What has changed (again) in HER2 testing of breast cancers

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Disclosures

- None

ISSUES

- **Changing guidelines / positivity rates**
- **Discordance between labs**
- **IHC vs FISH**

1998

First genetically engineered drug treatment for advanced breast cancer

1998

2019

HER2 Targeted Therapies

Metastatic

HER2 Targeted Therapies

Metastatic

Adjuvant

Neoadjuvant

What we have learned in 20 years

- **HER2 targeted therapy significantly improves outcome in metastatic, adjuvant and neoadjuvant settings**
- **However, this improvement is limited to HER2 positive cancers**
- **Definition of HER2 positivity has been a moving target, frustrating clinicians and pathologists alike**
- **Initial reported rates of 25%-30% is NOT correct. It is about 15%.**

Do HER2 negative tumors benefit from targeted therapies?

NSABP-31

Some patients tested positive at local hospitals and entered trial but were found to be HER2 negative on central testing

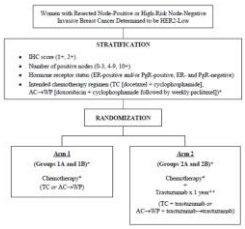
Do HER2 negative tumors benefit from targeted therapies?

Table 1. Relative Risks of Disease Progression and Death among Patients in the ACTH Group as Compared with the ACT Group.*

End Point and Central HER2 Assay†	ACT	ACTH	Relative Risk (95% CI)	P Value	P Value for the Interaction
	no. of events/total no. of events				
Disease progression					
HER2-positive	163/875	85/804	0.47 (0.37–0.62)	<0.001	0.47
HER2-negative	20/92	7/82	0.34 (0.14–0.80)	0.014	
Death					
HER2-positive	55/875	38/804	0.66 (0.43–0.99)	0.047	0.08
HER2-negative	10/92	1/82	0.08 (0.01–0.64)	0.017	

Paik et al, NEJM 2008

NSABP-47
Do women with HER2-low cancer improve DFS with targeted therapy?



NSABP-47
HER2 IHC 1+ or 2+

	Chemotherapy	Chemotherapy + Herceptin	*p*
Invasive Disease-free Survival	89.2%	89.6%	0.90
Recurrence-free Survival	92.2%	92.0%	0.97
Distant Recurrence-free Survival	92.7%	92.7%	0.55
Overall Survival	94.8%	94.8%	0.14

NSABP-47

Do women with HER2-low cancer improve DFS with targeted therapy?

NO

HER2 Testing Issues
Community vs Central Lab

18-26% of community based positive assays could not be confirmed in central lab

	Central HercepTest™ score†				
	0	1+	2+	3+	Total
Local HER2 testing					
IHC‡	8	9	12	81	110
FISH	1	1	0	7	9
Total	9	10	12	88	119

	Central FISH result§		
	Not amplified	Amplified	Total
Local HER2 testing			
IHC	37	73	110
FISH	3	6	9
Total	40	79	119

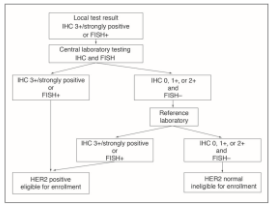
Paik et all JNCI 2002
Roche et al JNCI 2002

IHC vs FISH

HER2 Testing by Local, Central, and Reference Laboratories in Specimens From the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial

Perez et al JCO 2006

IHC vs FISH



IHC vs FISH

- **Discordance rate between local and central HER2 test results:**
  - **IHC: 18.4%**
  - **FISH: 11.9%**

Table 2. Concordance Between Central and Local Laboratories

Test at Local Laboratory	Specimens Confirmed by Central Testing* (No.)	Agreement With Central Laboratory		
		%	95% CI	Method
HercepTest	1,083	81.6	79.1% to 83.9%	HercepTest
Non-HercepTest	636	74.5	71.4% to 78.3%	HercepTest
FISH	813	88.1	85.6% to 90.2%	FISH

NOTE. HercepTest, DAKO, Carpinteria, CA.
Abbreviation: FISH, fluorescence in situ hybridization.
*Testing using the same method at both laboratories was not possible for 23 specimens.

