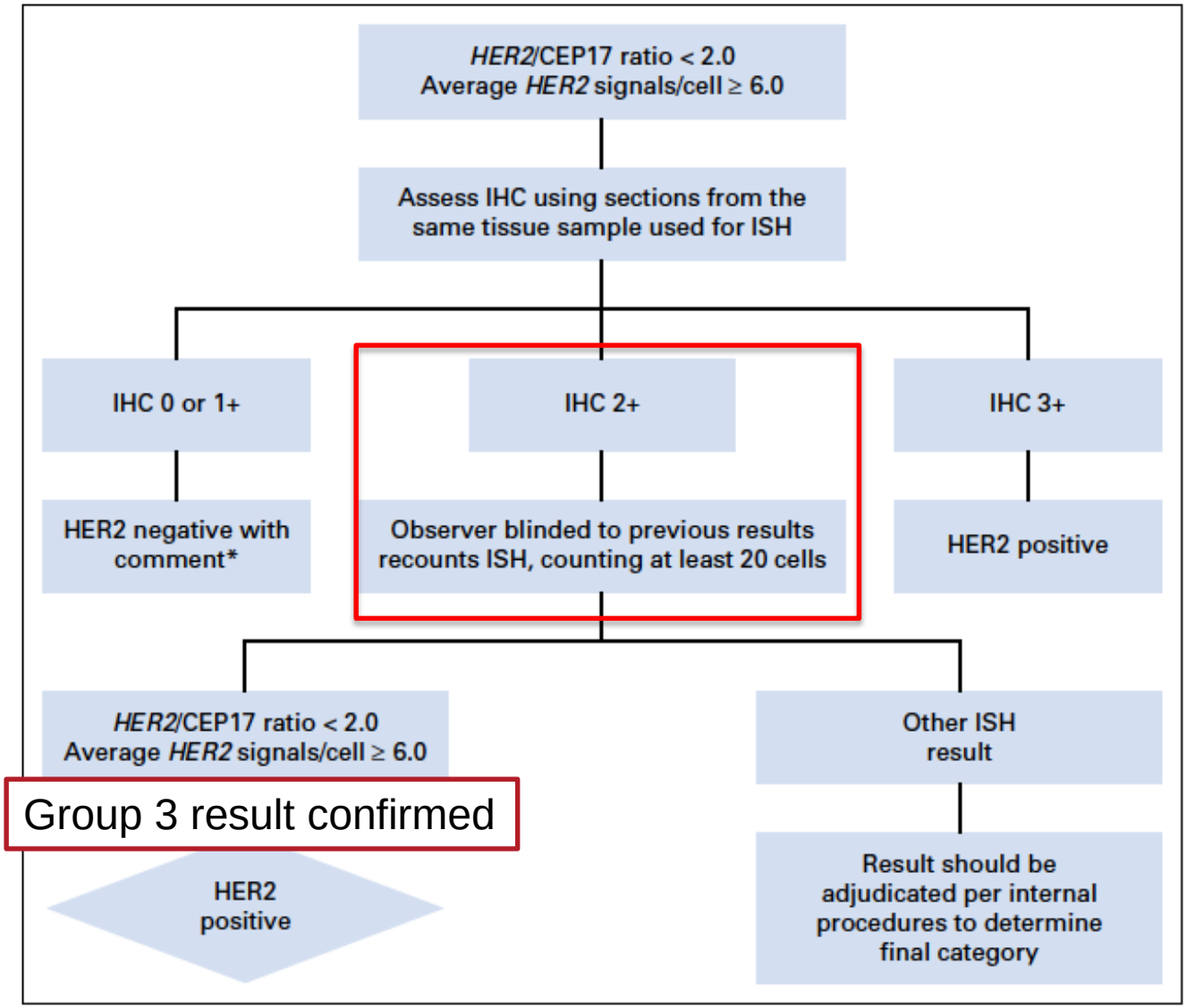


Fig 5. Clinical Question 4, group 3. (*) There are insufficient data on the efficacy of HER2-targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative. CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Example report for Group 3 Result

INTERPRETATION:

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

Concurrent IHC result: 2+

COMMENT (*not required unless negative*):

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.