Perez et al JCO 2006

Is FISH more reproducible than IHC?

- Breast Cancer International Research Group (BCIRG)
- ~2600 women, prospective, Herceptin based clinical trials
- Outside/Local labs vs Central Labs:
  - **79%** agreement between **local IHC** and **central FISH**
  - **77.5%** agreement between **local IHC** and **central IHC**
  - **92%** agreement between **local FISH** and **central FISH**

- CAP
  - 100% agreement between FISH labs
  - 72.3% agreement between IHC labs

What is HER2 Positive?

Initial Clinical Trials

HER2 positive defined as weak to moderate (2+) or strong (3+) circumferential membrane staining in >10% of the tumor cells

HER2 positive metastatic breast cancer:

- **Herceptin monotherapy effective in patients who failed treatment with prior chemotherapy**
- **Herceptin + chemotherapy is more effective than chemotherapy alone**

Despite targeted therapy companion diagnostic test we have had two decades of problems

HER2 Testing Issues

- **Antibody used in HercepTest and in the antibodies used in clinical trials (4D5 and CB11) are not the same.**
- **HercepTest was not evaluated in a clinical trail before its FDA approval**
- **It shows 79% concordance with clinical trials assay**
- **There was no standardization of pre-analytic factors (ischemic time, fixation time)**
- **Variations in testing, interpretation and reporting**

Early days of testing

- **FDA Criteria**
- 2007 ASCO/CAP Guidelines
- 2013 ASC0/CAP Guidelines
- 2018 Modifications to 2013 Guidelines

- **Lack of standardization**
  - **Preanlytical: ischemic time, fixation time**
  - **Analytic**
  - **Post-analytic**
- **High number of false positives**

- FDA Criteria
- **2007 ASCO/CAP Guidelines**
- **2013 ASCO0/CAP Guidelines**
- **2018 Modifications to 2013 Guidelines**

ASCO/CAP Guidelines

	Goal	FISH	IHC
2007 ASCO/CAP	**Reduce false positive results**	**Ratio >2.2 (dual probe) ≥6 HER2 (single probe)**	**>30%**
2013 ASCO/CAP	Reduce false negative results	Ratio >2.0 (dual probe) ≥6 HER2 (single probe)	>10%
2018 ASCO/CAP	Addresses issues with less common dual FISH pattern	Ratio >2.0 (dual probe) ≥6 HER2 (single probe)	>10%

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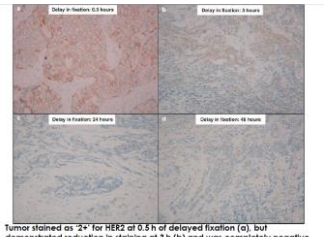
What has NOT changed?

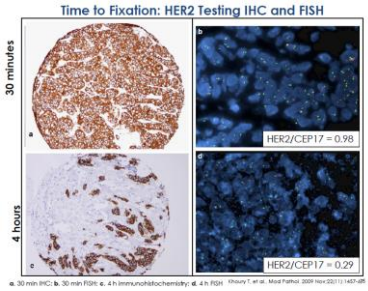
Specimen handling is <u>critical!</u>

- **Breast tissue undergoes ischemic changes from the minutes it is removed from the patient**
- **Enzymatic activity is not stopped until fixation begins**
- **Breast tissue should be cut and placed in 10% NBF within less than 1 hour of removed from the patient**



Tumor stained as '2+' for HER2 at 0.5 h of delayed fixation (a), but demonstrated reduction in staining at 3 h (b) and was completely negative at 24 h (c) and 48 h (d).

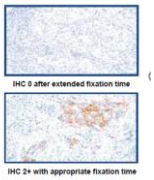
Yildiz-Aktas IZ, et al. Mod Pathol. 2012 Aug;25(8):1098-105.

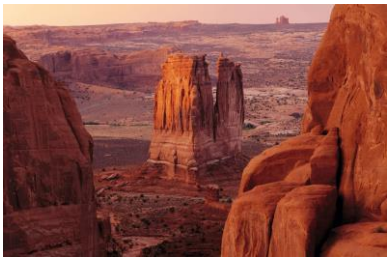


a. 30 min IHC; b. 30 min FISH; c. 4 h immunohistochemistry; d. 4 h FISH Khoury T, et al. Mod Pathol. 2009 Nov;22(11):1457-67.

Time in Fixation

- 6-72 hours
- Cores and excisions need similar time in fixation





2018 ASCO / CAP 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

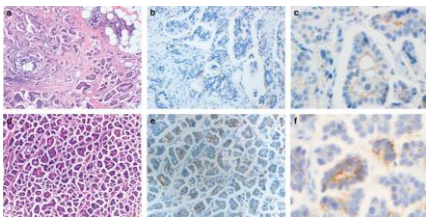
Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, Mitchell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

2018 ASCO / CAP Update

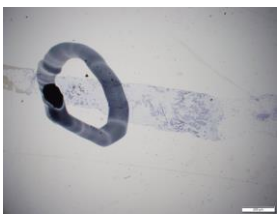
- **Clinical Question 1 :**
  - *What is the most appropriate definition for IHC 2+ (IHC equivocal)?*
  - 2013 HER2 Testing Update as invasive breast cancer showing "circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of tumor cells or complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells."
  - Revised / 2018 definition of IHC 2+(equivocal) is invasive breast cancer with "weak to moderate complete membrane staining observed in > 10% of tumor cells"

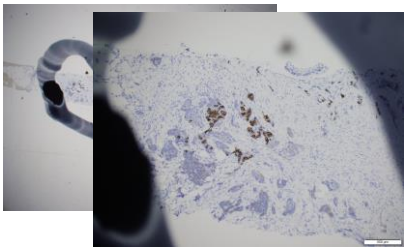
Uncommon patterns that are not covered by these definitions but should be considered 2+ / equivocal:

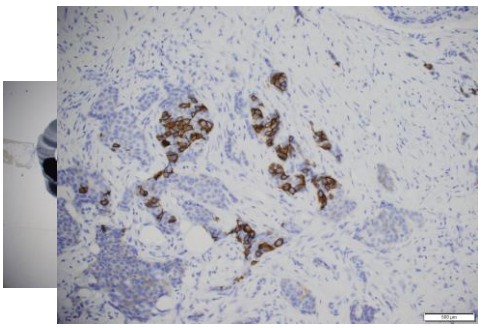
- Moderate to intense but incomplete (basolateral or lateral) staining but can be found to be HER2 amplified
  - Micropapillary carcinoma
- Intense ≤10% circumferential membrane staining

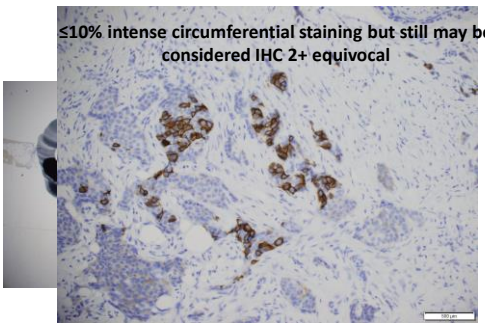


Micropapillary carcinoma with incomplete basolateral staining where HER2 FISH was amplified








≤10% intense circumferential staining but still may be considered IHC 2+ equivocal

2018 ASCO / CAP Update

- **Clinical Question 2**
  - *Must HER2 testing be repeated on a surgical specimen if initially negative test on core biopsy?*
  - HER2 testing ***may*** be repeated on the surgical specimen if initially negative on core biopsy

ASCO/CAP Guidelines

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2018 ASCO / CAP Update
FISH related questions

Clinical Question 3

Should invasive cancers with an *HER2*/chromosome enumeration probe 17 (CEP17) ratio of ≥2.0 but an average *HER2* copy number of <4.0 signals per cell be considered ISH positive?