Cell	HER2	CEP17
1	12	2
2	8	7
3	2	2
4	2	2
5	6	4
6	10	2
7	3	1
8	2	2
9	2	2
10	4	4
11	8	3
12	5	2
13	3	2
14	2	2
15	7	6
16	8	2
17	2	2
18	2	2
19	3	1
20	6	4
Mean	4.85	2.7
Ratio	1.79	

Test Case

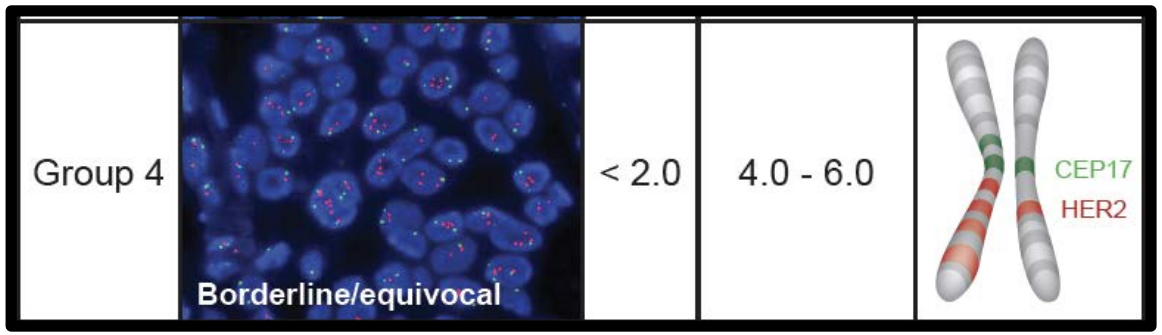
By the 2018 Update these HER2 FISH results are considered:

A. Positive

B. Negative

C. Equivocal

D. Additional work-up required



Cell	HER2	CEP17
1	12	2
2	8	7
3	2	2
4	2	2
5	6	4
6	10	2
7	3	1
8	2	2
9	2	2
10	4	4
11	8	3
12	5	2
13	3	2
14	2	2
15	7	6
16	8	2
17	2	2
18	2	2
19	3	1
20	6	4
Mean	4.85	2.7
Ratio	1.79	

Test Case

By the 2018 Update these HER2 FISH results are considered:

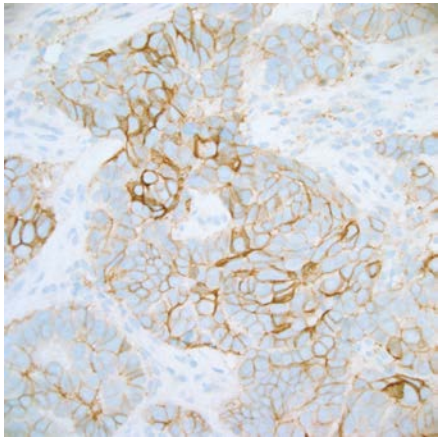
A. Positive

B. Negative

C. Equivocal

D. <u>Additional work-up required</u>

Concurrent IHC is 2+



Group 4 FISH Results

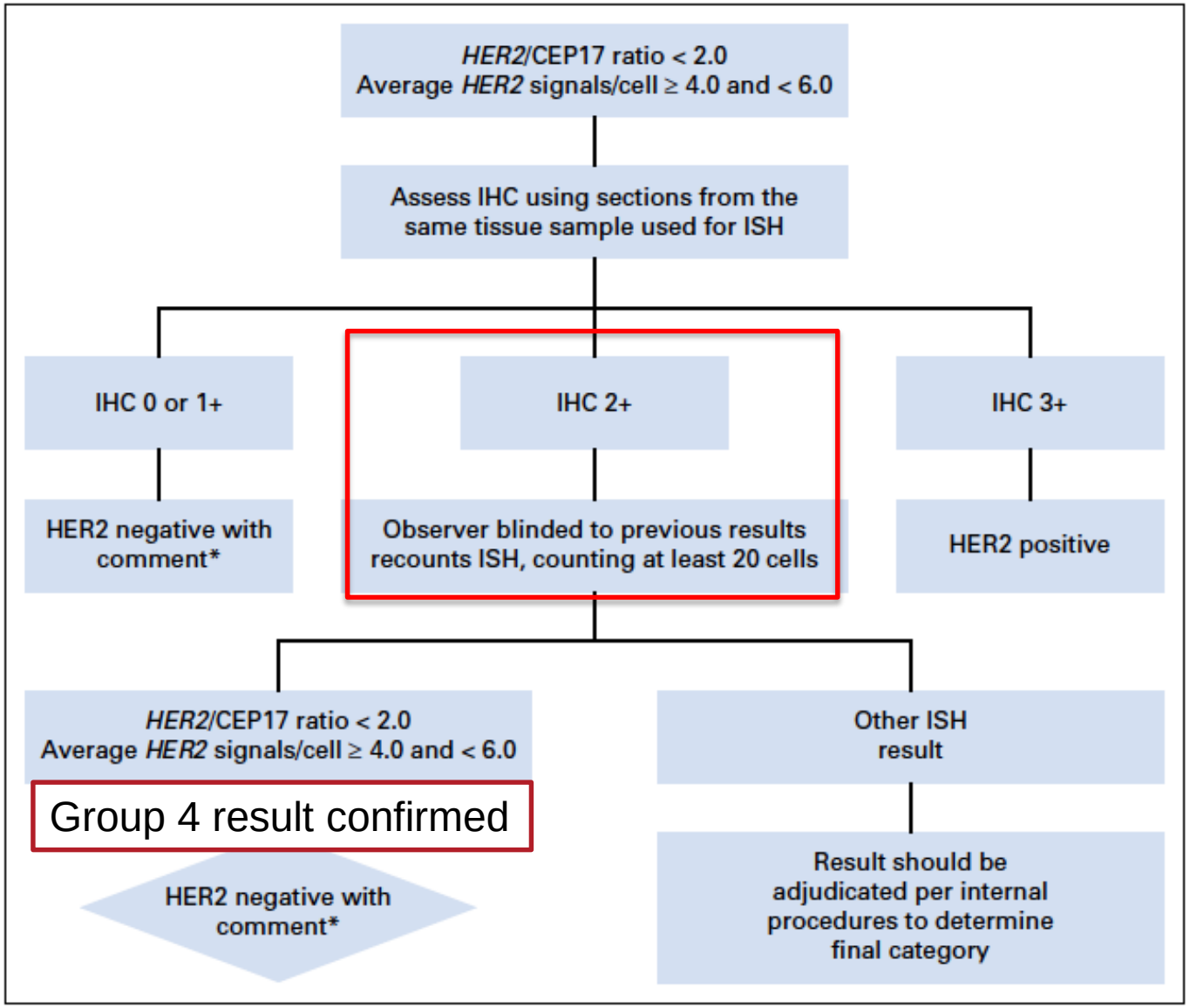


Fig 6. Clinical Question 5, group 4. (*) It is uncertain whether patients with an average of ≥ 4.0 and < 6.0 *HER2* signals per cell and a *HER2*/CEP17 ratio of < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH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

Example report for Group 4 Result

INTERPRETATION:

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

Concurrent IHC result: 2+

COMMENT (*not required unless negative*):

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.