Clinical Question 4

Should invasive cancers with an average *HER2* copy number of ≥6.0 signals per cell but a *HER2*/CEP17 ratio of <2.0 be considered ISH positive?

Clinical Question 5

What is the appropriate diagnostic workup for invasive cancers with an average HER2 copy number of ≥4.0 but <6.0 signals per cell and an *HER2*/CEP17 ratio of <2.0, and initially deemed to have an equivocal HER2 ISH test result?

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

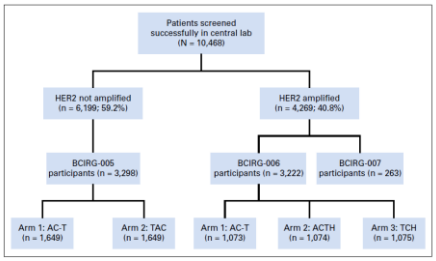
HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials

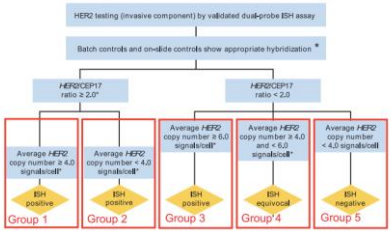
BCIRG

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, Valérie Bac, Yanling Ma, Ivonne Villalobos, Anuamika Campeau, Martina Mrsic, Mary-Ann Lindsay, and Dennis J. Slamon

HER2 FISH Groups of Breast Cancers Screened for Patient Enrollment Onto BCIRG Trials, 2000-2004

ASCO-CAP FISH Group	Description of HER2 FISH Category	No. of Cases (%)
1	Ratio ≥ 2.0, *HER2* average ≥ 4.0	4,269 (40.8)
2	Ratio ≥ 2.0, *HER2* average < 4.0	71 (0.7)
3	Ratio < 2.0, *HER2* average ≥ 6.0	55 (0.5)
4	Ratio < 2.0, *HER2* average ≥ 4.0, < 6.0	432 (4.1)
5	Ratio < 2.0, *HER2* average < 4.0	5,641 (53.9)
Total*		10,468* (100.0)





Group 1

HER2/CEP17≥2.0

Average HER2 signal / cell ≥ 4.0 **(FISH Positive)**

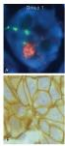


Table 1. Comparison of *HER2* Ratio and Average *HER2* Gene Copy Number and ASCO-CAP Groupings With Clinical Outcomes in BCIRG-006

HER2 FISH (*HER2*/CEP17) Ratio	*HER2* Copies per Cell	No. of Subjects	DFS Control, Events/No. of Subjects	DFS Trastuzumab, No. of Events/ Subjects	DFS, HR (95% CI)*	DFS *P* for Log-Rank Test*	OS Control	OS Trastuzumab	OS, HR (95% CI)*	OS *P* for Log-Rank Test*	ASCO-CAP FISH Group
≥ 2.0	≥ 4.0	46	4/18	6/28	1.16 (0.31 to 3.88)	.8663	2/16	4/28	1.19 (0.36 to 28.83)	.2830	Group 2
	< 4	3,109	251/1,031	391/2,078	0.71 (0.60 to 0.83)	< .0001	338/1,031	352/2,078	0.69 (0.59 to 0.80)	< .0001	Group 1
Total		3,155									

NOTE. The HRs are for trastuzumab treatment arms compared with control chemotherapy-only arm. There were too few patients (n = 5) accrued to BCIRG-006 with a *HER2* FISH ratio < 2.0 and *n* < 60 average *HER2* gene copy number/tumor cell for analysis of the HR.

Abbreviations: BCIRG, Breast Cancer International Research Group; CAP, College of American Pathologists; DFS, disease-free survival; *HER2* human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.

*Trastuzumab-containing treatment arms compared with control (chemotherapy alone) treatment arm.

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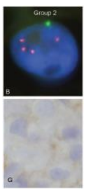
Group 2

HER2/CEP17≥2.0

Average HER2 signal / cell < 4.0 **(FISH Positive)**



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	< 4	3,109	251/1,031	391/2,078	0.71 (0.60 to 0.83)	< .0001	338/1,031	352/2,078	0.69 (0.59 to 0.80)	< .0001	Group 1
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*Trastuzumab-containing treatment arms compared with control (chemotherapy alone) treatment arm.

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Group 3

HER2/CEP17<2.0

Average HER2 signal / cell ≥ 6.0 **(FISH Positive)**

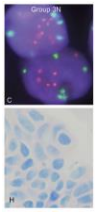

Table 3. Comparison of *HER2* Ratio and Average *HER2* Gene Copy Number and ASCO-CAP Groupings With Clinical Outcomes in BCIRG-005

HER2/FISH (*HER2*/CEP17) Ratio	*HER2* Copies per Cell	No. of Subjects	DFS, No. of Events	OS, No. of Events	DFS HR (95% CI) and *P* for Log-Rank Test*	OS HR (95% CI) and *P* for Log-Rank Test*	ASCO-CAP FISH Group
< 2.0	< 4.0	3,079	971	606	1.0 (reference)	1.0 (reference)	Group 5
	4.0-5.9	176	51	30	0.923 (0.697 to 1.224)	0.878 (0.609 to 1.267)	Group 4
					P = .5795	*P* = .4872	
	≥ 6	11	6	4	2.502 (1.121 to 5.583)	2.351 (0.879 to 6.284)	Group 3
					P = .0252	*P* = .0885	

NOTE. The hazard ratios are for each ASCO group compared with ASCO Group 5 taken as the reference. There were too few patients accrued to BCIRG-005 with a *HER2* FISH ratio ≥ 2.0 for analysis of DFS or OS.
Abbreviations: BCIRG, Breast Cancer International Research Group; CAP, College of American Pathologists; DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.
*Group 5 (reference) compared with each other group in BCIRG-005 (*HER2* not amplified breast cancers).

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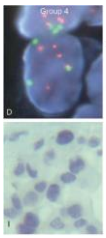
Group 4

HER2/CEP17<2.0

Average HER2 signal / cell ≥ 4.0 and <6.0 **(FISH Equivocal)**



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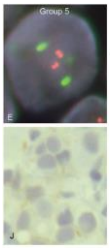
Group 5

HER2/CEP17<2.0

Average HER2 signal / cell < 4.0 **(FISH Negative)**



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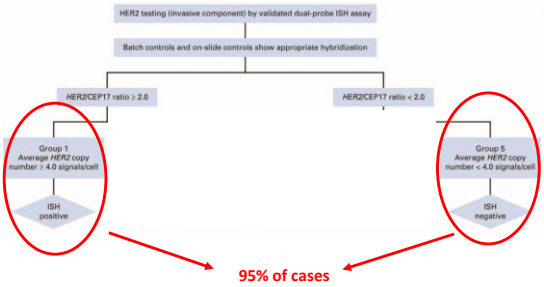
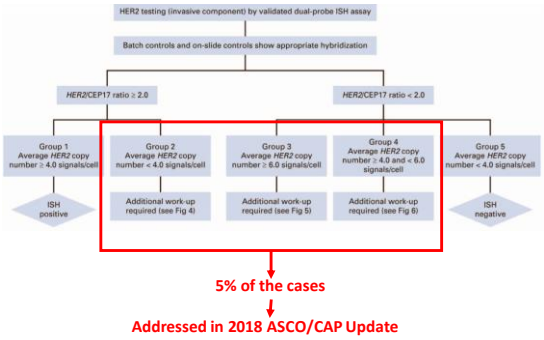
*Group 5 (reference) compared with each other group in BCIRG-005 (*HER2* not amplified breast cancers).