Cell	HER2	CEP17
1	12	2
2	8	2
3	2	2
4	2	2
5	6	2
6	10	1
7	3	2
8	2	2
9	2	2
10	4	2
11	8	1
12	5	2
13	3	2
14	2	2
15	7	2
16	8	2
17	2	2
18	2	2
19	3	1
20	6	2
Mean	4.85	1.85
Ratio	2.69	

Test Case

By the 2018 Update these HER2 FISH results are considered:

A. Positive

B. Negative

C. Equivocal

D. Additional work-up required

Same mean HER2 signals as last case but mean CEP17 is lower

Cell	HER2	CEP17
1	12	2
2	8	2
3	2	2
4	2	2
5	6	2
6	10	1
7	3	2
8	2	2
9	2	2
10	4	2
11	8	1
12	5	2
13	3	2
14	2	2
15	7	2
16	8	2
17	2	2
18	2	2
19	3	1
20	6	2
Mean	4.85	1.85
Ratio	2.69	

Test Case

By the 2018 Update these HER2 FISH results are considered:

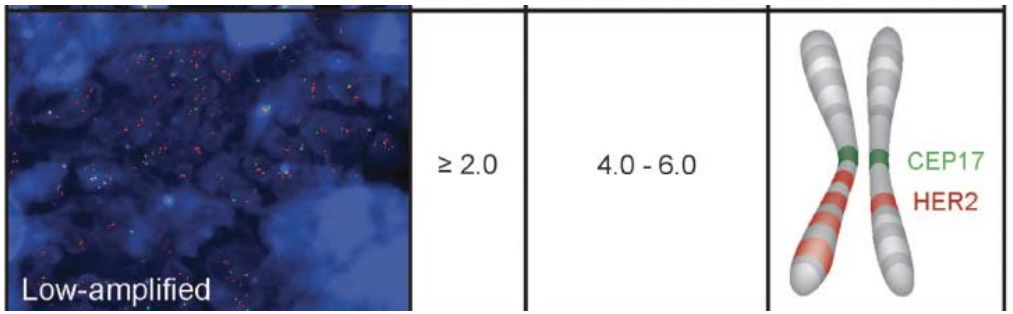
A. Positive

B. Negative

C. Equivocal

D. Additional work-up required

Same mean HER2 signals as last case but mean CEP17 is lower



Low Amplified Results: Correlate with IHC!

INTERPRETATION:

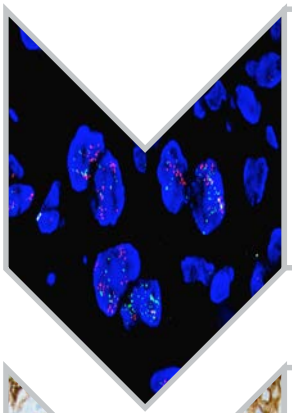
HER2 POSITIVE

Guidelines consider positive but good to correlate with IHC results

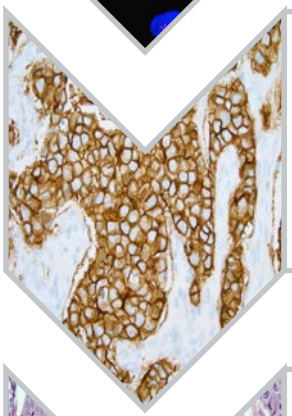
COMMENT:

This case has mildly elevated HER2 signals/cell with a ratio just above the threshold for positive. Because of this, the case was counted twice by two independent observers, whose scores were averaged for the final results. Samples with results near a threshold are statistically more likely to have variability on retesting. Of note, the IHC on this case was **. While these FISH results are considered HER2 positive by current 2018 CAP/ASCO HER2 Testing Guidelines, in the setting of such low level amplification without protein over-expression, this cancer may not behave like a typical HER2 positive cancer.**