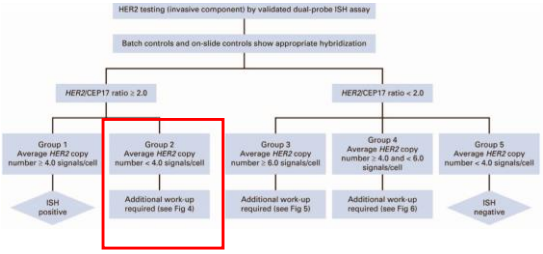
Press JCO 2016





2018 ASCO/CAP Update for Less Common FISH Patterns

- It is not based only on FISH but a combination of FISH and IHC testing.
- Requires review of IHC before designation of HER2 status (positive or negative)

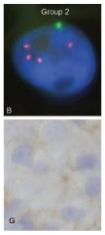


2018 ASCO / CAP Update

Clinical Question 3 (Group 2) :

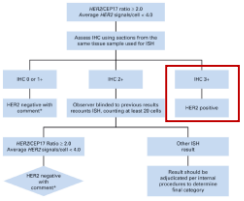
- FDA: trastuzumab regardless of HER2 copy number; 2013 ASCO/CAP considered these as positive
- Rare: 0.8% in HERA trial ; 0.7 % in BCIRG
- HERA trial : "Sample size insufficient to r/o benefit"

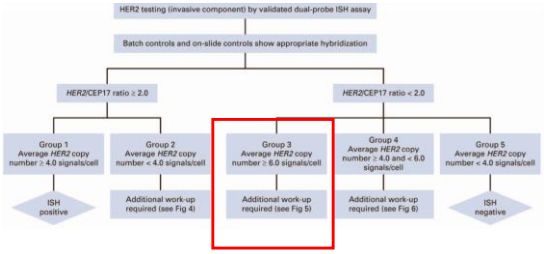
- Almost always HER2 negative by IHC
- Most are estrogen receptor (ER) positive



2018 ASCO / CAP Update

Clinical Question 3 (Group 2) :

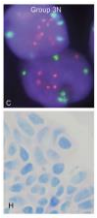




2018 ASCO / CAP Update

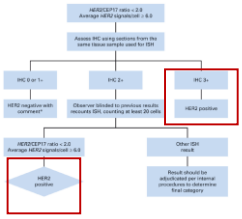
Clinical Question 4 (Group 3) :

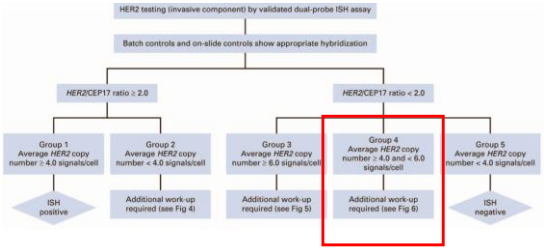
- Heterogeneous group: HER2 + and HER2-ive by IHC
  - HERA trial: 75% of 20 cases were IHC positive / 3+
  - Trial with three centers: 31% of 63 cases were IHC positive / 3+
  - USC: 8.3% of 48 cases were IHC positive / 3+



2018 ASCO / CAP Update

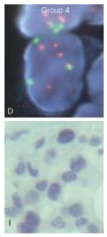
Clinical Question 4 (Group 3) :



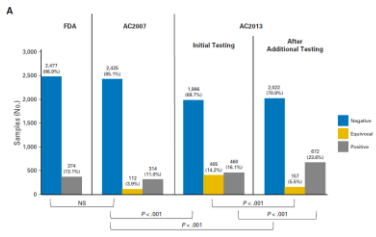


2013 ASCO/CAP
FISH Equivocal

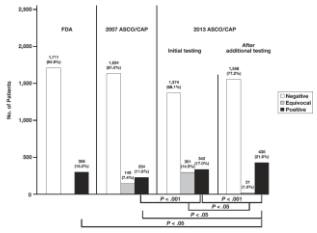
- **Mayo Clinic:** 14% of all FISH cases were equivocal→50% of which became positive with alternate probe (D17S122) increasing overall FISH positivity to ***23.6%***
- **ARUP** : 15% of all FISH cases were equivocal →30% of which became positive with alternate probe (RIA1) increasing overall FISH positivity to ***21.6%***
- ***Some labs used 4 or more FISH alternate probes, reported the positive one, increasing the overall FISH positivity rate even further***



Mayo Clinic



University of Utah / ARUP



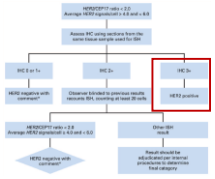
2018 ASCO / CAP Update

Clinical Question 5 (Group 4) :

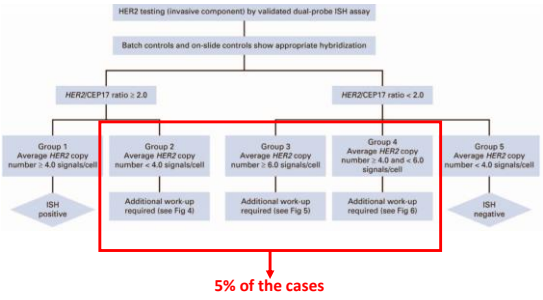
NO ALTERNATE PROBE !

2018 ASCO / CAP Update

Clinical Question 5 (Group 4) :



What to expect after 2018 ASCO/CAP Update?



2018 ASCO / CAP Update

Table 3. Distribution by Dual Fluorescent In Situ Hybridization (FISH) and Immunohistochemistry (IHC) Testing Results in Reported Data Sets[a]

Initial Test Results	Laboratory: HERA Central Laboratory[9]	BCIRG Central Laboratory[14]	USC Breast Cancer Analysis Laboratory[11]	Mayo Clinic Cytogenetics Laboratory[12]	UK NEQAS 2009-2014[5]	Stanford/ UCSF/ UNMC[14]
FISH distribution						
No.	6018	10 468	7526	2851	11 116	8068
Group 1 ratio ≥2.0; *HER2* ≥4.0	55.0 (≥6.0, 48.7; ≥4.0 and <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	0.9	1.3	1.7	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 and <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	23.9	76.7	69.6	73.4	78.8
IHC distribution						
No.	3089	4331	7526	1922	11 116	3027
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+)[8]	61.8	13.7	9.0	87.18	96.58	2+, 46.6
3+	36.2	22.4	8.4	2.5	1.3	3+, 15.3

2018 ASCO / CAP Update

Table 3. Distribution by Dual Fluorescent In Situ Hybridization (FISH) and Immunohistochemistry (IHC) Testing Results in Reported Data Sets[a]

Initial Test Results	Laboratory					
	HERA Central Laboratory[15]	BCIRG Central Laboratory[16]	USC Breast Cancer Analysis Laboratory[13]	Mayo Clinic Cytogenetics Laboratory[17]	UK NEQAS 2009-2016[9]	Stanford/ UCSF/ UWMC[14]
FISH distribution						
No.	6058	10 468	7526	2851	11 116	8068
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	0.9	0.5	0.4	1.2	0.7	1.4
Group 3 ratio <2.0; *HER2* ≥6.0	0.4	0.5	0.6	1.2	1.1	0.8
Group 4 ratio <2.0; *HER2* ≥4.0 and <6.0 (after alternative probe: pos, equivocal, neg)	1.9	4.1	4.0	14.2 (7.5, 5.5, 1.3)	7.6	5.2
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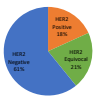
3+	36.2	22.4	8.4	2.5	1.3	3+, 15.3

In most labs , these three groups will be ~5-10% of all FISH cases. However, the proportion will be much higher in reference lab setting.

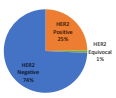
Almost 1/4th (127/521; 24.4%) of all HER2 FISH tests from primary or metastatic breast cancers at the University of Utah / ARUP Labs fell under the three groups (Groups 2,3, and 4)

2018 ASCO/CAP recommendations may result in some drop in HER2 FISH positivity rate which may be limited to reference labs.