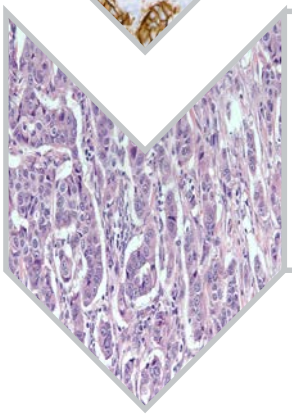
Steps for Analyzing HER2 FISH Results



- Review FISH Results:
  - Controls, where scored, counts, ratio, means



- Correlation:
  - Prior and Concurrent Results (Concurrent IHC only required for Groups 2-4), Histopath



- Additional work-up if needed:
  - Recounts for Groups 2-4 with 2+ IHC or close to threshold, Other concerns

REPORT

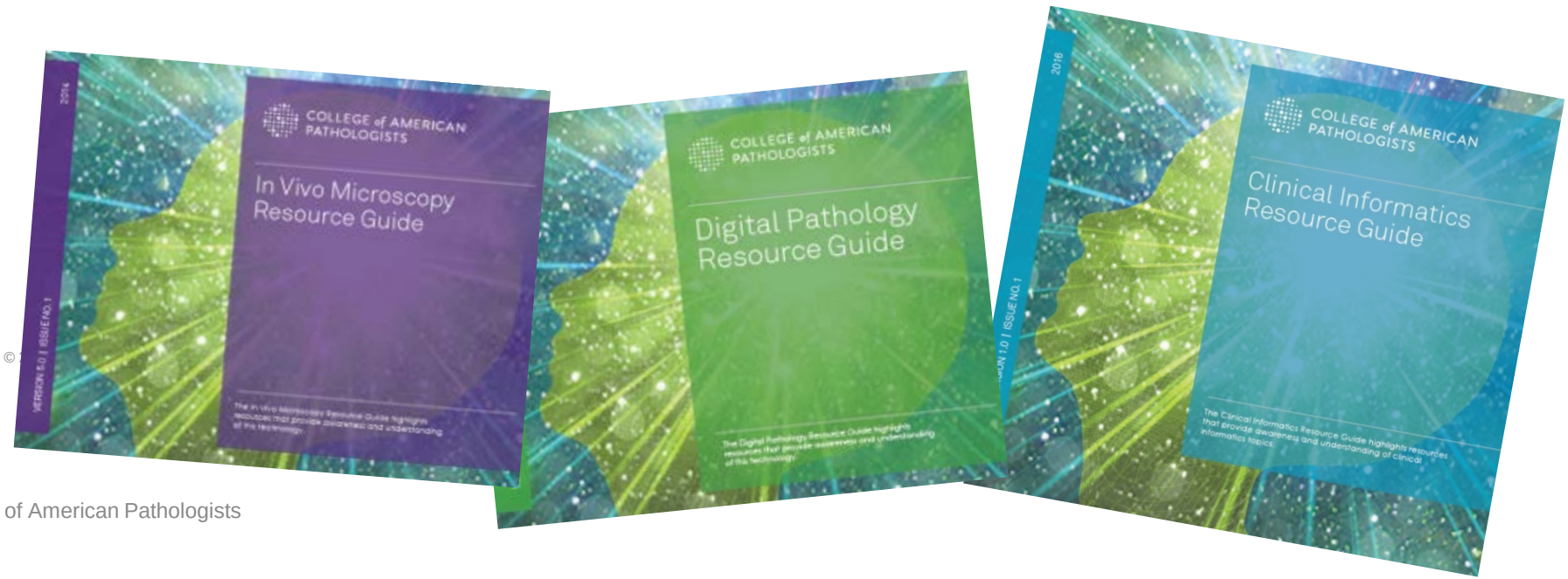
Comment on unusual or discordant results

Questions

- Questions?
- Comments?