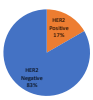
Reference Lab / ARUP HER2 FISH Results



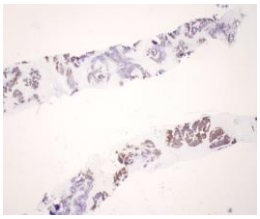
2013 ASCO/CAP
(before alternate probe)

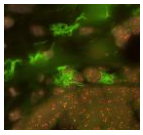


2013 ASCO/CAP
(after alternate probe)

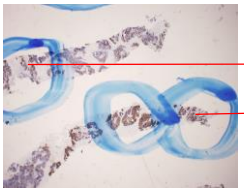


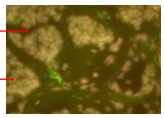
2018 ASCO/CAP





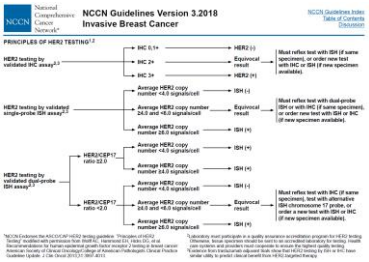
HER2/CEP17 Ratio <2.0
HER2 signal /cell ≥ 4.0 and <6.0
FISH Equivocal





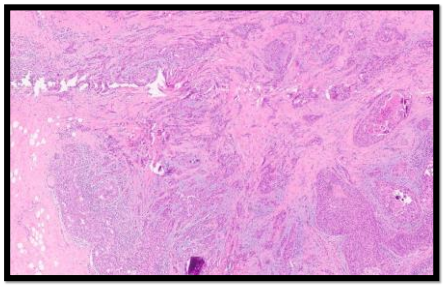
HER2/CEP17 Ratio >2.0
FISH Positive

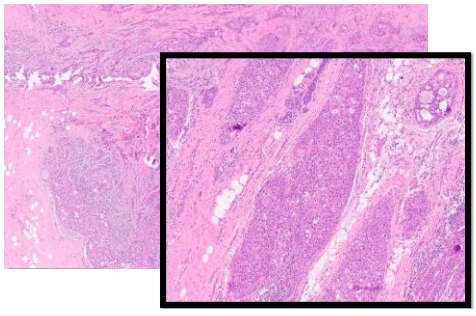
NCCN Guidelines NOT Updated

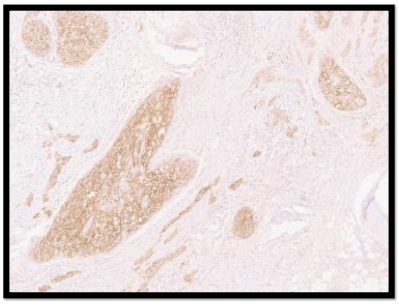


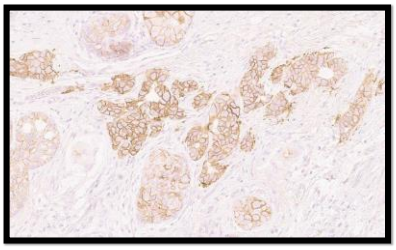
Common Problem in Interpretation of HER2 IHC

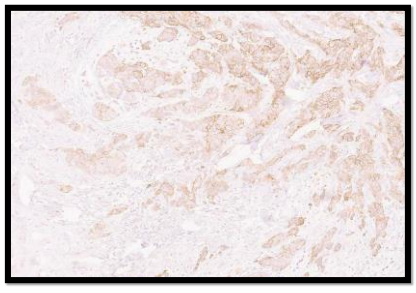
- Overcalling 2+ / Equivocal HER2 as positive (3+)
  - When there is heterogeneous IHC staining i.e. some areas look like 3+ and others 0-2+ → stop and think before calling it 3+
  - Most HER2 IHC positives (3+) are homogenously positive and you do not need a microscope to call it positive !











Lastly …

If you are using ink for breast cores to prevent specimen mix-up , avoid using orange ink as it auto- fluoresces and interferes with FISH interpretation.