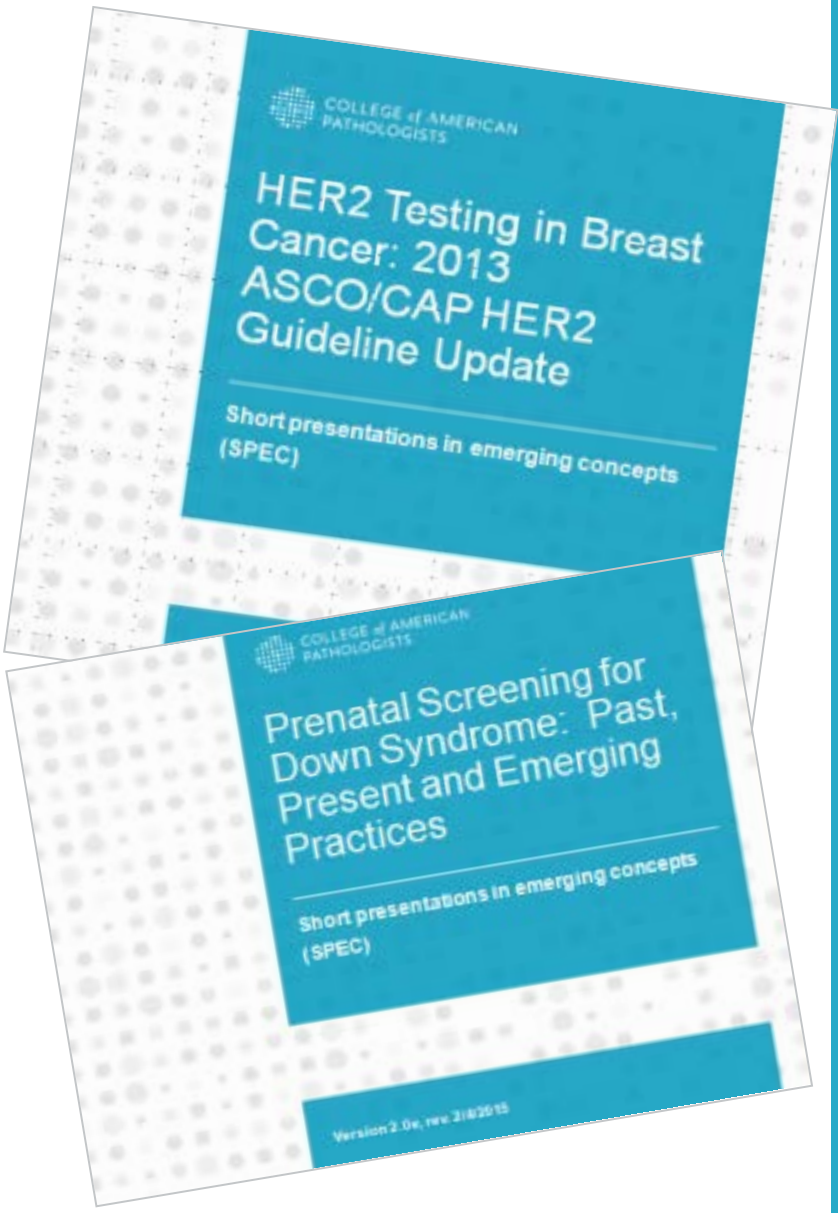
CAP's Pathology Resource Guide: Precision Medicine

- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **Short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **Recent topics include:**
  - **Microbiome**
  - **Biomarkers in Lung Cancer**
  - **MDS**
  - **Other emerging topics**
- **Access them at www.cap.org > Resources and Publications**



See, Test & Treat® brings cancer screenings to women in need!

- See, Test & Treat is a CAP Foundation-funded program that brings free, same-day cervical and breast cancer screening, diagnoses and follow-up care to women in medically underserved communities across the U.S.

- CAP member pathologists' partner with gynecologists, radiologists and other medical professionals to lead See, Test & Treat programs in hospitals, clinics and other facilities

- Women learn the importance of preventive care through annual exams, a Pap test, Mammogram and a healthy lifestyle

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Thank you for attending our webinar, "Latest Updates in HER2 Testing Breast Cancer Guidelines" by Kimberly H. Allison, MD

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